Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Catalytic Mechanism of DNA Cleavage in vitro and in vivo by Ruthenium Polypyridyl Complexes Containing Redox-Active Intercalating Ligands.

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T4 Ligase Assay

Digested reactions with pUC19 DNA, GSH, $\mathbf{4}^{4+}$ or $\mathbf{3}^{2+}$ and phosphate buffer were treated with T4 ligase enzyme and buffer. All reactions had a total volume of 20 µL. To serve as a control pUC19 plasmid DNA was digested with EcoRI for two hours. The EcoRI mixture containing 5 µL water, 8 µL of RE 10X buffer, 0.8 µL acetylated BSA, 4 µL of pUC19 plasmid DNA and 2 µL of EcoRI was heat inactivated at 65°C for ~20 minutes. Two reaction vials were made. Following heat inactivation 1.0 µL of T4 ligase 10X buffer and 0.5 µL of T4 DNA ligase was added to one of the two reaction vials to re-ligate the DNA for ~1 hour. Two reaction vials for both $\mathbf{3}^{2+}$ and $\mathbf{4}^{4+}$ were prepared as well. These reaction vials were prepared with samples prepped for the DNA agarose assay. However, to one of the vials containing $\mathbf{3}^{2+}$ and one containing $\mathbf{4}^{4+}$ was added 1.0 µL of T4 ligase 10X buffer and 0.5 µL of T4 DNA ligase. The reactions were digested for ~ 1 hour. All samples were then analyzed with DNA gel electrophoresis and 6X loading buffer.

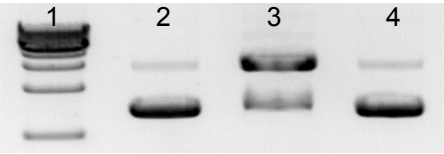


Figure S1. T4 ligase assay control.
Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 µM) cleavage products after incubation at 25 °C for 48 h with $[\mathbf{4}]^{4+}$ (12.8 µM), GSH (256 µM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Where lane 1: is a 1kb DNA ladder; lane 2: pUC19 DNA in buffer; lane 3: pUC19 DNA treated with EcoRI, and lane 4: pUC19 treated with T4 ligase after EcoRI treatment.

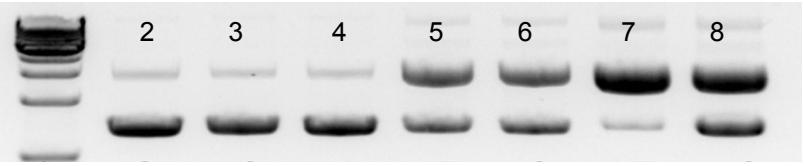
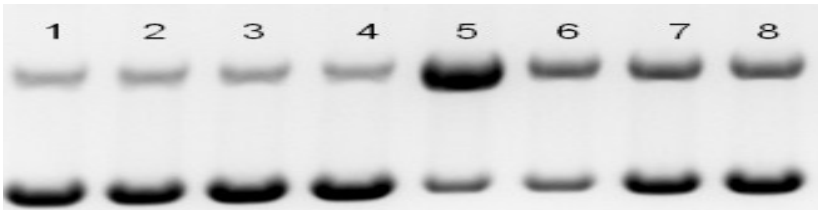


Figure S2.T4 ligase assay to show hydrolytic cleavage for $\mathbf{3}^{2+}$ and $\mathbf{4}^{4+}$
T4 ligase assay. Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 µM) cleavage products after incubation at 25 °C for 48 h with $\mathbf{4}^{4+}$ (12.8 µM), GSH (256 µM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Where lane 1: is a 1kb DNA ladder; lane 2: pUC19 DNA in buffer; lane 3: pUC19 DNA and $\mathbf{4}^{4+}$with buffer; lane 4:

: pUC19 DNA and $\mathbf{3}^{4+}$with buffer; lane 5: $\mathbf{4}^{4+}$, GSH and DNA; Lane 6: $\mathbf{3}^{2+}$, GSH and DNA; Lane 7: same as lane 5 treated with T4 ligase; lane 8: same as lane 6 treated with T4 ligase.

Figures of DNA Cleavage Activity of $\mathbf{3}^{2+}$ and $\mathbf{4}^{4+}$ with Inhibitors

A. Treated with $\mathbf{3}^{2+}$



B. Treated with $\mathbf{4}^{4+}$

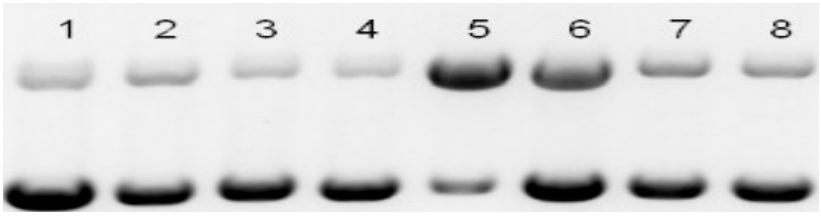
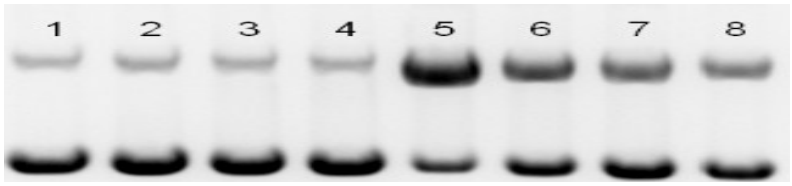


Figure S3. Effect of addition of varying concentrations of sodium benzoate to the DNA cleavage activity of $\mathbf{3}^{2+}$ (A. top gel) and $\mathbf{4}^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 ^{o}C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: sodium benzoate (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and sodium benzoate (2.1 mM); Lane 7: RPC, GSH, DNA and sodium benzoate (4.2 mM); Lane 8: RPC, GSH, DNA and sodium benzoate (6.4 mM). All reactions were carried out under aerobic conditions.

A. Treated with $\mathbf{3}^{2+}$

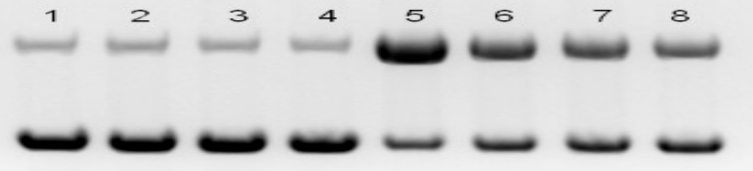


B. Treated with $\mathbf{4}^{4+}$

Figure S4. Effect of addition of varying concentrations of sodium formate to the DNA cleavage activity of $\mathbf{3}^{2+}$ (A. top gel) and $\mathbf{4}^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 ^{o}C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: sodium formate (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and sodium formate (2.1 mM); Lane 7: RPC,

GSH, DNA and sodium formate (4.2 mM); Lane 8: RPC, GSH, DNA and sodium formate (6.4 mM). All reactions were carried out under aerobic conditions.

A. Treated with **3**$^{2+}$



B. Treated with **4**$^{4+}$

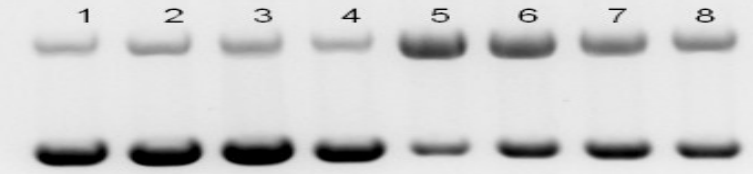
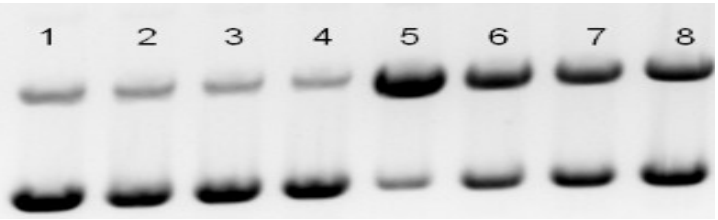


Figure S5. Effect of addition of varying concentrations of mannitol to the DNA cleavage activity of **3**$^{2+}$ (A. top gel) and **4**$^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 ^{o}C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4 mannitol (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and mannitol (2.1 mM); Lane 7: RPC, GSH, DNA and mannitol l (4.2 mM); Lane 8: RPC, GSH, DNA and mannitol (6.4 mM). All reactions were carried out under aerobic conditions.

A. Treated with **3**$^{2+}$



B. Treated with **4**$^{4+}$

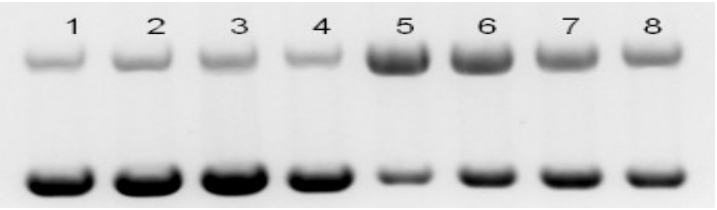
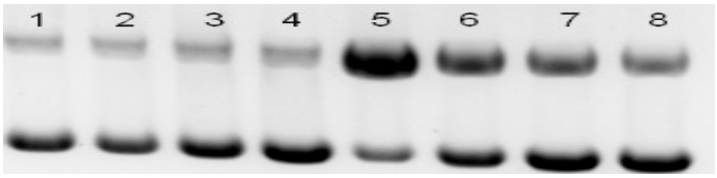


Figure S6. Effect of addition of varying concentrations of ethanol to the DNA cleavage activity of **3**$^{2+}$ (A. top gel) and **4**$^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 ^{o}C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: ethanol (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and ethanol l (2.1 mM); Lane 7: RPC, GSH, DNA and ethanol (4.2 mM); Lane 8: RPC, GSH, DNA and ethanol (6.4 mM). All reactions were carried out under aerobic conditions.

A. Treated with $\mathbf{3}^{2+}$



B. Treated with $\mathbf{4}^{4+}$

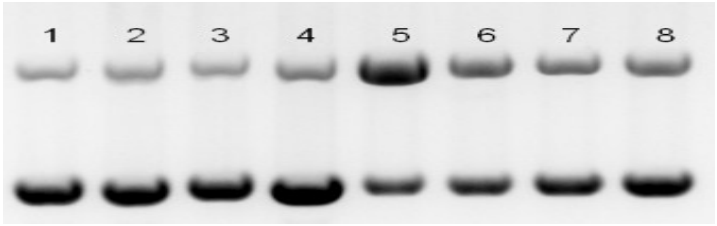
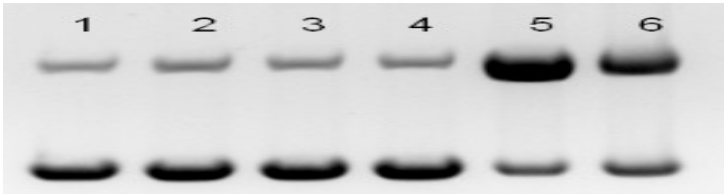


Figure S7. Effect of addition of varying concentrations of DMSO to the DNA cleavage activity of $\mathbf{3}^{2+}$ (A. top gel) and $\mathbf{4}^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 °C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: DMSO (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and DMSO (2.1 mM); Lane 7: RPC, GSH, DNA and DMSO (4.2 mM); Lane 8: RPC, GSH, DNA and DMSO (6.4 mM). All reactions were carried out under aerobic conditions.

A. Treated with $\mathbf{3}^{2+}$



B. Treated with $\mathbf{4}^{4+}$

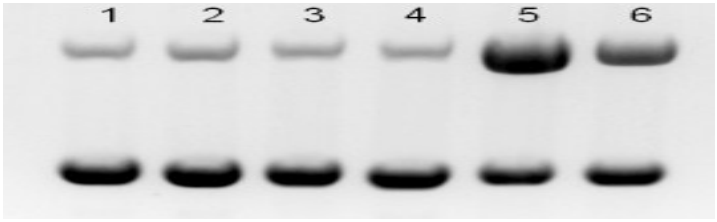
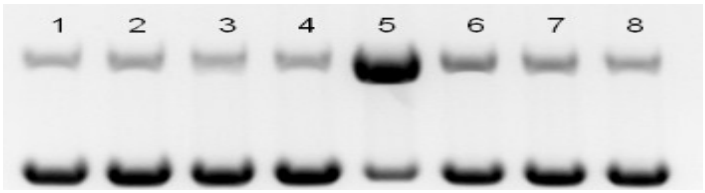


Figure S8. Effect of addition of varying concentrations of SOD to the DNA cleavage activity of $\mathbf{3}^{2+}$ (A. top gel) and $\mathbf{4}^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 °C for 48 h with RPC (12.8 μM), GSH (25 6 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: SOD (15 μg/mL) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and SOD (15 μg/mL). All reactions were carried out under aerobic conditions.

A. Treated with **3**$^{2+}$



B. Treated with **4**$^{4+}$

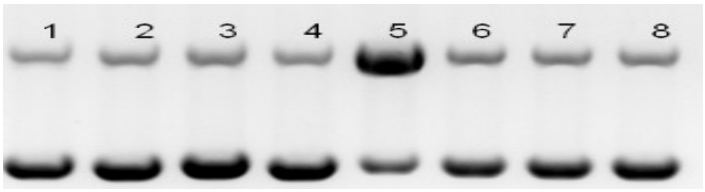
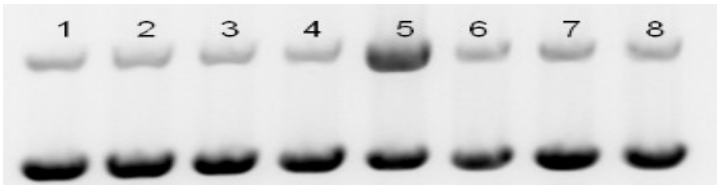


Figure S9. Effect of addition of varying concentrations of sodium pyruvate to the DNA cleavage activity of **3**$^{2+}$ (A. top gel) and **4**$^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 ^{o}C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: sodium pyruvate (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and sodium pyruvate (2.1 mM); Lane 7: RPC, GSH, DNA and sodium pyruvate (4.2 mM); Lane 8: RPC, GSH, DNA and sodium pyruvate (6.4 mM). All reactions were carried out under aerobic conditions.

A. Treated with **3**$^{2+}$



B. Treated with **4**$^{4+}$

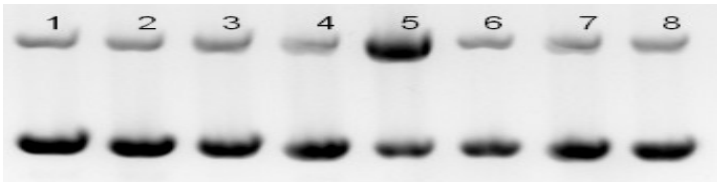


Figure S10. Effect of addition of varying concentrations of deferoxamine mesylate salt (DEF) to the DNA cleavage activity of **3**$^{2+}$ (A. top gel) and **4**$^{4+}$ (B. bottom gel). Agarose gel (1%) stained with ethidium bromide of supercoiled pUC18 DNA (154 μM) cleavage products after incubation at 25 ^{o}C for 48 h with RPC (12.8 μM), GSH (256 μM) in 50 mM Na_3PO_4/10 mM buffer (pH 7.2). Lane 1: DNA control; Lane 2: GSH and DNA; Lane 3: DNA and RPC; Lane 4: DEF (6.4 mM) DNA; Lane 5: RPC, DNA and GSH; Lane 6: RPC, GSH, DNA and DEF (2.1 mM); Lane 7: RPC, GSH, DNA and DEF (4.2 mM); Lane 8: RPC, GSH, DNA and DEF (6.4 mM). All reactions were carried out under aerobic conditions.

DNA Scission Experiment with HPLC Product Analysis

Experiments were conducted under the following conditions, (45.5 mL) 700 μM ctDNA, (4.1 mL) 58.3 μM (**4**$^{4+}$), (19.9 mL) 5.8 mM GSH, (30.5 mL) 50 mM phosphate, 10 mM NaCl Buffer at pH 7.4 digested at room temperature in air overnight , then heated at 90 ˚C for 1hr in a GC oven. The reaction was quenched with ice bath (dry ice/acetone), extracted with 20 mL dichloromethane (DCM) 3x, dried with magnesium sulfate ($MgSO_4$) and concentrated. Samples were resuspended in pure acetonitrile (MeCN) for HPLC analysis using an Agilent Infinity 1200 series HPLC.

The mobile phase of for HPLC was 0.1 TFA/MeCN 90/10, Flow Rate: 0.1mL/min ,Injection Vol: 10 μL, Stationary Phase: Zorbax Eclipse XDB-C18 4.6x150 column. The same method was conducted for **3**$^{2+}$.

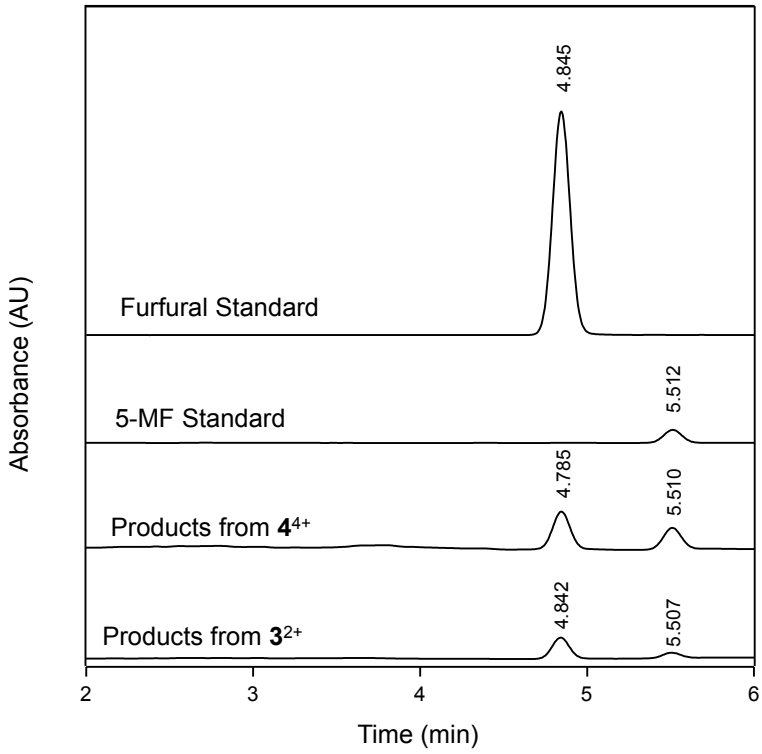


Figure S11 HPLC analysis of 5-MF and furfural at 280 nm, analyzed on a Agilent 1200 series with a mobile phase of 0.1 TFA/MeCN 90/10, Flow Rate: 0.1mL/min ,Injection Vol: 10 µL, Stationary Phase: Zorbax Eclipse XDB-C18 4.6x150 column.

Table S1 First reduction potential for $\mathbf{1}^{2+}$ – $\mathbf{8}^{4+}$ as obtained in acetonitrile with references

Compound	***Solvent***	***Couple***	***Potential (V) vs NHE****	***Ref***
$\mathbf{1}^{2+}$ $[Ru(phen)_3]^{2+}$	*MeCN*	$\mathbf{1}^{2+/+}$	-1.15 (1 e^-)	*1-5*
$\mathbf{2}^{2+}$ $[Ru(byp)_3]^{2+}$	*MeCN*	$\mathbf{2}^{2+/+}$	-1.11 (1 e^-)	*1, 2, 5*
$\mathbf{3}^{2+}$ $[(phen)_2Ru(tatpp)]^{2+}$	*MeCN*	$\mathbf{3}^{2+/3+}$	-0.11 (1 e^-)	*6, 7*
	MeCN	$\mathbf{3}^{+/3^0}$	-0.65 (1 e^-)	*6, 7*
$\mathbf{4}^{4+}$ $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$	*MeCN*	$\mathbf{4}^{4+/4^{3+}}$	-0.02 (1 e^-)	*4, 8*
	MeCN	$\mathbf{4}^{3+/4^{2+}}$	-0.51 (1 e^-)	*4, 8*
$\mathbf{5}^{2+}$ $[(phen)_2Ru(tatpq)Ru(phen)_2]^{4+}$	*MeCN*	$\mathbf{5}^{4+/5^{3+}}$	0.04 (1e^-)	*7*
$\mathbf{6}^{2+}$ $[(phen)_2Ru(dppz)]^{2+}$	*MeCN*	$\mathbf{6}^{2+/+}$	-0.73 (1 e^-)	*9, 10*
$\mathbf{7}^{2+}$ $[(phen)_2Ru(tpphz)]^{2+}$	*MeCN*	$\mathbf{7}^{2+/+}$	-0.76 (1 e^-)	*10-12*
$\mathbf{8}^{4+}$ $[(phen)_2Ru(tpphz)Ru(phen)_2]^{4+}$	*MeCN*	$\mathbf{8}^{4+/3+}$	-0.54 (1 e^-)	*12*

*All reduction potentials have been converted to NHE if not already done so in literature.

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