#### Supplementary Information

# Increasing the Cytotoxicity of Ru(II) Polypyridyl Complexes by tuning the Electronic Structure of Dioxo Ligands

Anna Notaro,<sup>a</sup> Marta Jakubaszek,<sup>a,b</sup> Nils Rotthowe,<sup>c</sup> Federica Maschietto,<sup>d</sup> Robin Vinck,<sup>a</sup> Patrick S. Felder,<sup>a</sup> Bruno Goud,<sup>b</sup> Mickaël Tharaud,<sup>e</sup> Ilaria Ciofini,<sup>d</sup> Fethi Bedioui,<sup>f</sup> Rainer F. Winter,<sup>c</sup> and Gilles Gasser<sup>a,\*</sup>

- <sup>a</sup> Chimie ParisTech, PSL University, CNRS, Institute of Chemistry for Life and Health Sciences, Laboratory for Inorganic Chemical Biology, F-75005 Paris, France.
- <sup>b</sup> Institut Curie, PSL University, CNRS UMR 144, Paris, France.
- <sup>c</sup> Department of Chemistry, University of Konstanz, Universitätsstrasse 10, D-78457 Konstanz, Germany.
- <sup>d</sup> Chimie ParisTech, PSL University, CNRS, Institute of Chemistry for Life and Health Sciences, Chemical Theory and Modelling Group, F-75005 Paris, France.
- <sup>e</sup> Université de Paris, Institut de physique du globe de Paris, CNRS, F-75005 Paris, France.
- <sup>f</sup> Chimie ParisTech, PSL University, CNRS, Institute of Chemistry for Life and Health Sciences, Team Synthèse, Electrochimie, Imagerie et Systèmes Analytiques pour le Diagnostic, F-75005 Paris, France.

\* Corresponding author: E-mail: <u>gilles.gasser@chimeparistech.psl.eu</u>; WWW: <u>www.gassergroup.com</u>; Phone: +33 1 44 27 56 02

#### **Table of Contents**

1)	Table S1. UV/Vis/NIR absorptions of the complexes in their native and electrochemically generated states in DMF.	S3
	0	
2)	Figure S1. NMR spectra of complesex 1-6	S3
3)	Figure S2. HPLC traces at 450 nm of complexes <b>1-6</b>	S16
4)	Figure S3. EPR spectra of a) <b>1</b> , b) <b>2</b> , c) <b>3</b> and d) <b>4</b> at -40 °C and -140 °C and e) <b>60x</b> at -140 °C	S18
5)	Table S2. Computed and Experimental g-Tensors	S19
6)	Figure S4. Votammograms recorded by CV and with the use of RDE for complexes <b>2-6</b>	S20
7)	Table S3. Electrochemical data for complexes 1-4.	S25
8)	Figure S5. UV/Vis/NIR-spectroelectrochemistry data for complex 1 in the presence of the reducing agent glutathione.	S26
9)	Table S4. Electrochemical data for complexes 5 and 6	S27
10)	0) Figure S6. Overlap of <sup>1</sup> H spectra of complexes <b>1-6</b> in DMSO over 96 h	
11)	1) Figure S7. Percentage concentration of complex 1 in human plasma, normalized with respect to the internal standard (caffeine) and plotted against time	
12)	Figure S8. Fluorometric cell viability assay	S35
13)	Table S5. IC <sub>50</sub> values for 3-methoxycatechol, 3-methylcatechol, 4-methylcatechol, 4-tertbutylcatechol, tetrabromocatechol and 4-nitrocatechol.	S57
14)	Figure S9. CellTiter Glo® viability Test	S58
15)	Figure S10. Cell Death Mechanism	S59
16)	) Figure S11. Cellular uptake mechanism of complex 1	
17)	) Figure S12. Oxygen consumption rates and different respiration parameters in HeLa cells alor or after treatment with various test compounds	
18)	Figure S13. Extracellular acidification rate and different parameters during glycolysis in HeLa cells alone or after treatment with various test compounds	S63
19)	Figure S14. Fuel flex assay in HeLa cells	S64

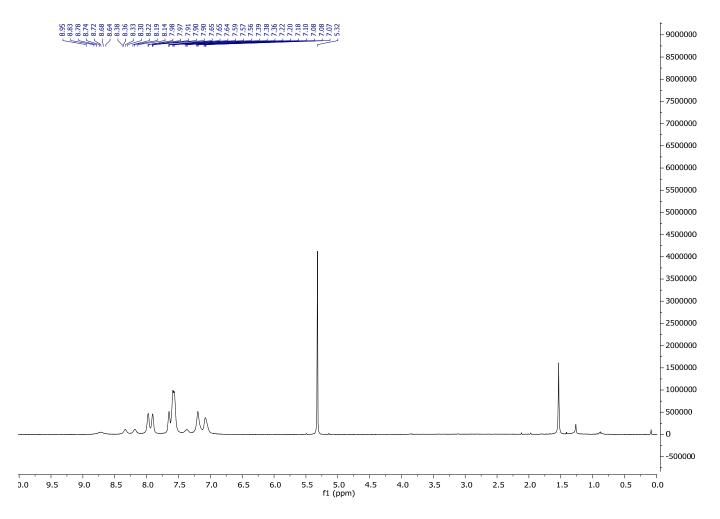
1) Table S1. UV/Vis/NIR absorptions of the complexes in their native and electrochemically generated states in DMF.

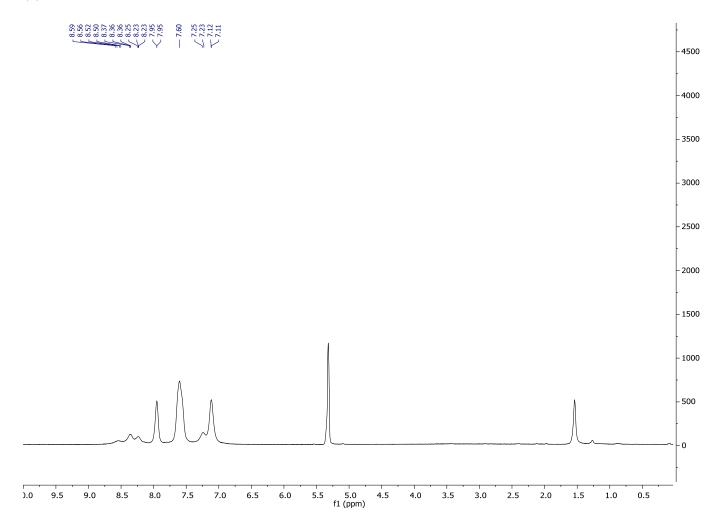
	oxidation		
compound		$\lambda_{\max}$	
	state		
		810(br)	
	0	650	
		535	
1	1+	885	
	_	510, 470	
	0.1	675	
	2+	405(sh)	
		350(sh)	
		810(br)	
	0	655	
		535	
2	1+	890	
		520, 470	
	2+	675	
		405(sh)	
		350(sh)	
3	1+	900	
		525, 490	
4	1+	895	
		525, 480	
	0	675(sh)	
	0	600, 505	
5		345(sh)	
3	1+	985-845	
		465, 410	
	2+ <sup>a</sup>	430 (sh), 390	
6	0	675(sh)	
U	0	590, 510(sh)	
		375	

Electrochemically generated species in DMF / 0.1M NBu<sub>4</sub>PF<sub>6</sub>,

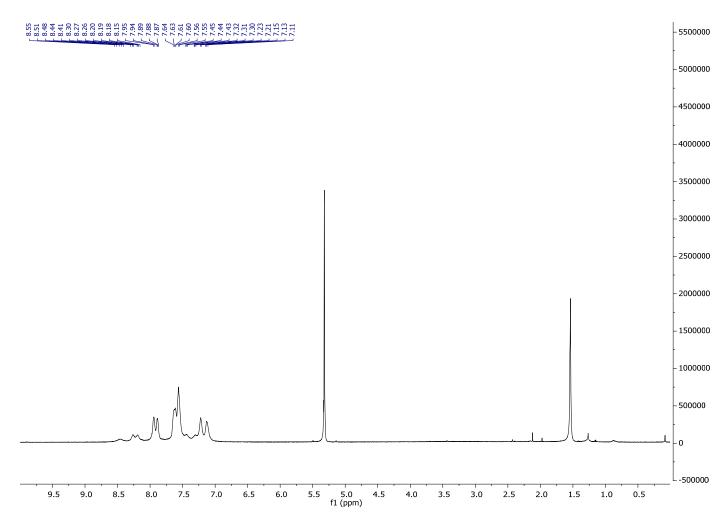
<sup>a</sup> irreversible

- 2) Figure S1. NMR spectra of complexes 1-6.
- (1),  ${}^{1}$ H, CD<sub>2</sub>Cl<sub>2</sub>, 500 MHz

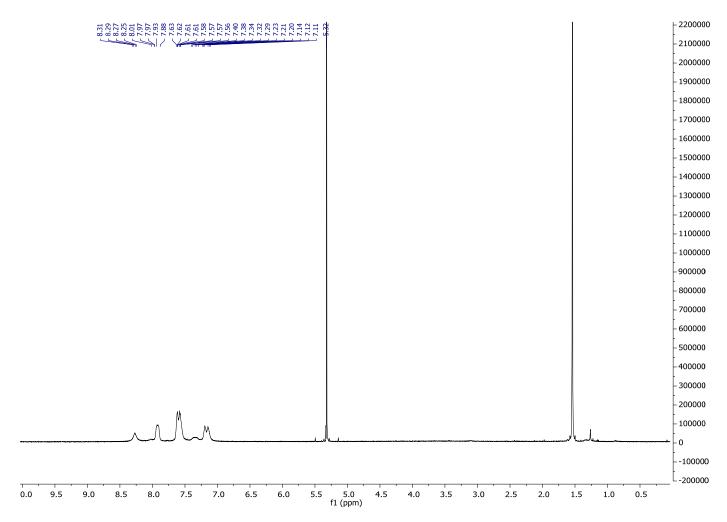


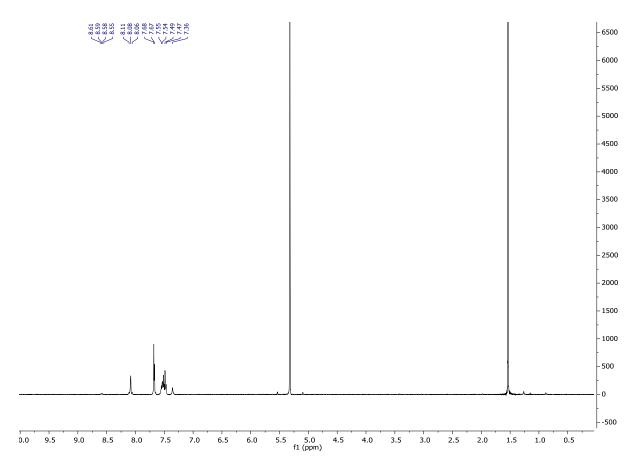


#### (3), <sup>1</sup>H, CD<sub>2</sub>Cl<sub>2</sub>, 500 MHz

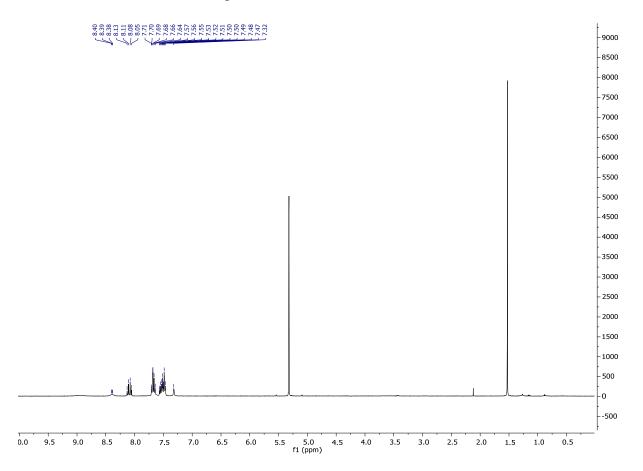


#### (4), <sup>1</sup>H, CD<sub>2</sub>Cl<sub>2</sub>, 500 MHz

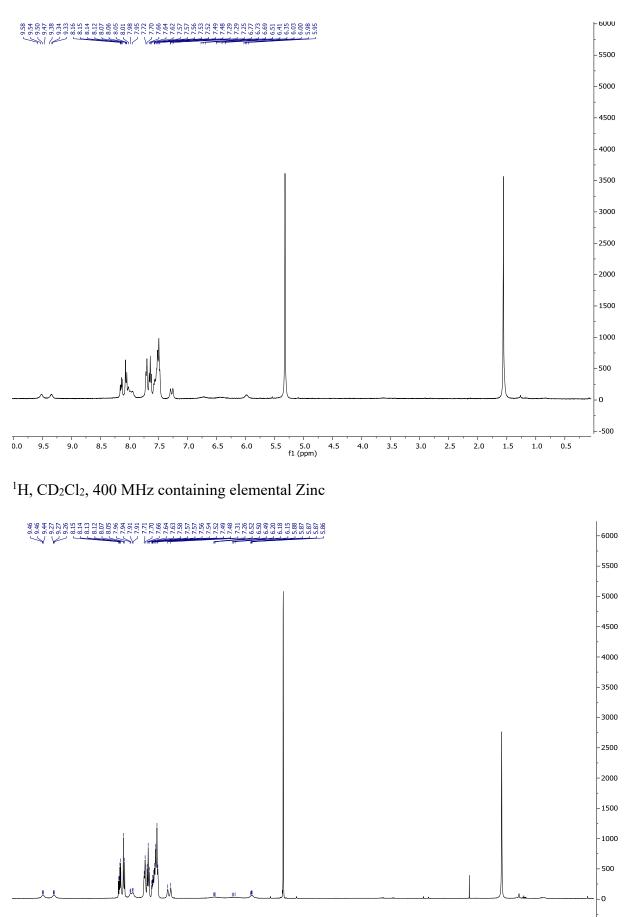




<sup>1</sup>H, CD<sub>2</sub>Cl<sub>2</sub>, 400 MHz containing elemental Zinc



#### (6), <sup>1</sup>H, CD<sub>2</sub>Cl<sub>2</sub>, 400 MHz



6.0

9.5

9.0

8.5

8.0

7.5

7.0

6.5

5.5

5.0 4.5 f1 (ppm) 4.0

3.5

3.0

2.5

2.0

1.5

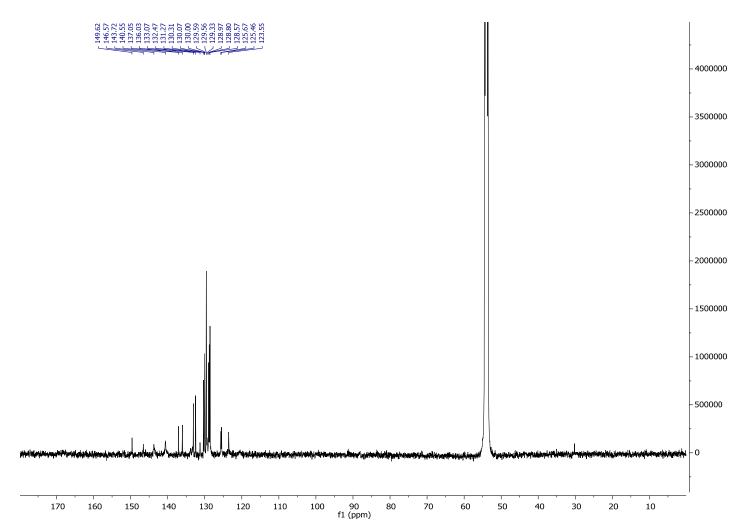
1.0

0.5

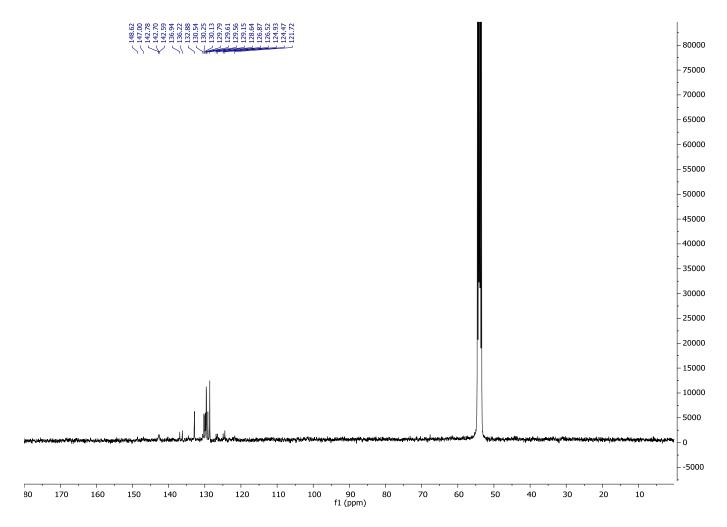
-500

0.0

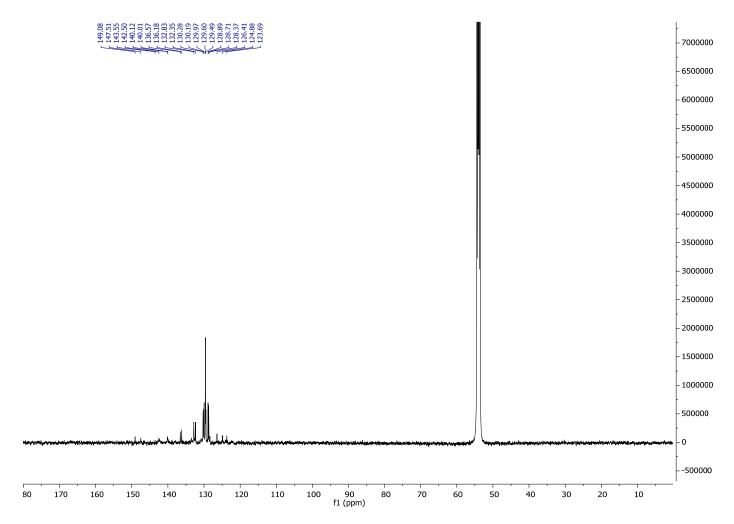
# (1), <sup>13</sup>C, CD<sub>2</sub>Cl<sub>2</sub>, 125 MHz



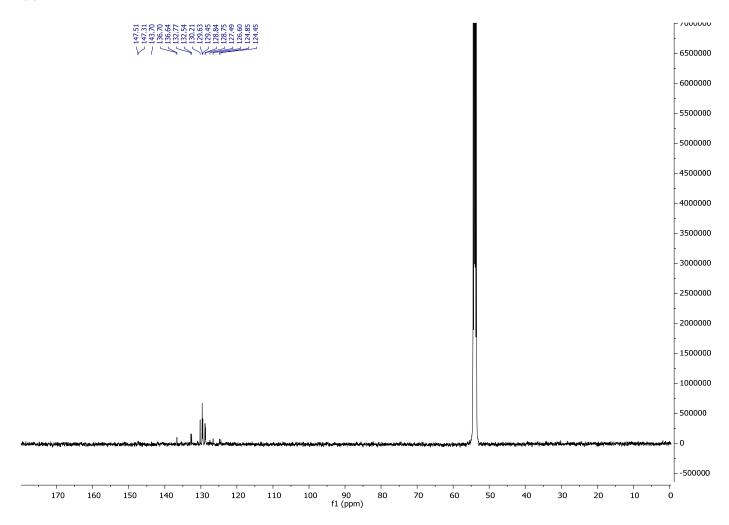
# (2), <sup>13</sup>C, CD<sub>2</sub>Cl<sub>2</sub>, 125 MHz



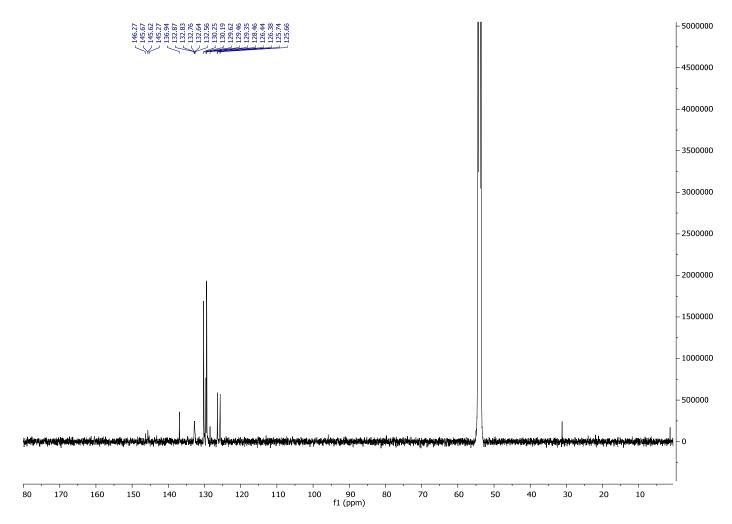
# (**3**), <sup>13</sup>C, CD<sub>2</sub>Cl<sub>2</sub>, 125 MHz



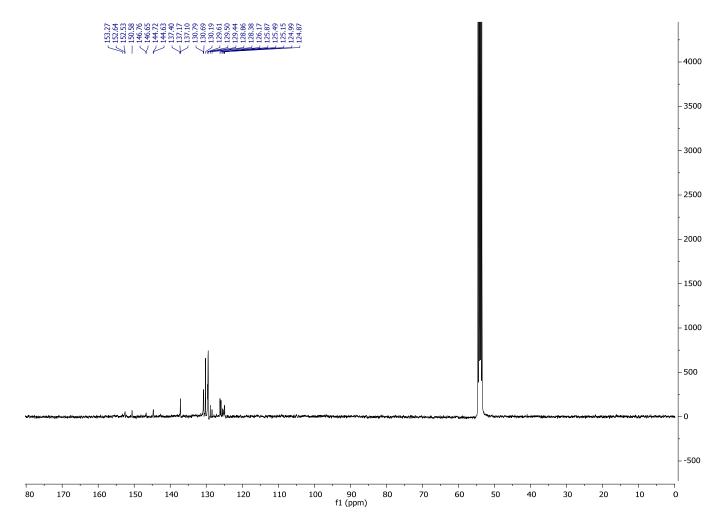
# (4), <sup>13</sup>C, CD<sub>2</sub>Cl<sub>2</sub>, 125 MHz



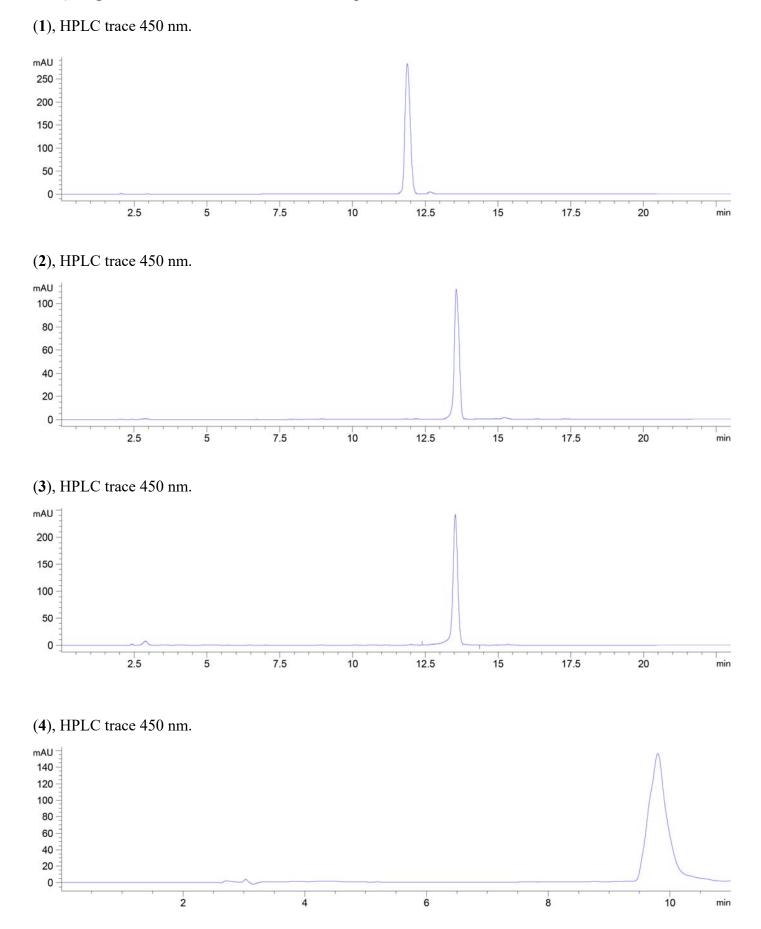
# (5), <sup>13</sup>C, CD<sub>2</sub>Cl<sub>2</sub>, 125 MHz



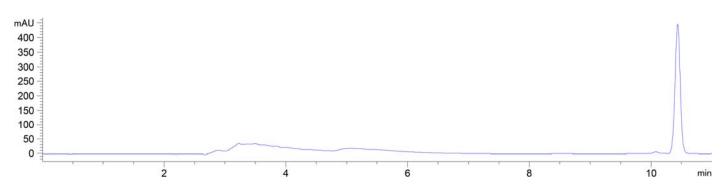
# (6), <sup>13</sup>C, CD<sub>2</sub>Cl<sub>2</sub>, 100 MHz



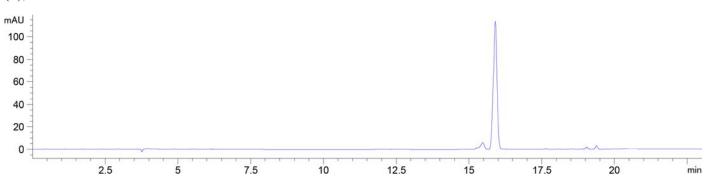
#### 3) Figure S2. HPLC traces at 450 nm of complexes 1-6.

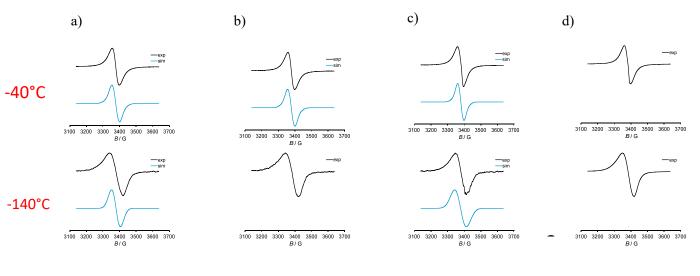


#### (**5**), HPLC trace 450 nm.

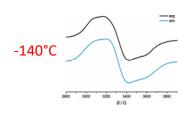


(**6**), HPLC trace 450 nm.







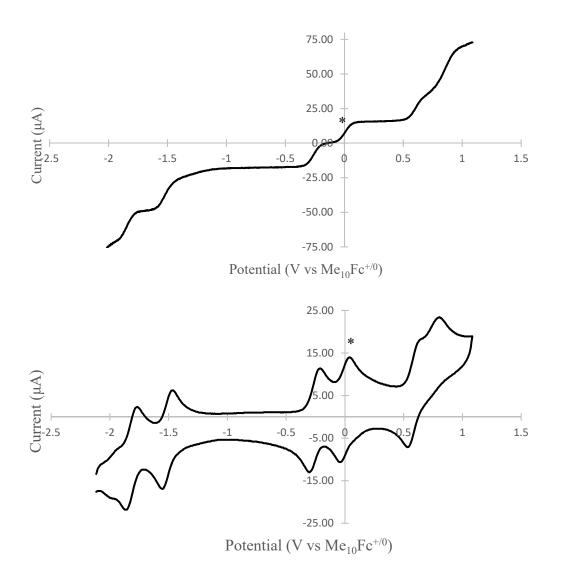


		B3LYP	exp.
(1)	giso	2.0055 (3.2)	1.9893 (-13.0)
	<b>g</b> 11	2.0020 (-0.3)	
	<b>g</b> 22	2.0070 (4.7)	
	<b>g</b> 33	2.0074 (5.0)	
	<b>g</b> 33 <b>-g</b> 11	0.0054	
	$< S^2 >$	0.759	
(3)	giso	2.0054 (3.1)	1.9872 (-15.1)
	<b>g</b> 11	2.0020 (-0.3)	
	<b>g</b> 22	2.0070 (4.7)	
	<b>g</b> 33	2.0071 (4.9)	
	<b>g</b> <sub>33</sub> - <b>g</b> <sub>11</sub>	0.0051	
	$< S^2 >$	0.759	
(5 <b>o</b> x)	giso	2.0089 (6.6)	2.019 (16.7)
	g11	2.0019 (-0.4)	
	<b>g</b> 22	2.0090 (6.6)	
	<b>g</b> 33	2.0159 (13.6)	
	<b>g</b> <sub>33</sub> - <b>g</b> <sub>11</sub>	0.0140	
	$< S^2 >$	0.759	
( <b>60</b> x)	giso	2.0076 (5.3)	2.032 (29.7)
	<b>g</b> 11	2.0038 (1.5)	1.870 (-132.3)
	<b>g</b> 22	2.0076 (5.3)	2.025 (22.7)
	<b>g</b> 33	2.0076 (8.9)	2.190 (187.7)
	g33-g11	0.0074	0.32
	$< S^2 >$	0.760	

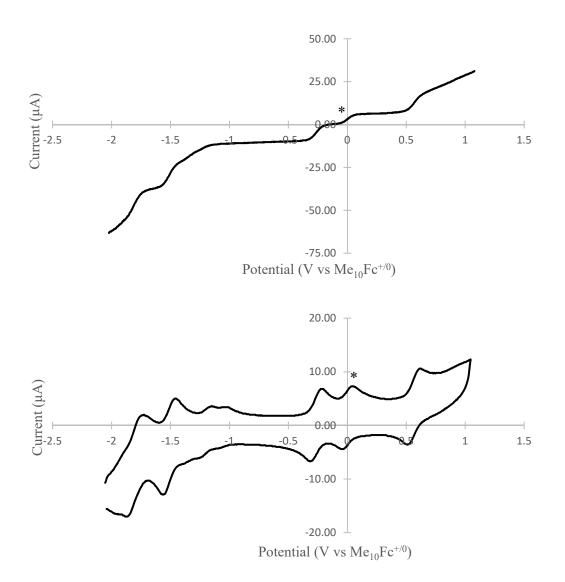
5) **Table S2**. Computed and Experimental *g*-Tensors (absolute g-values with *g*-shifts in ppt in parentheses).  $\langle S^2 \rangle$  values correspond to the expectation values of the Kohn-Sham determinant.

#### 6) Figure S4. Voltammograms recorded by CV and with the use of RDE of complexes 2-6.

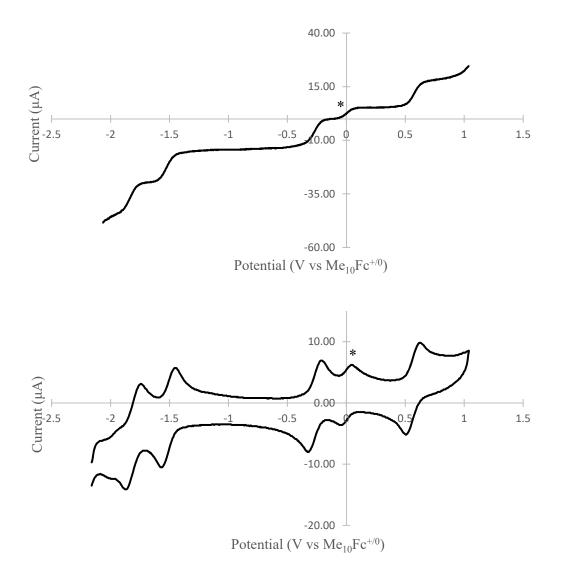
(2) (from -2.1 to +1 V) at a glassy carbon electrode in DMF (1 mM) containing  $Bu_4NPF_6$  (100 mM) as supporting electrolyte and decamethylferrocene as an internal standard (0.25 mM). Data were recorded versus saturated calomel electrode at scan rate of 100 mV/s and recalculated versus  $Me_{10}Fc^{0/+}$  potential value (-0.09 V and -0.09 V for RDE and CV respectively).



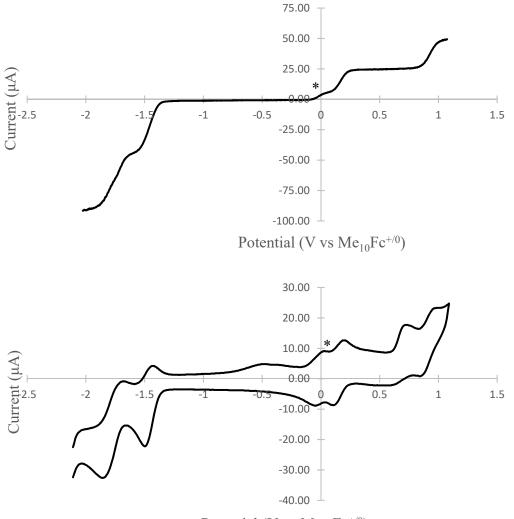
(3) (from -2.1 to +1 V) at a glassy carbon electrode in DMF (1 mM) containing Bu<sub>4</sub>NPF<sub>6</sub> (100 mM) as supporting electrolyte and decamethylferrocene as an internal standard (0.25 mM). Data were recorded versus saturated calomel electrode at scan rate of 100 mV/s and recalculated versus Me<sub>10</sub>Fc<sup>0/+</sup> potential value (-0.07 V and -0.05 V for RDE and CV respectively).



(4) (from -2.1 to +1 V) at a glassy carbon electrode in DMF (1 mM) containing Bu<sub>4</sub>NPF<sub>6</sub> (100 mM) as supporting electrolyte and decamethylferrocene as an internal standard (0.25 mM). Data were recorded versus saturated calomel electrode at scan rate of 100 mV/s and recalculated versus Me<sub>10</sub>Fc<sup>0/+</sup> potential value (-0.04 V and -0.04 V for RDE and CV respectively).

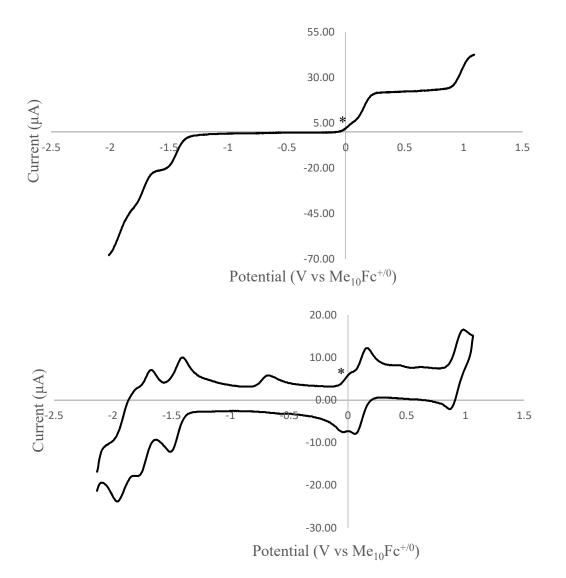


(5) (from -2.1 to +1 V) at a glassy carbon electrode in DMF (1 mM) containing Bu<sub>4</sub>NPF<sub>6</sub> (100 mM) as supporting electrolyte and decamethylferrocene as an internal standard (0.25 mM). Data were recorded versus saturated calomel electrode at scan rate of 100 mV/s and recalculated versus Me<sub>10</sub>Fc<sup>0/+</sup> potential value (-0.07 V and -0.09 V for RDE and CV respectively).



Potential (V vs Me<sub>10</sub>Fc<sup>+/0</sup>)

(6) (from -2.1 to +1 V) at a glassy carbon electrode in DMF (1 mM) containing  $Bu_4NPF_6$  (100 mM) as supporting electrolyte and decamethylferrocene as an internal standard (0.25 mM). Data were recorded versus saturated calomel electrode at scan rate of 100 mV/s and recalculated versus  $Me_{10}Fc^{0/+}$  potential value (-0.07 V and -0.09 V for RDE and CV respectively).

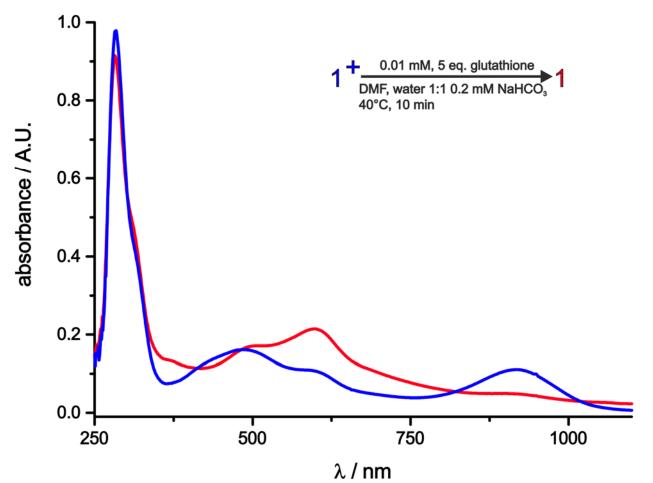


#### 7) Table S3. Electrochemical data for complexes 1-4

		Ph <sub>2</sub> Phen <sup>0/-</sup>	Ph <sub>2</sub> Phen <sup>0/-</sup>	sq/cat	Ru <sup>11/111</sup>
Ru-sq*	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.876 \pm 0.039$	-1.578 ± 0.035	$-0.249 \pm 0.010$	$0.647 \pm 0.018$
	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.816 \pm 0.015$	$-1.507 \pm 0.007$	$-0.209 \pm 0.002$	$0.623 \pm 0.005$
1	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.848 \pm 0.015$	$-1.537 \pm 0.008$	$-0.284 \pm 0.005$	$0.595 \pm 0.011$
I	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.798 \pm 0.001$	$-1.503 \pm 0.002$	$-0.251 \pm 0.001$	$0.602 \pm 0.002$
2	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.833 \pm 0.007$	$-1.497 \pm 0.012$	$-0.252 \pm 0.011$	$0.615 \pm 0.003$
L	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.813 \pm 0.002$	$-1.510 \pm 0.002$	$-0.256 \pm 0.001$	$0.592\pm0.004$
3	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.836 \pm 0.028$	$-1.472 \pm 0.070$	$-0.265 \pm 0.019$	$0.636 \pm 0.011$
5	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.797 \pm 0.002$	$-1.511 \pm 0.001$	$-0.264 \pm 0.002$	$0.569 \pm 0.003$
4	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.839 \pm 0.017$	$-1.515 \pm 0.005$	$-0.271 \pm 0.008$	$0.574\pm0.001$
4	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.807 \pm 0.006$	$-1.506 \pm 0.003$	$-0.265 \pm 0.002$	$0.567\pm0.004$

\* Values taken from[<sup>2</sup>] We however note that these experiments were performed on the same days. <sup>a</sup>  $E_{1/2} = half$ -wave potential in Volts. <sup>b</sup>  $E_{1/2} = (E_{Pa} + E_{Pc})/2$  in Volts.

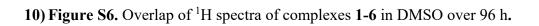
8) Figure S5. UV/Vis/NIR-spectroelectrochemistry data for complex 1 in the presence of the reducing agent glutathione.

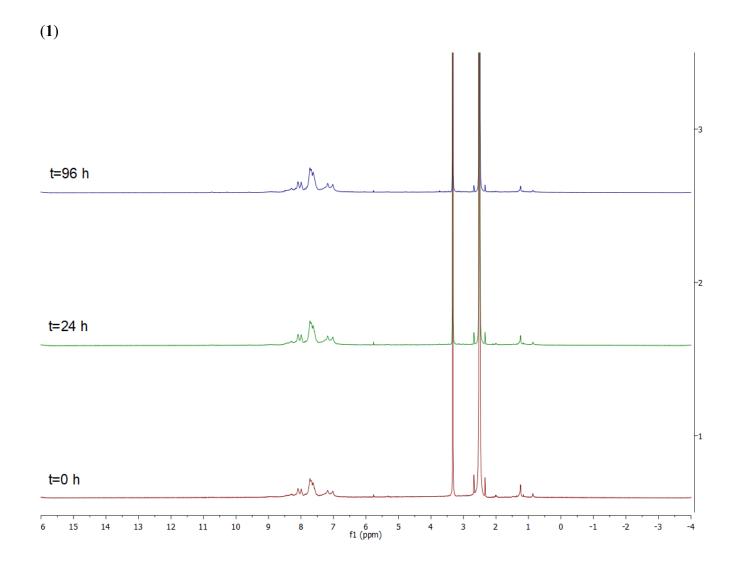


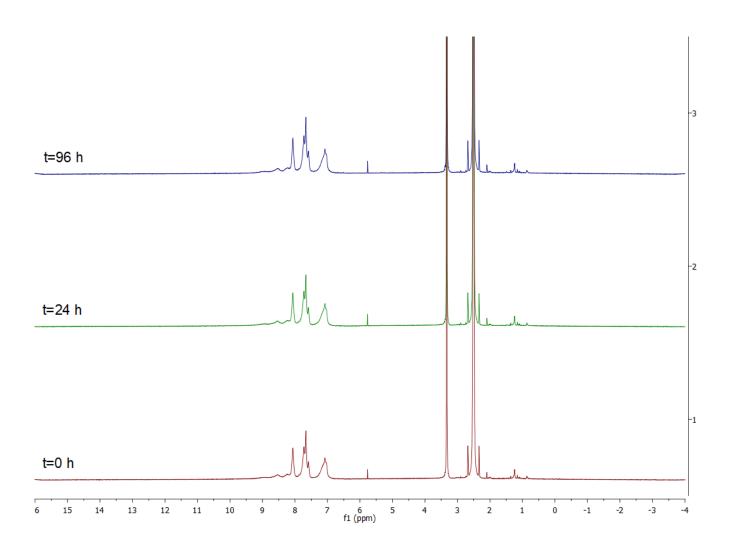
### 9) Table S4. Electrochemical data for complexes 5 and 6.

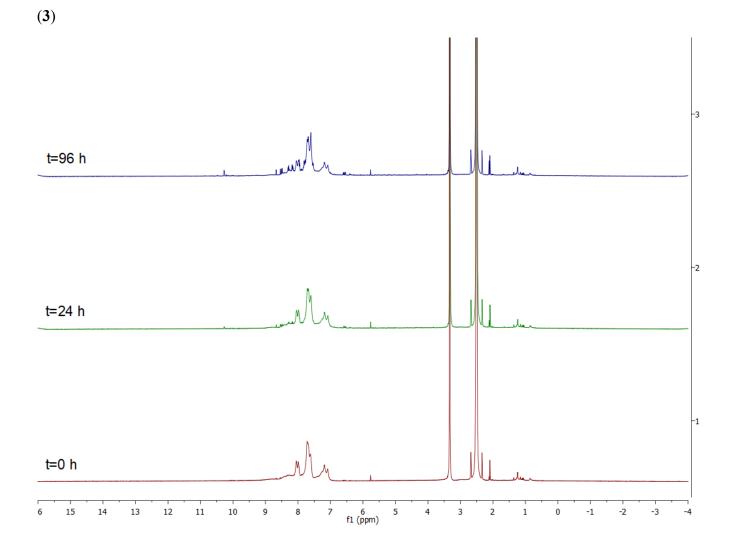
		Ph <sub>2</sub> Phen <sup>0/-</sup>	Ph <sub>2</sub> Phen <sup>0/-</sup>	Ox1	Ox2
5	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.746 \pm 0.003$	$-1.423 \pm 0.002$	$0.182\pm0.005$	$0.927\pm0.008$
	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.764 \pm 0.011$	$-1.456 \pm 0.003$	$0.162 \pm 0.001$	$0.910 \pm 0.003$
6	E <sub>1/2<sup>a</sup></sub> [V] (RDE)	$-1.737 \pm 0.009$	$-1.440 \pm 0.009$	$0.164 \pm 0.013$	$0.970 \pm 0.009$
Ū	E <sub>1/2<sup>b</sup></sub> [V] (CV)	$-1.734 \pm 0.006$	$-1.456 \pm 0.003$	$0.114 \pm 0.004$	$0.929\pm0.007$

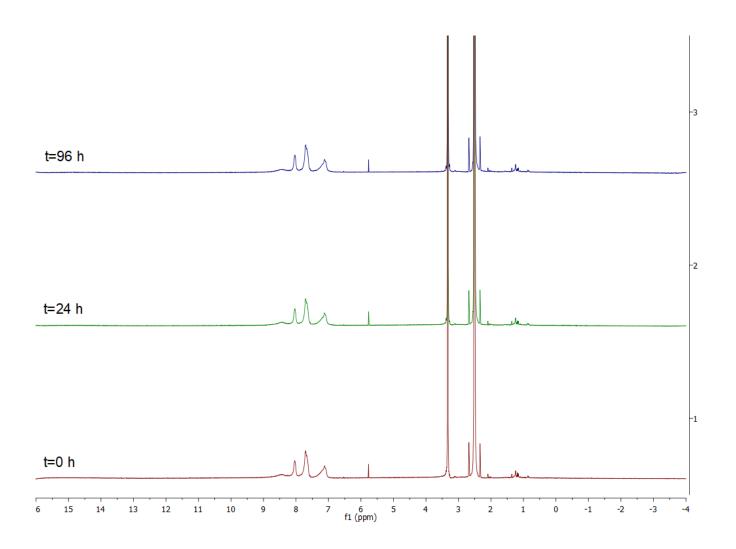
<sup>a</sup>  $E_{1/2}$  = half-wave potential in Volts. <sup>b</sup>  $E_{1/2}$  = ( $E_{Pa} + E_{Pc}$ )/2 in Volts.

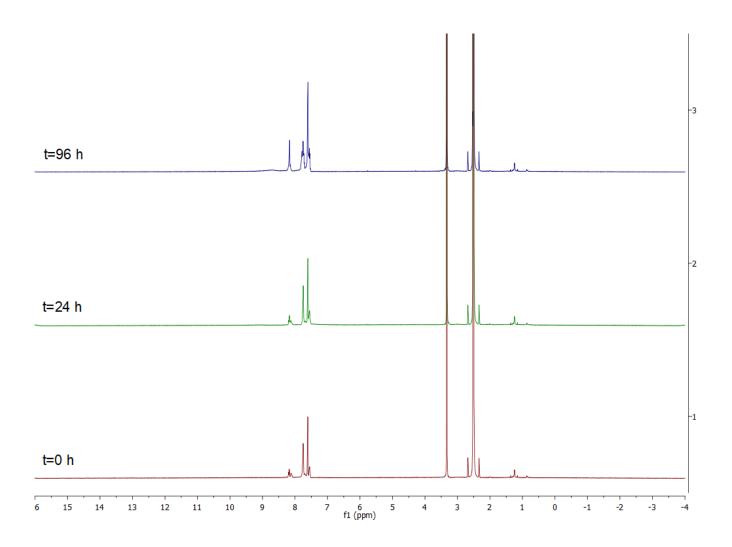


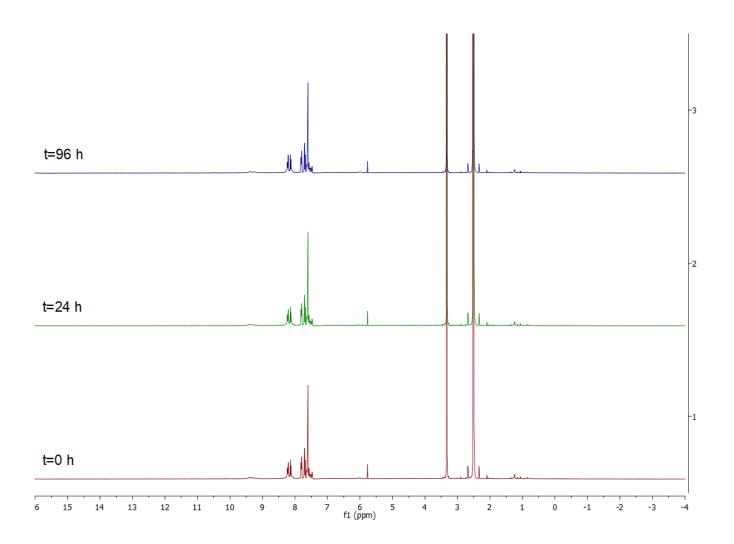




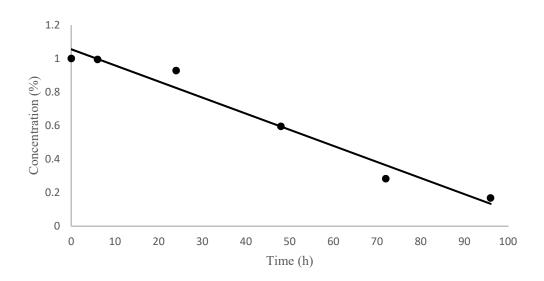


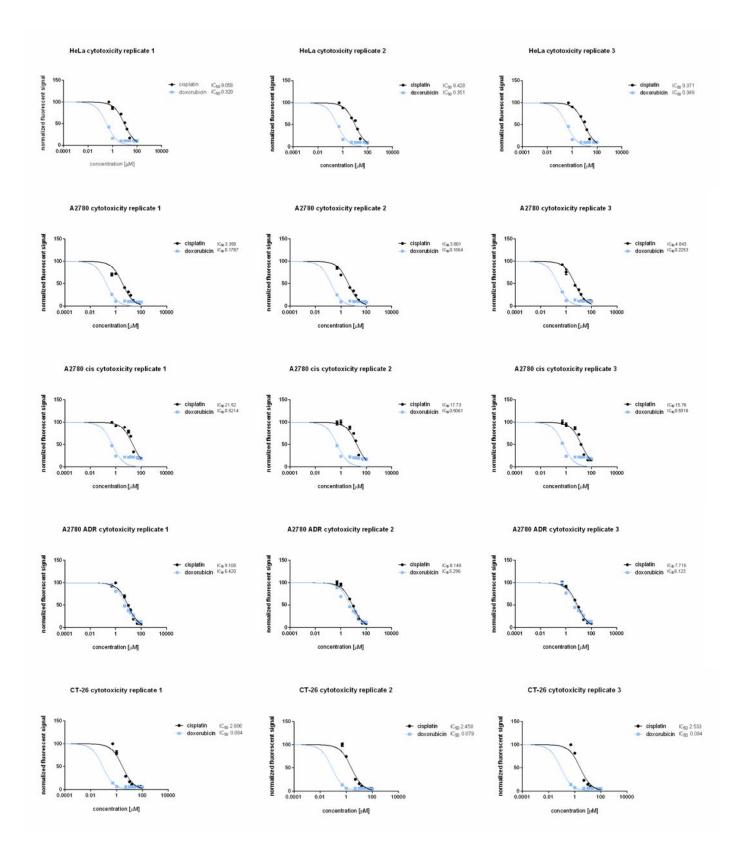






11) Figure S7. Percentage concentration of complex 1 in human plasma, normalized with respect to the internal standard (caffeine) and plotted against time.

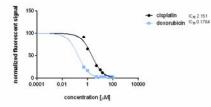


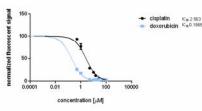


CT-26 LUC cytotoxicity replicate 1

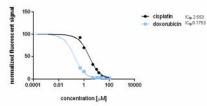
CT-26 LUC cytotoxicity replicate 2

CT-26 LUC cytotoxicity replicate 3

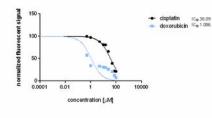


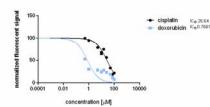


RPE-1 cytotoxicity replicate 2

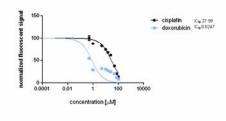


RPE-1 cytotoxicity replicate 1

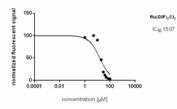




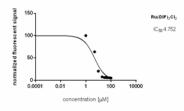
RPE-1 cytotoxicity replicate 3



HeLa cytotoxicity replicate 1



A2780 cytotoxicity replicate 1



A2780 ADR cytotoxicity replicate 1

concentration [µM]

100

10000

Ru(DIP)2CI2

IC<sub>50</sub>76.13

normalized fluorescent signal

15(

50

0.0001

0.01 ÷

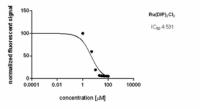
normalized fluorescent signal 10 50 0 0.01 4 concentration [µM]

HeLa cytotoxicity replicate 2

Ru(DIP)2CI2

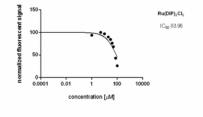
IC<sub>50</sub> 15.41

A 2780 cytotoxicity replicate 2

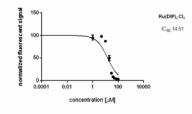


A2780 ADR cytotoxicity replicate 2

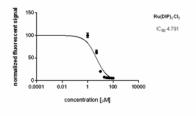
150



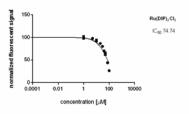
HeLa cytotoxicity replicate 3



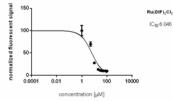
A 2780 cytotoxicity replicate 3



A2780 ADR cytotoxicity replicate 3



A 2780 cis cytotoxicity replicate 1



CT-26 cytotoxicity replicate 1

i

concentration (µM)

CT-26 LUC cytotoxicity replicate 1

Ru(DIP)2CI2

IC<sub>50</sub>8.615

Ru(DIP)2CI2

IC<sub>50</sub>7.075

10000

10000

100

normalized fluorescent signal

normalized fluorescent signal

150

100

50

0.0001

0.01

150

10

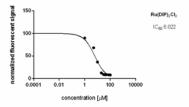
50

0.0001

0.01

A2780 cis cytotoxicity replicate 2

A 2780 cis cytotoxicity replicate 3



CT-26 cytotoxicity replicate 2

4 100 10000

concentration [µM]

CT-26 LUC cytotoxicity replicate 2

Ru(DIP)2CI2

IC<sub>50</sub> 8.393

Ru(DIP)2CI2

IC<sub>50</sub> 3.219

IC<sub>50</sub> 5.875

150

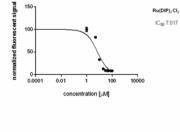
10

50

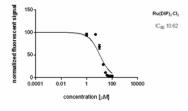
0.0001

0.01

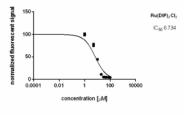
normalized fluorescent signal



CT-26 cytotoxicity replicate 3



CT-26 LUC cytotoxicity replicate 3



RPE-1 cytotoxicity replicate 3

Ru(DIP)2CI2

IC<sub>50</sub> 3.094

10000

signal

normalized fluorescent

150

10

50

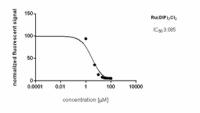
0.0001

0.01

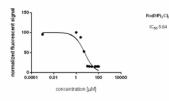
con

RPE-1 cytotoxicity replicate 1

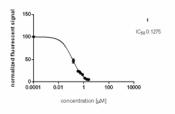
concentration [µM]



MRC-5 cytotoxicity replicate 1



HeLa cytotoxicity replicate 1



150

normalized fluorescent signal

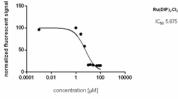
150-

100

50

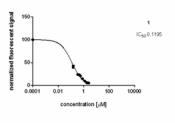
0

0.01



MRC-5 cytotoxicity replicate 2

HeLa cytotoxicity replicate 2

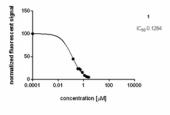


MRC-5 cytotoxicity replicate 3

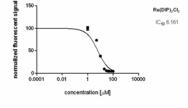
tration [µM]

ignal 150 Ru(DIP)2CI2 IC<sub>50</sub> 5.111 scent 10 normalized fluor 50 0.0001 0.01 10000 1 concentration [µM]





S37

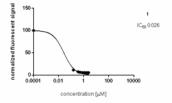


RPE-1 cytotoxicity replicate 2

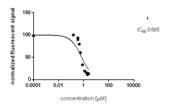
100 10000

ration [µM]

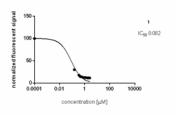
A 2780 cytotoxicity replicate 1



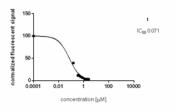
A 2780 ADR cytotoxicity replicate 1



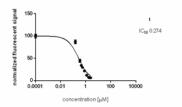
A2780 cis cytotoxicity replicate 1

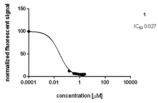


CT-26 cytotoxicity replicate 1



CT-26 LUC cytotoxicity replicate 1





A2780 cytotoxicity replicate 2

A2780 ADR cytotoxicity replicate 2

normalized fluorescent signal

normalized fluorescent signal

150

10

50 -

0.0001

0.01

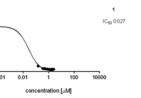
150

100

50

0.0001

0.01



100 10000

ntration [µM]

A2780 cis cytotoxicity replicate 2

1

IC<sub>50</sub> 0.665

1

IC<sub>50</sub> 0.067

normalized fluorescent signal 50 0.0001 0.01 100 10000 concentration [µM]

150

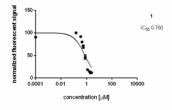
100

## A2780 ADR cytotoxicity replicate 3

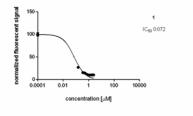
A 2780 cytotoxicity replicate 3

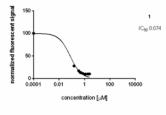
1

IC<sub>50</sub> 0.026



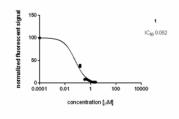
A2780 cis cytotoxicity replicate 3





CT-26 cytotoxicity replicate 2

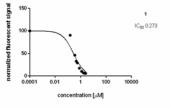
CT-26 cytotoxicity replicate 3



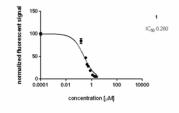
CT-26 LUC cytotoxicity replicate 2

concentration [µM]

100 10000



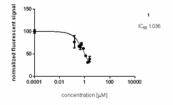
CT-26 LUC cytotoxicity replicate 3

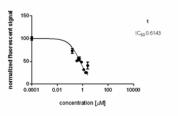


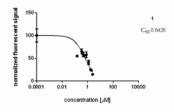
RPE-1 cytotoxicity replicate 1

RPE-1 cytotoxicity replicate 2

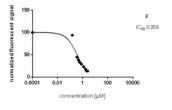
RPE-1 cytotoxicity replicate 3



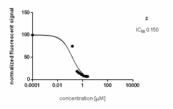




HeLa cytotoxicity replicate 1



A 2780 cytotoxicity replicate 1



A 2780 ADR cytotoxicity replicate 1

Ś

i

concentration (µM)

A2780 cis cytotoxicity replicate 1

100 10000

100 10000

entration (µM) con

2

IC<sub>50</sub> 1.22

2

IC<sub>50</sub> 0.311

normalized fluorescent signal

normalized fluorescent signal

150

100

50

0.0001

0.01

150

100

50

0.0001

0.01

normalized fluorescent signal 50 -

0.0001

0.01

150

100

A 2780 cytotoxicity replicate 2

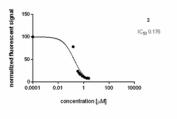
concentration [µM]

100

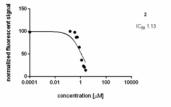
HeLa cytotoxicity replicate 2

2

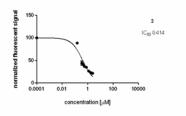
IC<sub>50</sub> 0.356

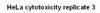


A2780 ADR cytotoxicity replicate 2



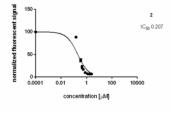
A2780 cis cytotoxicity replicate 2



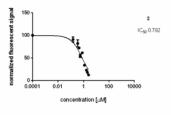


normalized fluorescent signal 150 2 IC<sub>50</sub> 0.346 50 0 0.01 10 concentration [µM]

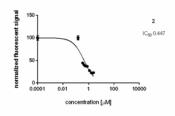




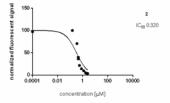
A2780 ADR cytotoxicity replicate 3



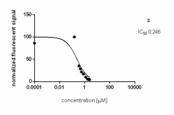
A 2780 cis cytotoxicity replicate 3

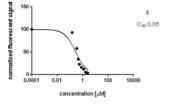


CT-26 cytotoxicity replicate 1



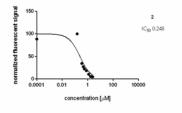
CT-26 LUC cytotoxicity replicate 1

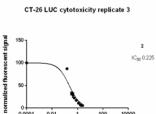




CT-26 cytotoxicity replicate 2

CT-26 LUC cytotoxicity replicate 2





CT-26 cytotoxicity replicate 3

100 10000

ntration [µM]

2

IC<sub>50</sub> 0.341

2

IC<sub>50</sub> 0.431

3

IC<sub>50</sub> 0.346

normalized fluorescent signal

150

10

50

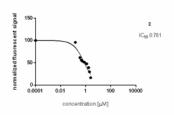
0.0001

0.01

conc

0.0001 0.01 i 100 concentration [µM]

RPE-1 cytotoxicity replicate 1



RPE-1 cytotoxicity replicate 2

normalized fluorescent signal

normalized fluorescent signal

150

100

50-

0.0001

150

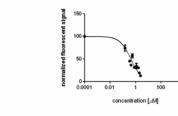
100

50-

0.0001

0.01

RPE-1 cytotoxicity replicate 3



normalized fluorescent signal

150

100

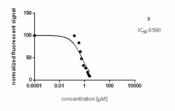
50

0.0001

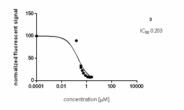
0.01

conc

HeLa cytotoxicity replicate 1



A 2780 cytotoxicity replicate 1

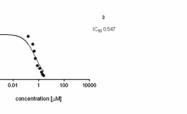


HeLa cytotoxicity replicate 2

1 100 10000

concentration [µM]

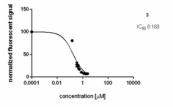
HeLa cytotoxicity replicate 3



2

IC<sub>50</sub> 0.787

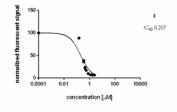
A 2780 cytotoxicity replicate 2



A 2780 cytotoxicity replicate 3

1 100 10000

ntration [µM]



A2780 ADR cytotoxicity replicate 1



A2780 ADR cytotoxicity replicate 3

IC<sub>50</sub> 1.38

3

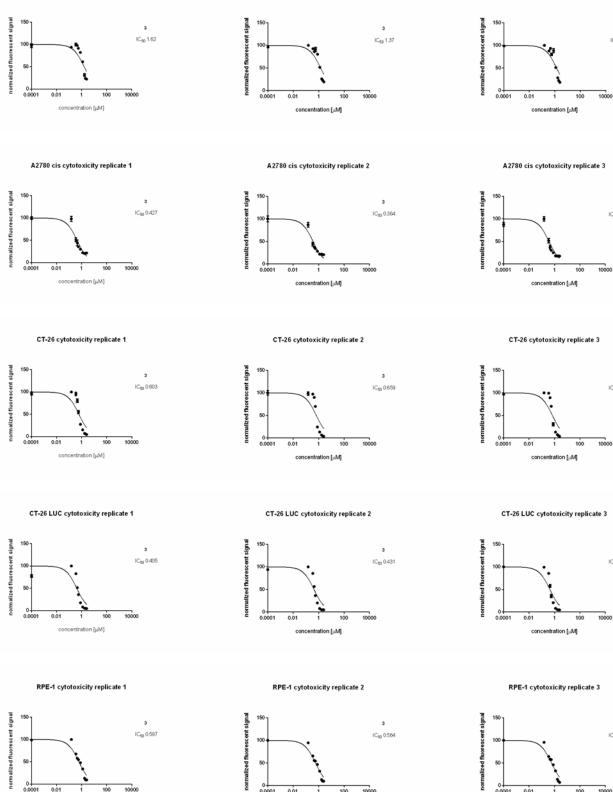
IC<sub>50</sub> 0.372

3

IC50 0.688

3

IC<sub>50</sub> 0.433



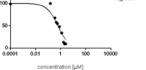
0.0001

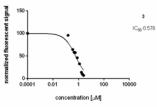
0.01

100 10000

1

concentration [µM]





HeLa cytotoxicity replicate 1

0.0001

0.01

concentration [µM]

10000 100

HeLa cytotoxicity replicate 2

HeLa cytotoxicity replicate 3

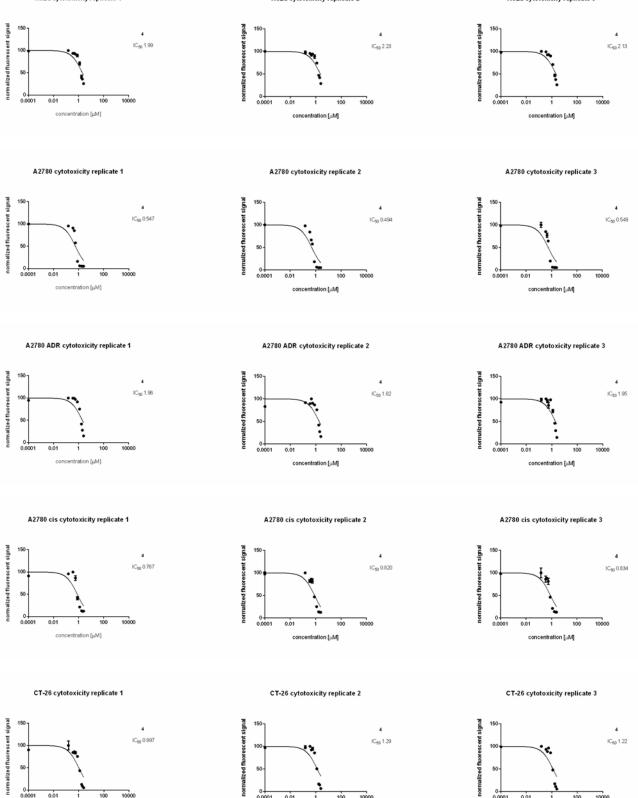
50

0.0001

0.01

100 10000

concentration [µM]



50-

0.0001

0.01

100 10000

concentration [µM]

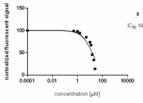
CT-26 LUC cytotoxicity replicate 1 CT-26 LUC cytotoxicity replicate 2 CT-26 LUC cytotoxicity replicate 3 normalized fluorescent signal 150 normalized fluorescent signal 150 normalized fluorescent signal 4 4 4 IC<sub>50</sub> 1.36 IC<sub>50</sub> 1.17 IC<sub>50</sub> 0.910 100 100 Ì∳i 50 50 50 ٩ ٩ 0.0001 0.0001 0.0001 0.01 100 1000 0.01 100 10000 0.01 1 100 10000 4 ntration [µM] concentration [µM] ntration [µM] conce RPE-1 cytotoxicity replicate 1 RPE-1 cytotoxicity replicate 2 RPE-1 cytotoxicity replicate 3 normalized fluorescent signal normalized fluorescent signal 150normalized fluorescent signal 150 4 4 4 IC<sub>50</sub> 3.43 IC<sub>50</sub> 2.52 IC<sub>50</sub> 2.95 Η 100 ł 50 50-50 • 0.0001 0.0001 0.0001 0.01 100 10000 1 0.01 100 10000 0.01 100 10000 con entration (µM) concentration [µM] concentration [µM] HeLa cytotoxicity replicate 1 HeLa cytotoxicity replicate 2 HeLa cytotoxicity replicate 3 normalized fluorescent signal normalized fluorescent signal 150 normalized fluorescent signal 150 5 5 5 IC<sub>50</sub> 10.37 IC<sub>50</sub> 10.27 IC<sub>50</sub> 10.74 100 100 50 50 2 0 ٩. 0.0001 0.0001 0.01 100 1000 0.01 100 0.01 10000 100 concentration [µM] concentration [µM] concentration [µM] A 2780 cytotoxicity replicate 1 A 2780 cytotoxicity replicate 2 A 2780 cytotoxicity replicate 3 normalized fluorescent signal 15 normalized fluorescent signal 150 normalized fluorescent signal 150 5 5 5 IC<sub>60</sub> 10.32 IC<sub>50</sub> 10.07 IC<sub>50</sub> 10.32 10 100 50 50 -50 ١. 0.0001 0.0001 0.0001 0.01 1 10000 100 0.01 100 0.01 1 100 100 concentration [µM] concentration [µM] concentration [µM] A2780 ADR cytotoxicity replicate 1 A2780 ADR cytotoxicity replicate 2 A2780 ADR cytotoxicity replicate 3 normalized fluorescent signal 150 150 5 5 IC<sub>50</sub> 14.99 IC<sub>50</sub> 14.28 IC<sub>50</sub> 15.78 100 100 100

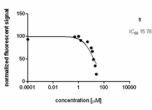
50-

0.0001

0.01 1 100 10000

concentration [µM]



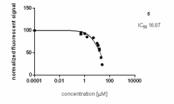


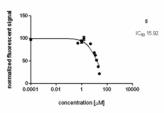
S43

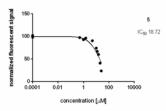
A2780 cis cytotoxicity replicate 1

A2780 cis cytotoxicity replicate 2

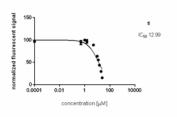
A 2780 cis cytotoxicity replicate 3



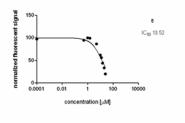




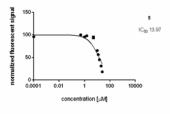
CT-26 cytotoxicity replicate 1



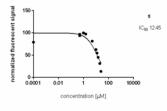
CT-26 cytotoxicity replicate 2

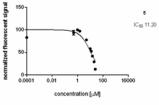


CT-26 cytotoxicity replicate 3



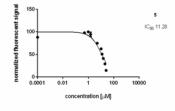
CT-26 LUC cytotoxicity replicate 1



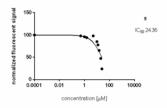


CT-26 LUC cytotoxicity replicate 2

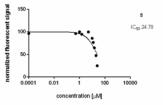




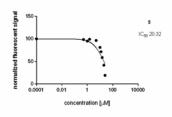
RPE-1 cytotoxicity replicate 1



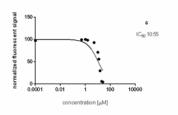
RPE-1 cytotoxicity replicate 2







HeLa cytotoxicity replicate 1





normalized fluorescent signal

150

100

50

0

0.01



2

i

concentration [µM]

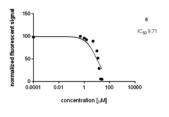
100

6

IC<sub>50</sub> 9.85

10000

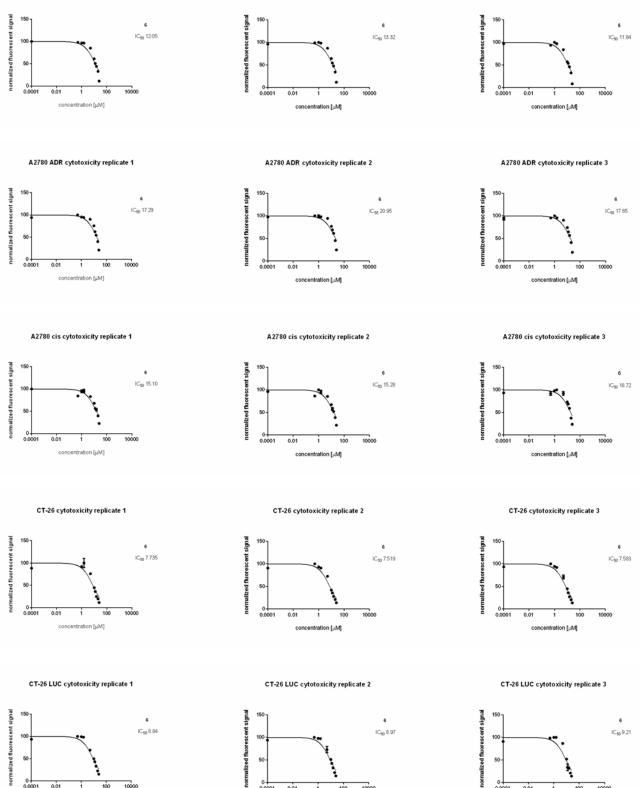
HeLa cytotoxicity replicate 3



A 2780 cytotoxicity replicate 1



A 2780 cytotoxicity replicate 3



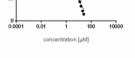
0.01

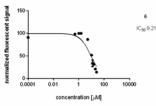
100

10000

i

concentration [µM]

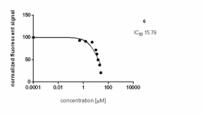




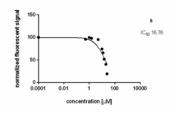
RPE-1 cytotoxicity replicate 1



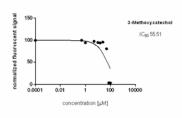
RPE-1 cytotoxicity replicate 3



150 100 0,0001 0,01 100 0,000 0,01 100 0,000 0,01 100 0,000 



HeLa cytotoxicity replicate 1





3-Methoxycatechol

IC<sub>50</sub> 60.68

10000

normalized fluorescent signal

150

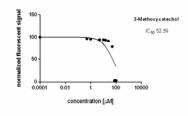
100

50

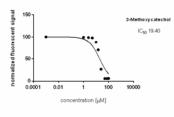
0.0

0.01

HeLa cytotoxicity replicate 3



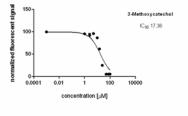
A 2780 cytotoxicity replicate 1



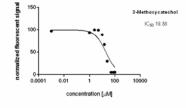
A2780 cytotoxicity replicate 2

ī

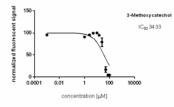
concentration [µM]



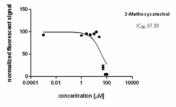
A 2780 cytotoxicity replicate 3



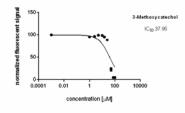
A2780 ADR cytotoxicity replicate 1



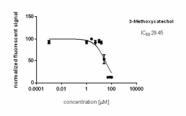
A2780 ADR cytotoxicity replicate 2







A2780 cis cytotoxicity replicate 1



A2780 cis cytotoxicity replicate 2

.

concentration [µM]

3-Methoxycatechol

IC<sub>50</sub> 29.15

normalized fluorescent signal

150

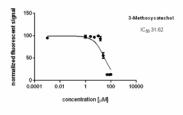
100-

50-

0

0.01 1 100 10000



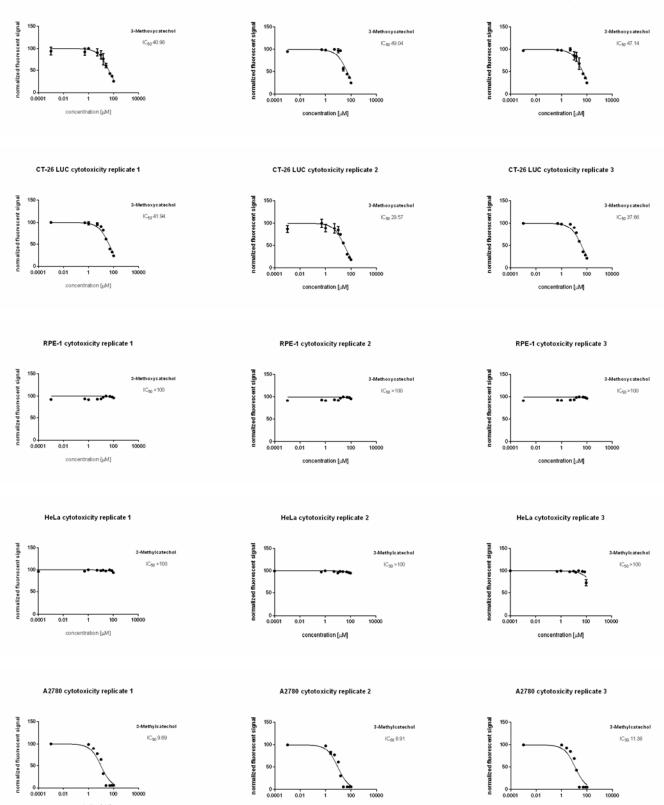


CT-26 cytotoxicity replicate 1

concentration [µM]

CT-26 cytotoxicity replicate 2

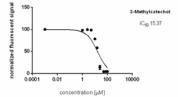
CT-26 cytotoxicity replicate 3



concentration [µM]

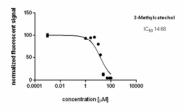
concentration [µM]

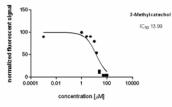
A2780 ADR cytotoxicity replicate 1



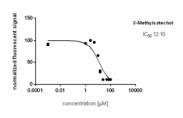
A2780 ADR cytotoxicity replicate 2

A 2780 ADR cytotoxicity replicate 3

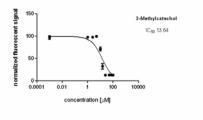




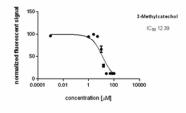
A2780 cis cytotoxicity replicate 1



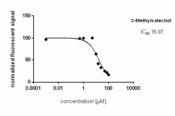
A2780 cis cytotoxicity replicate 2







CT-26 cytotoxicity replicate 1



CT-26 cytotoxicity replicate 2

3-Methylcatechol

IC<sub>50</sub> 17.12

3-Methylcatechol

IC<sub>50</sub> >100

normalized fluorescent signal

normalized fluorescent signal

150

100 -

50-

0

0.01

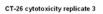
150

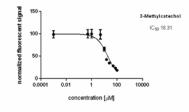
100

50

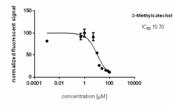
0

0.01





CT-26 LUC cytotoxicity replicate 1

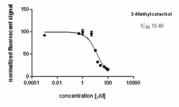


CT-26 LUC cytotoxicity replicate 2

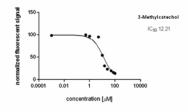
i

concentration [µM]

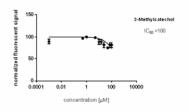
100 10000







RPE-1 cytotoxicity replicate 1





<del>1 144</del>

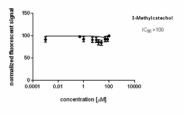
1

concentration [µM]

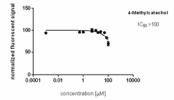
100

10000



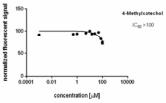


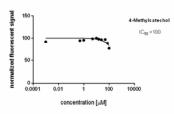
HeLa cytotoxicity replicate 1



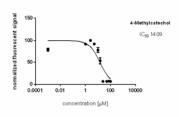
HeLa cytotoxicity replicate 2

HeLa cytotoxicity replicate 3

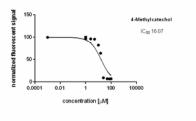




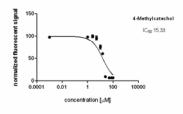
A 2780 cytotoxicity replicate 1



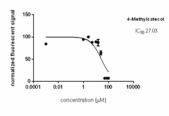
A 2780 cytotoxicity replicate 2



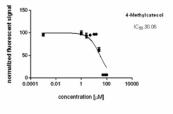
A 2780 cytotoxicity replicate 3



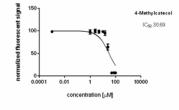
A2780 ADR cytotoxicity replicate 1



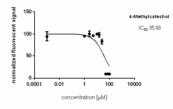
A2780 ADR cytotoxicity replicate 2



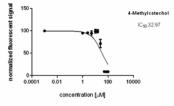
A2780 ADR cytotoxicity replicate 3



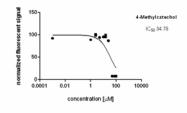
A2780 cis cytotoxicity replicate 1



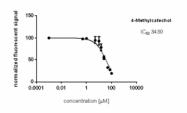
A 2780 cis cytotoxicity replicate 2



A2780 cis cytotoxicity replicate 3



CT-26 cytotoxicity replicate 1





normalized fluorescent signal

150

100

50 ·

0

0.01



.

i

concentration [µM]

Ś

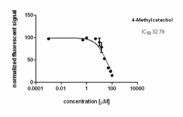
100

10000

4-Methylcatechol

IC<sub>50</sub> 35.51

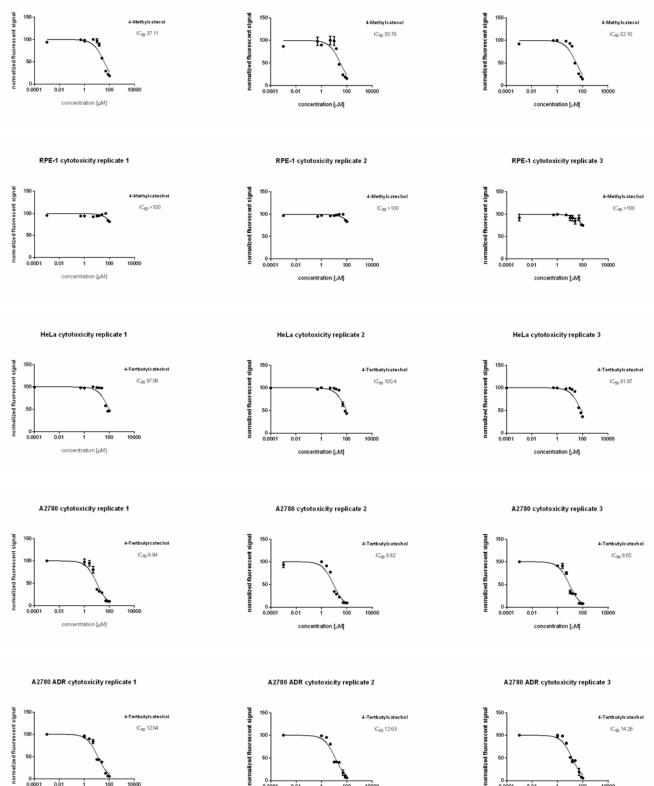


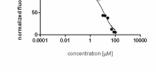


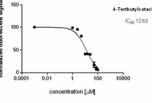
CT-26 LUC cytotoxicity replicate 1

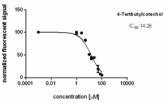


CT-26 LUC cytotoxicity replicate 3









A 2780 cis cytotoxicity replicate 1

4-Tertbutylcatechol

IC<sub>50</sub> 20.90

150

A 2780 cis cytotoxicity replicate 2

4-Tertbutylcatechol

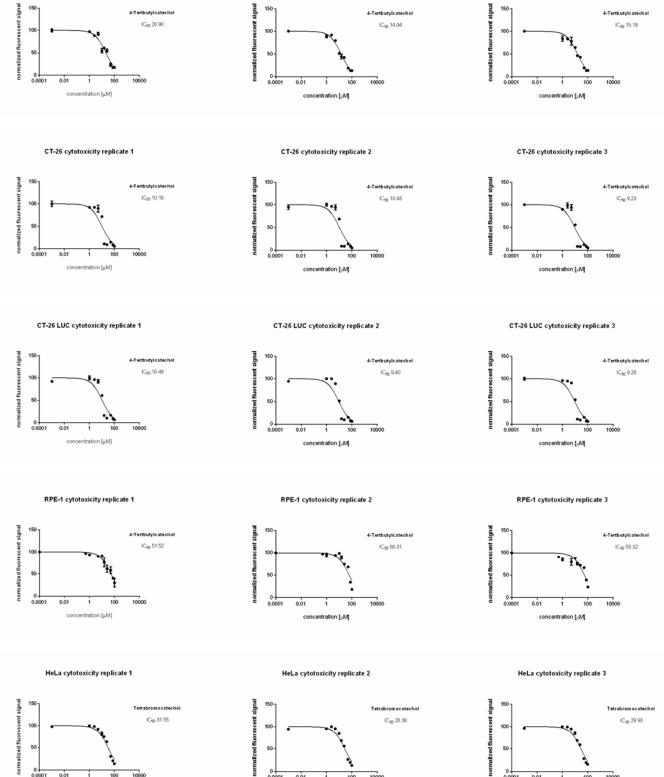
IC<sub>50</sub> 14.04

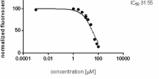
150

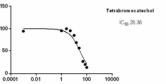
A2780 cis cytotoxicity replicate 3

4-Tertbutylcatechol

IC<sub>50</sub> 15.19







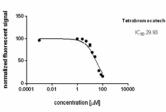
100

10000

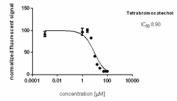
1

oncentration [µM]

0.01

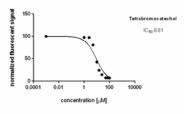


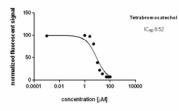
A 2780 cytotoxicity replicate 1



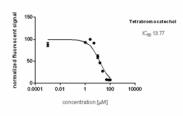
A 2780 cytotoxicity replicate 2

A2780 cytotoxicity replicate 3

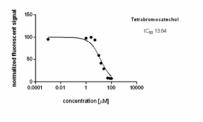




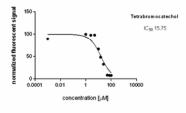
A2780 ADR cytotoxicity replicate 1



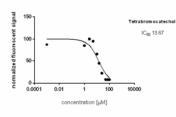
A2780 ADR cytotoxicity replicate 2



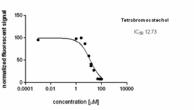
A2780 ADR cytotoxicity replicate 3



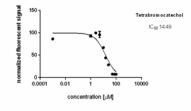
A2780 cis cytotoxicity replicate 1



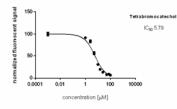
A 2780 cis cytotoxicity replicate 2



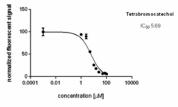
A 2780 cis cytotoxicity replicate 3



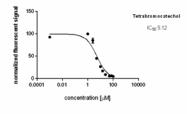
CT-26 cytotoxicity replicate 1



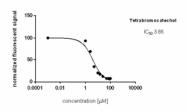
CT-26 cytotoxicity replicate 2







CT-26 LUC cytotoxicity replicate 1



CT-26 LUC cytotoxicity replicate 2

i

concentration [µM]

Tetrabromocatechol

IC<sub>50</sub> 3.43

10000

normalized fluorescent signal

150

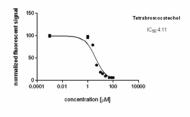
100

50

0

0.01

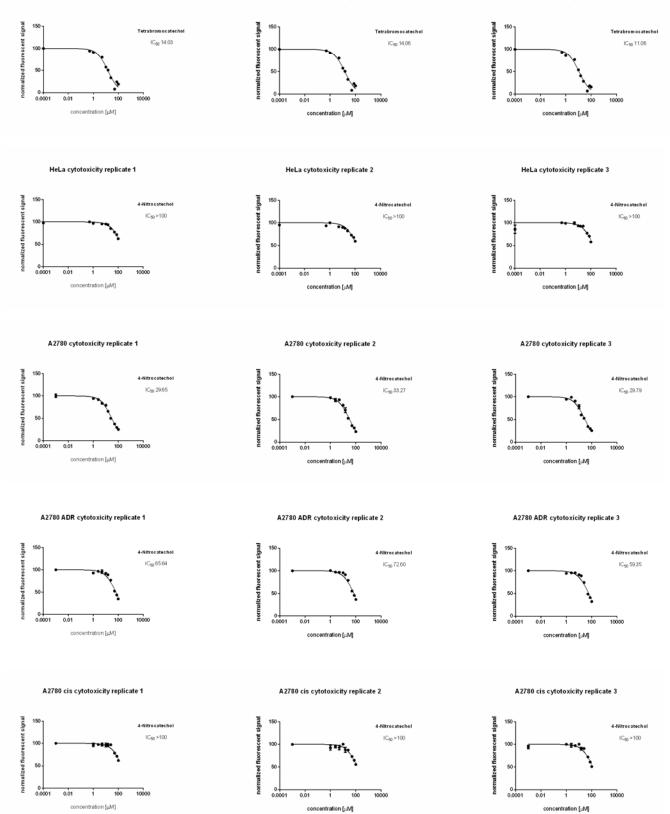




RPE-1 cytotoxicity replicate 1



RPE-1 cytotoxicity replicate 3



S53

CT-26 cytotoxicity replicate 1

0.01

100 10000

Concentration [µM]

CT-26 cytotoxicity replicate 2

CT-26 cytotoxicity replicate 3

0.0001

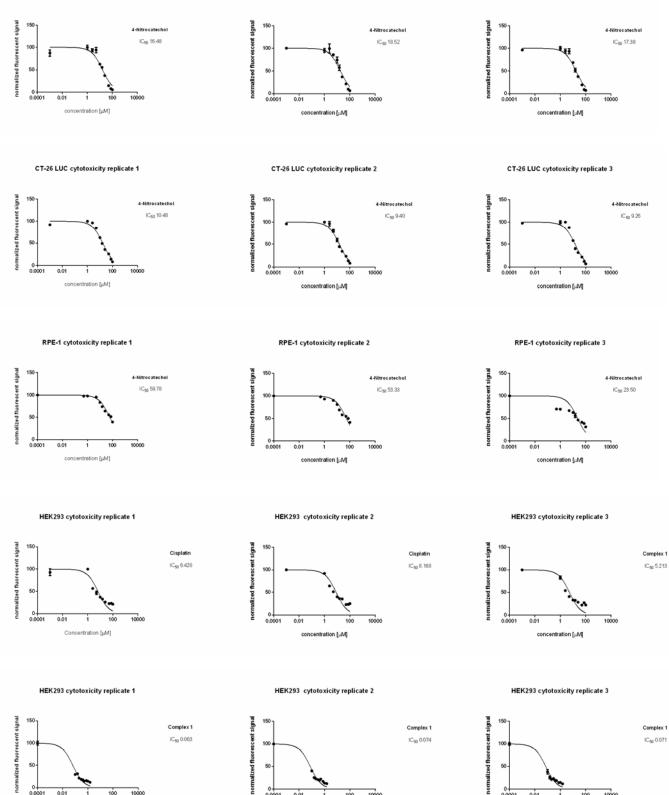
0.01

100

i

concentration [µM]

10000



0.0001

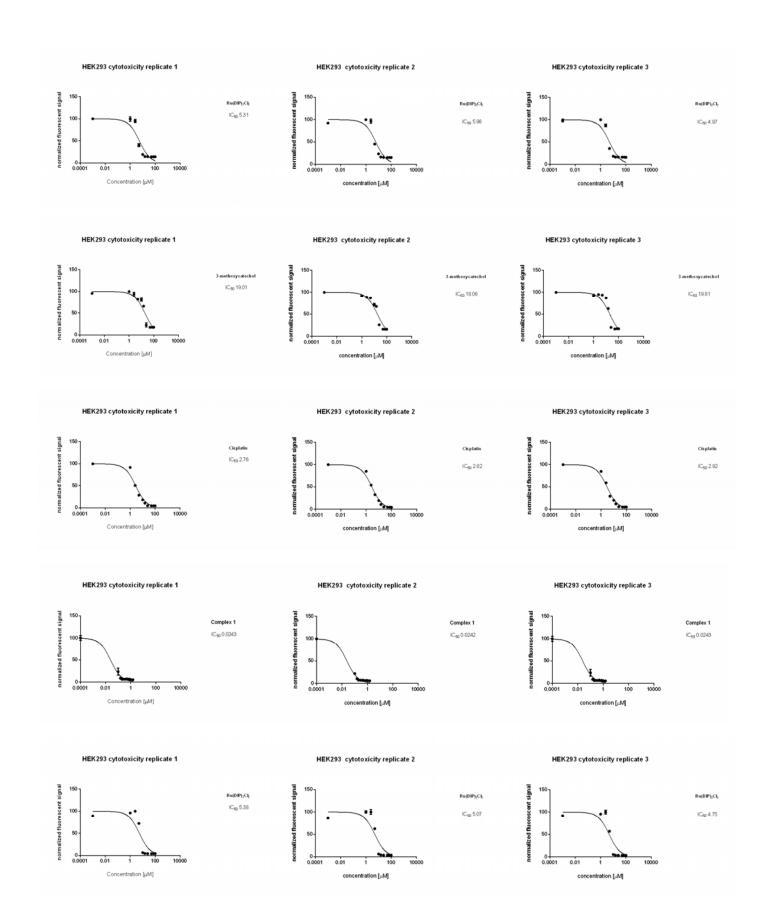
0.01

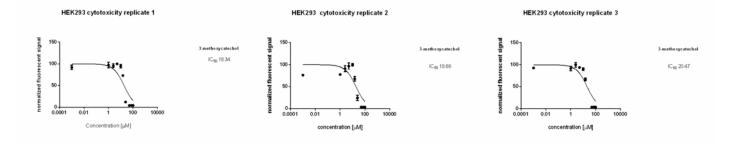
cond

100 10000

i

ntration [µM]

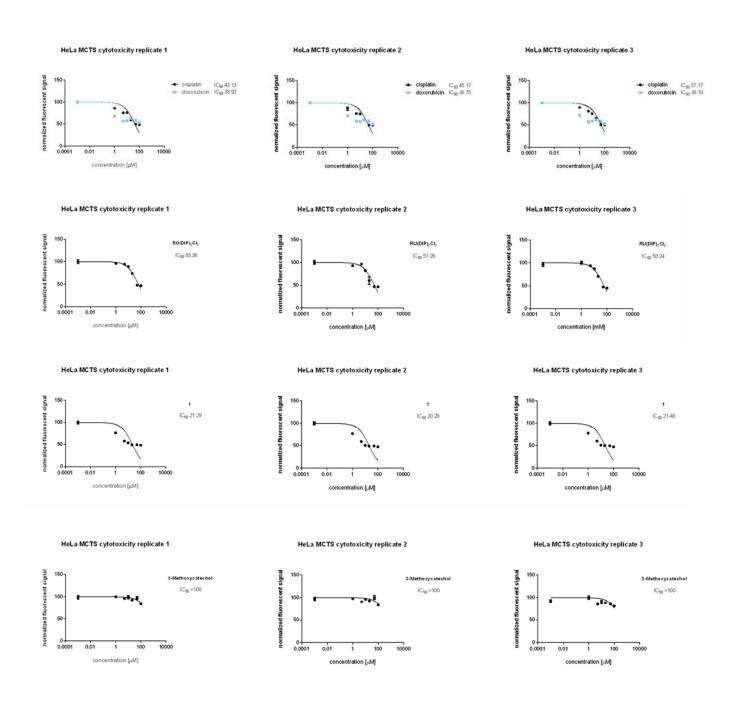




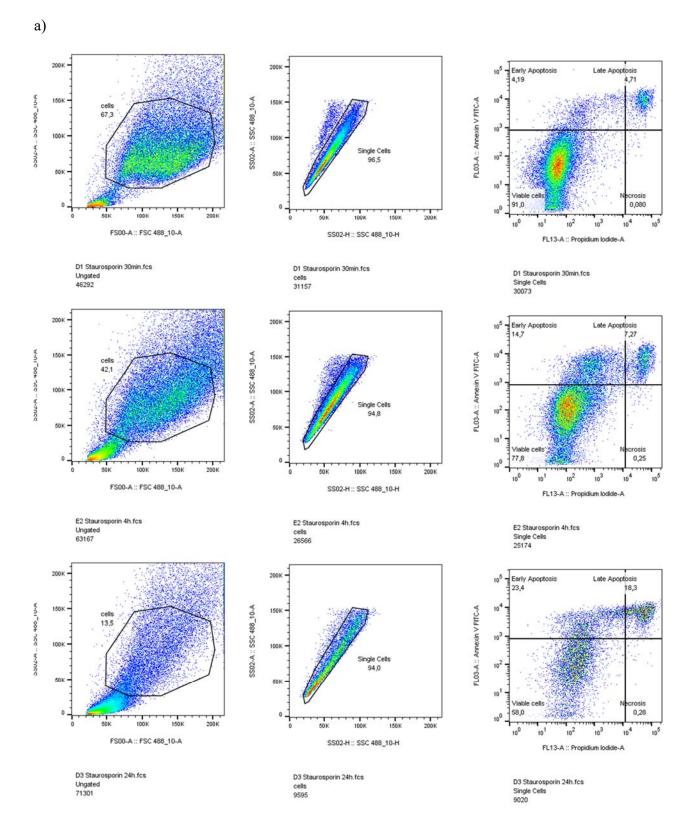
13) **Table S5.** IC<sub>50</sub> values for 3-methoxycatechol, 3-methylcatechol, 4-methylcatechol, 4-tertbutylcatechol, tetrabromocatechol and 4-nitrocatechol.

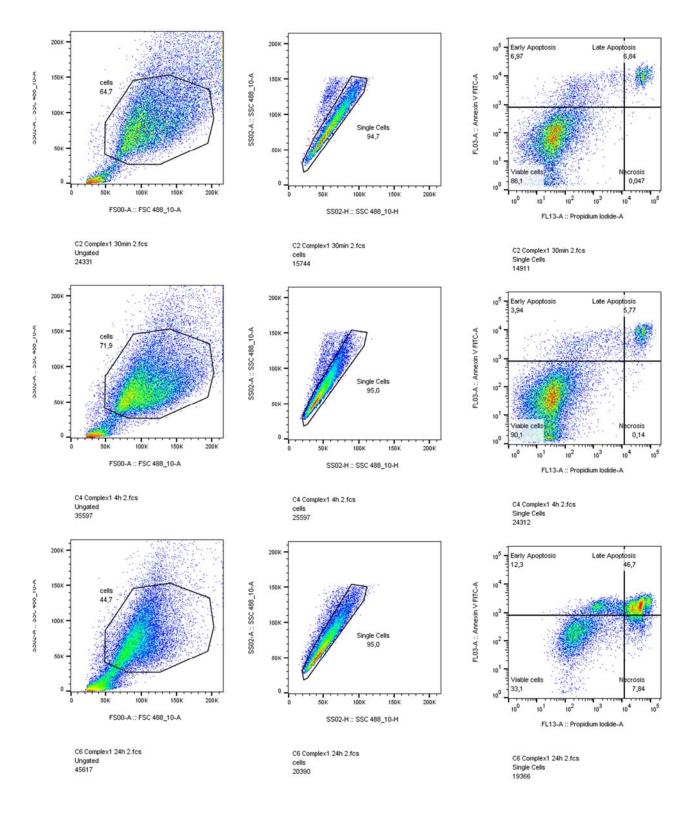
IC <sub>50</sub> (μM)	HeLa	A2780	A2780 ADR	A2780 cis	CT-26	CT-26 LUC	RPE-1
3-methoxycatechol	$56.19\pm4.18$	18.71 ± 1.17	$36.54 \pm 1.94$	30.07 ± 1.35	$45.72\pm4.21$	$36.39\pm 6.28$	>100
3-methylcatechol	>100	$9.99 \pm 1.26$	$14.68\pm0.69$	$12.71\pm0.82$	$17.47\pm0.73$	$12.13\pm1.40$	>100
4-methylcatechol	>100	15.16 ± 1.0	$29.27 \pm 1.96$	$34.56 \pm 1.49$	$34.37 \pm 1.41$	$33.33 \pm 3.4$	>100
4-tertbutylcatechol	$93.14\pm9.8$	$9.14\pm0.7$	$12.89 \pm 1.20$	$16.71 \pm 3.67$	$9.94\pm0.63$	$9.72\pm0.67$	$55.05\pm4.64$
Tetrabromocatech ol	$29.95 \pm 1.60$	$8.75\pm0.20$	$14.39 \pm 1.18$	$13.63 \pm 0.88$	$5.53\pm0.37$	$3.80\pm0.34$	13.5 ± 1.7
4-nitrocatechol	>100	$30.90\pm2.05$	$65.86\pm6.62$	>100	$17.46\pm1.02$	$15.31 \pm 1.0$	45 ± 19

## 14) Figure S9. CellTiter Glo® viability Test

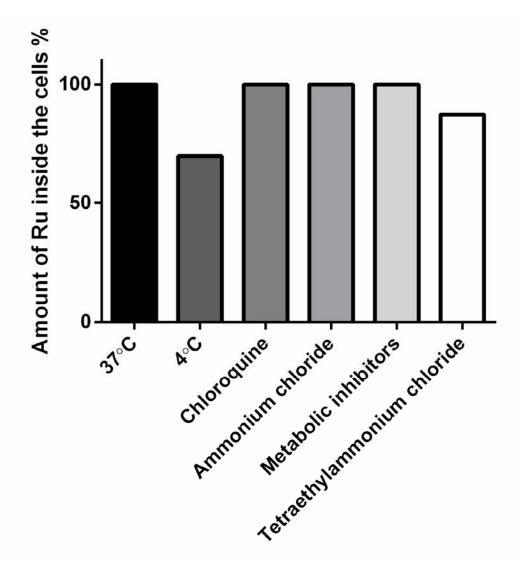


15) **Figure S10.** Cell Death Mechanism: Dot plots of staurosporin (a) and complex 1 (b) after 30 min, 4 h and 24 h treatment.

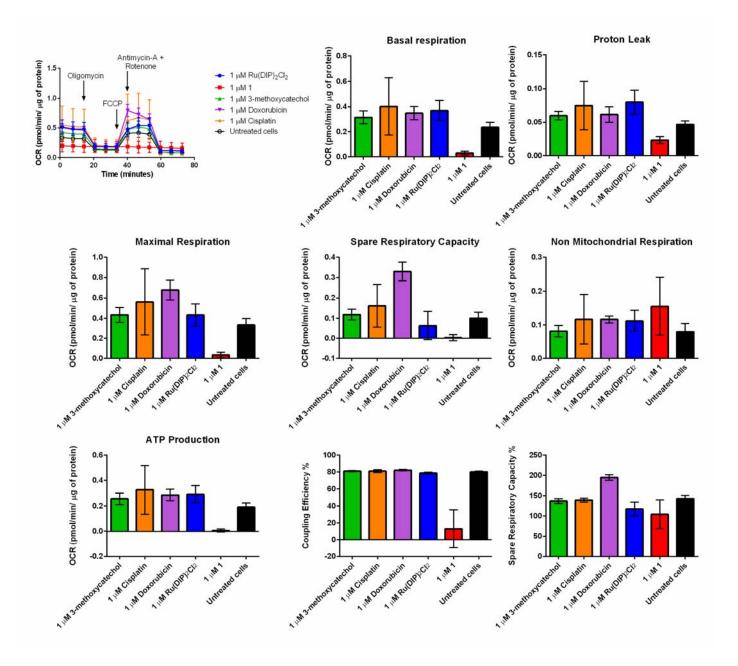




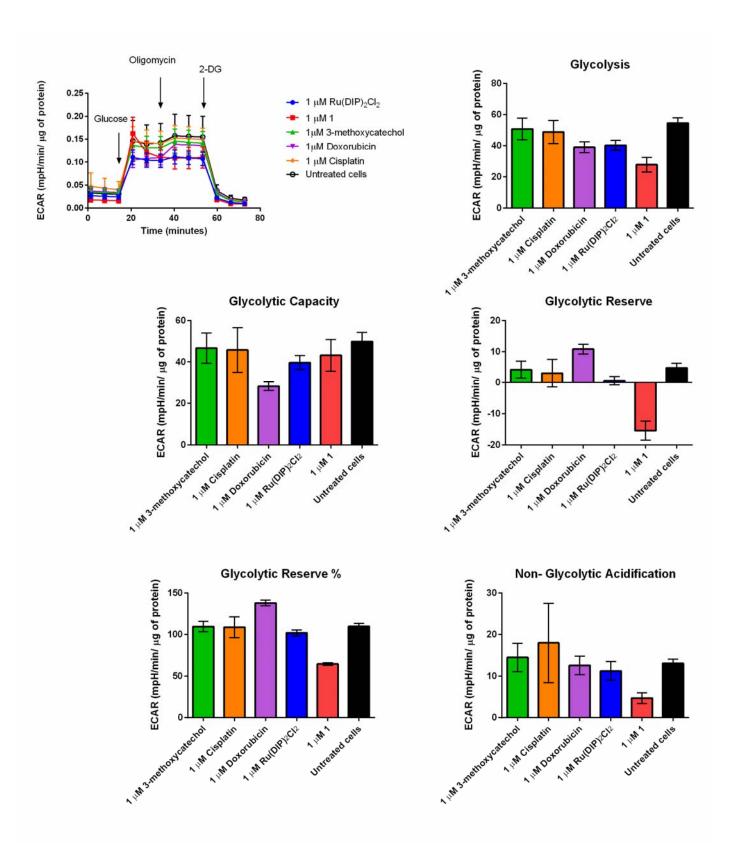
16) Figure S11. Cellular uptake mechanism of complex 1. Accumulation of ruthenium in HeLa cells in presence of different inhibitors and conditions: low temperature (4°C), blocked cellular metabolism (2-Deoxy-D-glucose, oligomycin), blocked endocytic pathways (chloroquine or ammonium chloride), blocked cation transporters (tetraethylammonium chloride). Cells were pre-treated with uptake inhibitors and then incubated with 1 (2 h, 5 μM). Amounts of ruthenium were measured using ICP-MS.



17) **Figure S12.** Oxygen consumption rates and different respiration parameters in HeLa cells alone or after treatment with various test compounds.



18) **Figure S13.** Extracellular acidification rate and different parameters of glycolysis in HeLa cells alone or after treatment with various test compounds.



19) Figure S14. Fuel flex assay in HeLa cells. Dependency studies were performed by adding the inhibitor for the target pathway in port A and inhibitors for the other two pathways in port B while capacity studies were done using the reverse sequence. UK-5099 (20  $\mu$ M), BPTES (30  $\mu$ M) and etomoxir (40  $\mu$ M) were used as the inhibitors for the fuela pathways run by glucose, glutamine and fatty acids.

