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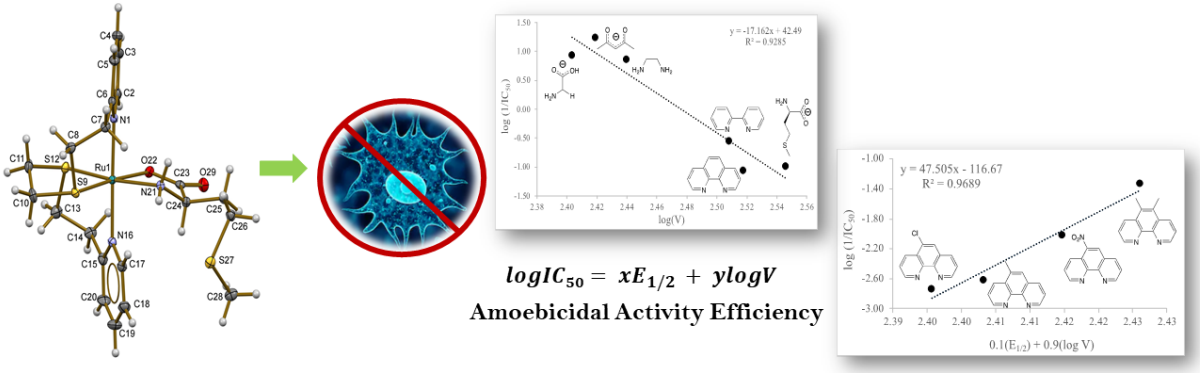

$$logIC_{50} = xE_{1/2} + ylogV$$

Amoebicidal Activity Efficiency

Identification of descriptors for structure-activity relationship in ruthenium (II) mixed compounds with antiparasitic activity

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Abstract

Herein is presented the synthesis, characterization, electrochemical studies, DFT calculations and *in vitro evaluation of amoebicidal activity* in trophozoites of *Entamoeba histolytica* of twenty ruthenium (II) mixed compounds with general formulae: [Ru(*pdto*)(E-E)]Cl_x (E-E bidentate, either neutral or negatively charged ligands). For compounds under study, O-O, N-O and N-N auxiliary donor ligands demonstrate to have a crucial impact on the electronic properties and that it is possible to modulate the antiparasitic activity. Among analyzed complexes, only four present a better performance compared to typically used metronidazole drug (IC_{50}< 6.80 μmol/L) to treat amoebiasis disease. For studied compounds, structure-activity relationships are strongly determined by either the redox potential ($\boldsymbol{E}_{1/2}$) of Ru^{II}/Ru^{III} and calculated molar volume ($\boldsymbol{V}$) of the complexes.

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Keywords: ruthenium (II) mixed complexes, amebiasis, amoebicidal activity, SAR studies, DFT calculations.

1. Introduction

Amebiasis is a harmful tropical disease responsible for approximately 100,000 worldwide annual deaths [1-5]. It is caused by the protozoan parasite *Entamoeba histolytica*, an important pathogen with major global impact [6,7] that colonizes the human gut through contaminated water or food and might cause life-threatening illness such as liver abscesses and hemorrhagic colitis [7-10]. After malaria (*Plasmodium malaria*), amebiasis is the second leading cause of death due to a protozoan parasite [11]. To face this serious public health care issue, nitroimidazoles derived compounds have been used for the last decades since they are considered to be the most effective against amebiasis disease [12-14]. In particular, metronidazole, tinidazole, ornidazole and secnidazole are effective drugs for treating amebiasis [15]. Nevertheless, irritation of the gastric mucus lining, vomiting, diarrhea, blood in urine, headache, and with less frequently a central nervous system toxicity are important side effects [16,17]. In addition, there is an increasing concern in the development of *E. histolytica* strains resistance to these drugs [18].

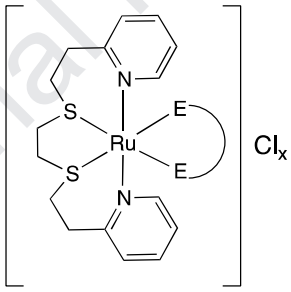
As an alternative to the widely nitroimidazole-type treatments, metal containing compounds arise as an attractive, versatile and powerful option to mitigate this disease [19-22]. In fact, medicinal inorganic chemistry is a rapidly growing field with a direct impact on the diagnosis and therapy of several diseases. In recent years, novel metal-based anticancer drugs have been developed to reduce toxicity, to increase clinical efficacy and to broaden the scope of activity [23-26]. The rational design of these metal-centered molecules allows tuning some important features, such as coordination modes, geometries

and redox behavior and thus, enhances the capability to participate in biological processes. It has been reported that these systems are useful to interact with DNA and other important biomolecules, to promote reactive oxygen species (ROS) generation, and to induce cellular apoptosis [27-35]. Additionally, divalent complexes of the first-row transition metals (Mn, Fe, Co, Ni, Cu and Zn) and Ru have shown excellent amoebicidal activity [36,37]. In particular, for some Ru^{II} chiral heteroleptic complexes with amoebicidal in vitro and in vivo activity, it has been recently demonstrated that trophozoites exposed to these compounds die through an apoptotic pathway triggered by ROS production without apparent cytotoxic effect to human peripheral blood lymphocytes. Furthermore, generated ROS shown a direct relationship with the redox potential as well as with the IC_{50} values. As the redox potential becomes more positive, a decrease in ROS generation and an increase in the IC_{50} value have been recorded [37]. Additionally, another advantage of these inorganic metal-based compounds over nitroimidazoles is their high solubility in water, which would help in their use for treatment when the parasite has migrated to a vital organ or when the ameba has become cystic [38,39].

Furthermore, *pdto* (1,8-bis-2-pyridyl-3,6-dithioctane) is a flexible open-chain tetradentated ligand with nitrogen and sulfur as donor atoms [40,41]. In addition, several complexes have been found to be stable showing uni to four-dentate coordination modes [42-44]. *Pdto* related systems have been proposed to act as biological materials (interaction with DNA and cyclodextrins), conductive polymers, and SOD mimetics [44-47]. Besides, octahedral Ru complexes have been suggested to play an important role in medicinal inorganic chemistry acting as anticancer, antimicrobial, antifungal and antiparasitic agents [48-51].

In the last decade, various heteroleptic compounds of Ru^{II} with *pdto* and different auxiliary ligands have been synthesized, characterized and tested showing an important *in vitro* and *in vivo* amoebicidal activity. In particular, acetylacetonate, glycinate and ethylenediamine Ru-*pdto* complexes show a relationship between biological activity, ROS production and redox potential [52].

The aim of this work is to find a structure-activity relationship in ruthenium (II) mixed compounds with antiparasitic activity. To this purpose, twenty complexes of the type [Ru(*pdto*)(E-E)]Cl_x (E-E bidentate, either neutral or negatively charged ligands, Scheme 1) were synthesized, characterized by diverse experimental techniques (IR, ^{1}H-NMR, UV-vis, *Log* ***P*** and redox potential, $\boldsymbol{E}_{1/2}$) and DFT studies and then, evaluated *in vitro* in trophozoites of *Entamoeba histolytica.*



Scheme 1. Molecular structure of [Ru(*pdto*)(E-E)]Cl_x complexes.

To assess the relationship between the *in vitro* antiparasitic activity and the structure of these complexes, several experimental and computational parameters were taken into account. The best structure-activity correlation results arise when redox potential (experimental and calculated) and theoretical molar volume are used, (alone and combined). These results could serve to estimate the antiparasitic activity of ruthenium (II) mixed compounds with only a few electronic and molecular descriptors. Furthermore, these

results could also promote the use of theoretical calculations to predict biological activity of different complexes.

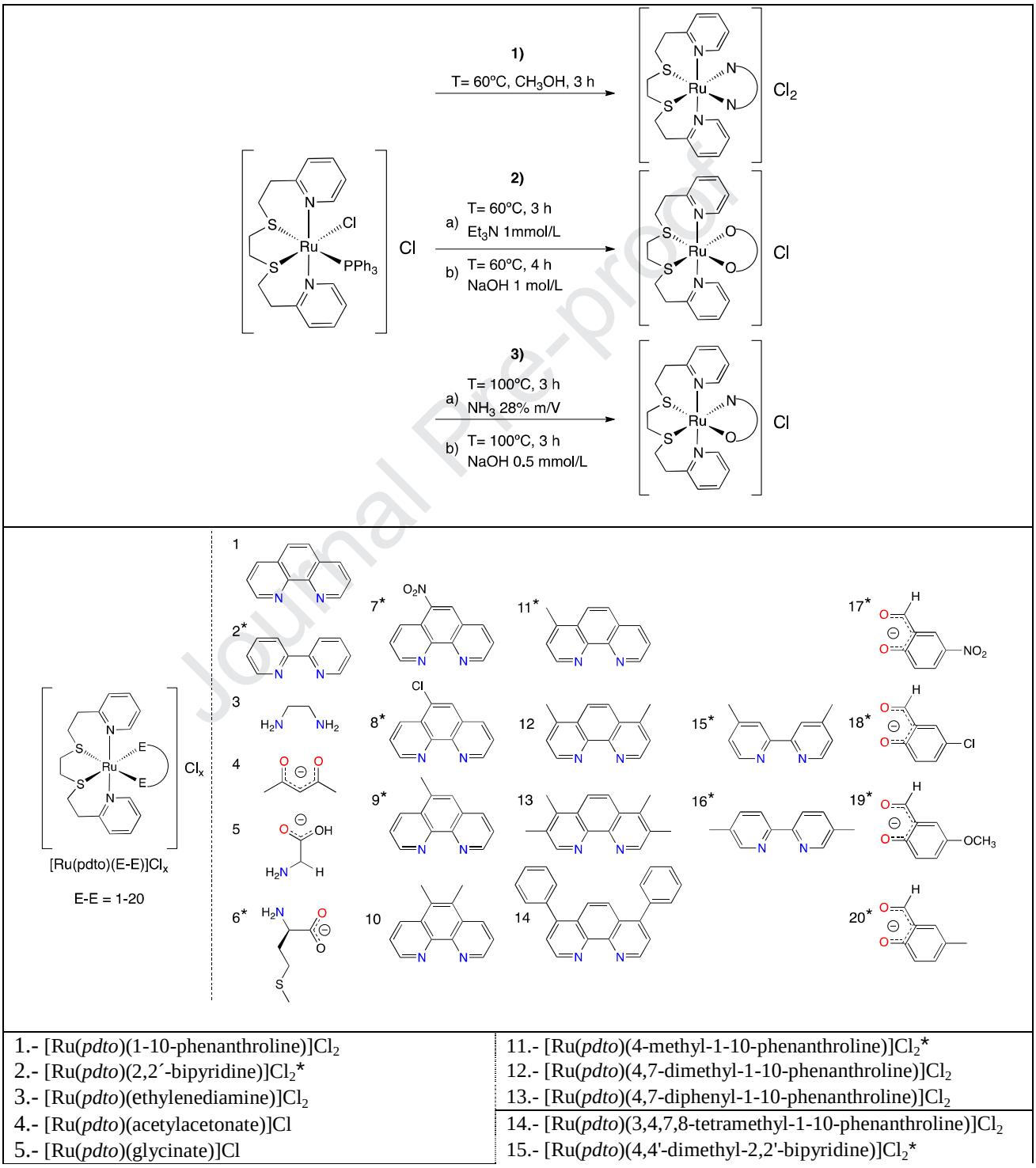
2. Results and discussion

2.1.- Chemistry

Scheme 2 presents the three general synthetic routes followed to obtain the complexes under study. In all the cases, [Ru(*pdto*)Cl(PPh_3)]Cl was the precursor and only slight changes are observed between different paths. The general formulae of these complexes is [Ru(*pdto*)(E–E)]Cl_x, in which *pdto* ligand remains unchanged and coordinated to Ru, and only the secondary ligand varies (E-E). All auxiliary E-E ligands are bidentate and can be either neutral or negatively charged ligands. The molecular structures of E-E ligands are also presented in Scheme 2. It is possible to distinguish N-N donors (synthetic path 1; E-E: 1 to 3 and 7 to 16), O-O donors (synthetic path 2.a corresponds to E-E: 4 and 2.b concerns E-E: 17 to 20) and mixed N-O donors (synthetic path 3.a and 3.b correspond to E-E: 5 and 6, respectively).

To study these complexes, four main groups were established. The first one contains pristine auxiliary ligands, *i.e.* without any structural modification (1 to 6: 1-10-phenanthroline, 2,2'-bipyridine, ethylenediamine, acetylacetonate, glycinate and methioninate). The second group includes the 1,10-phenanthroline substituted compounds (7 to 14). For this group, an additional categorization was done: compounds 7 to 10 are 1,10-phenanthroline substituted in position 5, while complexes 11 to 14 are substituted in position 4. The third group correspond to the systems containing the two substituted 2,2'-bipyridine ligands (15 and 16) and the last group is related to the salicylaldehydate compounds (17-20). As it can be seen in Scheme 2, systems 2, 6 to 9, 11 and 15 to 20 are

marked with star indicating that they have been synthesized and characterized for the very first time, meanwhile, the other 8 complexes (1, 3-5, 10, 12-14) have been already been presented in other studies [37,42]. For compounds analyzed, racemic mixtures were tested.



1.- [Ru(*pdto*)(1-10-phenanthroline)]Cl_2
2.- [Ru(*pdto*)(2,2´-bipyridine)]Cl_2*
3.- [Ru(*pdto*)(ethylenediamine)]Cl_2
4.- [Ru(*pdto*)(acetylacetonate)]Cl
5.- [Ru(*pdto*)(glycinate)]Cl

11.- [Ru(*pdto*)(4-methyl-1-10-phenanthroline)]Cl_2*
12.- [Ru(*pdto*)(4,7-dimethyl-1-10-phenanthroline)]Cl_2
13.- [Ru(*pdto*)(4,7-diphenyl-1-10-phenanthroline)]Cl_2
14.- [Ru(*pdto*)(3,4,7,8-tetramethyl-1-10-phenanthroline)]Cl_2
15.- [Ru(*pdto*)(4,4'-dimethyl-2,2'-bipyridine)]Cl_2*

6.- [Ru(*pdto*)(methioninate)]Cl*	16.- [Ru(*pdto*)(5,5'-dimethyl-2,2'-bipyridine)]Cl_2*
7.- [Ru(*pdto*)(5-nitro-1-10-phenanthroline)]Cl_2*	17.- [Ru(*pdto*)(5-nitro-salicylaldehydate)]Cl*
8.- [Ru(*pdto*)(5-chloro-1-10-phenanthroline)]Cl_2*	18.- [Ru(*pdto*)(5-chloro-salicylaldehydate)]Cl*
9.- [Ru(*pdto*)(5-methyl-1-10-phenanthroline)]Cl_2*	19.- [Ru(*pdto*)(5-methoxy-salicylaldehydate)]Cl*
10.- [Ru(*pdto*)(5,6-dimethyl-1,10-phenanthroline)]Cl_2	20.- [Ru(*pdto*)(5-methyl-salicylaldehydate)]Cl*

Scheme 2. The twenty heteroleptic complexes of Ru^{II} under study.

2.2.- Spectroscopic and electrochemical characterization

The complete characterization data for the new compounds can be founded in the S1 section of the Supplementary Material. IR spectra analysis show that significant shifts occur for complexes compared to the free ligands, *i.e. pdto* (30-50 cm^{-1}) and E-E secondary ligands (50-100 cm^{-1}), suggesting that they are properly coordinated to Ru atom, as previously reported [42]. Furthermore, for the systems under examination, λ_{max} transitions correspond to the metal-ligand charge transfer, around 330-360 nm. In all cases, compounds present high molar extinction coefficient values as expected for M-L charge transfer (ε from 2402 to 17862 mol $L^{-1}cm^{-1}$). ^{1}H-NMR, ^{13}C-NMR, COSY and HSQC analysis were also done to characterize the complexes. Additionally, conductivity evaluations are in accordance with expected global charge of the complexes (1:1 or 1:2 electrolyte behavior). Finally, single crystals for complexes 2, 5 and 15 were obtained and analyzed by X-ray diffraction technique (Section S2: Figures S1-S4 and Tables S1-S5).

Figure 1 shows the voltammogram of [Ru(*pdto*)(5,5'-dimethyl-2,2'-bipyridine)]PF_6 compound acquired with a glassy carbon electrode from open circuit potential to cathodic direction. It is important to signal that, Cl^- counter ion was exchanged by PF_6^- to avoid Cl^- oxidation to Cl_2 interference. This same methodology was employed for all analyzed voltammograms. Two reduction peaks I_C, II_C and their respective oxidation waves I_A, II_A, were observed. Electronic transfer I_a is associated with the [Ru^{II}(*pdto*)(L)]/[Ru^{III}(*pdto*)(L)] process, wave II_C correspond to [Ru^{II}(*pdto*)(L)]/[Ru^{II}(*pdto*)(L^-)] reduction. Half wave

potentials values ($\boldsymbol{E}_{1/2}$) for process I and II are 1.03 and -1.85 V/Fc^{+}-Fc, respectively. Although, remain compounds present different redox behavior, for this study, only Ru^{III}/Ru^{II} redox values were taken into account. For all the other compounds under study, half wave potentials are shown in Table 1.

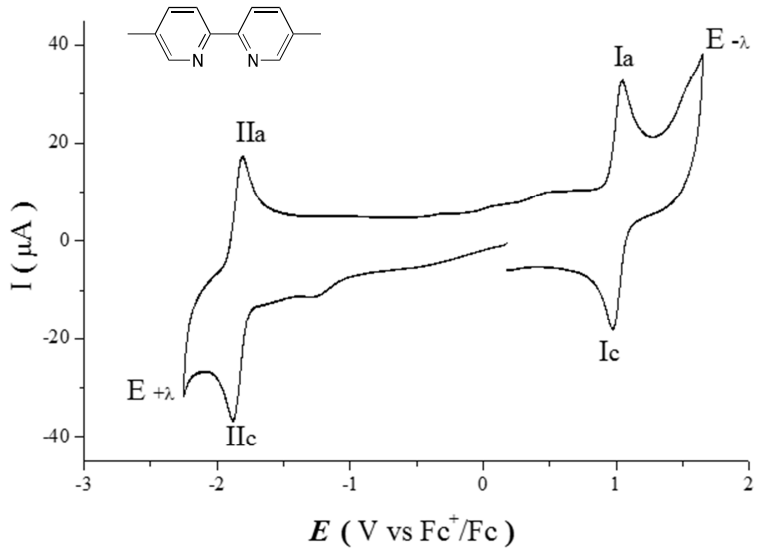


Figure 1. Cyclic voltammogram of 0.001 mol/L [Ru(*pdto*)(5,5'-dimethyl-2,2'-bipyridine)](PF_6) , 0.2 mol/L $TBABF_4$ in MeCN, v= 0.3 V/s.

Table 1 present redox potential values for ruthenium (II) mixed compounds. It has been already proposed that redox potential could play a critical role in modulating the biological activity, at least for copper and ruthenium systems [53-55]. For complexes under study, $\boldsymbol{E}_{1/2}$ for Ru^{III}/Ru^{II} redox pair, are in range between 0.33 and 1.15 V/Fc^{+}-Fc values. It is clear that compounds containing O-O and N-O donors as auxiliary ligands present lower $\boldsymbol{E}_{1/2}$ values ($\boldsymbol{E}_{1/2}$ = 0.33-0.91 V/Fc^{+}-Fc) compared to N-N secondary ligands ($\boldsymbol{E}_{1/2}$ = 1.03-1.15 V/Fc^{+}-Fc). This suggests that the former group is easier to oxidize than the latter, which corresponds to better reducing systems. It is worthy to note that [Ru(*pdto*)(ethylendiamine)]Cl_2 ($\boldsymbol{E}_{1/2}$ = 0.85 V/Fc^{+}-Fc) is the only exception and one

plausible explanation refers to the very small size and a complete saturated degree of ethylendiamine ligand. In addition, in Table 1, experimental partition coefficient (***P***) values are presented. Although, *Log* ***P*** is a very useful descriptor to classify hydrophilic behavior of organic molecules, for systems containing metal atoms and in these particular cases, no trends are observed. *Log* ***P*** behavior is complicated to analyze, and values range from -1.79 to 0.13, but no tendencies were detected. *Log* ***P*** results suggest that for metallodrugs systems, this parameter might not be the most accurate and useful.

Table 1. Redox potential ($\boldsymbol{E}_{1/2}$) and partition coefficient values (*Log* ***P***)

Compounds	$\boldsymbol{E}_{1/2}$ Ru^{II}/Ru^{III} (V/ Fc^{+}-Fc)	*Log* ***P*** (octanol/water)
[Ru(*pdto*)(1-10-phenanthroline)]Cl_2	1.08	-1.25
[Ru(*pdto*)(2,2'-bipyridine)]Cl_2	1.09	-1.05
[Ru(*pdto*)(ethylenediamine)]Cl_2	0.85	-1.33
[Ru(*pdto*)(acetylacetonate)]Cl	0.44	-0.24
[Ru(*pdto*)(glycinate)]Cl	0.58	-1.32
[Ru(*pdto*)(methioninate)]Cl	0.55	-1.41
[Ru(*pdto*)(5-nitro-1-10-phenanthroline)]Cl_2	1.15	-0.72
[Ru(*pdto*)(5-chloro-1-10-phenanthroline)]Cl_2	1.15	-0.85
[Ru(*pdto*)(5-methyl-1-10-phenanthroline)]Cl_2	1.02	-1.17
[Ru(*pdto*)(5,6-dimethyl-1,10-phenanthroline)]Cl_2	1.08	-0.87
[Ru(*pdto*)(4-methyl-1-10-phenanthroline)]Cl_2	1.10	-1.39
[Ru(*pdto*)(4,7-dimethyl-1-10-phenanthroline)]Cl_2	1.13	-0.95
[Ru(*pdto*)(4,7-diphenyl-1-10-phenanthroline)]Cl_2	1.06	-0.82
[Ru(*pdto*)(3,4,7,8-tetramethyl-1-10-phenanthroline)]Cl_2	1.01	-1.16
[Ru(*pdto*)(4,4'-dimethyl-2,2'-bipyridine)]Cl_2	1.04	-1.79
[Ru(*pdto*)(5,5'-dimethyl-2,2'-bipyridine)]Cl_2	1.03	-1.62
[Ru(*pdto*)(5-nitro-salicylaldehydate)]Cl	0.33	0.13
[Ru(*pdto*)(5-chloro-salicylaldehydate)]Cl	0.45	0.13
[Ru(*pdto*)(5-methoxy-salicylaldehydate)]Cl	0.69	-0.10
[Ru(*pdto*)(5-methyl-salicylaldehydate)]Cl	0.91	0.06

2.3.- DFT calculations

DFT geometry optimizations were performed for Ru^{II} complexes assuming a distorted octahedral arrangement with low multiplicity (singlets or doublets) as it has been previously observed and reported [37, 42]. In Figure S5, geometry optimizations and Molecular Electrostatic Potential (MEP) maps are presented and in Table S6, bond lengths

and angles for these optimized geometries are reported. In general, the values are in accordance with the X-ray structures.

Table 2 reports some calculated parameters: molar volume (***V***, cm^3/mol), electron affinity (EA, eV) and Ru^{III}/Ru^{II} redox potential ($\boldsymbol{E}_{1/2(calc)}$). The values of simulated redox potential are compared to the experimental results ($\boldsymbol{E}_{1/2(exp)}$) showing a strong correlation (R^2=0.93, Figure S6 in the Supporting Information). This is important to corroborate the computational employed strategy. It is noteworthy that also electron affinity presents an important linear correlation when compared to experimentally founded redox potentials (R^2=0.92, Figure S6). The best electron acceptor system is [Ru(*pdto*)(4,7-diphenyl-1-10-phenanthroline)]Cl_2 and results imply that 1-10-phenanthroline systems and 2,2'-bipyridine are much better electron acceptor systems compared to oxygen donor ligands (group of salicylaldehydate compounds). One possible explanation could arise from the well-known 1-10-phenanthroline and 2,2'-bipyridine electron delocalization and planarity properties. Furthermore, the obtained molar volume values are in accordance with the expected size of auxiliary ligands. In general, bulkier systems correspond to the substituted 1-10-phenanthroline systems, followed by the substituted 2,2'-bipyridine and finally the salicylaldehydate complexes.

Table 2. Calculated and experimental physicochemical properties of Ru^{II}-*pdto* complexes.

Compounds	***V*** (cm^3/mol)	EA (eV)	$\boldsymbol{E}_{1/2\ calc}$ (V)	$\boldsymbol{E}_{1/2\ exp}$ (V/Fc^+-Fc)
[Ru(*pdto*)(1-10-phenanthroline)]Cl_2	329.45	20.42	1.11	1.08
[Ru(*pdto*)(2,2'-bipyridine)]Cl_2	322.44	19.55	1.00	1.09
[Ru(*pdto*)(ethylenediamine)]Cl_2	275.53	15.79	0.68	0.85
[Ru(*pdto*)(acetylacetonate)]Cl	262.57	6.87	0.28	0.44
[Ru(*pdto*)(glycinate)]Cl	253.22	11.31	0.41	0.58
[Ru(*pdto*)(methioninate)]Cl	351.80	10.04	0.43	0.55
[Ru(*pdto*)(5-nitro-1-10-phenanthroline)]Cl_2	359.19	16.93	1.06	1.15
[Ru(*pdto*)(5-chloro-1-10-phenanthroline)]Cl_2	342.10	19.79	1.12	1.15
[Ru(*pdto*)(5-methyl-1-10-phenanthroline)]Cl_2	360.58	19.31	0.92	1.02
[Ru(*pdto*)(5,6-dimethyl-1,10-phenanthroline)]Cl_2	376.45	19.49	0.98	1.08
[Ru(*pdto*)(4-methyl-1-10-phenanthroline)]Cl_2	347.96	19.81	0.89	1.10
[Ru(*pdto*)(4,7-dimethyl-1-10-phenanthroline)]Cl_2	359.38	19.24	0.95	1.13
[Ru(*pdto*)(4,7-diphenyl-1-10-phenanthroline)]Cl_2	427.33	22.46	0.99	1.06
[Ru(*pdto*)(3,4,7,8-tetramethyl-1-10-phenanthroline)]Cl_2	403.71	18.17	0.76	1.01
[Ru(*pdto*)(4,4'-dimethyl-2,2'-bipyridine)]Cl_2	341.24	19.45	0.92	1.04
[Ru(*pdto*)(5,5'-dimethyl-2,2'-bipyridine)]Cl_2	368.83	19.47	0.9	1.03
[Ru(*pdto*)(5-nitro-salicylaldehydate)]Cl	332.41	5.51	0.47	0.33
[Ru(*pdto*)(5-chloro-salicylaldehydate)]Cl	296.62	7.47	0.40	0.45
[Ru(*pdto*)(5-methoxy-salicylaldehydate)]Cl	354.75	10.31	0.76	0.69
[Ru(*pdto*)(5-methyl-salicylaldehydate)]Cl	309.13	17.04	0.87	0.91

2.4.- Antiparasitic activity

The half inhibitory concentration (IC_{50}, μmol/L) results for the *in vitro* evaluation of *Entamoeba histolytica* parasite and ruthenium (II) mixed compounds are presented in Table 3 and Figure 2. Metronidazole is the most common drug for treating amebiasis and its IC_{50} is also presented for comparison purposes (IC_{50}= 6.80 μmol/L and *Log* $(1/IC_{50})$= -0.83). The obtained values present a wide range due to the strong modulation activity of the auxiliary ligand donor nature. In addition, the size, the global charge, the number of aromatic rings, the planarity and the electro donating and releasing groups of each complex play a crucial role in the amoebicidal activity. In this regard, IC_{50} values varies from 0.06 to 556.00 μmol/L. Comparing the established groups, substituted 2,2'-bipyridine are better than substituted 1,10-phenanthroline compounds but worse than salicylaldehydate systems. The best family correspond to the complexes containing pristine ligands. 1,10-

phenanthroline and 2,2'-bipyridine are better than their substituted ligands and it is worthy to note that among studied compounds, the more effective systems compared to metronidazole drug reference belong to this non substituted ligand group. In fact, 2,2'-bipyridine, ethylendiamine, acetylacetonate and glycinate compounds present a lower IC_{50} value compared to metronidazole (IC_{50} = 3.70, 0.14, 0.06 and 0.12 μmol/L, respectively vs 6.80 μmol/L for metronidazole, dark green bars in Figure 2: $Log\ (1/IC_{50}) > -0.83$). From all the analyzed complexes, [Ru(*pdto*)(acetylacetonate)]Cl is the more effective to *in vitro* treat *Entamoeba histolytica* (IC_{50} = 0.06 μmol/L). This system is characterized for having a small, charged, and O-O donor auxiliary ligand. In general, $[Ru(pdto)(E\text{-}E)]^{2+}$ are more hydrophilic systems compared to $[Ru(pdto)(E\text{-}E)]^{+}$, and thus, the cross through the cellular membrane could be more difficult. This is in accordance with the MEP diagrams (Figure S5), in which the electron density distribution for $[Ru(pdto)(methioninate)]^{+}$ ($[Ru(pdto)(E\text{-}E)]^{+}$) present a more homogenous distribution and less positive values. Some exceptions can be observed, principally due to the size or the positions of substituents ($[Ru(pdto)(2,2'\text{-bipyridine})]^{2+}$, IC_{50}= 3.70 μmol/L and $[Ru(pdto)(5,6\text{-dimethyl-1,10-phenanthroline})]^{2+}$, IC_{50}= 22 μmol/L). Furthermore, 10 complexes present a similar IC_{50} value compared to metronidazole (Figure 2, green bars: $-0.83 > Log\ (1/IC_{50}) > -1.50$) but metal containing compounds offer some advantages: high solubility in water, *a priori* no resistance and no harmful side-effects to development of *E. histolytica*, and ROS production, which increase apoptosis mechanism [36,37,57,58]. Hence, 14 synthesized compounds might be suitable and considered as candidates to metallo-drugs against amebiasis disease. Finally, in order to verify the antiparasitic effect of analyzed compounds, a UV-vis experiment measuring their stability in aqueous solution was performed. Results indicate that compounds are stable at

different times (0, 12, 24, 48 and 72 hours) and this might suggest that [Ru(*pdto*)(E-E)]Cl_x systems are responsible for the biological activity (Figure S7).

Table 3. *IC_{50}* value tested in trophozoites of *Entamoeba histolytica.*

Compounds	*IC_{50}* (µmol/L)	*Log (1/IC_{50})*
Metronidazole	**6.80**	**-0.83**
[Ru(*pdto*)(1-10-phenanthroline)]Cl_2	12.00	-1.08
[Ru(*pdto*)(2,2'-bipyridine)]Cl_2	**3.70**	**-0.57**
[Ru(*pdto*)(ethylenediamine)]Cl_2	**0.14**	**0.85**
[Ru(*pdto*)(acetylacetonate)]Cl	**0.06**	**1.22**
[Ru(*pdto*)(glycinate)]Cl	**0.12**	**0.92**
[Ru(*pdto*)(methioninate)]Cl	10.00	-1.00
[Ru(*pdto*)(5-nitro-1-10-phenanthroline)]Cl_2	106.00	-2.02
[Ru(*pdto*)(5-chloro-1-10-phenanthroline)]Cl_2	556.00	-2.74
[Ru(*pdto*)(5-methyl-1-10-phenanthroline)]Cl_2	422.00	-2.62
[Ru(*pdto*)(5,6-dimethyl-1,10-phenanthroline)]Cl_2	22.00	-1.34
[Ru(*pdto*)(4-methyl-1-10-phenanthroline)]Cl_2	152.00	-2.18
[Ru(*pdto*)(4,7-dimethyl-1-10-phenanthroline)]Cl_2	48.00	-1.68
[Ru(*pdto*)(4,7-diphenyl-1-10-phenanthroline)]Cl_2	11.00	-1.04
[Ru(*pdto*)(3,4,7,8-tetramethyl-1-10-phenanthroline)]Cl_2	117.00	-2.07
[Ru(*pdto*)(4,4'-dimethyl-2,2'-bipyridine)]Cl_2	17.00	-1.23
[Ru(*pdto*)(5,5'-dimethyl-2,2'-bipyridine)]Cl_2	17.50	-1.24
[Ru(*pdto*)(5-nitro-salicylaldehydate)]Cl	9.00	-0.95
[Ru(*pdto*)(5-chloro-salicylaldehydate)]Cl	11.00	-1.22
[Ru(*pdto*)(5-methoxy-salicylaldehydate)]Cl	13.00	-1.11
[Ru(*pdto*)(5-methyl-salicylaldehydate)]Cl	19.20	-1.28

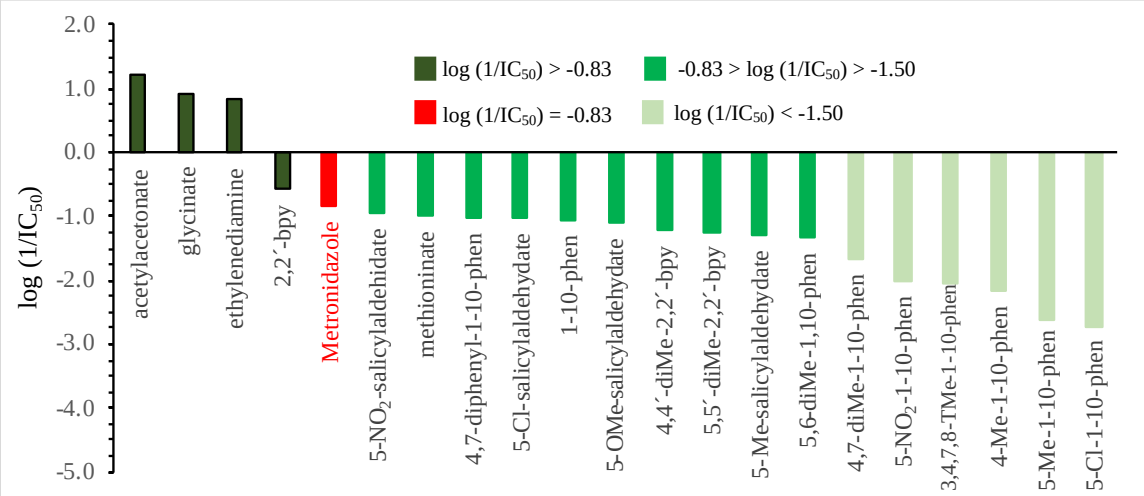


Figure 2. *Log (1/IC_{50})* values for [Ru(*pdto*)(E-E)]Cl_x and metronidazole (reference value) tested in trophozoites of *Entamoeba histolytica.*

2.5.- Physicochemical indexes related to amoebicidal activity

Structure-activity relationships are a very powerful tool to explain and to predict particular biological behaviors according to some physicochemical or steric parameters. For organic molecules, there are well-known geometric and electronic factors that are commonly used to *a priori* assess the activity. In many cases, this allows to rationally design molecular systems with *ad hoc* desired properties and enhanced activity. For compounds containing metal atoms, these relationships are often very difficult to obtain. In the present work, to establish a proper approach relating structure and biological activity, several parameters were taken into account, but only the redox potential and the calculated molar volume were accurate to describe the observed antiparasitic activity. Figure 3 presents the results for each group of compounds under study. To rationalize the obtained results, an equation considering the two parameters ($\boldsymbol{E}_{1/2}$ and $\boldsymbol{V}$) was proposed:

$$Log\ (1/IC_{50}) = x\ \boldsymbol{E}_{1/2} + y\ Log\ \boldsymbol{V}$$

Where x and y are the normalized fractions, *i.e.* $x + y = 1$, corresponding to the redox potential and molar volume, respectively. The proposed equation implies that depending on the group studied, the relative importance of the two parameters varies. For example, pristine auxiliary ligands are only correlated to the molar volume (R^2= 0.9285; Figure 3.a), and for salicylaldehydate complexes, the redox potential alone correctly fits (R^2= 0.9646; Figure 3.e). For the former, the most active compound is the acetylacetone derivative, and for the latter, the best amebicide agent is the one with the lowest redox potential value. This same behavior has been already described for other Ru-*pdto* complexes and amoebicidal activity [37]. For all the other families the ratio of the redox potential and the molar volume oscillates, indicating that both parameters are important and contribute to explain the antiparasitic activity. For both 1,10-phenanthroline groups (Figures 3.b and 3.c, R^2=0.9689

and R^2=0.9850, respectively), the volume contribution is more important than $\boldsymbol{E}_{1/2}$ and the bulkier the complex the more efficient to inhibited *Entamoeba histolytica*. In this family the compound that shows the major ability to inhibit the growth of *E. hist.* is the 4,7-diphenyl-1,10-phenanthroline. Contrarily, for 2,2'-bipyridine groups (Figure 3.d, R^2=0.9912) the smaller the complex, the more active against *Entamoeba histolytica*. The ligand with the best amebicide effect is the unsubstituted bipyridine that is the most oxidizing ligand. Since the values of simulated and experimental redox potential show a strong correlation, it is possible to employ both to describe the antiparasitic efficiency (Figure S8). On the other hand, although, lipophilicity indicator *Log* ***P*** has been reported to be one crucial factor to determine the activity of organic molecules, for complexes results do not show a clear trend and additional studies are required to properly evaluate the importance of it. Those results should be considered for further rationalize designing of ruthenium (II) mixed compounds with *pdto* and enhanced antiparasitic activity.

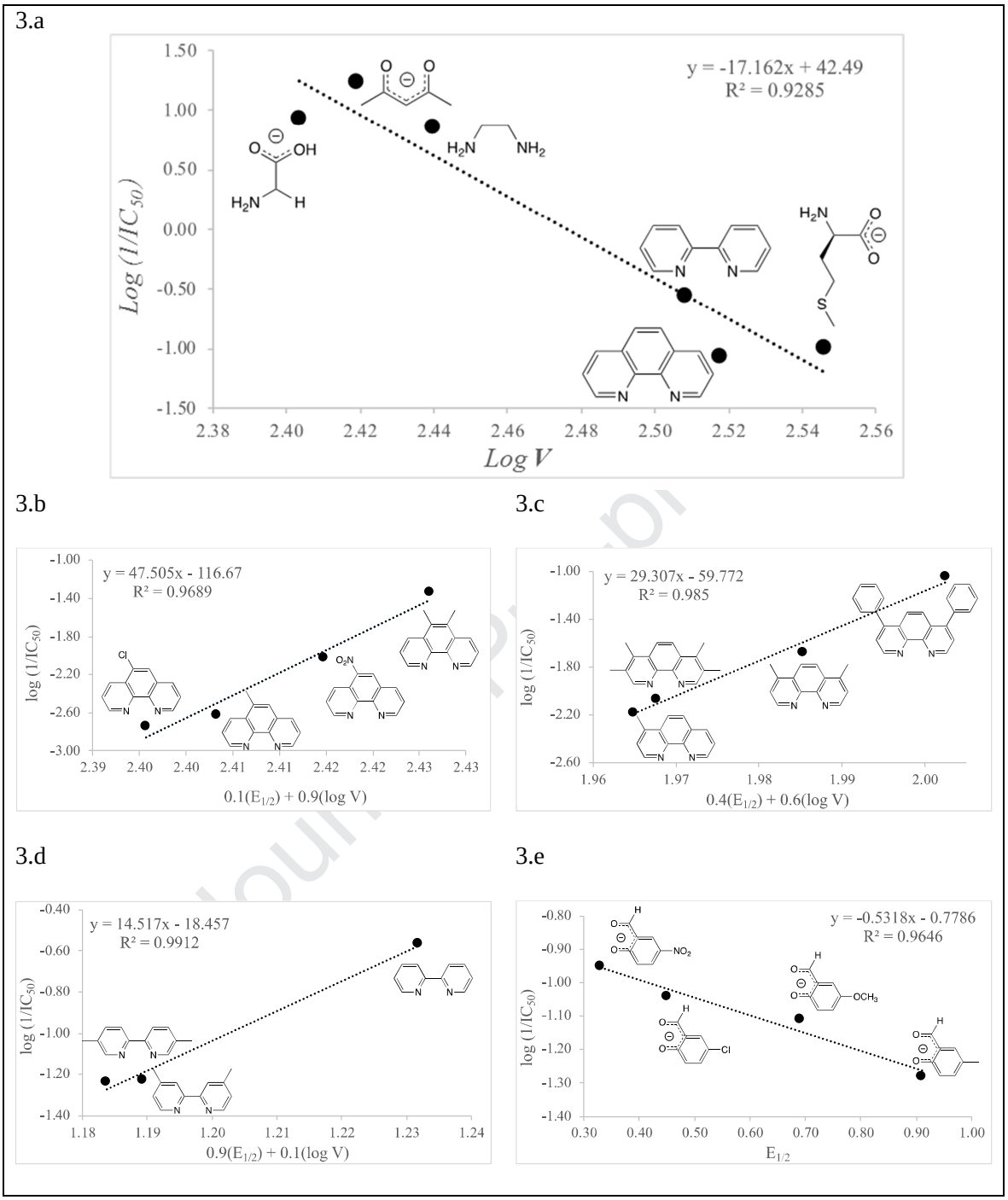


Figure 3. $E_{1/2}$ and *Log* ***V*** as descriptors to explain *in vitro* growth inhibition of *Entamoeba histolytica* results.

3. Conclusions

Twenty ruthenium (II) mixed compounds of the type [Ru(*pdto*)(E-E)]Cl_x have been synthesized, characterized and *in vitro* evaluated against *E. histolytica.* Twelve of them are reported for the first time (2, 6, 7-9, 11, 15-20). Cyclic voltammetry results indicate that complexes containing O-O and N-O donors are easier to oxidized than N-N donor ligands. In general, antiparasitic activity shows that O-O and N-O are better than N-N donor ligands. Among the complexes under study, only four of them ([Ru(*pdto*)(2,2'-bipyridine)]Cl_2, [Ru(*pdto*)(ethylenediamine)]Cl_2, [Ru(*pdto*)(acetylacetonate)]Cl and [Ru(*pdto*)(glycinate)]Cl) are better than the reference metronidazole drug to treat amoebiasis disease (IC_{50} < 6.80 μmol/L). Moreover, experimental redox potential values are in good agreement with biological effect. Additionally, DFT electronic and steric indicators provide useful information to describe the biological activity against *E. histolytica.* In this regard, $\boldsymbol{E}_{1/2}$ and theoretical molar volume are the most important parameters to explain the effectivity of these compounds to inhibit *in vitro E. histolytica* trophozoite parasite. Those results indicate that computational studies could be a powerful tool to predict the antiparasitic activity of Ru^{II}-*pdto* mixed compounds.

4. Experimental section

4.1.- Materials and methods

The solvents (J. T. Baker) and reagents (Sigma-Aldrich) used did not require further purification. Elemental analysis was performed on an EAGER 200 analyzer (EAGER 200 CHNS/method). The infrared spectra obtained in a range of 4000-400 cm^{-1} were acquired in a Nicolet Avatar 320 FT-IR device using KBr pads. The spectra of MNR ^{1}H and ^{13}C, COSY and HSQC were acquired in a VARIAN VNMRS 400 MHz equipment, using an

internal standard of tetramethylsilane (TMS) and using methanol-D_4 as solvent. The Packard 5484A diode matrix spectrophotometer was used to acquire UV-vis spectra with methanol as a solvent, in a range of 190 to 8000 nm. The conductivities were measured with a JENWAY 4330 conductivity meter, the conductivity data were obtained at concentrations of $1x10^{-3}$ mol/L in methanol solutions at 25 ° C.

Diffraction measurements were made on an Oxford Diffraction Gemini-Atlas diffractometer with a CCD area detector, the source of molybdenum monochromatic radiation was λ=0.71073 Å at a temperature of 130K. The structures were solved by direct methods using the SHELXS-97-2 program package. The molecular structure graphs were generated using the ORTEP3 program for Windows and Mercury 3.10.3.

All electrochemical measurements were made in acetonitrile (HPLC grade) with a CH Instruments electrochemical Workstation CH760E potentiostat with a conventional three electrode array. Experimental determinations of the partition coefficient ***P*** were made using the shake flask method in the octanol-water system. The determination of ***P*** was carried out by absorption spectroscopy in the UV-visible region.

4.2.- Synthesis

The 1,8-bis-(2-pyridyl)-3,6-dithioctane (*pdto*) and the dichlorotris(triphenylphosphine) ruthenium (II) raw material, as well as the precursor [$RuCl_2(PPh_3)_3$] were synthesized from as previously reported [42].

Precursor of the mixed compounds of ruthenium(II): chlorine chloride(1,8-bis-(2-pyridyl)-3,6-dithioctane)(triphenylphosphine) ruthenium(II), [Ru(Cl(*pdto*)(PPh_3)]Cl. The *pdto* is dissolved in methanol and added to a suspension of [$RuCl_2(PPh_3)_3$] in methanol. The mixture is heated at reflux for 3 h under constant stirring, the solution is concentrated for subsequent precipitation using ethyl ether. The precipitate that forms is yellow, filtered and

washed with cold ethyl ether, leaving it to dry under vacuum. Its purification is carried out by means of a chromatographic column made with neutral alumina using CH_2Cl_2 as solvent and a mixture CH_2Cl_2:MeOH (5: 1) as eluent.

Synthesis of mixed coordination compounds of ruthenium(II). The synthesis of the mixed compounds of ruthenium(II) with *pdto* and phenanthrolines, bipyridines, ethylenediamine, acetylacetonate and glycinate, were carried out according to that already reported in previous works, placing equimolar amounts of the precursor [RuCl(*pdto*)(PPh_3)]Cl and the respective bidentate ligands, in reflux with appropriate solvents and the time required according to the type of compound. Purification is carried out by means of a chromatographic column made with neutral alumina as support and CH_2Cl_2 as solvent; and a mixture CH_2Cl_2:MeOH (5:1) as eluent for all compounds.

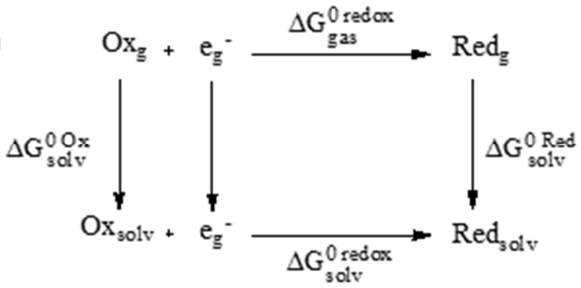
The synthesis of compounds containing different monosubstituted salicylaldehydates in position 5 [RuCl(*pdto*)(5-R-salal)]Cl (where R = methyl, methoxy, chlorine, nitro) is presented below. To obtain 1 mmol of the coordination compounds, [RuCl(*pdto*)(PPh_3)]Cl (precursor) was dissolved in methanol. Subsequently, a certain amount of the bidentate salicylaldehyde ligand (1: 1 stoichiometric ratio) was weighed, which was dissolved in methanol and 1 mL of a 1 mol/L aqueous NaOH previously prepared was added; both solutions were mixed and heated at reflux for 4 h, under constant stirring. The solution was concentrated to precipitate with ethyl ether, obtaining a fine brick red precipitate. It was filtered, washed with ether and dried under vacuum. Purification is carried out by means of a chromatographic column made with neutral alumina as support and CH_2Cl_2 as solvent.

4.3.- Computational details

DFT calculations were performed with Gaussian 09 package [56], using M06 functional [57] in conjunction with SDD basis set to describe Ru atom and Los Alamos LanL2DZ for

all the other atoms (H, C, N, O and S) [58-60]. This methodology has been already used to study metal containing systems [61-65]. All structures were confirmed as minima on the potential energy surface through the vibrational frequency analysis (0 imaginary frequencies). Molar volume was obtained with a single point calculation based on optimized structures and Molecular Electrostatic Potential (MEP) were plotted as a guide to assess charge distribution and global reactivity.

To calculate redox potentials, we followed previous reports that have proven to be useful for transition metal complexes [66-78], and in which geometries of ferrocene (Cp_2Fe) and ferrocenium ($[Cp_2Fe]^+$) in the eclipsed conformation (D_5h) were taken into account and optimized in gas phase. Gas phase and solvation ΔG energies were calculated by the same level of theory and using SMD continuum solvation model [69] and MeCN to simulate the same environment of the electrochemical experiments. To obtain gas phase and solvation free energies of all species, a thermochemical analysis at 298.15 K and 1 atm was performed following the next cycle:



Redox potential was determined through the free energy changes and according to the one electron exchange of Nernst equation:

$$\Delta G^{0\,redox}_{solv} = \Delta G^{0\,redox}_{gas} + \Delta G^{0\,Red}_{S} - \Delta G^{0\,Ox}_{S} \quad (1)$$

$$\Delta G^{0\,redox}_{solv} = -FE^{0}_{calc} \quad (2)$$

Where F is the Faraday constant. The values are doubly referenced first, to the standard hydrogen electrode that, which in MeCN has a value of 4.6 V [70] and second to a value of 0.68 V for Cp_2Fe estimated by the same level of theory to be in agreement with the experimental measurements.

4.4.- Amoebicidal activity

For the determination of the mean inhibitory concentration (IC_{50}), amibian viability assays were performed for *Entamoeba histolytica* trophozoites of the HM1: IMSS strain, where it was analyzed using the vital exclusion method with the trypan blue marker. 100 µL are taken every 24 h for 72 h of each of the tubes and 1 µL of marker is added. Samples are incubated at room temperature for 5 minutes and viable cell counts are performed in a hematocytometer. For the calculation of the average inhibitory concentration value IC_{50}, a multivariable analysis is used such as the Stat graph 2010 program.

4.5.- Electrochemical studies

All electrochemical experiments were carried out in solutions 0.001 mol/L of sample and 0.1 mol/L of terabutylammonium hexafluorophosphate (Bu_4NPF_6) in anhydrous MeCN. The measurements were performance in a CH Instruments Electrochemical Workstation CH760E potentiostat. A conventional three electrode array was used: 3 mm diameter carbon glassy disk as working electrode, platinum wire as counter-electrode and Ag/AgCl was the reference. According to IUPAC recommendations [71], Voltammograms were referenced with ferrocene (99.9% purity) in an internal setting. Due to the chloride oxidation interferes with the Ru^{II}/Ru^{III} process, the electrochemical measures were made with the PF6- salts of the complexes and this do not affect the magnitude of the metallic center redox potential. The solutions were bubbled with nitrogen prior and between each experiment.

*4.6.- Log **P** determinations*

The experimental determination of this parameter is made from a standard solution with a known concentration (0.05 mmol/L) of the test sample dissolved in water, an aliquot of 1 mL (aqueous phase) is taken, placed in a falcon tube of 10 mL An equivalent volume (1 mL) of 1-octanol grade HPLC (organic phase) was also added to said tube. Both phases are vigorously stirred with a vortex type apparatus, for 10 minutes constantly and maintaining a temperature between 24.5 to 25 °C, this to promote the distribution of the compound in both phases. An emulsion was obtained, which was separated with the help of a centrifuge. The time used for the separation was 3 minutes at 1600 revolutions per minute, sufficient time in which a complete separation of both phases was observed.

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Identification of descriptors for structure-activity relationship in ruthenium (II) mixed compounds with antiparasitic activity

Highlights

1. Molar volume and redox potentials are the best descriptors of amoebicidal activity.
2. Twenty Ru^{II} mixed complexes were synthesized and fully characterized.
3. X-ray diffraction structures of three complexes are presented, intermolecular interactions stabilize the crystal conformation.
4. Computational estimations of redox potential, molar volume are in agreement with experimental values.
5. Four complexes shows remarkable efficacy against *E. hist.* compared to amebiasis drug metronidazole.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: