


Please cite the Published Version

Britten, NS and Butler, JA  (2022) Ruthenium Metallotherapeutics: Novel Approaches to Combating Parasitic Infections. *Current Medicinal Chemistry*, 29 (31). pp. 5159-5178. ISSN 0929-8673

DOI: <https://doi.org/10.2174/0929867329666220401105444>

Publisher: Bentham Science Publishers

Version: Accepted Version

Downloaded from: <https://e-space.mmu.ac.uk/631758/>

Additional Information: This is an Author Accepted Manuscript of an article published in *Current Medicinal Chemistry*, by Bentham Science Publishers.

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

1 **Ruthenium metallotherapeutics: novel approaches to combatting**
2 **parasitic infections.**

3

4 Nicole S. Britten¹ and Jonathan A. Butler^{1*}

5

6 ¹Faculty of Science and Engineering, Manchester Metropolitan University, Manchester,
7 United Kingdom

8

9

10

11 *Corresponding author

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

13

14

15

16

17

18

19

20

21

22

23

24

25 **Abstract**

26 Human parasitic infections cause a combined global mortality rate of over one million people per annum and
27 represent some of the most challenging diseases for medical intervention. Current chemotherapeutic strategies
28 often require prolonged treatment, coupled with subsequent drug-induced cytotoxic morbidity to the host, while
29 resistance generation is also a major concern. Metals have been used extensively throughout the history of
30 medicine, with more recent applications as anticancer and antimicrobial agents. Ruthenium metallotherapeutic
31 antiparasitic agents are highly effective at targeting a range of key parasites, including the causative agents of
32 malaria, trypanosomiasis, leishmaniasis, amoebiasis, toxoplasmosis and other orphan diseases, while
33 demonstrating lower cytotoxicity profiles than current treatment strategies. Generally, such compounds
34 also demonstrate activity against multiple cellular target sites within parasites, including inhibition of enzyme
35 function, cell membrane perturbation, and alterations to metabolic pathways, therefore reducing the opportunity
36 for resistance generation. This review provides a comprehensive and subjective analysis of the rapidly developing
37 area of ruthenium metal-based antiparasitic chemotherapeutics, in the context of rational drug design and potential
38 clinical approaches to combatting human parasitic infections.

39

40 **Running title:** Ruthenium-based compounds as antiparasitic agents.

41

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

43

44

45

46

47

48

49

50

51

52

53 **1. Introduction**

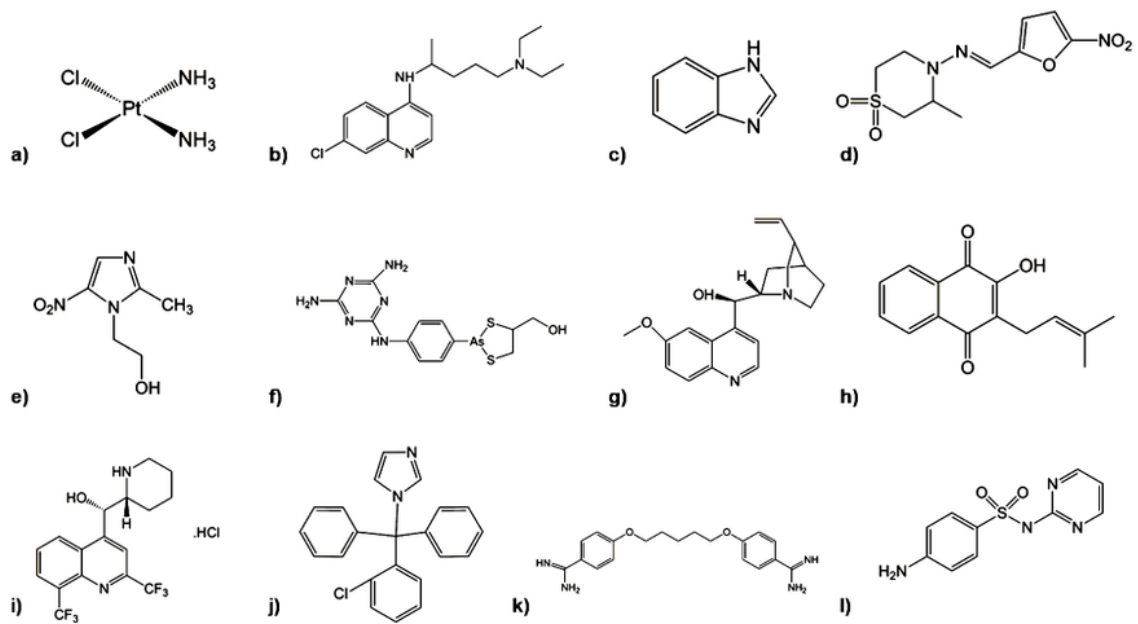
54 Ruthenium (Ru) is a transition metal found in Group 8 of the periodic table, with an atomic mass of 101.07.
55 Specifically, Ru is among the platinum group of transition metals, also known as precious metals, due to their
56 rarity. Ru is currently used in chemistry as a catalyst for various reactions [1] as well as in histology as a
57 polycationic stain called Ruthenium Red. It is typically used to stain mucopolysaccharide structures but may also
58 be used to trace cellular mechanisms for their study [2].

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

70 In the 1960s, the antimicrobial activity of Ru (II) mononuclear complexes was first studied against Gram-
71 positive, Gram-negative, and acid-fast bacteria [5]. The initial compound, $[\text{Ru}(\text{phen})_3]^{2+}$, was found to have little
72 to no activity against all bacteria. However, the addition of methyl groups to the bidentate phenanthroline
73 ligands, creating $[\text{Ru}(\text{Me}_4\text{Phen})_3]^{2+}$, caused a significant increase in antibacterial activity, most notably against
74 Gram-positive bacteria. It was also demonstrated that resistance generation was less likely when compared to
75 traditional antibiotics [5]. Subsequent studies discovered that the antibacterial effects were due to the compound
76 binding to the major groove of DNA [6]. More recently, dinuclear poly pyridyl ruthenium(II) compounds have
77 been investigated, mainly due to their higher affinity for DNA, with subsequent increased DNA binding ability.
78 These compounds were found to be highly active against a number of bacteria [7]. A diverse range of other
79 antibacterial Ru-based compounds have now been synthesised, all featuring a common Ru-based elemental core
80 [8].

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

83 chloroquine (Fig. 1b) in the form of $[\text{RuCl}_2(\text{chloroquine})]_2$. [9] This demonstrated significantly increased activity
 84 when compared to chloroquine alone [9]. Ru-based compounds are considered a potential treatment option due
 85 to lower host cytotoxicity, effective biodistribution, and different mechanisms of antiparasitic action compared to
 86 current chemotherapeutics [10].
 87 Current antiparasitic treatment options often cause significant side effects [11], and require prolonged treatment
 88 regimens [12]. One study showed 89.8% of 176 patients treated with benznidazole (Fig. 1c) and nifurtimox (Fig.
 89 1d) for trypanosomiasis experienced ad-verse effects, with mucocutaneous and digestive symptoms being
 90 recorded, respectively [12]. Metronidazole (Fig. 1e) which is used to treat amoebiasis is tolerated well in the
 91 host, however minor side effects such as nausea, diarrhoea, vomiting, and mouth dryness are common and
 92 potentially result in premature termination of treatment [13]. Indeed, longer-term use of metronidazole is linked
 93 to more serious side effects involving neurological disorders such as peripheral neuropathy. Melarsoprol (Fig.
 94 1f) treatment for African trypanosomiasis has a 50% fatality rate due to complications such as posttreatment
 95 reactive encephalopathy (P-TRE) that can occur 1-10 days after starting treatment in 5-10% of patients caused
 96 by the rapid destruction of the parasite within the central nervous system [14].



97
 98 Fig. (1). Current antiparasitic treatments (a) Cisplatin, (b) Chloroquine, (c) Benznidazole, (d) Nifurtimox, (e)
 99 Metronidazole, (f) Melarsoprol, (g) Quinine, (h) Lapachol, (i) Mefloquine, (j) Clotrimazole, (k) Pentamidine, (l)
 100 Sulfadiazine.

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

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

108

109 **2. Methods**

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

117 Studies were considered eligible if there was evidence of antiparasitic activity through the inclusion of half-
118 maximal inhibitory concentration (IC₅₀) or half-maximal effective concentration (EC₅₀) values against the
119 respective parasites. Secondary parameters included synergy with current treatment options, the potential for
120 disease progression, and the mechanism of anti-parasitic activity. Inclusion of publications was limited to those
121 written in English and authored as full manuscripts with no restriction on publication year.

122

123 **3. Malaria**

124 *Plasmodium falciparum* is one of the main species in the Plasmodium genus that causes malaria [15], affecting
125 228 million people worldwide and causing 405,000 deaths in 2018 [16, 17]. As one of the most prevalent
126 diseases in Africa, there are five human infective *Plasmodium* species, including *P. falciparum*, *Plasmodium*
127 *knowlesi*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax*. Although parasitic treatments and
128 prophylactic therapy are available, the overall global infective trend is increasing, from 214 million to 219
129 million cases in 2015 and 2017, respectively [15]. Malaria typically is asymptomatic, with few experiencing
130 symptoms such as head and muscle aches, fever, fatigue, and chills allowing for undetected progression to fatal

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

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

149 Chloroquine is a common treatment option for malarial diseases. Ru-based compounds coordinated with
150 chloroquine analogues and a, N,O-chelating salicyladimate ligand demonstrate the highest anti-plasmodial
151 activity to date [25]. One such compound, [Ru(II)-chloroquine]₂, demonstrated a 4.5-fold increase in in vitro
152 activity compared to chloroquine diphosphate [26]. Likewise, a trinuclear complex comprised of [Ru(p-
153 cymene)Cl₂]₂ containing polypyridyl ester ligands (monodentate donors) (Fig. 2a) and benzene-1,3,5-
154 tricarboxylic acid tripyridin-4-ylmethyl ester also proved to be effective anti-plasmodial compounds with low
155 cytotoxicity against HEK cell lines, proving to have a 4.5 fold increased effect on the parasites than the currently
156 used drug chloroquine [27] (Table 1).

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

161 of antiparasitic activity, where one example, $\eta^6\text{-iPrC}_6\text{H}_4\text{MeRu}(\mu\text{-Cl})\text{Cl}_2$, where $\text{R}_1 = \text{Ferrocene}$, $\text{R}_2 = \text{CH}_3$, $\text{X} =$
162 Si (Fig. 2b), demonstrated half-maximal inhibitory concentration (IC_{50}) values of $7.81 \pm 0.56 \mu\text{M}$ against *P.*
163 *falciparum* strain NF54 (Table 1). However, organosilane thiosemicarbazone Ru complexes have been shown to
164 be more selective against the parasite than non infected cell lines and in this example, the addition of silicon
165 improved differential toxicity and potency [19].

166 RAPTA complexes contain a monodentate 1,3,5-triaza-7-phosphaadamantane (PTA) and η^6 arene ligand coupled
167 to a Ru core to form $[\text{Ru}(\eta^6\text{-p-arene})\text{Cl}_2(\text{P-TA})]$. These compounds can protonate in low pH environments, such
168 as the conditions seen within the digestive vacuole of the parasite, thus making these ideal antiparasitic agents. A
169 series of RAPTA 7-chloroquino-line derivatives were synthesised with all demonstrating IC_{50} values of <0.40
170 μM and between 1.5 and $4.5\mu\text{M}$ against the chloroquine (Fig. 1b) sensitive *P. falciparum* NF54 and resistant *P.*
171 *falciparum* K1 strains respectively [18]. The lowest IC_{50} values were observed following exposure to $(\eta^6\text{-p-}$
172 $\text{cymene})(\text{N-(2-((5-fluoro-2-hydroxyphenyl)methylimino)propyl)-7-chloroquinolin-4-amine})\text{PTA}$ ruthenium(II)
173 hexafluorophosphate (Fig. 2c), where values of $0.10 \mu\text{M} \pm 0.069$ (against strain NF54) and $3.8 \mu\text{M} \pm 0.68$
174 (against strain K1) were observed, compared to chloroquine alone ($0.031 \mu\text{M} \pm 0.004$ and $0.36 \mu\text{M} \pm 0.07$
175 respectively). Observed cytotoxicity against a CHO cell line model with this derivative was $>100 \mu\text{M}$ [18]. The
176 protonation of complexes at low pH represents a promising drug delivery system for novel metal-chloroquine
177 metallotherapeutic agents.

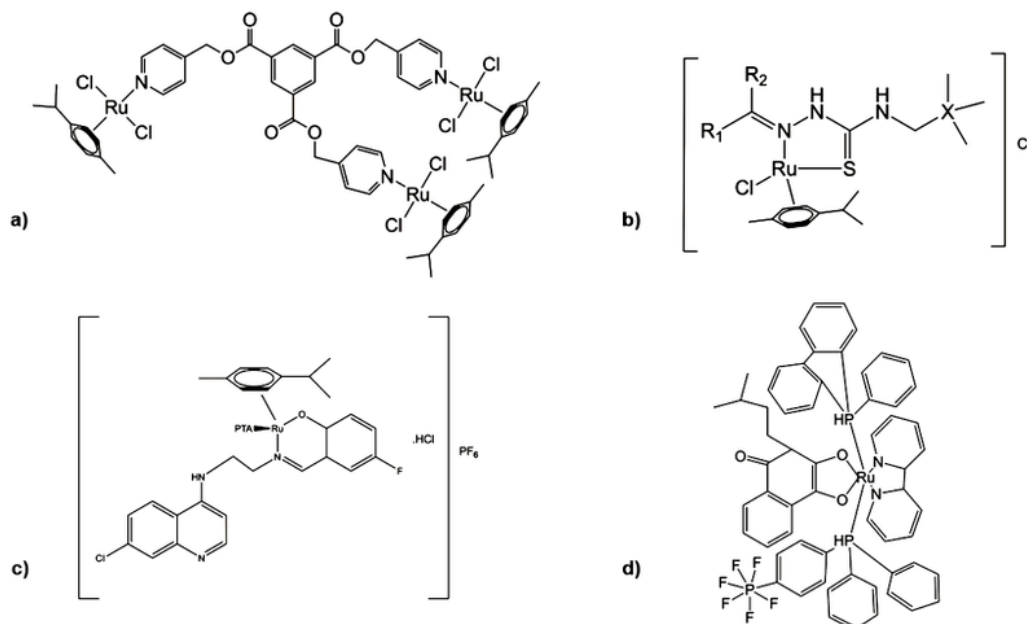
178 In a further study, $\text{RuCl}_2(\text{Lap})(\text{dppb})$ complexes were found to be more potent and have more selective
179 antiparasitic efficacy compared to lapachol (Fig. 1h) alone, which failed to inhibit *P. falciparum*. Compounds
180 such as $[\text{Ru}(\text{Lap})(\text{PPh}_3)_2(\text{phen})]\text{PF}_6$ (Fig. 2d) and $[\text{RuCl}_2(\text{Lap})(\text{dppb})]$ were found to be 50 times more potent
181 than lone lapachol and only 5 times less potent than the currently used drug mefloquine (Fig.1i) making it ideal
182 for use [11].

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

188 Overall, the presence of Ru compounds coordinated with traditional treatment options improves the effectiveness
189 of anti-plasmodial compounds. The addition of ligands such as chloride or η^6 arene considerably improves drug
190 specificity to the Plasmodium para-site with minimal cytotoxic effects on the host, which demonstrates the

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

194



195
 196 **Fig. (2).** Ruthenium-based compounds which demonstrate antiparasitic activity against *Plasmodium* species. (a)
 197 $[\text{Ru}(\eta^6\text{-p-cymene})\text{Cl}_2]_2$ with benzene-1,3,5-tricarboxylic acid, tripyridin-4-ylmethyl ester, [27] (b) $\eta^6\text{-}$
 198 $i\text{PrC}_6\text{H}_4\text{MeRu}(\mu\text{-Cl})\text{Cl}_2$, where $\text{R}_1 = \text{Ferrocene}$, $\text{R}_2 = \text{CH}_3$, $\text{X} = \text{Si}$ [19] (c) $(\eta^6\text{-p-cymene})(\text{N}-2\text{-}((5\text{-fluoro-2-}$
 199 $\text{hydroxyphenyl})\text{methylimino})\text{propyl})\text{-7-chloroquinolin-4-amine})\text{PTA}$ ruthenium(II) hexafluorophosphate [18]
 200 (d) $[\text{Ru}(\text{Lap})(\text{PPh}_3)_2(\text{phen})]\text{PF}_6$. [34]

201

202 4. Trypanosomiasis

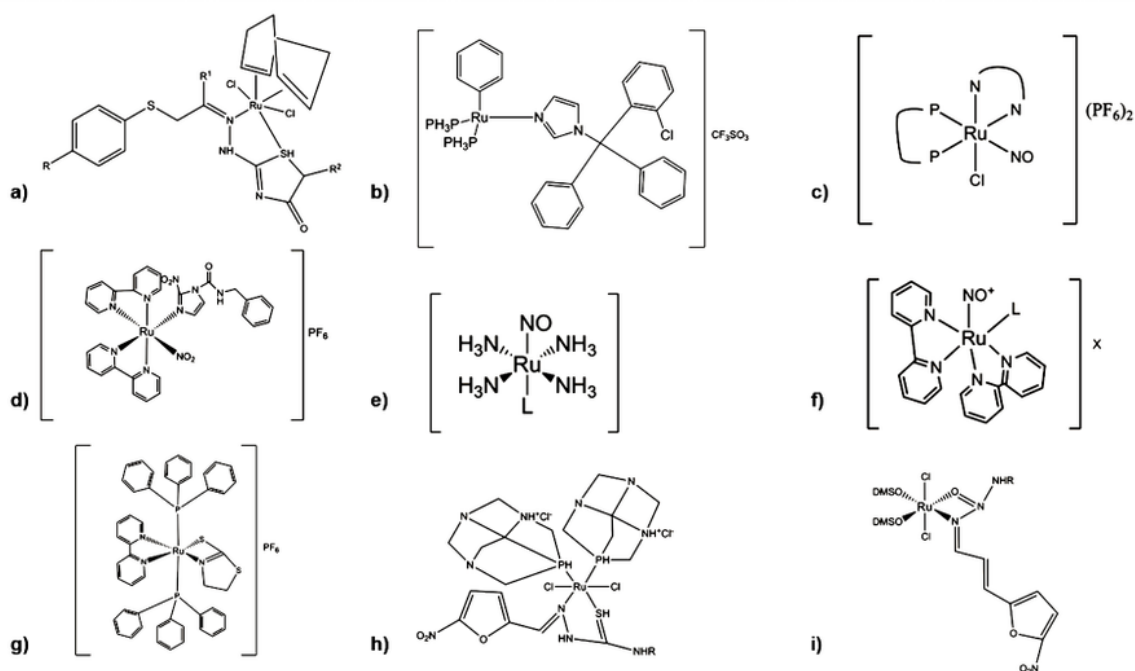
203 4.1 American trypanosomiasis

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

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

215 Current treatments for infection with *T. cruzi* generally target enzymes present within the parasite such
 216 as trypanothione reductase, superoxide dismutase, cysteine protease [39, 40] DNA topoisomerase [35], and
 217 kinase protease [40]. These enzymes are vital to the metabolic processes of the parasite and therefore disrupting
 218 these represents a key target for treatment [35]. Specific drug target sites include lanosterol 14-demethylase and
 219 transsialidase enzymes [41]. A cysteine protease termed cruzain also represents a potential unique drug target.
 220 This enzyme is responsible for the proteolytic activity, survival, and growth of the parasite. Nitric Oxide (NO)
 221 inactivates cruzain by S-nitrosylation of the binding site, therefore, it has been proposed that Ru-nitrosyl-
 222 containing compounds could demonstrate selective and pleiotropic activity by inhibiting the action of
 223 glyceraldehyde 3-phosphate dehydrogenase [42]. Furthermore, compounds with a general formula
 224 $[\text{RuCl}_2(\text{ATZ})(\text{COD})]$ (COD = 1,5-cyclo-octadi-ene) have high activity against the trypomastigote form of *T.*
 225 *cruzi* with an IC_{50} range between 3.3 and 27.2 μM after 24-hour exposure, whereas benznidazole is effective at
 226 5.0 μM . One of the more active compounds is dichloro[2-(para-bromophenylthio-(Z)-ethylidene)hydrazone-1,3-
 227 thiazol-4(5H)-one] cyclooctadiene ruthenium(II) (Fig. 3a) which had an IC_{50} value against trypomastigotes of
 228 5.5 μM could prove more effective than current treatment options [43].

229



231 **Fig. (3). Ruthenium-based compounds which demonstrated antiparasitic activity against *Trypanosoma***
 232 ***cruzi*.** (a) [RuCl₂(ATZ)(COD)], where R = Br, R₁ = H, R₂ = H [43], (b) [RuCp(PPh₃)₂(CTZ)](CF₃SO₃) [46], (c)
 233 ct-[RuCl(NO)(dpp-b)(5,5'-mebpy)](PF₆)₂ [42], (d) cis-[Ru(NO₂)(bpy)₂(Bz)](PF₆) [34], (e) trans-
 234 [RuII(NO)(NH₃)₄(L)]X₃, where L = imidazole co-ordinated through nitrogen (imN) or imidazole coordinated
 235 through carbon (imC), pyridine (py), L-histidine (L-hist), sulphite (SO₃²⁻), pyrazine (pz), nicotinamide (nic), 4-
 236 picoline (4-pic), triethyl-phosphite ([P(OEt)₃]), isonicotinamide (isn), isonicotinicacid (ina), X = BF₄⁻, Cl⁻ or
 237 PF₆⁻ [47], (f) cis-[RuII(NO)(bpy)₂(L)]X₃, where L = imidazole (imN), 1-methylimidazole (1-miN), or sulfite ion
 238 (SO₃²⁻) and X = BF₄⁻, Cl⁻ or PF₆⁻ [50], (g) trans-[Ru(tzdt)(PPh₃)₂(bpy)]PF₆ [10], (h) [RuCl₂(HL)(HPTA)₂]Cl₂
 239 [37], (i) [RuII(Cl)₂(dmsO)₂L] [56].

240

241 **Table 1:** Comparison of IC₅₀ values of Ru metallotherapeutics against respective parasite and mammalian cell
 242 lines (with cell type in brackets), coupled with the proposed mechanisms of antiparasitic activity for each
 243 compound.

244

Compound	IC ₅₀ of parasite (μM)	IC ₅₀ Cell cytotoxicity (μM)	Activity	Reference
Malaria				
[Ru(<i>p</i> -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>p</i> -C ₆ H ₄ Me)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 - <i>p</i> -cymene)(<i>N</i> -(2-((5-fluoro-2-hydroxyphenyl)methylimino)propyl)-7-chloroquinolin-4-amine)PTA ruthenium(II) hexafluorophosphate	0.10 ± 0.069 (NF54)	>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				
<i>mer</i> -[RuCl ₃ (dmsO)(H ₂ O)(tntp)]·2H ₂ O	43.2 ± 3.5	2150 ± 172.0 (J774)	Inhibit proliferation and Fe-SOD.	[35]
<i>trans</i> -[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	>200	Induce oxidative stress, interacts with DNA.	[37]
	(epimastigotes)	(murine macrophage RAW 264.7)		
	85.2 ± 1.9			
	(trypomastigotes)			
African trypanosomiasis				
[RuCl(η ⁶ - <i>p</i> -cym)(1,10-phenanthroline-5,6-dione)][PF ₆]	0.19 ± 0.5	1.26 ± 0.78 (HL60)	Coil and kink plasmid	[58]
[RuCl ₂ (η ⁶ - <i>p</i> -cym)(phenanthridine)]	165.0 ± 45.5	>100 (HL60)	Affects DNA replication	
[RuCl(η ⁶ - <i>p</i> -cym)(5-amine-1,10-phenanthroline)][PF ₆]	2.7 ± 0.3	44.63 ± 7.35 (HL60)	Knots and kinks plasmids at sharp angles.	
[Ru ₂ (<i>p</i> -cymene) ₂ (L1) ₂ Cl ₂]	2.9	>100 (J774)	Inhibit α-14 C demethylase	[60]
[Ru ₂ (<i>p</i> -cymene) ₂ (L4) ₂ Cl ₂]	0.5	26 (J774)		
Leishmaniasis				
<i>cis</i> -[RuII(η ² -O ₂ CR)(dppm) ₂ PF ₆ , where R = 4-bu-tylbenzoate (bbato)]	7.52	8.73	Interact with DNA covalently	[61]
	(<i>L. amazonensis</i>)	(RAW 264.7 macrophages)		
	9.09			
	(<i>L. braziliensis</i>)			
<i>cis.fac</i> -[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(η ⁶ - <i>p</i> -cymene)Cl ₂ (CTZ)]	LD ₅₀ 0.015 ± 0.004	>7.5		[30]
	(<i>L. major</i>)	(Human osteoblast)		
[Ru(Lap)(PPh ₃) ₂ (Me-bipy)][PF ₆ .CH ₃ OH]	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)		
<i>cis</i> -[Ru(bpy) ₂ SO ₃ (NO)]PF ₆	30 - 60	Not toxic at 10-60 (BALB)	NO donor	[64]
	(<i>L. amazonensis</i>)			
[RuCl(CTZ)(η ⁶ - <i>p</i> -cymene)(PPh ₃)]PF ₆	0.24 ± 1.65	Cytotoxic at 1. (murine macrophages)	Decrease flagella length, mitochondria swelling and leakage, decreased cell size	[68]
[RuCl(KTZ)(η ⁶ - <i>p</i> -cymene)(PPh ₃)]PF ₆	0.08 ± 2.62			
	(<i>L. amazonensis</i>)			
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 - <i>p</i> -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 - <i>p</i> -cymene)(tBu ₂ acac)(P(OEt) ₃)] [BF ₄]	41.1	10 (HFF)		
[(η ⁶ - <i>p</i> -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 - <i>p</i> -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 - <i>p</i> -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.	

245

246 The sterol biosynthesis pathway is another potential drug target site eliciting differential toxicity as it is
247 unique to the *T. cruzi* parasite. Compounds containingazole functional groups have demonstrated inhibitory
248 activity against cytochrome P450 14DM (CYP450), which is responsible for the enzymatic reaction of lanosterol
249 14 α -demethylation through binding to the N3 site of the imidazole group within the enzyme [36]. To address this
250 potential target, Sanchez-Delgado *et al.* [44] combined the sterol biosynthesis inhibiting properties of the
251 antifungal agent clotrimazole (CTZ) with Ru as the central metal ion to produce [RuCl₂(CTZ)₂]. This exhibited
252 90% growth inhibition of *T. cruzi* at 10⁻⁵ M with no cytotoxicity observed in mammalian vero cell lines.
253 Another study found [RuCl₂(CTZ)₂] to be 10 fold more active against *T. cruzi* than CTZ alone. However, this
254 compound was found to have low solubility, therefore bipy (bpy = 2,2'-bipyridine) ligands were used instead of
255 chloride, but this resulted in reduced anti-protozoal activity [44]. The original compound is therefore thought to
256 hydrolyse inside the parasite releasing the CTZ ligand to inhibit the activity of CYP450, leaving the remaining

257 compound $[\text{RuCl}_2]$ to interact with the DNA. Subsequent studies found that a Ru(II) compound containing p-
258 cymene and CTZ ligands had an LD_{50} of 0.1 μM , which is 58-fold higher activity than free CTZ and 6 fold
259 higher than the Ru compound with just the CTZ ligand [30]. In comparison, the addition of a ketoconazole
260 ligand to form Ru-KTZ (where KTZ = ketoconazole) exhibited higher solubility and lower cytotoxicity to host
261 cells, but had reduced antiparasitic activity than Ru-p-cymene-CTZ [36, 45]. A further compound with a CTZ
262 ligand, $[\text{RuCp}(\text{PPh}_3)_2(\text{CTZ})](\text{CF}_3\text{SO}_3)$ (Fig. 3b), was additionally found to exhibit a high IC_{50} value of 0.25 μM
263 against epimastigotes, which is 30-fold higher than the commonly used drug nifurtimox.
264 $[\text{RuCp}(\text{PPh}_3)_2(\text{CTZ})](\text{CF}_3\text{SO}_3)$ also demonstrated molecular inhibition towards the biosynthetic pathway where
265 squalene is converted to squalene oxide [46].

266 The antiparasitic activity of compounds containing both NO and phosphine ligands coordinated to Ru has also
267 been explored. One example, *ct*- $[\text{RuCl}(\text{NO})(\text{dpp-b})(5,5\text{-mebipy})](\text{PF}_6)_2$ (Fig. 3c), was the most effective in a
268 series and resulted in a significant increase in NO release with subsequent induced intracellular vacuole
269 formation. Using murine infection modelling, *ct*- $[\text{RuCl}(\text{NO})(\text{dppb})(5,5\text{-mebipy})](\text{PF}_6)_2$ proved to be a more
270 efficient treatment than the standard benznidazole with an EC_{50} of $2.1 \pm 0.6 \mu\text{M}$ (Table 1). The compound was
271 found to cause parasitic shrinking, cell membrane fragmentation and discontinuity in 76% of the parasitic cells,
272 mitochondria swelling, and nuclear membrane loss leading to necrosis of the parasitic cell. Multiple mechanisms
273 of antiparasitic activity on differing areas of the cell could prove beneficial in reducing resistance generation.
274 When *ct*- $[\text{RuCl}(\text{NO})(\text{dpp-b})(5,5\text{-mebipy})](\text{PF}_6)_2$ was used synergistically with benznidazole at 75 $\mu\text{mol}/\text{Kg}$ and
275 38 $\mu\text{mol}/\text{Kg}$, 100% survival rate and lower parasitaemia was observed than with the individual treatments [42].

276 The compound *trans*- $[\text{Ru}(\text{Bz})(\text{NH}_3)_4\text{-SO}_2](\text{CF}_3\text{SO}_3)_2$ has been shown to exhibit antiparasitic effects at low
277 concentrations. It is also capable of catalysing nitrite to nitrosyl conversion at a low concentration of 0.4 $\mu\text{mol}/\text{kg}$
278 and decreasing the number of parasites in the heart. *Cis*- $[\text{Ru}(\text{NO}_2)(\text{bpy})_2(\text{Bz})](\text{PF}_6)$ (Fig. 3d) also demonstrated
279 high anti-trypanocidal activity and low cytotoxicity in mouse cells. Following mouse infection studies, less
280 damage to the heart, less inflammation, and fewer parasites residing in the myocardium was observed following
281 treatment, which was more effective than benznidazole [34]. Compounds reported by Toledo *et al.*, 2005 [47]
282 with a general formula *trans*- $[\text{RuII}(\text{NO})(\text{NH}_3)_4(\text{L})]\text{X}_3$ (where L = imidazole coordinated through nitrogen (imN)
283 or imidazole coordinated through carbon (imC)), pyridine (py), L-histidine (L-hist), sulphite (SO_3^{2-}), pyrazine
284 (pz), nicotinamide (nic), 4-picoline (4-pic), triethylphosphite ($[\text{P}(\text{OEt})_3]$), isonicotinamide (isn), isonicotinic acid
285 (i-na), X = BF_4^- , Cl^- or PF_6^-) (Fig. 3e) donate NO, have high solubility and are resistance to oxidation
286 reactions [36, 47]. Antiparasitic assays with *trans*- $[\text{RuI}(\text{NO})(\text{NH}_3)_4(\text{L})]\text{X}_3$ compounds exhibited an IC_{50} value

287 of 244 μM (Table 1) [48], however the compounds were highly toxic to vero cell lines and less effective than
288 current treatment options. Many other trans compounds with the same formula were found to be as effective as
289 sodium nitroprussid (SNP) (NO donor reference), with 60% of those with the compound $[\text{Ru}(\text{NO})\text{isn}]$ (where isn
290 = isonicotinamide) surviving for more than 120 days in murine modelling. The glycolysis pathway for ATP
291 production is also vital in *T. cruzi* [49] and a study conducted by Silva *et al.*, 2010 [50] confirmed that Ru
292 compound *cis*- $[\text{RuII}(\text{NO})(\text{bpy})_2(\text{L})]\text{X}_3$, where L = imidazole (imN), 1-methylimidazole (1-miN), or sulfite ion
293 (SO_3^{2-}) and X = BF_4^- , Cl^- or PF_6^- (Fig. 3f) [50], affected the glyceraldehyde-3-phosphatedehydrogenase enzyme
294 which plays a vital part in this pathway [49]. Compounds such as *cis*- $[\text{Ru}(\text{NO}_2)(\text{bpy})_2(\text{Bz})](\text{PF}_6)$ exhibited high
295 efficacy at low concentrations, which was characterised by the ability to release NO into the intracellular
296 compartments of the parasite, with no demonstrable cytotoxicity against host cells. Such compounds with high
297 efficacy should be considered in further drug studies [34].

298 The compound, *trans*- $[\text{Ru}(\text{Bz})(\text{NH}_3)_4\text{SO}_2](\text{CF}_3\text{SO}_3)_2$, has also been found to mimic iron [35], binding to
299 transferrin and albumin and has shown high hydrosolubility against *T. cruzi* than benznidazole alone [51].
300 Compound *mer*- $[\text{RuCl}_3(\text{dmsO})(\text{H}_2\text{O})(\text{tmt-p})]\cdot 2\text{H}_2\text{O}$ exhibited a 21-fold higher activity than benznidazole,
301 proving to be highly selective against *T. cruzi* and displayed the ability to inhibit proliferation. The compound is
302 lipophilic as hydrophilic Tmt-p can cross the membrane ($\log P_{\text{o/w}} = -1.65$) and active up-take facilitates transport
303 through the cell membrane. The antioxidant enzyme Fe-SOD was significantly inhibited at 50% following the
304 addition of *mer*- $[\text{RuCl}_3(\text{dmsO})(\text{H}_2\text{O})(\text{tmt-p})]\cdot 2\text{H}_2\text{O}$, while decreased inhibition of human CuZn-SOD was
305 observed, showing that these compounds exhibited target specificity without affecting comparable human
306 enzymes [35].

307 Other Ru-based compounds were evaluated for their toxicity using cisplatin as a reference, where the results
308 showed toxicity against cancer cells and it was hypothesized that these compounds interact with ctDNA by
309 forming ternary complexes [52]. A Ru complex *trans*- $[\text{Ru}(\text{tzdt})(\text{PPh}_3)_2(\text{bipy})]\text{PF}_6$ (Fig. 3g) displayed the highest
310 antiparasitic activity against *T. cruzi* in a concentration-dependent manner compared to the other agents
311 examined in this study and had a high selectivity index. Additionally, the compound displayed a high ctDNA
312 binding constant of $4.9 \times 10^3 \text{ M}^{-1}$. It was further suggested that the presence of bipy ligands and a net molecular
313 positive charge contributed to the overall antiparasitic efficacy. At $0.1 \mu\text{M}$, this compound had similar activity to
314 benznidazole and reduced the number of parasites infecting macrophages. Synergistic studies with benznidazole
315 showed a further reduction in macrophage infection rate [10].

316 The compound $[\text{RuCl}_2(\text{HL}_4)(\text{HPTA})_2]\text{Cl}_2$, 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 $84.2 \pm 1.3 \mu\text{M}$ and $85.2 \pm 1.9 \mu\text{M}$ 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 provide 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 compound's overall effect. The lipophilicity of these
325 compounds increases as the N-substituent changes from hydrogen to phenyl. $[\text{RuCl}_2(\text{HL}_4)(\text{HPTA})_2]\text{Cl}_2$ 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 $[\text{RuIICl}_2(\text{dmsO})_2\text{L}$ (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

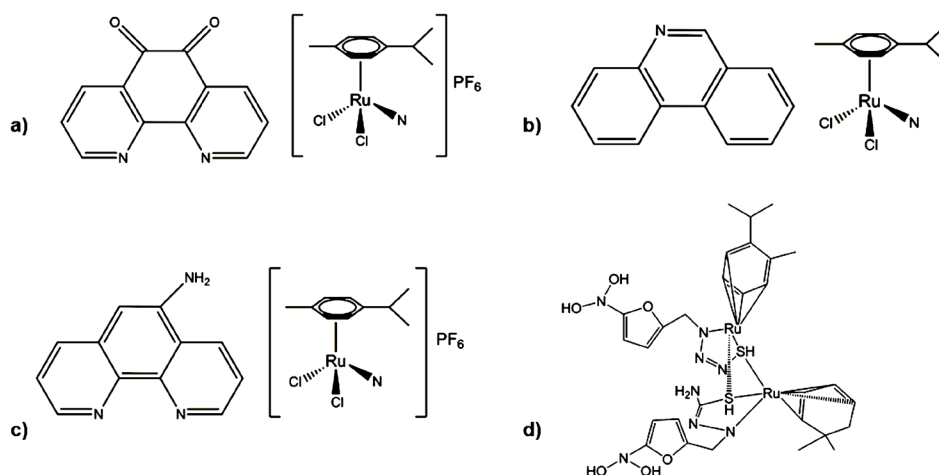
339

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, parasites can cross the blood-brain barrier causing
344 significant morbidity and mortality. Melarsoprol B and Eflornithine are some of the drugs commonly used for

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

347 A series of Ru compounds were synthesised to target DNA replication within *T. brucei* by intercalating
348 polycyclic aromatic ligands between β -form helix base pairs, allowing subsequent interaction with the parasitic
349 DNA [59]. $[\text{RuCl}(\eta^6\text{-p-cym})(1,10\text{-phenanthroline-5,6-dione})][\text{PF}_6]$ (Fig. 4a) caused coiling and kinking of
350 DNA, $[\text{RuCl}_2(\eta^6\text{-p-cym})(\text{phenanthridine})]$ (Fig.4b) caused knots and kinks with sharper angles; $[\text{Ru}-\text{Cl}(\eta^6\text{-p-}$
351 $\text{cym})(5\text{-amine-1,10-phenanthroline})][\text{PF}_6]$ (Fig. 4c) knotted and created breaking points thus affecting DNA
352 replication, preventing essential proteins being made leading to parasite death. $[\text{RuCl}(\eta^6\text{-p-cym})(1,10\text{-}$
353 $\text{phenanthroline-5,6-dione})][\text{PF}_6]$ was the most potent of the compounds examined, with an IC_{50} of 190 nM
354 (Table 1) and had similar mammalian cytotoxicity profiles to the well-characterized anti-cancer platinum-based
355 metallotherapeutic cisplatin, rendering it viable for use in humans [57].



356
357 **Fig. (4). Ruthenium metallotherapeutics which demonstrated antiparasitic activity against *Trypanosoma***
358 ***brucei*, (a) $[\text{RuCl}(\eta^6\text{-p-cym})(1,10\text{-phenanthroline-5,6-dione})][\text{PF}_6]$, (b) $[\text{RuCl}_2(\eta^6\text{-p-cym})(\text{phenanthridine})]$, (c)**
359 **$[\text{RuCl}(\eta^6\text{-p-cym})(5\text{-amine-1,10-phenanthroline})][\text{PF}_6]$ [57], (d) $[\text{Ru}_2(\text{p-cymene})_2(\text{L})_2]\text{X}_2$. [60]**

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

364 demonstrated a lower IC₅₀ than previously documented [Ru₂(p-cymene)₂(L)₂]X₂ complexes, where L = 5-
365 nitrofuryl containing thiosemicarbazones and X = Cl⁻ or PF₆⁻ (Fig. 4d) against *T. brucei brucei* strain number
366 427 [60]. The mechanism of antiparasitic action of the CTZ complex involves the inhibition of α-14 C
367 demethylase (an essential enzyme in the sterol membrane biosynthesis pathway), causing the conversion of
368 lanosterol to ergosterol to cease and lanosterol to accumulate, altering the permeability of the cell wall.

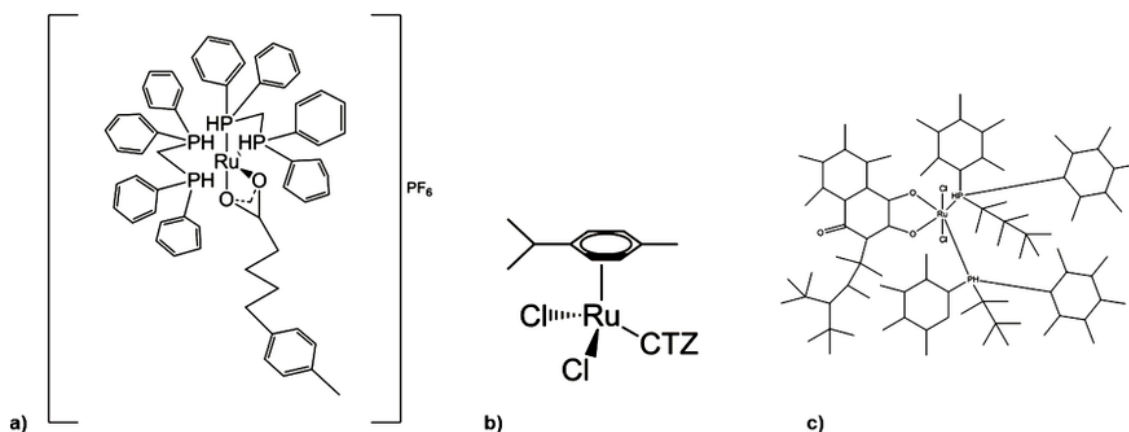
369

370 5. Leishmaniasis

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

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

388 Compounds with a general formula *cis*-[RuII(η²-O₂CR)(dppm)₂]PF₆, where dppm = *bis*(diphenylphos-
389 phino)methane and R = 4-butylbenzoate (bbato) (Fig.5a), have demonstrated high levels of activity towards
390 different *Leishmania* species except *L. braziliensis* and were also found to have low cytotoxicity (10%) against
391 host macrophages [61]. Exchanging chloride groups for chelating ligands increased the biological activity of the
392 compound by increasing the overall molecular positive charge, therefore permitting covalent interactions with
393 DNA more readily.



395

396 **Fig. (5). Ruthenium metallotherapeutics which demonstrated antiparasitic activity against *Leishmania***
 397 **species, (a) cis -[RuII(η^2 -O₂CR)(dppm)₂]PF₆ [61], (b) [Ru(η^6 -p-cymene)Cl₂(CTZ)] [30], (c) [RuCl₂(Lap)(dppb)]**
 398 **[11].**

399 Superoxide dismutase (SOD) antioxidant enzymes are found within mitochondria and are differentiated by metal
 400 cofactors. SOD with iron cofactors has also been the target for the development of novel anti-leishmania
 401 treatments [67]. The compound *cis, fac*-[RuCl₂(dmsO)₃(tmp)] demonstrated a 3-fold increase in anti-leishmania
 402 activity compared to meglumine antimoniate against *L. brasiliensis* and proved to be less toxic to macrophages
 403 and more lipophilic. This compound was also found to display high selectivity to Fe--SOD and caused 70% of
 404 SOD activity [35]. A further compound, [Ru(η^6 -p-cymene)Cl₂(CTZ)] (Fig. 5b), demonstrated high efficacy
 405 against *L. major* compared to treatment with CTZ alone and was also less toxic to the human host proving to be
 406 a promising compound for progression to further studies [30]. Lapachol-Ru complexes have also been explored
 407 and were biologically more active than the free ligand as they have the ability to inhibit *L. amazonensis*
 408 promastigote proliferation [11].

409 Two compounds [Ru(Lap)(PPh₃)₂(Me-bipy)]PF₆.CH₃OH and [RuCl₂(Lap)(dppb)] (Fig. 5c) had similar
 410 antiparasitic activity to amphotericin B but were shown to be non-cytotoxic and exhibit higher selectivity
 411 indexes [11]. As previously discussed, some Ru compounds act as NO donor/releasing compounds, such as *cis*-
 412 [Ru(bpy)₂SO₃(NO)]PF₆, which also has high water solubility to enable entry into parasitic cells, while

413 maintaining low cytotoxicity to host cells thus making them ideal candidates against *Leishmania* species [64].
414 The increase in intracellular NO levels caused by NO-re-leasing compounds has been shown to result in a
415 decrease in promastigote levels to the point of eliminating the infection, while maintaining host macrophage
416 activity during the treatment course [64]. Further studies using electron microscopy showed that Ru-based
417 compounds such as $[\text{RuCl}(\text{CTZ})(\eta^6\text{-p-cymene})(\text{PPh}_3)]\text{PF}_6$ and $[\text{RuCl}(\text{KTZ})(\eta^6\text{-p-cymene})(\text{PPh}_3)]\text{PF}_6$ have
418 physical effects on *Leishmania* species. These observations included reduced flagella length, swelling of the
419 mitochondria with subsequent leakage, kinetoplast disorganisation, abnormal condensation of chromatin, the
420 appearance of vacuoles containing cellular debris and an overall reduction in cell size. Additionally, membrane
421 protrusions were visible, but further work is required to demonstrate the in vivo effects of these compounds [68].

422

423 **6. Amoebiasis**

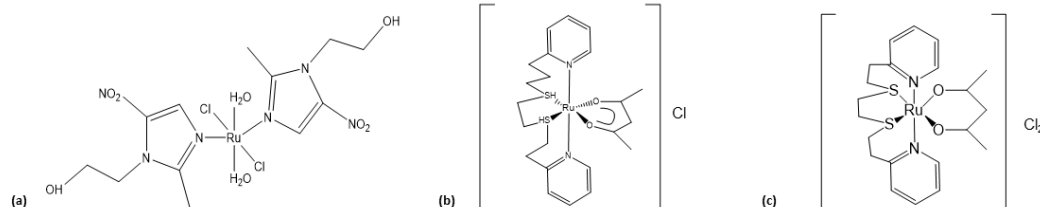
424 Amoebiasis is caused by the protozoan *Entamoeba histolytica* and is an amoebic dysentery disease that affects
425 50% of the population in developing countries and can lead to liver abscess formation and results in over
426 100,000 deaths annually, being the 4th leading cause of parasitic death [69]. Additionally, it is the second
427 leading cause of death by a protozoan parasite after malaria. Infection occurs through the consumption of
428 contaminated food sources [70]. Current first-line drug treatments such as metronidazole and nitroimidazole
429 generate nitroso free radicals to combat infection, however these cause unwanted side effects and an increase in
430 resistance [69]. Metronidazole is commonly used in high dosages for prolonged periods causing host peripheral
431 neuropathy with sensory disturbances and resistance is now emerging due to treatment stipulations [71].
432 Likewise, treatment with nitroimidazoles results in significant host morbidities such as irritation of the gastric
433 mucus lining, headache, vomiting, diarrhoea, haematuria and occasional toxicity within the central nervous
434 system [70].

435 Ru (III) ionic derivatives of compounds become active in a hypoxic environment, such as the colon, due to the
436 subsequent reduction and conversion into the more biologically active Ru (II) state. Given *E. histolytica*
437 colonises the colon, Ru (III)-based compounds have been explored as treatment options for amoebiasis infections
438 [72].

439 Under aerobic conditions, a reduction reaction with metronidazole treatment generates nitro radicals which bind
440 to DNA and enzymes within the parasite. Metals are thought to reduce the likelihood of enzymatic degradation
441 of metronidazole, therefore combined with the Ru metallotherapeutic, $[\text{Ru}(\text{metronidazole})_2(\text{Cl})_2(\text{H}_2\text{O})_2]$ (Fig.
442 6a), this exhibited an IC_{50} value of $0.51\ \mu\text{M}$ against *E. histolytica* which was lower than metronidazole alone

443 (IC₅₀ = 1.81 μM) showing that combining Ru compounds with metronidazole produced greater effects against
444 parasites [71].

445



446

447 **Fig. (6). Ruthenium metalloterapeutics which demonstrated antiparasitic activity against *Entamoeba***
448 ***histolytica*, (a) [Ru(metronidazole)₂(Cl)₂(H₂O)₂] [71], (b) [Ru(acac)(pdto)]Cl [69], (c)**
449 **[Ru(pdto)(acetylacetonate)]Cl [70].**

450

451 As previously discussed, Ru-based compounds are known to interact with DNA and bidentate ligands. In a study
452 by Toledano-Magaña *et al.*, 2017 [69], trophozoites of *E. histolytica* were targeted with [Ru(acac)(pdto)]Cl,
453 where pdto = 2,2'-[1,2-ethanediybis-(sulfanediy-2,1-ethanediy)]dipyridine and acac = acetylacetonate (Fig. 6b),
454 and a 50-100-fold increase in efficacy was observed when compared to traditional treatments. This compound
455 caused rounding of the parasite and nuclear membrane damage, thus inducing apoptosis in 90% of amoeba cells.
456 In murine models, 100% of parasites were eliminated after 24 hours with a dose of 5 mg/kg administered every
457 12 hours. Crucially, there was no evidence of parasitic infection after 5 days post treatment and there was a
458 reduction in observed inflammation within the liver [69].

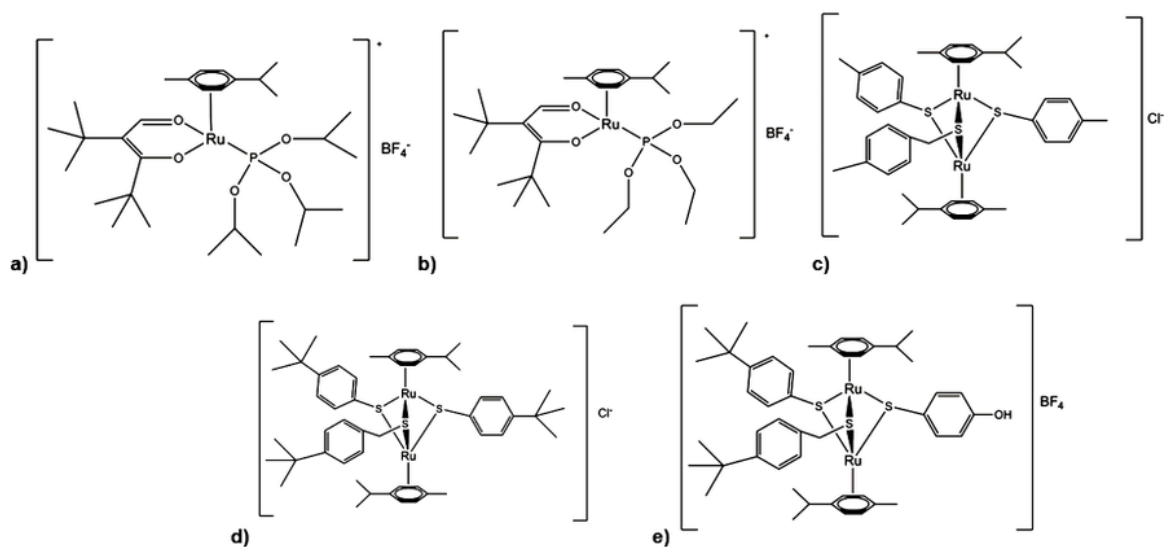
459 In another study [70], twenty Ru compounds were tested with a general formula of [Ru(pdto)(E-E)]Cl_x(E-E)
460 bidentate, either neutral or negatively charged ligands). [Ru(pdto)(acetylacetonate)]Cl (Fig. 6c) was the most
461 effective of the treatments studied with an IC₅₀ of 0.06 μmol/L due to the metal having high water solubility.
462 Low mammalian cytotoxicity was observed, despite an increase in the production of parasitic intra-cellular ROS.
463 Ru compounds with an O-O and N-O donor were more readily oxidised and exhibited better antiparasitic activity
464 than N-N ligands [70].

465

466 **7. Toxoplasmosis**

467 Toxoplasmosis is caused by the parasite *Toxoplasma gondii*, which when latent will reside within the
468 host central nervous system. Transmission can be conveyed through food, water, and the placenta where it can
469 replicate within the mammalian gut [73]. In the acute form, tachyzoites, fast replication allows the parasite to
470 invade and target host cells and additionally secrete proteins to allow survival and immune evasion. Colonisation
471 of monocytes results in hypermigration, permitting systemic dissemination through the host [74, 75]. This
472 includes invading epithelial cells within the blood-brain barrier where parasitic replication causes cells to lyse
473 enabling entry to the brain parenchyma [76]. Conditions caused by *T. gondii* include myocarditis, encephalitis,
474 and blindness, and treatment is normally through the administration of sulfadiazine (Fig.11) and pyrimetham,
475 with leucovori supplementation which inhibits dihydrofolate reductase [77, 78]. Treatment for toxoplasmosis is
476 complex as tachyzoites can establish drug-resistant bradyzoite cysts leading to persistent infection [79]. As 80%
477 of primary infections are asymptomatic, there is significant potential for disease progression [80].

478 Metallotherapeutic Ru compounds could provide an alternative treatment option, in addition to
479 combatting potentially drug-resistant forms of the disease. Two hydrolytically stable Ru phosphite compounds
480 with additional hydrocarbon exteriors $[\text{Ru}(\eta^6\text{-p-cymene})(\text{tBu}_2\text{acac})(\text{P}(\text{OiPr})_3)][\text{BF}_4]$ (Fig. 7a) and $[\text{Ru}(\eta^6\text{-p-}$
481 $\text{cymene})(\text{tBu}_2\text{acac})(\text{P}(\text{OEt})_3)][\text{BF}_4]$ (Fig. 7b) were synthesised for use against *T. gondii* and demonstrated IC_{50}
482 values of 18.7 and 41.1 nM respectively. However, $[\text{Ru}(\eta^6\text{-p-cymene})(\text{tBu}_2\text{acac})(\text{P}(\text{OiPr})_3)][\text{BF}_4]$ required a
483 prolonged treatment course of between 22 and 27 days of tachyzoites in a human foreskin fibroblast model.
484 These compounds functioned at multiple sites within the parasite and impaired the metabolic activity of the
485 tachyzoites by creating lipid-containing or empty inclusions, in addition to causing a distorted nuclear
486 membrane. While highly effective, the observed duration of treatment in vitro may be prohibitively long for in
487 vivo applications [81].



489

490 **Fig. (7). Ruthenium metalloterapeutics which demonstrated antiparasitic activity against *Toxoplasma***
 491 ***gondii*.** (a) $[\text{Ru}(\eta^6\text{-p-cymene})(\text{tBu}_2\text{acac})(\text{P}(\text{OiPr})_3)]^+[\text{BF}_4]^-$, (b) $[\text{Ru}(\eta^6\text{-p-cymene})(\text{tBu}_2\text{acac})\{\text{P}(\text{OEt})_3\}]^+[\text{BF}_4]^-$ [82],
 492 (c) $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pr}^i)\text{Ru}_2(\mu\text{-Cl})\text{Cl}_2]\text{S}_4\text{-C}_6\text{H}_4\text{CH}_3$. (d) $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pr}^i)\text{Ru}_2(\mu\text{-Cl})\text{Cl}_2]\text{S}_4\text{-C}_6\text{H}_4\text{Bu}^t$, (e) $[(\eta^6\text{-}p\text{-}$
 493 $\text{Me-C}_6\text{H}_4\text{Pr}^i)_2\text{Ru}_2(\mu_2\text{-SCH}_2\text{-C}_6\text{H}_4\text{-R})_2\text{Cl}_2]_2$ Where SCH_2R is $4\text{-C}_6\text{H}_4\text{CH}_3$. [82]

494

495 Compounds $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pri})\text{Ru}_2(\mu\text{-Cl})\text{Cl}_2]\text{SR}$ (where R is $4\text{-C}_6\text{H}_4\text{CH}_3$) (Fig. 7c), $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pri})\text{Ru}_2(\mu\text{-}$
 496 $\text{Cl})\text{Cl}_2]\text{SR}$ (where R is $4\text{-C}_6\text{H}_4\text{But}$) (Fig.7d), and $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pri})_2\text{Ru}_2(\mu_2\text{-SCH}_2\text{-C}_6\text{H}_4\text{-R})_2\text{Cl}_2]_2$ (where
 497 SCH_2R is $4\text{-C}_6\text{H}_4\text{CH}_3$) (Fig. 7e) were the most effective compounds in a study by Basto *et al.* (2017) [82], where
 498 IC_{50} values were observed of 34nM, 62 nM, and 1.2 nM respectively. However, $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pri})\text{Ru}_2(\mu\text{-}$
 499 $\text{Cl})\text{Cl}_2]\text{S}_4\text{-C}_6\text{H}_4\text{CH}_3$ was cytotoxic to human foreskin fibroblasts (HFF) with an IC_{50} against the parasite of 800
 500 nM whereas $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pri})\text{Ru}_2(\mu\text{-Cl})\text{Cl}_2]\text{S}_4\text{-C}_6\text{H}_4\text{But}$ had an IC_{50} of $>1\text{mM}$. Interestingly, compound $[(\eta^6\text{-}p\text{-}$
 501 $\text{Me-C}_6\text{H}_4\text{Pri})_2\text{Ru}_2(\mu_2\text{-SCH}_2\text{-C}_6\text{H}_4\text{-R})_2\text{Cl}_2]_2$ demonstrated the lowest IC_{50} against the parasite of 1.2 nM but was
 502 the least cytotoxic to cell lines with an observed IC_{50} against HFF of $>5\text{mM}$ indicating that this has the potential
 503 for future therapeutic applications. Furthermore, $[(\eta^6\text{-}p\text{-Me-C}_6\text{H}_4\text{Pri})_2\text{Ru}_2(\mu_2\text{-SCH}_2\text{-C}_6\text{H}_4\text{-R})_2\text{Cl}_2]_2$ interacted
 504 with TgTEF1 α , which is specific to the importation of mitochondrial tRNA [83, 84], and was able to inhibit the
 505 invasion of host cells by the parasite [85]. Although biologically relevant IC_{50} values were observed, it was
 506 further shown that the three compounds in this series acted in a parasitostatic manner, which might limit the

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

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

513

514 **8. Orphan Diseases**

515 **8.1 Lymphatic filariasis**

516 *Setaria cervi* is the causative agent of filariasis in the bovine host where it resembles the antigenic pattern and
517 nocturnal periodicity as the human parasite *Wuchereria bancrofti* [86]. Some Ru-based com-pounds have been
518 found to inhibit topoisomerase II in *Setaria cervi* with varying efficacy depending on the number of
519 uncoordinated and coordinated nitrogen atoms and mononuclear or binuclear configuration. These compounds
520 include the carbon monoxide-releasing molecules [RuH(CO)(PPh₃)₂(paa)]BF₄, [RuH(CO) (PPh₃)₂(pbp)]PF₆,
521 [RuH(CO)(PPh₃)₂(-μ-bbp)RuH(CO)-(PPh₃)₂](BF₄)₂, [RuH(CO)(PPh₃)₂(η²-tptz)]BF₄, [RuH(CO)(PPh₃)₂(η²-
522 bppz)]BF₄ and [RuH(CO)(PPh₃)₂(η²-bp-pz)]PF₆, which have all been found to exhibit inhibitory activity against
523 topoisomerase II [87].

524 **8.2 Schistosomiasis**

525 *Trans*-[Ru(bpy)₂(NO)SO₃](PF₆)-PF₆ has been investigated due to the NO-releasing potential of the molecule
526 with a view to treating infections caused by the parasitic flatworm *Schistosoma mansoni* [88]. Both eggs and
527 worms were eliminated upon treatment and infection-associated liver inflammation was also reduced. The NO
528 released by the donor stimulated the production of ROS and caused nitration and nitrosilation to render the
529 parasite nonviable [89]. The action of *trans*-[Ru(bpy)₂(NO)SO₃](PF₆)-PF₆ increased mitochondrial NADH
530 oxidation and subsequently caused the opening of permeability transition pores, releasing cytochrome c, causing
531 cell death [89]. Another two compounds, Rubb12-tri and Rubb7-tnl, both demonstrated high adult parasite
532 mortality (EC₅₀ values 3.489 ± 0.532 μM and 6.829 ± 0.625 μM, respectively) and reduced egg hatching upon
533 incubation at 50 μM due to an increase in the inhibition of acetylcholinesterase [90].

534 **8.3 Strongyloidiasis**

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

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

539 **8.4 Trichuriasis**

540 In over 100 countries, 4.5 billion people are at risk from a range of parasitic infections causing chronic and
541 insidious effects to the host rather than death [92]. Acetylcholinesterases are the main virulence factor of the
542 parasitic *Trichuris sp.* nematodes, which cause trichuriasis and target human nerve cells. Compounds based on
543 Ru polypyridal complexes such as $[\text{Ru}(\text{phen})_2(\text{bxbg})]^{2+}$ (where phen = 1,10 phenanthroline, bxbg = bis(o-
544 xylene)bipyridine glycoluril) have been found to inhibit the action of these enzymes, proving to be a promising
545 therapy [93]. These compounds exhibit their activity by electrostatically and hydrophobically interacting with
546 the peripheral anionic site on the enzyme and not interacting directly with the active site, thus proving less toxic
547 to mammalian cells. Compounds such as Rubb7-tl and Rubb12-tl demonstrated a range of activity against
548 nematodes of 18-76%, with the greatest inhibitory action against both worms and faecal eggs of *T. muris* as
549 shown by murine modelling [94]. As *T. muris* studies are used as a model for *T. trichuris* infection in humans
550 [95], it is possible that Rubb type compounds could be effective against human disease.

551

552 **9. Conclusion**

553 The development of effective antiparasitic chemotherapy remains a challenge, with very few novel therapeutics
554 being developed. Indeed, many current treatment options were identified and/or developed over forty years ago.
555 Identifying novel drug targets within parasites further remains a challenge due to the complexity of the
556 eukaryotic cell and requirements for differential toxicity within the host. Due to the length of treatment required
557 to resolve parasitic infections, coupled with reduced differential toxicity leads to significant drug-induced
558 morbidity. Many Ru metallotherapeutics described in this review exhibit low IC_{50} values and demonstrate high
559 selectivity indices. Due to the propensity for Ru coordination chemistry, it is possible to use intelligent drug
560 design to modify lead drug candidates to improve efficacy and minimize off-target effects. Although some Ru-
561 based compounds are cytotoxic to mammalian cells and have not yet been tested on murine models, the initial
562 results (Table 1) are promising with most compounds exhibiting low cytotoxicity against cell lines with low
563 concentrations of the compound required to produce significant antiparasitic effects. The most promising
564 current lead Ru therapeutic candidates include RAPTA 7-chloroquinoline derivatives and
565 $[\text{Ru}(\text{Lap})(\text{PPh}_3)_2(\text{phen})]\text{PF}_6$ for the treatment of malaria, *trans*- $[\text{Ru}(\text{tzdt})(\text{PPh}_3)_2(\text{bipy})]\text{PF}_6$ for treatment of
566 American trypanosomiasis, $[\text{RuCl}(\eta^6\text{-p--cym})(5\text{-amine-1,10-phenanthroline})][\text{PF}_6]$ for African trypanosomiasis,

567 *cis-fac*-[RuCl₂(dms_o)₃(tmt_p)] 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.

573 **Consent for Publication**

574 Not applicable.

575 **Funding**

576 None.

577 **Conflict of interest**

578 The author declares no conflict of interest, financial or otherwise.

579 **Acknowledgment**

580 The authors wish to thank Dr. Anthony J. Slate (University of Bath, UK) for providing constructive feedback on
581 the completed manuscript and assistance with graphical artwork.

582 **References**

583 [1] Hayes, AM; Morris, DJ; Clarkson, GJ; Wills, M. A class of ruthenium (II) catalyst for asymmetric transfer
584 hydrogenations of ketones. *J Am Chem Soc.* **2005**, *127*(20), 7318-9.

585 [2] Luft, JH. Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and
586 mechanism of action. *Anat Rec.* **1971**, *171*(3), 347-68.

587 [3] Shulman, A; Dwyer, FP. Metal chelates in biological systems. *Chelating agents and metal chelates.* **1964**
588 *1*,383-439.

589 [4] Allardyce, CS; Dyson, PJ. Ruthenium in medicine: current clinical uses and future prospects. *Platinum*
590 *Metals Reviews.* **2001**; *45*:62.

591 [5] Dwyer, FP; Reid, IK; Shulman, A; Laycock, GM; Dixson, S; THE BIOLOGICAL ACTIONS OF 1, 10-
592 PHENANTHROLINE AND 2, 2'-BIPYRIDINE HYDROCHLORIDES, QUATERNARY SALTS AND

593 METAL CHELATES AND RELATED COMPOUNDS: 1. BACTERIOSTATIC ACTION ON SELECTED
594 GRAM-POSITIVE, GRAM-NEGATIVE AND ACID-FAST BACTERIA. *Aust J Exp Biol Med Sci.* **1969**
595 *47(2)*,203-18.

596 [6] Metcalfe, C; Thomas, JA. Kinetically inert transition metal complexes that reversibly bind to DNA. *Chem*
597 *Soc Rev.* **2003**, *32(4)*,215-24.

598 [7] Li, F; Collins, JG; Keene, FR. Ruthenium complexes as antimicrobial agents. *Chem Soc Rev.* **2015**,
599 *44(8)*,2529-42.

600 [8] Southam, HM; Butler, JA; Chapman, JA; Poole, RK. The microbiology of ruthenium complexes. *Adv Microb*
601 *Physiol.* **2017** *1*;71, 1-96.

602 [9] Martínez, A; Rajapakse, CS; Naoulou, B; Kopkalli, Y; Davenport, L; Sánchez-Delgado, RA. The mechanism
603 of antimalarial action of the ruthenium (II)–chloroquine complex [RuCl₂ (CQ)] *2*. *JBIC J. Biol. Inorg. Chem.*
604 **2008**, *13(5)*, 703-12.

605 [10] Corrêa, RS; da Silva, MM; Graminha, AE; Meira, CS; Dos Santos, JA; Moreira, DR; Soares, MB; Von
606 Poelhsitz, G; Castellano, EE; Bloch Jr, C; Cominetti, MR. Ruthenium (II) complexes of 1, 3-thiazolidine-2-
607 thione: Cytotoxicity against tumor cells and anti-Trypanosoma cruzi activity enhanced upon combination with
608 benznidazole. *J. Inorg. Biochem.* **2016**, *1*:156, 153-63.

609 [11] Barbosa, MI; Correa, RS; de Oliveira, KM; Rodrigues, C; Ellena, J; Nascimento, OR; Rocha, V.P; Nonato,
610 F.R; Macedo, T.S; Barbosa-Filho, J.M; and Soares, M.B. Antiparasitic activities of novel ruthenium/lapachol
611 complexes. *J. Inorg. Biochem.* **2014**, *1*;136, 33-39.

612 [12] Jackson, Y; Wyssa, B; Chappuis, F. Tolerance to nifurtimox and benznidazole in adult patients with chronic
613 Chagas' disease. *J. Antimicrob. Chemother.* **2020**, *75(3)*, 690-696.

614 [13] Agarwal, A; Kanekar, S; Sabat, S; Thamburaj, K. Metronidazole-induced cerebellar toxicity. *Neurol Int.*
615 **2016**, *8(1)*, 4-6.

616 [14] Fairlamb, AH; Horn D. Melarsoprol resistance in African trypanosomiasis. *Trends Parasitol.* **2018**, *34(6)*,
617 481-92.

618 [15] Talapko, J; Škrlec, I; Alebić, T; Jukić, M; Včev, A. Malaria: the past and the present. *Microorganisms.*
619 **2019**, *7(6)*, 179.

- 620 [16] WHO. 2019. World malaria report 2019. Available: [https://www.who.int/malaria/publications/world-](https://www.who.int/malaria/publications/world-malaria-report-2019/en/)
621 [malaria-report-2019/en/](https://www.who.int/malaria/publications/world-malaria-report-2019/en/).
- 622 [17] Yan, SL; Wakasuqui, F; Wrenger, C. Point-of-care tests for malaria: Speeding up the diagnostics at the
623 bedside and challenges in malaria cases detection. *Diagnostic Microbiology and Infectious Disease*. **2020**, *3*,
624 115122.
- 625 [18] Stringer, T; Quintero, MA; Wiesner, L; Smith, GS; Nordlander, E. Evaluation of PTA-derived ruthenium
626 (II) and iridium (III) quinoline complexes against chloroquine-sensitive and resistant strains of the *Plasmodium*
627 *falciparum* malaria parasite. *J Inorg Biochem*. **2019**, *191*, 164-173.
- 628 [19] Adams, M; de Kock, C; Smith, PJ; Land, KM; Liu, N; Hopper, M; Hsiao, A; Burgoyne, AR; Stringer, T;
629 Meyer, M; Wiesner, L. Improved antiparasitic activity by incorporation of organosilane entities into half-
630 sandwich ruthenium (II) and rhodium (III) thiosemicarbazone complexes. *Dalton Trans*. **2015**, *44*(5), 2456-
631 2468.
- 632 [20] Coppée, R; Sabbagh, A; Clain, J. Structural and evolutionary analyses of the *Plasmodium falciparum*
633 chloroquine resistance transporter. *Sci Rep*. **2020**, *10*(1), 1-5.
- 634 [21] Rylands, LI; Welsh, A; Maepa, K; Stringer, T; Taylor, D; Chibale, K; Smith, GS. Structure-activity
635 relationship studies of antiplasmodial cyclometallated ruthenium (II), rhodium (III) and iridium (III) complexes
636 of 2-phenylbenzimidazoles. *Eur Jo Med Chem*. **2019**, *161*, 11-21.
- 637 [22] Cutillas, N; Yellol, GS; de Haro, C; Vicente, C; Rodríguez, V; Ruiz, J. Anticancer cyclometalated
638 complexes of platinum group metals and gold. *Coord Chem Rev*. **2013**, *257*(19-20), 2784-2797.
- 639 [23] Thangavel, P; Viswanath, B; Kim, S. Recent developments in the nanostructured materials functionalized
640 with ruthenium complexes for targeted drug delivery to tumors. *Int J Nanomed*. **2017**, *12*, 2749.
- 641 [24] Souza, NB; Aguiar, AC; Oliveira, AC; Top, S; Pigeon, P; Jaouen, G; Goulart, MO; Krettli, AU.
642 Antiplasmodial activity of iron (II) and ruthenium (II) organometallic complexes against *Plasmodium falciparum*
643 blood parasites. *Memórias do Instituto Oswaldo Cruz*. **2015**, *110*(8), 981-988.
- 644 [25] Ekengard, E; Glans, L; Cassells, I; Fogeron, T; Govender, P; Stringer, T; Chellan, P; Lisensky, GC; Hersh,
645 WH; Doverbratt, I; Lidin, S. Antimalarial activity of ruthenium (II) and osmium (II) arene complexes with
646 mono-and bidentate chloroquine analogue ligands. *Dalton Trans*. **2015**, *44*(44), 19314-19329.

647 [26] Sánchez-Delgado, RA; Navarro, M; Pérez, H; Urbina, JA. Toward a novel metal-based chemotherapy
648 against tropical diseases. 2. Synthesis and antimalarial activity in vitro and in vivo of new ruthenium- and
649 rhodium- chloroquine complexes. *J Med Chem.* **1996**, *39*(5), 1095-1099.

650 [27] Chellan, P; Land, KM; Shokar, A; Au, A; An, SH; Taylor, D; Smith, PJ; Riedel, T; Dyson, PJ; Chibale, K;
651 Smith, GS. Synthesis and evaluation of new polynuclear organometallic Ru (II), Rh (III) and Ir (III) pyridyl ester
652 complexes as in vitro antiparasitic and antitumor agents. *Dalton Trans.* **2014**, *43*(2), 513-526.

653 [28] Franz, AK; Wilson, SO. Organosilicon molecules with medicinal applications. *J Med Chem.* **2013**, *56*(2),
654 388-405.

655 [29] Blunder, M; Hurkes, N; Spirk, S; List, M; Pietschnig, R. Silanetriols as in vitro inhibitors for AChE.
656 *Bioorganic & medicinal chemistry letters.* **2011**, *21*(1), 363-365.

657 [30] Martínez, A; Carreon, T; Iniguez, E; Anzellotti, A; Sánchez, A; Tyan, M; Sattler, A; Herrera, L;
658 Maldonado, R.A; Sánchez-Delgado, R.A. Searching for new chemotherapies for tropical diseases: Ruthenium-
659 clotrimazole complexes display high in vitro activity against *Leishmania major* and *Trypanosoma cruzi* and low
660 toxicity toward normal mammalian cells. *J Med Chem.* **2012**, *55*(8), 3867-3877.

661 [31] Hillard, EA; Pigeon, P; Vessières, A; Amatore, C; Jaouen, G. The influence of phenolic hydroxy
662 substitution on the electron transfer and anti-cancer properties of compounds based on the 2-ferrocenyl-1-
663 phenyl-but-1-ene motif. *Dalton Trans.* **2007**, *43*, 5073-5081.

664 [32] WHO. (2020). Chagas disease (also known as American trypanosomiasis). Available:
665 [https://www.who.int/news-room/fact-sheets/detail/chagas-disease-\(american-trypanosomiasis\)](https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)).

666 [33] Radisic, MV; Repetto, SA. American trypanosomiasis (Chagas disease) in solid organ transplantation.
667 *Transpl Infect Dis.* **2020**, e13429.

668 [34] Sesti-Costa, R; Carneiro, ZA; Silva, MC; Santos, M; Silva, GK; Milanezi, C; da Silva, RS; Silva, JS.
669 Ruthenium complex with benzimidazole and nitric oxide as a new candidate for the treatment of chagas disease.
670 *PLoS Negl. Trop. Dis.* **2014**, *2*;8(10), e3207.

671 [35] Fandzloch, M; Arriaga, JM; Sánchez-Moreno, M; Wojtczak, A; Jezierska, J; Sitkowski, J; Wiśniewska, J;
672 Salas, JM; Łakomska, I. Strategies for overcoming tropical disease by ruthenium complexes with purine analog:
673 Application against *Leishmania* spp. and *Trypanosoma cruzi*. *J. Inorg. Biochem.* **2017**, *1*;176, 144-155.

674 [36] Ong, YC; Roy, S; Andrews, PC; Gasser, G. Metal compounds against neglected tropical diseases. *Chem.*
675 *Rev.* **2018**, 3;119(2), 730-796.

676 [37] Sarniguet, C; Toloza, J; Cipriani, M; Lapier, M; Vieites, M; Toledano-Magaña, Y; García-Ramos, JC; Ruiz-
677 Azuara, L; Moreno, V; Maya, JD; Azar, CO. Water-soluble ruthenium complexes bearing activity against
678 protozoan parasites. *Biol. Trace Elem. Res.* **2014**, 159(1), 379-392.

679 [38] Romero, AH; López, SE. In silico molecular docking studies of new potential 4-phthalazinyl-hydrazones on
680 selected Trypanosoma cruzi and Leishmania enzyme targets. *J Mol Graphics Modell.* **2017**, 76, 313-329.

681 [39] Moreira, DR; de Oliveira, AD; de Moraes Gomes, PA; de Simone, CA; Villela, FS; Ferreira, RS; da Silva,
682 AC; dos Santos, TA; de Castro, MC; Pereira, VR; Leite, A. Conformational restriction of aryl
683 thiosemicarbazones produces potent and selective anti-Trypanosoma cruzi compounds which induce apoptotic
684 parasite death. *Eur. J. Med. Chem.* **2014**, 75, 467-478.

685 [40] Das, P; Alam, MN; Paik, D; Karmakar, K; De, T; Chakraborti, T. Protease inhibitors in potential drug
686 development for Leishmaniasis. *Indian J Biochem Biophys.* **2013**, 50(5), 3363-3376

687 [41] Magalhaes Moreira, DR; Lima Leite, AC; Santos, RR; Soares, MB. Approaches for the development of new
688 anti-Trypanosoma cruzi agents. *Curr Drug Targets.* **2009**, 10(3), 212-231.

689 [42] Bastos, TM; Barbosa, MI; da Silva, MM; da C. Júnior, JW; Meira, CS; Guimaraes, ET; Ellena, J; Moreira,
690 DR; Batista, AA; Soares, MB. Nitro/nitrosyl-ruthenium complexes are potent and selective anti-trypanosoma
691 cruzi agents causing autophagy and necrotic parasite death. *Antimicrob Agents Chemother.* **2014**, 58(10), 6044-
692 6055.

693 [43] Donnici, CL; Araújo, MH; Oliveira, HS; Moreira, DR; Pereira, VR; de Assis Souza, M; de Castro, MC;
694 Leite, AC. Ruthenium complexes endowed with potent anti-Trypanosoma cruzi activity: Synthesis, biological
695 characterization and structure–activity relationships. *Bioorg Med Chem.* **2009**, 17(14), 5038-5043.

696 [44] Sánchez-Delgado, RA; Navarro, M; Lazard, K; Atencio, R; Capparelli, M; Vargas, F; Urbina, JA; Bouillez,
697 A; Noels, AF; Masi D. Toward a novel metal based chemotherapy against tropical diseases 4. Synthesis and
698 characterization of new metal-clotrimazole complexes and evaluation of their activity against Trypanosoma
699 cruzi. *Inorganica Chim Acta.* **1998**, 275, 528-540.

700 [45] Iniguez, E; Sánchez, A; Vasquez, MA; Martínez, A; Olivas, J; Sattler, A; Sánchez-Delgado, RA;
701 Maldonado, RA. Metal–drug synergy: new ruthenium (II) complexes of ketoconazole are highly active against
702 Leishmania major and Trypanosoma cruzi and nontoxic to human or murine normal cells. *J Biol Inorg Chem.*
703 **2013**, *18*(7), 779-790.

704 [46] Rodriguez Arce, E; Sarniguet, C; Moraes, TS; Vieites, M; Tomaz, AI; Medeiros, A; Comini, MA; Varela, J;
705 Cerecetto, H; Gonzalez, M; Marques, F. A new ruthenium cyclopentadienyl azole compound with activity on
706 tumor cell lines and trypanosomatid parasites. *J Coord Chem.* **2015**, *68*(16), 2923-2937.

707 [47] Toledo, JC; Neto, BD; Franco, DW. Mutual effects in the chemical properties of the ruthenium metal center
708 and ancillary ligands upon coordination. *Coord Chem Rev.* **2005**, *249*(3-4), :419-431.

709 [48] Silva, JJ; Osakabe, AL; Pavanelli, WR; Silva, JS; Franco, DW. In vitro and in vivo antiproliferative and
710 trypanocidal activities of ruthenium NO donors. *Br J Pharmacol.* **2007**, *152*(1), 112-121.

711 [49] Verlinde, CL; Hannaert, V; Blonski, C; Willson, M; Périé, JJ; Fothergill-Gilmore, LA; Opperdoes, FR;
712 Gelb, MH; Hol, WG; Michels, PA. Glycolysis as a target for the design of new anti-trypanosome drugs. *Drug*
713 *Resist Updat.* **2001**, *4*(1), 50-65.

714 [50] Silva, JJ; Guedes, PM; Zottis, A; Balliano, TL; Nascimento Silva, FO; Franca Lopes, LG; Ellena, J; Oliva,
715 G; Andricopulo, AD; Franco, DW; Silva, JS. Novel ruthenium complexes as potential drugs for Chagas's
716 disease: enzyme inhibition and in vitro/in vivo trypanocidal activity. *Br J Pharmacol.* **2010**, *160*(2), 260-269.

717 [51] Hartinger, CG; Zorbas-Seifried, S; Jakupec, MA; Kynast, B; Zorbas, H; Keppler, BK. From bench to
718 bedside—preclinical and early clinical development of the anticancer agent indazolium trans-[tetrachlorobis (1H-
719 indazole) ruthenate (III)](KP1019 or FFC14A). *J Inorg Biochem.* **2006**, *100*(5-6), 891-904.

720 [52] Anitha, P; Chitrapriya, N; Jang, YJ; Viswanathamurthi, P. Synthesis, characterization, DNA interaction,
721 antioxidant and anticancer activity of new ruthenium (II) complexes of thiosemicarbazone/semicarbazone
722 bearing 9, 10-phenanthrenequinone. *J Photochem Photobiol B.* **2013**, *129*, 17-26.

723 [53] Demoro, B; Rossi, M; Caruso, F; Liebowitz, D; Olea-Azar, C; Kemmerling, U; Maya, JD; Guiset, H;
724 Moreno, V; Pizzo, C; Mahler, G. Potential mechanism of the anti-trypanosomal activity of organoruthenium
725 complexes with bioactive thiosemicarbazones. *Biol Trace Elem Res.* **2013**, *153*(1), 371-381.

726 [54] Bergamo, A; Gaiddon, C; Schellens, JH; Beijnen, JH; Sava, G. Approaching tumour therapy beyond
727 platinum drugs: status of the art and perspectives of ruthenium drug candidates. *J Inorg Biochem.* **2012**, *106*(1),
728 90-99.

729 [55] Gambino, D; Otero, L. Perspectives on what ruthenium-based compounds could offer in the development of
730 potential antiparasitic drugs. *Inorg Chim Acta.* **2012**, *393*, 103-114.

731 [56] Otero, L; Smircich, P; Vieites, M; Ciganda, M; Severino, PC; Terenzi, H; Cerecetto, H; Gambino, D; Garat,
732 B. DNA conformational changes and cleavage by ruthenium (II) nitrofurylsemicarbazone complexes. *J Inorg*
733 *Biochem.* **2007**, *101*(1), 74-79.

734 [57] Miserachs, HG; Cipriani, M; Grau, J; Vilaseca, M; Lorenzo, J; Medeiros, A; Comini, MA; Gambino, D;
735 Otero, L; Moreno, V. Antitumor and antiparasitic activity of novel ruthenium compounds with polycyclic
736 aromatic ligands. *J Inorg Biochem.* **2015**, *150*, 38-47.

737 [58] Ong, YC; Gasser, G. Organometallic compounds in drug discovery: Past, present and future. *Drug Discov*
738 *Today Technol.* **2019**, 1-8.

739 [59] de Souza, W; Rodrigues, JC. Sterol biosynthesis pathway as target for anti-trypanosomatid drugs.
740 *Interdiscip Perspect Infect Dis.* **2009**, *2009*, 1-20.

741 [60] Demoro, B; Sarniguet, C; Sánchez-Delgado, R; Rossi, M; Liebowitz, D; Caruso, F; Olea-Azar, C; Moreno,
742 V; Medeiros, A; Comini, MA; Otero, L. New organoruthenium complexes with bioactive thiosemicarbazones as
743 co-ligands: potential anti-trypanosomal agents. *Dalton Trans.* **2012**, *41*(5), 1534-1543.

744 [61] Costa, MS; Gonçalves, YG; Nunes, DC; Napolitano, DR; Maia, PI; Rodrigues, RS; Rodrigues, VM; Von
745 Poelhsitz, G; Yoneyama, KA. Anti-Leishmania activity of new ruthenium (II) complexes: Effect on parasite-host
746 interaction. *J Inorg Biochem.* **2017**, *175*, 225-231.

747 [62] Sabzevari, S; Mohebbi, M; Hashemi, SA. Mucosal and mucocutaneous leishmaniasis in Iran from 1968 to
748 2018: a narrative review of clinical features, treatments, and outcomes. *Int J Dermatol.* **2020**, *59*(5), 606-612.

749 [63] Torres-Guerrero, E; Quintanilla-Cedillo, MR; Ruiz-Esmenjaud, J; Arenas, R. Leishmaniasis: a review.
750 *Fl000Res.* **2017**, 6.

751 [64] Marcusso Orsini, T; Kawakami, NY; Panis, C; Fortes dos Santos Thomazelli, AP; Tomiotto-Pellissier, F;
752 Cataneo, AH; Kian, D; Megumi Yamauchi, L; Gouveia Junior, FS; de França Lopes, LG; Cecchini, R.

753 Antileishmanial activity and inducible nitric oxide synthase activation by RuNO complex. *Mediators Inflamm.*
754 **2016**, *2016*.

755 [65] Zaki, M; Hairat, S; Aazam, ES. Scope of organometallic compounds based on transition metal-arene
756 systems as anticancer agents: starting from the classical paradigm to targeting multiple strategies. *RSC Adv.*
757 **2019**, *9*(6), 3239-3278.

758 [66] Marković, K; Milačić, R; Marković, S; Kladnik, J; Turel, I; Ščančar, J. Binding kinetics of ruthenium
759 pyridone chemotherapeutic candidates to human serum proteins studied by HPLC-ICP-MS. *Molecules.* **2020**,
760 *25*(7), 1512.

761 [67] Van Assche, T; Deschacht, M; da Luz, RA; Maes, L; Cos, P. Leishmania–macrophage interactions: Insights
762 into the redox biology. *Free Radic Biol Med.* **2011**, *51*(2), 337-351.

763 [68] Colina-Vegas, L; Godinho, JL; Coutinho, T; Correa, RS; de Souza, W; Rodrigues, JC; Batista, AA;
764 Navarro, M. Antiparasitic activity and ultrastructural alterations provoked by organoruthenium complexes
765 against *Leishmania amazonensis*. *New J Chem.* **2019**, *43*(3), 1431-1439.

766 [69] Toledano-Magana, Y; García-Ramos, JC; Torres-Gutierrez, C; Vazquez-Gasser, C; Esquivel-Sanchez, JM;
767 Flores-Alamo, M; Ortiz-Frade, L; Galindo-Murillo, R; Nequiz, M; Gudino-Zayas, M; Laclette, JP. Water-
768 soluble ruthenium (II) chiral heteroleptic complexes with amoebicidal in vitro and in vivo activity. *J Med Chem.*
769 **2017**, *60*(3), 899-912.

770 [70] Cedillo-Gutiérrez, EL; Hernández-Ayala, LF; Torres-Gutiérrez, C; Reina, M; Flores-Alamo, M; Carrero,
771 JC; Ugalde-Saldívar, VM; Ruiz-Azuara, L. Identification of descriptors for structure-activity relationship in
772 ruthenium (II) mixed compounds with antiparasitic activity. *Eur J Med Chem.* **2020**, *189*, 112084.

773 [71] Athar, F; Husain, K; Abid, M; Agarwal, SM; Coles, SJ; Hursthouse, MB; Maurya, MR; Azam, A. Synthesis
774 and Anti-Amoebic Activity of Gold (I), Ruthenium (II), and Copper (II) Complexes of Metronidazole. *Chem*
775 *Biodivers.* **2005**, *2*(10), 1320-1330.

776 [72] Nagaraja, S; Ankri, S. Target identification and intervention strategies against amebiasis. *Drug Resist*
777 *Updat.* **2019**, *44*, 1-4.

778 [73] Tyebji, S; Seizova, S; Hannan, AJ; Tonkin, CJ. Toxoplasmosis: A pathway to neuropsychiatric disorders.
779 *Neurosci Biobehav Rev.* **2019**, *96*, 72-92.

780 [74] Kanatani, S; Fuks, JM; Olafsson, EB; Westermark, L; Chambers, B; Varas-Godoy, M; Uhlén, P; Barragan,
781 A. Voltage-dependent calcium channel signaling mediates GABAA receptor-induced migratory activation of
782 dendritic cells infected by *Toxoplasma gondii*. *PLoS Pathog.* **2017**, *13*(12), e1006739.

783 [75] Weidner, JM; Kanatani, S; Uchtenhagen, H; Varas-Godoy, M; Schulte, T; Engelberg, K; Gubbels, MJ; Sun,
784 HS; Harrison, RE; Achour, A; Barragan, A. Migratory activation of parasitized dendritic cells by the protozoan
785 *Toxoplasma gondii* 14-3-3 protein. *Cell microbiol.* **2016**, *18*(11), 1537-1550.

786 [76] Konradt, C; Ueno, N; Christian, DA; DeLong, JH; Pritchard, GH; Herz, J; Bzik, DJ; Koshy, AA; McGavern,
787 DB; Lodoen, MB; Hunter, CA. Endothelial cells are a replicative niche for entry of *Toxoplasma gondii* to the
788 central nervous system. *Nat microbiol.* **2016**, *1*(3), 1-8.

789 [77] Hopper, AT; Brockman, A; Wise, A; Gould, J; Barks, J; Radke, JB; Sibley, LD; Zou, Y; Thomas, S.
790 Discovery of selective *Toxoplasma gondii* dihydrofolate reductase inhibitors for the treatment of toxoplasmosis. *J*
791 *med chem.* **2019**, *62*(3), 1562-1576.

792 [78] Sharma, M; Chauhan, PM. Dihydrofolate reductase as a therapeutic target for infectious diseases:
793 opportunities and challenges. *Future Med Chem.* **2012**, *4*(10), 1335-1365.

794 [79] Alday, PH; Doggett, JS. Drugs in development for toxoplasmosis: advances, challenges, and current status.
795 *Drug Des Devel Ther.* **2017**, *11*, 273.

796 [80] Robert-Gangneux, F; Dardé, ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin*
797 *Microbiol Rev.* **2012**, *25*(2), 264-296.

798 [81] Barna, F; Debache, K; Vock, CA; Küster, T; Hemphill, A. In vitro effects of novel ruthenium complexes in
799 *Neospora caninum* and *Toxoplasma gondii* tachyzoites. *Antimicrob Agents Chemother.* **2013**, *57*(11), 5747-
800 5754.

801 [82] Basto, AP; Müller, J; Rubbiani, R; Stibal, D; Giannini, F; Süss-Fink, G; Balmer, V; Hemphill, A; Gasser,
802 G; Furrer, J. Characterization of the activities of dinuclear thiolato-bridged arene ruthenium complexes against
803 *Toxoplasma gondii*. *Antimicrob Agents Chemother.* **2017**, *61*(9), e01031-17.

804 [83] Bouzaidi-Tiali, N; Aeby, E; Charriere, F; Pusnik, M; Schneider, A. Elongation factor 1a mediates the
805 specificity of mitochondrial tRNA import in *T. brucei*. *EMBO J.* **2007**, *26*(20), 4302-4312.

806 [84] Esseiva, AC; Naguleswaran, A; Hemphill, A; Schneider, A. Mitochondrial tRNA import in *Toxoplasma*
807 *gondii*. *J Biol Chem*. **2004**, 279(41), 42363-42368.

808 [85] Wang, S; Zhang, Z; Wang, Y; Gadahi, JA; Xu, L; Yan, R; Song, X; Li, X. *Toxoplasma gondii* elongation
809 factor 1-alpha (TgEF-1 α) is a novel vaccine candidate antigen against toxoplasmosis. *Front microbiol*. **2017**, 8,
810 168.

811 [86] Gupta, S; Bhandari, YP; Reddy, MV; Harinath, BC; Rathaur, S. *Setaria cervi*: immunoprophylactic
812 potential of glutathione-S-transferase against filarial parasite *Brugia malayi*. *Exp Parasitol*. **2005**, 109(4), 252-
813 255.

814 [87] Chandra, S; Ruhela, D; Deb, A; Vishwakarma, RA. Glycobiology of the *Leishmania* parasite and emerging
815 targets for antileishmanial drug discovery. *Expert Opin Ther Targets*. **2010**, 14(7), 739-757.

816 [88] Boscá, L; Zeini, M; Través, PG; Hortelano, S. Nitric oxide and cell viability in inflammatory cells: a role
817 for NO in macrophage function and fate. *Toxicol*. **2005**, 208(2), 249-258.

818 [89] Pavanelli, WR; da Silva, JJ; Panis, C; Cunha, TM; de Abreu Oliveira, FJ; de Menezes, MC; Costa, IC; da
819 Silva Thomé, G; da Silva, FO; de Sousa, EH; de França Lopes, LG. Nitric Oxide Donors with Therapeutic
820 Strategic in Experimental Schistosomiasis *Mansoni*. *Am J Immunol*. **2014**, 10(4), 225.

821 [90] Sundaraneedi, MK; Tedla, BA; Eichenberger, RM; Becker, L; Pickering, D; Smout, MJ; Rajan, S;
822 Wangchuk, P; Keene, FR; Loukas, A; Collins, JG. Polypyridylruthenium (II) complexes exert anti-schistosome
823 activity and inhibit parasite acetylcholinesterases. *PLoS Negl Trop Dis*. **2017**, 11(12), e0006134.

824 [91] Ruano, AL; López-Abán, J; Fernández-Soto, P; de Melo, AL; Muro, A. Treatment with nitric oxide donors
825 diminishes hyperinfection by *Strongyloides venezuelensis* in mice treated with dexamethasone. *Acta Trop*. **2015**,
826 152, 90-95.

827 [92] Pullan, RL; Smith, JL; Jasrasaria, R; Brooker, SJ. Global numbers of infection and disease burden of soil
828 transmitted helminth infections in 2010. *Parasit Vectors*. **2014**, 7(1), 1-9.

829 [93] Vyas, NA; Bhat, SS; Kumbhar, AS; Sonawane, UB; Jani, V; Joshi, RR; Ramteke, SN; Kulkarni, PP; Joshi,
830 B. Ruthenium (II) polypyridyl complex as inhibitor of acetylcholinesterase and A β aggregation. *Eur J Med*
831 *Chem*. **2014**, 75, 375-381.

- 832 [94] Sundaraneedi, M; Eichenberger, RM; Al-Hallaf, R; Yang, D; Sotillo, J; Rajan, S; Wangchuk, P; Giacomini,
833 PR; Keene, FR; Loukas, A; Collins, JG. Polypyridylruthenium (II) complexes exert in vitro and in vivo
834 nematocidal activity and show significant inhibition of parasite acetylcholinesterases. *Int J Parasitol Drugs Drug*
835 *Resist.* **2018**, 8(1), 1-7.
- 836 [95] Klementowicz, JE; Travis, MA; Grecis, RK. *Trichuris muris*: a model of gastrointestinal parasite infection.
837 *Semin Immunopathol.* **2012**, 34(6), 81528.
- 838