## **Supplementary Information**

for

## Fine-tuning the cytotoxicity of ruthenium(II) arene compounds to enhance selectivity against breast cancers

Sarah A.P. Pereira<sup>1,2</sup><sup>†</sup>, Jan Romano-deGea<sup>2</sup><sup>†</sup>, Ana Isabel Barbosa<sup>1</sup>, Sofia A. Costa Lima<sup>1</sup>, Paul J. Dyson<sup>2\*</sup>, M. Lúcia M. F. S. Saraiva<sup>1\*</sup>

<sup>1</sup> LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal.

<sup>2</sup> Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

<sup>†</sup> These authors contributed equally to this work.

\* Corresponding authors E-mail address: paul.dyson@epfl.ch; lsaraiva@ff.up.pt Tel.: +351 220428674; Fax: +351 226093483

# Index

1. Synthesis and characterization of the screened compounds	3
2. Solubility, partition coefficient and conductivity of 11CA	12
3. Stability in solution	14
4. Cytotoxicity studies	21
5. Apoptosis effect of compounds tested in breast cancer cell lines	27
6. Reactivity with model biomolecules	
7. Circular dichroism	
8. Tandem mass spectrometry of 11Cl and DNA oligomers	
9. Molecular docking	43
10. NMR spectra of the synthesized compounds	46

#### 1. Synthesis and characterization of the screened compounds

All organometallic manipulations were carried out under a nitrogen atmosphere using standard Schlenk techniques. CH<sub>2</sub>Cl<sub>2</sub> was dried catalytically under nitrogen using a solvent purification system, manufactured by Innovative Technology Inc. All other solvents were dried over molecular sieves (3 Å) and saturated with nitrogen prior to use.  $[Ru(\eta^{6}-toluene)Cl_{2}]_{2}^{1}, [Ru(\eta^{6}-toluene)(PPh_{3})Cl_{2}]^{2}$  and complexes 1<sup>3</sup>, 2<sup>3</sup>, 3<sup>4</sup>, 4<sup>5</sup>, 5<sup>6</sup>, 6 <sup>6</sup>, 7<sup>7</sup>, 8<sup>8</sup>, and 9<sup>2</sup> were prepared according to reported procedures and the characterization data were in agreement with those reported. Compounds  $11BF_4^9$  and  $11PF_6^2$  have been previously reported, but in this work, they have been prepared following a different synthetic route. All chemicals were either of reagent or analytical grade and used as purchased from commercial sources without additional purification. RuCl<sub>3</sub>·3H<sub>2</sub>O was obtained from Precious Metals Online. NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer at room temperature unless otherwise stated. Chemical shifts are reported in ppm relative to SiMe<sub>4</sub> ( $\delta = 0$ ) and coupling constants (J) are reported in Hz. The following abbreviations were used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublets. High-resolution mass spectra (HRMS)were acquired by the MS service at EPFL, using either a Thermo Orbitrap Elite instrument with an LTQ-Orbitrap analyser or a Waters XEVO G2-S QTOF instrument with a QTOF analyser.



Figure S1. Structures of the studied ruthenium arene compounds.

[Ru( $\eta^6$ -toluene)(PPh<sub>3</sub>)(PTA)Cl]PF<sub>6</sub> (10)

 $PF_6$  A suspension of  $[Ru(\eta^6-toluene)(PPh_3)Cl_2]$  (200 mg, 0.38 mmol, 1.0 eq.), 1,3,5-triaza-7-phosphaadamantane (90 mg, 0.57 mmol, 1.5 eq.) and ammonium hexafluorophosphate (99 mg, 0.61 mmol, 1.6 eq.) in a 1:1 mixture of MeOH:CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was stirred at 45°C for 1 h. The solvent was removed under reduced pressure, and the residue was taken in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). The organic phase was washed with H<sub>2</sub>O (2 × 15 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent removed under reduced pressure. The solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and the product was precipitated by the addition of pentane, filtered, washed with cold diethyl ether (2 × 5 mL), and dried under vacuum. Light yellow powder (106 mg, 35%). Light orange crystals suitable for X-ray diffraction were obtained by slow diffusion at 4°C of pentane into a solution of **10** in CH<sub>2</sub>Cl<sub>2</sub>.

<sup>1</sup>**H NMR (400 MHz, DMSO-***d*<sub>6</sub>**) \delta**: 7.66 – 7.53 (m, 9H), 7.54 – 7.44 (m, 6H), 6.60-6.55 (m, 1H) , 6.29 (d, <sup>3</sup>*J* = 5.9 Hz, 1H), 6.21 (d, <sup>3</sup>*J* = 6.3 Hz, 1H), 5.56-5.51 (m, 1H) , 4.35-4.27 (m, 4H), 4.25-4.21 (m, 2H), 4.21-4.19 (m, 1H) , 4.09 (ddd, <sup>2,2,4</sup>*J* = 14.8, 3.6, 1.5 Hz, 3H), 3.74 (ddd, <sup>2,2,4</sup>*J* = 14.8, 3.8, 1.6 Hz, 3H), 2.12 (d, <sup>4</sup>*J* = 1.1 Hz, 3H) ppm. <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 37.9 (d, <sup>2</sup>*J* = 52.8 Hz), -34.8 (d, <sup>2</sup>*J* = 52.8 Hz), -139.4 (hept, <sup>1</sup>*J*<sub>FP</sub> = 711.5 Hz) ppm.

<sup>19</sup>F{<sup>1</sup>H} NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ: -72.2 (d,  ${}^{1}J_{PF} = 711.5$  Hz) ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 134.1 (d, <sup>2</sup>*J* = 9.8 Hz), 133.3 (d, <sup>1</sup>*J* = 48.2 Hz), 131.3, 128.8 (d, <sup>3</sup>*J* = 9.8 Hz), 117.5 (d, <sup>2</sup>*J* = 4.4 Hz), 97.2 (d, *J* = 8.1 Hz), 97.1 (d, <sup>2</sup>*J* = 3.6 Hz), 91.8 (d, <sup>2</sup>*J* = 8.6 Hz), 91.0, 83.1, 71.5 (d, <sup>3</sup>*J* = 7.4 Hz), 53.2 (d, <sup>1</sup>*J* = 15.3 Hz), 18.3 ppm.

<sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-*d*) δ: 31.6 (d, <sup>2</sup>*J* = 54.0 Hz), -40.7 (d, <sup>2</sup>*J* = 54.0 Hz) ppm.

HRMS (nanochip-ESI/LTQ-Orbitrap) m/z:  $[M - PF_6^-]^+$  Calcd for  $C_{31}H_{35}ClN_3P_2Ru^+$  648.1033; Found 648.1014.

#### $[Ru(\eta^6-toluene)(PPh_3)_2Cl]Cl (11Cl)$

CI"...Ru

`PPh<sub>3</sub>

CI 75 mg of 11PF<sub>6</sub> (0.08 mmol) were dissolved in MeOH (50 mL). Amberlite<sup>®</sup> IRA-900 chloride form (5 g) was added, and the mixture was stirred for 12 h at room temperature. The solids were filtered off

and another portion of Amberlite<sup>®</sup> IRA-900 chloride form (5g) was added to the filtrate. The suspension was stirred for 12 h more at room temperature. Then, the solids were removed by filtration and the filtrate concentrated under reduced pressure. The residue was dissolved in EtOH (5 mL), and the product was precipitated by the addition of Et<sub>2</sub>O/hexane (2:1), filtered, and dried under vacuum. Counterion exchange was monitored and confirmed by the absence of PF<sub>6</sub> signals in the <sup>31</sup>P and <sup>19</sup>F NMR spectra and by the appearance of a signal in the <sup>35</sup>Cl NMR spectrum (bound chloride was not observed due to the quadrupolar moment of the nuclei). Dark orange crystalline powder (133 mg, 71 %). Light orange crystals suitable for X-ray diffraction were obtained by slow diffusion at 4°C of pentane into a solution of **11**Cl in CH<sub>2</sub>Cl<sub>2</sub>.

<sup>1</sup>H NMR (400 MHz, Chloroform-d) δ: 7.45 – 7.33 (m, 18H), 7.31 – 7.22 (m, 12H), 5.93 – 5.88 (m, 2H), 5.86 – 5.81 (m, 1H), 4.65 (d, <sup>3</sup>J = 5.5 Hz, 2H), 2.07 (s, 3H) ppm.
<sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-d) δ: 22.8 ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, Chloroform-*d*)  $\delta$ : 134.19 (t, <sup>2</sup>*J* = 4.6 Hz), 134.15 (d, <sup>1</sup>*J* = 48.0 Hz), 130.9, 128.6 (t, <sup>3</sup>*J* = 5.1 Hz), 122.9 (t, <sup>2</sup>*J* = 2.8 Hz), 99.3, 94.5 (t, <sup>2</sup>*J* = 4.9 Hz), 84.9, 19.5 ppm.

<sup>1</sup>H NMR (400 MHz, Methanol-*d*<sub>4</sub>)  $\delta$ : 7.52 – 7.38 (m, 18H), 7.34 – 7.25 (m, 12H), 5.93 – 5.85 (m, 2H), 5.02 (d, <sup>3</sup>*J* = 6.1 Hz, 2H), 4.66 – 4.59 (m, 1H), 2.15 (s, 3H) ppm. <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Methanol-*d*<sub>4</sub>)  $\delta$ : 22.1 ppm. <sup>35</sup>Cl NMR (39 MHz, Methanol-*d*<sub>4</sub>)  $\delta$ : -31 ppm.

**HRMS (nanochip-ESI/LTQ-Orbitrap) m/z:**  $[M - Cl^{-}]^{+}$  Calcd for  $C_{43}H_{38}ClP_2Ru^{+}$  753.1175; Found 753.1155.

## General Procedure for the synthesis of bis-triphenylphosphine ruthenium(II)toluene cationic compounds (11CA)

A suspension of  $[Ru(\eta^6-toluene)(PPh_3)Cl_2]$  (100 mg, 0.19 mmol, 1.0 eq.), triphenylphosphine (55 mg, 0.21 mmol, 1.1 eq.) and the silver salt of the desired counterion (0.23 mmol, 1.2 eq.) in a 1:1 mixture of MeOH:CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was stirred at 45°C for 6 h while protected from light. The mixture was filtered through celite to remove silver salts. The solvent was removed under reduced pressure, and the residue dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL). Finally, the product was precipitated by the addition of pentane, filtered, washed with cold diethyl ether (2 × 5 mL), and dried under vacuum.

[Ru( $\eta^6$ -toluene)(PPh<sub>3</sub>)<sub>2</sub>Cl]BF<sub>4</sub> (11BF<sub>4</sub>)

BF<sub>4</sub> Light yellow powder (137 mg, 86%)

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$ : 7.44 – 7.34 (m, 18H), 7.31 <sup>CI,...,Ru</sup><sub>Ph<sub>3</sub>P</sub> – 7.22 (m, 12H), 5.84 – 5.75 (m, 2H), 5.41 – 5.33 (m, 1H), 4.63 (d, <sup>3</sup>J = 6.0 Hz, 2H), 2.07 (s, 3H) ppm.

<sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-d) δ: 22.4 ppm.

<sup>19</sup>F{<sup>1</sup>H} NMR (**376** MHz, Chloroform-*d*) δ: -152.61, -152.67 ppm.

<sup>11</sup>B{<sup>1</sup>H} NMR (128 MHz, Chloroform-*d*) δ: -0.9 ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, Chloroform-*d*)  $\delta$ : 133.99 (d, <sup>1</sup>*J* = 48.3 Hz), 133.98 (t, <sup>2</sup>*J* = 4.6 Hz), 130.9, 128.5 (t, <sup>3</sup>*J* = 5.0 Hz), 122.7 (t, <sup>2</sup>*J* = 1.5 Hz), 98.7, 94.4 (t, <sup>2</sup>*J* = 4.9 Hz), 83.7, 19.3 ppm.

HRMS (nanochip-ESI/LTQ-Orbitrap) m/z:  $[M - BF_4^-]^+$  Calcd for  $C_{43}H_{38}ClP_2Ru^+$  753.1175; Found 753.1154.

[Ru( $\eta^6$ -toluene)(PPh<sub>3</sub>)<sub>2</sub>Cl]OTf (11OTf)



<sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$ : 7.44 – 7.34 (m, 18H), 7.30 – 7.21 (m, 12H), 5.84 – 5.76 (m, 2H), 5.32 – 5.25 (m, 1H), 4.67 (d, <sup>3</sup>*J* = 6.0 Hz, 2H), 2.08 (s, 3H) ppm. <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-*d*)  $\delta$ : 22.4 ppm.

<sup>19</sup>F{<sup>1</sup>H} NMR (376 MHz, Chloroform-d) δ: -78.0 ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (201 MHz, Chloroform-*d*)  $\delta$ : 134.1 (d, <sup>1</sup>*J* = 46.9 Hz), 134.1 (t, <sup>2</sup>*J* = 4.6 Hz), 131.0, 128.6 (t, <sup>3</sup>*J* = 5.0 Hz), 122.8 (t, <sup>2</sup>*J* = 2.8 Hz), 121.0 (q, <sup>1</sup>*J*<sub>FC</sub> = 321.5 Hz), 98.7, 94.6 (t, <sup>2</sup>*J* = 5.0 Hz), 83.9, 19.4 ppm.

HRMS (nanochip-ESI/LTQ-Orbitrap) m/z:  $[M - OTf^-]^+$  Calcd for C<sub>43</sub>H<sub>38</sub>ClP<sub>2</sub>Ru<sup>+</sup> 753.1175; Found 753.1155.

#### $[Ru(\eta^6-toluene)(PPh_3)_2Cl]PF_6$ (11PF<sub>6</sub>)



CI....Ru

Ph₂P

PPh<sub>3</sub>

CI. Ru

Ph₂P

PF<sub>6</sub> Dark yellow powder (151 mg, 89 %). Orange crystals suitable for X-ray diffraction were obtained by slow diffusion at 4°C of pentane into a solution of 11PF<sub>6</sub> in CH<sub>2</sub>Cl<sub>2</sub>.

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$ : 7.44 – 7.35 (m, 18H), 7.30 – 7.21 (m, 12H), 5.80 – 5.71 (m, 2H), 5.10 – 5.03 (m, 1H), 4.67 (d, <sup>3</sup>*J* = 6.0 Hz, 2H), 2.08 (s, 3H) ppm. <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-*d*)  $\delta$ : 22.2, -144.4 (hept, <sup>1</sup>*J*<sub>FP</sub> = 713.0 Hz) ppm. <sup>19</sup>F{<sup>1</sup>H} NMR (376 MHz, Chloroform-*d*)  $\delta$ : -72.8 (d, <sup>1</sup>*J*<sub>PF</sub> = 713.0 Hz) ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, Chloroform-*d*)  $\delta$ : 134.1 (d, <sup>1</sup>*J* = 44.1 Hz), 134.0 (t, <sup>2</sup>*J* = 4.6 Hz), 131.1, 128.6 (t, <sup>3</sup>*J* = 5.1 Hz), 122.8, 98.5, 94.7, 83.8, 19.4 ppm.

HRMS (nanochip-ESI/LTQ-Orbitrap) m/z:  $[M - PF_6^-]^+$  Calcd for  $C_{43}H_{38}ClP_2Ru^+$  753.1175; Found 753.1155.

#### $[Ru(\eta^6-toluene)(PPh_3)_2Cl]SbF_6$ (11SbF<sub>6</sub>)

SbF<sub>6</sub> Light red crystalline powder (133 mg, 71 %). Intense yellow crystals suitable for X-ray diffraction were obtained by slow diffusion at 4°C of pentane into a solution of **11**SbF<sub>6</sub> in CH<sub>2</sub>Cl<sub>2</sub>.

<sup>1</sup>**H NMR (400 MHz, Chloroform-***d***) δ:** 7.44 – 7.35 (m, 18H), 7.30 – 7.21 (m, 12H), 5.78 – 5.70 (m, 2H), 4.89 – 4.84 (m, 1H), 4.71 (d, <sup>3</sup>*J* = 6.0 Hz, 2H), 2.10 (s, 3H) ppm.

#### <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-d) δ: 22.1 ppm.

<sup>19</sup>F{<sup>1</sup>H} NMR (376 MHz, Chloroform-d) δ: -106.27 – -139.07 (m) ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, Chloroform-*d*)  $\delta$ : 134.03 (d, <sup>1</sup>*J* = 48.3 Hz), 134.01 (t, <sup>2</sup>*J* = 4.6 Hz), 131.1, 128.6 (t,  ${}^{3}J = 5.1$  Hz), 122.7 (t,  ${}^{2}J = 2.7$  Hz), 98.2, 94.8 (t,  ${}^{2}J = 5.0$  Hz), 83.5, 19.3 ppm.

**HRMS** (nanochip-ESI/LTQ-Orbitrap) m/z:  $[M - SbF_6^-]^+$  Calcd for C<sub>43</sub>H<sub>38</sub>ClP<sub>2</sub>Ru<sup>+</sup> 753.1175; Found 753.1159.

 $[Ru(\eta^6-toluene)(PPh_3)_2Cl]BPh_4$  (11BPh<sub>4</sub>)



CI....Ru

BPh<sub>4</sub> Light orange crystalline needles (158 mg, 78 %). Light Orange crystals suitable for X-ray diffraction were obtained by slow diffusion at 4°C of pentane into a solution of 11BPh<sub>4</sub> in CH<sub>2</sub>Cl<sub>2</sub>.

<sup>1</sup>H NMR (400 MHz, Chloroform-d) δ: 7.42-7.35 (m, 8H), 7.34 – 7.26 (m, 12H), 7.24 -7.13 (m, 18H), 6.91-6.85 (m, 8H), 6.82-6.76 (m, 4H), 5.11 - 4.99 (m, 2H), 4.43 (d,  ${}^{3}J = 6.0$  Hz, 2H), 3.73 - 3.63 (m, 1H), 1.98 (s, 3H) ppm.

<sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-*d*) δ: 22.4 ppm.

<sup>11</sup>B{<sup>1</sup>H} NMR (128 MHz, Chloroform-d) δ: -6.8 ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (201 MHz, Chloroform-d)  $\delta$ : 134.9, 134.2 (t, <sup>2</sup>J = 4.6 Hz), 134.2 (d, <sup>1</sup>J = 50.8 Hz), 131.0, 128.7 (t,  ${}^{3}J$  = 5.1 Hz), 128.5, 122.9 (d,  ${}^{2}J$  = 1.3 Hz), 119.6, 115.9, 99.3, 94.5, 85.5, 19.5 ppm.

HRMS (nanochip-ESI/LTQ-Orbitrap) m/z: [M – BPh<sub>4</sub><sup>-</sup>]<sup>+</sup> Calcd for C<sub>43</sub>H<sub>38</sub>ClP<sub>2</sub>Ru<sup>+</sup> 753.1175; Found 753.1153.

 $[Ru(\eta^6-toluene)(PPh_3)_2Cl]NO_3$  (11NO<sub>3</sub>)

NO<sub>3</sub> A suspension of [Ru( $\eta^6$ -toluene)(PPh<sub>3</sub>)Cl<sub>2</sub>] (100 mg, 0.19 mmol, 1.0 eq.), triphenylphosphine (55 mg, 0.21 mmol, 1.1 eq.) and NaNO<sub>3</sub> PPh<sub>3</sub> (19 mg, 0.23 mmol, 1.2 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was stirred at room temperature for 24 h. The mixture was filtered through celite to remove the solids. The filtrate was concentrated under reduced pressure (5 mL), and the product was

precipitated by the addition of pentane, filtered, washed with cold diethyl ether  $(2 \times 5)$ mL), and, finally, dried under vacuum. Light yellow cream powder (66 mg, 43%). <sup>1</sup>H NMR (400 MHz, Chloroform-d) δ: 7.33 – 7.27 (m, 18H), 7.20 – 7.12 (m, 12H),

5.80 - 5.76 (m, 2H), 5.61 - 5.54 (m, 1H), 4.54 (d,  ${}^{3}J = 5.8$  Hz, 2H), 1.97 (s, 3H) ppm.

#### <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, Chloroform-*d*) δ: 22.6 ppm.

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, Chloroform-*d*)  $\delta$ : 134.2 (t, <sup>2</sup>*J* = 4.6 Hz), 134.1 (d, <sup>1</sup>*J* = 46.4 Hz), 131.0, 128.6 (t, <sup>3</sup>*J* = 5.1 Hz), 122.9, 99.0, 94.5 (t, <sup>2</sup>*J* = 4.0 Hz), 89.1, 19.5 ppm. HRMS (nanochip-ESI/LTQ-Orbitrap) m/z: [M – NO<sub>3</sub><sup>-</sup>]<sup>+</sup> Calcd for C<sub>43</sub>H<sub>38</sub>ClP<sub>2</sub>Ru<sup>+</sup> 753.1175; Found 753.1156.

Single clear light orange or intense yellow crystals of **10**, **11**Cl, **11**OTf, **11**PF<sub>6</sub>, **11**SbF<sub>6</sub> and **11**BPh<sub>4</sub> were obtained and measured. Suitable crystals with dimensions  $0.09 \times 0.08 \times 0.05 \text{ mm}^3$  (**10**),  $0.14 \times 0.12 \times 0.08 \text{ mm}^3$  (**11**Cl),  $0.12 \times 0.09 \times 0.06 \text{ mm}^3$ (**11**OTf),  $0.51 \times 0.09 \times 0.06 \text{ mm}^3$  (**11**PF<sub>6</sub>),  $0.19 \times 0.12 \times 0.06 \text{ mm}^3$  (**11**SbF<sub>6</sub>) and  $0.10 \times 0.03 \times 0.03 \text{ mm}^3$  (**11**BPh<sub>4</sub>) were selected and mounted on a XtaLAB Synergy R, DW system, HyPix-Arc 150 diffractometer or on a SuperNova, Dual, Cu at home/near, AtlasS2 diffractometer. The crystals were kept at a steady *T* = 140.00(10) K during data collection. The structures were solved with the **ShelXT** (Sheldrick, 2015) solution program using dual methods and by using **Olex2** 1.5 (Dolomanov et al., 2009) as the graphical interface. The models were refined with **ShelXL** 2018/3 (Sheldrick, 2015) using full matrix least squares minimization on *F*<sup>2</sup>.

	<b>11</b> Cl	<b>11</b> BF <sub>4</sub> <sup>a</sup>	11OTf	<b>11</b> PF <sub>6</sub>	11SbF <sub>6</sub>	<b>11</b> BPh <sub>4</sub>
Ru1-Cl1	2.391(2)	2.389(3)	2.4069(4)	2.396(1)	2.4020(9)	2.3913(6)
Ru1-P1	2.362(2)	2.399(3)	2.3759(4)	2.3643(14)	2.379(1)	2.3632(6)
Ru1-P2	2.375(1)	2.384(3)	2.3730(4)	2.3668(13)	2.373(1)	2.3803(6)
Ru1-C1	2.327(6)	2.257(4)	2.3326(18)	2.321(5)	2.332(4)	2.321(2)
Ru1-C2	2.278(5)	2.247(4)	2.2932(19)	2.267(6)	2.260(4)	2.266(2)
Ru1-C3	2.242(6)	2.219(4)	2.2169(18)	2.256(5)	2.225(4)	2.260(2)
Ru1-C4	2.222(7)	2.214(5)	2.2191(17)	2.215(5)	2.219(3)	2.243(3)
Ru1-C5	2.215(7)	2.305(5)	2.2311(17)	2.223(5)	2.212(4)	2.227(3)
Ru1-C6	2.282(7)	2.347(4)	2.2709(17)	2.307(5)	2.285(4)	2.302(2)
Ru1-C <sub>avg</sub>	2.26(4)	2.26(5)	2.26(5)	2.26(4)	2.26(5)	2.27(4)
Cl1-Ru1-P1	89.76(6)	85.3(1)	91.682(14)	89.78(4)	91.94(3)	87.68(2)
Cl1-Ru1-P2	85.60(5)	89.3(1)	86.740(14)	89.06(5)	86.10(3)	88.86(2)
P1-Ru1-P2	98.35(5)	100.3(1)	97.894(14)	97.05(5)	97.44(3)	98.34(2)

*Table S1.* Selected bond lengths (Å) and angles (°) for 11Cl, 11BF<sub>4</sub> <sup>*a*</sup>, 11OTf, 11PF<sub>6</sub>, 11SbF<sub>6</sub> and 11BPh<sub>4</sub>. <sup>*a*</sup> Extracted from reference <sup>9</sup>.

Compound	10	<b>11</b> Cl	<b>11</b> OTf	<b>11</b> PF <sub>6</sub>	11SbF <sub>6</sub>	11BPh <sub>4</sub>
Formula	C <sub>31</sub> H <sub>35</sub> ClF <sub>6</sub> N <sub>3</sub> P <sub>3</sub> Ru	C43.5H43Cl3O2P2Ru	C44H38ClF3O3P2RuS	$C_{44}H_{40}Cl_3F_6P_3Ru$	C43H38ClF6P2RuSb	C <sub>67</sub> H <sub>58</sub> BClP <sub>2</sub> Ru
$D_{calc.}$ / g cm <sup>-3</sup>	1.653	1.507	1.558	1.508	1.721	1.363
$m/\text{mm}^{-1}$	6.739	6.335	5.705	6.174	1.319	3.798
Formula Weigh	t 793.05	867.14	902.26	983.09	988.94	1072.40
Colour	clear light orange	clear light orange	clear intense yellow	clear intense yellow	wclear intense orange	clear light orange
Shape	prism-shaped	irregular-shaped	prism-shaped	needle-shaped	prism-shaped	prism-shaped
Size/mm <sup>3</sup>	$0.09 \times 0.08 \times 0.05$	0.14×0.12×0.08	0.12×0.09×0.06	$0.51 \times 0.09 \times 0.06$	0.19×0.12×0.06	0.10×0.03×0.03
<i>T</i> /K	140.00(10)	140.00(10)	139.99(10)	140.00(10)	140.00(10)	140.00(10)
Crystal System	orthorhombic	triclinic	monoclinic	orthorhombic	monoclinic	monoclinic
Flack Parameter	r 0.143(7)			-0.014(4)		-0.0220(16)
Space Group	$P2_{1}2_{1}2_{1}$	<i>P</i> -1	$P2_{1}/c$	$P2_{1}2_{1}2_{1}$	$P2_{1}/c$	$P2_{1}$
a/Å	9.30092(8)	10.8812(5)	12.47399(8)	15.10287(19)	12.3243(4)	10.47706(6)
<i>b</i> /Å	10.67827(8)	11.0631(5)	10.42115(6)	16.6186(2)	10.3143(3)	16.55786(10)
c/Å	32.0935(2)	16.8995(8)	30.03284(18)	17.2511(3)	30.4650(9)	15.24231(9)
$a/^{\circ}$	90	74.198(4)	90	90	90	90
$b/^{\circ}$	90	78.406(4)	99.8863(6)	90	99.752(3)	98.9266(5)
$g/^{\circ}$	90	82.343(4)	90	90	90	90
$V/Å^3$	3187.46(4)	1910.88(15)	3846.10(4)	4329.83(10)	3816.6(2)	2612.17(3)
Ζ	4	2	4	4	4	2
Z'	1	1	1	1	1	1
Wavelength/Å	1.54184	1.54184	1.54184	1.54184	0.71073	1.54184
Radiation type	Cu K <sub>a</sub>	Cu K <sub>a</sub>	Cu K <sub>a</sub>	Cu K <sub>a</sub>	Mo Ka	Cu K <sub>a</sub>

Table S2. Crystallographic data for 10, 11Cl, 11OTf, 11PF<sub>6</sub>, 11SbF<sub>6</sub>, and 11BPh<sub>4</sub>.

$Q_{min}/^{\circ}$	2.754	2.759	2.987	3.693	2.749	2.935
$Q_{max}/^{\circ}$	76.220	75.557	76.592	72.474	29.599	76.069
Measured Refl's	s.33963	12093	40600	19149	28482	59800
Indep't Refl's	6575	12093	7930	8383	9225	10685
Refl's I≥2s(I)	6374	10369	7431	7948	6469	10430
$R_{ m int}$	0.0282		0.0176	0.0279	0.0827	0.0268
Parameters	463	492	571	543	553	650
Restraints	240	29	250	61	288	1
Largest Peak/ Å <sup>-3</sup>	/e0.638	1.950	0.669	1.071	0.945	0.239
Deepest Hole/ Å <sup>-3</sup>	/e-0.346	-1.801	-0.381	-0.510	-1.274	-0.398
GooF	1.038	1.054	1.028	1.049	0.989	1.044
$wR_2$ (all data)	0.0517	0.2149	0.0583	0.1025	0.0947	0.0428
$wR_2$	0.0513	0.2007	0.0574	0.0998	0.0821	0.0426
$R_1$ (all data)	0.0226	0.0825	0.0245	0.0404	0.0835	0.0190
$R_1$	0.0215	0.0725	0.0226	0.0376	0.0503	0.0181
<b>CCDC</b> number	2201998	2210246	2193598	2210241	2210245	2202259

#### 2. Solubility, partition coefficient and conductivity of 11CA

0.5 mg of compounds **10** and **11**CA were dispersed in 0.5 mL of MilliQ water, and, after sonication, the mixtures were stirred at 25°C for 1 h. The saturated solutions were filtered to remove any insoluble solids, dissolved in 0.5 mL of MilliQ water, and analyzed using ICP-MS. Samples were submitted to acidic digestion with 2 mL of concentrated HNO3 (69%, ROTIPURAN Supra, Roth) in PP digestion vials using a heating block system (DigiPREP Jr. 15ml, 40 Pos, SCP Science). The digestion program was the following: 15 min to heat to 100°C and keep at 100°C for 60 min. After the digestion, the sample volumes were precisely adjusted to 5 mL with MilliQ water. Samples were further diluted 30 times with 2% HNO3 solution and their Ru content was analyzed by ICP-MS using standard mode on NexIon 350 D ICP-MS instrument (PerkinElmer). Yttrium was added as an internal standard at a concentration of 2 ppb to all the solution and Ru quantitation was performed using an external calibration curve with standards in the 0.05-50 ppb range. All measurements were performed in triplicates.

The *n*-octanol-water partition ratio is the most common way of identifying the lipophilicity of a compound. This assay was performed following the Shake-flask method <sup>10</sup>. To a 0.1 mM of **11**CA in a water-saturated n-octanol solution was added n-octanolsaturated MilliQ water in different organic: aqueous ratios (1:1; 1:2 and 2:1). The mixture was shaken for 2 minutes, and after that, the solution was centrifuged to separate the organic and aqueous phase. Aliquots of each phase were taken and analyzed separately by ICP-MS. Samples were submitted to acidic digestion with 2 mL of concentrated HNO<sub>3</sub> (69%, ROTIPURAN Supra, Roth) in PP digestion vials using a heating block system (DigiPREP Jr. 15ml, 40 Pos, SCP Science). The digestion program was the following: 15 min to heat up to 100°C and keep at 100°C for 60 min. After the digestion, the sample volumes were precisely adjusted to 5 mL with MilliQ water. Samples were further diluted 30 times with 2% HNO3 solution and their Ru content was analyzed by ICP-MS using standard mode on NexIon 350 D ICP-MS instrument (PerkinElmer). Yttrium was added as an internal standard at a concentration of 2 ppb to all the solution and Ru quantitation was performed using an external calibration curve with standards in the 0.05-50 ppb range. The partition coefficient was calculated as  $P_{ow} =$  $[Ru]_{org_f}/([Ru]_{org_i}-[Ru]_{org_f})$ , where  $[Ru]_{org_i}$  and  $[Ru]_{org_f}$  are the concentrations of ruthenium in the organic phase before and after mixing with the aqueous phase, respectively. All measurements were performed in triplicates.

The molar conductivity ( $\Lambda_M$ , reported as  $S \cdot cm^2 \cdot mol^{-1}$ ) of solutions of **11**CA in acetonitrile (1 mM) were measured in a Radiometer Copenhagen CDM92 equipped with a conductivity cell CDC641T glass platinum electrode at room temperature (25 °C). The conductivity of the pure solvent was used as standard controls.

	Solubility	Solubility (uM)	ΙωσΡ	Conductivity
	(µg/mL)	Solubility (µ11)	Logi ow	(S·cm²/mol)
1	40 x10 <sup>3 4</sup>	77 x10 <sup>3</sup>	-1.85 11	
2	25 x10 <sup>3 4</sup>	$43 \text{ x} 10^3$		
3	$> 10 \text{ x} 10^3 ^{12}$	$> 20 \text{ x} 10^3$		
4			-1.5 13	
5	$\sim 10 \text{ x} 10^{3} ^{14}$	$\sim 22 \text{ x} 10^3$	>-1.8 <sup>13</sup>	
6				
7	$> 48 \text{ x} 10^3$	$> 100 \text{ x} 10^{3}$ <sup>7</sup>		
8		$> 25 \text{ x} 10^3 ^{15}$	-1.57 8	
9	$34\pm4$	$65\pm8$		
10	$475\pm102$	$547 \pm 117$		
11Cl	$416\pm70$	$528\pm88$	$1.48\pm0.29$	128
11NO3	$218\pm34$	$268\pm42$	$1.57\pm0.29$	141
<b>11</b> BF4	$4 \pm 1$	$5\pm 2$	$1.60\pm0.20$	124
<b>11</b> 0Tf	$1.0\pm0.1$	$1.1\pm0.1$	$1.75\pm0.18$	173
<b>11</b> PF <sub>6</sub>	$0.6\pm0.2$	$0.6\pm0.2$	$1.91\pm0.23$	150
<b>11</b> SbF <sub>6</sub>	below LOD $^*$	below LOD *	$2.01\pm0.07$	186
<b>11</b> BPh <sub>4</sub>	below LOD *	below LOD *	> 3.5	71

*Table S3.* Solubility in water at 25 °C, n-octanol/water partition coefficient and molar conductivity of the 11CA. \* below limit of detection = 5 ppt.

**Table S4.** Molar Gibbs energies of hydration of selected counteranions calculated using the method described in reference <sup>16</sup>. The radius, r; width of hydration shell,  $\Delta r$ ; number of water molecules in this shell, n; the neutral contribution,  $\Delta G_{neutral}$ ; and the electrostatic contribution,  $\Delta G_{ell+2}$ 

			∆G <sub>hyd</sub>			
	[11]	CA		[ <b>11</b> ] <sup>+</sup> +	CA	
	<b>r</b> (nm)	n	$\Delta \mathbf{r}$ (nm)	ΔG <sub>neutral</sub> (KJ/mol)	ΔG <sub>el1+2</sub> (KJ/mol)	$\Delta G_{ m hyd}^*$ calc $( m KJ/mol)$
Cl	0.168	2.1	0.050	+60	-331	-291
NO <sub>3</sub>	0.200	1.8	0.034	+72	-293	-245
$\mathbf{BF_4}^-$	0.211	1.7	0.029	+76	-281	-231
OTf	0.230	1.6	0.024	+84	-263	-206
PF <sub>6</sub>	0.242	1.5	0.021	+90	-252	-191

SbF <sub>6</sub>	0.252	1.4	0.018	+95	-243	-178
BPh₄ <sup>−</sup>	0.421	0.9	0.004	+217	-150	+67

#### 3. Stability in solution

Stability studies were performed on **11**CA over 72 h at 37 °C. The stability of the complexes **11**CA was studied via ESI-MS. The complexes were dissolved in DMSO and the solution was diluted with MilliQ water to reach 0.1% DMSO in water (final concentration, 10  $\mu$ M). Aliquots were taken and injected into the spectrometer without any further sample dilution or preparation. The stability of **11**Cl was also monitored over 72 h via <sup>1</sup>H and <sup>31</sup>P NMR spectroscopy. The complex was fully dissolved in *d*<sub>6</sub>-DMSO, diluted with D<sub>2</sub>O to reach 10% *d*<sub>6</sub>-DMSO in D<sub>2</sub>O (1 mM final concentration) and, finally, the pD was adjusted to 7.2 ± 0.1 (neutral conditions). Furthermore, the stability of **11**Cl and **11**BPh<sub>4</sub> was also assessed in 100% *d*<sub>6</sub>-DMSO (1 mM final concentration) as a highly coordinating solvent.



Figure S2. Stability of 11Cl in milliQ water with 0.1% DMSO monitored by ESI-MS.



Figure S3. Stability of 11NO<sub>3</sub> in milliQ water with 0.1% DMSO monitored by ESI-MS.



*Figure S4. Stability of 11BF*<sub>4</sub> *in milliQ water with 0.1% DMSO monitored by ESI-MS.* 



Figure S5. Stability of 110Tf in milliQ water with 0.1% DMSO monitored by ESI-MS.



*Figure S6. Stability of 11PF*<sup>6</sup> *in milliQ water with 0.1% DMSO monitored by ESI-MS.* 



*Figure S7. Stability of 11SbF*<sup>6</sup> *in milliQ water with* 0.1% *DMSO monitored by ESI-MS.* 



*Figure S8. Stability of 11BPh*<sup>4</sup> *in milliQ water with 0.1% DMSO monitored by ESI-MS.* 



*Figure S9. Stability of 11Cl* ( $\blacktriangle$ ) *in a D*<sub>2</sub>*O*:*d*<sub>6</sub>*-DMSO mixture (90:10) at pD* = 7.2 ± 0.2 *monitored by* <sup>1</sup>*H NMR.* 

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*Figure S10.* Stability of 11Cl ( $\blacktriangle$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO mixture (90:10) at pD = 7.2 ± 0.2 monitored by <sup>31</sup>P NMR.



**Figure S11.** Stability of 11Cl ( $\blacktriangle$ ) in d<sub>6</sub>-DMSO monitored by <sup>1</sup>H NMR.





*i*<sup>14</sup> *i*<sup>3</sup> *i*<sup>2</sup> *i*<sup>1</sup> *i*<sup>0</sup> *y s f i*<sup>6</sup> *s 4 s z i f igure S*13. *Stability of 1*1*BPh*<sub>4</sub> ( $\blacktriangle$ ) *in d*<sub>6</sub>-*DMSO monitored by* <sup>1</sup>*H NMR*.



## 4. Cytotoxicity studies





Compound **10** L929 - 72h





Compound 11CI L929 - 72h









Compound **10** MDA-MB-231 - 24h

Compound 10 MDA-MB-231 - 72h



Compound 11CIMDA-MB-23 - 24h



Compound 11CIMDA-MB-231 - 72h







Compound 11NO<sub>3</sub> L929 - 72h



Compound **11**PF<sub>6</sub> L929 - 24h





Compound 11NO<sub>3</sub> MCF-7 - 24h



Compound 11PF<sub>6</sub> MCF-7 - 24h 150 100 50 20 40 60 $[11PF_6]$  (µM)



Compound 11NO 3 MDA-MB-231 - 24h



Compound 11NO3 MDA-MB-231 - 72h

Compound 11PF<sub>6</sub> MDA-MB-231 - 24h



Compound 11PF<sub>6</sub> MDA-MB-231 - 72h





Compound **11**SbF<sub>6</sub>L929 - 72h







Compound 11SbF<sub>6</sub> MDA-MB-231 - 24h



Compound 11SbF<sub>6</sub> MDA-MB-231 - 72h



Compound **11**BF<sub>4</sub> L929 - 24h



Compound  $11BF_4L929 - 72h$ 



Compound 11BF<sub>4</sub> MCF-7 - 24h



Compound  $11BF_4$  MDA-MB-231 - 24h



Compound  $11BF_4$  MDA-MB-231 - 72h





Figure S15. Dose-response curves of 10 and 11CA over an exposure of 24 h and 72 h.

**Table S5** – In-vitro antiproliferative activities of compounds 1 - 11CA against breast cancer (MCF-7 and MDA-MB-231), and non-tumoral adherent mouse fibroblast connective tissue (L929) cell lines after 24 h of exposure. See Table S2 for the full statistical analysis. \*The SI (selectivity index) was calculated as  $IC_{50}$  non-cancerous L929 divided by the average of the  $IC_{50}$  of the breast cancers cell lines.

Compound	IC <sub>50</sub> (µM) (average ± standard deviation) 24 h						
Compound –	<b>MCF-7</b>	<b>MDA-MB-231</b>	L929	- 51			
1	$275\pm12$	$236\pm7$	$511\pm31$	2.0			
2	$403\pm9$	$286\pm7$	$537\pm29$	1.6			
3	$463\pm19$	$357\pm7$	$749\pm45$	1.8			
4	$474\pm19$	$384\pm16$	$723\pm29$	1.7			
5	$526\pm19$	$497\pm12$	$612\pm30$	1.2			
6	$376\pm15$	$388 \pm 12$	$670\pm60$	1.8			
7	$407\pm17$	$513\pm16$	$570\pm18$	1.2			
8	$213\pm8$	$284\pm7$	$572\pm33$	2.3			
9	$304\pm19$	$164 \pm 2$	$544\pm12$	2.3			
10	$128\pm30$	$113\pm14$	$74\pm5$	0.6			
<b>11</b> Cl	$4.0\pm0.3$	$5.5\pm0.6$	$5.3\pm0.7$	1.1			
<b>11</b> NO <sub>3</sub>	$5.9\pm0.5$	$4.6\pm0.3$	$3.7\pm 0.4$	0.7			
<b>11</b> BF <sub>4</sub>	$5.4\pm0.6$	$4.3\pm0.4$	$3.8\pm 0.4$	0.8			
11OTf	$4 \pm 1$	$4\pm 2$	$5.8\pm0.7$	1.5			
<b>11</b> PF <sub>6</sub>	$3.0\pm0.3$	$5.0\pm0.1$	$10 \pm 1$	2.5			
$11SbF_6$	$11 \pm 2$	$21\pm2$	$7.0\pm0.4$	0.4			
11BPh <sub>4</sub>	$51\pm5$	$100\pm 8$	$59\pm5$	0.8			

**Table S6** – Statistical significance of the  $IC_{50}$  values between L929, MCF-7, and MDA-MB-231 cell lines at 24 h and 72 h.

	Statistical significance at 24 h			Statistical significance 72 l		
_ Compounds	L929 / MCF-7	L929 / MDA-MB- 231	MCF-7 / MDA-MB- 231	L929 / MCF-7	L929 / MDA-MB- 231	MCF-7 / - MDA-MB- 231
1	****	****	****	****	***	****
2	****	****	****	****	****	Ø
3	****	****	****	****	****	****
4	****	****	****	****	****	Ø
5	****	**	****	****	****	****
6	****	Ø	****	****	****	****
7	****	****	****	****	****	****
8	****	****	****	****	****	Ø
9	****	* * * *	****	****	****	****

10	****	Ø	****	****	Ø	****
<b>11</b> Cl	Ø	Ø	Ø	****	Ø	****
<b>11</b> NO <sub>3</sub>	Ø	Ø	Ø	Ø	Ø	Ø
<b>11</b> BF <sub>4</sub>	Ø	Ø	Ø	****	Ø	****
<b>11</b> OTf	Ø	Ø	Ø	****	****	****
<b>11</b> PF <sub>6</sub>	****	Ø	****	Ø	Ø	Ø
$11SbF_6$	***	****	****	****	Ø	****
11BPh <sub>4</sub>	****	****	* * * *	****	****	****

Ø, no statistical difference; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001

**Table S7.**  $IC_{50}$  values of the sodium counteranion salts tested in the selected cell lines over an exposure of 24 h and 72 h.

Compounds	IC50 in L9 Mean	29 cell line ± SD*	IC50 in MC Mear	IC50 in MCF-7 cell line Mean ± SD*		IC <sub>50</sub> in MDA-MB-231 cell line Mean ± SD*	
	24 h	72h	24 h	72h	24 h	72h	
NaCl	$782\pm55$	$274\pm31$	> 2000	$280\pm33$	> 2000	$398\pm 38$	
NaNO <sub>3</sub>	> 1000	$331\pm57$	> 2000	$262\pm38$	> 2000	$334\pm23$	
NaBF <sub>4</sub>	$923\pm77$	$386\pm 49$	> 2000	$343\pm39$	> 2000	$347\pm19$	
NaOTf	> 1000	$159\pm20$	> 2000	$279\pm30$	> 2000	$354\pm27$	
NaPF <sub>6</sub>	$759\pm77$	$297\pm24$	> 2000	$311\pm27$	> 1000	$329\pm17$	
NaSbF <sub>6</sub>	$693\pm53$	$180\pm22$	> 1000	$234\pm24$	$689\pm61$	$236\pm13$	
NaBPh <sub>4</sub>	$186\pm11$	$55\pm4$	$217\pm44$	$102\pm14$	$441\pm 61$	$149\pm13$	





*Figure S16.* Apoptosis profiles of *11*CA in MCF-7 and MDA-MB-231 cell lines after 24 h. Dot-plot graphs illustrated the viable cells (the lower left quadrant), early-phase apoptotic cells (the lower right quadrant), late-phase apoptotic or dead cells (the upper right quadrant), and the necrotic cells (the upper left quadrant).

#### 6. Reactivity with model biomolecules

The interaction between **11**Cl and different model biomolecules (*aminoacids*: glutamic acid, histidine and cysteine; *nucleobase*, dGMP) was studied by <sup>1</sup>H and <sup>31</sup>P NMR spectroscopy over 72 h in a D<sub>2</sub>O:*d*<sub>6</sub>-DMSO mixture (90:10) at 37 °C. The complex and the different nucleophiles in a 1:3 ratio were fully dissolved in *d*<sup>6</sup>-DMSO, diluted with D<sub>2</sub>O to reach 10% DMSO in D<sub>2</sub>O (final concentration, 1 mM) and, finally, the pD was adjusted to  $7.2 \pm 0.1$ . The interaction with Cys was studied 20% *d*<sub>4</sub>-methanol in D<sub>2</sub>O, to avoid the oxidation of the thiol caused by *d*<sub>6</sub>-DMSO <sup>17</sup> Furthermore, the interaction was monitored by ESI-MS after 72 h. **11**Cl and the model biomolecules (1:3) were dissolved in DMSO and the solution was diluted with MilliQ water to reach 0.1% DMSO in water (final concentration, 10 µM). Aliquots were taken and injected into the spectrometer without any further sample dilution or preparation.

The catalytic activity of **11**Cl in transfer hydrogenation (NAD<sup>+</sup>/NADH) and glutathione oxidation reactions (GSH/GSSG) at 37 °C was monitored by <sup>1</sup>H and <sup>31</sup>P NMR spectroscopy over 8 h. Complex **11**Cl was dissolved in  $d_4$ -methanol/D<sub>2</sub>O (5:1 v/v, 1.4 mM). In a typical experiment, 3 mL of a solution containing complex **11**Cl (0.44 mM) and either NAD<sup>+</sup> (0.88 mM, 2 eq.) and sodium formate (11.02 mM, 25 eq.), for transfer hydrogenation; or reduced glutathione (1.32 mM, 3 eq.), for oxidation, were added to a 10-mm NMR tube. The pD was adjusted to 7.2 ± 0.1. <sup>1</sup>H NMR spectra and <sup>31</sup>P NMR spectra were recorded at 310 K every 300 s for 8 h.



*Figure S17.* Interaction of 11Cl ( $\blacktriangle$ ) with His ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO mixture (90:10) monitored by <sup>1</sup>H NMR.



*Figure S18.* Interaction of 11Cl ( $\blacktriangle$ ) with His ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO mixture (90:10) monitored by <sup>31</sup>P NMR.



*Figure S19. ESI-MS spectra of 11Cl and of its interaction with His in milliQ water with* 0.1% DMSO after 72 h.



*Figure S20.* Interaction of 11Cl ( $\blacktriangle$ ) with Glu ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO mixture (90:10) monitored by <sup>1</sup>H NMR.



140 120 100 80 60 20 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200 -220 -240 *Figure S21.* Interaction of 11Cl ( $\blacktriangle$ ) with Glu ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO mixture (90:10) monitored by <sup>31</sup>P NMR.



*Figure S22. ESI-MS spectra of 11Cl and of its interaction with Glu in milliQ water with 0.1% DMSO after 72 h.* 



*Figure S23.* Interaction of 11Cl ( $\blacktriangle$ ) with Cys ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>4</sub>-methanol mixture (80:20) monitored by <sup>1</sup>H NMR.

t = 0 h
t = 2 h
t = 8 h
t = 24 h
t = 48 h
t = 72 h
25 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -2 Figure S24. Interaction of $11Cl(\blacktriangle)$ with Cys ( $\bullet$ ) in a $D_2O:d_4$ -methanol mixture (80:20)

monitored by <sup>31</sup>P NMR.



*Figure S25. ESI-MS spectra of 11Cl and of its interaction with Cys in milliQ water with* 0.1% DMSO after 72 h.



**Figure S26.** Interaction of IICl ( $\blacktriangle$ ) with dGMP ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO m. monitored by <sup>1</sup>H NMR.



-240 140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200 -220 *Figure 27.* Interaction of 11Cl ( $\blacktriangle$ ) with dGMP ( $\bullet$ ) in a D<sub>2</sub>O:d<sub>6</sub>-DMSO mixture (90:10) monitored by <sup>31</sup>P NMR.



*Figure S28. ESI-MS spectra of 11Cl and of its interaction with dGMP (1:3) in milliQ water with 0.1% DMSO after 72 h.* 



*Figure S29.* <sup>1</sup>*H* NMR monitoring of the NAD<sup>+</sup>/NADH ( $\bullet$ ) transfer hydrogenation by 11Cl ( $\blacktriangle$ ).



3.5 23.0 22.5 22.0 21.5 21.0 20.5 20.0 19.5 -8.5 -9.0 -9.5 -10.0 -10.5 -11.0 -11.5 -12.0 -12.5 -13.0 -13.5

*Figure S30.* <sup>31</sup>*P NMR* monitoring of the NAD<sup>+</sup>/NADH ( $\bullet$ ) transfer hydrogenation by **11***Cl* ( $\blacktriangle$ ).



*Figure S31.* <sup>1</sup>*H* NMR monitoring of the GSH ( $\bullet$ ) oxidation by 11Cl ( $\blacktriangle$ ).



*Figure S32.* <sup>31</sup>*P NMR* monitoring of the GSH ( $\bullet$ ) oxidation by 11*Cl* ( $\blacktriangle$ ).

**Table S8.** Protein quenching constant  $(K_{sv})$ , bimolecular quenching rate  $(k_q)$ , binding constant  $(K_b)$ , and number of binding sites (n) for the interaction of **11**Cl with BSA.

Protein	$K_{SV}(10^4 \mathrm{M}^{-1})$	$k_q (10^{12} \mathrm{M}^{-1})$	$K_b (10^5 \mathrm{M}^{-1})$	n
BSA	$1.13\pm0.08$	$1.88\pm0.14$	$5.6\pm0.3$	1.4

**Table S9.** Protein quenching constant  $(K_{sv})$ , bimolecular quenching rate  $(k_q)$ ,  $c_{50}$ , and apparent binding constant  $(K_{app})$  for the interaction between **11**Cl and ctDNA from the DAPI and MG competitive binding assay.

Probe	$K_{SV} (10^3 \mathrm{M}^{-1})$	$k_q (10^{12} \mathrm{M}^{-1})$	<i>С</i> <sub>50</sub> (10 <sup>-4</sup> М)	$K_{app} (10^5 \mathrm{M}^{-1})$
DAPI	$8.8\pm0.6$	$3.3\pm0.3$	1.1	$4.4\pm0.3$
MG	$4.8\pm0.7$	$0.48\pm0.07$	2.1	$0.43\pm0.04$

### 7. Circular dichroism



*Figure S33.* Circular dichroism spectra of dsDNA incubated with DAPI (left), MG (center) and PI (right).

Table S10. Summary of the CD band changes on dsDNA induced by DAPI, MG, and PI.

Probe	210 nm		220 nm		246 nm		268–280 nm	
			Hydrogen bonding		Helicity		Base-pair stacking interactions	
<b>DAPI</b> Minor groove	Hypochromic, ∆↓	Red shift, λ↑	Hyperchromic, ∆ ↑	Blue shift, λ↓	Unchanged	Unchanged	268 nm − Hyperchromic, Δ ↑ 280 nm − Hypochromic, Δ ↓	Unchanged
<b>MG</b> Major groove	Hypochromic, $\Delta \downarrow$	Blue shift, λ↓	Hyperchromic, $\Delta \uparrow$	Blue shift, λ↓	Hypochromic, $\Delta \downarrow$	Unchanged	Unchanged	Unchanged
<b>PI</b> Intercalation			Unchanged	Blue shift, λ↓	Hyperchromic, Δ↑	Unchanged	Hyperchromic, $\Delta \uparrow$	Unchanged



*Figure S34.* Induced circular dichroism spectra of dsDNA incubated with DAPI (left), MG (center) and PI (right).

#### 8. Tandem mass spectrometry of 11Cl and DNA oligomers

11Cl was incubated with 14-mer ssDNA 5'-3' (ATACATGGTACATA) and 6-mer dsDNA 5'-3' (AGGCAG) (10 µM) in a 3:1 ratio at 37 °C in MilliQ (pH 7). After 24 and 72 h, respectively, the solution is centrifuged with an Amicon® Ultra 0.5 mL centrifugal filter 3kDa MWCO to remove the unreacted metal complex and excess salts. Mass spectra were recorded on an LTQ Orbitrap Elite FTMS instrument (Thermo Scientific) operating in negative mode with a HESI-II probe in an Ion Max ion Source interface. The ionization voltage was set at -1.4 kV and the ion transfer capillary temperature at 120 °C. For tandem MS data analysis, FTMS spectra were obtained in the 160-2000 m/z range in the reduced profile mode with a resolution set to 120k. In all spectra, 10 microscan was acquired with a maximum injection time value of 2000 ms. High-resolution mass spectra of the ruthenated oligonucleotides were analyzed using the automated tool termed Analysis of Oligonucleotide Modifications from Mass Spectra (Aom<sup>2</sup>s) <sup>18</sup>. Aom<sup>2</sup>s was used to calculate theoretical MS and MS/MS ions from oligonucleotide sequences with desired metal adducts. Subsequently, Aom<sup>2</sup>s automatically matches theoretical isotopic patterns to the experimental isotopically resolved mass spectrum and generates graphic representations of the oligonucleotide as a fragment map. Aom<sup>2</sup>s input parameter included the loss of protons  $((H^+)-1-10)$ ; variables groups for MS analysis included the different ruthenium adducts Ru<sup>2+</sup>, RuCl<sup>+</sup>, [Ru(PPh<sub>3</sub>)]<sup>2+</sup>, [Ru(PPh<sub>3</sub>)Cl]<sup>+</sup>, [Ru(PPh<sub>3</sub>)2]<sup>2+</sup>,  $[\operatorname{Ru}(\operatorname{PPh}_3)_2\operatorname{Cl}]^+$ ,  $[\operatorname{Ru}(\eta^6-\operatorname{toluene})(\operatorname{PPh}_3)]^{2+}$ ,  $[\operatorname{Ru}(\eta^6-\operatorname{toluene})(\operatorname{PPh}_3)_2]^{2+}$  and  $[\operatorname{Ru}(\eta^6-\operatorname{toluene})(\operatorname{PPh}_3)_2]^{2+}$ toluene)(PPh<sub>3</sub>)<sub>2</sub>Cl]<sup>+</sup>; ions (K<sup>+</sup> and Na<sup>+</sup>); and modifiable charge (0 to +2). MSMS of ruthenium-containing adducts were fragmented by collision-induced dissociation (CID) in the linear ion trap using an isolation window of 8 Da, with product ion detected in the Orbitrap with a resolution set to 120 K. Variables groups for MS/MS analysis included the different ruthenium adducts  $Ru^{2+}$ ,  $[Ru(PPh_3)]^{2+}$ ,  $[Ru(\eta^6-toluene)]^{2+}$  and  $[Ru(\eta^6-toluene)]^{2+}$ toluene)(PPh<sub>3</sub>)]<sup>2+</sup>. Top and bottom zone widths were selected according to the isotope distribution of Ru (-2.5 to 7.5) and allowed experimental mass error and minimal similarity to be set to 10 ppm and 70%, respectively.



**Figure S35.** MS<sup>2</sup> CID fragmentation of the isolated ruthenium-bound (A), ruthenium-free (B) and intact (C) 14-mer ssDNA ions. Fragment labels in black and gray have an average similarity above and below 90%, respectively.





С

*Figure S36. MS*<sup>2</sup> *CID fragmentation of the isolated 14-mer ssDNA ions:ruthenium-bound (A), ruthenium-free (B) and intact oligonucleotide (C). Fragment labels in black and gray have an average similarity above and below 90%, respectively.* 



41



**Figure S37.**  $MS^2$  CID fragmentation of the isolated 6-mer dsDNA ions (left and right figures correspond to the 5'-3' and the 3'-5' strands, respectively): ruthenium-bound (A, B), ruthenium-free (C,D) and intact (E,F). Fragment labels in black and gray have an average similarity above and below 90%, respectively.



**Figure S38.** Detailed analysis of the fragmentation of the intact, ruthenium-bound and ruthenium-free 14-mer oligonucleotide isolated ions:  $\blacksquare$  terminal fragments;  $\blacksquare$  internal fragments; and  $\blacksquare$  not fragments. Terminal fragmentation mechanisms of type a,  $\blacksquare$ ; b,  $\blacksquare$ ; c,  $\blacksquare$ ; d,  $\blacksquare$ ; w,  $\blacksquare$ ; x,  $\blacksquare$ ; y,  $\blacksquare$ ; and z,  $\blacksquare$ . Internal fragmentation mechanisms of type W/A,  $\blacksquare$ ; W/B,  $\blacksquare$ ; and Y/A,  $\blacksquare$ . Fragmentation mechanisms including base loss  $\blacksquare$ .

42



**Figure S39.** Detailed analysis of the fragmentation of the intact, ruthenium-bound and ruthenium-free 6-mer oligonucleotide isolated ions:  $\blacksquare$  terminal fragments;  $\blacksquare$  internal fragments; and  $\blacksquare$  not fragments. Terminal fragmentation mechanisms of type a,  $\blacksquare$ ; b,  $\blacksquare$ ; c,  $\blacksquare$ ; d,  $\blacksquare$ ; w,  $\blacksquare$ ; x,  $\blacksquare$ ; y,  $\blacksquare$ ; and z,  $\blacksquare$ . Internal fragmentation mechanisms of type W/A,  $\blacksquare$ ; W/B,  $\blacksquare$ ; and Y/A,  $\blacksquare$ . Fragmentation mechanisms including base loss  $\blacksquare$ .

#### 9. Molecular docking

The docking files were prepared using AutoDock Tools. After removing the solvent molecules, cocrystallized ligands, and cofactors, all the hydrogens atoms were added to DNA, Gasteiger charges were calculated, and then non-polar hydrogen atoms were merged. The free torsion tree was assigned, and the ruthenium atom parameters were added. A grid box was created with 96 × 96 × 96 points and a resolution of 0.375 Å (which is roughly a quarter of the length of a carbon-carbon single bond), in order to include the entire DNA fragment. Standard AutoDock force fields were used to calculate the energies and the best conformation was optimized by applying a Lamarckian Genetic Algorithm (LGA). With an initial population size of 300 and several evaluations of 2500000, the GA was allowed to run up to 27000 generations to find one best individual. Each experiment was set to 100 GA runs. The gene mutation and crossover rate were fixed at 2% and 80%, respectively. Clustering was performed to analyze the convergence of the simulations. The resulting poses were visualized with Visual Molecular Dynamics (VMD) <sup>19</sup> (University of Illinois, USA) and Discovery Studio Visualizer (BIOVIA, Dassault Systèmes).



*Figure S40.* Docked conformations for  $11^+$  interacting with 5T4W, minor groove (top); and 1G3X, intercalator (bottom).

*Table S11.* Binding energy for DAPI and MG for the different DNA structures.

PDB structure	1BNA	5T4W	1G3X
DAPI	-9.49 kcal/mol	-9.61 kcal/mol	-9.95 kcal/mol
MG	-9.29 kcal/mol	-9.27 kcal/mol	-10.57 kcal/mol



**Figure S41.** 3D (top) and 2D (bottom) interaction maps of the docking results of  $11^+$  interacting with the minor (left) and the major (right) groove of 1BNA. Charge-charge (red), van der Waals (green),  $\pi$ -anion (orange),  $\pi$ -sigma (purple), T-shaped  $\pi$ - $\pi$  (pink), and  $\pi$ -HBD (light green). Blue shading indicates solvent accessibility.

## 10.NMR spectra of the synthesized compounds



Figure S42. <sup>1</sup>H NMR spectra of 10 in DMSO-d<sub>6</sub>.



Figure S43. <sup>31</sup>P NMR spectra of 10 in DMSO-d<sub>6</sub>.



Figure S44. <sup>19</sup>F NMR spectra of 10 in DMSO-d<sub>6</sub>.



Figure S45. <sup>13</sup>C NMR spectra of 10 in DMSO-d<sub>6</sub>.



Figure S46. <sup>31</sup>P NMR spectra of 10 in chloroform-d.



Figure S47. <sup>1</sup>H NMR spectra of 11Cl in chloroform-d.



Figure S48. <sup>31</sup>P NMR spectra of 11Cl in chloroform-d.



Figure S49. <sup>13</sup>C NMR spectra of 11Cl in chloroform-d.



Figure S50. <sup>1</sup>H NMR spectra of 11Cl in methanol-d<sub>4</sub>.



Figure S51. <sup>31</sup>P NMR spectra of 11Cl in methanol-d<sub>4</sub>.



Figure S52. <sup>37</sup>Cl NMR spectra of 11Cl in methanol-d<sub>4</sub>.



*Figure S53.* <sup>1</sup>*H NMR spectra of* **11***PF*<sub>6</sub> *in chloroform-d.* 



Figure S54. <sup>31</sup>P NMR spectra of 11PF<sub>6</sub> in chloroform-d.



Figure S55. <sup>19</sup>F NMR spectra of 11PF<sub>6</sub> in chloroform-d.



Figure S56. <sup>13</sup>C NMR spectra of 11PF<sub>6</sub> in chloroform-d.



Figure S57. <sup>1</sup>H NMR spectra of 11SbF<sub>6</sub> in chloroform-d.



Figure S58. <sup>31</sup>P NMR spectra of 11SbF<sub>6</sub> in chloroform-d.

-110.48 -113.65 -113.65 -113.65 -113.65 -113.65 -113.65 -133.00 -136.20
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Figure S59. <sup>19</sup>F NMR spectra of 11SbF<sub>6</sub> in chloroform-d.



*Figure S60.* <sup>13</sup>*C NMR spectra of* **11***SbF*<sub>6</sub> *in chloroform-d.* 



*Figure S61.* <sup>1</sup>*H NMR spectra of 110Tf in chloroform-d.* 



Figure S62. <sup>31</sup>P NMR spectra of 11OTf in chloroform-d.



Figure S63. <sup>19</sup>F NMR spectra of 11OTf in chloroform-d.



Figure S64. <sup>13</sup>C NMR spectra of 11OTf in chloroform-d.



*Figure S65.* <sup>1</sup>*H NMR spectra of* **11***BF*<sub>4</sub> *in chloroform-d.* 



Figure S66. <sup>31</sup>P NMR spectra of 11BF<sub>4</sub> in chloroform-d.



Figure S67. <sup>19</sup>F NMR spectra of 11BF<sub>4</sub> in chloroform-d.



Figure S68. <sup>11</sup>B NMR spectra of 11BF<sub>4</sub> in chloroform-d.



Figure S69. <sup>13</sup>C NMR spectra of 11BF<sub>4</sub> in chloroform-d.



Figure S70. <sup>1</sup>H NMR spectra of 11BPh<sub>4</sub> in chloroform-d.



Figure S71. <sup>31</sup>P NMR spectra of 11BPh<sub>4</sub> in chloroform-d.



Figure S72. <sup>11</sup>B NMR spectra of 11BPh<sub>4</sub> in chloroform-d.



Figure S73. <sup>13</sup>C NMR spectra of 11BPh<sub>4</sub> in chloroform-d.



Figure S74. <sup>1</sup>H NMR spectra of 11NO<sub>3</sub> in chloroform-d.



Figure S75. <sup>31</sup>P NMR spectra of 11NO<sub>3</sub> in chloroform-d.



Figure S76. <sup>13</sup>C NMR spectra of 11NO<sub>3</sub> in chloroform-d.

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