Inhibitors of the mitochondrial calcium uniporter for the treatment of disease.

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KEYWORDS biological calcium trafficking, mitochondrial calcium uniporter, mitochondrial disease, ruthenium coordination compounds

ABSTRACT

The mitochondrial calcium uniporter (MCU) is a protein located in the inner mitochondrial membrane that is responsible for mitochondrial Ca^{2+} uptake. Under certain pathological conditions, dysregulation of Ca^{2+} uptake through the MCU results in cellular dysfunction and apoptotic cell death. Given the role of the MCU in human disease, researchers have developed small-molecule compounds capable of inhibiting mitochondrial calcium uptake as tools for understanding the role of this protein in cell death. Herein we describe recent findings on the role of the MCU in mediating pathological conditions and the search for small-molecule inhibitors of this protein for potential therapeutic applications.

INTRODUCTION.

Mitochondria are critical for the regulation of cellular respiration and energy production within eukaryotes. These organelles also serve a complementary function of buffering intracellular calcium (Ca^{2+}) levels. Mitochondria effectively uptake these ions to restore equilibrium Ca^{2+} concentrations when cytosolic levels are elevated. This mitochondrial Ca^{2+} ($_mCa^{2+}$) uptake is mediated by the highly selective and inwardly rectifying mitochondrial calcium uniporter (MCU)[1]. Although $_mCa^{2+}$ uptake is essential for signaling and bioenergetic processes, overload of mitochondria with these ions triggers the release of cytochrome *c*, overproduction of reactive oxygen species (ROS), mitochondrial swelling, and opening of the mitochondrial permeability transition pore (mPTP), all of which contribute to apoptotic cell death[2]. Over the past two decades, a significant number of studies have shown that this type of dysregulation of $_mCa^{2+}$ levels, caused in part by improper MCU activity, can have deleterious effects on cellular function, which manifest a number of serious pathological conditions[3,4••,5]. As such, the MCU has arisen as a potential therapeutic target for the treatment of diseases related to mitochondrial dysfunction such as neurodegeneration, ischemia/reperfusion injury, and cancer[6].

THE MITOCHONDRIAL CALCIUM UNIPORTER (MCU) COMPLEX.

Although the calcium-buffering capabilities of the mitochondria have been known for over 50 years, the precise identity of the MCU as the major Ca^{2+}-transporter remained elusive until 2011[1,7]. A series of combined efforts involving NMR spectroscopy[8·,9], cryo-EM[9–14], and x-ray crystallography[13••,15,16,17•,18] have elucidated the structure of this membrane-bound transporter and its regulatory machinery. The pore-forming subunit of the MCU contains 351 amino acid residues with both the N- and C-terminal domains located in the matrix of the

mitochondria. The two transmembrane domains, TM1 and TM2, are connected by a solvent-exposed loop with a highly conserved DXXE motif, which is essential for Ca^{2+} transport, located in the upper helix of TM2 (Figure 1).

Although initial structural studies suggested that the MCU complex exists as a pentamer comprising 5 identical subunits[9], more recent studies have clarified that this protein complex actually assumes a tetrameric, dimer of dimers assembly[10–13]. Tight regulation of MCU-mediated $_m Ca^{2+}$ uptake is carried out by the associated protein MICU1[19] and its homologues MICU2[17] and MICU3[20•]. These regulatory proteins contain EF-hands, which enable them to sense Ca^{2+} ions and tune $_m Ca^{2+}$ uptake through the MCU[17,18,21,22]. Three additional proteins in the MCU complex, EMRE[14], MCUb[23], and MCUR1[24] also exhibit important regulatory roles in restricting or enhancing $_m Ca^{2+}$ uptake.

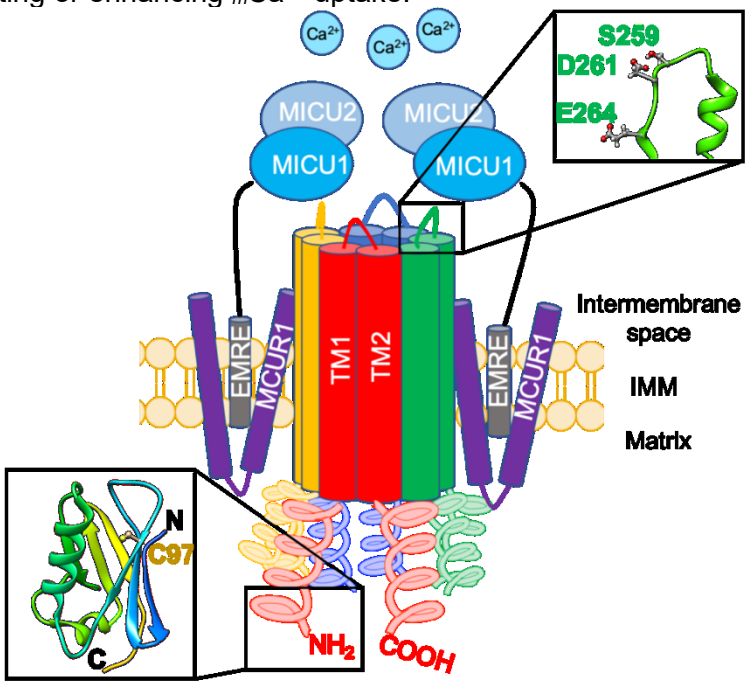

Figure 1. Topology diagram of human MCU showing the pore-forming subunit, the relevant regulator proteins MICU1/2, MCUR1, and EMRE and the orientation of the MCU in the inner mitochondrial membrane (IMM). Insets depict (left) the location of C97 in the crystal structure of the N-terminal domain (NTD; residues 72 – 189; PDB 5KUJ) and (right) location of the DXXE motif and S259 in the solvent accessible region of the MCU pore (PDB 5ID3).

THE MCU, MITOCHONDRIAL Ca^{2+}, AND DISEASE.

Neurodegenerative and Neuromuscular Disorders.

A number of neurodegenerative diseases exhibit improper handling of $_m Ca^{2+}$[25–28]. In Alzheimer's Disease (AD), for example, accumulation of amyloid-β (Aβ) plaques in brain tissue leads to increased $_m Ca^{2+}$ uptake in neurons and cell death via excitotoxicity[29,30]. As such, approaches to modulate $_m Ca^{2+}$ levels have been suggested as a therapeutic strategy for the prevention of AD[31]; inhibition of $_m Ca^{2+}$ uptake through the MCU was recently shown to inhibit Aβ-induced $_m Ca^{2+}$ overload and apoptosis in vitro[32•].

Parkinson's Disease (PD) is caused by α-synuclein aggregate accumulation in the brain, which causes $_m Ca^{2+}$ overload, overproduction of ROS, and death of dopaminergic neurons[33]. It was recently reported that the integrity of the MCU complex is compromised in early onset PD, as reflected by the degradation of MICU1 by the protein ligase Parkin, leading to increased

$_m Ca^{2+}$ uptake and apoptosis[34]. Supporting this conclusion, genetic knockdown of the MCU rescues dopaminergic neurons from PD-mediated cell death[35•].

Another neurodegenerative disease, amyotrophic lateral sclerosis (ALS), is also directly linked to $_m Ca^{2+}$ overload. Disrupted regulation of glutamate in neurons and astrocytes leads to $_m Ca^{2+}$ overload and cell death[36]. MCU expression in neurons varies over the progression of ALS; presymptomatic neurons upregulate the MCU, presumably to counter the high cytosolic Ca^{2+} influx, whereas late stage neurons show reduced expression to compensate for the cytotoxic $_m Ca^{2+}$ overload[37].

In addition to neurodegenerative disease, the MCU complex has been identified to play a major role in neuromuscular disease. Loss or mutation of MICU1 induces myopathy, learning difficulties, and progressive movement disorders[38]. These symptoms can prove lethal and appear to be a primary result of defective Ca^{2+} signaling, $_m Ca^{2+}$ overload, and a fragmented mitochondrial network[39,40]. Taken together, these findings emphasize the central role of the MCU in neurological disease and suggest that enforcing proper regulation of $_m Ca^{2+}$ uptake could be a powerful therapeutic strategy.

Ischemia/Reperfusion Injury and Ischemic Stroke

Ischemia/Reperfusion injury (IRI), which arises from the rapid restoration of oxygenated blood to oxygen-deficient, or ischemic, tissue, occurs in situations such as heart failure, organ transplant, stroke or ischemic brain injury[2]. Under ischemic conditions, oxygen-deficient cells employ anaerobic glycolysis as the primary metabolic pathway, which leads to the production of lactic acid and a concomitant decrease in cytosolic pH. Simultaneously, the mitochondrial membrane potential ($\Delta\Psi_m$) is diminished due to the cessation of oxygen-dependent oxidative phosphorylation. The drop in cytosolic pH sequentially activates Na^+/H^+ and Na^+/Ca^{2+} exchanger proteins, leading to a net increase in cytosolic Ca^{2+} levels. Upon reperfusion, the return of oxygen leads to rapid restoration of the $\Delta\Psi_m$ as oxidative phosphorylation resumes, generating a surge of ROS. This restoration of $\Delta\Psi_m$ provides a strong driving force for the entry of the cytosolic Ca^{2+} into the mitochondria via the MCU, triggering mitochondrial calcium overload, cell death, and the characteristic tissue damage associated with IRI[41].

Based on the contributing role of $_m Ca^{2+}$ overload in IRI, the MCU represents a potential therapeutic target for this condition. Surprisingly, the constitutive knockout of the MCU either specifically in the heart[42] or globally does not protect cardiac[43] or brain[44] tissues from IRI. By contrast, acute chemical inhibition or conditional knockout of the MCU in adult animals does confer the expected protective effects[43,45••,46]. These results suggest that the role of $_m Ca^{2+}$ in IRI may be more complex than originally expected and may indicate that alternative, as-of-yet undiscovered means of handling mitochondrial bioenergetics exist.

Cancer

Mitochondria and $_m Ca^{2+}$ levels play an important role in tumorigenesis and cancer biology. The MCU is highly expressed in certain forms of colorectal, breast, pancreatic, stomach, and prostate cancers. Additionally, various components of the MCU regulatory machinery show variable levels of expression and mutation in different cancer types; the significance of these mutations, however, is still not fully understood[47•].

The role of the MCU complex has most extensively been studied in the context of breast and colorectal cancers. Some models of breast cancer show high expression of the MCU channel, which facilitates metastasis in vivo. Similarly, overactivation of the MCU in colorectal cancer by receptor-interacting protein kinase 1, RIPK1, promotes cancer proliferation[48]. This hypothesis was confirmed by the knockdown and inhibition of the MCU, which drastically reduces cancer progression[49].

Recent work has also suggested that overexpression of components of the MCU complex may contribute to chemo-resistance in cancer cells[50]. In pancreatic and colon

cancers, overexpression of MICU1 and MICU2 decreases $_m$Ca^{2+} levels and prevents $_m$Ca^{2+} overload-induced apoptosis[51,52]. The role of the MCU in cancer is only beginning to be studied, but it is clear that $_m$Ca^{2+} regulation is fundamental to cancer cell growth. Given the seemingly contradictory roles of the MCU and $_m$Ca^{2+} in different cancer types, further investigations are required to decipher the function of this transporter in cancer.

REGULATION OF $_m$Ca^{2+} UPTAKE BY SMALL-MOLECULE INHIBITORS

Given the importance of $_m$Ca^{2+} dynamics in the pathological conditions described above, there has been a strong interest in developing small-molecule inhibitors of the MCU for use as therapeutic agents or tools for studying the role of this transporter in human disease. In recent years there have been a handful of studies aimed at identifying small molecules capable of inhibiting MCU-mediated Ca^{2+} uptake (Figure 2, Table 1).

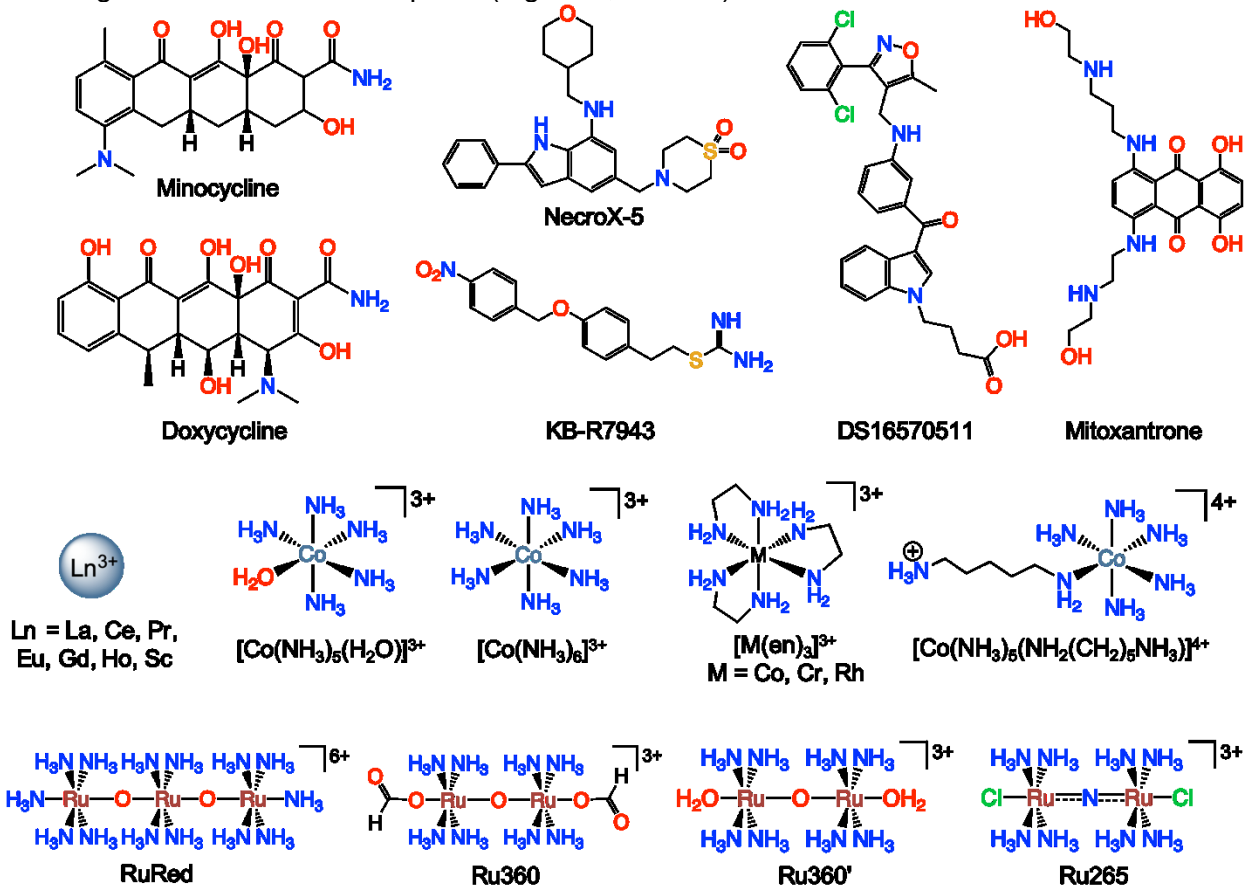

Figure 2. Structures of MCU inhibitors discussed in this work.

The organic molecules mitoxantrone[53•] and DS16570511[54•] were identified from distinct libraries comprising over 120,000 compounds to be potent inhibitors of the MCU. The MCU-inhibitory activity of several other small molecules was recognized serendipitously as a secondary function. For example, the necrosis inhibitor NecroX-5[55–57•], the Na$^+$/Ca^{2+} exchange inhibitor KB-R7943[58], and the antibiotics minocycline[59,60] and doxycycline[60,61] all possess MCU-inhibitory properties. Of these organic compounds, DS16570511 is the most potent, as reflected by its 50% MCU-inhibitory concentration of 860 nM in isolated mitochondria[54·]. This compound protects perfused rat hearts from $_m$Ca^{2+} overload, demonstrating its potential as a therapeutic agent for diseases related to $_m$Ca^{2+} dysregulation.

In comparing these organic MCU inhibitors, there is no apparent structure-activity relationship (SAR) that would be predictive of their inhibitory activities. Furthermore, these compounds are generally nonselective for the MCU, as reflected by their ability to induce off-target biological effects. For example, mitoxantrone is a cardiotoxic anticancer agent that inhibits human topoisomerase II[62,63]. Additionally, DS16570511 has recently been shown to depolarize mitochondria and induce mPTP opening[64]. These results highlight the challenge in finding compounds that can inhibit the MCU selectively in the absence of additional biological perturbations, which can complicate analysis of results and compromise their therapeutic viability.

Table 1. MCU inhibitors discussed in this work, their MCU-inhibitory activity, and observed off-target biological effects.

Compound	IC_{50} (μM)[a]	IC_{50} determination conditions	Off-target effects	Ref
Mitoxantrone	8.3	Yeast mitochondria[b]	Topoisomerase II inhibition, DNA binding, cardiotoxicity	[53•,62,63]
DS16570511	0.860	Isolated mitochondria[c]	Mitochondrial depolarization, cell death	[54•,64]
NecroX-5	ND[d]	–	Necrosis inhibition, ROS scavenging	[55–57•]
Minocycline	ND	Isolated mitochondria	Antibiotic activity, mitochondrial depolarization, Ca^{2+} binding, membrane binding	[59,60]
Doxycycline	ND	Isolated mitochondria	Antibiotic activity, altered cell metabolism and proliferation	[60,61]
KB-R7943	5.5	Permeabilized HeLa cells	Na^+/Ca^{2+} exchanger inhibition, inhibition of mitochondrial complex I	[58]
Ln^{3+} salts	0.02	Isolated mitochondria	Membrane binding, localization to bone tissue	[65–68]
$[Co(NH_3)_5(H_2O)]^{3+}$	0.54	Isolated mitochondria	ND	[69]
$[Co(NH_3)_6]^{3+}$	1.66	Isolated mitochondria	mucopolysaccharide channel inhibition	[69,70]
$[Co(en)_3]^{3+}$	0.053	Isolated mitochondria	ND	[70]
$[Cr(en)_3]^{3+}$	0.490	Isolated mitochondria	ND	[70]
$[Rh(en)_3]^{3+}$	0.360	Isolated mitochondria	ND	[70]
$[Co(NH_3)_5(NH_2(CH_2)_5NH_3)]^{4+}$	0.250	Isolated mitochondria	ND	[70]
RuRed	0.0036	Isolated mitochondria	Membrane binding, broad spectrum ion channel inhibition, induction of seizures	[70–87]
Ru360	0.227	Yeast mitochondria	Membrane binding	[6–8,32,88–95]
Ru360'	0.038	Yeast mitochondria	ND	[96]

Ru265	0.0025	Permeabilized HeLa cells	None observed	[97•]

a. Concentration required for 50% MCU inhibition
b. Yeast genetically modified to express the MCU and its regulator EMRE
c. Mitochondria isolated from mammalian cell lines
d. Not determined

Inorganic salts and coordination complexes have also been shown to inhibit $_m Ca^{2+}$ uptake. The trivalent lanthanide ions, which have ionic radii and coordination preferences comparable to Ca^{2+}, can bind to mitochondria and competitively inhibit $_m Ca^{2+}$ uptake[65–68]. Several transition metal coordination complexes, bearing ammine (NH_3) or amine ligands, have also been demonstrated to inhibit $_m Ca^{2+}$ uptake. Most notably, complexes of Co^{3+}, Cr^{3+}, and Rh^{3+} inhibit Ca^{2+} uptake in isolated mitochondria at nanomolar concentrations without negatively affecting $\Delta\Psi_m$[69,70]. Like the organic compounds discussed above, the SAR for these coordination complexes is lacking, given that complexes with diverse coordination environments appear to exhibit MCU-inhibitory activity. Moreover, these coordination complexes have not been evaluated in intact cellular systems.

Ruthenium Red (RuRed) and Ruthenium 360 (Ru360)

The most well-known and widely employed inhibitor of the MCU is the trinuclear oxo-bridged complex ruthenium red (RuRed)[71,80]. This +6 cation contains a nearly linear Ru–O–Ru–O–Ru core, with the remainder of the ruthenium coordination spheres supported by neutral NH_3 ligands (Figure 2). The +6 charge is a result of its mixed valent ground state, which arises from two formally Ru^{3+} centers and one Ru^{4+} center[71]. RuRed, named for its intense red color (ε_{532nm} = 85,900 M^{-1} cm^{-1}), was first synthesized in 1892[82] and found use as a cytological stain shortly after[81,83].

The widespread use of RuRed as a cytological stain led to the discovery that this compound inhibits $_m Ca^{2+}$ uptake by the MCU without negatively affecting mitochondrial respiration or Ca^{2+} efflux[84–87]. Furthermore, researchers have shown that RuRed can mitigate tissue damage due to IRI[74] and reduce cancer cell migration[75]. Despite the potential utility of RuRed as a $_m Ca^{2+}$ uptake inhibitor, its purification has always been a challenging matter. In fact, nearly all commercial sources of RuRed supply this compound in poor purity (<80%)[78]. Thus, most commercial formulations of RuRed actually contain mixtures of several different ruthenium ammine complexes. Not surprisingly, commercial formulations of RuRed have exhibited poor selectivity for the MCU, often showing inhibitory activity for other ion channels as well[76].

One of the minor impurities found within most formulations of RuRed is a binuclear oxo-bridged complex, called ruthenium 360 (Ru360). This compound, named for its intense UV-vis spectral absorption at 360 nm, is the active component of RuRed mixtures that is responsible for the perceived MCU-inhibitory activity[90–92]. This discovery was consistent with the fact that samples of highly purified RuRed are actually less active inhibitors than impure samples[78]. Inhibition of the MCU by Ru360 is selective and does not interfere with sarcoplasmic reticulum and cytosolic Ca^{2+} dynamics, Na^+/Ca^{2+} exchanger activity, or L-type Ca^{2+} channels[92].

In contrast to RuRed, Ru360 contains only two Ru centers bridged by a single oxo ligand. In addition to the bridging oxo, each Ru bears 4 ammine ligands and an axial formate ligand (Figure 2). Ru360 is paramagnetic and mixed valent, formally containing a Ru^{4+} and Ru^{3+} center[91]. Isolation of Ru360 can be achieved via a low-yielding synthesis that requires tedious ion exchange chromatographic purification[91]. Our group has developed synthetic methods for the preparation of a functional analogue of Ru360, which we call Ru360', where the axial formate ligands have been replaced with water ligands[96]. Because the axial formate

ligands of Ru360 undergo a fairly rapid aquation reaction, we have found that the aqua ligands of Ru360' have no negative impact on its MCU-inhibitory activity.

Given the high potency and selectivity of Ru360 for inhibiting $_m Ca^{2+}$ uptake, this commercially available complex has been widely employed for the study of calcium-dependent cellular processes and as a therapeutic agent for the prevention of IRI[93,94]. Ru360 was also shown to prevent glutamate-induced excitotoxicity in cortical neurons[89], and prevent Aβ-induced apoptosis by reducing oxidative stress in microglia[32]. Despite the apparent success of Ru360 in these studies, there are several reported concerns regarding the cell permeability of this reagent. For example, it has been noted that this compound binds the exterior of cell membranes and has low cell permeability[92], properties that are further reflected by its low accumulation in myocardial tissue in vivo[94] and highly variable results in biological assays[89].

Although Ru360 is widely used to study $_m Ca^{2+}$ in biological systems, surprisingly little is known regarding its mechanism of action. A series of recent site-directed mutagenesis experiments[7,10,13,21,22], NMR studies[8·], and molecular dynamics simulations[8·] suggest that Ru360 inhibits $_m Ca^{2+}$ uptake through interactions with conserved DXXE motif of the solvent exposed loop of the MCU that spans the TM1 and TM2 domains. Mutations of specific aspartate (D261) and serine (S259) residues in human MCU (Figure 1) maintain Ca^{2+}-uptake activity but reduce the inhibitory effects of Ru360, suggesting these residues are intimately involved in the inhibitory activity of Ru360. The exact nature of these interactions, however, remain unknown.

Ruthenium 265

Our group has recently reported the synthesis, characterization, and biological activity of a new ruthenium-based MCU inhibitor, called Ru265[97••]. This compound is structurally similar to Ru360 in that it contains two bridged Ru centers bearing ammine ligands. In contrast to Ru360, however, Ru265 is bridged by a nitrido (N^{3-}) ligand and both ruthenium centers attain the +4 oxidation state (Figure 2). Nitrido-bridged ruthenium complexes can be easily obtained by subjecting the nitrido-bridged precursor complex $K_3[Ru_2(\mu\text{-}N)Cl_8(OH_2)_2]$ to ligand substitution reactions. As such, Ru265 could be synthesized cleanly in moderate yields without the need for chromatographic purification.

Like Ru360, Ru265 is a potent inhibitor of $_m Ca^{2+}$ uptake in both isolated mitochondria and permeabilized cell systems. The most striking aspect of Ru265, in comparison to Ru360, is its high cell permeability. Ru265 is taken up by cells over twice as effectively as Ru360. Furthermore, Ru265 is relatively non-toxic to HEK293 kidney cells and does not alter other aspects of mitochondria or Ca^{2+} trafficking, such as the rate of cytosolic Ca^{2+} clearance, the energetics of the $\Delta\Psi_m$, the efflux of $_m Ca^{2+}$, and the operation of the Na^+/Ca^{2+} exchanger. Given the high selectivity and good cell permeability of Ru265, it was shown to protect neonatal rat ventricular myocytes from simulated IRI, prevent downstream mitochondrial swelling, mPTP opening, and cell death[97••].

Site-directed mutagenesis studies on the MCU were carried out to elucidate the mechanism of action of Ru265. As noted above in our discussion of Ru360, cells that express a mutated form of human MCU with a S259A mutation are somewhat resistant to Ru360 inhibition. By contrast, this same mutation had no effect on the inhibitory activity of Ru265, suggesting that there may exist subtle differences in the way that these complexes interact with the MCU. Somewhat surprisingly, the mutation of a cysteine residue (Cys97, Figure 1) located on the matrix-residing NTD conferred resistance to Ru265 but not Ru360. This cysteine residue is an important redox sensor for the MCU, and thus this mutation may suggest that redox activation of the MCU is critical for the inhibitory activity of Ru265[97••]. Further studies are required to more fully understand this compound's mechanism of action. Our initial studies on this compound have demonstrated its utility as a therapeutic agent for diseases associated with $_m Ca^{2+}$ overload, which is a consequence of its potent MCU-inhibitory activity and good cell permeability[97••].

Conclusions and Outlook

Recent advances in understanding the structure and function of the MCU complex have highlighted the central role of this transporter in bioenergetic processes and pathological conditions. As such, modulating $_m Ca^{2+}$ levels and MCU activity have been identified as promising targets for prevention or treatment of diseases such as IRI, neurodegeneration, and cancer[98]. Towards this goal, several groups have developed small molecules capable of inhibiting Ca^{2+} uptake through the MCU and preventing $_m Ca^{2+}$ overload-induced cell damage. Many of these inhibitors, however, lack cell permeability or selectivity for MCU inhibition and have off-target biological effects. Despite the promise of MCU inhibitors as therapeutic candidates, it should be noted that the administration of RuRed in vivo induces a complex seizure response in rats[77]. This phenomenon may either be a side effect of MCU inhibition or a consequence of other bioactive impurities within the RuRed formulation. Further studies are required to understand the biological implications of using MCU inhibitors as potential therapeutic agents.

The discovery of the greatly improved bioactivity of Ru265 compared to Ru360 underscores the power of inorganic complexes as novel tools for understanding the role of the MCU in biological systems. Metal complexes can be easily modified through substitution reactions to give diverse structures, allowing clear understanding of SARs. Furthermore, the low toxicity of Ru265 in contrast to many other cytotoxic ruthenium compounds, highlights how the coordination environment around a metal center can strongly influence the biological activity of metal-based compounds, and that coordination compounds are promising candidates as effective MCU inhibitors.

Competing Interests

The authors declare no conflict of interests.

Acknowledgements:

This research in the Wilson laboratory is supported by the United States National Science Foundation (NSF; CHE-1750295). J. J. Woods is supported by an NSF Graduate Research Fellowship (DGE-1650441).

References and recommended reading:

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest
•• of outstanding interest

1. De Stefani D, Raffaello A, Teardo E, Szabò I, Rizzuto R: **A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter**. *Nature* 2011, **476**:336–340.

2. Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL: **Mitochondrial Dysfunction and Myocardial Ischemia-Reperfusion: Implications for Novel Therapies**. *Annu Rev Pharmacol Toxicol* 2017, **57**:535–565.

3. Mammucari C, Gherardi G, Rizzuto R: **Structure, Activity Regulation, and Role of the Mitochondrial Calcium Uniporter in Health and Disease**. *Front Oncol* 2017, **7**:139.

••4. Arduino DM, Perocchi F: **Pharmacological modulation of mitochondrial calcium homeostasis**. *J Physiol* 2018, **596**:2717–2733.

This review describes phenotypic analysis after genetic manipulation of the MCU and its regulatory components and comment on recent progress in modulating mitochondrial Ca^{2+} homeostasis.

5. Nemani N, Shanmughapriya S, Madesh M: **Molecular regulation of MCU: Implications in physiology and disease**. *Cell Calcium* 2018, **74**:86–93.

6. Wang W, Karamanlidis G, Tian R: **Novel targets for mitochondrial medicine**. *Sci Transl Med* 2016, **8**:326rv3.

7. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK: **Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter**. *Nature* 2011, **476**:341–345.

•8. Cao C, Wang S, Cui T, Su X-C, Chou JJ: **Ion and inhibitor binding of the double-ring ion selectivity filter of the mitochondrial calcium uniporter**. *PNAS* 2017, **114**:E2846–E2851.
This manuscript uses NMR spectroscopy in conjunction with molecular dynamics simulations to probe the site of binding of Ru360 with the MCU.

9. Oxenoid K, Dong Y, Cao C, Cui T, Sancak Y, Markhard AL, Grabarek Z, Kong L, Liu Z, Ouyang B, et al.: **Architecture of the Mitochondrial Calcium Uniporter**. *Nature* 2016, **533**:269–273.

10. Nguyen NX, Armache JP, Lee C, Yang Y, Zeng W, Mootha VK, Cheng Y, Bai X, Jiang Y: **Cryo-EM structure of a fungal mitochondrial calcium uniporter**. *Nature* 2018, **559**:570–574.

11. Yoo J, Wu M, Yin Y, Herzik MA, Lander GC, Lee S-Y: **Cryo-EM structure of a mitochondrial calcium uniporter**. *Science* 2018, **361**:506–511.

12. Baradaran R, Wang C, Siliciano AF, Long SB: **Cryo-EM structures of fungal and metazoan mitochondrial calcium uniporters**. *Nature* 2018, **559**:580–584.

••13. Fan C, Fan M, Orlando BJ, Fastman NM, Zhang J, Xu Y, Chambers MG, Xu X, Perry K, Liao M, Feng, L: **X-ray and cryo-EM structures of the mitochondrial calcium uniporter**. *Nature* 2018, **559**:575–579.
The authors report the first X-ray crystal structure of the MCU channel and uncover key residues in the MCU pore for mitochondrial Ca^{2+} uptake and RuRed inhibition.

14. Wang Y, Nguyen NX, She J, Zeng W, Yang Y, Bai X, Jiang Y: **Structural Mechanism of EMRE-Dependent Gating of the Human Mitochondrial Calcium Uniporter**. *Cell* 2019, **177**:1252–1261.

15. Lee Y, Min CK, Kim TG, Song HK, Lim Y, Kim D, Shin K, Kang M, Kang JY, Youn H-S, et al.: **Structure and function of the N-terminal domain of the human mitochondrial calcium uniporter**. *EMBO Rep* 2015, **16**:1318–1333.

16. Lee SK, Shanmughapriya S, Mok MCY, Dong Z, Tomar D, Carvalho E, Rajan S, Junop

MS, Madesh M, Stathopulos PB: **Structural Insights into Mitochondrial Calcium Uniporter Regulation by Divalent Cations**. *Cell Chem Biol* 2016, **23**:1157–1169.

•17. Kamer KJ, Jiang W, Kaushik VK, Mootha VK, Grabarek Z: **Crystal structure of MICU2 and comparison with MICU1 reveal insights into the uniporter gating mechanism**. *PNAS* 2019, **116**:3546–3555.
This article describes the X-ray crystal structure of MICU2 and describe the mechanism by which MICU1/2 regulate Ca^{2+} uptake through the MCU.

18. Xing Y, Wang M, Wang J, Nie Z, Wu G, Yang X, Shen Y: **Dimerization of MICU Proteins Controls Ca^{2+} Influx through the Mitochondrial Ca^{2+} Uniporter**. *Cell Rep* 2019, **26**:1203–1212.

19. Kamer KJ, Sancak Y, Fomina Y, Meisel JD, Chaudhuri D, Grabarek Z, Mootha VK: **MICU1 imparts the mitochondrial uniporter with the ability to discriminate between Ca^{2+} and Mn^{2+}**. *PNAS* 2018, **115**:E7960–E7969.

•20. Patron M, Granatiero V, Espino J, Rizzuto R, De Stefani D: **MICU3 is a tissue-specific enhancer of mitochondrial calcium uptake**. *Cell Death Differ* 2019, **26**:179–195.
This paper investigates the role of MICU3 in regulating mitochondrial calcium uptake in neurons.

21. Paillard M, Csordás G, Huang K-T, Várnai P, Joseph SK, Hajnóczky G: **MICU1 Interacts with the D-Ring of the MCU Pore to Control Its Ca^{2+} Flux and Sensitivity to Ru360**. *Mol Cell* 2018, **72**:778–785.

22. Phillips CB, Tsai C-W, Tsai M-F: **The conserved aspartate ring of MCU mediates MICU1 binding and regulation in the mitochondrial calcium uniporter complex**. *Elife* 2019, **8**:e41112.

23. Raffaello A, De Stefani D, Sabbadin D, Teardo E, Merli G, Picard A, Checchetto V, Moro S, Szabò I, Rizzuto R: **The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit**. *EMBO J* 2013, **32**:2362–2376.

24. Vais H, Tanis JE, Müller M, Payne R, Mallilankaraman K, Foskett JK: **MCUR1, CCDC90A, Is a Regulator of the Mitochondrial Calcium Uniporter**. *Cell Metab* 2015, **22**:533–535.

25. Nam E, Han J, Suh J-M, Yi Y, Lim MH: **Link of impaired metal ion homeostasis to mitochondrial dysfunction in neurons**. *Curr Opin Chem Biol* 2018, **43**:8–14.

26. Pchitskaya E, Popugaeva E, Bezprozvanny I: **Calcium signaling and molecular mechanisms underlying neurodegenerative diseases**. *Cell Calcium* 2018, **70**:87–94.

27. Lee K-S, Huh S, Lee S, Wu Z, Kim A-K, Kang H-Y, Lu B: **Altered ER–mitochondria contact impacts mitochondria calcium homeostasis and contributes to neurodegeneration in vivo in disease models**. *PNAS* 2018, **115**:E8844–E8853.

28. Devine MJ, Kittler JT: **Mitochondria at the neuronal presynapse in health and disease**. *Nat Rev Neurosci* 2018, **19**:63–80.

29. Frere S, Slutsky I: **Alzheimer's Disease: From Firing Instability to Homeostasis Network Collapse**. *Neuron* 2018, **97**:32–58.

30. Calvo-Rodriguez M, Hernando-Perez E, Nuñez L, Villalobos C: **Amyloid β Oligomers Increase ER-Mitochondria Ca^{2+} Cross Talk in Young Hippocampal Neurons and Exacerbate Aging-Induced Intracellular Ca^{2+} Remodeling**. *Front Cell Neurosci* 2019, **13**:22.

31. Hung CH-L, Ho Y-S, Chang RC-C: **Modulation of mitochondrial calcium as a pharmacological target for Alzheimer's disease**. *Ageing Res Rev* 2010, **9**:447–456.

•32. Xie N, Wu C, Wang C, Cheng X, Zhang L, Zhang H, Lian Y: **Inhibition of the mitochondrial calcium uniporter inhibits Aβ-induced apoptosis by reducing reactive oxygen species-mediated endoplasmic reticulum stress in cultured microglia**. *Brain Res* 2017, **1676**:100–106.
The authors demonstrate the therapeutic potential of inhibiting the MCU for the preventative treatement of Alzheimer's Disease.

33. Ludtmann MHR, Abramov AY: **Mitochondrial calcium imbalance in Parkinson's disease**. *Neurosci Lett* 2018, **663**:86–90.

34. Matteucci A, Patron M, Reane DV, Gastaldello S, Amoroso S, Rizzuto R, Brini M, Raffaello A, Calì T: **Parkin-dependent regulation of the MCU complex component MICU1**. *Sci Rep* 2018, **8**:14199.

•35. Soman S, Keatinge M, Moein M, Da Costa M, Mortiboys H, Skupin A, Sugunan S, Bazala M, Kuznicki J, Bandmann O: **Inhibition of the mitochondrial calcium uniporter rescues dopaminergic neurons in *pink1*$^{-/-}$ zebrafish**. *Eur J Neurosci* 2017, **45**:528–535.
The authors demonstrate the therpeutic potential of regulating MCU activity as a treatment for neurodegenerative disease.

36. King AE, Woodhouse A, Kirkcaldie MTK, Vickers JC: **Excitotoxicity in ALS: Overstimulation, or overreaction?** *Exp Neurol* 2016, **275**:162–171.

37. Tadić V, Adam A, Goldhammer N, Lautenschlaeger J, Oberstadt M, Malci A, Le TT, Sengupta S, Stubendorff B, Keiner S, Witte OW, Grosskreutz J: **Investigation of mitochondrial calcium uniporter role in embryonic and adult motor neurons from G93A^{hSOD1} mice**. *Neurobiol Aging* 2019, **75**:209–222.

38. Logan C V., Szabadkai G, Sharpe JA, Parry DA, Torelli S, Childs A-MM, Kriek M, Phadke R, Johnson CA, Roberts NY, Bonthron DT, Pysden KA, Whtye T, Muneanu I, Foley AR, Wheway G, Szymanska K, Natarajan S, Abdelhamed ZA, Morgan JE, Roper H, Santen GW, Niks EH, Pol WL, Lindhout D, Raffaello A, De Stefani D, Dunnen, JT, Sun Y, Ginjaar I, Sewry CA, Hurles M, Rizzuto R, Duchen MR, Muntoni F, Sheridan E: **Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling**. *Nat Genet* 2014, **46**:188–193.

39. Bhosale G, Sharpe JA, Koh A, Kouli A, Szabadkai G, Duchen MR: **Pathological consequences of MICU1 mutations on mitochondrial calcium signalling and bioenergetics**. *Biochim Biophys Acta* 2017, **1864**:1009–1017.

40. Tufi R, Gleeson TP, von Stockum S, Hewitt VL, Lee JJ, Terriente-Felix A, Sanchez-Martinez A, Ziviani E, Whitworth AJ: **Comprehensive Genetic Characterization of Mitochondrial Ca^{2+} Uniporter Components Reveals Their Different Physiological Requirements In Vivo**. *Cell Rep* 2019, **27**:1541–1550.

41. Shintani-Ishida K, Inui M, Yoshida K ichi: **Ischemia-reperfusion induces myocardial infarction through mitochondrial Ca^{2+} overload**. *J Mol Cell Cardiol* 2012, **53**:233–239.

42. Rasmussen TP, Wu Y, Joiner MA, Koval OM, Wilson NR, Luczak ED, Wang Q, Chen B, Gao Z, Zhu Z, Wagner B, Soto J, McCormick ML, Kutschke W, Weiss RM, Yu L, Boudreau RL, Abel ED, Zhan F, Spitz DR, Buetttner GR, Song L-S, Zingman LV, Anderson ME: **Inhibition of MCU forces extramitochondrial adaptations governing physiological and pathological stress responses in heart**. *PNAS* 2015, **112**:9129–9134.

43. Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E, Finkel T: **The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter**. *Nat Cell Biol* 2013, **15**:1464–1472.

44. Nichols M, Elustondo PA, Warford J, Thirumaran A, Pavlov E V, Robertson GS: **Global ablation of the mitochondrial calcium uniporter increases glycolysis in cortical neurons subjected to energetic stressors**. *J Cereb Blood Flow Metab* 2017, **37**:3027–3041.

••45. Nichols M, Pavlov E V, Robertson GS: **Tamoxifen-induced knockdown of the mitochondrial calcium uniporter in Thy1-expressing neurons protects mice from hypoxic/ischemic brain injury**. *Cell Death Dis* 2018, **9**:606.
The authors demonstrate that conditional knockdown of the MCU in mice subjected to hypoxic brain injury induces a protective effect, which is in direct contrast to previous studies that show constitutive knockout of the MCU fails to reduce tissue damage, suggesting the therapeutic potential of MCU inhibitors for the prevention of IRI.

46. Kwong JQ, Lu X, Correll RN, Schwanekamp JA, Vagnozzi RJ, Sargent MA, York AJ, Zhang J, Bers DM, Molkentin JD: **The Mitochondrial Calcium Uniporter Selectively Matches Metabolic Output to Acute Contractile Stress in the Heart**. *Cell Rep* 2015, **12**:15–22.

•47. Vultur A, Gibhardt CS, Stanisz H, Bogeski I: **The role of the mitochondrial calcium uniporter (MCU) complex in cancer**. *Pflügers Arch J Physiol* 2018, **470**:1149–1163.
A thorough review on the current knowledge of the role of the MCU in cancer.

48. Zeng F, Chen X, Cui W, Wen W, Lu F, Sun X, Ma D, Yuan Y, Li Z, Hou N, Zhao H, Bi X, Zhao J, Zhou J, Zhang Y, Xiao R-P, Cai J, Zhang X: **RIPK1 binds MCU to mediate induction of mitochondrial Ca^{2} uptake and promotes colorectal oncogenesis**. *Cancer Res* 2018, **78**:2876–2885.

49. Yu C, Wang Y, Peng J, Shen Q, Chen M, Tang W, Li X, Cai C, Wang B, Cai S, Meng X, Zou F: **Mitochondrial calcium uniporter as a target of microRNA-340 and promoter**

of metastasis via enhancing the Warburg effect. *Oncotarget* 2017, **8**:83831–83844.

50. Chakraborty PK, Mustafi SB, Xiong X, Dwivedi SKD, Nesin V, Saha S, Zhang M, Dhanasekaran D, Jayaraman M, Mannel R, Moore K, McMeekin S, Yang D, Zuna R, Ding K, Tsiokas L, Bhattacharya R, Mukherjee P: **MICU1 drives glycolysis and chemoresistance in ovarian cancer**. *Nat Commun* 2017, **8**:14634.

51. Chen L, Sun Q, Zhou D, Song W, Yang Q, Ju B, Zhang L, Xie H, Zhou L, Hu Z, Yao H, Zheng S, Wang W: **HINT2 triggers mitochondrial Ca^{2+} influx by regulating the mitochondrial Ca^{2+} uniporter (MCU) complex and enhances gemcitabine apoptotic effect in pancreatic cancer**. *Cancer Lett* 2017, **411**:106–116.

52. Bustos G, Cruz P, Lovy A, Cárdenas C: **Endoplasmic Reticulum–Mitochondria Calcium Communication and the Regulation of Mitochondrial Metabolism in Cancer: A Novel Potential Target**. *Front Oncol* 2017, **7**:199.

•53. Arduino DM, Wettmarshausen J, Vais H, Navas-Navarro P, Cheng Y, Leimpek A, Ma Z, Delrio-Lorenzo A, Giordano A, Garcia-Perez C, Médard G, Kuster B, García-Sancho J, Mokranjac D, Foskett JK, Alonso MT, Perocchi F: **Systematic Identification of MCU Modulators by Orthogonal Interspecies Chemical Screening**. *Mol Cell* 2017, **67**:711–723.

The authors report a unique high-throughput drug discovery strategy to identify mitoxantrone as an effective MCU inhibitor out of a library of ~700 compounds. Molecular dynamics simulations are employed to understand the mechanism by which mitoxantrone inhibits the MCU.

•54. Kon N, Murakoshi M, Isobe A, Kagechika K, Miyoshi N, Nagayama T: **DS16570511 is a small-molecule inhibitor of the mitochondrial calcium uniporter**. *Cell Death Discov* 2017, **3**:17045.

High throughput screening techniques identify DS16570511 as a potent inhibitor of the MCU out of 120,000 compounds screened.

55. Kim HJ, Koo SY, Ahn B-H, Park O, Park DH, Seo DO, Won JH, Yim HJ, Kwak H-S, Park HS, Chung CW, Oh TL, Kim SH: **NecroX as a novel class of mitochondrial reactive oxygen species and $ONOO^-$ scavenger**. *Arch Pharm Res* 2010, **33**:1813–1823.

56. Thu VT, Kim HK, Long LT, Lee SR, Hanh TM, Ko TH, Heo HJ, Kim N, Kim SH, Ko KS, et al.: **NecroX-5 prevents hypoxia/reoxygenation injury by inhibiting the mitochondrial calcium uniporter**. *Cardiovasc Res* 2012, **94**:342–350.

•57. Park J-H, Kim HK, Jung H, Kim KH, Kang MS, Hong JH, Yu BC, Park S, Seo S-K, Choi IW, Kim SA, Kim N, Han J, Park SG: **NecroX-5 prevents breast cancer metastasis by AKT inhibition via reducing intracellular calcium levels**. *Int J Oncol* 2017, **50**:185–192.

This paper demonstrates the potential of MCU inhibitors as tools to inhibit breast cancer metastasis.

58. Santo-Domingo J, Vay L, Hernández-Sanmiguel E, Lobatón CD, Moreno A, Montero M, Alvarez J: **The plasma membrane Na^+/Ca^{2+} exchange inhibitor KB-R7943 is also a potent inhibitor of the mitochondrial Ca^{2+} uniporter.** *Br J Pharmacol* 2007, **151**:647–654.

59. Antonenko YN, Rokitskaya TI, Cooper AJL, Krasnikov BF: **Minocycline chelates Ca^{2+}, binds to membranes, and depolarizes mitochondria by formation of Ca^{2+}-dependent ion channels**. *J Bioenerg Biomembr* 2010, **42**:151–163.

60. Schwartz J, Holmuhamedov E, Zhang X, Lovelace GL, Smith CD, Lemasters JJ: **Minocycline and doxycycline, but not other tetracycline-derived compounds, protect liver cells from chemical hypoxia and ischemia/reperfusion injury by inhibition of the mitochondrial calcium uniporter**. *Toxicol Appl Pharmacol* 2013, **273**:172–179.

61. Ahler E, Sullivan WJ, Cass A, Braas D, York AG, Bensinger SJ, Graeber TG, Christofk HR: **Doxycycline Alters Metabolism and Proliferation of Human Cell Lines**. *PLoS One* 2013, **8**:e64561.

62. Smith PJ, Morgan SA, Fox ME, Watson J V: **Mitoxantrone-DNA binding and the induction of topoisomerase II associated DNA damage in multi-drug resistant small cell lung cancer cells**. *Biochem Pharmacol* 1990, **40**:2069–2078.

63. Nägele H, Castel MA, Deutsch O, Wagner FM, Reichenspurner H: **Heart transplantation in a patient with multiple sclerosis and mitoxantrone-induced cardiomyopathy**. *J Hear Lung Transplant* 2004, **23**:641–643.

64. Payne R, Li C, Fernandez-Garcia E, Vais H, Foskett K: **The MCU Inhibitor DS16570511 has Off-Target Effects on Mitochondrial Membrane Potential**. *Biophys J* 2019, **116**:270a.

65. Doggenweiler CF, Frenk S: **Staining Properties of Lanthanum on Cell Membranes**. *PNAS* 1965, **53**:425–430.

66. Mela L: **Inhibition and activation of calcium transport in mitochondria. Effect of lanthanides and local anesthetic drugs**. *Biochemistry* 1969, **8**:2481–2486.

67. Mela L: **Interactions of La^{3+} and local anesthetic drugs with mitochondrial Ca^{++} and Mn^{++} uptake**. *Arch Biochem Biophys* 1968, **123**:286–293.

68. Reed KC, Bygrave FL: **The Ihibition of Mitochondrial Calcium Transport by Lanthanides and Ruthenium Red**. *Biochem J* 1974, **140**:143–155.

69. Crompton M, Andreeva L: **On the interactions of Ca^{2+} and cyclosporin A with a mitochondrial inner membrane pore: a study using cobaltammine complex inhibitors of the Ca^{2+} uniporter**. *Biochem J* 1994, **302**:181–185.

70. Unitt JF, Boden KL, Wallace A V, Ingall AH, Coombs ME, Ince F: **Novel Cobalt Complex Inhibitors of Mitochondrial Calcium Uptake**. *Bioorg Med Chem* 1999, **7**:1891–1896.

71. Fletcher JM, Greenfield BF, Hardy CJ, Scargill D, Woodhead JL: **Ruthenium red**. *J Chem Soc* 1961, 2000–2006.

72. Leperre A, Millart H, Prévost A, Trenque T, Kantelip J, Keppler B: **Compared effects of ruthenium red and cis-$[Ru(NH_3)_4Cl_2]Cl$ on the isolated ischaemic-reperfused rat**

heart. *Fundam Clin Pharmacol* 1995, **9**:545–553.

73. Ferrari R, Lisa F di, Raddino R, Visioli O: **The effects of ruthenium red on mitochondrial function during post-ischaemic reperfusion**. *J Mol Cell Cardiol* 1982, **14**:737–740.

74. Grover GJ, Dzwonczyk S, Sleph PG: **Ruthenium Red Improves Postischemic Contractile Function in Isolated Rat Hearts**. *J Cardiovasc Pharmacol* 1990, **16**:783–789.

75. Tang S, Wang X, Shen Q, Yang X, Yu C, Cai C, Cai G, Meng X, Zou F: **Mitochondrial Ca^{2+} uniporter is critical for store-operated Ca^{2+} entry-dependent breast cancer cell migration**. *Biochem Biophys Res Commun* 2015, **458**:186–193.

76. Hajnóczky G, Csordás G, Das S, Garcia-Perez C, Saotome M, Sinha Roy S, Yi M: **Mitochondrial calcium signalling and cell death: Approaches for assessing the role of mitochondrial Ca^{2+} uptake in apoptosis**. *Cell Calcium* 2006, **40**:553–560.

77. García-Ugalde G, Tapia R: **Convulsions and wet-dog shakes produced by systemic or intrahippocampal administration of ruthenium red in the rat**. *Exp Brain Res* 1991, **86**:633–640.

78. Broekemeier KM, Krebsbach RJ, Pfeiffer DR: **Inhibition of the mitochondrial Ca^{2+} uniporter by pure and impure ruthenium red**. *Mol Cell Biochem* 1994, **139**:33–40.

79. Carrondo MA, Griffith WP, Hall JP, Skapski AC: **X-ray structure of $[Ru_3O_2(NH_3)_{14}]^{6+}$, cation of the cytological reagent Ruthenium Red**. *Biochim Biophys Acta* 1980, **627**:332–334.

80. Luft JH: **Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action**. *Anat Rec* 1971, **171**:347–368.

81. Luft JH: **Ruthenium red and violet. II. Fine structural localization in animal tissues**. *Anat Rec* 1971, **171**:369–415.

82. Joly A: **Composés ammoniacaux dérivés du sesquichlorure de ruthénium**. *Compt Rendu Acad Sci* 1892, **115**:1299–1301.

83. Mangin L: **Sur l'emploi du rouge de ruthénium en anatomie végétale**. *Compt Rendu Acad Sci* 1893, **116**:653–656.

84. Moore CL: **Specific inhibition of mitochondrial Ca^{++} transport by ruthenium red**. *Biochem Biophys Res Commun* 1971, **42**:298–305.

85. Vasington FD, Gazzotti P, Tiozzo R, Carafoli E: **The effect of ruthenium red on Ca^{2+} transport and respiration in rat liver mitochondria**. *Biochim Biophys Acta* 1972, **256**:43–54.

86. Rossi CS, Vasington FD, Carafoli E: **The effect of ruthenium red on the uptake and release of Ca^{2+} by mitochondria**. *Biochem Biophys Res Commun* 1973, **50**:846–852.

87. Griffiths EJ: **Use of ruthenium red as an inhibitor of mitochondrial Ca^{2+} uptake in single rat cardiomyocytes**. *FEBS Lett* 2000, **486**:257–260.

88. Santo-Domingo J, Demaurex N: **Calcium uptake mechanisms of mitochondria**. *Biochim Biophys Acta* 2010, **1797**:907–912.

89. Abramov AY, Duchen MR: **Mechanisms underlying the loss of mitochondrial membrane potential in glutamate excitotoxicity**. *Biochim Biophys Acta* 2008, **1777**:953–964.

90. Ying W-L, Emerson J, Clarke MJ, Rao Sanadi D: **Inhibition of Mitochondrial Calcium Ion Transport by an Oxo-Bridged Dinuclear Ruthenium Ammine Complex**. *Biochemistry* 1991, **30**:4949–4952.

91. Emerson J, Clarke MJ, Ying WL, Sanadi DR: **The component of "ruthenium red" responsible for inhibition of mitochondrial calcium ion transport. Spectra, electrochemistry, and aquation kinetics. Crystal structure of μ-O-$[(HCO)_2(NH_3)_4Ru]_2Cl_3$**. *J Am Chem Soc* 1993, **115**:11799–11805.

92. Matlib MA, Zhou Z, Knight S, Ahmed S, Choi KM, Krause-Bauer J, Phillips R, Altschuld R, Katsube Y, Sperelakis N, Bers DM: **Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca^{2+} uptake into mitochondria in vitro and in situ in single cardiac myocytes**. *J Biol Chem* 1998, **273**:10223–10231.

93. García-Rivas GDJ, Guerrero-Hernández A, Guerrero-Serna G, Rodríguez-Zavala JS, Zazueta C: **Inhibition of the mitochondrial calcium uniporter by the oxo-bridged dinuclear ruthenium amine complex (Ru360) prevents from irreversible injury in postischemic rat heart**. *FEBS J* 2005, **272**:3477–3488.

94. García-Rivas GJ, Carvajal K, Correa F, Zazueta C: **Ru360, a specific mitochondrial calcium uptake inhibitor, improves cardiac post-ischaemic functional recovery in rats in vivo**. *Br J Pharmacol* 2006, **149**:829–837.

95. Storey NM, Lambert DG: **Mitochondrial pharmacology turns its sights on the Ca^{2+} uniporter**. *Cell Death Discov* 2017, **3**:17064.

96. Nathan SR, Pino NW, Arduino DM, Perocchi F, MacMillan SN, Wilson JJ: **Synthetic Methods for the Preparation of a Functional Analogue of Ru360, a Potent Inhibitor of Mitochondrial Calcium Uptake**. *Inorg Chem* 2017, **56**:3123–3126.

••97. Woods JJ, Nemani N, Shanmughapriya S, Kumar A, Zhang M, Nathan SR, Thomas M, Carvalho E, Ramachandran K, Srikantan S, Stathopulo PB, Wilson JJ, Madesh M: **A Selective and Cell-Permeable Mitochondrial Calcium Uniporter (MCU) Inhibitor Preserves Mitochondrial Bioenergetics after Hypoxia/Reoxygenation Injury**. *ACS Cent Sci* 2019, **5**:153–166.

This report describes the synthesis and biological activity of Ru265, a novel inhibitor of the MCU that is both highly cell-permeable and selective for MCU inhibtion. Preliminary biological investigations demonstrate this compound is capable of preventing IRI in isolated neonatal rat ventricular myocytes and preserving cell function.

98. Giorgi C, Agnoletto C, Bononi A, Bonora M, De Marchi E, Marchi S, Missiroli S, Patergnani S, Poletti F, Rimessi A, Suski JM, Wieckowski MR, Pinton P: **Mitochondrial calcium homeostasis as potential target for mitochondrial medicine**. *Mitochondrion* 2012, **12**:77–85.