1521-0103/380/1/47–53$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

https://doi.org/10.1124/jpet.121.000798
J Pharmacol Exp Ther 380:47–53, January 2022

The Journal of PHARMACOLOGY And Experimental Therapeutics

Minireview

The Role of Ruthenium Compounds in Neurologic Diseases: A Minireview

Fátima Virgínia Gama Justi, Gabriella Araújo Matos, Juan de Sá Roriz Caminha, Cássia Rodrigues Roque, Edinilton Muniz Carvalho, Márcio Wilker Soares Campelo, Ludmila Belayev, Luiz Gonzaga de França Lopes, and Reinaldo Barreto Oriá

Department of Morphology and the Institute of Biomedicine, School of Medicine (F.V.G.J., G.A.M., J.d.S.R.C., C.R.R., R.B.O.) and Department of Organic and Inorganic Chemistry (E.M.C., L.G.d.F.L.), Federal University of Ceará, Fortaleza, Brazil; Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, Louisiana (L.B.); and Laboratory of Experimental Vascular Surgery and Gene Expression, School of Medicine, Christus University Center (UNICHRISTUS), Fortaleza, Brazil (M.W.S.C.)

Received June 17, 2021; accepted October 25, 2021

ABSTRACT

Ruthenium compounds, nitric oxide donors in biologic systems, have emerged as a promising therapeutic alternative to conventional drugs in anticancer chemotherapy and as a potential neuroprotective agent with fewer cytotoxic effects. This minireview summarizes promising studies with ruthenium complexes and their roles in cancer, neuroinflammation, neurovascular, and neurodegenerative diseases. The up-to-date evidence supports that ruthenium-based compounds have beneficial effects against gliomas and other types of brain cancers, reduce motor symptoms in models of cerebral ischemia-reperfusion, and may act in the control of nociceptive and inflammatory events, such as those seen in early Alzheimer's disease. More studies are needed to fill many current knowledge gaps about the intricate and complex biologic effects and therapeutic-related mechanisms of ruthenium, stimulating further research.

SIGNIFICANCE STATEMENT

This minireview summarizes studies addressing the role of ruthenium compounds on neurological illnesses, focusing on brain cancer and neurovascular and neurodegenerative diseases. No such review is available in the literature.

Introduction

Many metal ions (e.g., copper, zinc, iron, and manganese) play a crucial role in various biologic processes, potentially influencing medical treatment (Arnesano and Natile, 2009). Preparations containing metal complexes have been used historically in clinical medicine, and over the past five decades, they have attracted the pharmaceutical industry's attention in the relentless search for new therapeutic approaches against cancer and neurologic diseases (Pricker, 1996; Thota et al., 2018; Englinger et al., 2019). These worrisome chronic illnesses are expected to increase dramatically with aging populations worldwide (Nolen et al., 2017; GBD 2016 Neurology Collaborators, 2019). One of the eldest and best known metallodrug-therapeutic approaches is platinum-based anticancer therapy with cisplatin or platinol (*cis*-$[Pt(NH_3)_2Cl_2]$), with antitumoral effects first reported by a pioneer study of Barnett Rosenberg and Loretta VanCamp in 1965 (Arnesano and Natile, 2009).

Several other metallodrugs have been used in empirical medicine throughout history, e.g., auranofin (based in gold) used in the treatment of rheumatoid arthritis, trisenox (based in arsenic) used in the treatment of acute promyelocytic leukemia, and sodium nitroprusside (based in iron) used in cardiovascular surgery and hypertensive emergency (Pricker, 1996). The suboptimal activity and

This study was supported by the National Council for Scientific and Technological Development (CNPq) [Projeto Universal n° 406585/2016-4] and Coordination for the Improvement of Higher Education Personnel (Capes) [CAPES PrInt n°: 88887.311938/2018-00].
dx.doi.org/10.1124/jpet.121.000798.

ABBREVIATIONS: Aβ, β-amyloid; AD, Alzheimer's disease; 8-HQ, 8-hydroxyquinoline; KP1019, indazolium *trans*-tetrachlorobis(1*H*-indazole) ruthenate(III); MCU, mitochondrial calcium uniporter; NAMI-A, imidazolium(imidazole)(dimethylsulfoxide)tetrachlororuthenate(III); NO, nitric oxide; PDT, photodynamic therapy; PrP, prion protein; ROS, reactive oxygen species; RR, ruthenium red; Ru, ruthenium; 106Ru, ruthenium-106 plaque radiotherapy; RuBi-GABA, ruthenium-bipyridine-trimethylphosphine gamma aminobutyric acid; RuBi-Glu, ruthenium-bipyridine-trimethylphosphine glutamate; Rut-bpy, ruthenium nitrosyl complex *cis*-$[Ru(bpy)_2(SO_3)(NO)]PF_6$; TLD1433, [Ru(II)(4,4′-dimethyl-2,2′-bipyridine(dmb))2(2-(2′,2″:5″,2‴-terthiophene)-imidazo[4,5-f][1,10]phe-nanthroline)]Cl_2; TQ-6, Ru(η6-cymene)2-(1H-benzoimidazol-2-yl)-quinoline Cl]BF4; TRPV, transient receptor potential cation channel vanilloid subfamily.

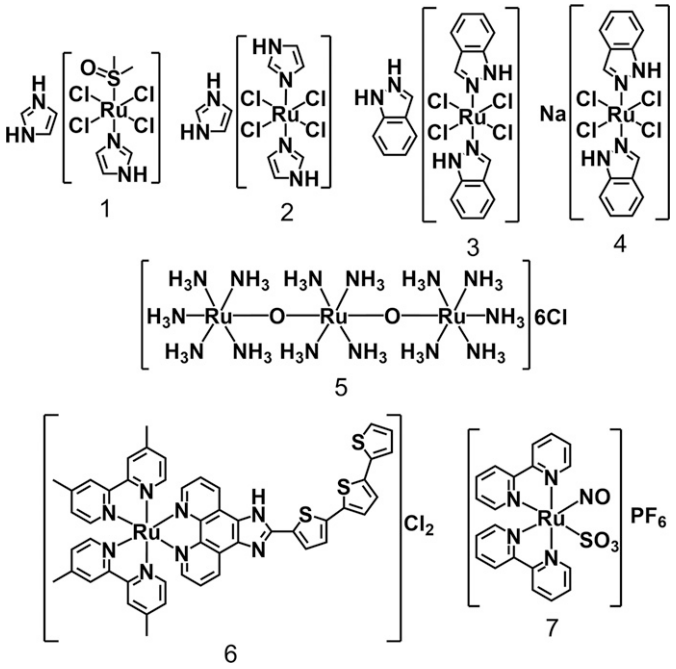

Fig. 1. Structures of ruthenium compounds studied for pharmacological purposes: NAMI-A (1) and KP418 (2) (imidazole-ruthenium complexes derivatives—anticancer activity), KP1019 (3) and KP1339 (4) (indazole-ruthenium complexes derivatives—anticancer activity), RR (5) (amine-ruthenium complex—calcium mitochondrial uniporter blocker), TLD1433 (6) (ruthenium polypyridine complex—treatment of nonmuscle invasive bladder cancer via photodynamic therapy, and [Ru(bpy)2(SO3)(NO)](PF6) (7) (ruthenium nitrosyl complex—vasodilation activity).

side effects of many cisplatinum compounds have stimulated the search for other anticancer therapies, among which are ruthenium (Ru) complexes.

The idea of using ruthenium compounds as pharmacological agents emerged from significant scientific breakthroughs between 1975 and 1985, such as the discoveries of their chemical activation by reduction (Clarke et al., 1980; Frasca et al., 1996), their ability to be delivered to cancer cells by transferrin, and their different forms of DNA binding, as compared with cisplatin (Som et al., 1983; Brabec, 2002; Brabec and Nováková, 2006). Such findings can be explained in part by some physicochemical and biological properties of ruthenium compounds [(Ru(II), Ru(III), and Ru(IV) are the most used in biologic conditions], including the rate of ligand exchange, the possibility of changing in oxidation state, the ability to mimic iron in binding biologic molecules, and low toxicity to normal cells.

Imidazolium(imidazole)(dimethylsulfoxide)tetrachlororuthenate(III) (NAMI-A) (Fig. 1) was the first ruthenium complex tested in clinical trials. However, in phase II studies, it showed limited efficacy, resulting in a halt in clinical development (Thota et al., 2018). Indazolium *trans*-tetrachlorobis(1*H*-indazole) ruthenate(III) (KP1019) (Fig. 1) was another ruthenium derivative that entered phase I clinical trial; however, its low solubility limited its progress in the later stages. Nevertheless, its sodium salt derivative, NKP1339 (Fig. 1), has advanced in clinical trials (ClinicalTrials.gov identifier: NCT01415297) (Thota et al., 2018).

Another example is [Ru(II)(4,4′-dimethyl-2,2′-bipyridine-(dmb))2(2-(2′,2″:5″,2‴-terthiophene)-imidazo[4,5-f][1,10]-phe-nanthroline)]Cl_2 (TLD1433) (Fig. 1), a ruthenium-based complex, currently in clinical trials (phase I/II, for treatment of nonmuscle invasive bladder cancer via photodynamic therapy; ClinicalTrials.gov identifier: NCT03945162) (Imberti et al., 2020).

In the last decade, there has been a substantial increase in research involving ruthenium-based substances (Dragutan et al., 2015). Several publications highlight the significant advances of ruthenium-based complexes and their chemical-biologic applications in medicine, catalysis, nanoscience, redox, and photoactive materials (Thota et al., 2018; de Sousa et al., 2017). The main properties that make ruthenium complexes a valuable and versatile platform in biology are charge variation, metal-ligand interaction, different coordination geometries, Lewis acid properties, partially filled d-shell, and redox activity (Haas and Franz, 2009). Another advantage of Ru complexes is the possibility to include them in some nanomaterials, which may benefit anticancer therapy (Englinger et al., 2019; Zhu et al., 2018).

Ruthenium-based complexes have stood out in different therapeutic areas. In addition to their potential use as anticancer agents, ruthenium complexes have also shown promising results in the field of neurology, acting as neuroprotective agents (Campelo et al., 2012).

In this minireview, we summarize studies addressing the role of ruthenium compounds on neurologic illnesses, focusing on brain cancer and neurovascular and neurodegenerative diseases.

Ruthenium and Neuro-Oncology

Cell Biology. The use of polypyridine Ru(II) complexes as photosensitizers in the photodynamic therapy (PDT) technique is well established since through its binding with albumin and/or transferrin in plasma serum it can be easily transported into cancer cells through receptors (Imberti et al., 2020; Abreu et al., 2017; Kaspler et al., 2016).

The polypyridyl Ru complex with taurine ligand has been shown to have intracellular affinity in cancer cells and a great capacity for reactive oxygen species (ROS) production, making it an effective photosensitizer for treating brain cancer. The photosensitizer, which contains a source of light and tissue oxygen, is one of the components in the PDT used for some types of brain tumors, including glioblastoma, specifically stimulating ROS production, leading to the death of the target cells (Du et al., 2017).

Therapeutic Approach. Some forms of ruthenium are used in radiotherapy devices to treat cancer, inducing a death signal to neoplastic cells or as a radioactive source for reducing and even eliminating tumors. Ruthenium-106 plaque radiotherapy (106Ru) is a variation of brachytherapy (treatment based on placing a plaque with a concave surface from a radioactive source close to or next to the tumor), widely used in small intraocular tumors (up to 6 mm), such as diffuse choroidal hemangioma associated with Sturge-Weber syndrome (Cho et al., 2018). A study of 20 patients with diffuse choroidal hemangioma associated with Sturge-Weber Syndrome treated with 106Ru plates resulted in tumor regression and resolution of serous retinal detachments; in several cases, there was a return of visual stability (Kubicka-Trząska et al., 2015).

Ruthenium-based nanomaterials are also being developed, and their chemotherapy effects are continually being explored. A recent study carried out with the mesoporous ruthenium nanosystem RBT@ MRN-SS-Tf/Ap demonstrated the therapeutic potential of this system against gliomas. The study reported the capacity of this nanosystem to cross the

blood-brain barrier reaching the target cells when activated by light (808 nm laser irradiation), generating ROS. This mechanism was responsible for the observed antitumor effect. Such a study reveals a promising strategy in PDT for brain cancer (Zhu et al., 2018).

Gliomas and glioblastomas are aggressive tumors with autophagic characteristics and high drug resistance. A comparison made between a combination of ruthenecarborane derivative plus 8-hydroxyquinoline (8-HQ) linked by ester bond and these same compounds alone (free carboxylic acid and 8-HQ), in mouse astrocytoma C6 cells and U251 human glioma, showed promising results by inhibiting the autophagy mechanism of U251 glioma cells, as well as making them unfeasible even under conditions of glucose deprivation (where 8-HQ loses activity) (Drača et al., 2021).

Ruthenium Complexes in Neurovascular Diseases

Cell Biology. The antioxidant potential of ruthenium complexes and their vasodilation properties have been recognized in the literature. Such effects may be potentially beneficial to improve the treatment of neurovascular diseases, especially hypertension-related morbidities. Several studies carried out with ruthenium red (RR) confirmed a blocking effect to different calcium channels, which is important to reduce cerebral ischemia-reperfusion–related tissue injury. The pathophysiology of this process is dependent on a massive release of intracellular Ca^{2+} and unbalanced calcium cell metabolism, which ultimately leads to neuronal cell death (Hamilton and Lundy, 1995; Scorza et al., 2020).

RR blocks the intracellular ryanodine receptor in the sarcoplasmic reticulum, thus inhibiting the calcium-induced calcium release. It also contributes to the reduction of mitochondrial fission by reducing the expression of dinamine-related protein 1 and by blocking mitochondrial calcium uniporter (MCU) located in the mitochondria (Saklani et al., 2010). MCU block reduces early brain damage after subarachnoid hemorrhage (Liang et al., 2014; Tonin et al., 2014; Yan et al., 2015).

In addition, RR favors reducing the volume of the infarction area and improves the scavenging of ROS, released in various types of oxidative stress–related tissue injuries (Sun et al., 2013). RR also induces less pronounced mitochondrial respiratory complex dysfunction with preserved ATP production, crucial in cellular processes that require energy expenditure, diminishing the deleterious effects seen with nervous myelin sheath disruption in ischemia-reperfusion injury, by blocking the transient receptor potential cation channel vanilloid subfamily (TRPV) members 1 and 4 (Hamilton et al., 2016). Finally, RR has been proposed to significantly reduce transient focal cerebral ischemia-reperfusion–related motor symptoms by inhibiting modulator 1 of calcium homeostasis channel in mice (Cisneros-Mejorado et al., 2018).

In previous studies carried out with ruthenium nitrosyl complex *cis*-$[Ru(bpy)_2(SO_3)(NO)]PF_6$ (Rut-bpy) (Fig. 2), this ruthenium compound was found to induce a pronounced relaxant effect in the rabbit corpus cavernosum and aortic vascular smooth muscle due to the release of intracellular nitric oxide (NO) and soluble guanylate cyclase activation (Cerqueira et al., 2008). Rats that suffered cerebral ischemia-reperfusion, when preconditioned with Rut-byp, showed a decrease in total cerebral infarction area and improved hippocampal neuronal viability in an initial phase of ischemia-reperfusion (Campelo et al., 2012).

Rut-byp may show a neuroprotective effect primarily by inhibiting nuclear factor kappa B signaling. This transcription factor regulates a downstream proinflammatory cytokine cascade and stabilizes blood pressure in the transition from ischemia to reperfusion. Thus, Rut-bpy may be a strong candidate for future clinical studies to treat cerebrovascular diseases (Campelo et al., 2012).

Recently, Ru(η6-cymene)2-(1H-benzoimidazol-2-yl)-quinoline Cl]BF4 (TQ-6), a ruthenium(II) complex, was found to reduce microglial activation in a model of focal brain ischemia-reperfusion in mice and to improve platelet activation. TQ-6 was able to diminish inducible nitric oxide synthase and cyclooxygenase 2 expression, diminish nuclear factor kappa B p65 phosphorylation, and reduce oxidative stress in lipopolysaccharide-activated microglia (Chih-Hsuan Hsia et al., 2020).

Ruthenium Compounds in Neuropathic Pain

Cell Biology. RR has been shown to reverse neural-related side effects induced by the antineoplastic paclitaxel (Taxol) in Wistar rats, presumably by reducing the activation of TRPV1 receptors. Paclitaxel increases the expression of TRPV1 receptors in the dorsal ganglion root, causally linked to the mechanisms of thermal hyperalgesia. RR being a nonselective antagonist of transient receptor potential receptors, when administered in a single dose of 3 mg/kg s.c. after 14 days from the start of treatment with paclitaxel, has been shown to significantly inhibit thermal hyperalgesia, assessed by the tail flick test (Hara et al., 2013).

Chiba and coworkers used RR in the same dose (3 mg/kg s.c.) to treat the dose-dependent vincristine-induced neuropathy (Chiba et al., 2017). Vincristine, a vinca alkaloid antineoplastic compound, acts similarly to paclitaxel, causing upregulation of TRPV1 receptors but differing in inducing allodynia and mechanical hyperalgesia evaluated by the Von Frey test. Qu and coworkers indicated that RR inhibited the expression of TRPV4 in the dorsal ganglia of Wistar rats, measured by Western blot (Qu et al., 2016).

Intrathecal RR injection at doses of 1 nmol/L, 10 nmol/L, and 100 nmol/L to neuropathic pain–induced rats (due to dorsal ganglion compression) reduced nerve spontaneous ectopic discharge compared with saline controls, therefore improving pain sensitivity (Qu et al., 2016; Qu et al., 2016; Qu et al., 2016; Qu et al., 2016) with a reduction in the number of TRPV4-positive neurons in dorsal ganglion (Qu et al., 2016). Reduced expression of TRPV4 (2–4 hours), p38 (1–8 hours), and phosphorylated p38 (1–4 hours) was detected in the dorsal ganglia of RR-treated Wistar rats compared with neuropathic controls without treatment. In addition, a less pronounced reduction in p38-positive neurons (only seen in medium-sized neurons) was found compared with controls. RR may be an interesting candidate for a therapeutic approach against pathologic conditions where the TRPV4 and p38 pathways are involved via neuropathic-related mechanosensitive and nonmechanosensitive channels (Qu et al., 2016).

Treatment with RR once a day (3 mg/kg i.p., dose 1 or 6 mg/kg i.p., dose 2) in Swiss mice, after experimentally induced chronic cerebral hypoperfusion by double common carotid occlusion, attenuates cognitive impairments compared with the untreated group, as evaluated by Morris water maze test. RR treatment could reduce the escape latency time and time

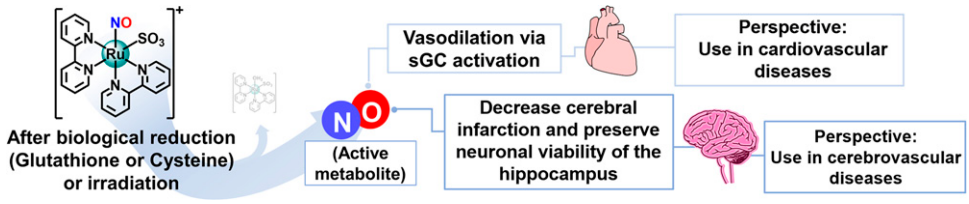

Fig. 2. Summary of studies evaluating the ruthenium capacity to affect neuroinflammatory conditions through either biologic reduction (glutathione or cysteine) or irradiation, releasing nitric oxide as an active metabolite. The nitric oxide promotes vasodilatation via guanilate ciclase activation, decreasing cerebral infarction and preserving neuronal viability of the hippocampus, thus resulting in good perspectives for future studies and clinical application. sGC, soluble Guanylate Cyclase

spent in the target quadrant, thus mitigating learning and memory deficits induced by chronic cerebral hypoperfusion (Singh and Sharma, 2016). RR treatment reduces levels of thiobarbituric acid in the brain, improves the levels of glutathione, and restores the levels of superoxide dismutase and the activity of its reduced isoform. RR also significantly decreased acetylcholinesterase activity, thus potentially rescuing cholinergic activity (Singh and Sharma, 2016).

The modulation of rianodine receptors, by antagonists such as RR, reduces sustained calcium release and neuronal death in individuals with ischemic conditions, fostering brain protection and from the inhibition of mechanisms linked to MCU channels, which plays a fundamental role in ischemic brain damage (Singh and Sharma, 2016).

Furthermore, Córdova and coworkers have shown that RR administered as a pretreatment at a dose of 3 mg/kg intraperitoneally to mice significantly reduced by 51% the nociception induced by intraplantar injection of menthol (a selective alcohol agonist for transient receptor potential melastatin 8 channels) (Córdova et al., 2011).

Ruthenium-bipyridine-trimethylphosphine glutamate (RuBi-Glu) and ruthenium-bipyridine-trimethylphosphine gamma aminobutyric acid (RuBi-GABA) were used to monitor neural activity in epilepsy, a neurologic condition characterized by seizures and abnormal neural activity. Electrical stimulation and optogenetic technology are commonly used methods in epilepsy research. Gao and colleagues, using 16-channel microelectrode arrays to evaluate a potential neural activity modulation by photolysis, could confirm that RuBi-Glu induced neuronal excitation, whereas RuBi-GABA caused inhibition of neuronal activity. The signal amplitudes had a peak of 242 μV during seizures and decreased later to 112 μV. (Gao et al., 2019). RuBi-GABA complex significantly inhibited nerve spikes related to epileptic triggers, thus preventing the occurrence of seizures in a model of epilepsy in rats (Gao et al., 2019).

Of note, ruthenium-based compounds have been shown to elicit a potential arachidonate 5-lipoxygenase inhibitory activity (Freitas et al., 2015). Since blocking this enzyme promotes a reduction in leukotriene signaling, these compounds may influence nociceptive and inflammatory events.

Figure 2 summarizes studies evaluating the use of ruthenium as an agent for stabilizing or halting neuroinflammatory conditions.

Ruthenium Complexes in Neurodegenerative Diseases

Cell Biology. Ruthenium compounds have been used in diagnostic tools for neurodegenerative diseases, based on their aggregative properties to β-amyloid (Aβ) peptides.

Such effects may have been highlighted when luminescent water-soluble metal complex *cis*-$[Ru(phen)_2(3,4Apy)_2]^{2+}$ (RuApy, 3,4Apy = 3,4-diaminopyridine, phen = 1,10-phenanthroline) was tested in mouse pheochromocytoma PC12 cells to investigate its in vitro effect on the aggregation of Aβ1–40 and its fragments Aβ1–28, Aβ11–22, and Aβ29–40. The complex did not show toxicity at concentrations of up to 60 μM for PC12 cells and did not interfere with the aggregation of Aβ fragments; however, it affected the aggregation of Aβ1–40 generated in the early stages, protecting PC12 cells while maintaining their viability (Cali et al., 2021).

Diagnostics. Different ruthenium complexes have been used for the early detection of neurodegenerative diseases, such as Parkinson's and Alzheimer's, and may be potential candidates for therapeutic strategies.

Previous studies have reported that the dispersion of single-walled carbon nanotubes in the presence of water-soluble polypyridyl complexes with extended planar π system ligands assists in the solubilization of such carbon nanotubes through π-π interactions. This strategy has helped identify aggregates of amyloid-β fibrils, commonly associated with the onset of Alzheimer's disease (Cook et al., 2011).

Another biotarget for ruthenium complexes is the infectious protein particles called prions. The conformational conversion of a cellular prion protein (PrP) into its abnormal PrPSc isoform can be involved in the pathophysiology of several potentially fatal neurodegenerative and infectious diseases (Atkinson et al., 2016).

Ruthenium complexes NAMI-A–based KP1019, KP1019-2, and KP418 (Fig. 1) interact electronically with PrP106–126, effectively inhibiting its aggregation. Such studies revealed the KP1019 complex as having the best results (Wang et al., 2015). These complexes also were found with less cellular toxicity than platinum- and gold-based compounds.

Alzheimer's disease (AD), which features profound cognitive and memory impairments in the elderly, has been associated with the accumulation of extracellular amyloid plaques (Aβ) and intracellular neurofibrillary tangles in the brain. There has been growing interest in the biochemical phases of Aβ peptide aggregation due to its implications for the development and progression of AD (Singh and Sharma, 2016).

The diagnosis of AD is currently performed only by brain tissue biopsy or autopsy. Biochemical compounds that may assist in recognition of the first stages of Aβ aggregation can effectively support early diagnosis and facilitate AD therapy for patients with initial symptoms. Aβ aggregation is commonly studied in vitro, using a variety of techniques. Yin and coworkers synthesized gold nanostars Ru@Pen@PEG-AuNS, modified with Ru(II) complex, to act as luminescent probes in drug delivery tracking (Yin et al., 2016). The complexed

TABLE 1
Summary of findings from the literature on the mechanism of action, biokinetics, and potential therapeutic application of ruthenium compounds.

Ruthenium compound	Mechanism of action and biokinetics	Potential therapeutic use
RuBi-GABA	Inhibits neuronal activity Significantly inhibits nerve peaks related to epileptic triggers Prevents occurrence of seizures in a rat model of epilepsy	Epilepsy treatment
RuBi-Glu	Induces neuronal excitation through a photoactivation of dendrites and neuronal circuits with visible or two-photon light sources	Affects neural activity
Rut-bpy	Inhibits inflammation in experimental models through the donation of NO Induces a marked relaxing effect on rabbit penile corpus cavernosum and aorta vascular smooth muscle	Potential use in stroke and anti-hypertensive Potential use in male reproduction
RR	Inhibits tumor growth, with a marked inhibition of mitochondrial $Ca2^{+}$ Capsaicin antagonist by blocking ion channels coupled to the vanilloid receptor (TRPV1 and 4) and ryanodine antagonist	Potential use in stroke Potential antioxidant and antinociceptive effect
TLD1433	Nontoxic ruthenium is used in photodynamic therapy as a photosensitizer inducing cancer cell apoptosis through the release of ROS	Treatment of nonmuscle invasive bladder cancer through PDT
Polypyridine Ru(II) complexes	Photosensitizers are used in PDT, easily transported to cancer cells via receptors	Treatment of nonmuscle invasive bladder cancer through PDT
106Ru	Emits radiation in the form of high electrons from beta particles, providing a high dose for tumors up to 5 mm thick	Widely used in small intraocular tumors
RBT@ MRN-SS-Tf/Ap	Ruthenium-based nanomaterials with the therapeutic potential against gliomas, for the capacity to cross the blood-brain barrier, a promising strategy in PDT	Anticancer treatment
KP1019	Cytotoxic agent Internalized through transferrin receptors Reduces to Ru(II) and presumably affects the DNA of tumoral cells via the mitochondrial pathway Induces apoptosis in colorectal tumor cell lines	Anticancer treatment
NAMI-A	NAMI-A is a noncytotoxic, antimetastatic drug Released ruthenium ions interact with proteins binding to carboxylate groups of two aspartate residues	Induces potent and selective cytotoxic effects on several leukemia cell lines
Rutenecarborane derivative plus 8-HQ	Effectively inhibit tumoral cells cytoprotective autophagy, likely via a different mechanism than that of quinolyl-containing organic scaffolds, i.e., inhibition of early stages of autophagosome formation	Anticancer treatment
RuApy	RuApy complex can interfere with the aggregation process of the full-length Aβ1–40 in biologic environment, reducing its cellular toxicity, probably interacting with Aβ at its surface	Neuroprotection against Aβ1–40 aggregation in the early stages
TQ-6	Decreases microglia activation in a model of focal brain ischemia-reperfusion in mice and improves platelet activation	Potential use in stroke Potential antioxidant

RuApy, *cis*-$[Ru(phen)_2(3,4Apy)_2]^{2+}$ (RuApy, 3,4Apy = 3,4-diaminopyridine, phen = 1,10-phenanthroline).

materials inhibited the formation of Aβ fibrils and dissociated the preformed fibrous Aβ under near-infrared irradiation. In addition, Ru@Pen@PEG-AuNS had an excellent neuroprotective effect on cell toxicity induced by Aβ through the application of near-infrared irradiation.

Silva and coworkers developed the complex *cis*-$[Ru(phen)_2(3,4Apy)_2]^{2+}$ (3,4Apy=3,4-aminopyridine and phen=phenanthroline) and investigated its properties in vitro (Silva et al., 2016). These authors reported no toxic effects in Neuro2A cells and documented a protective effect against ROS ($OH^{\bullet}$ radical) and an inhibitory effect on the activity of cholinesterase enzymes.

The complex *cis*-$[Ru(phen)_2(3,4Apy)_2]^{2+}$ is luminescent in aqueous solution, allowing in vitro imaging of neuronal cells and the direct observation of the structural evolution of Aβ monomers to protofibrils ($A\beta 1-40$) and globular oligomers ($A\beta 15-21$) in real time, with no apparent loss of luminescence. Thus, these molecules prove to be a viable tool in cell

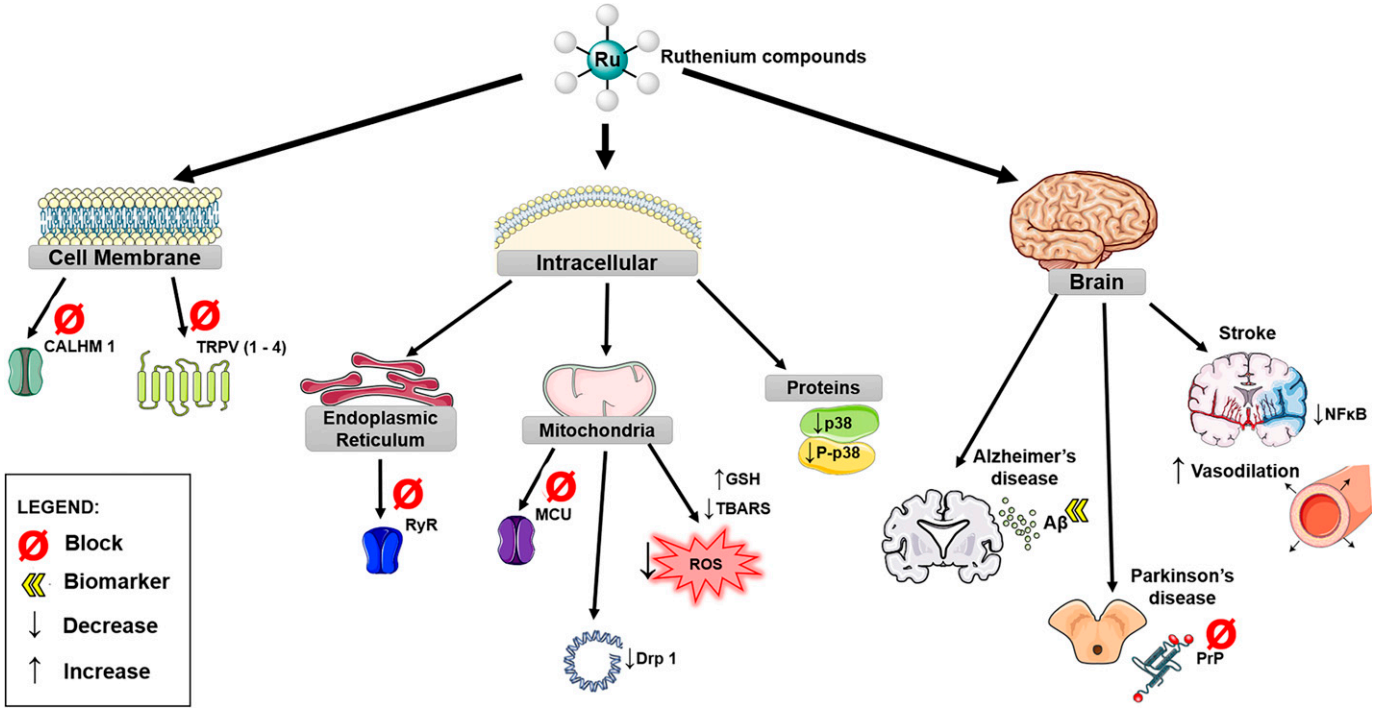

Fig. 3. Overview of mechanisms of action of ruthenium compounds in neurologic diseases. Ruthenium can bind to CALHM1 and TRPV1 and 4 receptors and enter the target cells. Ruthenium compounds are beneficial by donating NO and causing NO-related vasodilatation in experimental stroke models, improving infarction area, and reducing oxidative stress. Ruthenium compounds have been used in diagnostic approaches for detecting amyloid peptides (Aβ) and tracking the progression of Alzheimer's disease. ø illustrates the blockade of receptors and bindings, « means fluorescent marking for diagnosis, and the arrows ↓ and ↑ show decreased and increased activity, respectively. CALHM1, modulator 1 of calcium homeostasis channel; Drp1, dinamine-related protein 1; GSH, glutathione; NFκB, nuclear factor kappa B; RyR, rianodine receptors; TBARS, thiobarbituric acid.

imaging studies of Aβ accumulation, allowing the investigation of the biochemical stages of amyloid proteins in neuronal cells (Silva et al., 2016).

The tau protein plays a role in stabilizing microtubules in neuronal axons, conspicuously occurring in the central nervous system. The hyperphosphorylation of tau leads to insoluble hyperphosphorylated cell aggregates called neurofibrillary tangles, a hallmark in AD pathogenesis (Alonso et al., 2018; Barbier et al., 2019).

The $[Ru(phen)_2(dppzidzo)]^{2+}$ complex ion (dppzidzo = dipyrido-[3,2-a:2',3'-c]-phenazine-imidazolone and phen = phenanthroline), developed by Gao and coworkers (Gao et al., 2015) was used as a new luminescent tracing probe for aggregation of the R3 tau peptide. Through interaction with the short tau filament R3, the ruthenium complex provided useful information about tau aggregation (Gao et al., 2015).

Hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]Cl_3$, 98%) and Tris-(2,2'-bipyridyl)-ruthenium(II) chloride hexahydrate ($[Ru(bpy)_3]Cl_2 \bullet 6H_2O$, 98%) are also Ru-based systems that have been used in aid of AD diagnosis. Rapid electrochemical detection of Cu^{2+} and dopamine (candidate biomarkers of AD) in body fluids may be helpful for early AD diagnosis. Electrochemistry and electrochemiluminescence by using a vitreous carbon electrode modified by a silica nanochannel membrane/glass carbon electrode have been tested with ruthenium-based compounds. Tris-(2,2'-bipyridyl)-ruthenium(II) chloride hexahydrate ($[Ru(bpy)_3]Cl_2 \bullet 6H_2O$, 98%) ameliorates silica nanochannel membrane/glass carbon electrode sensitivity and improved antifouling capacity in biofluids, such as human blood and artificial cerebrospinal fluid, avoiding interference/noise from cells, proteins, and other large and small molecules, with consistent electrophysiological signals (Zhou et al., 2018).

A summary of the mechanisms of action, biokinetics, and potential therapeutic use of various ruthenium compounds is shown in Table 1.

Final Considerations

Ruthenium compounds have become widely studied in their various presentations because of desirable antioxidant, anti-inflammatory, vasodilatory, and photosensitizing activities (Fig. 3). Despite the preclinical benefits of ruthenium compounds in cancer and neurologic diseases, up to now, no ruthenium compound has been approved for clinical use in patients. Ongoing clinical trials are promising in identifying safe and efficacious ruthenium compounds for therapeutic and diagnostic practice. This minireview summarizes findings about several promising ruthenium candidates' for clinical use. Although evidence is mounting, more studies are needed to dissect the protective mechanisms of ruthenium-based compounds on neurologic diseases.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Gama Justi, Araújo Matos, de Sá Roriz Caminha, Rodrigues Roque, Muniz Carvalho, Soares Campelo, Belayev, Gonzaga de França Lopes, Barreto Oriá.

References

Alonso AD, Cohen LS, Corbo C, Morozova V, ElIdrissi A, Phillips G, and Kleiman FE (2018) Hyperphosphorylation of tau associates with changes in its function beyond microtubule stability. *Front Cell Neurosci* **12**:338. DOI: 10.3389/fncel.2018.00338.

Arnesano F and Natile G (2009) Mechanistic insight into the cellular uptake and processing of cisplatin 30 years after its approval by FDA. *Coord Chem Rev* **253**:2070–2081. DOI: 10.1016/j.ccr.2009.01.028.

Atkinson CJ, Zhang K, Munn AL, Wiegmans A, and Wei MQ (2016) Prion protein scrapie and the normal cellular prion protein. *Prion* **10**:63–82. DOI: 10.1080/19336896.2015.1110293.

Barbier P, Zejneli O, Martinho M, Lasorsa A, Belle V, Smet-Nocca C, Tsvetkov PO, Devred F, and Landrieu I (2019) Role of tau as a microtubule-associated protein: structural and functional aspects. *Front Aging Neurosci* **11**:204.

Brabec V (2002) DNA modifications by antitumor platinum and ruthenium compounds: their recognition and repair. *Prog Nucleic Acid Res Mol Biol* **71**:1–68. DOI: 10.1016/s0079-6603(02)71040-4.

Brabec V and Nováková O (2006) DNA binding mode of ruthenium complexes and relationship to tumor cell toxicity. *Drug Resist Updat* **9**:111–122. DOI: 10.1016/j.drup.2006.05.002

Cali MP, Pereira LMB, Teodoro MD, Sellani TA, Rodrigues EG, and Carlos RM (2021) Comparison of Aβ (1-40, 1-28, 11-22, and 29-40) aggregation processes and inhibition of toxic species generated in early stages of aggregation by a water-soluble ruthenium complex. *J Inorg Biochem* **215**:111314. DOI: 10.1016/j.jinorgbio.2020.111314.

Campelo MWS, Oriá RB, Lopes LG, Brito GA, Santos AA, Vasconcelos RC, Silva FO, Nobrega BN, Bento-Silva MT, and Vasconcelos PR (2012) Preconditioning with a novel metallopharmaceutical NO donor in anesthetized rats subjected to brain ischemia/reperfusion. *Neurochem Res* **37**:749–758. DOI: 10.1007/s11064-011-0669-x.

Cerqueira JBG, Silva LFG, Lopes LGF, Moraes MEA, and Nascimento NRF (2008) Relaxation of rabbit corpus cavernosum smooth muscle and aortic vascular endothelium induced by new nitric oxide donor substances of the nitrosyl-ruthenium complex. *Int Braz J Urol* **34**:638–645, discussion 645–646.

Chiba T, Oka Y, Sashida H, Kanbe T, Abe K, Utsunomiya I, and Taguchi K (2017) Vincristine-induced peripheral neuropathic pain and expression of transient receptor potential vanilloid 1 in rat. *J Pharmacol Sci* **133**:254–260. DOI: 10.1016/j.jphs.2017.03.004.

Cho Y, Chang JS, Yoon JS, Lee SC, Kim YB, Kim JH, and Keum KC (2018) Ruthenium-106 brachytherapy with or without additional local therapy shows favorable outcome for variable-sized choroidal melanomas in Korean patients. *Cancer Res Treat* **50**:138–147. DOI: 10.4143/crt.2016.391.

Cisneros-Mejorado A, Gottlieb M, Ruiz A, Chara JC, Pérez-Samartín A, Marambaud P, and Matute C (2018) Blockade and knock-out of CALHM1 channels attenuate ischemic brain damage. *J Cereb Blood Flow Metab* **38**:1060–1069. DOI: 10.1177/0271678X17713587.

Clarke MJ, Bitler S, Rennert D, Buchbinder M, and Kelman AD (1980) Reduction and subsequent binding of ruthenium ions catalyzed by subcellular components. *J Inorg Biochem* **12**:79–87. DOI: 10.1016/s0162-0134(00)80045-8.

Cook NP, Torres V, Jain D, and Martí AA (2011) Sensing amyloid-β aggregation using luminescent dipyridophenazine ruthenium(II) complexes. *J Am Chem Soc* **133**:11121–11123. DOI: 10.1021/ja204656r.

Córdova MM, Werner MF. de P., Silva MD, Ruani AP, Pizzolatti MG, and Santos ARS (2011) Further antinociceptive effects of myricitrin in chemical models of overt nociception in mice. *Neurosci Lett* **495**:173–177. DOI: 10.1016/j.neulet.2011.02.007.

de Sousa AP, Carvalho EM, Ellena J, Sousa EHS, de Sousa JR, Lopes LGF, Ford PC, and Holanda AKM (2017) Photochemical studies of cis-$[Ru(bpy)_2(4\text{-}bzpy)(CO)](PF_6)_2$ and cis-$[Ru(bpy)_2(4\text{-}bzpy)(Cl)](PF_6)$: blue light-induced nucleobase binding. *J Inorg Biochem* **173**:144–151. DOI: 10.1016/j.jinorgbio.2017.05.006.

Drača D, Marković M, Gozzi M, Mijatović S, Maksimović-Ivanić D, and Hey-Hawkins E (2021) Ruthenacarborane and quinoline: a promising combination for the treatment of brain tumors. *Molecules* **26**:3801. DOI: 10.3390/molecules26133801.

Dragutan I, Dragutan V, and Demonceau A (2015) Editorial of Special Issue Ruthenium complex: the expanding chemistry of the ruthenium complexes. *Molecules* **20**:17244–17274. DOI: 10.3390/molecules200917244.

Du E, Hu X, Roy S, Wang P, Deasy K, Mochizuki T, and Zhang Y (2017) Taurine-modified Ru(ii)-complex targets cancerous brain cells for photodynamic therapy. *Chemical Commun (Camb)* **53**:6033–6036. DOI: 10.1039/c7cc03337k

Englinger B, Pirker C, Heffeter P, Terenzi A, Kowol CR, Keppler BK, and Berger W (2019) Metal drugs and the anticancer immune response. *Chem Rev* **119**:1519–1624. DOI: 10.1021/acs.chemrev.8b00396.

Frasca D, Ciampa J, Emerson J, Umans RS, and Clarke MJ (1996) Effects of hypoxia and transferrin on toxicity and DNA binding of ruthenium antitumor agents in HeLa cells. *Met Based Drugs* **3**:197–209. DOI: 10.1155/MBD.1996.197.

Freitas CS, Roveda Jr ACJ, Truzzi DR, Garcia AC, Cunha TM, Cunha FQ, and Franco DW (2015) Anti-inflammatory and anti-nociceptive activity of ruthenium complexes with isonicotinic and nicotinic acids (niacin) as ligands. *J Med Chem* **58**:4439–4448. DOI: 10.1021/acs.jmedchem.5b00133.

Gao F, Xiao G, Song Y, Wang M, Li Z, Zhang Y, Xu S, Xie J, Yin H, and Cai X (2019) Recording of neural activity with modulation of photolysis of caged compounds using microelectrode arrays in rats with seizures. *IEEE Trans Biomed Eng* **66**:3080–3087. DOI: 10.1109/TBME.2019.2900251.

Gao X, Wang L, Huang H-L, Wang L-L, Yao J-L, Shi S, and Yao T-M (2015) Molecular "light switch" [Ru(phen)2dppzidzo](2+) monitoring the aggregation of tau. *Analyst (Lond)* **140**:7513–7517. DOI: 10.1039/c5an01624j.

GBD 2016 Neurology Collaborators (2019) Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* **18**:459–480. DOI: 10.1016/S1474-4422(18)30499-X

Haas KL and Franz KJ (2009) Application of metal coordination chemistry to explore and manipulate cell biology. *Chem Rev* **109**:4921–4960. DOI: 10.1021/cr900134a.

Hamilton MG and Lundy PM (1995) Effect of ruthenium red on voltage-sensitive Ca++ channels. *J Pharmacol Exp Ther* **273**:940–947.

Hamilton NB, Kolodziejczyk K, Kougioumtzidou E, and Attwell D (2016) Proton-gated Ca(2+)-permeable TRP channels damage myelin in conditions mimicking ischaemia. *Nature* **529**:523–527. DOI: 10.1038/nature16519.

Hara T, Chiba T, Abe K, Makabe A, Ikeno S, Kawakami K, Utsunomiya I, Hama T, and Taguchi K (2013) Effect of paclitaxel on transient receptor potential vanilloid 1 in rat dorsal root ganglion. *Pain* **154**:882–889. DOI: 10.1016/j.pain.2013.02.023.

Imberti C, Zhang P, Huang H, and Sadler PJ (2020) New designs for phototherapeutic transition metal complexes. *Angew Chem Int Ed Engl* **59**:61–73. DOI: 10.1002/anie.201905171.

Kaspler P, Lazic S, Forward S, Arenas Y, Mandel A, and Lilge L (2016) A ruthenium(II) based photosensitizer and transferrin complexes enhance photo-physical properties, cell uptake, and photodynamic therapy safety and efficacy. *Photochem Photobiol Sci* **15**:481–495. DOI: 10.1039/c5pp00450k

Kubicka-Trząska A, Karska-Basta I, Oleksy P, and Romanowska-Dixon B (2015) Management of diffuse choroidal hemangioma in Sturge-Weber syndrome with ruthenium-106 plaque radiotherapy. *Graefes Arch Clin Exp Ophthalmol* **253**:2015–2019. DOI: 10.1007/s00417-015-3061-8.

Liang N, Wang P, Wang S, Li S, Li Y, Wang J, and Wang M (2014) Role of mitochondrial calcium uniporter in regulating mitochondrial fission in the cerebral cortexes of living rats. *J Neural Transm (Vienna)* **121**:593–600. DOI: 10.1007/s00702-014-1166-6

Nolen SC, Evans MA, Fischer A, Corrada MM, Kawas CH, and Bota DA (2017) Cancer-incidence, prevalence and mortality in the oldest-old. A comprehensive review. *Mech Ageing Dev* **164**:113–126. DOI: 10.1016/j.mad.2017.05.002.

Pricker SP (1996) Medical uses of gold compounds: past, present and future. *Gold Bull* **29**:53–60. DOI: 10.1007/BF03215464.

Qu Y-J, Zhang X, Fan Z-Z, Huai J, Teng Y-B, Zhang Y, and Yue S-W (2016) Effect of TRPV4-p38 MAPK pathway on neuropathic pain in rats with chronic compression of the dorsal root ganglion. *BioMed Res Int* **2016**:6978923. DOI: 10.1155/2016/6978923.

Saklani R, Jaggi A, and Singh N (2010) Pharmacological preconditioning by milrinone: memory preserving and neuroprotective effect in ischemia-reperfusion injury in mice. *Arch Pharm Res* **33**:1049–1057. DOI: 10.1007/s12272-010-0711-6.

Scorza FA, Menezes-Rodrigues FS, Olszewer E, Errante PR, Tavares JGP, Scorza CA, Ferraz HB, Finsterer J, and Caricati-Neto A (2020) The mitochondrial calcium uniporter: a new therapeutic target for Parkinson's disease-related cardiac dysfunctions? *Clinics (São Paulo)* **75**:e1299

Silva DES, Cali MP, Pazin WM, Carlos-Lima E, Salles Trevisan MT, Venâncio T, Arcisio-Miranda M, Ito AS, and Carlos RM (2016) Luminescent Ru(II) phenanthroline complexes as a probe for real-time imaging of Aβ self-aggregation and therapeutic applications in Alzheimer's disease. *J Med Chem* **59**:9215–9227. DOI: 10.1021/acs.jmedchem.6b01130.

Singh P and Sharma B (2016) Reversal in cognition impairments, cholinergic dysfunction, and cerebral oxidative stress through the modulation of ryanodine receptors (RyRs) and cysteinyl leukotriene-1 (CysLT1) receptors. *Curr Neurovasc Res* **13**:10–21. DOI: 10.2174/1567202612666151026105610.

Som P, Oster ZH, Matsui K, Guglielmi G, Persson BR, Pellettieri ML, Srivastava SC, Richards P, Atkins HL, and Brill AB (1983) 97Ru-transferrin uptake in tumor and abscess. *Eur J Nucl Med* **8**:491–494. DOI: 10.1007/BF00598908.

Sun F-C, Shyu H-Y, Lee MS, Lee MS, and Lai YK (2013) Involvement of calcium-mediated reactive oxygen species in inductive GRP78 expression by geldanamycin in 9L rat brain tumor cells. *Int J Mol Sci* **14**:19169–19185. DOI: 10.3390/ijms140919169.

Thota S, Rodrigues DA, Crans DC, and Barreiro EJ (2018) Ru(II) compounds: next-generation anticancer metallotherapeutics? *J Med Chem* **61**:5805–5821. DOI: 10.1021/acs.jmedchem.7b01689.

Tonin AM, Amaral AU, Busanello EN, Gasparotto J, Gelain DP, Gregersen N, and Wajner M (2014) Mitochondrial bioenergetics deregulation caused by long-chain 3-hydroxy fatty acids accumulating in LCHAD and MTP deficiencies in rat brain: a possible role of mPTP opening as a pathomechanism in these disorders? *Biochim Biophys Acta* **1842**:1658–1667. DOI: 10.1016/j.bbadis.2014.06.011.

Wang X, Zhu D, Zhao C, He L, and Du W (2015) Inhibitory effects of NAMI-A-like ruthenium complexes on prion neuropeptide fibril formation. *Metallomics* **7**:837–846. DOI: 10.1039/c5mt00029g

Yan H, Zhang D, Hao S, Li K, and Hang C-H (2015) Role of mitochondrial calcium uniporter in early brain injury after experimental subarachnoid hemorrhage. *Mol Neurobiol* **52**:1637–1647. DOI: 10.1007/s12035-014-8942-z.

Yin T, Xie W, Sun J, Yang L, and Liu J (2016) Penetratin peptide-functionalized gold nanostars: enhanced BBB permeability and NIR photothermal treatment of Alzheimer's disease using ultralow irradiance. *ACS Appl Mater Interfaces* **8**:19291–19302. DOI: 10.1021/acsami.6b05089.

Zhou L, Ding H, Yan F, Guo W, and Su B (2018) Electrochemical detection of Alzheimer's disease related substances in biofluids by silica nanochannel membrane modified glassy carbon electrodes. *Analyst (Lond)* **143**:4756–4763. DOI: 10.1039/C8AN01457D.

Zhu X, Zhou H, Liu Y, Wen Y, Wei C, Yu Q, and Liu J (2018) Transferrin/aptamer conjugated mesoporous ruthenium nanosystem for redox-controlled and targeted chemo-photodynamic therapy of glioma. *Acta Biomater* **82**:143–157. DOI: 10.1016/j.actbio.2018.10.012.

Address correspondence to: Reinaldo Barreto Oriá, Institute of Biomedicine, School of Medicine, Federal University of Ceará. Rua Coronel Nunes de Melo, 1315 – Rodolfo Teófilo, 60430-270, Fortaleza, Ceará, Brazil. E-mail: oria@ufc.br