

## SUPPLEMENTARY INFORMATION

### **Organoruthenium(II) Complexes Bearing an Aromatase Inhibitor: Synthesis, Characterization, *in Vitro* Biological Activity and *in Vivo* Toxicity in Zebrafish Embryos**

*Golara Golbaghi, Mohammad Mehdi Haghdooost, Debbie Yancu, Yossef López de los Santos, Nicolas Doucet, Shunmoogum A. Patten, J. Thomas Sanderson and Annie Castonguay\**

*INRS - Institut Armand-Frappier, Université du Québec, 531 boul. des Prairies, Laval, Quebec, H7V 1B7, Canada*

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**Table S1.** Crystallographic data and structure refinement for complexes **2-5**.

	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
empirical formula	C <sub>64</sub> H <sub>64</sub> BCIN <sub>10</sub> Ru	C <sub>65</sub> H <sub>60</sub> BCIN <sub>5</sub> PRu	C <sub>8</sub> H <sub>8</sub> O <sub>5</sub> Ru	C <sub>25</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub> Ru
formula weight	1120.58	1089.48	285.21	560.57
crystal size	0.2 x 0.16 x 0.02 mm	0.24 x 0.12 x 0.04 mm	0.24 x 0.22 x 0.07 mm	0.32 x 0.07 x 0.03 mm
crystal system, space group	triclinic, <i>P</i> -1	monoclinic, <i>P</i> 2 <sub>1</sub> / <i>c</i>	triclinic, <i>P</i> -1	Monoclinic, C2/ <i>c</i>
unit cell dimensions	a = 13.7319(6)Å	a = 38.2764(15) Å	a = 6.2877(2)Å	a = 32.8261(11)Å
	b = 14.5443(6)Å	b = 9.9847(4)Å	b = 7.8586(2)Å	b = 9.1294(2)Å
	c = 15.9097(7)Å	c = 31.5850(13) Å	c = 8.9078(2)Å	c = 16.3868(4)Å
	α = 113.327(2)°	α = 90°	α = 75.191(1)°	α = 90°
	β = 99.435(2)°	β = 114.361(2)°	β = 89.895(1)°	β = 95.992(1)°
	γ = 91.374(2)°	γ = 90°	γ = 78.879(1)°	γ = 90°
volume	2864.4(2) Å <sup>3</sup>	10996.3(8) Å <sup>3</sup>	417.02(2) Å <sup>3</sup>	4884.0(2) Å <sup>3</sup>
Z, Calculated density	2, 1.299 Mg m <sup>-3</sup>	8, 1.316 Mg m <sup>-3</sup>	2, 2.271 Mg m <sup>-3</sup>	8, 1.525 g/cm <sup>3</sup>
F(000)	1168.0	4528.0	280.0	2288.0
μ	2.031 mm <sup>-1</sup>	2.235 mm <sup>-1</sup>	15.224 mm <sup>-1</sup>	5.554 mm <sup>-1</sup>
temperature	100 K	150 K	100 K	100 K
wavelength	1.34139Å (GaK <sub>α</sub> )	1.34139Å (GaK <sub>α</sub> )	1.54178 Å (CuK <sub>α</sub> )	1.54178 Å (CuK <sub>α</sub> )
index ranges	-17 ≤ h ≤ 17	-47 ≤ h ≤ 47	-7 ≤ h ≤ 7	-40 ≤ h ≤ 40
	-18 ≤ k ≤ 18	-12 ≤ k ≤ 12	-9 ≤ k ≤ 9	0 ≤ k ≤ 10
	-20 ≤ l ≤ 20	-39 ≤ l ≤ 39	-10 ≤ l ≤ 10	0 ≤ l ≤ 20
2θ range for data collection	5.36 to 121.424°	4.106 to 114.104°	10.284 to 143.67°	10.06 to 144.67°
reflections collected/unique	76314/13153	80612/22364	21675/1584	4865/4865
data/parameters/restraints	13153/702/0	22364/1402/288	1584/135/6	4865/322/0
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.043	1.021	1.134	1.030
Final R indices [ <i>I</i> > 2σ( <i>I</i> )] <sup>a,b</sup>	R1 = 0.0280	R1 = 0.0540	R1 = 0.0383	R1 = 0.0366
	wR2 = 0.0741	wR2 = 0.1321	wR2 = 0.1012	wR2 = 0.1076
R indices (all data)	R1 = 0.0322	R1 = 0.0540	R1 = 0.0383	R1 = 0.0370
	wR2 = 0.0759	wR2 = 0.1394	wR2 = 0.1012	wR2 = 0.1084
Largest diff. peak and hole	1.11 and -0.35 e Å <sup>-3</sup>	1.12 and -0.85 e Å <sup>-3</sup>	1.96 and -2.02 e Å <sup>-3</sup>	1.35 and -1.07 Å <sup>-3</sup>
CCDC deposition no.	1840870	1840871	1840872	1840873

$$^a R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|. \quad ^b wR_2 = \{\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]\}^{1/2}.$$

**Table S2.** Selected bond lengths (Å) and angles (deg) of complexes **2-5**.

<b>2</b>		<b>3</b>	
bond length/angle	Å/deg	bond length/angle	Å/deg
Ru1-N1	2.1024 (12)	Ru1-N1 (Ru1'-N1')	2.114 (4) (2.091 (5))
Ru1-N2	2.1104 (12)	Ru1-P1 (Ru1'-P1')	2.352 (1) (2.346 (3))
Ru1-Cl1	2.3961	Ru1-Cl1 (Ru1'-Cl1')	2.401 (2) (2.380 (1))
Ru-arene <sub>centroid</sub>	1.662	Ru-arene <sub>centroid</sub>	1.699 (1.747)
N1-Ru1-N2	87.14 (5)	N1-Ru1-P1 (N1'-Ru1'-P1')	90.5 (1) (89.6 (2))
N1-Ru1-Cl1	86.15 (3)	N1-Ru1-Cl1 (N1'-Ru1'-Cl1')	87.00 (1) (84.6 (3))
N2-Ru1-Cl1	85.45 (3)	P1-Ru1-Cl1 (P1'-Ru1'-Cl1')	88.27 (5) (84.0 (3))

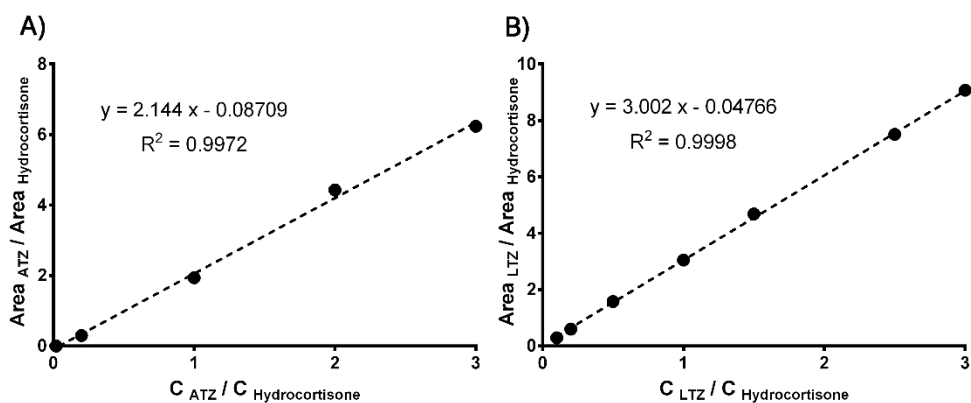
<b>4</b>		<b>5</b>	
bond length/angle	Å/deg	bond length/angle	Å/deg
Ru1-O1	2.086 (3)	Ru1-N1	2.1130 (15)
Ru1-O2	2.096 (3)	Ru1-O1	2.0847 (13)
Ru1-O3	2.143 (3)	Ru1-O2	2.0788 (13)
Ru-arene <sub>centroid</sub>	1.643	Ru-arene <sub>centroid</sub>	1.653
O1-Ru1-O2	78.31 (12)	O1-Ru1-O2	78.53 (6)
O1-Ru1-O3	82.78 (13)	O1-Ru1-N1	86.20 (6)
O2-Ru1-O3	85.97 (13)	O2-Ru1-N1	85.71 (6)

**Table S3.** Anastrozole release after incubation (48 h) of a 150  $\mu\text{M}$  solution of complexes **1**, **2**, **3**, and **5** in water (DMSO 0.5%). Data are reported as mean  $\pm$  SD resulting from 3 independent experiments.

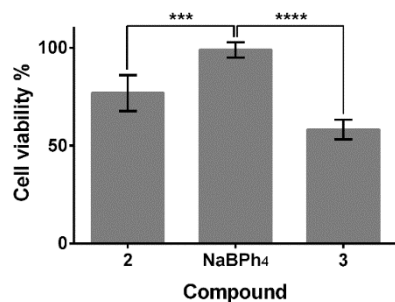
Complex	<b>1</b>	<b>2</b>	<b>3</b>	<b>5</b>
Anastrozole release (%)	7% ( $\pm 2$ )	15% ( $\pm 2$ )	3% ( $\pm 1$ )	15% ( $\pm 3$ )

**Table S4.** Anastrozole (or letrozole) release after incubation (1.5 h) of a 10  $\mu\text{M}$  solution of complexes **1**, **2**, **3**, **5** and Ru-LTZ in DMEM/F-12 (DMSO 0.1%). Data are reported as mean  $\pm$  SD resulting from 3 independent experiments.

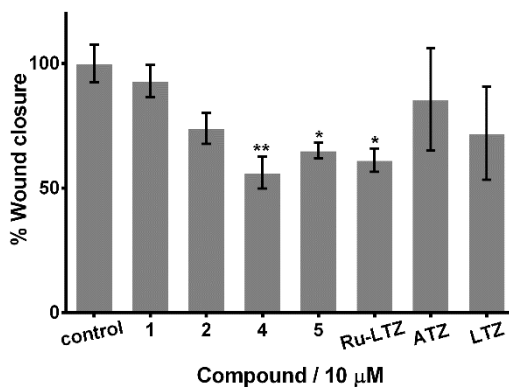
Complex	<b>1</b>	<b>2</b>	<b>3</b>	<b>5</b>	Ru-LTZ
Equivalent(s) of released inhibitor	1.54 (0.05) / 2	0.42 (0.03) / 2	0.04 (0.00) / 1	0.81 (0.05) / 1	2.00 (0.06) / 2



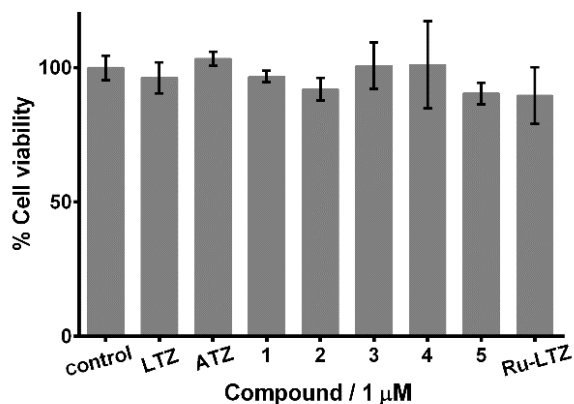
**Figure S1.** Calibration curves of extracted A) anastrozole (ATZ) and B) letrozole (LTZ) from phenol red free DMEM/F-12 (DMSO final concentration: 0.1%). Hydrocortisone was used as an external standard.



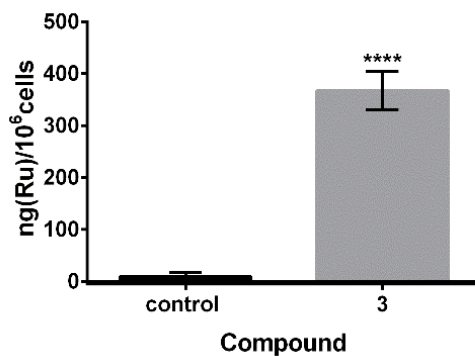
**Figure S2.** Cell viability determined by the SRB assay in T47D cancer cells treated with a 25  $\mu$ M solution of **2**, **3** and NaBPh<sub>4</sub> (48 h). All values are expressed as means (from three independent experiments)  $\pm$  SD relative to the carrier: media containing 0.25% DMSO and the negative control: growth media containing 25% DMSO (0%).



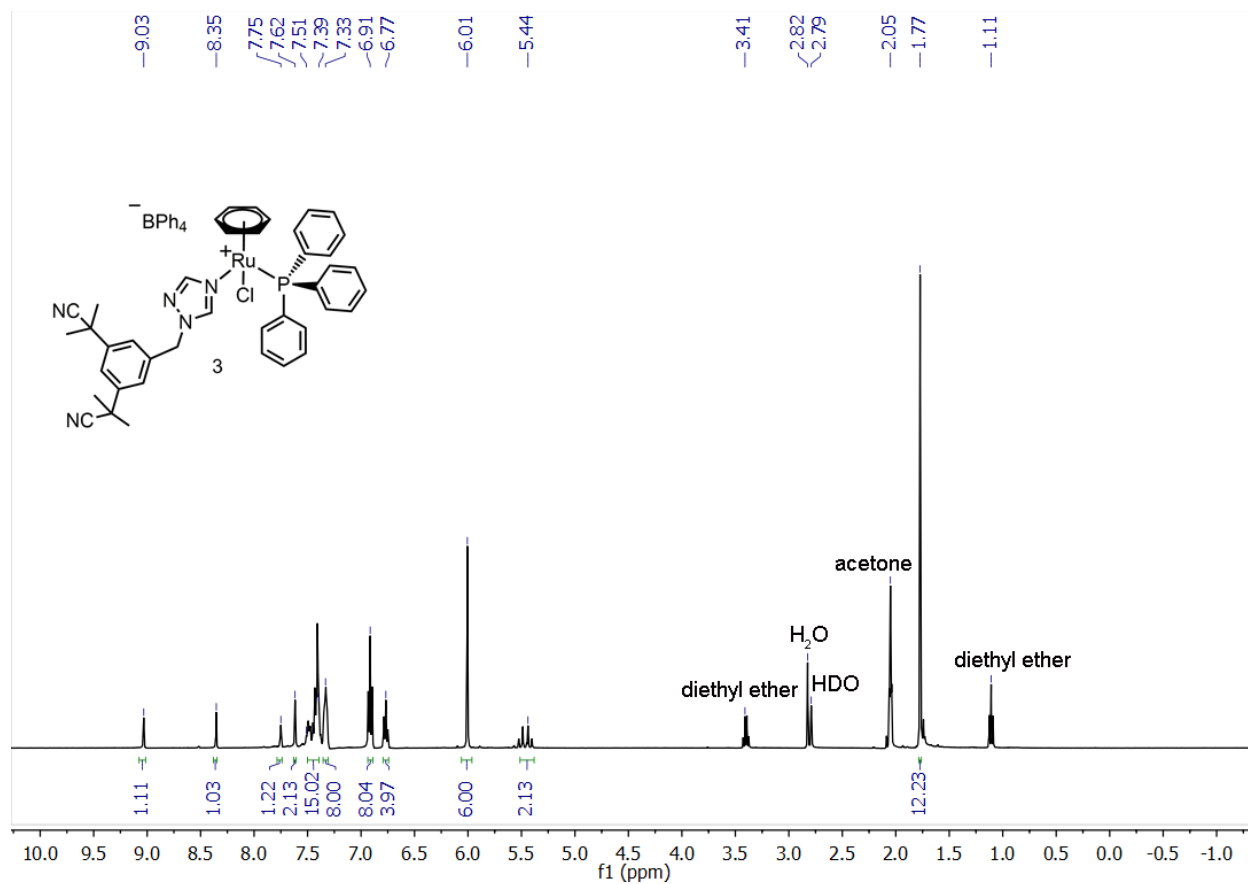
**Figure S3.** Migration inhibition assessment of MCF-7 cells after exposure to 10  $\mu$ M solutions of **1**, **2**, **4**, **5**, Ru-LTZ, anastrozole (ATZ) and letrozole (LTZ) over 48 h. Error bars shown in the graph represent the standard error of mean (n=8). Significant differences: \* $p$  < 0.05; \*\* $p$  < 0.01.



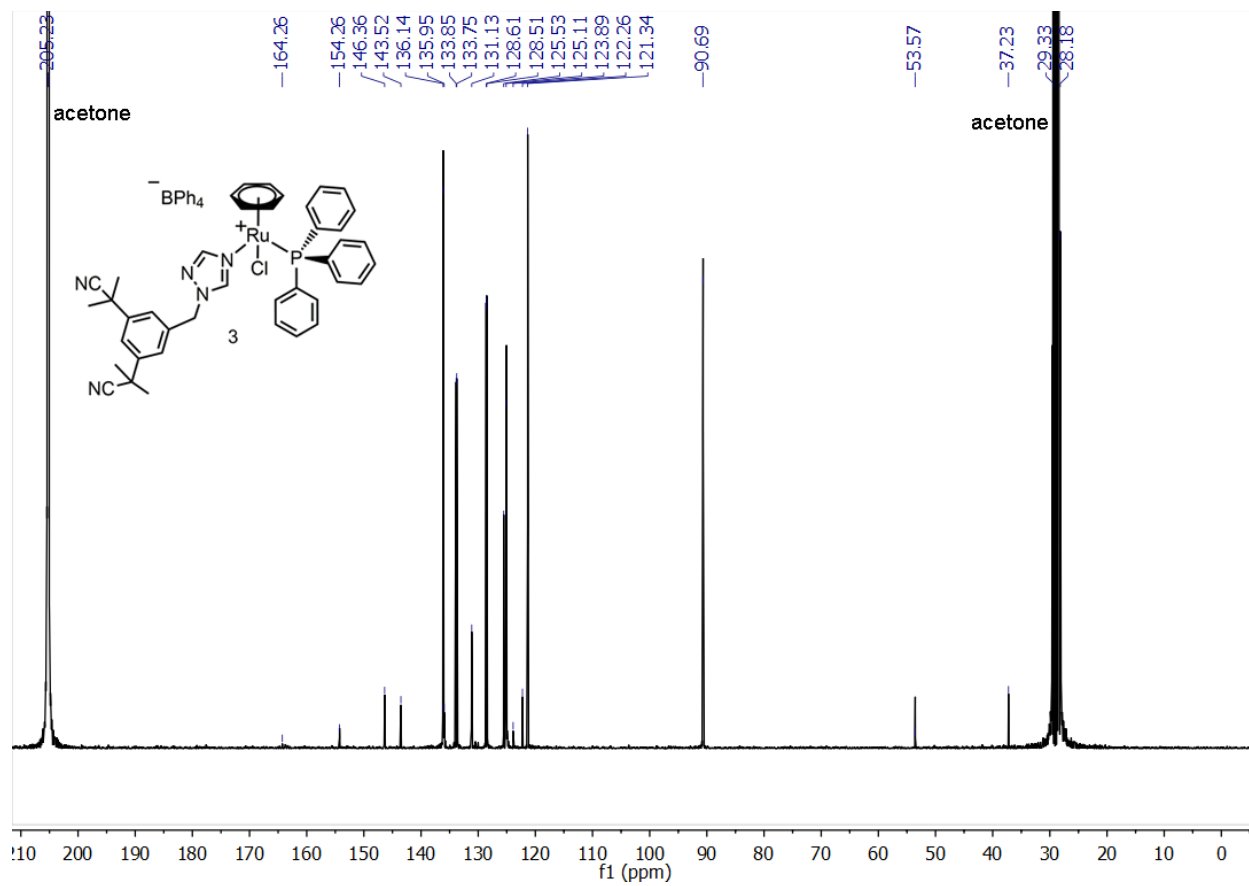
**Figure S4.** Cell viability determined by the SRB assay in H295R cancer cells treated with 1 μM **1-5**, Ru-LTZ, anastrozole (ATZ) and letrozole (LTZ) (48 h). All values are expressed as means (from three independent experiments) ± SD relative to the carrier: media containing 0.25% DMSO and the negative control: growth media containing 25% DMSO (0%).



**Figure S5.** Ruthenium cellular uptake determined by ICP-MS after exposure of H295R cells to a 10 μM solution of **3** for 1.5 h. Error bar in the graph represent the standard deviation.

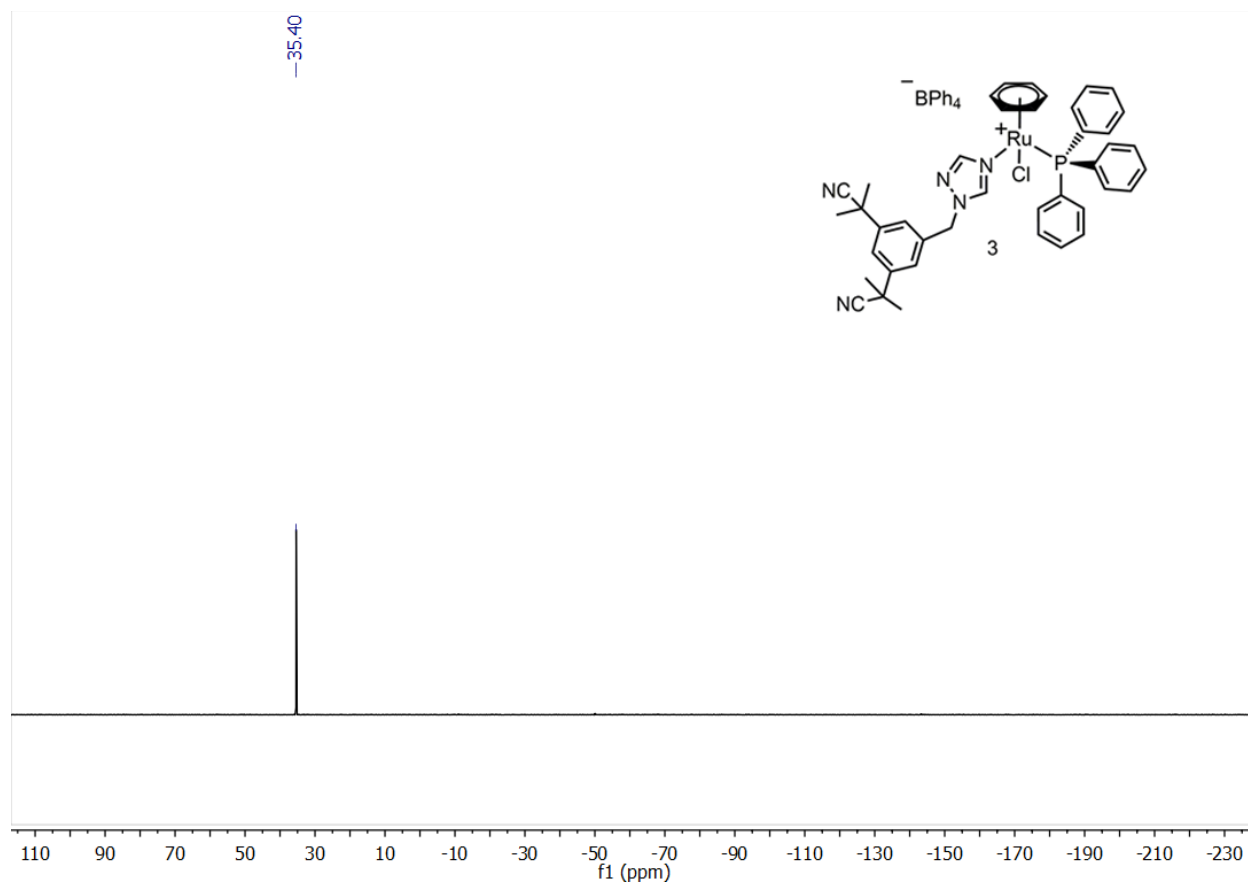


**Figure S6.** <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>CO, 400 MHz) spectrum of **3**.



**Figure S7.**  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $(\text{CD}_3)_2\text{CO}$ , 100 MHz) of **3**.





**Figure S8.**  $^{31}\text{P}\{^1\text{H}\}$  NMR  $(\text{CD}_3)_2\text{CO}$ , 200 MHz of **3**.