NITRIC OXIDE DONORS

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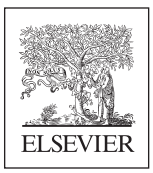
Novel Biomedical Applications and Perspectives

Edited by

AMEDEA BAROZZI SEABRA
Center for Natural and Human Sciences,
Federal University of ABC (UFABC),
Santo André, São Paulo, Brazil



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LIST OF CONTRIBUTORS

José L.B. Alves
Health Sciences Center, Federal University of Paraiba, João Pessoa, Paraíba, Brazil

Camille M. Balarini
Health Sciences Center, Federal University of Paraiba, João Pessoa, Paraíba, Brazil; Biotechnology Center, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

Divya Bhatnagar
New Jersey Center for Biomaterials and Rutgers, The State University of New Jersey, Piscataway, NJ, United States

Valdir A. Braga
Biotechnology Center, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

Harinath Chakrapani
Department of Chemistry, Indian Institute of Science Education and Research Pune, Maharashtra, India

Mathilde Champeau
Institute of Chemistry, University of Campinas, UNICAMP, São Paulo, Brazil

Allen L. Chen
Tulane University School of Medicine, New Orleans, LA, USA

Josiane C. Cruz
Biotechnology Center, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

Roberto S. da Silva
School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Vinod B. Damodaran
New Jersey Center for Biomaterials and Rutgers, The State University of New Jersey, Piscataway, NJ, United States

Marcelo Ganzarolli de Oliveira
Institute of Chemistry, University of Campinas, UNICAMP, São Paulo, Brazil

Nelson Durán
Institute of Chemistry, Biological Chemistry Laboratory, University of Campinas, Campinas, São Paulo, Brazil; Brazilian Nanotechnology National Laboratory (LNNano-CNPEM), Campinas, Brazil

Pascale L. Duvalsaint
Department of Physiology, Pharmacology, and Neuroscience, Sophie Davis School of Biomedical Education, City University of New York School of Medicine, New York, NY, United States

Kathleen Fontana
Department of Chemistry and Biochemistry, University of Windsor, Windsor, ON, Canada

Maria S. França-Silva
Biotechnology Center, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

Megan C. Frost
Department of Biomedical Engineering, Michigan Technological University, Houghton, MI, United States

Marisol Godínez-Rubí
Departamento de Microbiología y Patología, Universidad de Guadalajara, Jalisco, México

Serap Gur
Department of Pharmacology, School of Pharmacy, Ankara University, Ankara, Turkey; Department of Pharmacology, Tulane University Health Sciences Center, New Orleans, LA, United States

Tania Henríquez
Department of Microbiology and Mycology, Institute of Biomedical Sciences, University of Chile, Santiago, Chile

Kitty Ka Kit Ho
School of Chemistry, University of New South Wales, Sydney, NSW, Australia

Philip J. Kadowitz
Department of Pharmacology, Tulane University Health Sciences Center, New Orleans, LA, United States

Khosrow Kashfi
Department of Physiology, Pharmacology, and Neuroscience, Sophie Davis School of Biomedical Education, City University of New York School of Medicine, New York, NY, United States

Naresh Kumar
School of Chemistry, University of New South Wales, Sydney, NSW, Australia

Samuel K. Kutty
School of Chemistry, University of New South Wales, Sydney, NSW, Australia

Tassia Joi Martins
Faculty of Philosophy, Sciences and Literature of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Massimiliano Marvasi
Middlesex University London, The Burroughs, London, United Kingdom

Bulent Mutus
Department of Chemistry and Biochemistry, University of Windsor, Windsor, ON, Canada

Laísa Bonafim Negri
School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Daniel Ortuño-Sahagún
Instituto de Investigación en Ciencias Biomédicas, Departamento de Biología Molecular y Genómica. Universidad de Guadalajara, Jalisco, México

Loyanne C.B. Ramos
School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Govindan Ravikumar
Department of Chemistry, Indian Institute of Science Education and Research Pune, Maharashtra, India

Melissa M. Reynolds
Department of Chemistry and School of Biomedical Engineering, Colorado State University, Fort Collins, CO, United States

Heather Rubin
Department of Chemistry and School of Biomedical Engineering, Colorado State University, Fort Collins, CO, United States

Amedea Barozzi Seabra
Center for Natural and Human Sciences, Federal University of ABC (UFABC), Santo André, São Paulo, Brazil; Institute of Chemistry, Biological Chemistry Laboratory, University of Campinas, Campinas, São Paulo, Brazil

CHAPTER 1

Nitric Oxide Derivative Ruthenium Compounds as NO-Based Chemotherapeutic and Phototherapeutic Agents

Laísa Bonafim Negri[1], Tassia Joi Martins[2], Loyanne C.B. Ramos[1] and Roberto S. da Silva[1]

[1]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[2]Faculty of Philosophy, Sciences and Literature of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

INTRODUCTION

In the context of chemistry, the term "complex" means a central atom surrounded by a set of linkers. The central atom is a metal center that performs the function of a Lewis acid capable of receiving electrons; this central atom combines with ligands that act as Lewis base which are capable of donating electrons. Among all the atoms, metallic elements are the most abundant and include species of blocks s, d, and f as well as some elements of block p (aluminum, gallium, indium, thallium, tin, lead, and bismuth). One of the most important characteristics of metals is their tendency to display stable oxidation states within each block, which allows extraction of these metals from their ores and facilitates their handling in the laboratory. Elements belonging to block d probably constitute one of the most versatile classes of compounds. Their diverse chemical properties have given rise to a wide range of compounds with application in several fields including medicinal chemistry (Atkins et al., 2009).

Medicinal Inorganic Chemistry originated in 1908 when Paul Ehrlich introduced the first studies on the structure–activity relationship of arsenic compounds (Atkins et al., 2009; Beraldo et al., 2008). Since then, several studies involving coordination compounds have been carried out aiming at their clinical applications. Essentially, the development of metal complexes for chemotherapeutic purposes involves hydrolysis, protein binding, membrane transportation, and interaction with the molecular target (Schwietert and MCccue, 1999). Many commercially available metal-based drugs have been successfully employed in the clinical setting. Among these drugs, platinum compounds such as cisplatin and its analogs (carboplatin and oxaliplatin) stand out in the treatment of ovarian cancer, head and neck cancer, bladder cancer, cervical cancer, and lymphomas (van Rijt and Sadler, 2009). The cytotoxicity of these compounds

is related to the binding of platinum to a specific site in the DNA of the tumor cell, which triggers a cascade of reactions that culminate in cell death by apoptosis. Despite the wide use of these compounds in cancer therapy, severe side effects such as nausea, bone marrow suppression, and toxicity to the kidneys have emerged in patients (van Rijt and Sadler, 2009). These difficulties have motivated scientists to find different ways to employ metal-based compounds as drugs. One strategy involves applying metal ions that carry ligands with biological activity. Nitric oxide (NO) delivery agents are probably one of the best-known representatives of this class of complexes. Nitrosyl (NO^+) coordination compounds have found therapeutic application and have been the object of many studies since the 19th century (Szczepura and Takeuchi, 1990). Sodium nitroprusside ($Na_2[Fe(CN)_5(NO)]$) is an example of a metal complex that bears a nitrosyl ligand in the metal coordination sphere and can therefore release NO, being a useful compound in blood pressure control (Moncada et al., 1991; Stochel et al., 1998). However, this compound is only applied in medical emergency because it can also release cyanide (CN^-) in a reaction secondary to the reaction of pharmacological interest (Thomas et al., 2009). Researchers have therefore sought to control NO release from coordination compounds and make these complexes clinically feasible. In this sense, Flitney et al. (1996) conducted photochemical experiments with clusters such as $[Fe_4S_4(NO)_4]$ and $[Fe_4S_3(NO)_7]^-$. Photolysis at certain wavelengths promoted NO release. In addition, light induction and electrochemical reduction of coordinated nitrosyl could be interesting approaches that take the low affinity of the NO^0 ligand for some metal ions into account. For this reason, the search for nitrosyl ruthenium complexes capable of releasing NO in the organism via external stimulus has been quite intense. Togniolo et al. (2001) described spectroscopic and photochemical studies of the complex *cis*-$[RuCl(bpy)_2(NO)](PF_6)_2$ and demonstrated NO release in aqueous medium upon irradiation with laser at 355 nm ($\phi_{NO} = 0.98\,mol\,Einstein^{-1}$). Bonaventura et al. (2004) reported the physicochemical and photochemical properties of the complex *trans*-$[RuCl([15]aneN_4)(NO)]Cl_2$ in physiological pH. This ruthenium compound produced NO under reduction or light irradiation at 355 nm. Based on these results, our group has published numerous papers on nitrosyl ruthenium complexes. This chapter will address these publications and shall contribute to the description of the physicochemical and photochemical properties of nitrosyl ruthenium complexes for controlled NO release and their use in biological and physiological processes.

NITROSYL RUTHENIUM COMPLEXES AS NO DELIVERY AGENTS

Basic physical and chemical properties of some NO derivative species

NO is a paramagnetic molecule with an unpaired electron located in an antibonding π-orbital, as shown in Fig. 1.1.

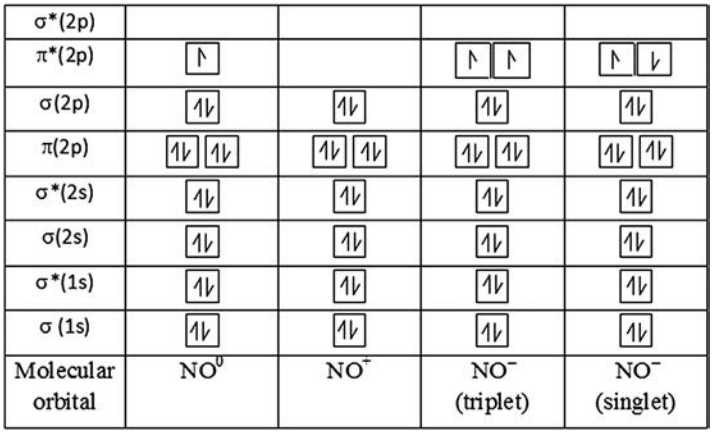


Figure 1.1 Electron configuration of some nitric oxide derivative species.

Scheme 1.1 Dimerization reaction of NO.

In aqueous solution, NO has solubility and diffusion rate of $1.9 \times 10^{-3}\,\text{mol}\,\text{L}^{-1}$ and about $50\,\mu\text{M}\,\text{s}^{-1}$, respectively (Ignarro, 2000; Wink et al., 1996). This colorless gas is stable in its pure form and, despite its radical nature, the formation of a dimer is thermodynamically unfavorable. Indeed, the energy involved (ΔH) in such dimerization (Scheme 1.1) corresponds to about $-2.6\,\text{kcal}\,\text{mol}^{-1}$, and the term $-T\Delta S$ is $+4.3\,\text{kcal}\,\text{mol}^{-1}$ at 1 atm and 300 K, which gives a positive Gibbs free energy ($\Delta G = \Delta H - T\Delta S$) and shows that the reaction does not occur spontaneously under these conditions (Beckman et al., 1996). Low temperatures decrease the term $-T\Delta S$, the free energy becomes negative, and dimerization at lower temperatures is thus spontaneous.

NO tends to react quickly with some transition metals to establish a σ-bond between the metal and either nitrogen or oxygen (Richter-addo and Legzdins, 1992). In some cases and depending on the metal ion, in addition to forming the σ-bond, the d-orbital in the metal may interact with the empty $\pi^{\star}$ orbital in the NO derivative ligand. This interaction elicits back-bonding as in the case of the nitrosyl ligand (NO^{+}) (Fig. 1.2).

NO has rich biochemical diversity as a result of three known forms, namely NO^{+}, NO^{0}, and NO^{-} (nitroxyl), all of which present different physicochemical properties (Table 1.1).

In biological medium, nitrogen oxide derivative species react with oxygen to generate reactive oxygen and nitrogen species (RONS), as described for NO (Scheme 1.2).

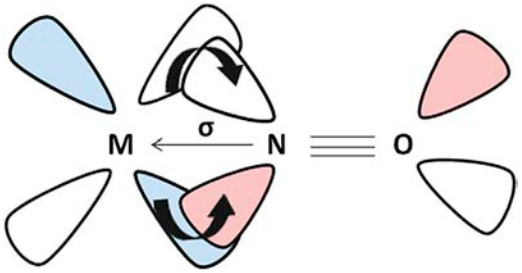


Figure 1.2 The back-bonding representation between metal ion and nitrosyl ligand.

Table 1.1 Bond length, vibrational energy, and reduction potential of free NO^+, NO^0, and NO^- species

	NO^+	NO^0	NO^-
Bond distance N(O) (Å)	1.06	1.15	1.26
$\nu_{(NO)}$ (cm^{-1})	2377	1875	1470
E vs ENH (V)	−1.20[a]	–	−0.39[b]

Source: Hughes (1999).
[a] $NO^{+/0}$.
[b] $NO^{0/-}$.

$$NO^0 + O_2 \rightarrow ONOO^\bullet \quad (1)$$

$$NO^0 + ONOO^\bullet \rightarrow ONOONO \rightarrow 2NO_2 \quad (2)$$

$$2NO_2 + 2NO^0 \rightarrow 2N_2O_3 \quad (3)$$

$$2N_2O_3 + H_2O \rightarrow 4NO_2^- + 4H^+ \quad (4)$$

$$4NO^0 + O_2 + 2H_2O \rightarrow 4NO_2^- + 4H^+ \quad (5)$$

Scheme 1.2 NO oxidation reaction steps originating RONS (Ignarro, 2000).

Although NO^+, NO^0, and NO^- all have therapeutic implications (Shoman and Aly, 2016; Cheung et al., 2000), this chapter will focus on the biological mechanism related to NO delivery agents like $[Ru^{II}N_4L'NO^+]^{n+}$, paying special attention to the effect of L on the Ru-(N). This chapter will also consider biological properties of these complexes such as vasodilation, trypanocidal action, and cytotoxicity activities.

RUTHENIUM-NITROGEN OXIDE DERIVATIVES AS NO SOURCES

Several ruthenium-nitrogen oxide derivative compounds have been described as NO delivery agents (de Lima et al., 2014). Table 1.2 contains compounds that represent these agents and which will be the basis of this chapter. The physicochemical and photochemical characteristics of these compounds depend mainly on the interaction between Ru(II) and the ligands "L" in $[RuN_4L'NO_x]^{n+}$ as well as on the strength of the bond established between the metal ion and the nitrogen oxide derivative ligand. In general, all the ruthenium compounds discussed here are stable both in the solid state and in aqueous solution.

NITROSYL RUTHENIUM COMPLEXES AS NO DELIVERY AGENT BY REDUCTION PROCESS

Nitrosyl ruthenium complexes of the $[Ru^{II}N_4L'NO^+]^{n+}$ type are stable as complex salt but undergo pH-dependent electrophilic attack by hydroxide ion in aqueous solution to generate nitroruthenium(II) species (Sauaia and da Silva, 2003a) (Scheme 1.3).

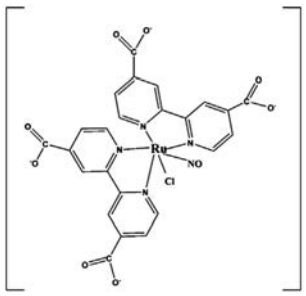
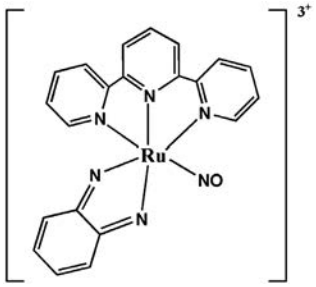
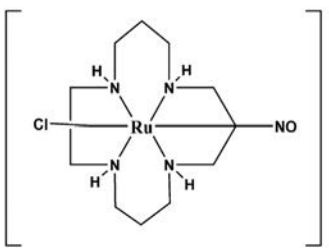
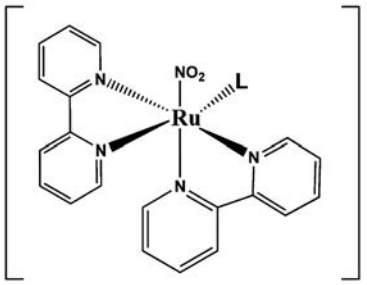
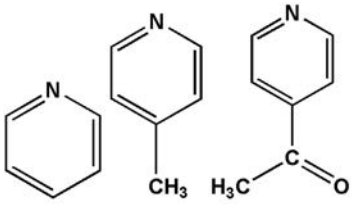
Systematic tuning of the electrophilic character of coordinated NO^+ through modulation of the ancillary ligand "L" in $[RuN_4L'NO^+]^{n+}$ is possible (Cândido et al., 2015; Sauaia and da Silva, 2003a). The equilibrium constant for the conversion of nitrosyl species into nitro species depends on the π-acceptor character of ligand "L," and this rate constant decreases with increasing electron density in the nitrosyl ligand. On the steady state pK_{NO} affords as a result of this electrophilic attack. Fig. 1.3 shows the effect of pH on UV–visible spectrum of *cis*-$[Ru(bpy)_2(py)(NO)]^{3+}$ as representative of nitrosyl ruthenium(II) compounds. The pK_{NO} may help to describe the molecular formula of ruthenium-NO derivative species as a function of pH ($\{Ru^{II}\text{-}NO^+\}^{3+}$ or $\{Ru^{II}\text{-}NO_2^-\}^+$), as observed in Fig. 1.4 and Table 1.3 for some $[Ru^{II}N_4L'NO^+]^{n+}$ compounds.

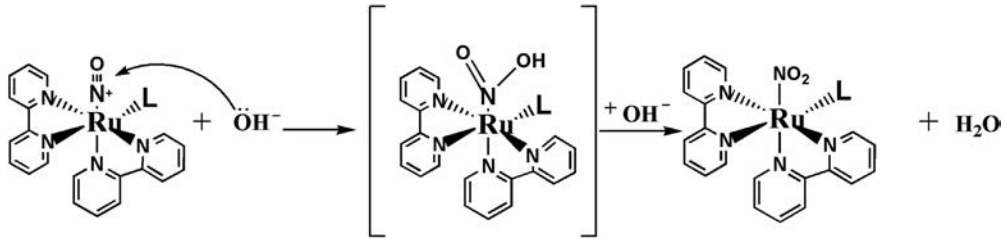
The electrochemical reduction of $\{Ru^{II}\text{-}NO^+\}$ compounds is fascinating. Fig. 1.5 brings representative cyclic voltammograms (CV) of $[Ru^{II}N_4L'NO^+]^{n+}$ species. The CV of *cis*-$[Ru(bpy)_2(pz)(NO)]^{3+}$ in nonaqueous or aqueous medium showed two cathodic and anodic peaks namely "1" and "2" attributed to $NO^{+/0}$ and $NO^{0/-}$ process, respectively.

In aqueous solution, the reduction process is followed by chemical reaction centered on the NO derivative species. Fig. 1.6 depicts the controlled potential reduction of nitrosyl ruthenium compounds monitored by spectroelectrochemistry; spectral changes are evident.

The first electrolytic potential reduction conducted in the presence of the NO-sensor raises the peak current, which is consistent with increasing NO concentration (Fig. 1.6). These studies aid description of the electrochemical mechanism related to the $[Ru^{II}N_4L'NO^+]^{n+}$ species (Scheme 1.4). This mechanism leads to the assumption that nitrosyl ruthenium complexes release NO after the first reduction step.

Table 1.2 Chemical structure of some ruthenium-nitrogen oxide derivative species

Chemical structure	Chemical formula
	*cis*-[RuCl(H-dcbpy)$_2$(NO)]
	[Ru(bqdi)(terpy)(NO)]$^{3+}$
	*trans*-[RuCl([15]-ane$_4$)(NO)]$^{2+}$
  **L_1** **L_2** **L_3**	*cis*-[Ru(NO$_2$)L(bpy)$_2$]$^+$ **L_1 =** pyridine **L_2 =** 4-picoline **L_3 =** 4-acetylpyridine



Scheme 1.3 Representative reaction of nitrosyl ruthenium complex with hydroxide ion.

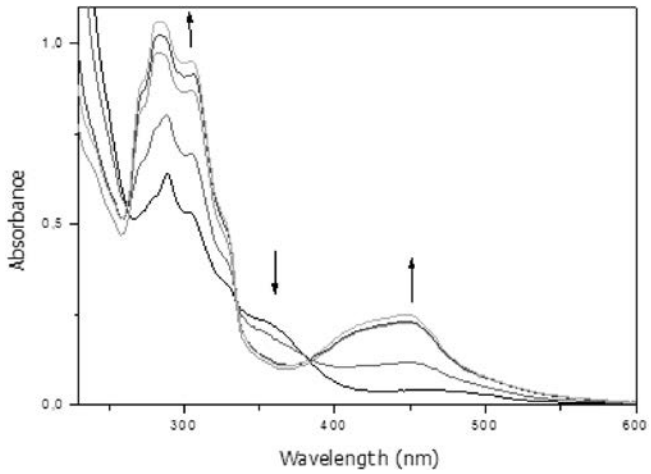


Figure 1.3 Spectral profile change with pH for the conversion of $[Ru(bpy)(terpy)(NO)]^{3+}$ in $[Ru(NO_2)(bpy)(terpy)]^{+}$. pH = 2.48, 4.30, 5.72, 7.16, and 10.29.

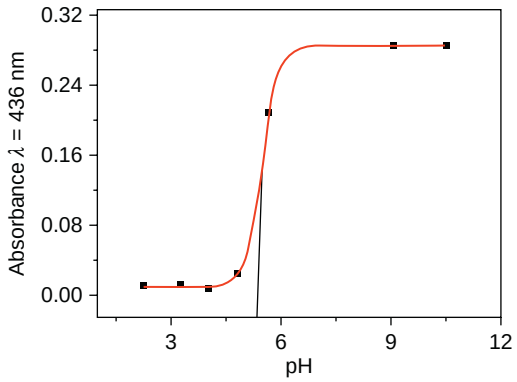


Figure 1.4 Effect of pH on the metal ligand charge transfer band of nitro ruthenium(II) species $[Ru(bpy)_2(2\text{-}tFmepy)(NO)]^{+}$.

Table 1.3 pK_{NO} values for some nitrosyl ruthenium compounds

Compounds	pK_{NO}
$[Ru(bpy)_2(4\text{-pic})(NO)]^{3+}$	3.30
$[Ru(bpy)_2(py)(NO)]^{3+}$	3.40
$[Ru(bpy)_2(4\text{-acpy})(NO)]^{3+}$	3.80
$Ru(bdqi\text{-}COOH)(terpy)(NO)]^{3+}$	4.30
$[Ru)(bpy)_2(2\text{-pic})(NO]^{3+}$	5.47
$[Ru(bpy)_2(2\text{-tFmepy})(NO)]^{3+}$	5.75
$[Ru(bpy)_2(2\text{-me-}4\text{-ampy})(NO)]^{3+}$	6.03

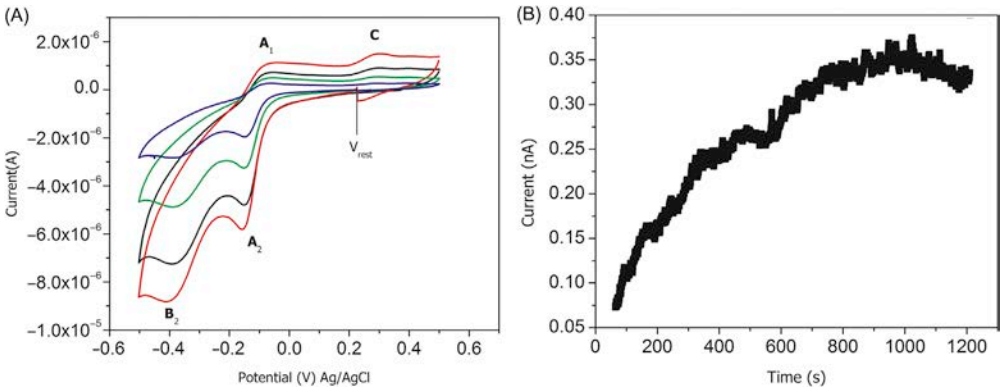


Figure 1.5 Cyclic voltammogram (A) and chronoamperogram (B) of $[Ru(terpy)(py\text{-}SH)_2(NO)]^{3+}$ complex in buffer solution (pH = 2.03) with KCl (0.10 mol cm^{-3}) added as support electrolyte. ν = 200, 100, 50, and 20 mV s^{-1}.

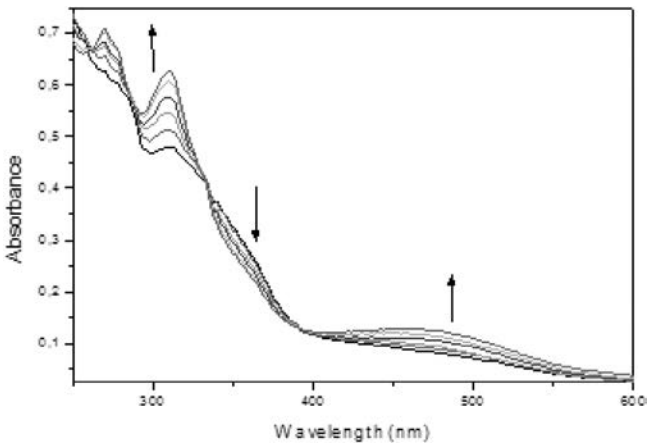
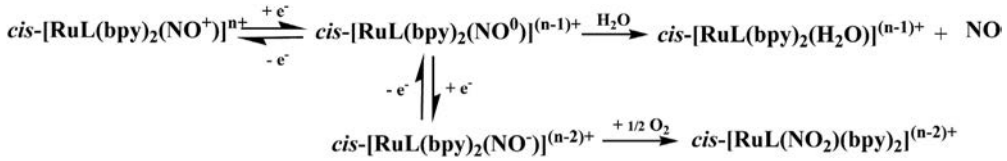


Figure 1.6 Spectral profile change with time of $[Ru(bdqi)(terpy)(NO)]^{3+}$ complex in buffer solution (pH = 4.50) after electrolysis at −0.30 V versus Ag/AgCl.



Scheme 1.4 The electrochemical reduction mechanism of *cis*-[RuL(bpy)$_2$(NO)]$^{n+}$ complex with nitric oxide release.

Synergistic effect between ruthenium complex and nitric oxide as a tool to increase cytotoxicity

We have investigated the cytotoxicity activity of several nitrosyl ruthenium species (Maranho et al., 2009). One of the example is [Ru(bdqi)(terpy)(NO)]Cl$_3$ as NO delivery agent and the effect of this complex on the melanoma cancer B16F10 cell line in aqueous solution as well as in solid lipid nanoparticles (SLN). The high affinity of ruthenium for NO is a marked feature of ruthenium chemistry. To understand the effect of NO as anticancer agent better, we have also evaluated the effect of [Ru(bdqi)(terpy)(H$_2$O)]$^{2+}$ complex from a cytotoxic point of view. Fig. 1.7 illustrates the cell viability results obtained for these compounds in B16F10 cell line.

Only the complex [Ru(bdqi)(terpy)(H$_2$O)]$^{2+}$ at very high concentration (250 μM) and after 48 hours of incubation exerts significant cytotoxicity. These results point to the dual action of [Ru(bdqi)(terpy)(NO)]$^{3+}$—NO release and formation of aquaruthenium(II) species. Intracellular reduction of [Ru(bdqi)(terpy)(NO)]$^{3+}$ should underlie NO release as judged by cytosolic NO concentration measurements (Fig. 1.8).

The intracellular NO release from [Ru(bdqi)(terpy)(NO)]$^{3+}$ gives rise to the formation of [Ru(bdqi)(terpy)(H$_2$O)]$^{2+}$. Considering the described cytotoxicity of NO (Heinrich, 2013), decreased cell viability for [Ru(bdqi)(terpy)(NO)]$^{3+}$ should be expected. Based on the data from Fig. 1.7, it was not observed. It may be due to cellular uptake of nitrosyl ruthenium complex once encapsulation of [Ru(bdqi)(terpy)(NO)]Cl$_3$ into SLN increases cytotoxicity considerably (Fig. 1.9; Table 1.3).

In vitro cellular death with different concentrations of the nitrosyl ruthenium complex in B16F10 cancer cell line can be observed in Table 1.4. Clearly the SLN improve cellular uptake of ruthenium complex, which is followed by change on the biochemical parameters proportioned by intracellular chemical reactions with [Ru(bdqi)(terpy)(NO)]Cl$_3$. The biochemical pathway is under investigation but apparently one of the imminent process involves the DNA interaction with [Ru(bdqi)(terpy)(H$_2$O)]$^{2+}$. It may guide discovering of new cancer drugs based on synergistic effect of NO and ruthenium compounds.

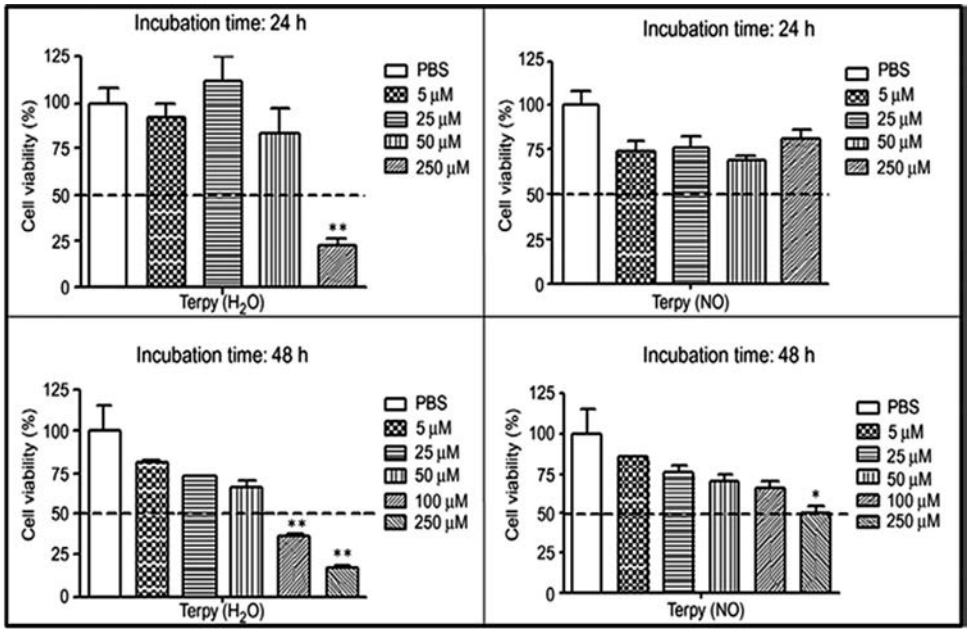


Figure 1.7 Comparation between $[Ru(bdqi)(terpy)(NO)]Cl_3$ and $[Ru(H_2O)(bdqi)(terpy)]^{2+}$ complexes in murine melanoma cells in different concentrations and incubation time. $^*p < 0.05$ and $^{**}p < 0.01$, different from control (Heinrich, 2013).

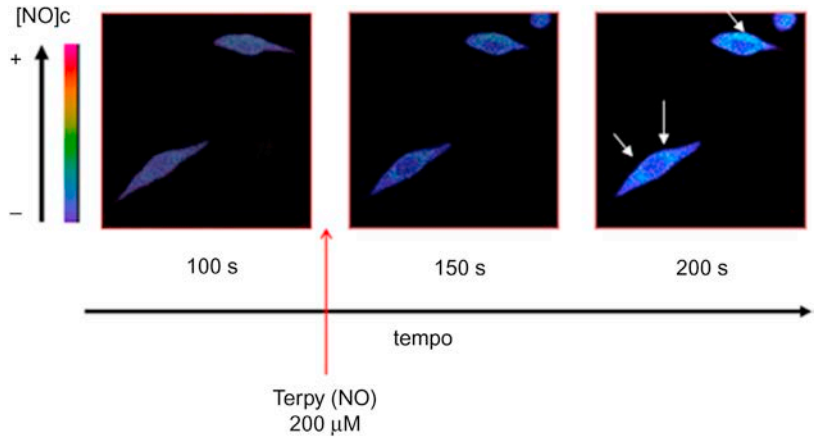


Figure 1.8 Cytosolic concentration of nitric oxide in murine melanoma cells (B16F10), using a fluorescent probe, DAF-2 DA (5 μM), after NO release from $[Ru(bdqi)(terpy)(NO)]^{3+}$ complex (Heinrich, 2013).

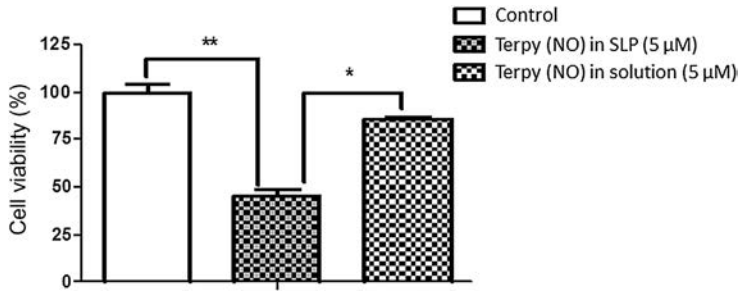


Figure 1.9 Cell viability in B16F10 cell of $[Ru(bdqi)(terpy)(NO)]Cl_3$ complex in aquo solution and $[Ru(bdqi)(terpy)(NO)]Cl_3$ encapsulated in solid lipid nanoparticles. *$p < 0.05$ and **$p < 0.01$, different from control (Heinrich, 2013).

Table 1.4 Cell viability (%) in murine melanoma cells (B16F10) of $[Ru(bdqi)(terpy)(NO)]Cl_3$ and $[Ru(bdqi)(terpy)(H_2O)]^{2+}$ complexes in solution and encapsulated in SLP in different concentration and incubation time (Heinrich, 2013)

% Cell viability in B16F10 cell line

$[Ru^{II}L_4L'NO^+]^{n+}$	$[Ru(bdqi)(terpy)(H_2O)]^{2+}$			$[Ru(bdqi)(terpy)(NO)]^{3+}$			$[Ru(bdqi)(terpy)(NO)]^{3+}$ encapsulated in SLN
Concentration (μM)	**4 hours**	**24 hours**	**48 hours**	**4 hours**	**24 hours**	**48 hours**	**4 hours**
5	100	100	80	100	75	86	45
25	110	110	75	110	75	75	–
50	100	100	70	100	75	70	–
100	–	–	30	–	–	65	–
250	85	25	20	100	75	50	–

PHOTO-NITRIC OXIDE RELEASE PROMOTED BY RUTHENIUM COMPLEXES

NO plays a critical role in biological medium: it can act for example as a vasorelaxant agent in blood vessels (Marchesi et al., 2012). The concentration–relaxation dependent (pD_2) and the maximum relaxant effect (E_{max}) depend on the molecular structure of the vasorelaxant agent. The NO derivative ligand bound to ruthenium also induces this process in isolated vessels of normotensive and hypertensive rats by producing NO after stimulation (Table 1.5).

Table 1.5 The vasorelaxation induced by nitric oxide from nitrosyl ruthenium complexes donors (Lunardi et al., 2009)

Complex $[Ru^{II}L_4L'NO^+]^{n+}$	pD_2	E_{max} (%)
trans-$[RuCl([15]\text{-}ane_4)(NO)]^{2+}$	5.03 ± 0.2	98.35 ± 1.22
$[Ru(bdqi)(terpy)(NO)]^{3+}$	6.47 ± 0.13	102.38 ± 0.38
cis-$[RuL(bpy)_2(NO)]^{n+}$	6.62 ± 0.12	101.2 ± 3.7

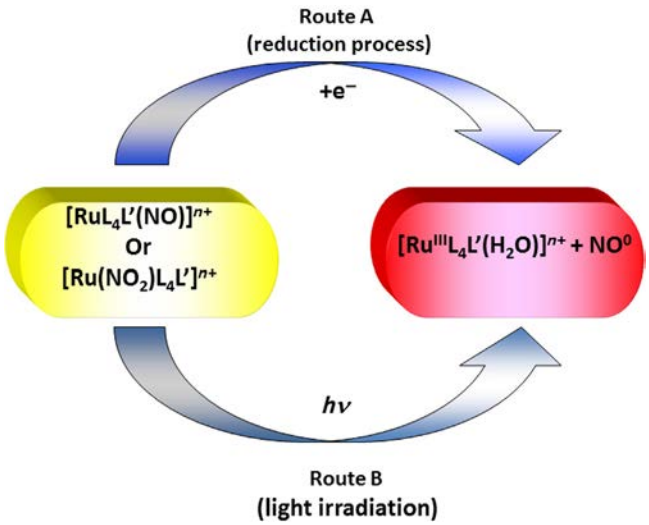


Figure 1.10 Ruthenium complexes as NO deliver agents.

Two mechanisms explain NO release from these ruthenium species (Fig. 1.10).

In route "a," the nitrosyl ruthenium complex releases NO by reduction in complexes of the $[RuL_4L'NO^+]^{n+}$ type or by oxygen transfer reaction in complexes of the $[Ru(NO_2)L_4L']^{n+}$ type. Fig. 1.11 summarizes the extensively explored pharmacologic mechanism of NO during vasorelaxation (de Lima et al., 2014).

Essentially, NO activates guanylate cyclase, which mediates vasorelaxation. The literature presents discussions involving vasodilation by reduction of nitrosyl ruthenium complexes (da Silva et al., 2015). Photo-vasodilation employing those species is less common and will be the focus of this discussion. As described in Fig. 1.10, route "b," aqueous nitrogen oxide derivative bound to ruthenium produces NO under light irradiation. All the studied nitrosyl ruthenium species release NO by excitation of the metal ligand charge transfer band (MLCT) centered on $d\pi(Ru^{II})–\pi^\star(NO)$, which generally emerges in the ultraviolet region (de Lima et al., 2006). The photochemical

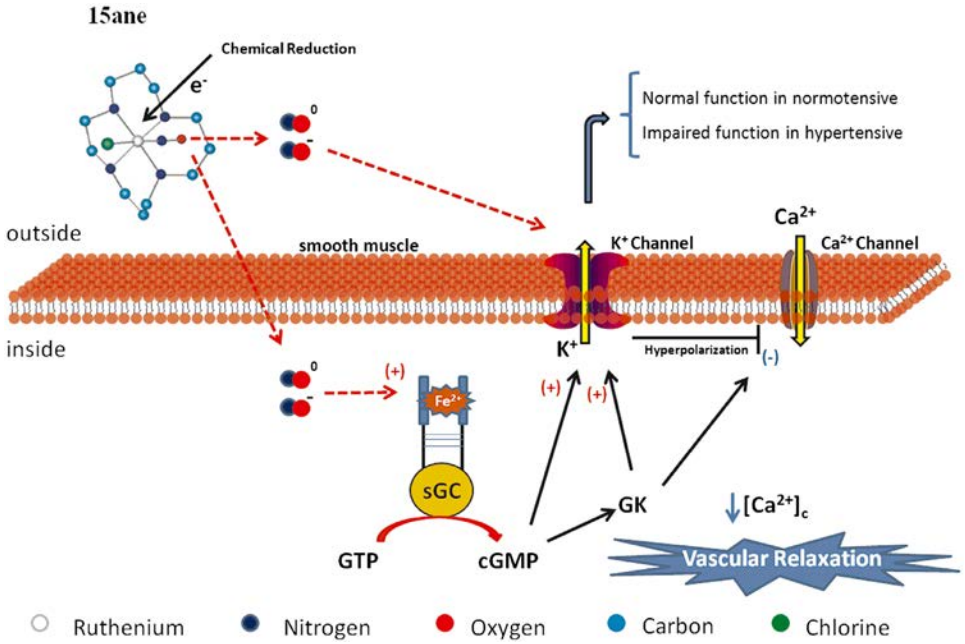


Figure 1.11 Proposed NO release and cellular mechanisms involved in the vasodilation promoted by *trans*-[RuCl(15-aneN_4)(NO)]$^+$ (15ane). The colored circles represent atoms in the chemical structure. The hydrogen atom has been omitted in the structure. sGC, soluble guanylyl cyclase; GK, G Kinase Protein; Ca^{2+}, calcium; K^+, potassium; $[Ca^{2+}]_c$, cytosolic calcium concentration. *Reproduced with permission from de Lima, R.G., Silva, B.R., da Silva, R.S., Bendhack, L.M., 2014. Ruthenium complexes as NO donors for vascular relaxation induction. Molecules 19, 9628–9654, MDPI Publishing Group.*

$$[Ru^{II}L_4L'(NO^+)]^{n+} \xrightarrow{h\nu} [Ru^{III}L_4L'(NO^0)]^{n+}$$

$$[Ru^{III}L_4L'(NO^0)]^{n+} + H_2O \longrightarrow [Ru^{III}L_4L'(H_2O)]^{n+} + NO$$

Scheme 1.5 The photochemical pathway of NO release from ruthenium complexes by UV light irradiation.

pathway described for these systems includes the formation of $[Ru^{III}L_4L'(H_2O)]^{n+}$, as shown in Scheme 1.5 (Sauaia et al., 2003c).

Nitrosyl ruthenium complexes rarely display bands in the visible region, which would result from contribution of the molecular orbital of NO^+. Photostimulation of those compounds has been providing be useful vasodilator agents for in vitro analysis, although there is very little possibility to apply this knowledge for in vivo assays due dangerous use of UV light. In this way, we have succeeded to produce ruthenium complex, which presents bands with strong absorption on visible region. The complex [Ru(phthalocyanine)(NO_2)(NO)] is a representative species of such systems.

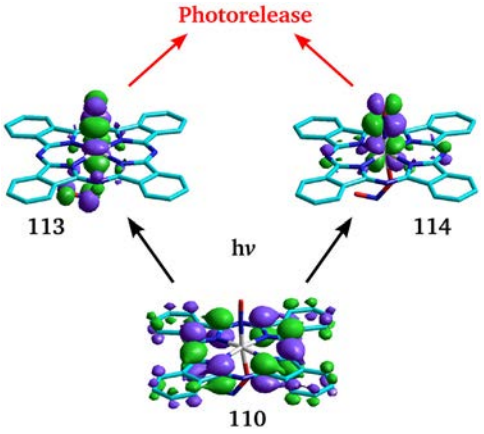
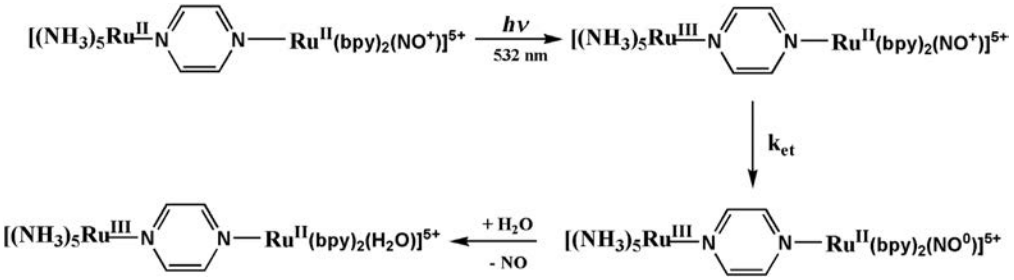


Figure 1.12 Optimized geometry for the B and Q transitions of [Ru(Pc)(ONO)(NO)] calculated by DFT and ZINDO/S. *Reproduced with permission from Carneiro, Z.A., de Moraes, J.C.B., Rodrigues, F.P., de Lima, R.G., Curti, C., da Rocha, Z.N., et al., 2011. Photocytotoxic activity of a nitrosyl phthalocyanine ruthenium complex—a system capable of producing nitric oxide and singlet oxygen. J. Inorg. Biochem. 105, 1035–1043, Elsevier Publishing Group.*



Scheme 1.6 Photo-induced electron transfer in ruthenium complexes to induce NO release (Sauaia et al., 2003b).

In aqueous solution, photolysis of this complex upon irradiation with light at 660 nm culminates in NO release. According to molecular orbital calculation (Fig. 1.12), the $\pi^{\star}$ orbital contributes to the transition centered in this region.

A similar mechanism occurs for some ruthenium compounds in which an antenna is responsible for the light absorption process. Photo-induced electron transfer is claimed to induce NO release (Scheme 1.6).

The binuclear ruthenium system illustrates how this system works for nitrosyl ruthenium compounds (Fig. 1.13) (Sauaia et al., 2003b).

The phthalocyanine–ruthenium compound as well as the binuclear ruthenium species have been proved to be useful tools as vasorelaxant agents by visible light irradiation (Carneiro et al., 2011). More recently, we have found be unnecessary have a covalent bond for photo-induced electron transfer between two ruthenium moieties.