

# Metal Coordination Complexes as Therapeutic Agents for Ischemia-Reperfusion Injury

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**ABSTRACT:** Ischemia-reperfusion injury (IRI), which describes the cell damage and death that occurs after blood and oxygen are restored to ischemic or hypoxic tissue, is a significant factor within the mortality rates of heart disease and stroke patients. At the cellular level, the return of oxygen triggers an increase in reactive oxygen species (ROS) and mitochondrial calcium ( $mCa^{2+}$ ) overload, which both contribute to cell death. Despite the widespread occurrence of IRI in different pathological conditions, there are currently no clinically approved therapeutic agents for its management. In this Perspective, we will briefly discuss the current therapeutic options for IRI and then describe in great detail the potential role and arising applications of metal-containing coordination and organometallic complexes for treating this condition. This Perspective categorizes these metal compounds based on their mechanisms of action, which include their use as delivery agents for gasotransmitters, inhibitors of  $mCa^{2+}$  uptake, and catalysts for the decomposition of ROS. Lastly, the challenges and opportunities for inorganic chemistry approaches to manage IRI are discussed.

## 1. INTRODUCTION

Researchers have leveraged the unique features of metal ions to develop metal-based small molecules for various applications within biology, marking an important research area within bioinorganic chemistry. In particular, the use of metal complexes with either therapeutic or diagnostic properties has led to the field of metals in medicine. Perhaps the most influential metal-based drug is the simple Werner coordination complex *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] known as cisplatin. This compound is a highly effective anticancer drug that is used clinically for several different cancer types, including ovarian and testicular.<sup>1</sup> The success of cisplatin subsequently led to the investigation and worldwide clinical approval of two other platinum-based drugs oxaliplatin<sup>2</sup> and carboplatin.<sup>3</sup> Mechanistically, these platinum-based drugs induce their anticancer properties through the formation of covalent DNA adducts, which inhibit transcription in cancer cells.<sup>4</sup> The success of these compounds has motivated significant efforts to develop new metal complexes as cytotoxic anticancer drugs with an emphasis on tuning their abilities to bind to DNA.<sup>5–7</sup> In fact, one could argue that the field of metals in medicine is dominated by such cytotoxic metal complexes with secondary aspects of research within the development of diagnostic agents like gadolinium-based magnetic resonance imaging (MRI) contrast agents.<sup>8</sup> An alternative role for metal complexes, however, is one that provides protective effects to cells, an approach that can lead to therapeutic agents for conditions like stroke and heart disease. The use of metal complexes as cytoprotective agents has only been scarcely explored with most efforts directed toward the development of superoxide dismutase (SOD) mimics for removing deleterious reactive oxygen species (ROS).<sup>9–12</sup> In this Perspective, we discuss the pathophysiological condition known as ischemia-

reperfusion injury (IRI) for which metal complexes can play an important therapeutic role.

IRI describes the irreparable cell death and tissue damage that is caused by the rapid reoxygenation and restoration of blood flow to hypoxic and ischemic organs (Figure 1). This process occurs after the medical intervention of stroke and heart failure, as well as in transplanted organs.<sup>13</sup> Although restoration of oxygen and blood is essential for immediate treatment, it also triggers the damaging effects of IRI that can negatively affect the success rates of these procedures and long-term patient survival. Thus far, there are no clinically approved drugs for the prevention or minimization of IRI. Consequently, IRI has been referred to as a “neglected therapeutic target,”<sup>14</sup> a concerning designation given its implication in heart failure and stroke, two of the leading causes of death in the United States.<sup>15</sup>

To develop therapeutic agents for this condition, an understanding of the cellular pathways that trigger its pathology are needed (Figure 1). When cells are deprived of oxygen, they switch their metabolic pathways from O<sub>2</sub>-dependent oxidative phosphorylation in the mitochondria to anaerobic glycolysis in the cytosol.<sup>13</sup> This change has two key implications on the intracellular environment. First, the mitochondrial membrane potential (MMP) is depolarized due to the lack of the transmembrane H<sup>+</sup> gradient that would

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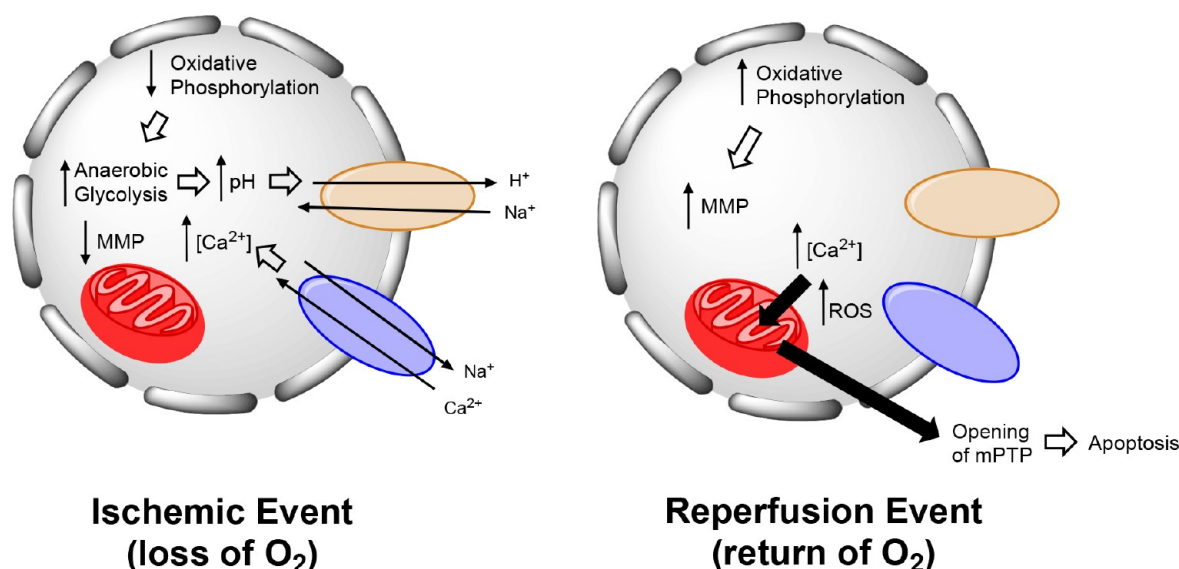


Figure 1. Intracellular biochemical events that contribute to IRI.

normally be generated by oxidative phosphorylation. Second, the production of lactic acid by anaerobic glycolysis leads to an excess of  $H^+$  ions in the cytosol, which are swapped for  $Ca^{2+}$  ions via the sequential operation of the  $Na^+/H^+$  and  $Na^+/Ca^{2+}$  exchanger proteins.<sup>16</sup> When reperfusion occurs, blood flow is restored, and oxygen is returned to cells. With oxygen levels suddenly elevated, the formation of ROS occurs, and the cell switches back to oxidative phosphorylation as its primary metabolic pathway, reestablishing the MMP.<sup>17</sup> Consequently, the return of the MMP provides a strong driving force for the cytosolic  $Ca^{2+}$  ions to enter the mitochondria via a transporter known as the mitochondrial calcium uniporter (MCU), triggering the phenomenon of mitochondrial  $Ca^{2+}$  ( $mCa^{2+}$ ) overload and subsequent opening of the mitochondrial permeability transition pore (mPTP) that leads to cell death.<sup>18</sup>

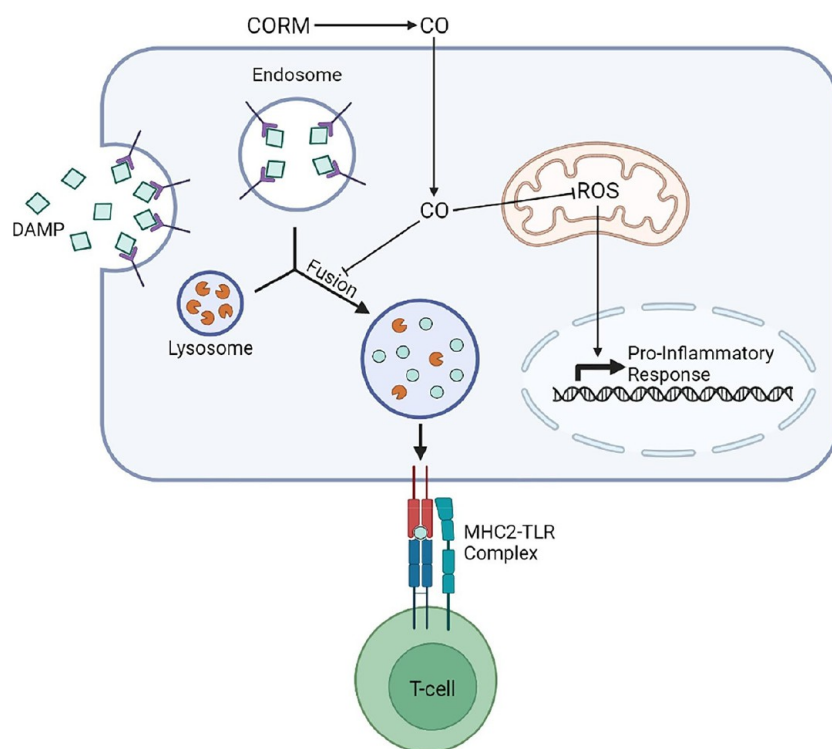
These cellular events that precede and trigger IRI provide opportunities and therapeutic targets to address this condition. The most commonly investigated drug candidate for IRI is the organic natural product cyclosporin A, which inhibits the opening of the mPTP.<sup>19</sup> Despite preclinical success with this compound, it has not advanced to clinical approval, due to its variable efficacies in trials<sup>20</sup> as well as side effects like neurotoxicity and chronic nephrotoxicity.<sup>21</sup> Thus, alternative drugs are needed, ideally ones that target different parts of the IRI pathway. In this Perspective, we will examine recent efforts to use the novel properties of metal-containing small molecules to develop therapeutic agents for IRI. To date, three main approaches have been investigated to leverage the unique properties of metal complexes. First, researchers have used coordination and organometallic complexes to deliver the gasotransmitters carbon monoxide (CO), nitric oxide (NO), and hydrogen sulfide ( $H_2S$ ), which are known to elicit cytoprotective effects at low concentrations. Second, metal complexes have been used as inhibitors of the MCU to prevent  $mCa^{2+}$  overload. Finally, the ability of metal complexes to cycle through different oxidation states has made them effective antioxidants that can catalytically decompose the harmful ROS produced during IRI. Each of these three strategies is discussed within this Perspective, highlighting the important role that the field of metals in medicine plays in the management of IRI.

## 2. GASOTRANSMITTER DELIVERY

The three toxic gases CO,  $H_2S$ , and NO have been recognized to be endogenously produced gasotransmitters that play an important role in regulating a variety of biological processes and give rise to anti-inflammatory, antiapoptotic, and antioxidant effects.<sup>22</sup> The appropriate application of these gasotransmitters can also protect against IRI.<sup>23</sup> In this section, an overview of the efficacy of these gasotransmitters as cytoprotective agents against IRI is given, followed by a highlight on specific recent examples of how metal complexes have been leveraged for their delivery to manage this pathological condition.

**2.1. Carbon Monoxide (CO).** CO is well-known for its toxicity. Concentrations above 10,000 ppm are lethal due to the ability of this gas to bind tightly to hemes, a property that stops mitochondrial respiration via inhibition of cytochrome *c* oxidase.<sup>24</sup> At lower concentrations, however, CO is produced endogenously and plays a vital role in cellular function and regulation.<sup>25,26</sup> The primary origin of endogenous CO is from the enzyme heme oxygenase-1 (HO-1), which is responsible for the catabolism of heme and is activated by a number of different cellular processes. The CO that is produced by HO-1-mediated heme decomposition provides a regulatory feedback loop in response to different biological stimuli.<sup>27,28</sup> The physiological importance of CO was demonstrated in HO-1-deficient mice, where the addition of this gasotransmitter exogenously was able to overcome the pathological effects associated with this defect.<sup>29,30</sup>

With the importance of endogenously produced CO recognized, researchers have also studied the biological and medicinal effects of exogenously administered CO. At low concentrations, this gasotransmitter has demonstrated therapeutic effects for a variety of pathophysiological conditions, such as cardiovascular disease, sepsis, and cancer, as well as beneficial properties for organ transplantation.<sup>31–33</sup> Notably, extensive *in vitro* and *in vivo* studies have shown that CO can protect against IRI.<sup>34–42</sup> These protective effects include the mitigation of apoptosis by decreasing the production of ATP and consequent lowering of mitochondrial ROS generation, the suppression of dendritic cell maturation, and the inhibition of toll-like receptor (TLR) activation by preventing endosomal

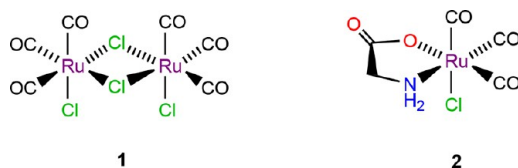


**Figure 2.** Mechanism of protection of CO against IRI. DAMPs (damage-associated molecular patterns) are molecules released in damaged or dying cells. Upon their release, these molecules trigger endosomal and lysosomal fusion and activate toll-like receptors (MHC2-TLR), which trigger a damaging inflammatory response. CO prevents lysosomal and endosomal fusion and can therefore attenuate this inflammatory response. In addition, CO decreases ATP production in the mitochondria, lowering the presence of ROS and slowing apoptosis. Reproduced with permission from ref 28. Copyright 2022 Elsevier Inc.

and lysosomal fusion (Figure 2).<sup>28</sup> However, challenges associated with the direct administration of gaseous CO at therapeutically beneficial levels indicate that alternative approaches are needed for its controlled delivery to hypoxic or ischemic tissue. As a means of accomplishing this goal, the development of CO-releasing molecules (CORMs), which comprise both organic and metal-containing complexes that act as prodrugs for this gasotransmitter, is an active field of biomedical research.<sup>43</sup> Because CO is a highly effective ligand for metal ions,<sup>44</sup> a property that is reflected by the long history of metal–carbonyl compounds dating back to the late 19th century,<sup>45</sup> coordination and organometallic complexes comprise a promising platform for the delivery of small, therapeutically relevant concentrations of this gasotransmitter for IRI.

Among the wide variety of metal–carbonyl complexes that have been investigated as CORMs,<sup>46</sup> CORM-2<sup>46</sup> (1, Chart 1), and CORM-3<sup>47</sup> (2, Chart 1) are arguably the most thoroughly studied with respect to their activities for the management of

**Chart 1.** Structures of CORM-2 (1) and CORM-3 (2) That Have Shown Protective Effects against IRI<sup>46,47a</sup>

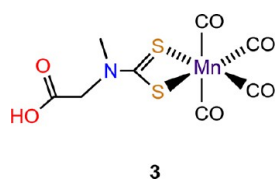


<sup>a</sup>These compounds release CO via ligand substitution displacement with solvent molecules.

IRI. Compound 1, a dinuclear, chlorido-bridged ruthenium (Ru) compound, showed a protective effect in several models of IRI by releasing CO into the extracellular milieu.<sup>48–55</sup> Despite the promising therapeutic effects of this complex, its low aqueous solubility and fast CO-release limited its use in further studies. A more soluble CORM, 2, is also able to reduce the effects of IRI.<sup>47,52,56–61</sup> The primary limitation of 2 for this application, however, arises from its challenging synthesis and solvent-dependent speciation.<sup>62</sup> Furthermore, recent studies suggest that some of the biological effects of this compound arise from the Ru byproducts rather than CO.<sup>63–66</sup> Based on these limitations, researchers have sought to develop CORMs with different transition metals<sup>27,67</sup> and metal-free CORMs.<sup>68,69</sup> The use of alternative metal CORMs, as specifically applied for IRI, is described below.

**2.1.1. Manganese CORMs.** Manganese (Mn) complexes with CO-releasing properties have been thoroughly investigated for biological applications over the past decade.<sup>27</sup> For many of these complexes, however, poor water solubility and fast CO release have limited their biomedical applications. The Mn(I) tetracarbonyl complex, CORM-401 (3, Chart 2), contains a bidentate dithiocarbamate ligand with a terminal carboxylic acid group that enhances water solubility while releasing 3 equiv of CO.<sup>70</sup> This compound exhibits protective effects against IRI both in vitro within H9c2 cardiomyocytes<sup>71</sup> and ex vivo within pig kidneys.<sup>72</sup> In these studies, treatment of the cells or tissue with 3 prior to subsection to IRI led to improvement in cell viability and tissue integrity. Within the pig kidney model, these protective effects were accompanied by a significant increase in blood CO levels, suggesting that this gasotransmitter is the active protecting agent.

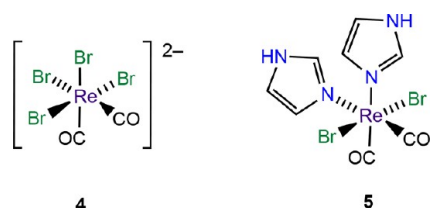
Chart 2. Structure of the Mn-Based CORM-401 (3) That Shows Protective Effects against IRI<sup>70a</sup>



<sup>a</sup>This compound releases CO by ligand substitution with water molecules in solution.

**2.1.2. Rhenium CORMs.** Although rhenium (Re) carbonyl complexes have been thoroughly investigated for their photochemical and catalytic properties, recent studies have revealed their potential for biomedical applications.<sup>73,74</sup> In particular, this class of compounds has found use as CORMs,<sup>75–78</sup> with several of them being specifically investigated as therapeutic agents for IRI. The Re(II) carbonyl complexes of the general formula *cis*-[Re(CO)<sub>2</sub>Br<sub>2</sub>L<sub>2</sub>]<sup>n-</sup> (Chart 3) are promising examples of Re-based CORMs for IRI.<sup>79</sup> The

Chart 3. Structures of Re(II) CORMs That Show Protective Effects against IRI<sup>79a</sup>

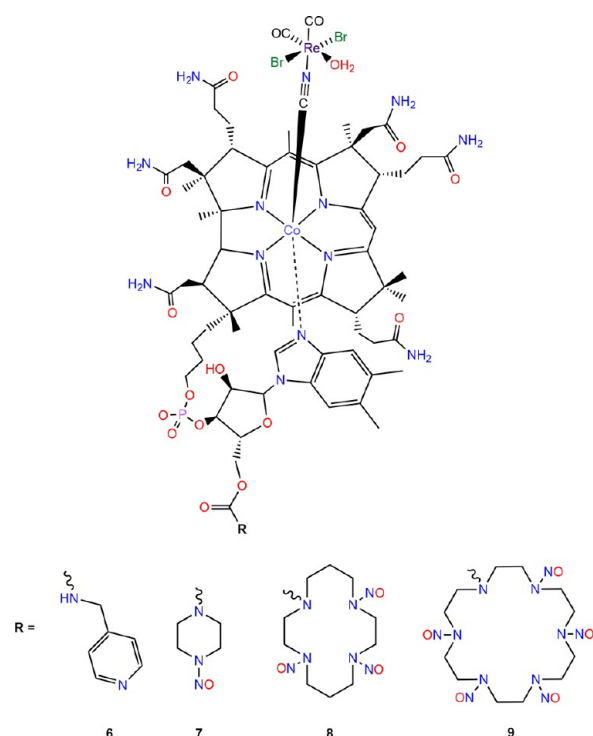


<sup>a</sup>The mechanism of CO-release of these complexes is still currently unknown, but is postulated to be through ligand substitution in physiological environments.

CO-release kinetics of these complexes under physiological conditions are comparable to those of the well-studied CORM-3 (2). For these complexes, L was altered to be different monodentate nitrogen-donor ligands to assess structure–activity relationships. Based on CO-release profiles, *cis*-[Re(CO)<sub>2</sub>Br<sub>4</sub>]<sup>2-</sup> (4, Chart 3) and *cis,trans*-[Re(CO)<sub>2</sub>Br<sub>2</sub>(Im)<sub>2</sub>] (5, Chart 3), where Im = imidazole, were identified to be the most promising complexes from this class. Accordingly, when neonatal rat ventricular cardiomyocytes in the presence or absence of these compounds were exposed to a short period of hypoxia followed by a brief reoxygenation period to model IRI, only 4 and 5 were able to increase cell survival relative to the untreated control. Importantly, cellular uptake studies revealed that these two compounds were not taken up by cells, suggesting that the delivery of extracellular CO is sufficient to elicit their cytoprotective effects. Based on these studies, it is clear that these Re(II) dicarbonyl complexes represent an important class of therapeutic agents for IRI.

Building upon the success of *cis*-[Re(CO)<sub>2</sub>Br<sub>4</sub>]<sup>2-</sup>, researchers sought to improve its biological properties by conjugating it to cyanocobalamin (vitamin B<sub>12</sub>) and an *N*-nitrosoamine-functionalized version of vitamin B<sub>12</sub>, adding to the growing number of multimetallic Re carbonyl complexes that have been investigated for biological applications.<sup>80,81</sup> In comparison to 4, which lacks the cyanocobalamin vector, compound 6 (Chart 4) exhibited improved stability in aqueous solution and demonstrated a more substantial cytoprotective effect within

Chart 4. Heteronuclear Co and Re Complexes Designed to Simultaneously Release CO and NO To Reduce Cell Death in Models of IRI<sup>82,83a</sup>

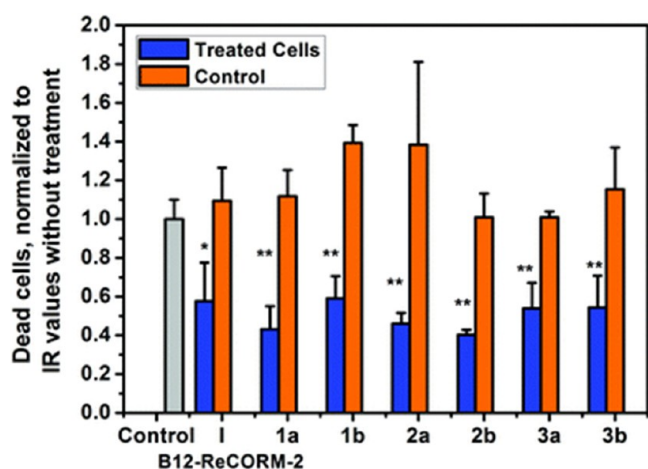


<sup>a</sup>The Re(II) component releases CO in a similar manner as compounds 4 and 5, whereas NO arises from the macrocyclic *N*-nitrosamines that can undergo protolytic denitrosation in water.

neonatal rat cardiomyocytes in a model of IRI.<sup>82</sup> To further increase the functionality of this compound class, *N*-nitrosamines were appended to the vitamin B<sub>12</sub> carriers to afford compounds 7–9 (Chart 4).

The presence of both the *N*-nitrosamine and Re carbonyl enables these complexes to be used for the release of both NO and CO, providing a dual-action basis for their therapeutic activities against IRI.<sup>83</sup> To assess the therapeutic efficacy of these compounds, 3T3 mouse fibroblast cells were incubated in the presence and absence of each complex and then exposed to a short period of hypoxia followed by a brief reoxygenation period. Cells that were treated with 7–9 demonstrated a 50% reduction in cell death compared to untreated cells. Despite these promising results, the cytoprotective effects of 7–9 were not greater than those of 4, the *N*-nitrosamine functionalized vitamin B<sub>12</sub> alone, or 6, which contains vitamin B<sub>12</sub> but lacks the *N*-nitrosamine (Figure 3). Although these results indicate that the dual NO and CO delivery strategy did not lead to significant enhancement of activity, it did highlight the value of vitamin B<sub>12</sub> as a conjugate that can improve the biological compatibility of CORMs.

**2.2. Hydrogen Sulfide (H<sub>2</sub>S).** Most commonly known as a toxic gas with a rotten egg smell, H<sub>2</sub>S has recently emerged as the third gasotransmitter, joining NO and CO in this important biological regulatory role.<sup>84</sup> Over the past two decades, endogenous H<sub>2</sub>S and related polysulfides (H<sub>2</sub>S<sub>n</sub>), produced via different enzymatic processes,<sup>85</sup> have been implicated in a wide variety of biological processes,<sup>86</sup> including cancer progression,<sup>87</sup> cellular metabolism,<sup>88</sup> neurological regulation,<sup>89,90</sup> cell death pathways,<sup>91</sup> and the regulation of



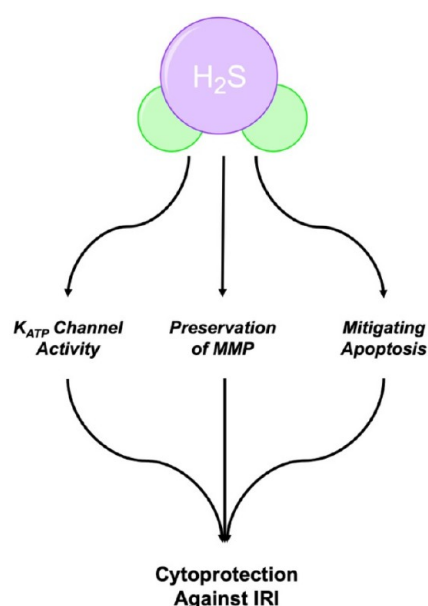
**Figure 3.** Protective effect of compounds 6–9 in an in vitro model of IRI. Blue traces represent cells treated with 30  $\mu\text{M}$  of each compound, whereas orange traces represent untreated cells. In this figure, compound 6 is B12-ReCORM-2, compound 7 is 1b, compound 8 is 2b, and compound 9 is 3b. The designations for 1a, 2a, and 3a are the vitamin B<sub>12</sub> conjugates without the appended *cis*-[Re(CO)<sub>2</sub>Br<sub>4</sub>]<sup>2-</sup>. Reproduced with permission from ref 83. Copyright 2016 Royal Society of Chemistry.

cardiovascular function.<sup>92</sup> Three H<sub>2</sub>S-generating enzymes, cystathionine- $\beta$ -synthase (CBS), cystathionine- $\gamma$ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST), are the primary producers of endogenous H<sub>2</sub>S and play a vital role in the aforementioned processes.<sup>93</sup> This importance has generated interest in the exploration of exogenous H<sub>2</sub>S as a therapeutic agent for pathological conditions such as stroke and IRI.<sup>94–96</sup>

With respect to the role of H<sub>2</sub>S in myocardial IRI, the endogenous production of H<sub>2</sub>S is vital to heart health.<sup>98</sup> Consequently, the infarct size of isolated rat hearts is larger when endogenous H<sub>2</sub>S production is inhibited, and the introduction of exogenous H<sub>2</sub>S reverses this effect.<sup>99</sup> In addition, the overexpression of CSE in isolated rat hearts reduces the effects of IRI, demonstrating that both endogenous and exogenous H<sub>2</sub>S can protect tissues from IRI.<sup>100</sup> H<sub>2</sub>S has additionally shown an attenuation of injury in ischemic stroke, demonstrating its efficacy in neurological systems.<sup>101–106</sup>

The cytoprotective properties of H<sub>2</sub>S in IRI are mediated by several targets (Figure 4).<sup>97</sup> The most well-documented mechanism is based on its activity on mitochondrial K<sub>ATP</sub> channels. This activation stimulates K<sup>+</sup> uptake in the mitochondria, which attenuates the MMP after reperfusion and diminishes the driving force for cytotoxic *m*Ca<sup>2+</sup> overload.<sup>107</sup> Furthermore, H<sub>2</sub>S inhibits cytochrome *c* oxidase,<sup>108</sup> which slows the production of ROS as reperfusion occurs and mitochondrial oxidative phosphorylation is restored.<sup>109</sup> A third cytoprotective mechanism of H<sub>2</sub>S arises from its ability to activate several important enzymes, like phosphatidylinositol-3-kinase (PI-3-kinase), which are involved in upregulated cell survival pathways and apoptosis prevention.<sup>110</sup>

Despite these beneficial effects, its toxicity at high concentrations and challenges associated with administering it as a gas have sparked significant research efforts to develop small molecules that can deliver H<sub>2</sub>S to biological systems at suitable concentrations for therapeutic use.<sup>95</sup> The design and implementation of stimuli-activated H<sub>2</sub>S donors has been particularly fruitful, yielding compounds that are triggered by

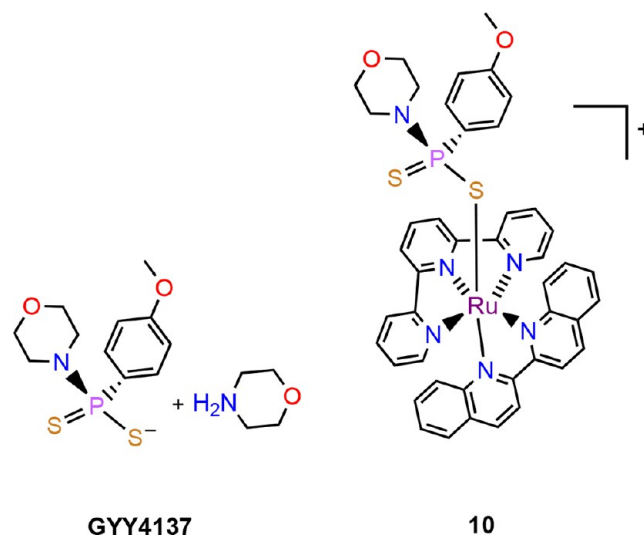


**Figure 4.** Mechanisms of H<sub>2</sub>S cytoprotection during events of IRI. Adapted with permission from ref 97. Copyright 2010 Elsevier, Inc.

external light,<sup>111–119</sup> ROS,<sup>120,121</sup> biological thiols,<sup>122–124</sup> enzymatic activity,<sup>125,126</sup> and pH.<sup>127</sup> Notably, in contrast to CORMs, which are mostly coordination and organometallic complexes, H<sub>2</sub>S donors are predominately organic compounds. The redox activity and acidity of H<sub>2</sub>S leads to challenges in affording metal-based donors of this gas that have only recently been addressed. Recent examples of metal-based H<sub>2</sub>S donors and their applications in IRI are described below.

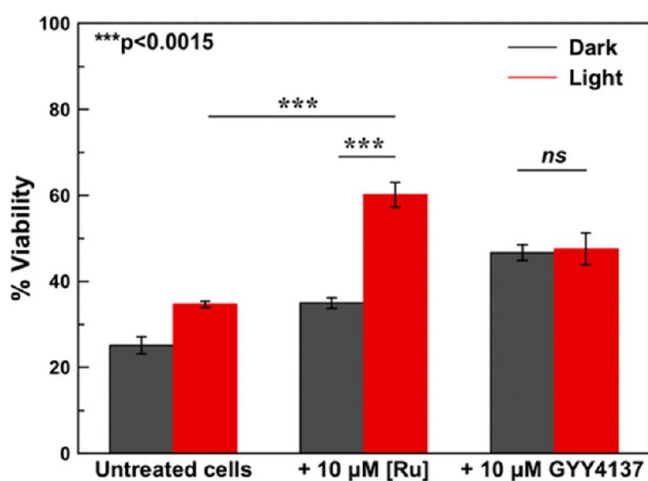
**2.2.1. Light-Activated H<sub>2</sub>S Delivery.** The organic compound morpholin-4-ium 4-methoxyphenyl-(morpholino)phosphino-dithioate (GY4137, Chart 5) is a well-studied H<sub>2</sub>S donor<sup>128,129</sup> that releases this gasotransmitter via hydrolysis over a time scale of several hours,<sup>130</sup> enabling its use for the

**Chart 5.** Structures of the H<sub>2</sub>S Donor GYY4137 and the Red Light-Activated Ru<sup>2+</sup> H<sub>2</sub>S Donor (10)<sup>a</sup>



<sup>a</sup>GY4137 releases H<sub>2</sub>S via hydrolysis of the P–S bonds. Compound 10 undergoes a photosubstitution reaction to release GYY4137, which subsequently hydrolyzes to produce H<sub>2</sub>S.<sup>132</sup>

treatment of IRI.<sup>131</sup> The hydrolysis of and H<sub>2</sub>S release from this compound occurs instantaneously upon dissolution in water, limiting the conditions in which it can be applied therapeutically. In an effort to control this H<sub>2</sub>S-release process, it was coordinated to a photoactive Ru<sup>2+</sup> polypyridyl complex (**10**, Chart 5).<sup>132</sup> When bound to the Ru<sup>2+</sup> center, the hydrolysis of GYY4137 is suppressed. Upon irradiation of **10** with red (631 nm) light, this H<sub>2</sub>S-donating ligand dissociates from the Ru<sup>2+</sup> coordination sphere, allowing it to undergo hydrolysis and release H<sub>2</sub>S in solution. In an in vitro model of IRI, H9c2 rat cardiomyoblast cells that were treated with **10** and irradiated with 631 nm light had substantially higher viability compared to untreated cells and treated cells not exposed to light (Figure 5), indicating that this light-activation



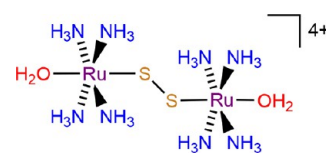
**Figure 5.** Protective effect of compound **10** ([Ru]) and GYY4137 in cells subjected to an in vitro model of IRI. Reproduced with permission from ref 132. Copyright 2018 American Chemical Society.

process operates in the cellular milieu as well. This complex, a red light-activated H<sub>2</sub>S donor, highlights the value of using metal complexes to leverage their photochemistry for this application.

**2.2.2. Reduction-Activated H<sub>2</sub>S Delivery.** The hypoxic nature of ischemic cells and tissues gives rise to a reducing environment that could potentially be leveraged for the activation of anti-IRI agents. In this context, the redox activity of metal complexes can be used to design compounds that are reduced under these conditions. This concept was investigated recently for the selective delivery of H<sub>2</sub>S from a Ru coordination compound. Upon chemical reduction of the dinuclear persulfide-bridged ( $\mu$ -S<sub>2</sub><sup>2-</sup>) Ru compound (**11**, Chart 6), the S–S bond is cleaved, leading to the release of H<sub>2</sub>S as a byproduct.<sup>134</sup> Importantly, it was demonstrated that this process occurs in the presence of biologically relevant reducing agents in aqueous solution, suggesting that this compound could be used for the therapeutic delivery of this gasotransmitter. To test this hypothesis, H9c2 rat cardiomyoblast cells were treated with **11** and then subjected to lethal hypoxia-reoxygenation. Under these conditions, the viability of the cells increased in the presence of higher concentrations of the complex. Thus, the initial hypoxic conditions found during IRI may be sufficient to activate this and related metal complexes by chemical reduction.

**2.3. Nitric Oxide (NO).** The biochemistry of NO has been studied extensively.<sup>135</sup> In particular, its protective effects

**Chart 6. Structure of a Redox-Activated Persulfide-Bridged Ru Complex (**11**)**<sup>134a</sup>



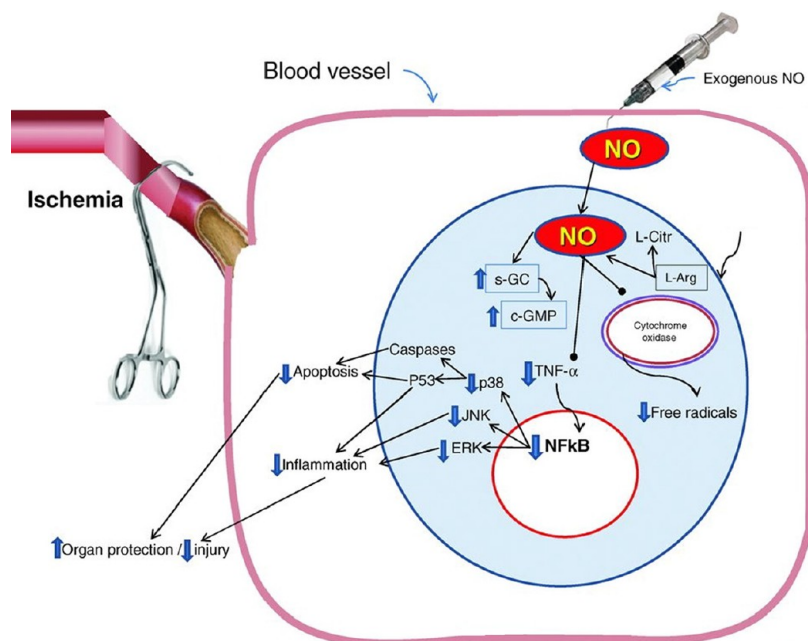
**11**

<sup>a</sup>Upon chemical reduction, the S–S bond is cleaved, leading to a terminal Ru–SH complex that releases H<sub>2</sub>S upon protonation in water.

against IRI have been widely reported, with both endogenously produced<sup>136–143</sup> and exogenously delivered<sup>139,144–146</sup> NO giving rise to these therapeutic properties. Although high concentrations of NO are cytotoxic, small doses have anti-inflammatory, antioxidant, and antiapoptotic effects.<sup>147</sup> The primary mechanism of protection of NO involves the inhibition of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), which is responsible for the activation of transcription factor NF- $\kappa$ B. This transcription factor triggers various events that lead to downstream apoptotic and inflammatory events. By suppressing TNF, these pathways are prevented, decreasing the damaging effects of IRI. In addition, NO acts as a radical scavenger and binds to cytochrome *c* oxidase, giving rise to its antioxidant properties. Finally, NO binds to and activates soluble guanylate cyclase (s-GC) in cells. This enzyme produces cyclic guanosine monophosphate (c-GMP), a messenger molecule that mitigates apoptosis through the modulation of caspase production (Figure 6).<sup>148</sup> In order to use NO as a therapeutic agent, it needs to be delivered in small, regulated quantities. To address this challenge, researchers have developed nitric oxide-releasing molecules (NORMs), which are capable of slow and sustained release of NO. Given the long-standing history and well-known photochemistry of metal nitrosyl complexes,<sup>149,150</sup> this compound class forms a promising basis for photoactivated NORMs for biological use.<sup>151,152</sup>

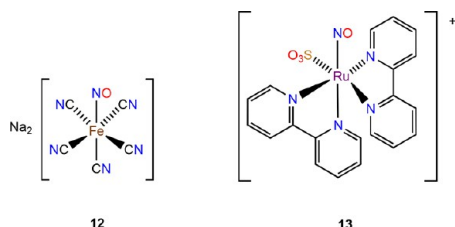
The longest used therapeutic NORM is arguably sodium nitroprusside, Na<sub>2</sub>[Fe(CN)<sub>5</sub>(NO)] (**12**, Chart 7). This iron (Fe) compound has been extensively applied in different models of IRI, demonstrating good efficacy in minimizing the damaging effects of this condition.<sup>153–157</sup> The release of NO from this complex is triggered by chemical reduction by biological reducing agents like cysteine and glutathione.<sup>158</sup> Compound **12** has been used in human patients for this condition as well.<sup>159</sup> Cancer patients with chest pains associated with myocardial ischemia were treated with a cotton pad soaked in 1.5 M **12** on the abdomen. Elevated levels of NO were detected in plasma taken from patients, and patients exposed to **12** showed a significantly lower death rate caused by acute myocardial infarction. These results suggest that NO can help reduce damage associated with IRI and that metal-based compounds are suitable delivery agents for this gas.

Despite the frequent use of the Fe-based **12**, researchers have more extensively studied Ru-based NORMs to leverage the greater inertness of this 4d transition metal. Although a large number of these Ru NORMs have been studied, in general, they have not yet been extensively applied to address IRI.<sup>160</sup> A notable example of one such NORM used for this



**Figure 6.** Mechanisms of protection against IRI by exogenous NO. NO lowers the production of free radicals by binding to cytochrome *c* oxidase. It also inhibits TNF- $\alpha$ , which deactivates NF- $\kappa$ B, shutting down several mitogen-activated protein kinases such as p38, extracellular signal-regulated kinases (ERK), and c-Jun N-terminal kinases (JNK). These kinases are linked to intensifying inflammation and apoptosis through the release of caspases and the tumor protein p53. Finally, NO activates s-GC, leading to an enhanced production of c-GMP. Reproduced with permission from ref 148. Copyright 2009 Taylor & Francis Group.

**Chart 7. Structures of Sodium Nitroprusside (12) and *cis*-[Ru(bpy)<sub>2</sub>(SO<sub>3</sub>)(NO)]<sup>+</sup> (13), an NO-Releasing Molecule with Demonstrated Neuroprotective Effect against IRI<sup>161a</sup>**



<sup>a</sup>These compounds release NO upon reaction with biological thiols.

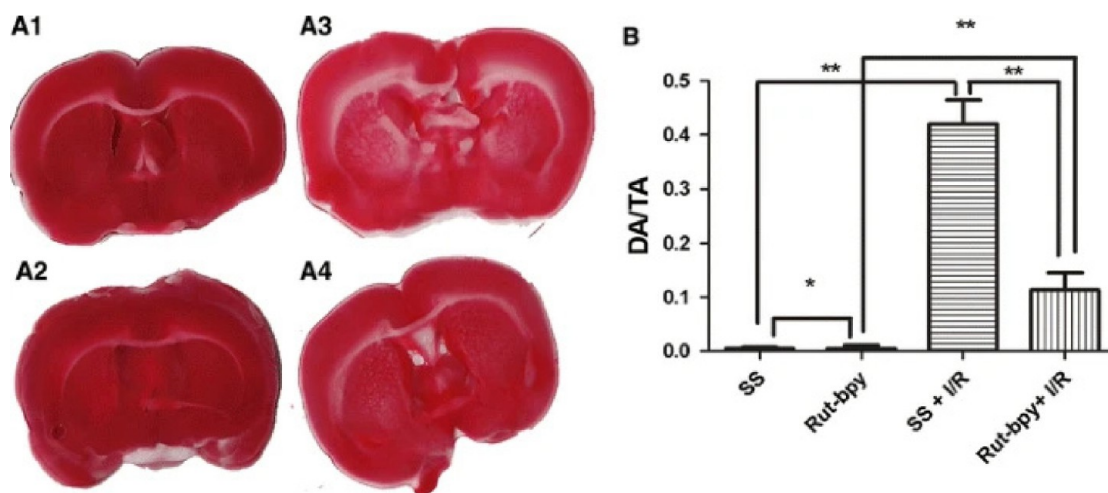
purpose is the Ru nitrosyl complex *cis*-[Ru(bpy)<sub>2</sub>(SO<sub>3</sub>)(NO)]<sup>+</sup> (13, Chart 7).<sup>161</sup> The protective effects of this compound against an *in vivo* model of cerebral IRI were investigated in rats. Cessation of arterial blood flow to the brain via occlusion of the carotid artery for 30 min, followed by a 60 min reperfusion period, led to the formation of significant infarct regions within the brain. In rats pretreated with this complex via intraperitoneal (*i.p.*) injection, however, the size of the infarct regions of 2,3,4-triphenyltetrazolium chloride (TTC)-stained coronal brain sections were significantly smaller, demonstrating the therapeutic viability of this compound (Figure 7). Furthermore, elevated levels of nitrite, an *in vivo* metabolite of NO, and decreased NF- $\kappa$ B expression in the hippocampus were detected in the treated rats, implicating NO to be the mediator of the observed biological effects.

### 3. PREVENTING MITOCHONDRIAL CALCIUM OVERLOAD

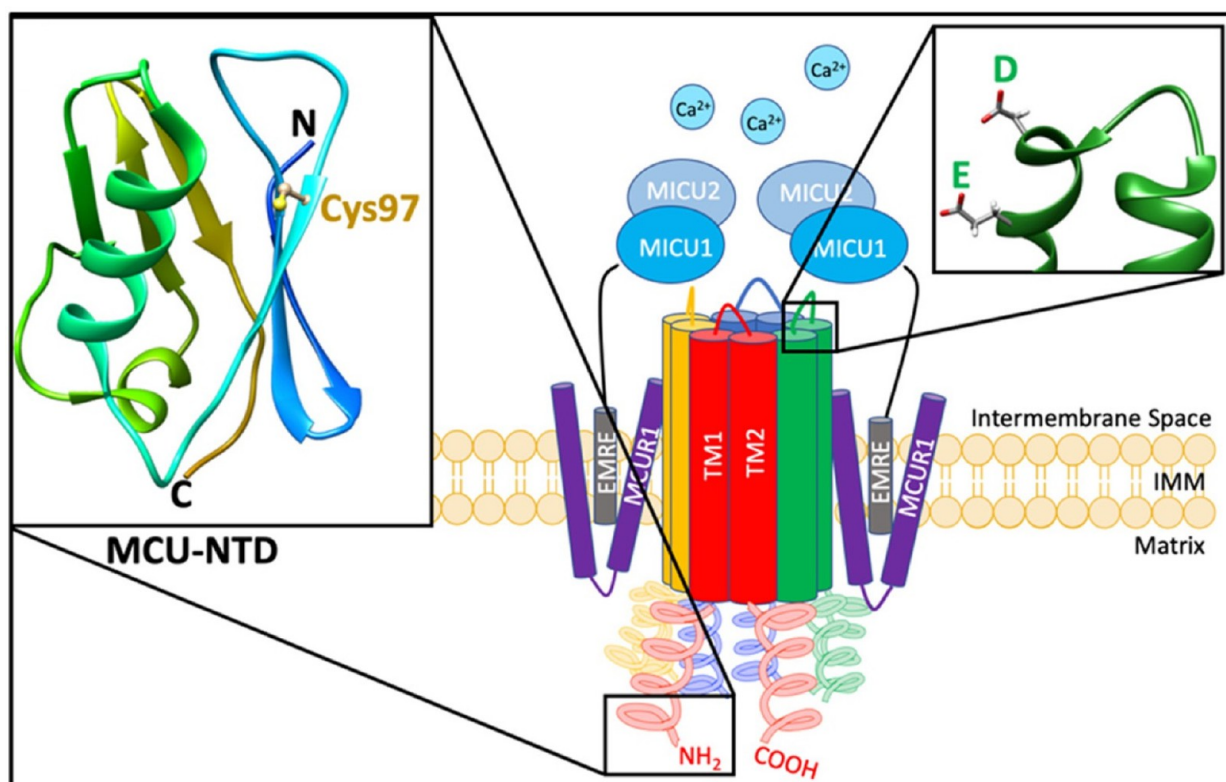
As mentioned in the Introduction, *m*Ca<sup>2+</sup> overload is one of the key intracellular processes that causes the harmful effects of IRI. Thus, inhibition of *m*Ca<sup>2+</sup> uptake to prevent *m*Ca<sup>2+</sup>

overload has been proposed and investigated as a therapeutic strategy to prevent IRI. These efforts have focused on targeting the MCU, a transmembrane protein complex that mediates Ca<sup>2+</sup> uptake into the mitochondria.<sup>162–166</sup> The tetrameric MCU complex<sup>167–170</sup> comprises the MCU subunit and the regulatory EMRE,<sup>171</sup> MICU1,<sup>172</sup> and MICU2<sup>173</sup> subunits. The EF-hand domains of the MICU1 and MICU2 subunits can recognize and respond to high cytosolic Ca<sup>2+</sup> concentrations, dissociating from the pore-forming MCU subunit to allow for *m*Ca<sup>2+</sup> uptake.<sup>173–176</sup> The MICU regulatory proteins interact with a highly conserved solvent-exposed DXXE motif at the pore of the MCU subunit (Figure 8).<sup>167,168</sup> This motif interacts directly with and mediates the uptake of Ca<sup>2+</sup> ions.<sup>177,178</sup> Although a number of organic compounds that possess MCU-inhibitory activities have recently been identified,<sup>179–184</sup> the earliest and most commonly used MCU inhibitors are metal coordination complexes. These complexes, as well as new discoveries in this area, are discussed below.

**3.1. Multinuclear Ruthenium Complexes.** The oxo-bridged trinuclear Ru complex ruthenium red<sup>186,187</sup> (14, Chart 8) was one of the first compounds discovered to inhibit *m*Ca<sup>2+</sup> uptake.<sup>188</sup> Although it was originally used as a cytological stain,<sup>189</sup> its MCU-inhibitory activity led to its widespread implementation in different biological studies,<sup>188,190–192</sup> and it was demonstrated to attenuate the downstream effects of IRI in an *ex vivo* model.<sup>193</sup> The use of 14 for these applications, however, has been limited by its poor purity, which has led to batch to batch variations in activity depending on the supplier.<sup>194–196</sup> An important observation from these studies was that the *m*Ca<sup>2+</sup> uptake-inhibitory properties of 14 actually decreased as the purity of the compound increased, implying that another species was primarily responsible for this property.<sup>197</sup> Accordingly, the dinuclear oxo-bridged complex, Ru360 (15, Chart 8), was identified as an impurity within 14 that possesses potent nM *m*Ca<sup>2+</sup> uptake inhibitory proper-



**Figure 7.** (A) TTC-stained coronal brain sections treated with either saline solution (SS) or compound **13** (Rut-bpy). Sections A1 (SS-treated) and A2 (**13**-treated) did not undergo IRI, while sections A3 (SS-treated) and A4 (**13**-treated) were exposed to an IRI model. (B) The calculated damaged area (DA) to total area (TA) ratio of each TTC-stained brain section. Reproduced with permission from ref 161. Copyright 2011 Springer Science Business Media, LLC.



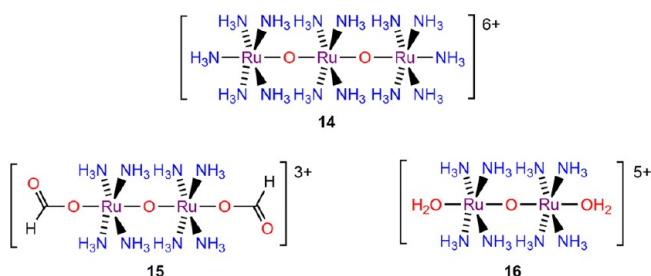
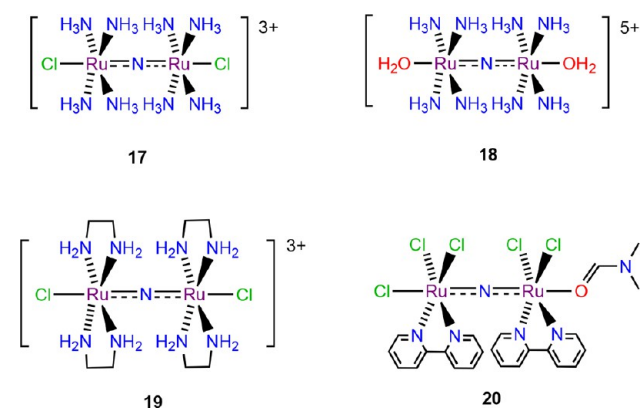
**Figure 8.** Topology of the MCU. Shown are the relevant regulatory proteins EMRE, MCUR1, MICU1, and MICU2 and the orientation of the transmembrane domains (TM1 and TM2) of the MCU within the inner mitochondrial membrane (IMM). The insets depict (left) the N-terminal domain of the MCU (MCU-NTD) and (right) the location of the DXXE motif in the MCU pore. Adapted with permission from ref 185. Copyright 2019 American Chemical Society.

ties.<sup>198–200</sup> Since this discovery, **15** has become the most frequently used MCU inhibitor, employed primarily as a tool to study  $m\text{Ca}^{2+}$  dynamics and regulation. Although its therapeutic potential for the prevention of IRI was demonstrated,<sup>201,202</sup> its more widespread applicability is limited by its poor cell permeability and stability.<sup>203</sup> The axial formate ligands of **15** undergo a rapid aquation reaction in buffered solutions, affording the diaqua-capped analogue

Ru360' (**16**, Chart 8), which can be synthesized independently.<sup>204</sup> The rapid aquation of **15** implies that **16** is the active inhibitor.

Given the high potency of **15**, analogues of this compound with improved stability and cell permeability were sought. The use of a bridging nitrido, rather than oxo, ligand afforded the compounds Ru265 (**17**, Chart 9)<sup>185</sup> and Ru265' (**18**, Chart 9).<sup>205</sup> These analogues retained the nM potency for MCU



**Chart 8. Structures of Previously Reported Multinuclear Oxo-Bridged Ru MCU Inhibitors**<sup>186,189,198–200,204</sup>**Chart 9. Structures of the Dinuclear Nitrido-Bridged Ru MCU Inhibitors**<sup>185,205,207</sup>

inhibition observed for **15**. Unlike **15**, these compounds were also able to inhibit the MCU in intact, non-permeabilized cells. A series of studies were carried out to study the origin of the enhanced cell permeability of **17** compared to **15**. These results suggest that this property is a consequence of the greater redox stability of **17**. Compound **15** is reduced by common biological reductants like glutathione, affording products with no MCU-inhibitory properties.<sup>205</sup> By contrast, **17** remains intact in the presence of reducing agents. Thus, the current working model is that extracellular reduction and decomposition of **15** forms species that are not cell permeable nor active MCU inhibitors. Importantly, like **15**, **17** interacts with the DXXE motif of the MCU, as evidenced by site-directed mutagenesis studies<sup>163,178</sup> and molecular docking,<sup>206</sup> indicating that both compounds have the same molecular target and mechanism of action.

Given the better cell permeability and enhanced stability of **17**, it was demonstrated to be an effective protective agent in *in vitro* hypoxia-reoxygenation assays within both rat ventricular myocytes<sup>185</sup> and primary cortical neurons.<sup>208</sup> In addition to preserving cell viability, **17** also showed no negative effects on mitochondrial integrity.<sup>185</sup> Furthermore, **17** was effective *in vivo*, substantially reducing the brain infarct size within mice that had been subjected to a model of ischemic stroke (Figure 9).<sup>208</sup> In addition to **17**, the ethylenediamine (en) (**19**, Chart 9) and 2,2'-bipyridine (bpy) (**20**, Chart 9) analogues of these compounds were investigated. The en analogue **19** was a substantially less effective MCU inhibitor than **17** with poorer cell permeability,<sup>185</sup> and **20** with bpy ligands was completely inactive with respect to MCU inhibition.<sup>207</sup> These results indicate that the equatorial ammine ligands are important for the MCU-inhibitory properties of this compound class.

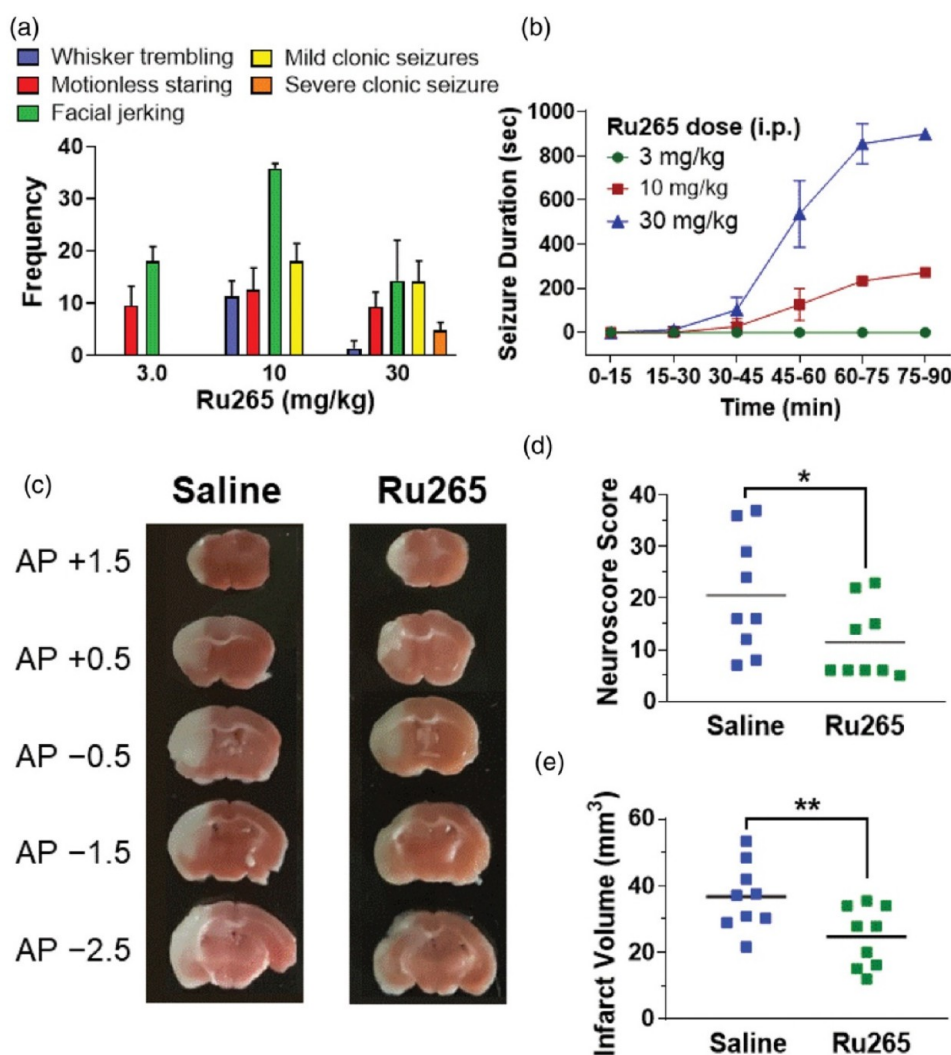
**3.2. Dinuclear Osmium Complexes.** Building upon the success of **17** as an MCU inhibitor, an analogue containing osmium (Os), instead of Ru, was investigated. The Os analogue, named Os245 (**21**, Chart 10), was also able to inhibit the MCU in intact cells and was stable toward biological reductants.<sup>209</sup> This compound protected primary cortical neurons exposed to oxygen-glucose deprivation, an *in vitro* model for IRI, without causing any negative effects on mitochondrial function. A significant difference between **17** and **21** arises within their axial ligand aquation kinetics. For **17**, the chlorido ligands are displaced by water under physiologically relevant conditions with a half-life of only 2.3 min, whereas the half-life for this process for **21** is 700.1 min. Accordingly, the diaqua analogue of **21**, Os245' (**22**, Chart 10), exhibits different MCU-inhibitory activity than its parent compound. Compound **22** is equally potent as **17** and **18**, and 100-fold more potent than **21**. These results suggest that the axial chlorido ligands, which remain bound to the Os centers on **21** for a substantial length of time, act to diminish the MCU-inhibitory activity of this compound class.<sup>205</sup> Molecular docking simulations support the higher potency of the diaqua compounds, as these coordinated water ligands are engaged in hydrogen-bonding interactions with acidic residues within the MCU pore entry.<sup>206,209</sup> Both of these nitrido-bridged Ru and Os dinuclear complexes are among the most potent MCU inhibitors reported to date, and their redox stability and cell permeability make them excellent therapeutic candidates for the prevention of IRI. Further functionalization of the axial sites of this compound class has also shown promise for the improvement of their delivery with added chemical functionalities,<sup>210–212</sup> suggesting that axial ligand modification is a viable pathway for identifying new lead compounds.

The major limitation of this compound class is their relatively low *in vivo* therapeutic window, which when exceeded causes seizures in mice (Figure 9). The *i.p.* injection of **17** in adult male mice caused seizures at doses of 10 mg kg<sup>-1</sup> or higher 45 min after treatment.<sup>208</sup> For **21**, the onset of seizures was observed at similar doses, but was delayed by nearly 30 min.<sup>209</sup> These side effects present challenges in the use of these compounds as therapeutic agents for IRI. Optimization of these compounds to increase their therapeutic windows is an important objective in using MCU inhibition as an approach for treating IRI.

#### 4. ROS SCAVENGERS

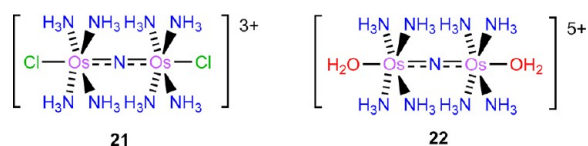
Ischemic cells and tissue are ill-equipped to handle the surge of oxygen that returns upon reperfusion. Consequently, ROS, which include hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (HO<sup>•</sup>), and superoxide (O<sub>2</sub><sup>•-</sup>), are produced by the reduction of dioxygen as undesired side products of the mitochondrial respiratory chain,<sup>213</sup> and contribute to the cell damage and death that is characteristic of IRI.<sup>214</sup> Although it has recently been recognized that low levels of ROS play key roles in cellular regulatory and signaling processes, high concentrations of these species damage critical biomolecules and lead to cell death.<sup>215</sup> As such, cells have evolved sophisticated enzymes, like catalases and SODs, that can decompose these ROS to prevent cellular damage.<sup>216,217</sup> Inspired by nature, researchers have developed small molecules with similar catalytic properties and applied them as therapeutic agents to decompose ROS and protect against IRI.<sup>218,219</sup>

SODs catalyze the dismutation of O<sub>2</sub><sup>•-</sup> into H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>,<sup>220,221</sup> whereas catalase facilitates the decomposition of

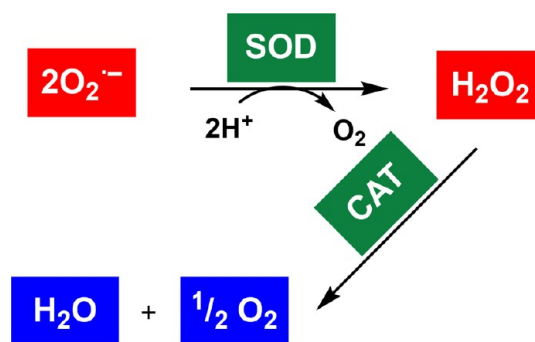


**Figure 9.** In vivo effects within mice treated with Ru265 (17). (a, b) Durations of seizures detected over 90 min after i.p. injection of 17 at varying concentration. (c) Representative TTC-stained brain sections after subjection to 24 h of hypoxic injury. (d, e) Neuroscores and infarct volumes in mice injected with saline or 3 mg kg<sup>-1</sup> 17 after 24 h of IRI. Reproduced with permission from ref 208. Copyright 2020 Sage Publications.

### Chart 10. Structures of the Dinuclear Nitrido-Bridged Os MCU Inhibitors<sup>209</sup>

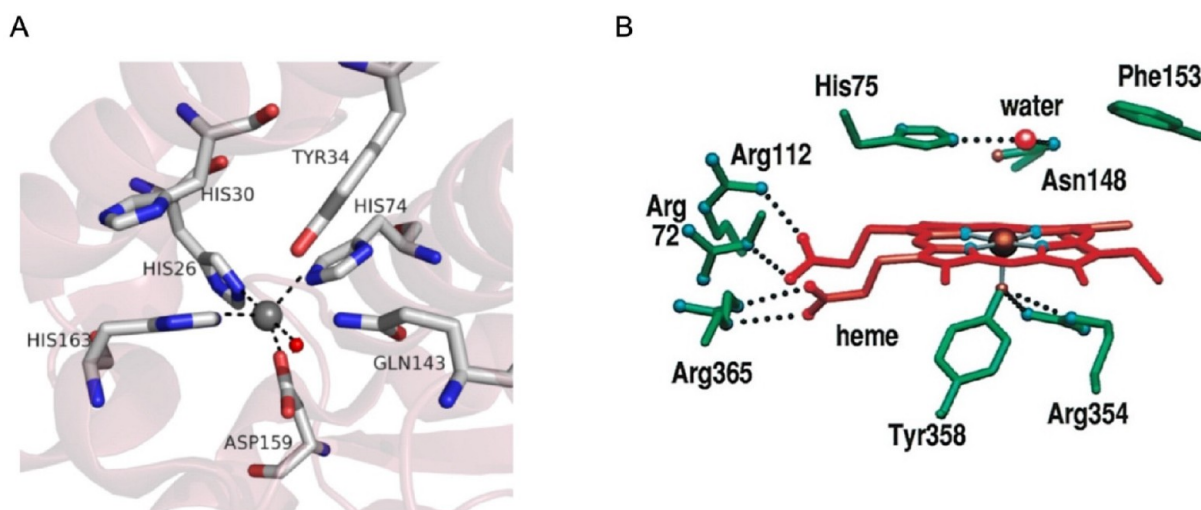


H<sub>2</sub>O<sub>2</sub> into O<sub>2</sub> and H<sub>2</sub>O (Figure 10).<sup>222</sup> Mammals express three types of SOD: manganese SOD (MnSOD), found primarily in the mitochondria, copper/zinc SOD (CuZnSOD), the major SOD in cells found within the nucleus and cytosol, and extracellular SOD (ECSOD), which has similar dinuclear Cu/Zn active sites as CuZnSOD.<sup>223,224</sup> Catalases in mammals are classified into three groups: monofunctional heme-containing catalases, heme-containing catalase peroxidases, and Mn-containing catalases.<sup>225</sup> The therapeutic potential of systems that can catalytically decompose ROS is evident in various studies that explored the effects of different expression profiles of SOD and catalase within animals. For example, overexpression of MnSOD within the hearts of transgenic mice rendered them substantially less susceptible to the damaging



**Figure 10.** Mechanism of ROS dismutation by the enzymes SOD and catalase (CAT).

effects of myocardial IRI.<sup>226</sup> With respect to catalase, its deficiency has been linked to a wide variety of diseases and disorders, including neurological disorders, cancer, and certain metabolic disorders.<sup>222</sup> Accordingly, the overexpression of catalase confers protective effects against IRI in vivo.<sup>227</sup> These studies suggest that the use of small-molecule SOD and



**Figure 11.** A. Active site of human MnSOD (PDB 1N0J, ref 227). Adapted with permission from ref 229. Copyright 2010 Elsevier Inc. B. Active site of human catalase (PDB 1DGF, ref 229). Adapted with permission from ref 230. Copyright 2000 Elsevier Inc.

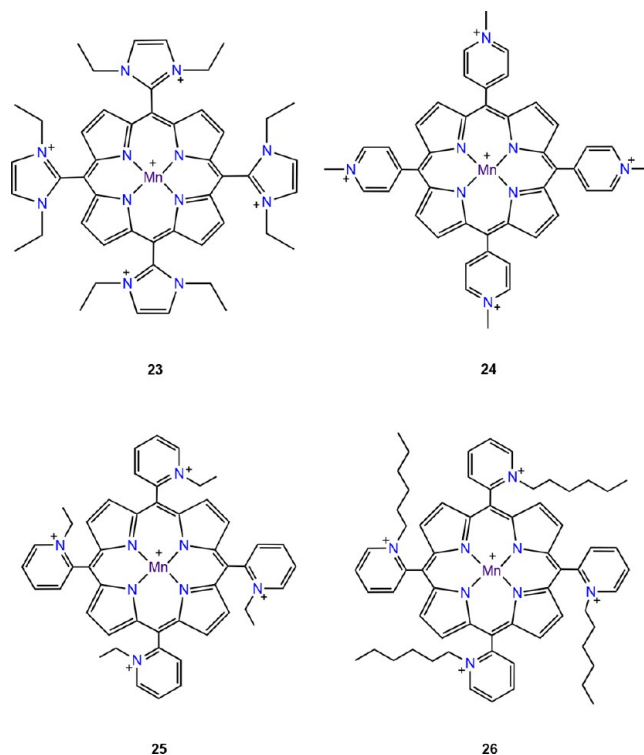
catalase mimics may be a therapeutically viable strategy for the management of IRI.

To design suitable small-molecule analogues, consideration of the metal-containing active sites of these enzymes is needed. With respect to SOD mimics, most efforts have focused on modeling MnSOD rather than CuZnSOD, due to the fact that the former provides a simpler mononuclear active site. The active site of human MnSOD (Figure 11a) comprises a Mn center in a trigonal bipyramidal geometry coordinated by three histidine and one aspartate residues, as well as a labile water molecule.<sup>228,229</sup> Human catalases are tetrameric proteins with a heme-Fe active site (Figure 11b). Unsurprisingly, the Fe center attains a five-coordinate, square pyramidal geometry, supported by the porphyrin donor atoms and an axial labile site occupied by water in the resting state.<sup>230</sup> As described below, small-molecule mimics of these enzymes have adopted similar primary coordination sphere features of their active sites.

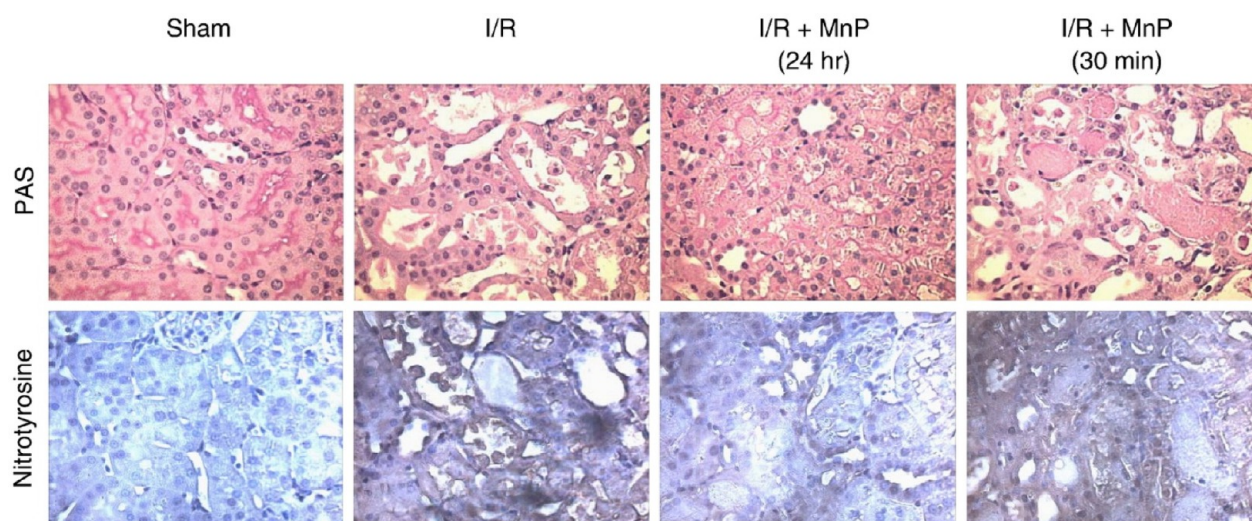
**4.1. Mn(III) Porphyrins.** A very common class of small-molecule SOD/catalase mimics are Mn(III) porphyrin complexes. The Mn(III) porphyrin, AEOL 10150 (**23**, Chart 11), was investigated in various clinical trials for its application as a catalytic antioxidant, displaying both SOD and catalase activities.<sup>231–235</sup> Compound **23** was protective in an in vitro model of neuronal oxygen-glucose deprivation and was also able to decrease the brain infarct size in mice subjected to an ischemic stroke model.<sup>236</sup> Other Mn(III) porphyrins (**24–25**, Chart 11) were found to attenuate the infarct size of ischemic brain tissue of rats.<sup>237</sup> These protective effects correlated with decreased ROS levels in the brain, indicating that their catalase and SOD activities are responsible for ameliorating the effects of IRI. Notably, the administration of these complexes both before and after the reperfusion gave rise to protective effects, suggesting that depletion of ROS can be therapeutic even after the IRI event.

Modifying the substituents of the porphyrin provides a versatile means of altering and improving the catalytic activities and therapeutic potentials of their Mn(III) complexes. Adding *N*-hexylpyridinium substituents onto the porphyrin (**26**, Chart 11) led to the discovery of a MnSOD mimic that has a rate of O<sub>2</sub><sup>•-</sup> dismutation on the same scale as native SOD enzymes.<sup>240</sup> This dramatic increase in rate compared to compounds **24** and **25** suggested that this complex afforded significant promise for

**Chart 11.** Structures of SOD/Catalase Mimics That Showed Attenuation of Neuronal IRI<sup>236–239</sup>



IRI. Researchers thus investigated the ability of this complex to attenuate the effects of IRI ex vivo with primary renal tissue<sup>238</sup> and in vivo, examining the effects of IRI on the spinal cord, with adult female rats.<sup>239</sup> In the ex vivo model, a dose of 50 μg kg<sup>-1</sup> of **26** for 30 min followed by a 40 min ischemic period and an 18 h reperfusion period showed minimal protection in renal tissue, but treatment with **26** for 24 h showed significant reduction in tissue damage (Figure 12).<sup>238</sup> Longer pretreatment periods of **26** afforded a greater attenuation of damage in the in vivo spinal cord model, indicating that the presence of this compound during the IRI event was vital. The measurement of ROS within the relevant tissues of the treated animals revealed them to be lower than those within untreated animals,

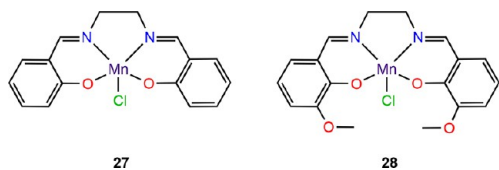


**Figure 12.** Renal tissue that has been stained with periodic acid-Schiff (PAS) and an anti-nitrotyrosine antibody after IRI (I/R) with and without pretreatment of **26** (MnP). Sham = No IRI. PAS staining demonstrates the presence of polysaccharides in tissue and was used to examine histopathological changes. The anti-nitrotyrosine antibody staining detects nitrotyrosine, a metabolite of tyrosine that arises from oxidative nitration (dark staining surrounding damaged white space within tissue). In both cases, the even distribution of the PAS stain is characteristic of undamaged tissue, whereas white regions indicate significant morphological damage. Furthermore, the lower intensity of the nitrotyrosine staining within the treated mice indicate less oxidative nitration occurred. Reproduced with permission from ref 238. Copyright 2007 Elsevier Inc.

supporting that the therapeutic activity of **26** is mediated by its antioxidant properties.

**4.2. Salen and Macrocyclic Complexes.** The well-known tetradentate Schiff base salen ligands afford metal complexes with SOD and catalase activities.<sup>241,242</sup> Two such Mn(III) salen complexes, EUK-8 (**27**, Chart 12) and EUK-134

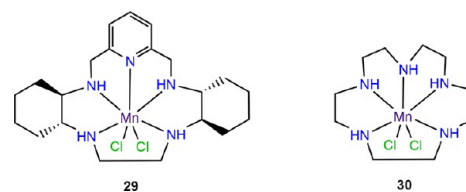
**Chart 12. Structures of EUK-8 (27) and EUK-134 (28), Salen Mn(III) Complexes That Can Attenuate the Effects of IRI**<sup>249,250</sup>



(**28**, Chart 12), possess exceptional SOD and catalase activities<sup>243,244</sup> that have been leveraged for a variety of biological applications.<sup>245–247</sup> Notably, these compounds have been applied for the management of IRI in various models and conditions.<sup>248–253</sup> Compound **27** was found to preserve normal cardiac and mitochondrial function in rats subjected to IRI.<sup>249</sup> Compound **28** was able to decrease the brain infarct size in rats subjected to an ischemic stroke more effectively than compound **27**, indicating that the methoxy groups improved the therapeutic potential, presumably due to the higher catalase activity of **28** compared to **27**.<sup>250</sup>

A series of pentaazamacrocyclic complexes of Mn(II) are also effective SOD mimics.<sup>254,255</sup> Most notably, the compound M40403 (**29**, Chart 13)<sup>256,257</sup> has progressed to clinical trials for the treatment of various pathological conditions related to elevated levels of ROS.<sup>258–261</sup> This complex has specifically demonstrated protective effects against models of IRI. Treatment of rats exposed to ischemia and sequential reperfusion periods with compound **29** led to smaller myocardial infarct sizes at concentrations of 1 mg kg<sup>-1</sup> or higher.<sup>262</sup> In addition, the compound SC-52608 (**30**, Chart

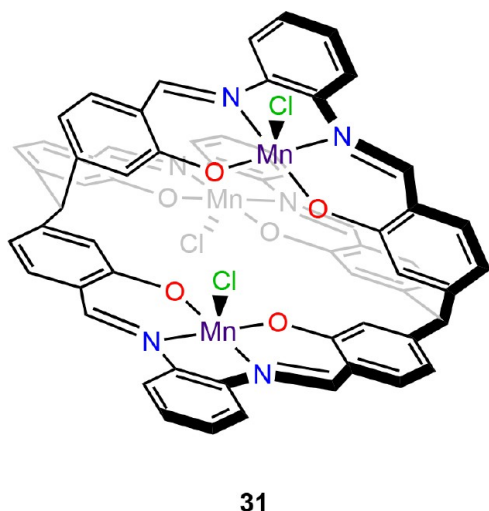
**Chart 13. Structures of M40403 (29) and SC-52608 (30), Macrocyclic Mn(II) Mimics of SOD/Catalase That Protect against IRI**<sup>256,257,263</sup>



**13**)<sup>263</sup> has shown a significant protective effect against IRI in both isolated rabbit hearts<sup>264</sup> and an in vivo model of myocardial IRI within dogs.<sup>265</sup> In both models, the myocardial infarct sizes were significantly smaller within those treated with **30** compared to the control.

A general limitation of many SOD mimics arises from their production of H<sub>2</sub>O<sub>2</sub>. If their catalase activity is not sufficiently fast to further decompose H<sub>2</sub>O<sub>2</sub>, this ROS can undergo redox chemistry with intracellular Fe, which catalyzes the Fenton reaction and produces highly toxic HO•.<sup>266,267</sup> Avoiding the production of HO• can be achieved by a more rapid succession of catalase activity, a feature that is lacking in some of the prior Mn complexes discussed, limiting their therapeutic potentials.<sup>268</sup> A recent study described a trinuclear Mn(III) salen complex, supported by a cryptand-like ligand (**31**, Chart 14).<sup>269</sup> This complex showed catalase activity and was able to decrease H<sub>2</sub>O<sub>2</sub> levels in vitro without producing HO•. Based on these promising results, this complex was evaluated in an in vivo ischemic stroke model. In rats treated with **31** via intracerebroventricular injection, the size of the brain infarct, as measured by post-mortem TTC staining and in vivo <sup>18</sup>F-fluodeoxyglucose ([<sup>18</sup>F]FDG) positron emission tomography (PET), was significantly smaller than that in untreated rats (Figure 13). Additionally, the large magnetic moment of the trinuclear compound **31** enabled its use as a MRI contrast agent. Compound **31** could be directly detected via MRI and was shown to be present throughout the brain with this

Chart 14. Structure of a Salen-Based, tri-Mn Metallocryptand (31) with Protective Effects against Ischemic Stroke<sup>269</sup>



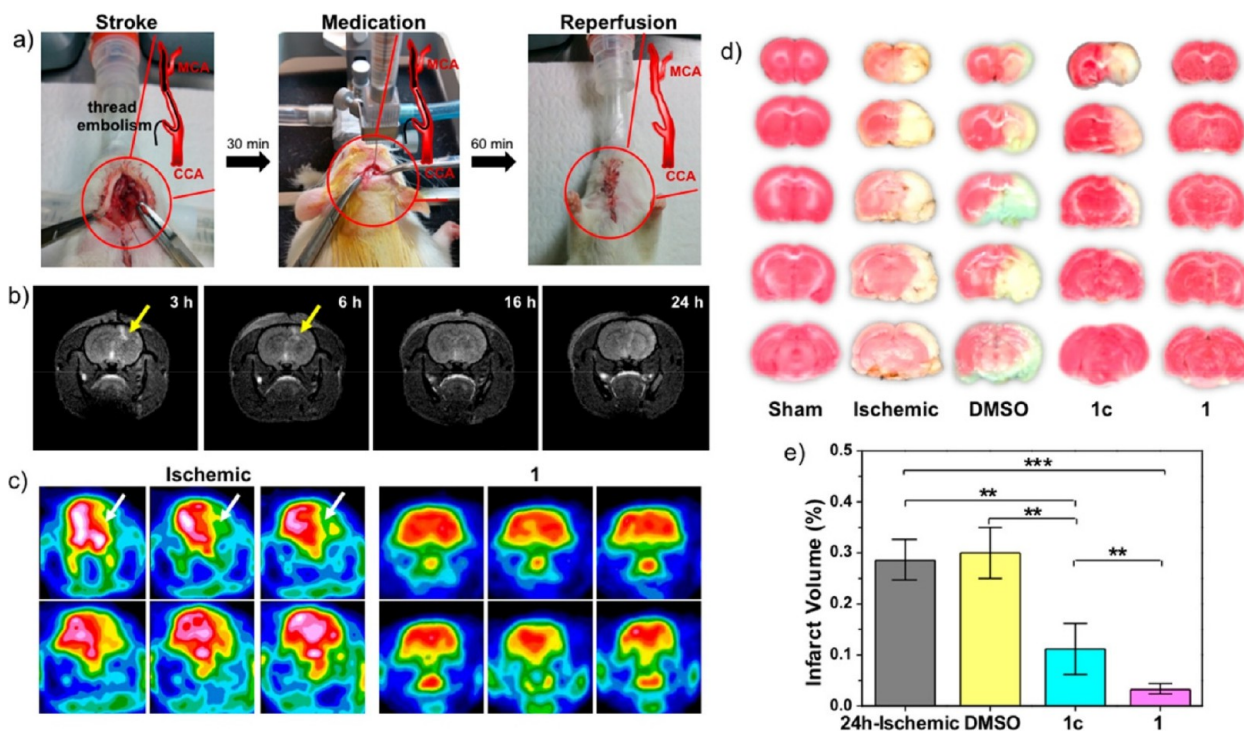
imaging technique. The dual antioxidant and imaging properties of **31** could render it valuable as a theranostic agents and further highlights the value of Mn complexes as MRI contrast agents.<sup>270,271</sup>

## 5. CONCLUSIONS

In this Perspective, we examined the potential of metal coordination complexes as therapeutic agents for IRI. Given the current lack of clinically approved preventative measures

for this condition, bioinorganic chemists can play an important role in this area by targeting the well-defined therapeutic strategies discussed in this manuscript. An important commonality between these three therapeutic approaches is that metal coordination complexes have unique properties that make them amenable for these applications. The release of CO, H<sub>2</sub>S, and NO from metal coordination complexes is a well validated strategy to deliver these compounds in biological settings because of the strong ligating properties of these gasotransmitters. Furthermore, stopping *m*Ca<sup>2+</sup> overload, another therapeutic approach for the prevention of IRI, is accomplished by blocking the MCU. In this context, the best inhibitors for the MCU are multimetallic Ru and Os complexes, and these have been demonstrated to successfully prevent cell death caused by IRI. Various other MCU inhibitors have been developed,<sup>272</sup> but few of these have been tested in models of IRI. Examining these complexes and others not yet reported can continue to expand this area. Finally, ROS scavengers have shown significant promise as therapeutic agents against IRI. In this case, metal complexes are uniquely suited for this application based on their ability to reversibly cycle through different oxidation states to catalytically decompose ROS.

The complexes all described within this Perspective exhibit protective effects against different models of IRI. The majority of these drug candidates have only been tested in vitro, with several key examples of compounds that have been evaluated in vivo and even in humans. Obviously, complexes in these latter categories have substantially more promise for further clinical advancement. Compound **12** has been used clinically for the management of blood pressure<sup>273–276</sup> and has recently



**Figure 13.** (a) Schematic of the operation and procedure of ischemic stroke and intracerebroventricular injection. (b) Biodistribution of compound **31** (shown as **1** in the figure) by MRI after surgery. (c) PET images of rat brain following tail vein injection of [<sup>18</sup>F]FDG after treatment with **31**. (d) Coronal brain sections of rats stained with TTC in the absence of IRI, with IRI, no treatment (DMSO), and treatment of compound **31** (**1**) and a mononuclear Mn-salen compound (**1c**). (e) Calculated brain infarct volumes under conditions described in (d). Reproduced with permission from ref 269. Copyright 2020 American Chemical Society.

been explored clinically for the treatment of IRI.<sup>159</sup> This study, although demonstrating promise for the treatment of IRI, did not show a consistent outcome in patients, indicating that further clinical studies need to be performed. The MnSOD mimics **23**<sup>231–235</sup> and **29**<sup>258–261</sup> have been investigated in clinical trials for a wide range of conditions, but have not yet been tested clinically for IRI. The strategy of MCU inhibition is the only approach presented in this manuscript that has not yet been applied in humans. The lack of work in this area may arise as a consequence of the fact that the MCU was only conclusively identified in 2011,<sup>163,164</sup> and as such it represents a relatively new drug target. In addition, as noted in Section 3, a major hurdle for the metal-based MCU inhibitors that must be overcome before human trials can be considered arises from their dose-limiting seizure-inducing activities within mouse models. Because ongoing studies suggest that this property arises from off-target effects,<sup>277</sup> efforts are required to improve their selectivity for the MCU in order to minimize their abilities to induce seizures. Therefore, like any drug candidate, improving the therapeutic window is an important objective for their advancement to clinical trials. In any case, this Perspective has shown that the groundwork for preventative strategies against IRI has been laid, and metal complexes have an important role in the management of this condition moving forward.

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### Notes

The authors declare no competing financial interest.

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