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Title Page

The Role of Ruthenium Compounds in Neurological Diseases: A Minireview

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Running Title Page

Running title: Ruthenium application in neurological diseases.
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List of abbreviations used in the paper:
106Ru, ruthenium-106 plaque radiotherapy;
5-LOX, 5-lipoxygenase;
Aβ, β-amyloid;
AChE, acetylcholinesterase;
AD, Alzheimer's disease;
ATP, adenosine triphosphate;
AuNS, gold nanostars;
CALHM1, modulator 1 of calcium homeostasis channel;
CCH, chronic cerebral hypoperfusion;
CICR, calcium-induced calcium release;
DNA, deoxyribonucleic acid;
Drp1, dinamine-related protein 1;
GSH, glutathione;
I;R, ischemia/reperfusion;
KP1019, indazolium trans-tetrachlorobis(1H-indazole) ruthenate(III)];
MCU, mitochondrial calcium uniporter;
MEAs, microelectrode arrays;
MWM, Morris Water Maze;
NAMI-A, imidazolium(imidazole)(dimethylsulfoxide)tetrachlororuthe-nate(III);
NFκB, nuclear factor kappa B;
NIR, near-infrared;
NO, nitric oxide;
PDT, photodynamic therapy;
PrP, prion protein;
ROS, reactive oxygen species;
RR, ruthenium red;
Ru, ruthenium;
RuBi-GABA, ruthenium-bipyridine-trimethylphosphine gamma aminobutyric acid;
RuBi-Glu, ruthenium-bipyridine-trimethylphosphine glutamate;
Rut-bpy, ruthenium nitrosyl complex cis-[Ru(bpy)2(SO3)(NO)]PF6;
RyR, rianodine receptors;
sGC, soluble guanylate cyclase;
SNM/GCE, silica nanochannel membrane/glass carbon electrode;
SNP, sodium nitroprusside;
SOD, superoxide dismutase;
SWCNTs, single-walled carbon nanotubes;
TBARS, thiobarbituric acid;
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)]Cl2;
TRPM8, transient receptor potential melastatin 8;
TRPV1, transient receptor potential cation channel V1.

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Abstract Page

Abstract: Ruthenium (Ru) compounds, nitric oxide donors in biological systems, have emerged as a promising therapeutical alternative to conventional drugs in anticancer chemotherapy and as a potential neuroprotective agent, with less 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 seen in early Alzheimer's disease. More studies are needed to fill many current knowledge gaps about the intricate and complex ruthenium biological effects and therapeutic-related mechanisms, stimulating further research.

Keywords: ruthenium-based compounds; biological application; neurological diseases; nitric oxide.

Significance Statement: In our minireview, we summarize studies addressing the role of ruthenium compounds on neurological illnesses, focusing on brain cancer, neurovascular and neurodegenerative diseases. No such review is available in the literature.

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1 Introduction

Many metal ions (e.g., copper, zinc, iron, and manganese) play a crucial role in various biological processes, potentially influencing medical treatment (Arnesano and Natile, 2009). Preparations containing metal complexes have been utilized 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 neurological 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)("Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016.," 2019). One of the eldest and most well-known metallodrug-therapeutical approaches is the 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 [Au]) used in the treatment of rheumatoid arthritis, Trisenox® (based in arsenic [As]) used in the treatment of acute promyelocytic leukemia, and sodium nitroprusside – SNP (based in iron [Fe]), used in cardiovascular surgery and hypertensive emergency (Fricker, 1996). The sub-optimal activity and side-effects of many cisplatinum-compounds have stimulated the search for other anti-cancer therapies, among which Ru-complexes.

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The idea of using ruthenium compounds as pharmacological agents evolves with significant scientific breakthroughs between 1975-1985, such as the discovery 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 to 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 the ruthenium compounds [(Ru(II), Ru(III), and Ru(IV) are the most used in biological conditions] including the rate of ligand exchange; the possibility of changing in oxidation state, its ability to mimic iron in binding biological molecules and, low toxicity to normal cells.

Imidazolium(imidazole)(dimethylsulfoxide)tetrachlororuthenate (III) (NAMI-A) (**Figure 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) (**Figure 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 (**Figure 1**), has advanced in clinical trials (ClinicalTrials.gov Identifier: NCT01415297) (Thota et al., 2018).

Another example is the [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) (**Figure 1**), a ruthenium-based complex, currently in clinical trials (phase I/II, for treatment of non-muscle 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-biological 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 therapy anticancer (Englinger et al., 2019, Zhu et al., 2018).

Ruthenium-based complexes have stood out in different therapeutic segments. 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 neurological illnesses, focusing on brain cancer, neurovascular and neurodegenerative diseases.

2.1 Ruthenium and neuro-oncology

2.1.1. Cell biology

The use of polypyridine Ru(II) complexes as photosensitizers in the 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 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 photodynamic therapy (PDT) utilized for some types of brain tumors, including the glioblastoma, specifically stimulating ROS production, leading to the death of the target cells (Du et al., 2017).

2.1.2. 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 continuously 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 (808nm 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, to 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).

2.2 Ruthenium complexes in neurovascular diseases

2.2.1. 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

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$[Ca_2^+]$i 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 (CICR). It also contributes to the reduction of mitochondrial fission by reducing the expression of Drp1 (dinamine-related protein 1), and by blocking MCU (calcium mitochondrial uniporter) 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 reactive oxygen species (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 potential receiver vanilloid channels (TRPVc) 1 and 4 (Hamilton et al., 2016). Finally, the RR has been proposed to significantly reduce the transient focal cerebral ischemia-reperfusion-related motor symptoms by inhibiting the modulator 1 of calcium homeostasis channel (CALHM1) in mice (Cisneros-Mejorado et al., 2018).

In previous studies carried out with the ruthenium nitrosyl complex *cis*-$[Ru(bpy)_2(SO_3)(NO)]PF_6$ (Rut-bpy) (**Figure 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 NO and

soluble guanylate cyclase (sGC) activation (Cerqueira et al., 2008). Rats that suffered cerebral ischemia/reperfusion (I/R), when preconditioned with Rut-byp, showed a decrease in total cerebral infarction area and improved hippocampal neuronal viability in an initial phase of I/R (Campelo et al., 2012).

Rut-byp may show a neuroprotective effect primarily by inhibiting nuclear factor kappa B (NFκB) signaling. This transcription factor regulates a downstream pro-inflammatory 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 microglia activation in a model of focal brain ischemia-reperfusion in mice, and improved platelet activation. TQ-6 was able to diminish inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) expression, nuclear factor kappa B (NF-κB) p65 phosphorylation, reduced oxidative stress in LPS-activated microglia (Chih-Hsuan Hsia et al, 2020).

2.3 Ruthenium compounds in neuropathic pain

2.3.1. 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 transient receptor potential cation channel V1 (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

non-selective antagonist of TRP receptors, when administered in a single dose of (3mg/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 co-workers used RR in the same dose (3mg/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 co-workers 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 to 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 h), p38 (1-8 h), and P-p38 (1-4 h) was detected in the dorsal ganglia of RR-treated Wistar rats compared to neuropathic controls without treatment. In addition, a less pronounced reduction in p38-positive neurons (only seen in medium-sized neurons) was found compared to controls. RR may be an interesting candidate for a therapeutical approach against pathological conditions where the TRPV4 and p38 pathways are involved via neuropathic-related mechano- and non-mechanosensitive channels (Qu et al., 2016).

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Treatment with RR once a day (D1- 3 mg/kg; i.p or D2 – 6 mg/kg; i.p) in Swiss mice, after experimentally-induced chronic cerebral hypoperfusion (CCH) by double common carotid occlusion, attenuates cognitive impairments compared to the untreated group, as evaluated by Morris Water Maze (MWM) test. RR treatment could reduce the escape latency time and time spent in the target quadrant, thus mitigating learning and memory deficits induced by CCH (Singh and Sharma, 2016). RR treatment reduces thiobarbituric acid (TBARS) levels in the brain, improves the levels of GSH, restored the levels of SOD, and the activity of its reduced isoform. RR also significantly decreased acetylcholinesterase (AChE) activity, thus potentially rescuing cholinergic activity (Singh and Sharma, 2016).

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

Furthermore, Córdova and co-workers have shown that RR administered as a pretreatment at a dose of 3mg/kg intraperitoneally to mice significantly reduced by 51% the nociception induced by intraplantar injection of menthol (a selective alcohol agonist for TRPM8 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 neurological condition characterized by seizures and abnormal neural activity. Electrical stimulation and optogenetic technology are commonly used methods in epilepsy research.

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Gao and colleagues using 16-channel microelectrode arrays (MEAs) to evaluate a potential neural activity modulation by photolysis could confirm that RuBi-Glu induced neuronal excitation while RuBi-GABA caused inhibition of neuronal activity. The signals amplitude had a peak of 242 μV during seizures and decreasing 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 (5-LOX) 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.

2.4 Ruthenium complexes in neurodegenerative diseases

2.4.1. Cell biology

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

Such effects could be highlighted when luminescent water-soluble metal complex cis-[Ru (phen) 2 (3.4Apy) 2] 2+ (RuApy, 3.4Apy = 3,4-day minopyridine, fen = 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).

2.4.2. Diagnostics

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

Previous studies have reported the dispersion of single-walled carbon nanotubes (SWCNTs) in the presence of water-soluble polypyridyl complexes with extended planar π systems ligand, assists in the solubilization of SWCNTs 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 bio-target 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 (**Figure 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.

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Alzheimer's disease (AD), which is featured by 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 employing 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 co-workers synthesized gold nanostars (AuNS) Ru@Pen@PEG-AuNS, modified with Ru (II) complex, to act as luminescent probes in drug delivery tracking (Yin et al., 2016). The complexed materials inhibited the formation of Aβ fibrils and dissociated the pre-formed fibrous Aβ under near-infrared (NIR) irradiation. In addition, Ru@Pen@PEG-AuNS had an excellent neuroprotective effect on cell toxicity induced by Aβ through the application of NIR irradiation.

Silva and co-workers 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, as well as; 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 (Aβ1−40) and globular

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oligomers (Aβ15−21) in real-time, with no apparent loss of luminescence. Thus, these molecules prove to be a viable tool in cell 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 co-workers (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 ($[Ru(bpy)_3]Cl_2•6H_2O$, 98%) are also Ru-based systems, which 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 (DEC) and electrochemiluminescence (ECL) by using a vitreous carbon electrode modified by a silica nanochannel membrane/glass carbon electrode (SNM/GCE) has been tested with ruthenium-based compounds. Tris-(2,2'-bipyridyl)-ruthenium(II) chloride hexahydrate ($[Ru(bpy)_3]Cl_2•6H_2O$, 98%) ameliorates SNM/GCE sensitivity and improved anti-fouling capacity in

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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 are shown in **Table 1**.

4 Final Considerations

Ruthenium compounds have become widely studied in their various presentations because of desirable antioxidant, anti-inflammatory, vasodilatory, and photosensitizing activities (**Figure 3**). Despite the pre-clinical benefits of ruthenium compounds in cancer and neurological 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 therapeutical and diagnostic practice. This minireview summarizes several promising ruthenium candidates' clinical use findings, both in clinical and laboratory studies. Although mounting evidence is evolving, more studies are needed to dissect the protective mechanisms of ruthenium-based compounds on neurological diseases.

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Conflict of Interest

The authors declare no conflict of interest.

Authorship Contributions

All authors wrote, revised, and contributed to the writing of the manuscript.

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Figure legends

Figure 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 non-muscle invasive bladder cancer via photodynamic therapy, and [Ru(bpy)2(SO3)(NO)](PF6) (7) (ruthenium nitrosyl complex - vasodilation activity).

Figure 2. Summary of studies evaluating the ruthenium capacity to affect neuroinflammatory conditions, either through biological 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.

Figure 3. Overview of ruthenium compounds mechanisms of action in neurological diseases. Ruthenium can bind to CALHM1 and TRPV1-4 receptors and enter the target cells. Ruthenium compounds are beneficial by donating nitric oxide (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

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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;

TRPV1-4, transient receptor potential cation channel V1 and 4;

RyR, rianodine receptors;

MCU, mitochondrial calcium uniporter;

GSH, glutathione;

TBARS, thiobarbituric acid;

ROS, reactive oxygen species;

Drp1, dinamine-related protein 1;

NFκB, nuclear factor kappa B;

PrP, prion protein.

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

Ruthenium Compound	Mechanism of Action and Biokinetics	Potential therapeutic use
RuBi-GABA	Inhibits neuronal activity Significantly inhibited nerve peaks related to epileptic triggers. Prevents occurrence of seizures in a rat model of epilepsy.	Epilepsy treatment
RuBi-Glu	Induced neuronal excitation through a photoactivation of dendrites and neuronal circuits with visible or two-photon light sources	Affects neural activity
Rut-bpy	Inhibit 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 (TRPV - 1 and 4) and ryanodine antagonist.	Potential use in stroke Potential antioxidant and antinociceptive effect
TLD1433	Non-toxic ruthenium used in photodynamic therapy as a photosensitizer inducing cancer cell apoptosis through the release of ROS.	Treatment of non-muscle invasive bladder cancer through photodynamic therapy (PDT)
Polypyridine Ru(II) complexes	Photosensitizers used in PDT, easily transported to cancer cells via receptors.	Treatment of non-muscle invasive bladder cancer through PDT
106Ru	This compound 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.	Anti-cancer treatment
KP1019	Cytotoxic agent. Internalized through transferrin receptors, Reduced to Ru (II) and presumably affecting the DNA of tumoral cells via the mitochondrial pathway. Induce apoptosis in colorectal tumor cell lines	Anti-cancer treatment
NAMI-A	NAMI-A is a non-cytotoxic, 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-hydroxyquinoline (8-HQ)	Effectively inhibit tumoral cells cytoprotective autophagy, likely via a different mechanism than that of quinolyl-containing organic	Anti-cancer treatment

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	scaffolds, i.e., inhibition of early stages of autophagosome formation	
RuApy	RuApy complex can interfere with the aggregation process of the full-length Aβ1–40 in biological environment, reducing its cellular toxicity, probably interacting with Aβ at its surface	Neuroprotection against Aβ1-40 aggregation in the early stages
TQ-6	Decrease microglia activation in a model of focal brain ischemia-reperfusion in mice, and improved platelet activation.	Potential use in stroke Potential antioxidant

RuBi-GABA, ruthenium-bipyridine-trimethylphosphine gamma aminobutyric acid;
RuBi-Glu, ruthenium-bipyridine-trimethylphosphine glutamate;
Rut-bpy, ruthenium nitrosyl complex cis-[Ru(bpy)2(SO3)(NO)]PF6;
RR, Ruthenium Red;
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)]Cl2;
106Ru, ruthenium-106 plaque radiotherapy;
KP1019, indazolium trans-tetrachlorobis(1H-indazole) ruthenate(III)];
NAMI-A, imidazolium(imidazole)(dimethylsulfoxide)tetrachlororuthe-nate(III);
RuApy, cis-[Ru (phen) 2 (3.4Apy) 2] 2+ (RuApy, 3.4Apy = 3,4-day minopyridine, fen = 1, 10-phenanthroline);
TQ-6, Ru(η6-cymene)2-(1H-benzoimidazol-2-yl)-quinoline Cl]BF4 .

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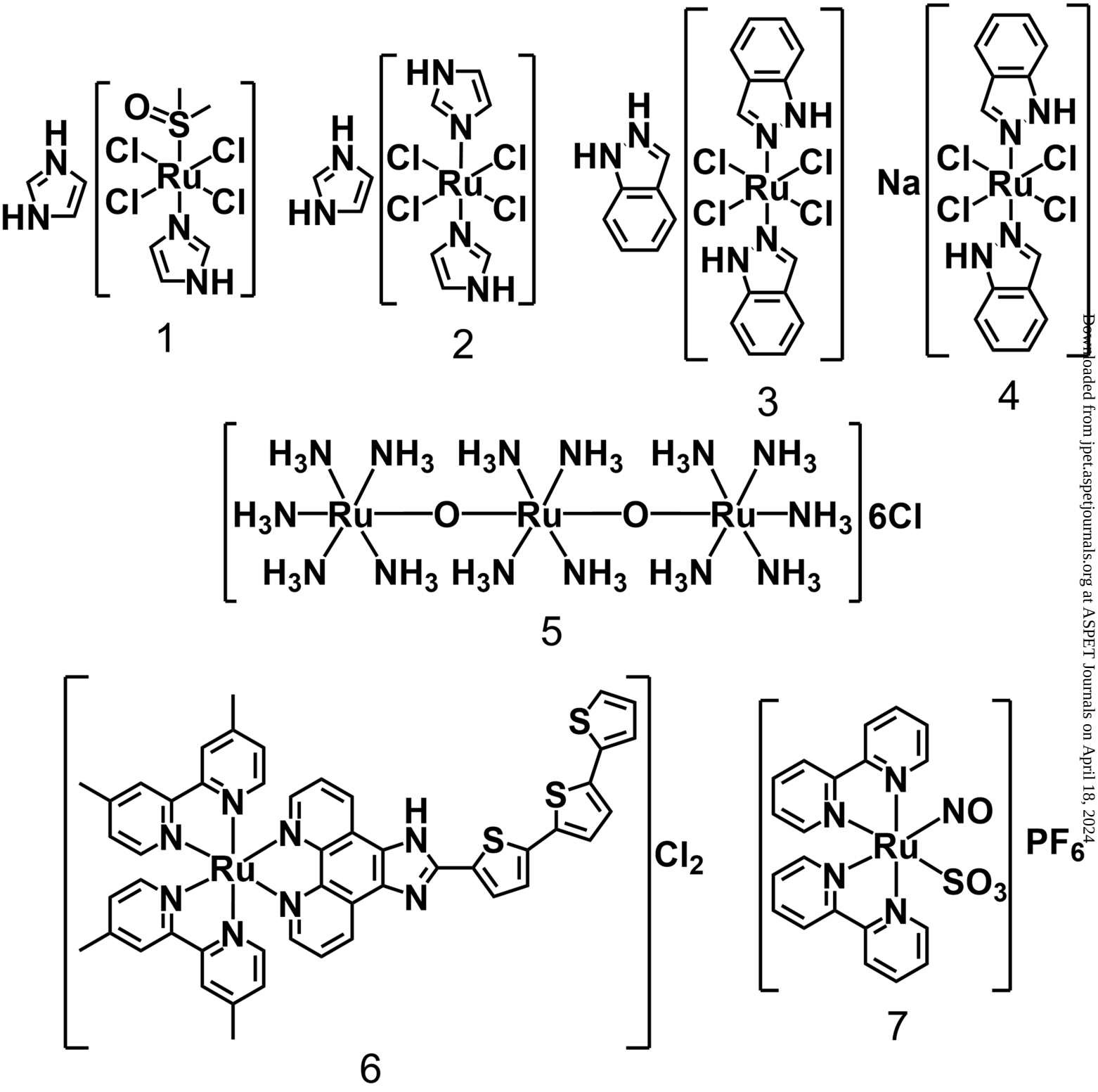
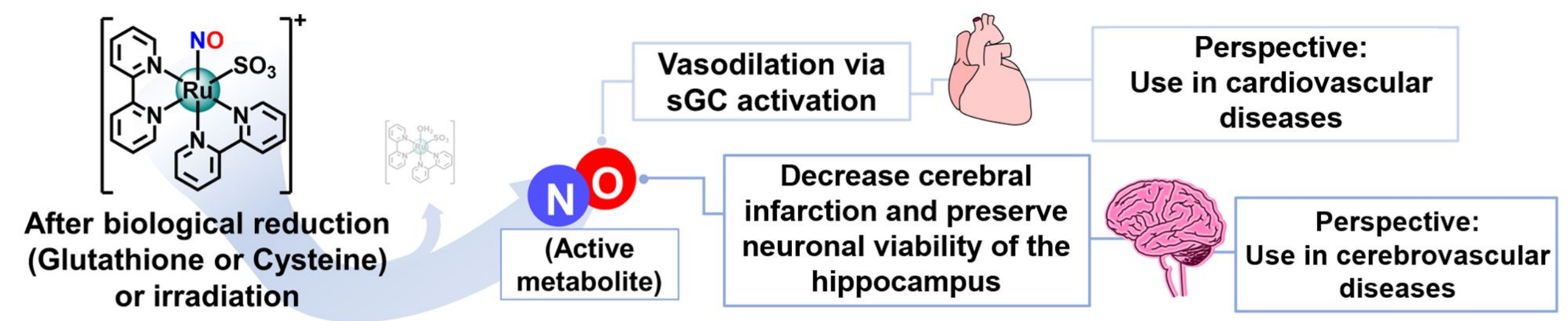


Figure 1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sGC activation
Perspective:
Use in cardiovascular
diseases
Decrease cerebral
infarction and preserve
neuronal viability of the
hippocampus
Perspective:
Use in cerebrovascular
diseases

Figure 2

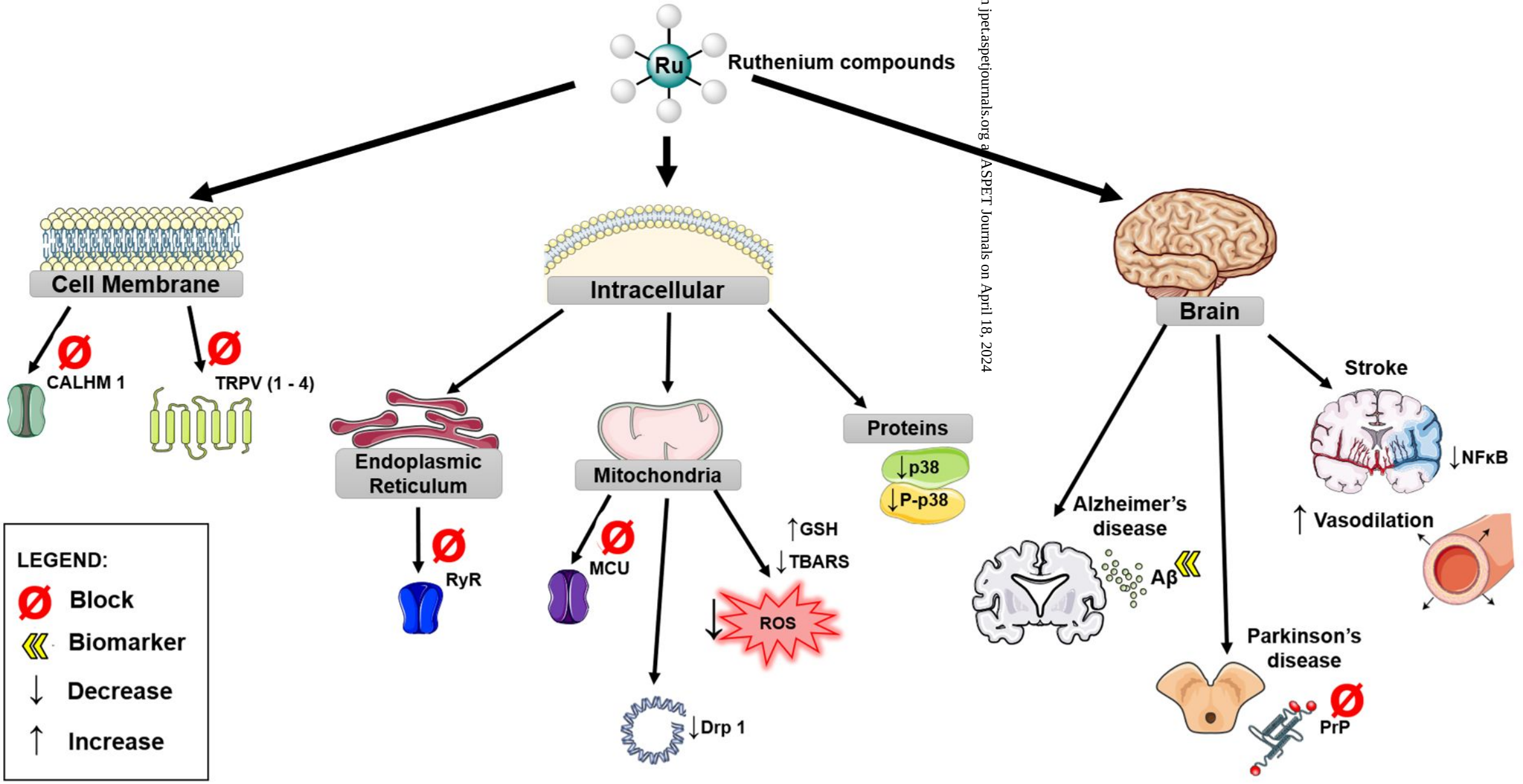

Figure 3