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Review

Biological activity of ruthenium nitrosyl complexes

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ABSTRACT

Nitric oxide plays an important role in various biological processes, such as neurotransmission, blood pressure control, immunological responses, and antioxidant action. The control of its local concentration, which is crucial for obtaining the desired effect, can be achieved with exogenous NO-carriers. Coordination compounds, in particular ruthenium(III) and (II) amines, are good NO-captors and -deliverers. The chemical and photochemical properties of several ruthenium amine complexes as NO-carriers *in vitro* and *in vivo* have been reviewed. These nitrosyl complexes can stimulate mice hippocampus slices, promote the lowering of blood pressure in several *in vitro* and *in vivo* models, and control *Trypanosoma cruzi* and *Leishmania major* infections, and they are also effective against tumor cells in different models of cancer. These complexes can be activated chemically or photochemically, and the observed biological effects can be attributed to the presence of NO in the compound. Their efficiencies are explained on the basis of the $[Ru^{II}NO^{+}]^{3+}/[Ru^{II}NO^{0}]^{2+}$ reduction potential, the specific rate constant for NO liberation from the $[RuNO]^{2+}$ moiety, and the quantum yield of NO release.

Contents

Introduction

Coordination compounds have long been used as metallopharmaceuticals [1–3]. There is a wide range of metal complexes that have biological activities and applications for diseases, such as cancer [4–6], cardiovascular problems [7], arthritis [8], and parasitosis [9], and some of these complexes are commercially available. The best known and most studied metal drugs are the anticancer compounds of platinum, which have been used since the antitumor activity of cisplatin (*cis*-diamminedichloridoplatinum(II), [*cis*-$Pt^{II}(NH_3)_2Cl_2$]) was discovered in the 1960s [3]. The continued development of metal complexes has sought to overcome the drug resistance and side effects of [*cis*-$Pt^{II}(NH_3)_2Cl_2$].

Iron complexes have been extensively used in the treatment of anemia, cancer, and cardiovascular problems. Iron(II) citrate is a classical anemia treatment [10]; ferrocenyl derivatives are anticancer drugs for breast cancer [11], while sodium nitroprusside (SNP)

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($Na_2[Fe^{II}(NO^+)(CN)_5]$) is a vasodilator drug that is able to deliver nitric oxide (NO) by reduction *in vivo* [1,12,13]. Although SNP is administered in the clinic [14], there are problems associated with its use, such as a susceptibility to photolysis and its oxidative breakdown through the action of the immune system, ultimately releasing cyanide [7].

Osmium compounds have not been well-studied as metal-based drugs, likely due to the higher cost of the metal, the increased difficulty associated with compound synthesis [15], and its high toxicity [16]. In addition, the ligand exchange rates in osmium compounds are not favorable on the timescale of cellular processes and are approximately 10^5 times lower than those of the corresponding ruthenium complexes [17]. However, the kinetic lability of osmium complexes can be tuned so that their anticancer activity approaches the range of cellular processes [18].

Metallopharmaceuticals can also be activated by irradiation with light [19–35]. Possibly, the best known examples of application are complexes designed for photodynamic therapy (PDT) [20], such as in the treatment of neoplastic tissues. The key components of PDT are a photosensitizer, light, and tissue oxygen. The absorption of light by a photosensitizer molecule can lead to energy transfer to activate another molecule, such as the conversion of O_2 to the excited singlet state ($^1O_2$) and other free radicals, such as $^{\cdot}OH$, $HO_2^{\cdot}$, $O_2^{\cdot-}$, which induce damage to membranes, DNA, and other cell structures [20,36].

Of the metallo drugs, ruthenium-based complexes have received considerable attention for medical applications [19,37,38]. Their kinetic behavior is similar to platinum with respect to cellular division processes [19,39,40], but their toxicity is lower than that of cisplatin [41], probably due to their ability to mimic iron and therefore bind to many biomolecules, such as human serum albumin and the iron transport protein transferrin [37,38].

According to its coordination sphere composition, ruthenium has several oxidation states: Ru(II), Ru(III), and Ru(IV). Most of these oxidation states are accessible under physiological conditions [37]. Ru(III) complexes serve as precursors to Ru(II) by reduction *in vivo* by biological reductants such as glutathione and ascorbic acid [19,37,42]. Ru(II) centers are more reactive than Ru(III) in substitution reactions and are able to coordinate rapidly and bind strongly to nitrogen and sulfur atoms available in biomolecules.

The biological activities of ruthenium complexes were first reported in the 1950s (Fig. 1a) [43–46] and have been reviewed elsewhere [19,47]. As an example, *fac*-$[Ru^{III}(NH_3)_3Cl_3]$ exhibits anticancer activity but is not soluble enough in aqueous solutions [48] for use in the clinic. Since then, a series of ruthenium complexes have been studied [49,50]. NAMI-A (*trans*-$[Ru^{III}$(dmso-*S*)(Im)$Cl_4]$ImH, dmso = dimethyl sulfoxide, Im = imidazole) (Fig. 1b) inhibits the formation and growth of metastases [51], and KP-1019 (*trans*-$[Ru^{III}(Ind)_2Cl_4]$IndH, Ind = indazole) (Fig. 1c) induces apoptosis in colorectal carcinoma cells [52]. Both are currently the most promising Ru(III) complexes in cancer treatment; NAMI-A is the first anticancer ruthenium compound to successfully complete Phase I Clinical Trials and will enter Phase II Trials, whereas KP-1019 is in Phase I Clinical Trials [53].

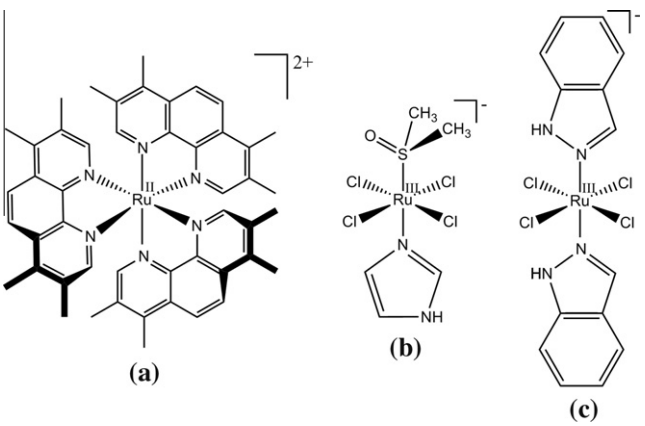

Fig. 1. (a) Dwyer compound, (b) NAMI-A, and (c) KP-1019 structures.

Ruthenium(III) and (II) tetraamines (d^5 and d^6 low spin) constitute a very interesting class of compounds for medicinal chemistry studies because of their water solubility, stability in aqueous medium, and low cytotoxicity [22,25,54–58]. The amines, which are oriented along the *x*- and *y*-axes of the pseudo-octahedral complexes, are inert ligands that enable substitution reactions to take place only in the *z* direction. Depending on the nature of the ligands and the ruthenium oxidation state, the photochemical reactivity can differ from the chemical one [21–25,59,60]. Thus, given the chemical and photochemical properties of this class of compounds [22–25,59–61], they are attractive candidates for developing a metallopharmaceutical platform.

In this respect, ruthenium compounds could be useful as delivery or scavenging agents for molecules that have a crucial role in physiology. Nitric oxide (NO), for instance, is an endogenous molecule that is produced by nitric oxide synthase (NOS) through the conversion of L-arginine to L-citrulline and NO [62]. There are three NOS isozymes: the endothelial and neuronal (eNOS and nNOS) isoforms, which are dependent on intracellular Ca^{2+} concentration and expressed constitutively in several tissues, and the inducible NOS (iNOS), which are Ca^{2+}-independent and activated by pro-inflammatory stimuli [63,64]. An important difference between the NOS isoforms is that the constitutive type produces low NO concentrations in the nmol L^{-1} range, while the inducible form produces NO in the μmol L^{-1} range [63]. Thus, nitric oxide plays an important role in a variety of physiological functions, such as blood pressure control [65], neurotransmission [66–68], immunological responses [55,69,70], and antioxidant action [71–73]. These effects are quite dependent on the local NO bioavailability and concentration [74]. Therefore, intense efforts have been devoted to developing compounds that deliver NO efficiently and in a controlled manner in physiological medium, including NONOates [75–77], *S*-nitrosothiols [78–80], and metal complexes [27,30,81–83], such as ruthenium(II) nitrosyl compounds [22–25,34,59,60,84–92], and also to developing scavengers of NO, such as ruthenium(III) edta and cyclam compounds [24,93–96].

Ruthenium tetraammine and tetraazamacrocyclic nitrosyl compounds [22,24,25,97–99] are of great interest as NO-donors due to their outstanding properties, as the low cytotoxicity against host cells, water solubility, stability to air oxidation and tailoring reactivity possibilities of the coordinated NO, by the choice of the axial ligand (L_2) in *trans*-$[Ru^{II}(NO^+)(L_1)_x(L_2)]^{n+}$ [25,97–100]. In addition, there is also the possibility to immobilize these complexes into matrices such as silica, organic inorganic hybrid materials, polyurethane, poly(vinyl alcohol), poly(methylmethacrylate), and dendrimers [23]. Many of these NO-donors have presented biological activities against several diseases. The challenges of providing a new platform based on nitric oxide are to overcome the resistance that some actual drugs and treatments have encountered, and to be site specific in order to minimize side effects.

trans-$[Ru^{II}(NO^+)(L_1)_x(L_2)]^{n+}$ complexes have been widely studied [21–23,25,61,101,102]. In these complexes, the L_2 ligand is very important because of its ability to modulate, based on its own properties (σ-donation, π-acceptation, orbital energy and symmetry), the reduction potential of the NO^+ ligand (E_{NO^+/NO^0})[1], the NO^+

[1] Considering the canonical form $[Ru^{II}$-$NO^+]$; other forms include $[Ru^{III}$-$NO^0]$ and $[Ru^{IV}$-$NO^-]$ for the $[RuNO]^6$ moiety [59,103].

Table 1
Properties of selected ruthenium nitro and nitrosyl complexes.

Complex	$E^{+}_{NO/NO}{}^{0}$ (V vs. NHE)	k_{-NO} (s^{-1})	(ν^{+}_{NO}) (cm^{-1})	λ_{irr} (nm)	φ_{NO} (mol/estein)	Ref.
trans-$[Ru^{II}(NO^{+})(NH_3)_4(isn)](BF_4)_3$	0.052	0.043	1923	330	0.07 ± 0.01[a]	[60,97]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(nic)](BF_4)_3$	0.072	0.025	1940	310	0.07 ± 0.01[b]	[60,97,101]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(L\text{-}hist)](BF_4)_3$	−0.108	0.140	1921	313	0.086 ± 0.004[c]	[60,98,101]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(py)](BF_4)_3$	0.012	0.060	1931	310	0.18 ± 0.01[d]	[60,97,101]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(4\text{-}pic)](BF_4)_3$	−0.008	0.090	1934	330	0.40 ± 0.01[e]	[60,101]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(imN)](BF_4)_3$	−0.118	0.160	1923	313	0.079 ± 0.003[c]	[60,98,101]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(imC)]Cl_3$	−0.298	5.100	1913	–	–	[111]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(pz)](BF_4)_3$	0.112	0.070	1942	313	0.23 ± 0.03[c]	[60,97]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(ina)](BF_4)_3$	0.061	–	1934	–	–	[55]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(4\text{-}Clpy)](BF_4)_3$	0.012	0.030	1928	–	–	[101]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(H_2O)](BF_4)_3$	−0.148	0.040	1912	–	–	[112,113]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(P(OEt)_3)](PF_6)_3$	0.142	0.980	1909	310	0.30 ± 0.05[b]	[60,102,114]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(P(OH)_3)]Cl_3$	−0.278	–	1903	–	–	[115]
trans-$[Ru^{II}(NO^{+})(cyclam)Cl](PF_6)_2$	−0.100	0.00062	1875	–	–	[93,112]
$[Ru^{II}(NO^{+})(Hedta)]$	−0.098	0.002	1846	–	–	[112]
trans-$[Ru^{II}(NO^{+})([15]aneN4)Cl]Cl$	0.473	–	1860	355	0.60[f]	[91]
$[Ru^{II}(NO^{+})(H_2O)(salen)]Cl$	–	–	1856	365	0.005 ± 0.001[g]	[91]
$[Ru^{II}(NO^{+})(salen)Cl]$	–	–	1844	365	0.13 ± 0.002[h]	[91]
$[Ru^{II}(NO^{+})(NH_3)_5]Cl_3$	−0.148	–	1913	–	–	[112]
trans-$[Ru^{II}(NO^{+})(NH_3)_4(SO_3)]Cl$	−0.138	–	1871	–	–	[98]
cis-$[Ru^{II}(NO^{+})(4\text{-}pic)(bpy)_2](PF_6)_3$	0.31	–	1944	332	0.179 ± 0.002[b]	[116,117]
cis-$[Ru^{II}(NO^{+})(py)(bpy)_2](PF_6)_3$	0.34	–	1947	334	0.169 ± 0.002[b]	[116,117]
cis-$[Ru^{II}(NO^{+})(4\text{-}acpy)(bpy)_2](PF_6)_3$	0.39	–	1943	336	0.079 ± 0.002[b]	[116,117]
cis-$[Ru^{II}(bpy)_2(imN)(NO_2)]^{+}$	–	–	–	412	–	[118]
cis-$[Ru^{II}(bpy)_2(isn)(NO_2)]^{+}$	0.34	–	1948	408	–	[118]
cis-$[Ru^{II}(bpy)_2(SO_3)(NO_2)]^{-}$	–			405	–	[118]
cis-$[Ru^{II}(NO^{+})(bpy)_2(imN)](PF_6)_3$	0.397	–	1944	325	–	[118]
cis-$[Ru^{II}(NO^{+})(bpy)_2(isn)](PF_6)_3$	0.537	–	1948	321	–	[118]
cis-$[Ru^{II}(NO^{+})(bpy)_2(SO_3)]PF_6$	0.057	–	1911	326	–	[118]
trans-$[Ru^{II}(NO^{+})(bpy)_2(SO_3)]^{+}$	−0.34	–	1881	320	–	[118]
[Ru(NO)-(NO2)pc]	–	–	1830	366	0.120 ± 0.004[g]	[119]

Ref. = reference ·pH.
[A] pH = 4.95.
[b] pH = 2.0.
[c] pH = 4.32.
[d] pH = 6.4.
[e] pH = 6.0.
[f] pH = 7.4 (phosphate buffer).
[g] Water.
[h] Acetonitrile.

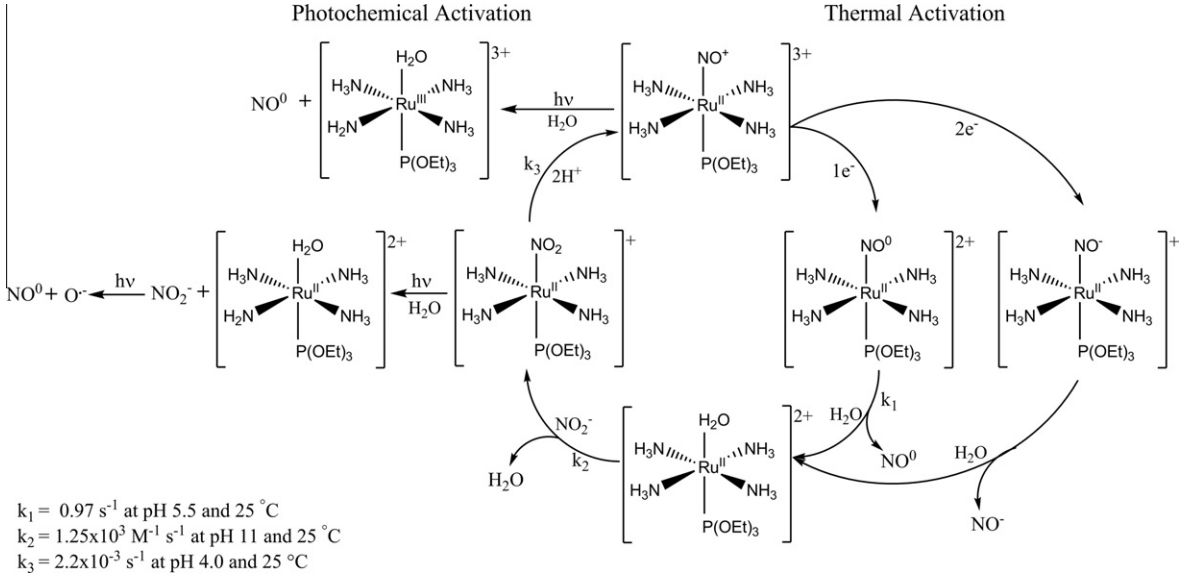

Fig. 2. Photochemical and thermal paths for nitrosyl (NO^{+}) ligand activation in *trans*-$[Ru^{II}(NO^{+})(NH_3)_4P(OEt)_3]^{3+}$.

stretching frequency (ν^{+}_{NO}), the specific rate constant of NO liberation (k_{-NO}) [22–25,61,101], and the chemical and photochemical reactivities of the complex as a whole [22–25,59,60] (Table 1). Therefore, the modulation of these chemical and photochemical

properties, promoted by L_2, is relevant for tailoring ruthenium compounds to selectively deliver or capture NO according to the characteristics of each target.

Nitric oxide activation on ruthenium(II) nitrosyl complexes

The *trans*-$[Ru^{II}(NO^+)(L_1)_x(L_2)]^{n+}$ complexes can be activated to release nitric oxide thermally and/or photochemically. Fig. 2 shows reactions involving these two activation paths for the *trans*-$[Ru^{II}(NO^+)(L_1)_x(L_2)]^{n+}$, exemplified by *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3]^{3+}$.

In the thermal pathway, the NO^+ ligand can be reduced by a one or two electron reductors [104], yielding the $[Ru^{II}(NO^0)]^{2+}$or $[Ru^{II}(NO^-)]^+$ moieties, respectively (Fig. 2). After reduction, neither nitric oxide [25,61,101] nor the nitroxyl anion (NO^-) [105] have an affinity for the metal center, and are released with rate constants dependent on the trans effect and trans influence promoted by the trans ligand (L_2).

Conceivably, the *trans*-$[Ru^{II}(H_2O)(L_1)_x(L_2)]^{(n-1)+}$ generated after NO or NO^-dissociation can react with nitrite (NO_2^-) present in the plasma ($114 \pm 11\ \mu mol\ L^{-1}$) [106], giving rise to *trans*-$[Ru^{II}(NO_2)(L_1)_x(L_2)]^{(n-2)+}$, which can be quantitatively converted in acid medium to *trans*-$[Ru^{II}(NO^+)(L_1)_x(L_2)]^{n+}$, regenerating the initial nitrosyl compound [107–109]. This catalytic cycle should be further explored as an NO buffer because the Ru(II) complexes would convert NO_2^- to NO^+ [108,109].

The ruthenium nitrosyl complexes can also be activated by light irradiation, yielding NO and either *trans*-$[Ru^{III}(H_2O)(L_1)_x(L_2)]^{n+}$ (at $pH \leqslant 2$) or *trans*-$[Ru^{III}(OH)(L_1)_x(L_2)]^{(n-1)+}$ (at $pH \geqslant 6$) as the only photoproducts [25,59,60] (Fig. 2). *trans*-$[Ru^{II}(NO_2)(NH_3)_4P(OEt)_3]^+$ is also a potential model of a photochemical NO-donor because, after irradiation, it produces *trans*-$[Ru^{II}(H_2O)(NH_3)_4P(OEt)_3]^{2+}$ and free NO_2^-, which, after a secondary photolysis, yields NO and $O^{\cdot-}$ [110].

Table 1 shows several ruthenium complexes and the properties that enable their use as NO-donors by thermal or photochemical activation.

Biological molecules and nitric oxide activation on ruthenium(II) nitrosyl complexes

The ability of ruthenium(II) nitrosyl compounds to be thermally activated by biological redactors to release NO was first investigated using mice hippocampi [120] and mitochondria [114,121].

Central nervous neurons can be excited by different factors, for instance, electrical stimulus, which can be used at high or low frequencies, resulting in long-term potentiation (LTP) [122] or long-term depression (LTD) [123] of the synaptic response. In the hippocampus, nNOS and eNOS are abundant [124], and NO produced in this area can act as a mediator in LTP [120]. Therefore, the *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3](PF_6)_3$ compound was investigated as an NO-donor in mice hippocampus slices *in vitro*, facilitating the population spike [120]. Other ruthenium(II) compounds, such as $[Ru^{II}(NO^+)(NH_3)_5]Cl_3$, $[Ru^{II}(NO^+)Cl_5]K_2$, *trans*-$[Ru^{II}(NO^+)(bpy)_2Cl]Cl_2$, *cis*-$[Ru^{II}(NO^+)(bpy)_2Cl](PF_6)_2$, *trans*-$[Ru^{II}(NO^+)(14ane)Cl]Cl_2{\cdot}4H_2O$, *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]Cl_2{\cdot}4H_2O$, which release NO at lower rate compared to the phosphite complex (see Table 1), and *trans*-$[Ru^{III}(cyclam)Cl_2]Cl_2{\cdot}2H_2O$ did not show activity. These findings were explained by the ability of the *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3](PF_6)_3$ compound to liberate NO rapidly (k_{-NO} = 0.98 s^{-1}, 25 °C) and by the NO^+/NO reduction potential, which is accessible under physiological conditions [102].

The mitochondria was used as a probe because it contains several components able to reduce nitrosyl complexes, such as NADH, complex I and II, FADH2 and FMNH2 [125]. Moreover, the electron transfer of these components in mitochondria increases in low oxygen conditions, being an important reduction route for the treatment of hypoxic cells. The *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{3+}$ (L = $P(OEt)_3$ or py) complexes were exposed to a mitochondrial suspension in which malate or succinate was used as the substrate and rotenone was used as an inhibitor of the electron transport chain to ensure that NADH was the only available reductant. The $[Ru^{II}NO^+]^{3+}/[Ru^{II}NO^0]^{2+}$ couple was followed by differential pulse polarography, which indicated that the nitrosyl compounds were consumed completely [114,121]. Under the same conditions, mitochondrial NADH oxidation was monitored by fluorescence [114,121]. The second-order specific rate constants for the NO^+/NO reduction in *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3]^{3+}$ by intact or homogenized mitochondria were of the same order of magnitude and were calculated as $2 \times 10^1\ L\ mol^{-1}\ s^{-1}$ at $T = 25 \pm 1$ °C. However, the reaction between pure NADH and *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3]^{3+}$ was much slower [114], suggesting the possible existence of an electron-transfer mediator that accelerates the electron flow from NADH to ruthenium(II) nitrosyl [114].

The impact of NO release from *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)]^{3+}$ on mitochondria was also evaluated. A decrease in membrane potential and ATP levels, the generation of reactive oxygen species (ROS) and the elicitation of mitochondrial permeability transition (MPT) were observed, indicating the potential involvement of NO in cell apoptosis, which could be a new anti-cancer strategy [121].

Another possible thermal activation route for ruthenium(II) nitrosyl compounds is reaction with ROS. Recent studies [126] have shown that superoxide (O_2^-), which is present in the respiratory chain [127], ischemia, and inflammatory situations [104], is able to react with *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{3+}$(L = $P(OEt)_3$, im*N*, nic, isn, 4-pic, or py) and $[Ru^{II}(NO^+)(Hedta)]^{3+}$ (reaction 1) with apparent bimolecular rate constants (k_{app}) ranging from 10^3 to $10^5\ L\ mol^{-1}\ s^{-1}$.

$$trans-[Ru^{II}(NO^+)(NH_3)_4L]^{3+} + O_2^- \xrightarrow{\cdot K_{app}} trans-[Ru^{II}(NO^0)(NH_3)_4L]^{2+} + O_2 \quad (1)$$

$$trans-[Ru^{II}(NO^0)(NH_3)_4L]^{2+} \rightarrow trans-[Ru^{II}(H_2O)(NH_3)_4L]^{2+} + NO^0 \quad (2)$$

The products (NO and the corresponding aquo complexes) were detected with an NO-selective electrode and differential pulse polarography [126]. These findings show that reduction by superoxide or possibly other ROS can also activate NO release in a biological medium.

Photochemical activation

With the advent of PDT as a treatment for certain cancers, combined with the biological importance of NO, light-activated NO donors have also gained attention. Light irradiation provides further control for NO release, such as targeting and dosage. Several different approaches and metal complexes, including ruthenium nitrosyls, have been proposed in this regard [20–25,27,29–32,34–36,59,60,81–83,87–92,109,110,119]. Compounds reported to be NO donors include nitrosyl complexes of ruthenium porphyrins of the type [Ru(P)(NO)(X)] (P = TPP, OEP; X = Cl^-, ONO^-). Exposure of both [Ru(TPP)(NO)(Cl)] and [Ru(OEP)(NO)(Cl)] to 366 nm light leads to photolysis of the Ru–N(O) bond [128]. In recent years, various exogenous NO donors have been synthesized to modulate NO concentrations in cellular environments to kill malignant cells [129]. Alternatives for improving the cancer treatment have been reported that use combined systems that produce NO and $^1O_2$ simultaneously upon irradiation with light, such as *cis*-[Ru(H-dcbpy$^-$)$_2$(Cl)(NO)] (H_2-dcbpy = 4,4′-dicarboxy-2,2′-bipyridine) and Na_4[Tb(TsPc)(acac)] (TsPc = tetrasulfonated phthalocyanines;

acac = acetylacetone), zinc phthalocyanine associated with $[Ru(NH{\cdot}NHq)(tpy)NO]^{3+}$, and 5,10,15,20-tetrakis(4-sulphonatophenyl)-21*H*,23*H*-porphyrin (TPPS) and a nitroaniline derivative [36,130,131]. However, several complexes, such as some ruthenium nitrosyls, have no absorption bands in the visible region [21–25], and it is necessary to use some artifice to promote the photoinduced release of NO, especially by irradiation with 600–1100 nm light, which corresponds to the phototherapeutic window [132–134] for greater penetration in the skin and could therefore be useful as a non-invasive clinical therapy. Nevertheless, in some instances, such as in dermatology, compounds activated by near-UV light are needed [135–137]. Several strategies to develop systems able to deliver NO by irradiation in the therapeutic window have been reported. One is the use of two-photon antenna systems, for which the focus is on designing compounds with antenna chromophores that have high absorption cross-sections at longer wavelengths and that sensitize NO photochemical labilization from the linked metal center [138]. An alternative means of broadening the absorption spectrum to the visible region, with the photochemical release of NO, is to modify the coordination sphere of the $\{M–NO\}^6$ complexes using conjugated heterocyclic ligands that can act as light antennae or to employ a second metal center that acts as a photosensitizer [82,83,118,119,139–142]. Examples of the modification of the coordination sphere of ruthenium complexes are the phthalocyanines, which absorb light at long wavelengths. The phthalocyanineruthenium(II) complex [Ru(NO)-(NO_2)pc] (pc = phthalocyanine) possesses an intense band ($\varepsilon = 2.75 \times 10^4$ L mol^{-1} cm^{-1}) at 690 nm in the electronic spectra, which, under irradiation with 660 nm light, is capable of releasing NO [119]. A nice strategy is the use of quantum dots (QD) that allow the attachment of the desired molecules at the surface of the nanocrystal and/or impart water solubility or biological specificity [143]. Quantum confinement effects permit shifts in the QD optical absorbance and photoluminescence by varying their size, suggesting that QDs should be tunable antennae for photoreaction sensitization [143].

Ruthenium nitrosyls are substitution inert at room temperature, but some release NO when exposed to light and/or by reduction [19–25,59,60,87–89,91,92,144]. Typically, upon exposure to light, one coordinated ligand (L) is replaced by a solvent molecule (Fig. 2) [22–25,59,60]. The number of complexes that release NO exclusively when triggered by light and are stable under physiological conditions is limited [34]. The limitation imposed by decomposition of a compound under physiological conditions can be overcome by encapsulating the complex in systems like micro and nanoparticles, for example, preventing degradation and yet allowing activation through light irradiation. Notably, the irradiation of ruthenium ammine and macrocycle nitrosyls generally does not lead to a mixture of several products but, rather, results in the release of NO and only one other product [22–25,59,60,89]. This is extremely interesting when applications are concerned. As an example, aqueous solutions of *trans*-$[Ru^{II}(NO^+)(NH_3)_4L](BF_4)_3$ (L = L-hist, im*N*, 4-pic, py, pz, or nic) complexes are photoactive toward near-UV (310–370 nm) excitation to give *trans*-$[Ru^{III}(NH_3)_4(L)(H_2O)]^{3+}$or *trans*-$[Ru^{III}(OH)(NH_3)_4(L)]^{2+}$, depending on the pH and pK_a of the aquo product, and NO as products (Fig. 2) [60]. The same behavior is observed for *trans*-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$, *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]Cl$, 1-(3-propylammonium)-1,4,8,11-tetraazacyclotetradecane, and $[Ru^{II}(NO^+)(salen)Cl]$ [59,87–89,91,92].

Table 2
Vasorelaxant effect induced by thermally activated ruthenium(II) nitrosyl complexes in aorta ring without endothelium.

Compound	Vasorelaxant effect (%)	Ref.
trans-$[RuII(NO^+)(NH_3)_4(P(OEt)_3)](PF_6)_3$[a]	100	[104]
trans-$[Ru^{II}(NO^+)(NH_3)_4(4\text{-pic})](BF_4)_3$[a]	99.4 ± 8.0	[104]
trans-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$[a]	100	[104]
trans-$[Ru^{II}(NO^+)(NH_3)_4(pz)](BF_4)_3$[a]	49.2 ± 8.0	[104]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$[a]	82.2 ± 5.8	[104]
trans-$[Ru^{II}(NO^+)(NH_3)_4(nic)](BF_4)_3$[a]	43.4 ± 5.2	[104]
trans-$[Ru^{II}(NO^+)(NH_3)_4(SO_3)]Cl$[a]	36.4 ± 4.7	[104]
$[Ru^{II}(NO^+)(NH_3)_5]Cl_3$[a]	37.0 ± 4.2	[104]
trans-$[Ru^{II}(NO^+)([15]aneN_4)Cl](PF_6)_2$[b]	100	[148]
cis-$[Ru(bpy)_2(py)(NO_2)]PF_6$[c]	100	[149]
$[Ru^{II}(NO^+)(Hedta)]$[d]	10.8 ± 6.3	[150]
$Na_2[Fe^{II}(NO^+)(CN)_5]$[e]	100	[149]

Ref. = reference;
Pre-contractor/complex concentration/time passed until reaches the relaxation(%):
[a] 1 μmol L^{-1} noradrenaline/3 μmol L^{-1}/7200 s.
[b] 0.1 μmol L^{-1} norepinephrine/100 μmol L^{-1}/595 s.
[c] 0.1 μmol L^{-1}phenylephrine/3 μmol L^{-1}/240 s.
[d] 5 μmol L^{-1} noradrenaline/1 μmol L^{-1}/10 min.
[e] 0.1 μmol L^{-1} norepinephrine/0.1 μmol L^{-1}/210 s.

Biological tests

Vasodilation and vasoconstriction

Endothelial cells play an important role in the vascular system through the production of vasoactive mediators, as nitric oxide, which is also known as the endothelium-derived relaxing factor (EDRF) [64,145]. In endothelial cells, eNOS is responsible for producing nitric oxide [146,147], and dysfunctions in its expression can result in a range of vascular diseases. Thus, metal complexes capable of delivering NO are desired not only for understanding their chemical reactions mechanisms but also for future medical applications, including an eventual alternative to sodium nitroprusside (SNP).

Table 2 lists the vasorelaxant effects of ruthenium(II) nitrosyl complexes and sodium nitroprusside in endothelium-denuded aorta ring of male Wistar. These experiments were performed in an organ bath (T = 37 °C, 95% O_2 and 5% CO_2) with Krebs solution and a transducer connected to aorta ring was used to detect tension [104,148–150]. Among the ruthenium tetraammines, *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^n$, only complexes in which L = P(OEt)$_3$, 4-pic, or py induced complete relaxation. Indeed, all of the ruthenium tetraammines in Table 2 induced relaxation effect, but it is important to note that the intensity and time course were different for each one. Because the autoxidation and diffusion of NO in muscle cells was the same for all compounds, the relaxant effect is closely related to the specific rate constant for NO liberation (k_{-NO}), which determines the time course of the vasorelaxant effect [104]. For instance, *trans*-$[Ru^{II}(NO^+)(NH_3)_4(P(OEt)_3)]^{3+}$ has the higher k_{-NO} (0.98 s^{-1}) value among the ruthenium tetraammines and exhibited the faster vasorelaxant effect, while $[Ru^{II}(NO^+)(Hedta)]^-$ (k_{-NO} = 0.002 s^{-1}) exhibited a slower time course. The plot of k_{-NO} against time for 50% relaxation effect displays a linear correlation, showing that the k_{-NO} value is directly related to the relaxant effect [104].

When administered to normotensive or hypertensive rats, the complex *trans*-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$ (cyclam-NO) (cyclam = 1,4,8,11-tetraazocyclotetradecane) induced a reduction in blood pressure, especially in hypertensive rats, that was 20 times longer than that resulting from SNP administration in either normotensive or hypertensive rats. The relaxation effect induced by cyclam-NO was completely inhibited by the unspecific GMPc inhibitor methylene blue and by c-PTIO and *trans*-$[Ru^{II}(tfms)(cyclam)Cl]^+$ [93], which reacts with NO to give *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$, indicating that the effect was due to NO release. Similarly, *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^+$ can be activated to deliver NO by chemical reduction [148] or by UV light irradiation

(λ = 355 nm) [151]. However, the time course of the relaxation effect is different for each activation pathway. For the thermal activation, the maximum effect was observed at 595 s, while it was observed at 50 s when light irradiation was used.

The *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$ and *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^+$ complexes can release NO photochemically; however, the pharmacological mechanism of vasorelaxation by these nitrosyl macrocyclic ruthenium complexes was impaired in rat aortas under light irradiation [89]. The *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^+$ complex gave the maximum relaxation (22.66 ± 1.60%, n = 9) after the 355 nm light irradiation, while *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$ failed to induce relaxation [89]. Diffusion of NO to the cell is one of the pharmacological mechanisms of vasorelaxation; using the nitrosyl macrocyclic ruthenium complex, the amount of NO generated by light irradiation of *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$ is not enough to activate this biological pathway [89]. Because both complexes release NO photochemically, the relaxation observed with *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^+$ is consistent with a larger quantum yield (ϕ_{NO} 0.60 mol einstein^{-1}, Table 1) of NO release by this complex, while the cyclam complex has a much lower quantum yield (ϕ_{NO} 0.16 mol einstein^{-1}, Table 1) and is unable to provide a sufficient concentration of NO for the relaxation [89]. As a result, relaxation was not observed when the cyclam nitrosyl ruthenium complex was used as an NO delivery agent with 355 nm light irradiation [89].

The nitrite ruthenium complex *cis*-$[Ru^{II}(bpy)_2(py)NO_2]^+$ is reported to exhibit [149] a fast relaxant effect (100% vasodilation in 240 s), with the same efficiency as SNP but a lower potency. According to the authors, the maximum relaxation requires 3 μmol L^{-1} *cis*-$[Ru^{II}(bpy)_2(py)NO_2]^+$ and just 0.1 μmol L^{-1} SNP [149]. Similar experiments were carried out with $NaNO_2$, and a concentration of 2 mmol L^{-1} was needed to equal the performance of *cis*-$[Ru^{II}(bpy)_2(py)NO_2]^+$ at 3 μmol L^{-1} [149]. It was also reported that this complex is not activated to release NO photochemically or thermally alone; NO is released only in the presence of aortic tissue [149]. Experiments with a soluble guanylylcyclase (sGC) inhibitor revealed that the relaxation effect was partially blocked (Table 3), showing that the conversion of nitrite into nitric oxide in the complex *cis*-$[Ru^{II}(bpy)_2(py)NO_2]^+$ could be catalyzed by the sGC enzyme [149].

Arterial blood pressure variations in adult male Wistar and Swiss rats were followed after intravenous administration of $[Fe^{II}(NO^+)(CN)_5]^{2-}$, *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3]^{3+}$, and $NaNO_2$, and combinations of these complexes with $NaNO_2$ in normotensive and hypertensive rats [54,152]. In normotensive rats, the ruthenium compounds resulted in dose-dependent decreases in blood pressure, with hypotensive responses of 3–26% for doses between 2.5 and 80 nmol kg^{-1}, while for SNP it was 11–32% under the same conditions. When acute hypertension was induced with 0.3 mg kg^{-1} L-NAME, both complexes resulted in similar blood pressure decreases. Moreover, $NaNO_2$ administered alone did not have a hypotensive effect, but when administrated in combination with *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3]^{3+}$ it increased the blood pressure lowering effect of this complex. However, this effect was not observed when $NaNO_2$ was co-administered with SNP [152]. This last observation strengthens the possibility that the reaction between *trans*-$[Ru^{II}(H_2O)(NH_3)_4P(OEt)_3]^{2+}$ and nitrite ions to yield *trans*-$[Ru^{II}(NO_2)(NH_3)_4P(OEt)_3]^+$ (Fig. 2) could occur *in vivo* and thus renew the NO source. The activation of SNP results in several products [7], and therefore it is not regenerated by $NaNO_2$ under these conditions.

The pathway by which ruthenium(II) nitrosyl compounds induce smooth muscle relaxation is also an important subject for study. The vasodilatation process promoted by NO seems to be mediated mainly by the soluble guanylylcyclase (sGC) stimulus, which catalyzes the production of the second messenger guanosine-3′,5′-monophosphate (cGMP) from guanosine triphosphate (GTP) [146,147,153]. However, there are reports [154–157] suggesting that the induced NO relaxation is also related to the direct activation of potassium channels and is thus independent of the cGMP stimulus.

The relaxation mechanism of the best known inorganic vasodilator, SNP, is not fully understood [160,161]. NO release from SNP is accompanied by cyanide liberation, which is undesirable due to cell toxicity and other effects, such as sGC activation [162,163]. In addition to the activation of sGC by NO release, SNP can react with proteins and thiols [164,165]. Indeed, experiments have shown [93,159] that the relaxation effects of SNP are only partially blocked in the presence of both Carboxy-PTIO (NO scavenger) and MB (sGC inhibitor) and thus are only partially related to the NO-cGMP pathway. However, there is evidence that K^+ channel activation may also be related to the observed SNP effects (Table 3) [93,159]. This last behavior was also observed for $[Ru^{II}(NO^+)(terpy)(bdq)]^{3+}$ under similar conditions [159].

Changes in the cytosolic calcium concentration ($[Ca^{2+}]c$) are a critical process in the relaxation of vascular smooth muscle cells [166,167]. The mechanism of NO-dependent relaxation of vascular smooth muscle cells involves a decrease in $[Ca^{2+}]c$ by inhibition of Ca^{2+} entry [168], and it was described that NO and cGMP may relax vascular smooth muscle cells by cGMP-dependent K^+ channel activation [169]. Another mechanism that operates by a direct effect of NO on Ca^{2+}-dependent K^+ channels [170] and the L-type calcium current [171] and depends on cGMP has been reported. Studies on other types of smooth muscle preparations indicate that NO/cGMP may decrease $[Ca^{2+}]c$ and reduce the sensitivity of contractile proteins to Ca^{2+}, thereby resulting in the relaxation of smooth muscle. Confocal microscopy assays have shown that NO donors like *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^+$, $[Ru^{II}(NO^+)(NH{\cdot}NHq)(terpy)]^{3+}$, and *cis*-$[Ru^{II}(NO^+)(bpy)_2Cl](PF_6)_2$ decrease $[Ca^{2+}]c$ in vascular smooth muscle cells [172,173]. A decrease in $[Ca^{2+}]c$, measured as the fluorescence intensity of the Ca^{2+}-sensitive dye Fluo-3AM, was observed after the addition of these NO donors [172,173].

In contrast to SNP, the relaxation effect of NO-donors such as *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{3+}$ and *trans*-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3]^{3+}$ was completely blocked by an NO scavenger and a sGC inhibitor

Table 3
Effects on the relaxation behavior induced by ruthenium(II) nitrosyl compounds in the presence of a sGC inhibitor, NO scavenger and K^+ channel blocker.

Compound	sGC inhibitor	Relaxation Effect	NO scavenger	Relaxation Effect	K^+ channel blocker	Relaxation Effect	Ref.
trans-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$	MB	Fully blocked	Carboxy-PTIO	Fully blocked	–	–	[93]
trans-$Ru^{II}(NO^+)(NH_3)_4(P(OEt)_3)](PF_6)_3$	MB	Fully blocked	Carboxy-PTIO	Fully blocked	–	–	[152]
cis-$[Ru^{II}(NO^+)(bpy)_2(Cl)]PF_6$	ODQ	Partly blocked	–	–	TEA	Partly blocked	[158]
$[Ru^{II}(NO^+)(terpy)(bdq)](PF_6)_3$	ODQ	Partly blocked	OHCbl	Partly blocked	TEA	Partly blocked	[159]
trans-$[Ru^{II}(NO^+)([15]aneN_4)Cl]Cl$	–	–	HbO_2	Fully blocked	TEA	Partly blocked	[148,151]
cis-$[Ru^{II}(bpy)_2(py)NO_2]PF_6$	ODQ	Partly blocked	–	–	–	–	[149]
$Na_2[Fe^{II}(NO^+)(CN)_5]$	MB	Partly blocked	Carboxy-PTIO	Partly blocked	TEA	Partly blocked	[93,159]

Ref. = reference; soluble Guanylyl Cyclase (sGC); Methylene Blue (MB); 1*H*-[1,2,4] oxadiozolo[4,3-*a*]quinoxaline-1-one (ODQ); Oxyhaemoglobin (HbO_2); Tetraethylammonium (TEA); Hydroxocobalamin (OHCbl).

(Table 3), strongly suggesting that their mechanism of action is related to the NO-cGMP pathway [93,152]. The vasorelaxant effects of *trans*-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^+$ were also fully blocked in the presence of the NO scavenger HbO_2, but the potassium channel also seemed to be involved [148,151].

In addition to the problems caused by low NO concentrations, it is important to highlight that also high levels of NO produced from the activation of the inducible nitric oxide synthase (iNOS) [174,175] have been implicated in a variety of diseases, such as septic shock, tissue injury, inflammatory bowel disease, and rheumatoid arthritis [176]. After stimulation by, for instance, bacterial lipopolysaccharide or inflammatory cytokines, iNOS remains active for hours and thus increases the endogenous NO concentration [174,175]. To treat these conditions, inhibitors of iNOS have been widely studied [177,178]. However, adverse effects and nonselective action have been observed [179].

Metal compounds (d^5, slow spin) that react rapidly with NO are an attractive alternative for use as nitric oxide scavengers. The reaction between the metal scavenger and NO is dependent on the concentrations of both; therefore, NO capture would be more effective in regions of high NO concentration than in low ones [95]. This action would follow a different pathway than iNOS inhibitors, the performance of which is NO concentration-independent.

$$[Ru^{III}(H_2O)(Hedta)]^- + NO^0 \overset{K_{+NO}}{\rightarrow} [Ru^{III}(NO^+)(Hedta)]^- + H_2O \qquad (3)$$

$[Ru^{III}(H_2O)(Hedta)]^-$ [180] and *trans*-$[Ru^{III}(OH)(cyclam)(Cl)]^+$ [93] are NO scavengers, $[Ru^{III}(H_2O)(Hedta)]^-$ ($k_{+NO} = 2 \times 10^7$ mol L^{-1} s^{-1}) is 8 orders of magnitude better than *trans*-$[Ru^{III}(OH)(cyclam)(Cl)]^+$ (k_{+NO} = 0.20 mol L^{-1} s^{-1}) at pH 7 [181], acting according to reaction 3. It is interesting to recall that the $[Ru^{II}(NO^+)(Hedta)]^-$, the product of reaction 3, releases nitric oxide very slowly after reduction (Table 1). The complexes $[Ru^{III}(L_1)(Hedta)]^n$ ($L_1 = H_2O$ or Cl^-) were already tested and were shown to be useful as alternatives to NOS inhibitors during NO overproduction [94–96].

Ruthenium nitrosyls as anti-infective agents

Neglected diseases and tuberculosis represent 12% of global diseases [182], affecting billions of poor and marginalized people in developing countries [182–186]. Among these diseases, two are caused by flagellates of the *Trypanosomatidae* family: American trypanosomiasis, commonly known as Chagas' disease, and leishmaniasis [187]. In both cases, the treatment is painful and based on drugs that have serious toxicity, side effects and poor efficacy, besides high cost and increase of the parasite resistance [187]. Because of this, are necessary additional incentives for the development of new treatments and chemotherapeutic agents that are more effective and less toxic against these pathogenic agents.

Chagas' disease is a parasitic infection caused by the flagellated protozoan *Trypanosoma cruzi* (*T. cruzi*), which is an organism endemic in 21 countries of Latin America. According to the World Health Organization (WHO) [185,188], there are 10 million people infected worldwide. It is estimated that Chagas' disease caused more than 10,000 deaths in 2008, making it the parasitic disease responsible for the largest number of deaths in Latin America.

The life-cycle of *T. cruzi* includes a passage through both vertebrate and invertebrate hosts and three forms of the parasite: the trypomastigote form (extracellular phase, that circulates in the blood), the amastigote form (intracellular phase) and the epimastigote form (found in the digestive tract of the vector) [187]. The Chagas' disease presents two phases: the acute phase, where the tripomastigote form is prominent and a high number of parasites circulate in the blood. When the disease is in this phase there are two available drugs for treatment: benznidazole (Bz) and nifurtimox (Nif). In the chronic phase, the amastigote form is prominent and the parasites are mainly in the heart and digestive muscle. In this phase the treatment using these drugs is not effective [189,190].

Leishmaniasis is caused by about 20 species of the flagellated protozoan of the genus *Leishmania*, and affects about 12 million people in 88 countries around the world. In Latin America, 90% of cases occur in Brazil [185]. The *Leishmania* parasites present two forms, the amastigotic (intracellular phase without a flagellum) and promastigotic (extracellular phase with a flagellum). The disease may be cutaneous, mucocutaneous or visceral, and the therapies mainly include pentavalent antimonials (Sb^V) (Pentostam and Glucantime) [190–192], amphotericin B or pentamidine. Other effective anti-infective drugs are miltefosine and paromomycin [192]. Some examples are shown in Fig. 3.

Nitric oxide has an important role in controlling infections caused by pathogenic agents such as *Trypanosoma* and *Leishmania* [193,194]. In this context, ruthenium(II) NO-donors, which could release this molecule in a controlled manner, would be useful for developing a platform of potential therapies for these diseases [55,56,195–198]. It is worthwhile to recall that the reduction of the NO^+ ligand occurs at potential values accessible to the reducing environment present in biological media, such as NADH [101], sulfhydryl groups (R-SH) [100] and ROS [126].

As can be seen in Table 4, the *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{n+}$ complexes exhibited *in vitro* cytotoxicity against host normal cells ($IC_{50}{}^{V79}$, where IC_{50} is the concentration in which 50% of activity is inhibited) in the range of 120 μmol L^{-1} (L = pz) to 2260 μmol L^{-1}

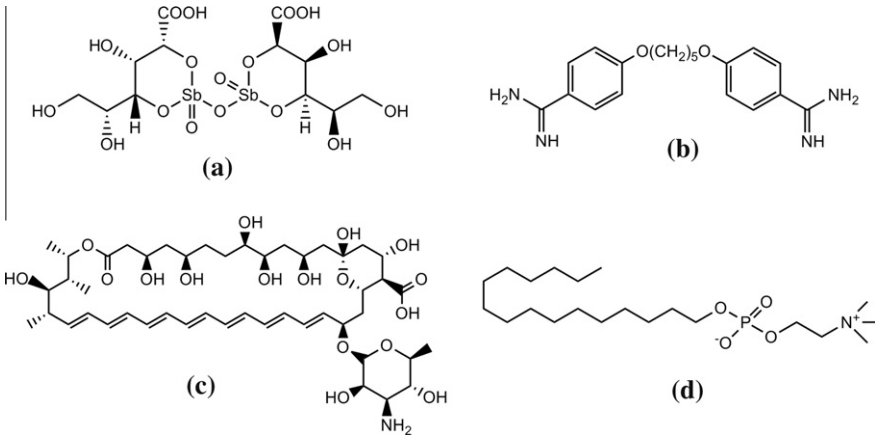

Fig. 3. Structures of (a) pentostam (antimonial) (b) pentamidine B (c) amphotericin and (d) miltefosine.

Table 4
Antiparasite activity of ruthenium(II) NO-donors, Bz, SNP, GV, $Na_2N_2O_3$, GSNO, and SNAC.

NO donor	Trypanocidal activity		Leis. activity	IC_{50}^{V79*}	LD_{50}^{*}	TI			Ref.
	IC_{50}^{try*}	IC_{50}^{epi*}	IC_{50}^{pro*}			try	epi	Leis.	
trans-$[Ru^{II}(NO^+)(NH_3)_4(pz)](BF_4)_3$	50	56	41	120	–	2	2	3	[55,56]
trans-$[Ru^{II}(NO^+)(NH_3)_4(L\text{-hist})](BF_4)_3$	51	78	95	414	125–250	8	5	4	[22,55,56,196]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$	52	86	36	646	125–250	12	7	18	[55,56,196]
trans-$[Ru^{II}(NO^+)(NH_3)_4(isn)](BF_4)_3$	77	67	280	743	125–250	10	11	3	[55,56,196]
trans-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$	75	90	42	930	125–250	12	10	22	[55,56,196]
trans-$[Ru^{II}(NO^+)(NH_3)_4(nic)](BF_4)_3$	158	136	>900	–	125–250	–	–	–	[22,55,56]
trans-$[Ru^{II}(NO^+)(NH_3)_4(ina)](BF_4)_3$	63	–	–	–	–	–	–	–	[55]
trans-$[Ru^{II}(NO^+)(NH_3)_4(4\text{-pic})](BF_4)_3$	177	–	38	–	125–250	–	–	–	[22,55,56]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imC)]Cl_3$	≈3400	–	>5000	–	–	–	–	–	[55,56]
trans-$[Ru^{II}(NO^+)(NH_3)_4P(OEt)_3](PF_6)_3$	60	328	82	2260	257	38	7	28	[22,55,56,152,196]
trans-$[Ru^{II}(NO^+)(NH_3)_4(SO_3)]Cl$	59	–	>300	1000	–	17	–	3	[55,56,196]
$[Ru^{II}(NO^+)(Hedta)]$	275	275	>900	–	>90	–	–	–	[22,55,56,150]
cis-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$	59	106[a]	–	–	250–500	–	–	–	[197]
cis-$[Ru^{II}(NO^+)(bpy)_2(1\text{-}miN)](PF_6)_3$	88	117[a]	–	–	250–500	–	–	–	[197]
cis-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$	85	121[a]	–	–	250–500	–	–	–	[197]
trans-$[Ru^{II}(NO^+)([15]aneN_4)Cl]^{2+}$				∼2000	2000–6000	–	–	–	[195]
trans-$[Ru^{II}(NO^+)(cyclam)(Cl)]Cl_2$	–	–	–	3000	–	–	–	–	[22]
Benznidazole (Bz)[b]	53	3180	–	–	–	–	–	–	[55]
Sodium nitroprusside (SNP)[b]	52	244	214	51	15	1	0.2	0.2	[55,56,196]
Gentian violet (GV)	536	–	–	–	–	–	–	–	[56]
Angeli's salt (AS)	–	–	158	–	–	–	–	–	[56]
GSNO	–	–	68	–	–	–	–	–	[198]
SNAC	–	–	54	–	–	–	–	–	[198]

Leis. = antileishmanial; Ref. = reference.

[*] IC values in μmol L^{-1} and LD values in μmol kg^{-1}; IC_{50}^{try} = corresponds to the concentration with 50% trypanocidal activity, after 24 h of incubation; IC_{50}^{epi} = corresponds to the concentration with 50% antiproliferative activity, after 24 h of incubation; IC_{50}^{pro} = corresponds to the concentration with 50% antiproliferative activity, after 24 h of incubation.

[a] IC_{50}^{epi} = corresponds to the concentration with 50% antiproliferative activity, after 72 h of incubation; IC_{50}^{V79} = concentration corresponding to 50% inhibition on mammalian V-79 cells.

[b] Used as a reference anti-parasitic drug; TI = Therapeutic Index: IC_{50}^{V79} / IC_{50}^{try}, IC_{50}^{V79} / IC_{50}^{epi} or IC_{50}^{V79} / IC_{50}^{pro}; Benznidazole (*N*-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide, $C_{12}H_{12}N_4O_3$); Sodium nitroprusside (sodium pentacyanonitrosylferrate(II), $Na_2[Fe(CN)_5NO]$); Gentian violet ($C_{25}H_{30}ClN_3$); Angeli's salt (disodium diazen-1-ium-1,2,2-triolate, $Na_2N_2O_3$)); GSNO = *S*-Nitrosoglutathione ($C_{10}H_{16}N_4O_7S$); SNAC = *S*-nitroso-*N*-acetyl-L-cysteine ($C_5H_8N_2O_4S$).

(L = $P(OEt)_3$). The complexes *trans*-$[Ru^{II}(NO^+)([15]aneN_4Cl)]^{2+}$ and *trans*-$[Ru^{II}(NO^+)(cyclam)(Cl)]^{2+}$ had IC_{50}^{V79} values of ∼2000 and 3000 μmol L^{-1}, respectively. From the *in vivo* analysis performed with female BALB/c mice, *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{n+}$ compounds were classified as moderately toxic [199]. *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{n+}$ and *cis*-$[Ru^{II}(NO^+)(bpy)_2L]^{n+}$ exhibited LD_{50} values between 125–500 μmol kg^{-1}, while for $[Ru^{II}(NO^+)(Hedta)]$ it was >90 μmol kg^{-1}. The *in vitro* and *in vivo* toxicities of these complexes were therefore lower than that of SNP (IC_{50}^{V79} = 51 μmol L^{-1} and LD_{50} = 15 μmol kg^{-1}), a well-known NO-donor.

Regarding to the anti-leishmanial activity, $[Ru^{II}(NO^+)(Hedta)]$ and *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{n+}$ exhibited IC_{50}^{pro} values in the range of 36 μmol L^{-1} (L = Im*N*) to 5000 μmol L^{-1} (L = im*C*) [56] (Table 4). Thus, the complexes where L = pz, L-hist, im*N*, py, 4-pic, or $P(OEt)_3$ were more effective against *Leishmania major* than the control SNP (IC_{50}^{pro} = 214 μmol L^{-1}) and $Na_2N_2O_3$ (Angeli's salt) (IC_{50}^{pro} = 158 μmol L^{-1}). Some analog compounds without an NO^+ group coordinated to the metal center showed lower anti-promastigote activity *in vitro* than the corresponding nitrosyl complexes: *trans*-$[Ru^{II}(NH_3)_4L(SO_4)]^+$ (L = im*N* (IC_{50}^{pro} = 81 μmol L^{-1}), py (IC_{50}^{pro} = 137 μmol L^{-1}), pz (IC_{50}^{pro} = 260 μmol L^{-1}), L-hist (IC_{50}^{pro} = 378 μmol L^{-1}), isn (IC_{50}^{pro} = 488 μmol L^{-1}), and nic (IC_{50}^{pro} = 5640 μmol L^{-1})) and *trans*-$[Ru^{II}(H_2O)(NH_3)_4(imN)]^{3+}$ (IC_{50}^{pro} = 181 μmol L^{-1}). This data strongly suggests that the NO released by the NO-donor complexes is responsible for the control of *L. major*.

For *in vivo* leishmaniasis assays, the compound in which L = im*N* was selected due to its high therapeutic index (TI = 18) [56]. Female BALB/c mice were inoculated subcutaneously with 1×10^5 *L. major* stationary phase promastigote forms and later treated with 0.5 μmol kg^{-1} day^{-1} of *trans*-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$ on a consecutive or alternate day doses during the fourth, fifth and sixth week after infection. Alternate day treatment had a lower efficiency (70% parasite growth inhibition) than consecutive day treatment (98% parasite growth inhibition). This nitrosyl complex inhibited parasite growth similarly to glucantime (a drug used in leishmaniasis treatment) but at a concentration 66-fold smaller.

Nitroxyl (HNO) also has relevant biological and pharmacological activities [56,200–203], and its influence was also investigated. *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{3+}$ complexes in which L = im*N* or $P(OEt)_3$ can undergo one- and two-electron reductions (Fig. 2), releasing NO and HNO in parallel reactions as a function of the cysteine concentration [56]. Thus, HNO activity against the *Leishmania* amastigote form was evaluated using $Na_2N_2O_3$ (Angeli's salt) and *trans*-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$ as a nitroxyl source [56]. According to the experimental data, the HNO generated exhibited similar *in vitro* inhibitory effect against the growth of intramacrophage amastigote forms by 49%, 49% and 44% at concentrations of 100, 33 and 11 μmol L^{-1}, respectively, suggesting that HNO can also contribute to kill the parasite.

The ruthenium(II) nitrosyl compounds also exhibited *in vitro* and *in vivo* anti-parasitic activity against the Chagas' disease Y strain parasite (Table 4). *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$, *cis*-$[Ru^{II}(NO^+)(bpy)_2(1\text{-}miN)](PF_6)_3$, *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$, and *trans*-$[Ru^{II}(NO^+)(NH_3)_4L](BF_4)_3$ (L = pz, isn, L-hist, im*N*, py, or nic) exhibited IC_{50}^{epi} values in the range of 56 (L = pz) to 136 μmol L^{-1} (L = nic) [55,56]. These results showed that the complexes were more effective anti-parasitics than SNP (IC_{50}^{epi} = 244 μmol L^{-1}) and Bz (IC_{50}^{epi} = 3180 μmol L^{-1}), both used as reference trypanocidal drugs for the clinical treatment of Chagas' disease.

Against trypomastigotes forms, the *trans*-$[Ru^{II}(NO^+)(NH_3)_4L]^{n+}$ (L = pz, L-hist, im*N*, SO_3, $P(OEt)_3$, or ina) and *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$ complexes exhibited IC_{50}^{try} values in the range of 50 (L = pz) to 63 μmol L^{-1} (L = ina), which are similar to SNP (IC_{50}^{try} = 52 μmol L^{-1}) and Bz (IC_{50}^{try} = 53 μmol L^{-1}) [55]. In

addition, *cis*-$[Ru^{II}(NO^+)(bpy)_2(1\text{-mi}N)](PF_6)_3$, *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)](PF_6)$, *trans*-$[Ru^{II}(NO^+)(NH_3)_4(isn)](BF_4)_3$, *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$, *trans*-$[Ru^{II}(NO^+)(NH_3)_4(nic)](BF_4)_3$, *trans*-$[Ru^{II}(NO^+)(NH_3)_4(4\text{-pic})](BF_4)_3$, and $[Ru^{II}(NO^+)(Hedta)]$ also had good trypanocidal activity (see Table 4) and were more effective than gentian violet (IC_{50}^{try} = 536 μmol L^{-1}), the standard drug recommended by the WHO for preventive blood treatment against *T. cruzi* [204].

Due to the antiproliferative activities and low cytotoxicity *in vitro* of *trans*-$[Ru^{II}(NO^+)(NH_3)_4(isn)](BF_4)_3$ and *trans*-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$, these compounds were selected for *in vivo* tests (acute model) [55]. Female Swiss mice were infected by injecting 1.0×10^3 bloodstream trypomastigotes per mouse and were treated with a single daily dose of 100 nmol kg^{-1} of each one of these complexes for 15 consecutive days. The treated mice exhibited lower parasitemia than the group treated with only phosphate-buffered saline (PBS). In addition, 60% of the mice treated with *trans*-$[Ru^{II}(NO^+)(NH_3)_4(isn)](BF_4)_3$ and 40% of the mice treated with *trans*-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$ survived for more than 120 days, whereas those treated with PBS died before the 18th day. Light microscopy revealed that the infected control mice had several nests of amastigotes in the myocardial tissue, whereas no nests were observed in the hearts of the mice treated with the ruthenium complexes.

To study dose-dependence, *trans*-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$ and *trans*-$[Ru^{II}(NO^+)(NH_3)_4(isn)](BF_4)_3$ [196] were intraperitoneally administered at concentrations of 10, 50, 100, 400, 1000 and 3000 nmol kg^{-1} for 15 consecutive days. The determined ideal dose was 400 nmol kg^{-1} for both compounds. Comparatively, the infected mice treated with a 400 nmol kg^{-1} dose of Bz did not exhibit protective effect against the animal death. Then, Swiss mice were intraperitoneally infected with 1.0×10^3 bloodstream trypomastigote per mouse and treated with a dose of 400 nmol kg^{-1} of either complex for 15 consecutive days. On the 15th day after infection, microscopy analysis revealed that the control mice (PBS) exhibited several nests of amastigotes in their hearts, whereas no nests were observed in the hearts of the mice treated with these nitrosyl complexes. Treatment with either 100 or 400 nmol kg^{-1} reduced myocarditis inflammation.

The *in vivo* trypanocidal activities of *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$ and *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$ were evaluated using a murine model of acute Chagas's disease [197]. BALB/c mice were treated 24 h after infection with a single daily dose of 385 nmol $kg^{-1}day^{-1}$ of *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$ or *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$, for 15 consecutive days. The mice treated with these compounds had lower levels of parasitemia than animals treated with PBS or Bz at the same dose. Furthermore, 80% and 60% of the mice treated with *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$ or *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$, respectively, survived for more than 60 days, while the mice treated with PBS or Bz, at the same dose, died within 20 days. Histological analysis of the control mice revealed the existence of several amastigote nests in their hearts, whereas a smaller number of nests were observed in the hearts of the mice treated with *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$ and *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$. The histological analysis showed a reduction in the myocarditis inflammation in the mice treated with these NO-donors.

Intending to understand the possible chemotherapeutic target of the above *cis*-ruthenium(II) nitrosyl in *T. cruzi* treatment, the complexes were assayed against the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [197]. The values of IC_{50}^{GAPDH} = 89 μmol L^{-1} for *cis*-$[Ru^{II}(NO^+)(bpy)_2(imN)](PF_6)_3$, IC_{50}^{GAPDH} = 97 μmol L^{-1} for *cis*-$[Ru^{II}(NO^+)(bpy)_2(1\text{-mi}N)](PF_6)_3$ and IC_{50}^{GAPDH} = 153 μmol L^{-1} for *cis*-$[Ru^{II}(NO^+)(bpy)_2(SO_3)]PF_6$ indicate substantial inhibitory effects against *T. cruzi* GAPDH. X-ray data suggest a possible mechanism of action of these compounds via S-nitrosylation of the active cysteine (Cys166) of GAPDH [197].

The *trans*-$[Ru^{II}(NO^+)(NH_3)_4L](X)_3$ (L = pz, 4-pic, isn, py, im*N*, inaH, $P(OH)_3$, or $P(OEt)_3$, X = BF_4, Cl, or PF_6), *trans*-$[Ru^{III}(NH_3)_4(SO_4)L]Cl$ (L = 4-pic, isn, py, nic, or inaH) and $[Ru^{II}(NO^+)(Hedta)]$ compounds were also evaluated for their ability to inhibit the *T. cruzi* enzyme cruzain. The preliminary results, at a concentration of 100 μmol L^{-1}, yielded IC_{50} values of 247 μmol L^{-1} for *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$ and 237 μmol L^{-1} for *trans*-$[Ru^{III}(NH_3)_4(SO_4)(py)]Cl$.

S-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetyl-L-cysteine (SNAC) can act as NO-donors [205] and also had activity [206,207] in *in vitro* experiments against the promastigote forms of the pathogens *L. major* and *L. amazonensis* [198]. GSNO had an IC_{50}^{pro} 68 μmol L^{-1} for *L. major* and *L. amazonensis*, and SNAC presented IC_{50}^{pro} = 54 and 181 μmol L^{-1} for *L. major* and *L. amazonensis*, respectively. These IC_{50}^{pro} values against the *L. major* parasite are slightly higher than the ones exhibited by *trans*-$[Ru^{II}(NO^+)(NH_3)_4L](BF_4)_3$, where L = 4-pic, imN, py, or pz (see Table 4).

Cancer

The anticancer activity of NO has also attracted considerable interest [22,33,208–210]. In this context, different forms of NO-donors, such as NONOates [211,212] and many ruthenium compounds, are promising candidates for anticancer drugs [22,33,41,121,209,213,214]. Although promising, the use of compounds that release NO in the body as a treatment against cancer is problematic because NO has contradictory effects in tumor cells [74,215–217]. NO can either stimulate or inhibit tumor growth, depending on the local concentration of the NO generated, its rate of release, the cell type, and other factors. For example, the exposure of PC12 cells to low concentrations of NO (between 20 and 100 nmol L^{-1}) significantly stimulated proliferation, whereas higher concentrations (between 200 and 600 nmol L^{-1}) inhibited proliferation [218]. While angiogenesis is promoted by low levels of NO [71], higher levels of NO overwhelm the cellular detoxification defenses and induce apoptosis [219]. NO also initiates leakage of cytochrome c from the mitochondrial membrane, which in turn activates several caspases (cysteine proteases) in a 'one-way' signaling cascade. In addition, cellular DNA is modified and often becomes fragmented. As a result, cellular homeostasis of protein synthesis, regulation, and degradation becomes unsustainable [219]. The resulting liquidation of cellular assets is referred to as NO-induced apoptosis [220,221].

Studies with ruthenium nitrosyl complexes with substituted pyridines have shown that these compounds are toxic against cancer cells and are also able to induce cell apoptosis [32,222–224]. As an example, 10–100 μmol L^{-1} of *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)]^{3+}$ induced apoptosis in liver carcinoma HepG2 cells [121]. Endogenous nitric oxide has also shown importance in the growth and vascularization of a rat carcinosarcoma (P22). The animals treated with nitric oxide synthase (NOS) inhibitors showed reducing of tumor growth of 60–75% [225].

As can be seen in Table 5, cytotoxicity activity, *in vitro*, of *cis*-$[Ru^{II}(NO^+)(dppp)(L)Cl_2]PF_6$ (dppp = 1,3-bis(diphenylphosphino)propane; L = py, 4-pic, 4-phenylpyridine, or dmso) against human breast carcinoma MDA-MB-231 tumor cell line yielded IC_{50} values of 19.0, 7.4, 7.1 and 12.1 μmol L^{-1}, respectively [213]. The IC_{50} value of cisplatin was 63 μmol L^{-1} under the same experimental conditions, demonstrating that the nitrosyl complexes were three- to nine-fold more active than cisplatin [213].

Recently, the *in vitro* and *in vivo* cytotoxicities of *trans*-$[Ru^{II}(NO^+)(NH_3)_4(L)]^{3+}$ (L = isn, 4-pic, nic, $P(OEt)_3$, im*N*, or py), *trans*-$[Ru^{II}(NO^+)(NH_3)_5]^{3+}$ and $[Ru^{II}(NO^+)Hedta]$were also evaluated, using murine melanoma B16F10-Nex2 cells (Table 5) supplied by American Type Culture Collection (ATCC) [57]. With the exception of $[Ru^{II}(NO^+)Hedta]$, the complexes were cytotoxic against murine

Table 5
NO-donor ruthenium complexes activity against cancer cells.

NO donor	Cells	IC_{50}	Ref.
cis-$[Ru^{II}(NO^+)(dppp)(py)Cl_2]PF_6$	MDA-MB-231	19.0 μmol L^{-1}	[213]
cis-$[Ru^{II}(NO^+)(dppp)(4\text{-pic})Cl_2]PF_6$	MDA-MB-231	7.4 μmol L^{-1}	[213]
cis-$[Ru^{II}(NO^+)(dppp)(4\text{-phen})Cl_2]PF_6$	MDA-MB-231	7.1 μmol L^{-1}	[213]
cis-$[Ru^{II}(NO^+)(dppp)(dmso)Cl_2]PF_6$	MDA-MB-231	12.1 μmol L^{-1}	[213]
trans-$[Ru^{II}(NO^+)(NH_3)_4(isn)](BF_4)_3$	B16F10-Nex2	1.0 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(4\text{-pic})](BF_4)_3$	B16F10-Nex2	6.0 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(nic)](BF_4)_3$	B16F10-Nex2	20.0 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(P(OEt)_3)](PF_6)_3$	B16F10-Nex2	33.0 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](BF_4)_3$	B16F10-Nex2	74.0 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$	B16F10-Nex2	86.0 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_5](BF_4)_3$	B16F10-Nex2	44.0 μmol L^{-1}	[57]
$[Ru^{II}(NO^+)Hedta]$	B16F10-Nex2	$>40 \times 10^2$ μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(inaH)](BF_4)_3$	B16F10	12.6 μmol L^{-1}	[222]
trans-$[Ru^{II}(NO^+)(NH_3)_4(4\text{-mapy})](BF_4)_3$	B16F10	347 μmol L^{-1}	[222]
trans-$[Ru^{II}(NO^+)(NH_3)_4(ina\text{-}Tat48\text{–}60)](BF_4)_3$	B16F10	49.0 μmol L^{-1}	[222]
trans-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$	B16F10	1.0 mmol L^{-1}	[32]
trans-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$	B16F10	6.8 mmol L^{-1}	[224]
trans-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$	Melan-a	0.9 mmol L^{-1}	[223]
trans-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$	Melan-a	9.8 mmol L^{-1}	[223]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](PF_6)_3$	A2058	335 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](PF_6)_3$	HCT	287 μmol L^{-1}	[57]
trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](PF_6)_3$	Siha	333 μmol L^{-1}	[57]

MDA-MB-231 = human breast carcinoma; B16F10 = murine melanoma; B16F10-Nex2 = murine melanoma subclone Nex2; melan-a = normal murine melanocyte; A2058 = human metastatic melanoma; HCT = human colorectal tumor; Siha = human cervical cancer.

melanoma B16F10-Nex2 in *in vitro* tests (IC_{50} values in the range of 1.0 (L = isn) to 86.0 μmol L^{-1} (L = py)). Against V79 cells, the IC_{50} values were higher than 646 μmol L^{-1}, showing that these cells were less sensitive to these complexes than the murine melanoma B16F10-Nex2 cells [57].

In vitro assays with B16F10 cell line treated with *trans*-$[Ru^{II}(NO^+)(NH_3)_4(L)]^{3+}$ (L = isonicotinic acid (inaH) or 4-(aminomethyl)pyridine (4-mapy)) were also performed [222]. The preliminary IC_{50} values obtained by the MTT cell viability assay were 12.6 $\mu mol^{-1}\ L^{-1}$ for L = inaH and 347 $\mu mol^{-1}\ L^{-1}$ for L = 4-mapy (Table 5). Compared to cisplatin, which has IC value of 33.3 $\mu mol^{-1}\ L^{-1}$ against this cell line [226], the first complex is very toxic, while the other is a little less. The difference in toxicity, however, can not be explained on the basis of the rate of release of NO from these nitrosyls. The estimated rate constants value, k_{-NO}, of release of NO for these complexes are similar and in the order of $10^{-2}\ s^{-1}$. The induction of apoptosis, measured by flow cytometry with annexin marking, using BD Pharmingen™ "PE Annexin V Apoptosis Detection Kit I", followed the same tendency. The complex with inaH induced apoptosis to 51% of the cancer cells, while for the 4-mapy complex this percentage was 13% [222]. These results illustrate the complexity of this subject and that other factors may be involved.

trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](PF_6)_3$ was evaluated *in vitro* against melanoma (A2058), colorectal (HCT), and cervical human (Siha) tumor cells [57] and showed inhibitory antitumor effects with IC_{50} values in the range of 287–335 μmol L^{-1} (Table 5). Also, an inhibitory effect on angiogenesis was observed for *trans*-$[Ru^{II}(NO^+)(NH_3)_4(ImN)](PF_6)_3$, as indicated by the *in vitro* cytotoxicity on human umbilical vein endothelial cells (HUVEC). At doses of 100 and 250 μmol L^{-1}, a 50 to 70% of reduction in the number of pro-angiogenic structures formed by HUVEC was observed, respectively. It is interesting to point out that even at concentrations as high as 0.5 mmol L^{-1}, 60% of the endothelial cells were still viable.

trans-$[Ru^{II}(NO^+)(NH_3)_4(imN)](PF_6)_3$ and *trans*-$[Ru^{II}(NO^+)(NH_3)_4(isn)](PF_6)_3$, which exhibited higher IC_{50} values *in vitro*, were assayed *in vivo* in the murine melanoma B16F10-Nex2 model using adult male C57Bl/6 mice [57]. In this assay, 5×10^4 B16F10-Nex2 cells were subcutaneously injected in the mice. For *trans*-$[Ru^{II}(NO^+)(NH_3)_4(ImN)](PF_6)_3$, two different treatments were applied 24 h after injection. In the first treatment, doses of 210 and 1050 ng kg^{-1} were inoculated intraperitoneally every other day for a 2-week period. Both doses inhibited tumor development in 40% of animals even 60 days after tumor cell inoculation. In the second treatment protocol, 210 ng kg^{-1} of the complex was injected intraperitoneally every day for one week and every other day on the second week. As a result, only 10% of the animals were protected against melanoma development. *trans*-$[Ru^{II}(NO^+)(NH_3)_4(isn)](PF_6)_3$ was injected intraperitoneally at 23 and 232 ng kg^{-1} doses every other day for 2 weeks. Sixty days after the injection of tumor cells, the lowest dose inhibited tumor development in 40% of the animals, while the highest dose protected only 10% of the animals. The above data suggest that lower doses and less aggressive protocols are more effective in controlling tumor development.

The cytotoxicity of *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)]^{3+}$, *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$, and $[Ru^{II}(NO^+)(Hedta)]$ were evaluated in murine melanocyte tumorigenic and non-tumorigenic cell lines (B16F10 and melan-a, respectively) [32,223,224,227] (Table 5) using MTT assay. Cytotoxicity was observed for both *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)]^{3+}$ ($IC_{50}^{B16-F10}$ = 1.0 mmol L^{-1} [32,224], $IC_{50}^{melan\text{-}a}$ = 0.9 mmol L^{-1} [223]), and *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$ ($IC_{50}^{B16-F10}$ = 6.8 mmol L^{-1} [224]; $IC_{50}^{melan\text{-}a}$ = 9.8 mmol L^{-1} [223]) at concentrations higher than 1×10^{-3} mol L^{-1}, causing cell death. The complex $[Ru^{II}(NO^+)(Hedta)]$ was cytotoxic in the presence of light, whereas in its absence, it had no cytotoxic effect on the cell line in the study. The behavior in the dark is consistent with the higher NO scavenging rate constant of the aquo complex ($k_f = 2.24 \times 10^7\ L\ mol^{-1}\ s^{-1}$), which is formed after the release of NO, than the release of NO from the nitrosyl complex ($k_{-NO} = 7.3 \times 10^{-3}\ s^{-1}$) at pH 7.4 [150,228], 53.9% for *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$ and 22.3% for $[Ru^{II}(NO^+)(Hedta)]$ [32,223,224,227]. In addition, the phototoxicity of these complexes was also evaluated against melan-a cells, with cell death values of 57.6% for *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)]^{3+}$, 47.0% for *trans*-$[Ru^{II}(NO^+)(cyclam)Cl]^{2+}$ and 17.7% for $[Ru^{II}(NO^+)(Hedta)]$ [32,223,224,227].

A mixture of *cis*-$[Ru^{II}(NO^+)(H\text{-}dcbpy^-)_2Cl]$ (H_2-dcbpy = 4,4′-dicarboxy-2,2′-bipyridine) and $Na_4[Tb(TsPc)(acac)]$ (TsPc = tetrasulfonated phthalocyanines; acac = acetylacetone) was reported

to simultaneously produce NO and $^1O_2$ under irradiation with light of $\lambda \geqslant 550$ nm, strongly inhibiting B16-F10 cell viability [131].

To increase selectivity and avoid side effects, it is necessary to tailor more specific ruthenium complexes to act at the desired target. A ruthenium nitrosyl NO-donor linked to a penetrating peptide called Tat48–60 [229–231] (Tat48–60 = GRKKRRQRRRPPQ, G = glycine, R = arginine, K = lysine, Q = glutamine, P = proline) was synthesized for this purpose. This 13 aminoacid sequence corresponds to the positions 48 to 60 of the cell penetrating Tat protein of the HIV virus [230–234], a protein that is important for virus replication [229,234]. The synthesized complex was *trans*-$[Ru^{II}(NO^+)(NH_3)_4(L)]^{3+}$, L = ina-Tat48–60 (ina-Tat48–60 = Tat48–60 plus inaH, leading to the sequence ina-GRKKRRQRRRPPQ). This complex was tested *in vitro* against the tumor cell line B16F10, with preliminary results similar to those of the complex with inaH; an IC_{50} value of 49 μmol L^{-1}, and 38% of induced apoptosis (Table 5) [222], which is close to that of cisplatin.

Immobilization of ruthenium nitrosyl complexes

The use of nanostructured materials as drug delivery systems has begun to impact medicine due to beneficial size-dependent physical and chemical properties [235] and targeted selectivity. As far as ruthenium nitrosyl complexes are concerned, this approach may also improve the *in vivo* residence time of ruthenium nitrosyl complexes, increase the number of species that could be carried by a single matrix, and avoid undesired side reactions, such as nucleophilic attack on the coordinated nitrosyl [23]. A wide range of materials can be used as matrices. The choice of the immobilization procedure depends on the structure of the complex, the material characteristics, and the desired properties. A review on the immobilization of ruthenium complexes has recently been published [23].

The use of NO-releasing silica nanoparticles as a therapeutic tool to improve NO delivery to human ovarian cancer cells has been reported [235]. It was shown that the NO-releasing nanoparticles exhibited enhanced growth inhibition of tumor cells when compared to both empty nanoparticles (control) and a previously reported small molecule NO donor, sodium 1-(pyrrolidium-1-yl)diazen-1-ium-1,2-diolate (PYRRO/NO) [235].

Incorporation of a ruthenium nitrosyl ($[Ru(NO^+)(Me_2bpb)(4\text{-}vpy)](BF_4)$) in polyHEMA hydrogel via a radical-induced copolymerization process has been described [236]. The transparent pHEMA nitrosyl-hydrogel conjugate readily releases NO upon exposure to low-intensity UV light (5–10 mW). Because 1-pHEMA can transfer NO to proteins like myoglobin quite readily, this material could be employed in the cellular environment for light-triggered NO delivery [236].

Photoinduced nitric oxide and singlet oxygen release from liposomal zinc phthalocyanine associated with the nitrosyl ruthenium complex $[Ru^{II}(NO^+)(NH{\cdot}NHq)(tpy)]^{3+}$ in neoplastic cells has been proposed in a PDT application using the B16F10 cell line [36]. The dark toxicity for three ZnPC preparations (ranging from 1.0 to 5.0 μmol L^{-1}) was evaluated at a fixed $[Ru^{II}(NO^+)(NH{\cdot}NHq)(tpy)]^{3+}$ complex concentration (0.05 mmol L^{-1}). As observed for all of the samples studied, no dark toxicity was observed above the cell viability basal level (90%). These results led to light activation studies (675 nm) to analyze the best combination of both drugs that could induce cell damage. The observed ideal complex formulation for inducing 90% cellular death was 5.0 μmol L^{-1} ZnPC and 0.05 mmol L^{-1} $[Ru^{II}(NO^+)(NH{\cdot}NHq)(tpy)]^{3+}$ [36].

The NO donors *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$, *trans*-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$, and $[Ru^{II}(NO^+)(Hedta)]$ were recently incorporated into polymeric micro and nanoparticles (MP) of poly(lactic-co-glycolic acid) (PLGA) (py-MP, cyclam-MP and Hedta-MP). This system was effective for complex transport and NO release in biological targets [32,223,224,227,237]. B16F10 melanoma cells were chosen as the biological model to evaluate the *in vitro* cytotoxicity of the ruthenium nitrosyl- and MP-loaded complex. The MTT test showed that the nitrosyl complex was non-toxic in low concentrations in solution or entrapped in microparticles, promoting a proliferative effect when solutions of this complex with concentrations lower than 1×10^{-4} mol L^{-1} were applied. Toxicity was observed only for the complexes in solution at higher concentrations (>1×10^{-4} mol L^{-1}), causing death (*trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$ $IC_{50} = 1.0 \times 10^{-3}$ mol L^{-1}; *trans*-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$ $IC_{50} = 6.8 \times 10^{-3}$ mol L^{-1}) [32]. The absence of cell death when using nitrosyl complexes entrapped in PLGA microparticles, which are able to release NO after reduction, indicated that the entrapped complex was not available to the medium and its reducing agents. The behavior of the NO donors was enhanced under light irradiation. Phototoxicity assays of the nitrosyl complexes were performed using the same cell lines and the MTT assay. After treating the B16F10 cells (10 min irradiation with 366 nm light) with the NO donor in solution (1×10^{-4} mol L^{-1}) in the presence of light, more cell damage was observed, leading to 63 ± 3% of cell death in the presence of *trans*-$[Ru^{II}(NO^+)(NH_3)_4(py)](BF_4)_3$, 53.8 ± 6.2% of cell death in the presence of *trans*-$[Ru^{II}(NO^+)(cyclam)Cl](PF_6)_2$ and 22.3 ± 8.2% of cell death in the presence of $[Ru^{II}(NO^+)(Hedta)]$. When encapsulated, py-MP produced 12 ± 6% of cell death, cyclam-MP 13.6 ± 6.4% of cell death and Hedta-MP 6.3 ± 7.6% of cell death. As the py-MP system is non-toxic without light irradiation, these results suggest that cell death was due to NO photorelease [22,32].

Nitrosyl ruthenium complex-loaded lipid carriers for topical administration, such as for skin cancer treatment, were also reported [129]. Lipid carrier systems were developed to circumvent problems related to the topical absorption of drugs, particularly positively-charged drugs, as is the case for the $[Ru^{II}(NO^+)(terpy)(bdqi)](PF_6)_3$ complex [129].

The PAMAM dendrimers (G_x) functionalized with $[Ru^{III}(H_2O)(edta)]^-$(G_x/RuH_2O) and $[Ru^{II}(NO^+)(edta)]^-$ (G_x/RuNO) were studied in vascular and trypanocidal models [150,238]. The initial experiments were performed using denuded normotensive rat aortic rings pre-contracted with noradrenaline. Similar to the observations for $[Ru^{II}(NO^+)(edta)]^-$, treatment with G_x/RuNO ($x = 0$ and 3.3 μmol L^{-1}) induced a slow delayed relaxation that began after 15 min (14.11 ± 6.25%, $n = 5$), inducing a maximal relaxation effect in the second hour (36.8 ± 6.5%, $n = 5$). The trypanocidal activity of G_0/RuNO *in vitro* was evaluated using a drug-resistant strain of *T. cruzi*. Active bloodstream trypomastigotes (*T. cruzi*) were not detected in infected mice after 24 h of incubation with the G_0/RuNO dendrimer at 1.0×10^{-3} mol L^{-1}. The G_0/RuNO dendrimer was slightly more effective against *T. cruzi* (100%) than $[Ru^{II}(NO^+)(edta)]^-$ (89%) [238].

Conclusions and perspectives

Nitrosyl complexes proved to be good NO-carriers. In particular, ruthenium(II) amines are very useful compounds because they are water soluble, stable in solution in the presence of oxygen, and can easily deliver NO after activation by chemical or photochemical pathways. The redox potential for $[Ru^{II}NO^+]^{3+}/[Ru^{II}NO^0]^{2+}$ is in the range of biological reductants, and the rate constant for release of NO from the $[Ru^{II}NO^0]^{2+}$ moiety can be modulated through coordination sphere tailoring. Photoactive nitrosyl complexes with regard to NO release can be obtained by judicious choice of the ligands, resulting in complexes with absorption bands in the region of the spectrum suitable for PDT.

Coordination compounds have been comparatively more explored as NO-donors than as NO-scavengers. In this respect, it

would be worthy to investigate the corresponding Ru(III) species of *trans*-$[Ru(NH_3)_4(L_1)(L_2)]^{n+}$, $L_1 = SO_4^{2-}$ or H_2O and L_2 = other ancillary ligand. Preliminary results from ours experiments with *trans*-$[Ru^{III}(SO_4)(NH_3)_4(4\text{-pic})]^+$ and *trans*-$[Ru^{III}(SO_4)(NH_3)_4(inaH)]^+$ in denuded aorta rings are promising [239]. The beneficial effects of NO-donors on some infections and cancer have been described, but very little is known about their *in vivo* mechanism of action. The metal nitrosyls react with ROS, acting as radical scavengers, and with thiols (RSH) [126]. In this case, NO and HNO could be formed. Whether NO and possibly HNO are released inside or outside of the cell is still an open question.

NO can act also as a pronociceptive mediator, and the nonsteroidal anti-inflammatory drugs (NSAIDs) used in the treatment of inflammatory diseases and pain exhibit several side effects. To circumvent these effects, new compounds called NO-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs) have been studied [240]. Correspondingly, nitrosyl ruthenium(II) tetraammines containing or lacking NSAID molecules in the coordination sphere should be investigated for use as analgesics.

Efforts are on-going to develop NO-carrier complexes for a specific desired target and to increase the residence time of these species in the blood. Studies are being performed in this direction using dendrimers and antibodies that specifically act on the receptors of breast cancer cells, for example, to kill the cancerous tissue without damaging healthy cells. Several approaches and strategies directed to make NO donors photochemically active in the therapeutic window were described, but they are non-exhaustive and other strategies can be designed, such as developing systems using the rare earths "upconversion", which would allow irradiation in longer wavelengths and activation of the NO release from higher energy states. The use of "caged" NO donors in matrices allows the arrival of the complex without being degraded which would be activated by irradiation, and are examples of targeting. Studies are under way to combine strategies of activation in the therapeutic window with "caged" NO donors in nano- and microparticles.

From this perspective of drug development, we see many possibilities, some of which are already under investigation, to improve the number and efficiency of complexes that have biological activity against many kinds of disease. There is still much to be explored on this field, but the most promising areas seem to be in the development of systems able to deliver NO upon light irradiation in the therapeutic window, drug delivery systems for controlled release of NO, and the linkage of the drugs to systems that carry them specifically to the local of interest, greatly improving its activity and causing few, or even none, side effects. With regard to photochemical therapy, it is conceivably possible to overcome the limitation to skin diseases by assembling a carrier, such an optical fiber with embedded complex, to be irradiated within vessels.

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