

# Structure-Activity Relationships of Metal-Based Inhibitors of the Mitochondrial Calcium Uniporter

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## Abstract

The mitochondrial calcium uniporter (MCU) is a transmembrane protein that is responsible for mediating mitochondrial calcium ( $m\text{Ca}^{2+}$ ) uptake. Given this critical function, the MCU has been implicated as an important target for addressing various human diseases. As such, there has been a growing interest in developing small molecules that can inhibit this protein. To date, metal coordination complexes, particularly multinuclear ruthenium complexes, are the most widely investigated MCU inhibitors due to both their potent inhibitory activities as well as their longstanding use for this application. Recent efforts have expanded the metal-based toolkit for MCU inhibition. This concept paper summarizes the development of new metal-based inhibitors of MCU and their structure-activity relationships in the context of improving their potential for the therapeutic use in managing human diseases related to  $m\text{Ca}^{2+}$  dysregulation.

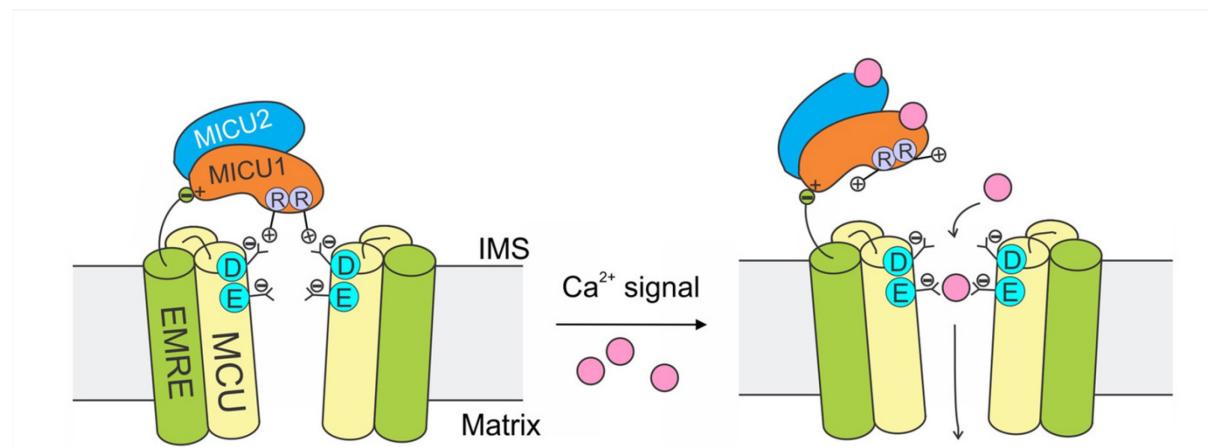
## Introduction

Intracellular calcium ions ( $\text{Ca}^{2+}$ ) play a key role in signal transduction for a wide range of biological events.<sup>[1,2]</sup> At any given instance, the level of intracellular  $\text{Ca}^{2+}$  is tightly controlled by a combination of metal-ion buffering biomolecules, importers, exporters, and exchangers. Alterations in the  $\text{Ca}^{2+}$  homeostasis contribute to various pathological conditions, reflecting the importance of its precise regulation.<sup>[3]</sup> The increase of intracellular  $\text{Ca}^{2+}$  levels is a consequence of both influx from extracellular milieu and  $\text{Ca}^{2+}$  release from endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR). One key organelle that maintains the  $\text{Ca}^{2+}$  homeostasis is the mitochondria, which act as a  $\text{Ca}^{2+}$  sink when excessive concentrations of this ion are present within the cytosol.<sup>[4,5]</sup> Mitochondrial calcium ( $m\text{Ca}^{2+}$ ) uptake is primarily mediated by a transmembrane protein known as the mitochondrial calcium uniporter (MCU).<sup>[6–8]</sup>

The MCU subunit comprises two transmembrane helices, which assemble as a tetramer to form a  $\text{Ca}^{2+}$ -selective pore (**Figure 1**).<sup>[9–12]</sup> These two transmembrane helices are connected by a highly conserved and solvent-exposed DXXE motif,<sup>[13]</sup> where D and E are aspartate and glutamate residues, respectively, and X refers to other amino acid residues that vary within different organisms. This motif functions as a  $\text{Ca}^{2+}$ -selective filter by directly interacting with this ion and cooperating with various regulatory proteins,<sup>[10,14–16]</sup> including MICU1,<sup>[17]</sup> MICU2,<sup>[18]</sup> and EMRE.<sup>[19]</sup> At low cytosolic  $\text{Ca}^{2+}$  concentrations, MICU1 and MICU2 block the entrance of the uniporter and subsequently inhibit the uptake of  $\text{Ca}^{2+}$ . At elevated  $\text{Ca}^{2+}$  concentrations, however, the  $\text{Ca}^{2+}$ -binding EF hand domains of MICU1 and MICU2 trigger a conformational change that leads to their dissociation from the pore entry, enabling  $\text{Ca}^{2+}$  uptake.<sup>[20]</sup>

Although MCU-mediated  $m\text{Ca}^{2+}$  uptake is critical for bioenergetics,<sup>[4,5]</sup> excessive  $\text{Ca}^{2+}$  influx through this transporter causes  $m\text{Ca}^{2+}$  overload, initiating a pathway that opens the

mitochondrial permeability transition pore (mPTP) and triggers irreversible cell damage and death.<sup>[21]</sup> This phenomenon of  $m\text{Ca}^{2+}$  overload plays a key role in a number of different pathological conditions,<sup>[22–24]</sup> including ischemia-reperfusion injury,<sup>[25,26]</sup> cancer,<sup>[27–29]</sup> and neurodegenerative disorders.<sup>[30–33]</sup> Therefore, preventing  $m\text{Ca}^{2+}$  overload via the chemical inhibition of the MCU represents a promising therapeutic strategy.<sup>[34]</sup> Although many organic compounds have been screened for this purpose,<sup>[35–37]</sup> metal coordination complexes, particularly those of ruthenium, remain the most prominent and well-studied inhibitors of the MCU.<sup>[38]</sup> In this concept paper, we will summarize the progress in the development of metal-based MCU inhibitors and their structure-activity relationships (SARs), with an emphasis on dinuclear ruthenium and osmium inhibitors. Readers are referred elsewhere for a more general overview of MCU inhibitors including both organic and inorganic drug candidates.<sup>[38]</sup>



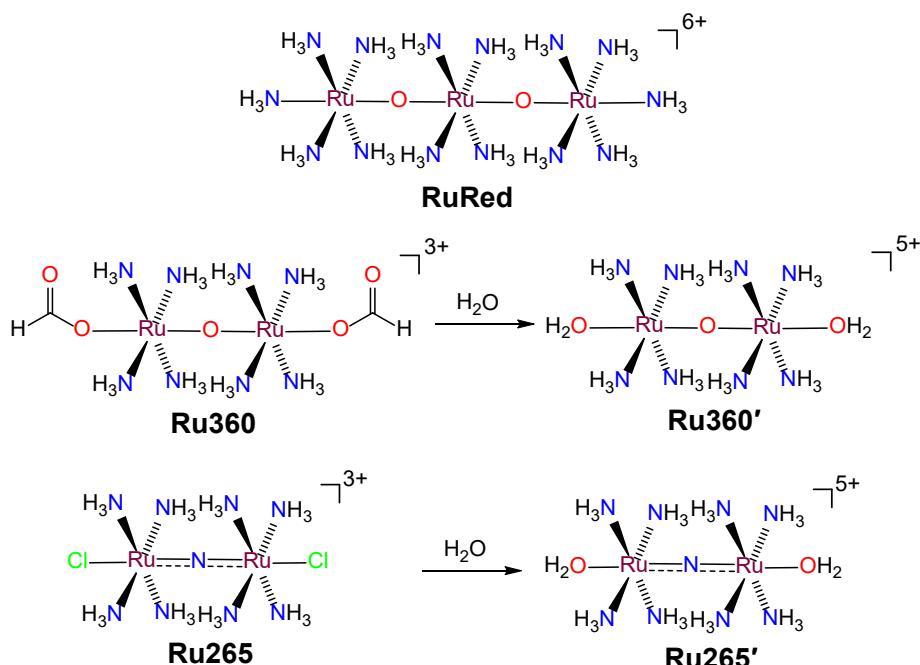
**Figure 1.** Representative topology diagram of the MCU complex. Only two subunits of the tetrameric MCU protein are shown for clarity. Pink balls represent  $\text{Ca}^{2+}$ . With low  $\text{Ca}^{2+}$  in the intermembrane space (IMS), MICU1 and MICU2 block  $m\text{Ca}^{2+}$  uptake. At elevated  $\text{Ca}^{2+}$  concentration, MICU1 and MICU2 bind this ion and undergo a conformational change that allows the passage of  $\text{Ca}^{2+}$  into the mitochondrial matrix through the uniporter. The highly conserved DXxE motif directly interacts with  $\text{Ca}^{2+}$  and serves as a cation-selective filter. Adapted from ref<sup>[16]</sup>. Copyright (Phillips et al.). The reference is distributed under a Creative Commons Attribution License, which permits the unrestricted use and redistribution provided the original author and source are credited.

## Ruthenium-Based Inhibitors

The most well-known MCU inhibitor is the oxo-bridged trinuclear ruthenium complex ruthenium red (RuRed, **Figure 2**).<sup>[39,40]</sup> This compound was initially used as a cytological stain,<sup>[41]</sup> but was later found to inhibit MCU-mediated  $m\text{Ca}^{2+}$  uptake.<sup>[42–45]</sup> Commercial sources of RuRed, however, contain a large amount of impurities (>20%).<sup>[46]</sup> The impurities comprise several different ruthenium ammine complexes, causing off-target effects such as the inhibition of other ion channels.<sup>[47]</sup> The discovery that the purification of RuRed actually leads to poorer MCU-inhibitory activities suggested that this property actually arose from a different complex present within commercial samples of RuRed.<sup>[46]</sup> Accordingly, it was later discovered that one of the impurities, Ru360 (**Figure 2**), is responsible for the MCU-inhibitory activity of RuRed.<sup>[48,49]</sup> The name Ru360 comes from its intense absorption at 360 nm. This oxo-bridged dinuclear ruthenium complex can potently and selectively inhibit the MCU in permeabilized cells at nanomolar concentrations (**Figure 3**).<sup>[50]</sup> Such activity has led to the extensive use of

Ru360 for studies of  $m\text{Ca}^{2+}$ -related pathological conditions including ischemia-reperfusion injury,<sup>[51,52]</sup> glutamate excitotoxicity,<sup>[53]</sup> and  $\text{A}\beta$ -induced apoptosis.<sup>[54]</sup>

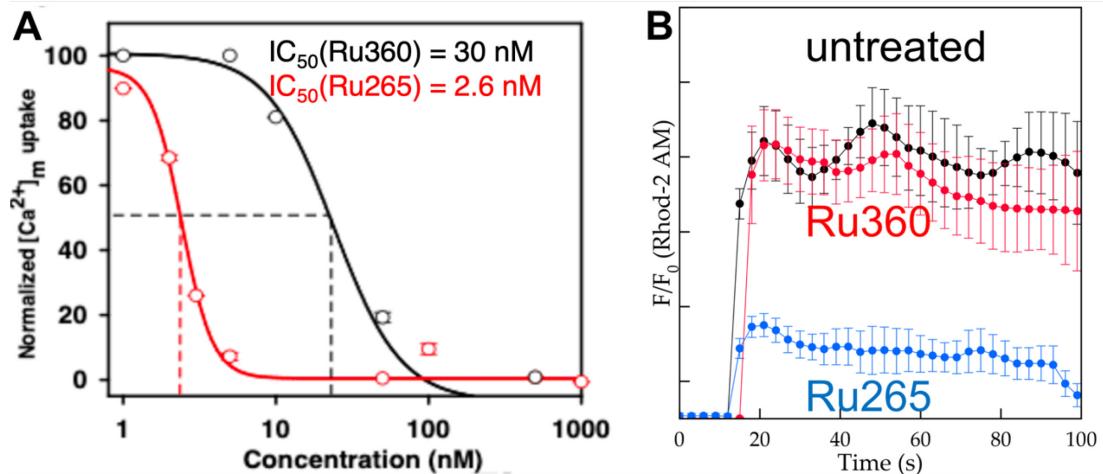
In aqueous solution, Ru360 readily undergoes rapid ligand substitution to form the diaqua-capped product, Ru360' (**Figure 2**).<sup>[49]</sup> Because this ligand substitution process starts almost instantaneously under physiological conditions, the aquated complex is presumed to be the active species that is responsible for inhibiting the MCU, a hypothesis that is also supported by the fact that the independently synthesized Ru360' is a highly potent MCU inhibitor.<sup>[55]</sup> Regarding the molecular target of these inhibitors, the current understanding is that Ru360 or Ru360' inhibits the MCU by interacting with the DXXE region of the MCU pore, as indicated by site-directed mutagenesis experiments,<sup>[10,15,16,56]</sup> molecular docking,<sup>[57]</sup> and NMR studies.<sup>[57]</sup> Notably, mutation of the D261 (**Figure 1**) and S259 residues in the human MCU attenuates the inhibitory activity of Ru360, suggesting that these residues are involved in its binding mode.



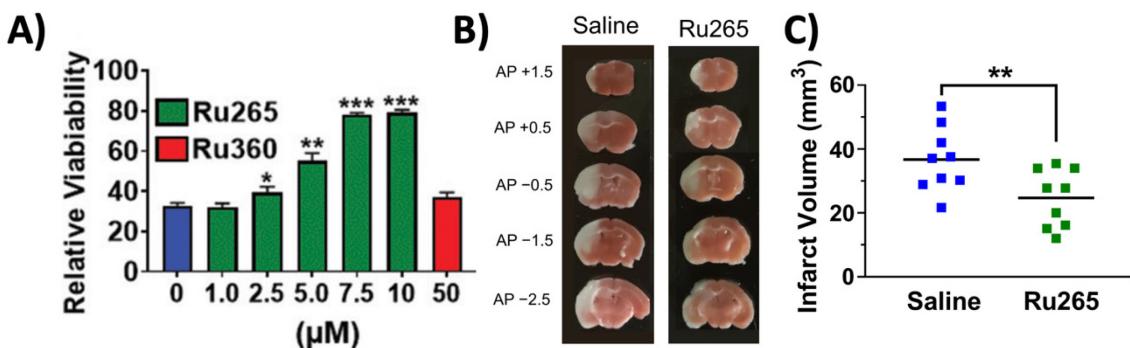
**Figure 2.** Structures of ruthenium-based MCU inhibitors RuRed, Ru360 and Ru265 and their aquated products Ru360' and Ru265', the presumed active species responsible for their inhibitory activities.

Despite the wide use of Ru360 for studying the  $m\text{Ca}^{2+}$  dynamics in permeabilized cells, the practical application of this complex in intact cells is significantly limited by its high affinity for the cell membrane,<sup>[50]</sup> low tissue accumulation in vivo,<sup>[52]</sup> and challenging purification procedures,<sup>[49]</sup> features which often lead to inconsistent results in biological assays.<sup>[53]</sup> To develop complexes with more favorable biological properties, our group synthesized structural analogues with various modifications. Our initial SAR studies found that dinuclear complexes are required for MCU-inhibitory activity, based on the observation that mononuclear ruthenium polyammine complexes including *cis*-[Ru(NH<sub>3</sub>)<sub>4</sub>Cl<sub>2</sub>]<sup>+</sup>, *trans*-[Ru(NH<sub>3</sub>)<sub>4</sub>Cl<sub>2</sub>]<sup>+</sup>, [Ru(NH<sub>3</sub>)<sub>5</sub>Cl]<sup>2+</sup>, and [Ru(NH<sub>3</sub>)<sub>6</sub>]<sup>3+</sup> do not inhibit  $m\text{Ca}^{2+}$  uptake in permeabilized cells at a relatively high concentration (10  $\mu\text{M}$ ).<sup>[58]</sup> Given these results, we next sought to explore the effects of changing the identity of bridging ligand. These efforts resulted in the nitrido-bridged analogue Ru265 (**Figure 2**), named for its strong absorbance at 265 nm,<sup>[59]</sup> which was synthesized based on a literature procedure.<sup>[60]</sup> In comparison to Ru360, Ru265 exhibits significantly greater cell uptake, enabling it to inhibit the MCU in both permeabilized (**Figure 3A**) and intact (**Figure**

**3B**) cells. Consequently, Ru265 was able to induce protective effects in both an in vitro hypoxia-reoxygenation injury model (**Figure 4A**) and in vivo model of ischemic stroke (**Figure 4B and 4C**).<sup>[61]</sup> It is worth noting at a high dose (10 mg/kg), Ru265 can cause seizure-like behaviors in mice. The cause of this dose-limiting effect is unclear and requires more investigations.



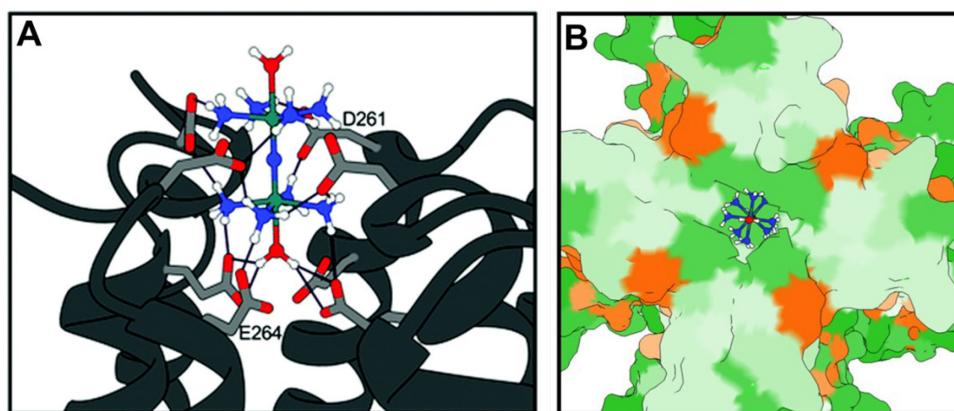
**Figure 3.** A. Dose-response curve of  $m\text{Ca}^{2+}$  uptake inhibition in permeabilized HEK293T cells. B. Representative  $m\text{Ca}^{2+}$  uptake in intact HeLa cells treated with 50  $\mu\text{M}$  of Ru360 or Ru265. The  $m\text{Ca}^{2+}$  uptake was stimulated by the addition of histamine and the degree of uptake was quantified by the fluorescence turn-on ( $F/F_0$ ) of the Rhod-2AM dye. Adapted from ref<sup>[59]</sup>. Copyright (2019), with the permission from the American Chemical Society (ACS). <https://pubs.acs.org/doi/10.1021/acscentsci.8b00773>. Further permission related to the material excerpted should be directed to the ACS.



**Figure 4.** A. Comparison of cell viability in cortical neuron cultures treated with Ru265 (green bar) or Ru360 (red bar) and subjected to 90 min of lethal oxygen-glucose deprivation, an in vitro model of ischemic stroke. The blue bar represents the relative viability of the untreated cells. B. Brain section of mice treated with saline (8 mL/kg) or Ru265 (3 mg/kg) and subjected to a model of ischemic stroke. C. Quantified size of the mice brain infarct. Ru265 decreases the infarct size compared to the saline control, suggesting that this compound has promising therapeutic potential for the management of ischemic stroke. Adapted from ref<sup>[61]</sup>. Copyright (Novorolsky et al., 2020), with permission from the SAGE Publishing.

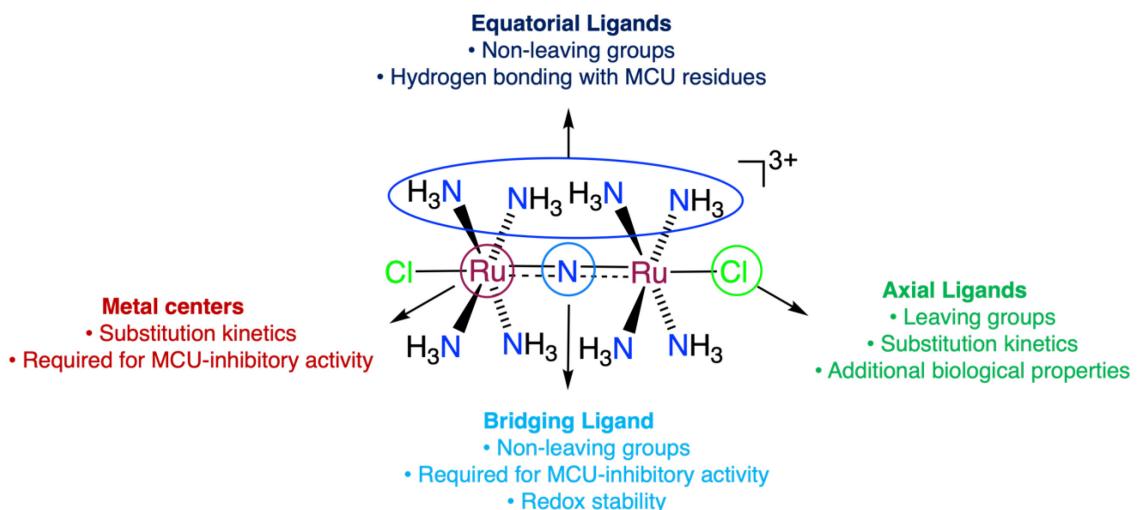
The mechanism of Ru265 is thought to be similar to that of Ru360. Under physiological conditions, Ru265 aquates to Ru265' (**Figure 2**) with a half-life of several minutes, suggesting that — like Ru360 — the diaqua product is the active MCU inhibitor.<sup>[58,62]</sup> With respect to the

nature of the interaction of this compound with the MCU, a D261A mutation within the DXXE region of the human MCU suppressed the inhibitory activity of Ru265. In addition, molecular docking studies suggest that Ru265' interacts with this region of the MCU pore by engaging in significant hydrogen-bonding interactions with D261 and E264 residues (**Figure 5**).<sup>[63]</sup> The observation that Ru265, but not Ru360, is effective in intact cells was puzzling given their structural similarities and the fact that they appear to target the same region of the MCU. To address this difference, we thoroughly compared their physical properties and identified redox activity to be a key factor.<sup>[58]</sup> Specifically, Ru360 is unstable in the presence of the biological reductants, leading to products that are not effectively internalized by cells or capable of inhibiting the MCU. By contrast, the strongly donating nitrido ligand of Ru265 makes this compound redox-inert, rendering it stable towards reduction within the biological milieu. Thus, Ru265 can be taken up by cells and remains intact intracellularly, enabling it to inhibit the MCU. This result highlights the importance of the bridging ligand, which controls the redox stability and consequently modulates the biological properties of this compound class.



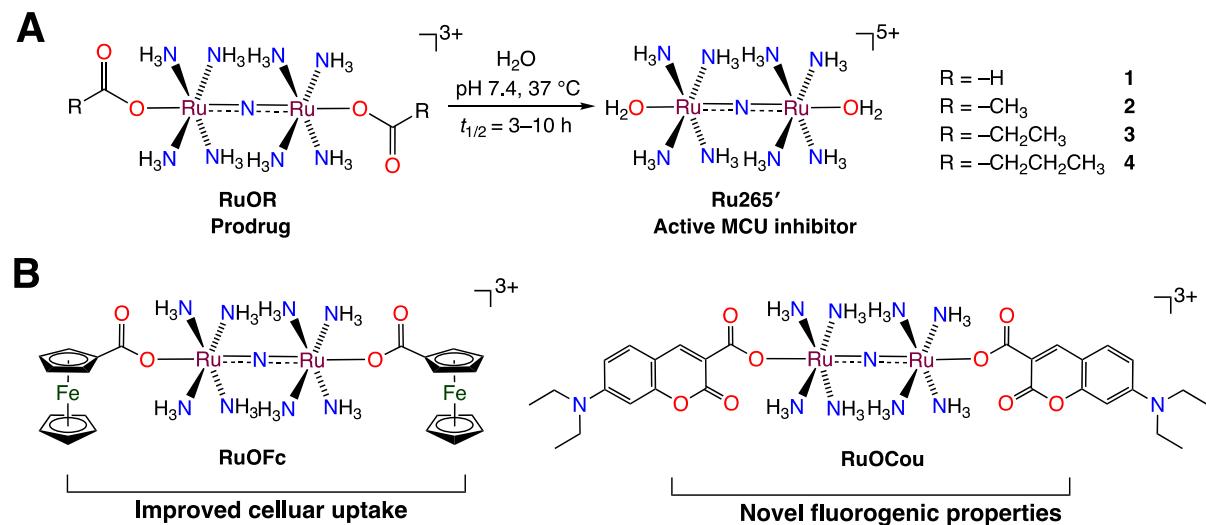
**Figure 5.** A. Molecular docking study of Ru265' in the MCU. Predicted hydrogen bonding interactions are shown as black lines. Atom colors: green = Ru, blue = N, red = O, white = H, grey = C. B. Top-down view of Ru265' docked into the MCU. The surface is colored by amino acid hydrophobicity (lime green = hydrophilic, orange = hydrophobic). Adapted from ref<sup>[63]</sup>. Copyright (2021), with permission from the Royal Society of Chemistry.

Capitalizing on the success of Ru265, this compound was further functionalized in order to fine-tune its pharmacological profile and to understand the role of each of its ligands on the overall biological activity (**Figure 6**). We first examined the importance of the ammine ligands on the MCU-inhibitory activity by synthesizing analogues of Ru265 with different equatorial ligands including chlorides,<sup>[64]</sup> polypyridines,<sup>[64]</sup> and ethylenediamine.<sup>[59]</sup> Complexes bearing the chloride and polypyridine ligands failed to block  $m\text{Ca}^{2+}$  uptake, whereas the ethylenediamine analogue showed MCU-inhibitory activity, albeit with less potency than Ru265. These results suggest that hydrogen-bonding donor ligands are required for inhibitory activity.

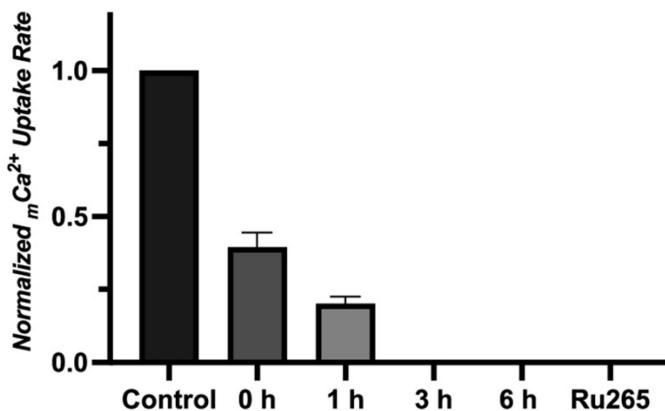


**Figure 6.** Different components of diruthenium-based MCU inhibitors.

In continuing an investigation of the SARs, modifications to the axial ligands of Ru265 were next investigated. Replacing the axial chlorides of Ru265 with carboxylates gave rise to the carboxylate-capped species **1–4** (Figure 7A).<sup>[65]</sup> Under physiological conditions, **1–4** underwent aquation with half-lives on the order of hours, a timescale that is significantly longer than that of the chloride-capped Ru265, for which this process occurs within a few minutes. In their intact forms, carboxylate-capped derivatives are less potent inhibitors of the MCU than Ru265. Nevertheless, upon aquation, the MCU-inhibitory properties of these complexes increases as they form the potent diaqua complex Ru265' (Figure 8). These results provided the first example of using this axial ligand modification strategy to afford MCU inhibitor prodrugs with improved biological stability (Figure 6).



**Figure 7.** Carboxylate-capped Ru265 derivatives. A. Alkyl carboxylate derivatives of Ru265 (**1–4**) are MCU inhibitor prodrugs with prolonged half-lives. B. RuOFc and RuOCou are Ru265 derivatives with functional axial ligands that introduce new features into the system.



**Figure 8.** Time-dependent normalized  $m\text{Ca}^{2+}$  uptake rate within permeabilized HEK293T cells treated with acetate-capped Ru265 derivative **2** (Figure 7A). The  $m\text{Ca}^{2+}$  uptake rate was obtained by monitoring the fluorescence response of the Calcium Green 5N sensor over time. Adapted from ref<sup>[65]</sup>. Copyright (2022), with permission from the American Chemical Society.

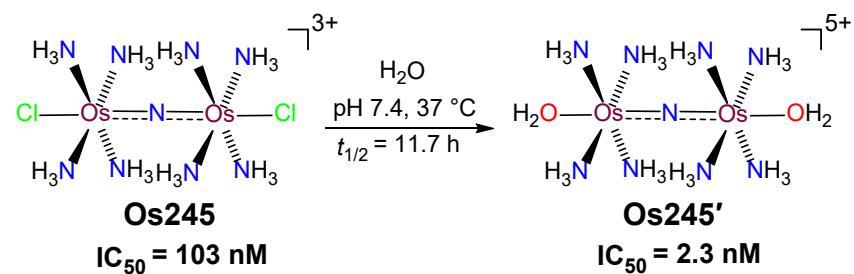
In addition to optimizing aquation kinetics and prodrug activation rates, the implementation of different axial ligands can be leveraged to impart additional functionality to this class of MCU inhibitors (Figure 7B). For instance, we have employed ferrocenecarboxylates as axial ligands. The ferrocene moieties are redox active and also highly lipophilic.<sup>[66]</sup> Accordingly, the resulting complex RuOFC exhibited reversible electrochemistry and was also substantially more lipophilic than Ru265. This latter property contributed to its enhanced cellular uptake, which was 10-fold higher than that of Ru265 under identical conditions. This improvement of uptake also resulted in a modest increase in MCU-inhibitory activity in intact cells. Another axial ligand-functionalized example is RuOCou, a Ru265 derivative containing coumarin fluorophores (Figure 7B).<sup>[67]</sup> In its intact form, this complex is not fluorescent because the Ru centers quench the radiative decay of the coumarin-based excited states. Upon aquation, however, the coumarin ligands are released concomitantly with Ru265', leading to an increase in both the fluorescence intensity and MCU-inhibitory activity. This turn-on fluorescence response enabled the aquation of RuOCou to be monitored in both HeLa cell lysates and live HeLa cells. Thus, the implementation of fluorescent axial ligands demonstrates RuOCou to be a fluorogenic prodrug, whose activation via aquation can be monitored by fluorescence spectroscopy and microscopy. Collectively, these studies demonstrate that axial modification of Ru265 is a promising approach for tuning the pharmacological properties and installing new functionalities for this class of MCU inhibitors.

## Osmium-Based Inhibitors

As reflected by the discussion above, the equatorial, bridging, and axial ligands within these dinuclear MCU inhibitors play important and distinct roles in modulating their biological activities. Likewise, it was expected that the nature and identity of the metal center would be critical in their MCU-inhibitory properties.

As the 5d congener of ruthenium, osmium can form structurally similar complexes, thus presenting a rational starting point for exploring the importance of the metal center within this compound class. Accordingly, the osmium analogue of Ru265, named Os245 based on its strong absorbance at 245 nm (Figure 9), was successfully synthesized<sup>[68]</sup> based on procedures within the literature.<sup>[60,69,70]</sup> Like Ru265, Os245 undergoes aquation to the diaqua-capped

Os245' under physiologically relevant conditions. However, this process is significantly slower (11.7 h) for Os245. This observation is consistent with the known high relative inertness of 5d transition metals compared to their 4d and 3d congeners. Furthermore, in contrast to Ru265, the MCU-inhibitory activities of the dichloride Os245 and its aqua analogue Os245' are different. Specifically, Os245' is two orders of magnitude more potent than Os245, achieving an inhibitory activity that is equivalent to that of Ru265. The difference in inhibitory activities between Os245 and Os245' indicates that the chloride ligands of Os245 are detrimental to MCU inhibition, presumably because they are less effective than water in engaging in relevant hydrogen-bonding interactions with this transporter. This result also highlights that the slow ligand substitution kinetics plays an important role in the biological activities of these osmium analogues. By contrast, the rapid aquation of Ru265 to Ru265' prevented the observation of different inhibitory properties of these complexes. Building on these results, we next evaluated the therapeutic potential of these osmium derivatives. Similar to Ru265, both Os245 and Os245' can protect primary cortical neurons against oxygen-glucose deprivation, an *in vitro* model for ischemic stroke. In vivo, however, a dose-limiting toxic side effect of seizure induction is observed when these compounds are administered to mice at a dose of 10 mg/kg. This side effect is identical to that observed for mice treated with Ru265.<sup>[61]</sup> The time for seizure onset induced by the osmium-complexes, however, is delayed compared to Ru265. This result highlights how the slow ligand substitution kinetics of Os245 also manifests in vivo. Thus, modifying ligand substitution kinetics via alteration of the metal center provides another means of generating complexes with distinct pharmacological properties and therapeutic profiles.

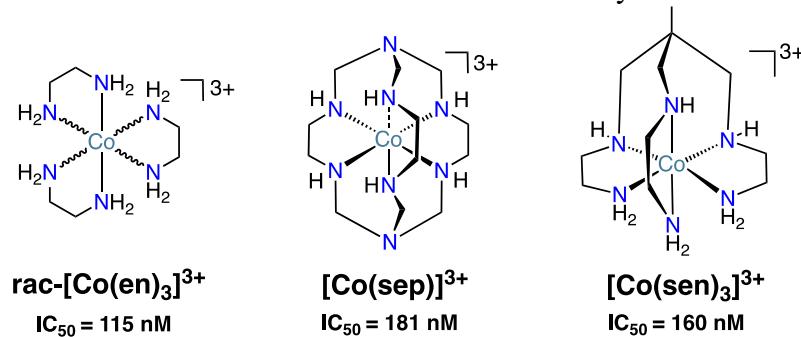


**Figure 9.** Structures of Os245 and its aquation product Os245' with their IC<sub>50</sub> values for *mCa*<sup>2+</sup> uptake inhibition in permeabilized HeLa cells.

## Other Metal-Based Inhibitors

Although the dinuclear complexes discussed above have been thoroughly studied for their MCU-inhibitory properties, early reports have also shown that mononuclear amine complexes of Co<sup>3+</sup>, Cr<sup>3+</sup>, and Rh<sup>3+</sup> inhibit *mCa*<sup>2+</sup> uptake in isolated mitochondria without negatively affecting the mitochondrial membrane potential.<sup>[71,72]</sup> The mechanism of action and SAR of these molecules, however, were not fully elucidated. Building upon these prior studies, we initiated an investigation on a series of different cobalt amine complexes that led to the identification of promising candidates that can inhibit *mCa*<sup>2+</sup> uptake in permeabilized cells with nanomolar potency (Figure 10).<sup>[63,73]</sup> These efforts revealed [Co(sen)]<sup>3+</sup> to be an MCU inhibitor that can operate in intact, non-permeabilized cells.<sup>[73]</sup> In addition, docking and site-directed mutagenesis studies revealed that these compounds most likely interact with the DXXE region of the MCU pore.<sup>[63]</sup> Thus, unsurprisingly, this critical motif appears to be a common target for most MCU inhibitors. Although these first-row transition metal complexes are generally much less potent than the ruthenium and osmium inhibitors mentioned above, their earth abundance, low cost, and facile syntheses make them attractive alternatives that warrant further investigations. Lastly, several trivalent lanthanide ions have also been reported

to inhibit  $m\text{Ca}^{2+}$  uptake in isolated mitochondria.<sup>[74–77]</sup> These ions have similar charge-to-ionic radius ratios and ligand donor atom preferences as  $\text{Ca}^{2+}$ , properties that enable them to inhibit the MCU in a competitive fashion. Off-target effects such as membrane binding and bone-localization have been reported for these lanthanide ions. Further studies are needed to further establish their mechanism of action and viability as MCU inhibitors in intact cells.



**Figure 10.** Structures of three cobalt-based MCU inhibitors with their  $\text{IC}_{50}$  values for  $m\text{Ca}^{2+}$  uptake inhibition in permeabilized HeLa cells.

## Conclusions

Although  $m\text{Ca}^{2+}$  plays a key role in normal cellular functions, dysregulation of its homeostasis, primarily through  $m\text{Ca}^{2+}$  overload, is a key contributor to various human diseases. As such, the MCU has arisen to be an important therapeutic target. Within recent years, various research groups have developed both inorganic and organic small molecules that inhibit MCU-mediated  $m\text{Ca}^{2+}$  uptake. In comparing these two classes of inhibitors, organic MCU inhibitors have typically been discovered via combinatorial screening efforts from libraries of compounds with known biological activities. As such, organic MCU inhibitors often possess alternative biological properties and have poorly defined SARs. By contrast, the metal-based inhibitors were initially discovered based on their MCU-inhibitory activities and have a pattern of SARs that is beginning to be elucidated, as summarized here. Dinuclear ruthenium inhibitors are the most commonly used, due in part to the initial discoveries of the inhibitory properties of RuRed and Ru360. As summarized here, Ru265 has emerged as a new state-of-art MCU inhibitor, which exhibits favorable cell permeability and redox stability compared to Ru360. These unique properties of Ru265 have been demonstrated in both *in vitro* and *in vivo* models to confer protective effects against ischemic injury.

Despite the potential of Ru265 and other metal-based MCU inhibitors, many fundamental questions regarding their mechanisms of action still remain. For instance, the exact nature of the interactions between these metal complexes and the DXXE residues of MCU pore is still inconclusive. In addition, little is known about the *in vitro* and *in vivo* fate of these metal complexes, despite the extensive studies on their behaviors in aqueous solutions. Further investigations are required in order to shed light on these important questions, which when answered will enable the design of improved analogues.

From the perspective of drug development, Ru265 requires significant optimization efforts. A key dose-limiting side effect of this compound and its analogues is the induction of seizure-like behaviors in mice.<sup>[61,68]</sup> This side effect has also been observed in animals treated with RuRed,<sup>[78,79]</sup> suggesting that a common mechanism is at play with these compounds. To overcome this drawback, identifying the origin of this side effect is critical, as that information will enable the design of compounds with optimized selectivity and pharmacokinetic properties.

Fortunately, as noted here, this compound class has many different possibilities for modification, which provides a means of rationally improving them. Specifically, as demonstrated in this review, the physical and biological properties of these dinuclear MCU inhibitors can be fine-tuned by altering axial, equatorial, and bridging ligands, as well as the metal centers. Importantly, the pharmacological profiles of these compounds can be rationally tailored for different therapeutic applications. For instance, most complexes reported in this review are not cytotoxic, which render them beneficial for cytoprotective applications such as managing ischemia-reperfusion injury and neurodegeneration. However, it is possible to intentionally confer these complexes with cytotoxic activity via their conjugation to bioactive ligands to enable their use as anticancer agents that simultaneously inhibit the MCU. Overall, these results highlight the power and versatility of synthetic coordination chemistry, which has facilitated the discovery of SARs and the expansion of this library of metal-based MCU inhibitors. Moving forward, modifying these Ru-based MCU inhibitors, based on the SARs outlined above, will enable improved compounds for different biological applications.

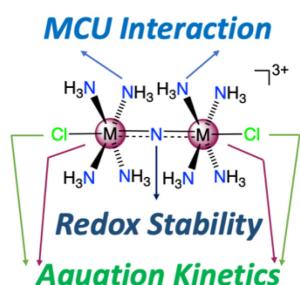
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**Keywords:** Bioinorganic chemistry • Ion channels • Osmium • Ruthenium • Structure-activity relationship

## Entry for Table of Contents



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