Ru(II)-Based Antimicrobials: Looking Beyond Organic Drugs

A.I. Ramos[1], T.M. Braga[2] and S.S. Braga*[,1]

[1]*Department of Chemistry, CICECO, University of Aveiro, 3810-193 Aveiro, Portugal*

[2]*Antibiotic Stress and Virulence of Enterococci Laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*

Abstract: This review deals with the bactericidal, anti-fungal and even anti-parasitary properties of ruthenium complexes, both inorganic and organometallic, establishing comparisons between these and the available commercial drugs.

The description is mostly composed of results found in the literature of the past two decades, complemented with relevant results from our group's research on antimicrobial ruthenium complexes.

The complexes are divided into five groups according to the kind of ligands, geometry and chemical nature.

The first group comprises ruthenium octahedral complexes with Schiff bases, the most well explored kind of ruthenium antimicrobials. The second group comprises complexes with planar ligands and an overall more flattened geometry, designed for DNA intercalation. In the following two groups, ruthenium complexes feature a particular functionality, which is, in one case, the presence of the PTA ligand for higher solubility in water, and, in the second, the mimicry of an active organic drug. Finally, a small section presents the most recent results on supramolecular antimicrobials comprising ruthenium, in particular a polymer and a cyclodextrin adduct.

Keywords: Ruthenium complexes, supramolecular compounds, antimicrobial activity, pathogenic bacteria, leishamaniasis.

1. INTRODUCTION

Antimicrobial metal complexes are known since the 1950's. The first reports are associated with studies on the mechanism of the bactericidal action of oxine (8-hydroxyquinoline), deemed to arise not from the organic compound itself, but rather from chelation products with copper and iron ions available in the medium, the chelates being the first known antimicrobial metal agents [1]. However, a significant growth in the number of publications on these complexes has spawned only in the two latest decades, which are covered by the present review.

Ruthenium complexes provide a rich platform for the design of antimicrobials, allowing to choose between different Ru oxidation states, ligands and coordination geometries. These will ultimately afford a variety of interactions with biological targets, which include DNA (for the metallointercalators in section 3 and several other species), and enzymes or protein receptors.

Ruthenium complexes can also be conceived with base on an organic ligand known to act on a particular biological target, to take advantage of the specific properties inherent to the transition metal center. This is the case of the biomimetic complexes described in section 5.

Finally, in the current panorama of scientific research, the efforts devoted to the synthesis of a stable- and pathogen-selective metallic drug may require not only the use of suitable, biomimetic ligands on the first co-ordination sphere, but should rather include a supramolecular organization. Two examples of such strategy are presented in section 6, one comprising a rutheno-organic polymer as a drug delivery agent for silver nanoparticles and another one describing a ruthenium complex with a second sphere capsule having drug carrier properties.

2. RUTHENIUM COMPLEXES OF SCHIFF BASES AND DIKETONATES

The vast majority of antimicrobial ruthenium complexes comprises at least one Schiff base ligand. Schiff bases are defined by a covalent linkage between a carbonyl group and a free amine to form an imine, and occur frequently in metabolic reactions: (i) in glycolysis, in the mechanism of aldolase, (ii) in the phosphogluconate pathway, in the mechanism of transaldolase and (iii) during amino acid degradation, in some transamination reactions.

Ruthenium Schiff base complexes in the literature are usually classified according to the oxidation state of the ruthenium core, typically Ru(II) or Ru(III); the same classification will be followed here.

2.1. Ru(II) Complexes

The number of reported compounds in this class with claimed bactericidal action rounds four tenths, all sharing an octahedral geometry.

The vast majority of complexes comprises a tridentate Schiff base ligand, with the general formula [Ru(η^3-Schiff)(CO)(axial)$_2$]. The Schiff occupies three positions on the Ru(II) equatorial plane, which is completed by the carbonyl

*Address correspondence to this author at the Department of Chemistry and CICECO, University of Aveiro, 3810-193, Aveiro, Portugal; Tel: +351 234401418; Fax: +351 234360084; E-mail: sbraga@ua.pt

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ligand (C≡O). The axial ligands may be triphenylphosphine/arsenine (PPh_3/$AsPh_3$) or comprise one of the previous and one pyridine (pyr) or one piperidine (pip). For an example, refer to Fig. (**1**), depicting the structure of the complex [Ru(dhatsc)(CO)(PPh_3)$_2$] (from single-crystal X-ray diffraction).

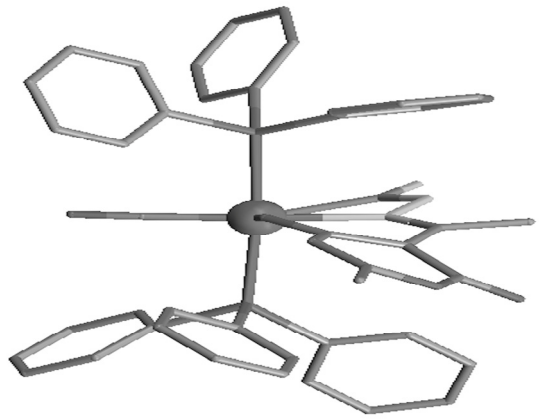
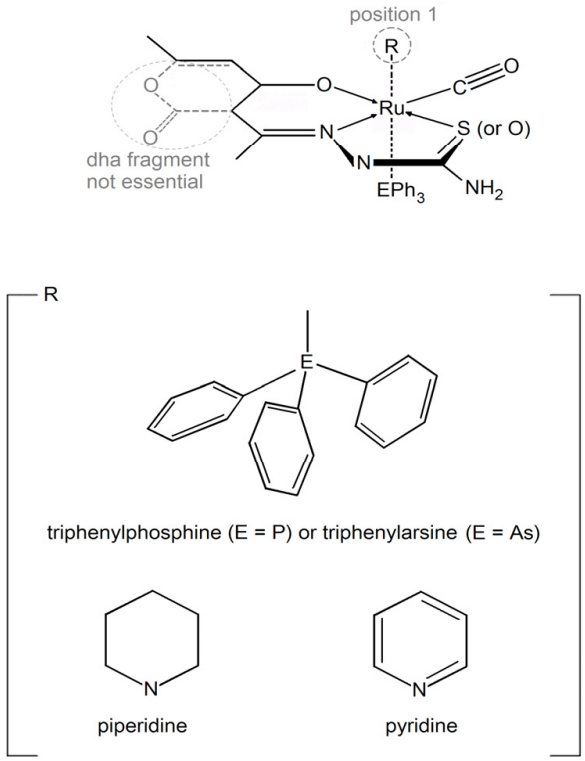


Fig. (1). The crystal structure of [Ru(dhatsc)(CO)(PPh_3)$_2$] (dhatsc = dehydroacetic acid thiosemicarbazone). Redrawn using Mercury [2] from the atomic coordinates of complex, available at the Cambridge Structural Database.

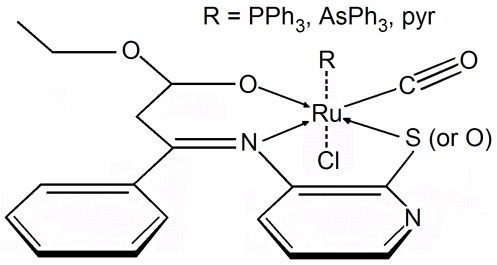
A conjectural rationale on the structures of a family of different Ru(II) complexes and their activities is presented in Scheme **1**. Of note is the fact that the different Schiff ligands used — dhatsc versus methyl- or ethyl-acetothiosemicarbazone (atsc) — exerted low influence on the antimicrobial activity, whilst the replacement of the axial ligand R was quite more relevant to the biological action, as the most effective bactericides were the complexes with one axial pyridine [3, 4]. Indeed, the inhibition halos of [Ru(atsc)(CO)(PPh_3)(pyr)] on *Escherischia coli* and *Bacillus subtilis* were very similar (though slightly smaller) to those of the reference drug ampicillin [3]. Furthermore, the Ru(dhatsc) complexes, besides their bactericidal action, also exhibited a moderate inhibition towards *Candida albicans* and *Aspergillus niger* [4].

A second family of complexes with modified Schiff base backbones was prepared by replacing the –HN=C(Y)–NH_2 (Y = S, O) moiety with an aryl, these are represented in Scheme **2**. However, their performances were poorer, with inhibition halos quite smaller than those of the reference drug amikacin. In fact, even the most active complex of this family has only reached 65% of the efficacy of the reference drug against *E. coli*, and 75% on *Staphylococcus aureus* [5].

In another family of complexes (Scheme **3**), introduction of the extra aromatic functionality was carried without altering the –HN=CO– function to avoid hampering the activity. The methoxyl (O-CH_3) groups on the aromatic rings had no particular influence on the overall inhibitory action of the complexes against the tested bacteria, *E. coli* and *B. subtillis* [6]. Of relevance was the presence of a chlorine on position 2 (see Scheme **3**); chlorinated complexes had slightly higher inhibition halos [6]. Noteworthy and in parallel with the complexes depicted in Scheme **1**, a pyridine in position 1 was beneficial towards activity: all pyridine-bearing complexes had activities nearly equivalent to the reference drug ampicillin [6]. The pyridine residue can then be deemed as a key ligand for the antimicrobial activity of Ru(II) Schiff complexes.

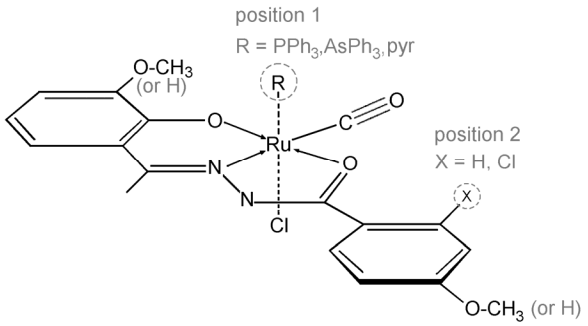


Scheme 1. A conjectural structure-activity rationale for the family of Ru(II) complexes with dhatsc or methyl- or ethyl-acetothiosemicarbazone as the Schiff ligand.



Scheme 2. Structure of a second family of complexes with modified Schiff base backbones, in which the –HN=C(Y)–NH_2 (Y = S, O) functionality was replaced with an aryl.

Ru(II) complexes with a tetradentate – η^4 – Schiff base ligand were also developed. The ligand derives from salicylalethylenediamine (salen) and lies on the equatorial plane; the axial positions are filled by dimethylsulfoxide (dmso). These complexes, two enantiomers, were found to inhibit selectively the growth of Gram-positive bacteria (tested: *B. subtilis*, *B. megaterium*, *B. cereus*, *S. aureus* and *Micrococcus luteus*) and had no activity on the tested Gram-negative bacteria. Noteworthy, the activity was enantiomer-



Scheme 3. Structural backbone of a family of Ru(II) complexes with an aromatic Schiff base in which the –HN=CO– function was kept intact.

related, with the *S*,*S*-enantiomer exhibiting the best bactericidal action [7].

2.2. Ru(III) Complexes

These complexes are all octahedral, as those of Ru(II), but present a wider variety of composition and coordination modes for the Schiff ligands. As a general observation, the activities of these complexes were parallel to those of the Ru(II) complexes, with none being able to surpass the activity of the commercially available antibiotics. Still, a few notable complexes have exhibited bactericidal activities very similar to the drug of reference, as follows.

The best bactericides had the general formula Ru(η^3-Schiff)(EPh_3)$_2$X (EPh_3 = triphenylphosphine/arsine, X = Cl or Br). Using acetophenalsemicarbazone derivatives as the Schiff base ligands, bactericidal activities have reached 80% of the effectiveness of ampicillin against *B. subtillis* and 75% against *E. coli* [8]. Complexes based on salicylbenzoylhy-drazone Schiff ligands were slightly more active against *E. coli*, reaching 70% of the effectiveness of streptomycin, than against *Bacillus spp* and *Pseudomonas spp* (average effectiveness of 65-67%) [9].

Other reported complexes, such as Ru(η^3-Schiff)(EPh_3)X_2 (X = Cl or Br) [10] and Ru(η^2-Schiff)$_2Cl_2$ [11], had low or moderate bactericidal effect in comparison with reference drug streptomycin. Of highlight is the complex Ru(η^2-Schiff)$_2Cl_2$ with η^2-Schiff = thiophene-acetalthiosemi-carbazone, which inhibited *B. macerans* with approximately 80% of the efficacy of streptomycin [11]. By replacing one of the chloride ligands with triphenylphosphine/arsine to afford Ru(η^2-Schiff)$_2$(EPh_3)X (X = Cl or Br), better activities were attained. Within this family, the most promising complexes exhibited inhibition halos of 24 to 26 mm, very close (almost within the experimental error) to that of ampicillin (30 mm) against *E. coli* and *B. subtilis* [12].

Finally, a Ru(III) complex with a tetradentate cyclic *bis*-pirimidino-tetraimine was found to inhibit *Pseudomonas aeruginosa* and *Xanthomonas campestris* with roughly 75% the efficacy of streptomycin. The complex was also effective against the two tested fungi species: it inhibited the growth of *Fusarium oxysporium* by 69% in comparison with the control plate (no drug) and of *Alternaria porri* by 79%; note that the reference drug captan had growth inhibition percentages of 78% and 90%, respectively [13].

2.3. Antifungal Ru Schiff Complexes

The first known cytotoxic ruthenium Schiff complexes of bases were antifungals, and, to date, there remain a few which were only reported to be active against fungi. Overall, they have the same general formula as those in subsection 2.1, with exception of the binuclear ruthenium compounds.

One family of these [Ru(η^3-Schiff)(CO)(axial)$_2$] complexes, where Schiff = salicylaldehyde amines was tested on a single microorganism, *A. flavus*, but showed only a moderate inhibition (from 50 to 63% the efficacy of bavistin) [14]. Two families of these complexes are described, one with Schiff = di-substituted cyclobutanylaminothiazole [15], and another where the Schiff base was formed from the condensation of anthranilic acid with acetylacetone, salicylaldehyde, *o*-vanillin or *o*-hydroxyacetophenone [16]; the complexes exhibited fungicidal action on *Aspergillus flavus*, *F. oxysporium* and *Rhizoctonia solani*, but only to a moderate extent, as the observed inhibition halos were small (diameters of 10 to 15 mm) compared to the reference drug bavistin (30-32 mm).

Binuclear complexes are also reported, in particular di-rutheno(III)thiobis(β-diketonates) [17], which were tested on the same microorganisms and, again, have only displayed moderate action, being unable to surpass that of bavistin.

3. RUTHENIUM COMPLEXES WITH PLANAR LIGANDS FOR DNA INTERCALATION

This section focuses on ruthenium metallointercalators: complexes which are able to bind and react with DNA [18]. These feature one or more planar aromatic heterocyclic ligands which can insert and stack between the base pairs of doublehelical DNA.

3.1. Background

The first DNA intercalation studies were carried out with tris(phenanthroline) complexes of ruthenium [19-23] and other transition metals [22, 24, 25], proposed to bind to DNA through three noncovalent modes: (i) electrostatically, (ii) binding hydrophobically against the minor groove, and (iii) partial intercalation of one of the phenanthroline ligands into the DNA base stack from the major groove side (Fig. **2**).

Ligands with a larger surface area available for intercalation have higher intercalative binding affinity. The best known and more extensively studied of these extended planar ligands is dipyrido[3,2-a:2',3'-c]phenazine or dppz, which stacks between the base pairs and is often used as stable anchor in the major groove. The bipyridyl and phenanthroline dppz-ruthenium complexes were found to intercalate relatively nonspecifically into B-form DNA with a slight preference for AT-rich regions [26].

One must note, however, that intercalative properties and cytotoxicity may not always go hand in hand and that the vast majority of these complexes remain to be tested for their potential biological action. So far, a direct relation between DNA intercalation and microbiological growth inhibition

was only demonstrated for some complexes of copper [27], gold [28] and vanadium [29].

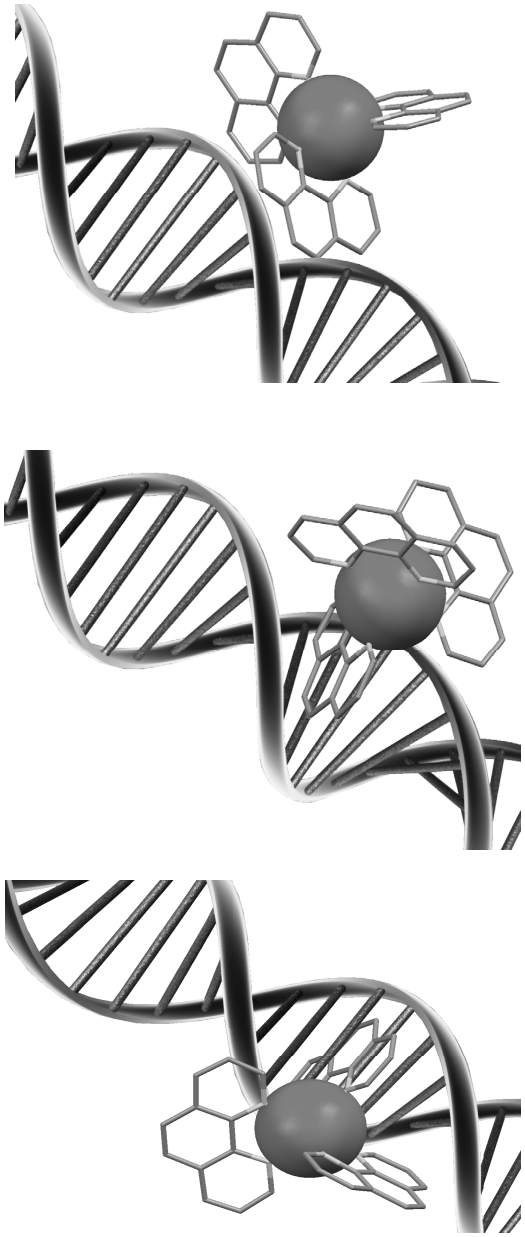


Fig. (2). The three proposed modes of interaction of a metallointercalator (herein represented by the $Ru(phen)_3$ cation) with DNA: **a**) electrostatic interaction; **b**) hydrophobic binding; **c**) partial intercalation in the base pairs.

Regarding ruthenointercalators, there are scarce reports of successful cytotoxic agents on human cancer cell lines [30]. The few known examples of antimicrobial and anti-leishmaniasis complexes are presented in the following subsections.

3.2. Antimicrobial Ruthenointercalators

This class is comprised entirely of ruthenium(II) polypyridyl complexes, from the basic tris-chelates, to those with one extended planar ligand, usually dppz or derivatives.

Three tris-chelate complexes were investigated as antimicrobials under photoactivation, with $Ru(bpy)_3Cl_2$ and $Ru(phen)_3Cl_2$ exhibiting low activity (MICs above 50 μg/ml against *Staphylococcus aureus* and above 2000 μg/ml against *Pseudomonas aeruginosa*) [30]. The introduction of two methoxyl groups on the bpy ring to afford 4,4'-dimethoxy-2,2'-bipyridine (dmob) strongly improved the activity, with $Ru(dmob)_3Cl_2$ displaying a MIC of 12.5 μg/ml against *S. aureus* and of 50 μg/ml against *P. aeruginosa*. Relevant photoactivated inhibition of *Candida albicans* growth was obtained with $Ru(phen)_3Cl_2$ (MIC of 12.5 μg/ml) and $Ru(dmob)_3Cl_2$ (MIC of 25 μg/ml) [31]. When compared to MIC values for antibiotics, these values are quite high, however future studies may lead to some promising results.

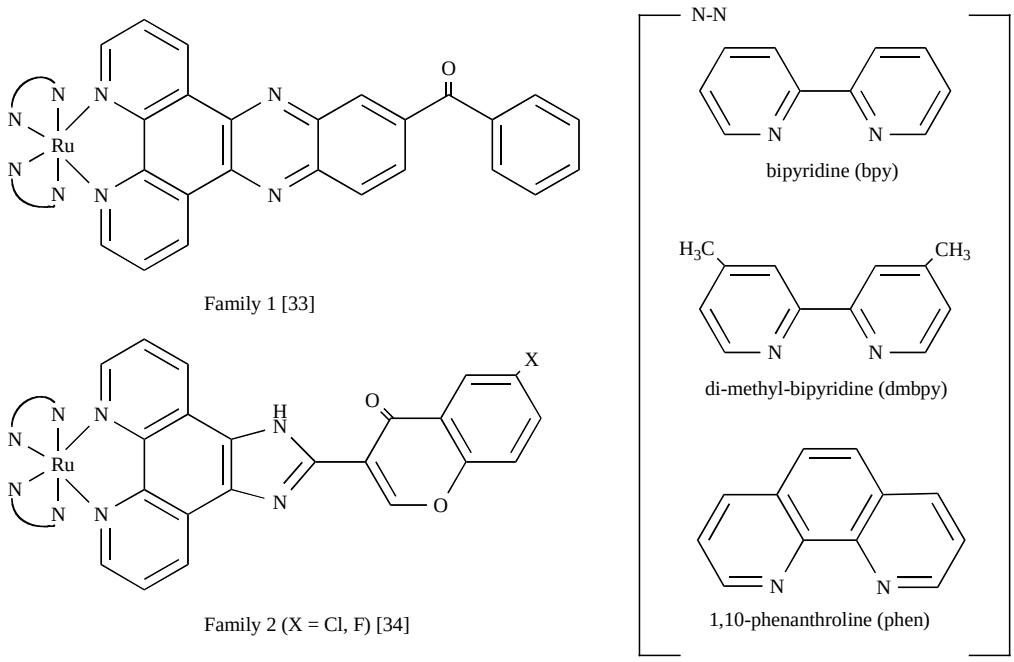
Complexes with dppz also have two smaller ligands, named ancillary, typically bpy, phen, or derivatives. The ancillary ligands may also influence the capability of interaction with DNA due to steric hindrance effects. Their planar geometry, though relatively extended, seems to favour their cellular uptake, proposed to occur by passive diffusion [32].

Typical examples of ruthenointercalators based on dppz derivatives and analogs are presented in Scheme **4**. The first family comprises ruthenium dppz phenylmethanone (pdppz) complexes with bpy, dmbpy or phen as ancillary ligands [33]. The second family of complexes was based on the same ancillary ligands and halogeno-chromenone-derivatized imidazophenanthrolines (ipc) as the intercalating moieties (in the place of dppz) [34].

Binding studies performed with calf-thymus DNA showed that the best results were obtained with phen as the ancillary ligand: all the complexes, $[Ru(phen)_2(pdppz)]^{2+}$, $[Ru(phen)_2(ipc\text{-}Cl)]^{2+}$ and $[Ru(phen)_2(ipc\text{-}F)]^{2+}$ bound strongly to DNA. In turn, interaction with the complexes bearing bpy or dmbpy was smaller or nearly non-existent (for $[Ru(dmbpy)_2(pdppz)]^{2+}$); the poorer results were associated with the bulky methyl groups on the dmbpy ligands. Although these tests were performed on non-bacterial DNA, results translate relatively well into the antimicrobial activities observed. In fact, all three complexes with ancillary phen displayed activities slightly higher than the reference drugs (streptomycin and fluconazole, respectively) against the tested bacteria and the pathogenic fungus *A. niger* whereas complexes with ancillary bpy or dmbpy had antimicrobial action similar or lower than the reference drugs [33, 34].

3.3. Polypiridyl Ruthenium Complexes Against Leishmaniasis

Leishmaniasis is a highly-debilitating protozoan infection arising from transmission of *Leishmania spp*. to humans by sandflies. In the search for new leishmanicidal drugs, some ruthenium(II) polypyridyl complexes were tested, based on the assumption that "every DNA interacting compound could have antiprotozoa activity" [35]. The complexes $[Ru(phen)_2(pdon)]^{2+}$, $[Ru(phen)_2(pda)]^{2+}$ (where pdon = phenanthroline-5,6-dione) and pda = phenanthroline-5,6-diamine) have displayed inhibitory action on *Leishmania (L) Mexicana* promastigotes, at the rates of 49 and 36%,



Scheme 4. Representation of the most common DNA-intercalating Ruthenium complexes bearing the ligand dppz or a derivative.

respectively, after 48h of incubation. The treated cells showed a high proportion of parasite fission forms, motility loss and abundant vacuolization, which constitute a good indication of toxicity and a promising result for future studies in experimental leishmaniasis models [36].

4. RUTHENIUM-PTA COMPOUNDS

4.1. Organoruthenium Complexes

Organometallic Ru(II) arene complexes, developed primarily by the groups of Sadler [37] and Dyson [38], are the most numerous group of cytotoxic Ru compounds [39]. Their activity has been directed and widely explored for tumour inhibition, which has hampered their application as bactericides. Indeed, a compound with favorable characteristics for tumor localization may not be ideal for selective localization in bacterial cells. Nonetheless, some of them present interesting antimicrobial activity, particularly RAPTA compounds.

The designation, from Ruthenium Arene PTA, was presented by P. Dyson when developing the first compounds of this family [38]. RAPTA defines all half-sandwich Ru(II) compounds with the hydrophilic ligand PTA (1,3,5-triaza-7-phosphaadamantane). One family of PTA compounds with *p*-cymene and different labile ligands – halogens (Cl, Br, I) or thiocyanate (SCN) –, was developed for cancer treatment [40] and subsequently tested on a selection of pathogenic bacteria, fungi and viruses [41]. Interestingly, the halogens revealed to play an important role in the inhibitory action. The bromide- and iodide-bearing compounds were almost inactive, with a low, non-specific antimicrobial action (likely as the result of their general toxicity) whilst those with Cl or SCN were antifungal against *Trycophyton mentagrophytes* and *Cladosporium resinae* (but had no action on the tested bacteria and viruses) [41].

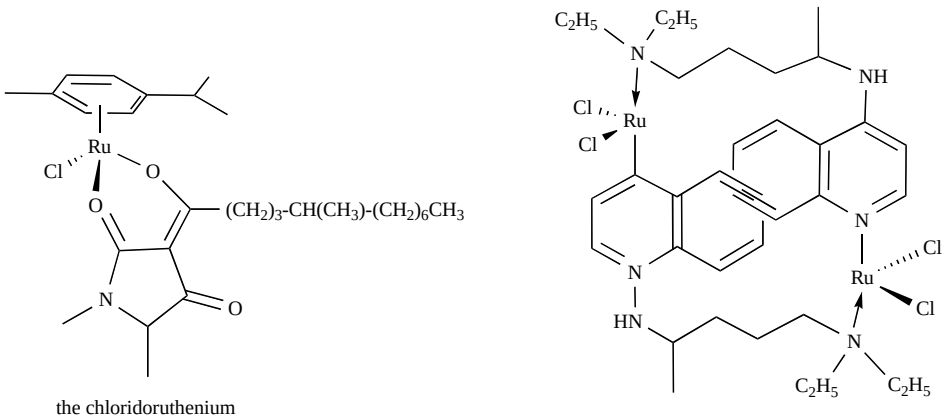
It is worth noting that *p*-cymene, though naturally occurring in bactericidal oils as oregano, cloves, thyme, geranium, etc, was proved the least active of these organic compounds when tested against 25 different genera of bacteria [42], and only mildly cytotoxic on *Bacillus cereus* [43]. We have thus chosen to classify *p*-cymene compounds outside the group of the drug analogs.

4.2. Inorganic Complexes

Two Tp–Ruthenium(II) PTA complexes were developed as inorganic analogs of the organometallic complexes in light of the similarities between the isoelectronic Cp (cyclopentadienyl) and Tp (Tp = hydridotris(pyrazolyl) borate). The presence of nitrogen atoms in the pyrazol groups of these complexes is highly advantageous; while three are bound to ruthenium forming $RuCl\{\kappa^3\text{-}(N,N,N)\text{-}Tp\}(PTA)_2$ and $RuCl\{\kappa^3\text{-}(N,N,N)\text{-}Tp\}(PTA)(PPh_3)$, the others are free and geometrically available to form interactions with hydrogen atoms, not only in water but in biological molecules as well [44]. The complexes were shown to inhibit the growth of *Micrococcus luteus*, *P. aeruginosa*, *E. coli*, *B. subtillis* and *Streptomyces* (*antibioticus*, *coelicolor* and *glaucescens*), but antimicrobial activities were low in comparison with those of reference antibiotics. For example, an inhibition halo against *M. luteus* of 21 mm in diameter can be obtained with 0.6 μg of erythromycin A or 1 μg of mitramycin, whereas the most active ruthenium compound required 20 μg to display the same inhibition halo diameter [44].

5. RUTHENIUM ANALOGS OF ORGANIC DRUGS

Organic drugs can benefit immensely from the association with a metal center. One known example is ferrocifen, the organometallic analog of tamoxifen with a wider range of antitumoral activity [45]. This strategy can also be successfully applied to the design of ruthenium



Scheme 5. Structures of the two ruthenium analogs of organic drugs described in this review.

antimicrobials, as illustrated by these two following examples (Scheme **5**).

5.1. (Melophlinato)(*p*-cymene)ruthenium(II) Chloride

Melophlins are tetramic acid derivatives with long alkyl chains, isolated from sea sponges of the genus *Melophlus* [46, 47]. In particular, melophlin C displayed pronounced antibacterial activity against *S. aureus* and was also able to inhibit the growth of *B. subtilis* and *C. albicans* [46].

The coordination of melophlin C to a bioactive metal as ruthenium (Scheme **5**, left) affords a synergistic effect. The complex Ru(*p*-cym)(mel-C) was able to inhibit the growth of the bacterium *M. luteus*, which is resistant to melophlin C, it also lowered the IC50 against human cancer cells to roughly one-half of the values found for free melophlin C [48].

5.2. *Bis*-(chloroquine) ruthenium(II) Dichloride

The continued use of the antimalarial drug chloroquine has led to the rise of resistant pathogens, which are becoming a pressing health issue in tropical countries [49].

In response to this need, the complex $[RuCl_2(chloroquine)]_2$ was developed, associating two units of the organic drug in a binuclear ruthenium complex (Scheme **5**, right). The synergy between the ruthenium centres and the active drug ligands has afforded enhanced action, both *in vitro* and *in vivo*. In fact, the inhibitory effect of $[RuCl_2(chloroquine)]_2$ on cultures of *Plasmodium berghei* was superior to that of chloroquine and *in vivo* tests performed on mice showed that, at the fourth day of infection, parasitemia levels were below 1% when using $[RuCl_2(chloroquine)]_2$, in comparison with a 4% rate for chloroquine-treated subjects [50].

6. SUPRAMOLECULAR RUTHENIUM ANTIMICROBIALS

6.1. Polymers

An antiseptic rutheno-organic polymeric material, composed of poly-{*trans*-$[RuCl_2(vpy)_4]$-sty-4-vpy} (vpy = vinylpyridine, sty = styrene), and impregnated with silver nanoparticles, was reported [51] and its inhibitory action was compared with those of the non-impregnated Ru-polymer and a nanosilver-loaded purely organic polymer analog (made of styrene-divinylbenzene-vinylpiridine). The Ru-polymer by itself was inert, with no intrinsic inhibitory action over the tested cultures, *E. coli* (ATCC-25922) and *S. aureus* (ATCC-25923) [51]. Interestingly though, ruthenium was demonstrated to play a vital role in the polymer's ability to release the impregnated silver nanoparticles onto the test solid microbiological cultures. In fact, the nanosilver-loaded Ru-polymer was effective against both pathogens whereas the nanosilver-loaded organic polymer had no inhibitory effect [52].

6.2. Cyclodextrin Adducts

Cyclodextrins (CDs) are ubiquitous drug carriers with solubilizing, taste ameliorating and stabilizing actions which

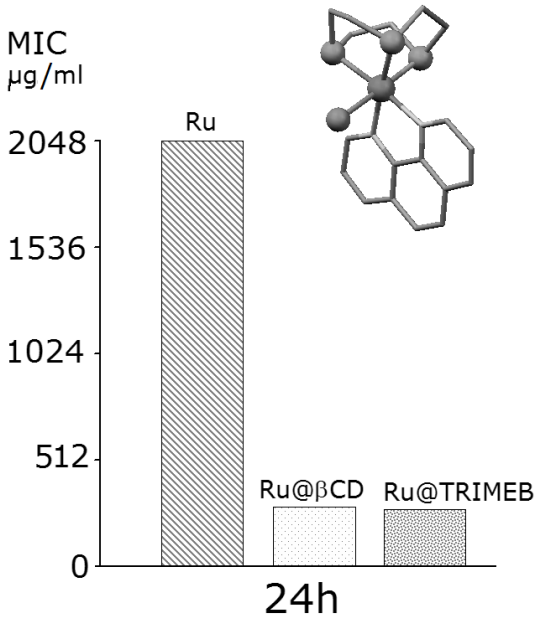


Fig. (3). The RuCl(trithiacyclononane)(phenanthroline)]Cl complex (schematics at the top right) and its antimicrobial action on the ATCC 29212 strain of *Enterococcus faecalis*, either in the free form or included into βCD and TRIMEB. MIC values were read at an incubation time of 24 h.

have recently been demonstrated to perform beyond their mere physico-chemical abilities and improve the biological activity of selected cytotoxic metal complexes, both on human cell lines [53, 54] and bacteria [55].

The complex [RuCl(trithiacyclononane)(phenanthroline)] Cl and its two inclusion compounds with native β-CD and the permethylated derivative TRIMEB were tested for their antimicrobial action on a selection of Gram-positive and Gram-negative bacterial strains implicated in human infections [55]. The free Ru(II) complex had only a mild activity (MICs of 2048 to 1024 mg/ml) which, upon inclusion into the cyclodextrins, was increased two to four times for most of the tested bacteria, and eight times for the ATCC 2912 strain of *Enterococcus faecalis* (Fig. **3**).

Bacterial resistance to cytotoxic agents is often dose-induced. Evidence of the cyclodextrins' ability to reduce the amount of drug needed for the antimicrobial action is an encouraging new strategy to fight bacterial resistance.

FINAL REMARKS

This paper presents a structured overview of the literature reported antimicrobial ruthenium compounds, ranging from inorganic and organometallic complexes to more elaborate supramolecular products, and aiming at a comprehensive comparison between their biological activities. In this matter, a word of caution must be presented regarding the methodologies used for antimicrobial studies, which exert a strong influence on the results. The antibiotic resistance patterns can be determined by several methods, such as minimum inhibitory concentration (MIC), E-test and disk diffusion, which must be performed according to the CLSI guidelines. The results vary according to the method, the first two affording more quantitative results and being more precise than the disk diffusion assay. Note also that the E-test has the limitation of being only applicable to antibiotics while the other two can be used for any desired compound.

The studies herein reviewed illustrate that work on the subject is still somewhat fragmented. The most commonly used methodology was the disk diffusion, followed by MIC determination, but within these two methods, we noticed that almost each research group used a somewhat different, and often non-standard, antimicrobial activity assessment methodology, sometimes altering the method from one paper to another. The non-uniformity of results has, thus, hampered a good comparison of results.

We were, nonetheless, able to sketch a general overview on how these compounds interact with bacteria and other pathogens. In fact, a handful of reports show highly promising results: within ruthenium complexes with Schiff bases, those bearing an axial pyridine ligand were able to match bactericidal action of reference antibiotics (in particular the ones depicted in Scheme **3** [6]), and within ruthenointercalators, $[Ru(phen)_2(d\text{-}dppz)]^{2+}$ (where d-dppz = derivatives of dppz) have surpassed slightly the activities of the drugs streptomycin and fluconazole [33, 34]; another complex, $[Ru(phen)_2(pdon)]^{2+}$, revealed to be a promising anti-leishmanic agent [36]. The use of well-known drugs as a scaffold for miomimetic metallopharmaceuticals was successfully used to afford a mellophlinatoruthenium complex able to overcome bacterial resistance [48] and a bis-chloroquineruthenium with enhanced antimalarial action [50]. Two punctual examples of antimicrobial supramolecular architectures are a ruthenopolymeric delivery system for silver nanoparticles [52] and a cyclodextrin-carried antimicrobial ruthenium complex [55]. Still, such promising new agents remain as scattered examples of the practical utility of chemical design tools, thus stressing the need for a systematization of these studies.

In our opinion, a promising and logical step for future works would be the implementation of a systematic trial-and-error approach for the molecular design, as used by pharmaceutical companies to probe a wide universe of metal based drugs. Regarding the activity assessment, it will also be of vital importance to determine the antimicrobial activity according to the CLSI guidelines, to establish a set of reference data for these compounds (currently unavailable), and to use the same culture media for the assays. It is well established that the activity of antibiotics suffers influence from the medium composition and its pH, so this may also be true for compounds of ruthenium and other metals.

CONFLICT OF INTEREST

None declared.

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