Cytotoxic Ru\textsuperscript{II}-p-cymene complexes of an anthraimidazoledione: halide dependent solution stability, reactivity and resistance to hypoxia deactivation

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1. Molecular docking

In this work we have chosen Erlotinib bound EGFR protein crystal structure (PDB: 1M17)\(^1\) from Brookhaven Protein Data Bank (RCSB), out of the large number of EGFR crystal structure, to predict the possible modes of binding of the ligand PAIDH. The PDB structure was optimized after removing the bound inhibitor, water and processed by applying the OPLS\(_{2005}\) force field by the protein preparation utility in Maestro Suite 2016-1 in Maestro (Schrödinger Suite 2016-1 Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2016; Impact, Schrödinger, LLC, New York, NY, 2016; Prime, Schrödinger, LLC, New York, NY, 2016).\(^2\) The erlotinib and the optimized ligand PAIDH were then prepared and minimized by means of OPLS\(_{2005}\).\(^3\)-\(^5\) The lowest energy conformer in ligprep output was docked in the EGFR tyrosine kinase active site (Coordinates of the site: X = 21.6, Y = 0.24, Z = 52.96) using extra precision (XP) within 10 Å cubic distance. The grid box used in the docking process is large enough to cover the entire active site.
**Fig. S1** $^1$H NMR of ligand PAIDH in CDCl$_3$, 400 MHz. Inset shows aromatic region where corresponding protons are designated accordingly.

**Fig. S2** $^1$H NMR of complex 1 in DMSO-$d_6$, 500 MHz.
Fig. S3 $^{13}$C NMR of complex 1 in DMSO-$d_6$, 125 MHz.

Fig. S4 $^{13}$C DEPT-135 NMR of complex 1 in DMSO-$d_6$. 
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Fig. S8 $^{13}$C DEPT-135 NMR of complex 2 in DMSO-$d_6$. 
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Fig. S10 $^1$H NMR of complex 3 in DMSO-$d_6$, 500 MHz.
Fig. S11 $^{13}$C NMR of complex 3 in DMSO-$d_6$, 125 MHz.

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**Fig. S19** $^1$H NMR kinetic experiments to study hydrolysis of complex 2 in PBS buffer solution (pH = 7.4) in presence of 110 mM NaCl. ‘!’ stands for the halide exchange forming 1 in solution. No arene loss is evident during the experiment for 3 days.

**Fig. S20** $^1$H NMR kinetic experiments to study hydrolysis of complex 2 in PBS buffer solution (pH = 7.4) in presence of 4 mM NaCl. ‘!’ denotes the peaks arising due to halide exchange to form 1. ‘*’ denotes the hydrolyzed peaks.
Fig. S21 \(^1\)H NMR kinetic experiments to study hydrolysis of complex 3 in PBS buffer solution (pH = 7.4) in presence of 110 mM NaCl. ‘!’ denotes the peaks arising due to halide exchange to form 1 and ‘#’ stands for the peak of arene after loss from the metal center. No hydrolyzed peaks were observed during the course of the experiment for 3 days.

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Fig. S30 ESI-MS spectrum of GSH interaction with complex 1 using 1:2.5 mole ratio of complex:GSH. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 24 h. The overall peak intensities show that there has been increase in GSH adduct intensity. Spectra represents experimental and simulated m/z values along with isotopic resolution of monocationic complexes- (A) PAIDH dissociated GSH bound metal complex, [Ru(GS)(p-cym)]\(^+\), (B) GSH adduct of halide dissociated complex, [Ru(PAIDH)(GS)(p-cym)]\(^+\) and (C) overall speciation (inset: sodiated GSH adduct of 1, [Ru(PAID)(GS)(p-cym)]Na\(^+\)). All the simulated spectra are represented in red colour. [PAID = anionic form of the ligand PAIDH and GS = anionic glutathione]
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Fig. S35 ESI-MS spectra of 9-ethylguanine(9-EtG) interaction with complex 1 using 1:1 mol ratio of complex:9-EtG. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 2 h, showing 9-EtG bound complex. Spectra represents m/z values along with isotopic resolution of monocationic complexes- (A) magnified view showing the halide dissociated 1, aquated species, molecular ion peak and (B) overall speciation with the 9-EtG bound 1 (inset: magnified view of the 9-EtG bound species along with the simulation). All the simulated spectra are represented in red colour. PAID = anionic form of the ligand PAIDH.
Fig. S36 ESI-MS spectrum of 9-ethylguanine(9-EtG) interaction with complex 1 using 1:1 mol ratio of complex:9-EtG. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 24 h, showing 9-EtG bound complex. The dehalogenated species concentration shows a ratio metric increase over a 24 h period.
**Fig. S37** ESI-MS spectrum of 9-ethylguanine (9-EtG) interaction with complex 2 using 1:1 mol ratio of complex:9-EtG. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 2 h, showing that the most intense m/z corresponds to 9-EtG adduct with 2.
Fig. S38 ESI-MS spectrum of 9-ethylguanine (9-EtG) interaction with complex 2 using 1:1 mol ratio of complex:9-EtG. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 24 h. The overall peak intensities show a decrease suggesting there may be degradation or precipitation of the complex from solution. ‘¥’ stands for uncharacterized polynuclear Ru species.
**Fig. S39** ESI-MS spectrum of 9-ethylguanine (9-EtG) interaction with complex 3 using 1:1 mol ratio of complex:9-EtG. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 2 h. The overall peak intensities show that there is hardly any adduct formation with 9-EtG. There is some ‘¥’ uncharacterized polynuclear Ru species in the same place where the dehalogenated species appears.
**Fig. S40** ESI-MS spectrum of 9-ethylguanine (9-EtG) interaction with complex 3 using 1:1 mol ratio of complex:9-EtG. Incubation in 2:8 v/v methanol and 4 mM aqueous NaCl, recorded after 24 h. The overall peak intensities show that there is formation of a small amount of adduct with 9-EtG. There is also some ‘¥’ uncharacterized polynuclear Ru species in the same place where the dehalogenated species appears.

<table>
<thead>
<tr>
<th>species</th>
<th>Exp. m/z</th>
<th>Calc. m/z</th>
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<tbody>
<tr>
<td>[Ru(PAID)(p-cym)]⁺</td>
<td>560.091</td>
<td>560.091</td>
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<tr>
<td>[Ru(PAIDH)(p-cym)Cl]⁺</td>
<td>596.068</td>
<td>596.068</td>
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<td>[Ru(PAIDH)(p-cym)I]⁺</td>
<td>688.004</td>
<td>688.004</td>
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<tr>
<td>[Ru(PAIDH)(p-cym)(9-EtG)]⁺</td>
<td>739.173</td>
<td>739.172</td>
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</table>
Fig. S41 9-Ethyl Guanine binding of 3 in DMSO-\textit{d}_6/D_2O 7:3 (\textit{v}/\textit{v}) mixture, recorded at different interval of time at 25 °C. Recording started after 5 minutes of dissolving 9-Ethyl guanine and complex 3 together. ‘Δ’ stands for the metal complex peaks and ‘%’ stands for the 9-Ethyl guanine peaks of the 9-EtG bound metal complex species.

Fig. S42 Cytotoxicity of HepG2 cells under normoxic condition in presence of different concentration of N-acetyl cysteine (NAC). Concentration taken from 100 µM to 1 mM.
Fig. S43 Plots of cell viability (%) vs. log of concentrations of 1-3 against HepG2 cell line after incubation for 48 h determined from MTT assays under normoxic condition. The plots provided are for one independent experiment (each concentration in triplicate) out of at least three independent experiments.

Fig. S44 Plots of cell viability (%) vs. log of concentrations of 1-3 against MIA PaCa-2 cell line after incubation for 48 h determined from MTT assays under normoxic condition. The plots provided are for one independent experiment (each concentration in triplicate) out of at least three independent experiments.

Fig. S45 Plots of cell viability (%) vs. log of concentrations of 1-3 against MDA-MB-231 cell line after incubation for 48 h determined from MTT assays under normoxic condition. The plots provided are for one independent experiment (each concentration in triplicate) out of at least three independent experiments.
Table S1 Cell cycle analysis of MIA PaCa-2 treated with 1-3, showing percentage of arrest in different phases of cell cycle.

<table>
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<tr>
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<th>Sub G1</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
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<tbody>
<tr>
<td>DMSO Control</td>
<td>0.11</td>
<td>59.26</td>
<td>15.33</td>
<td>25.73</td>
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<tr>
<td>1 (0.5µM)</td>
<td>0.97</td>
<td>64.38</td>
<td>11.12</td>
<td>23.58</td>
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<tr>
<td>1 (1.0µM)</td>
<td>0.93</td>
<td>68.31</td>
<td>11.31</td>
<td>19.57</td>
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<tr>
<td>2 (0.5µM)</td>
<td>1.44</td>
<td>66.88</td>
<td>11.61</td>
<td>20.28</td>
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<tr>
<td>2 (1.0µM)</td>
<td>0.79</td>
<td>68.89</td>
<td>10.87</td>
<td>19.67</td>
</tr>
<tr>
<td>3 (0.5µM)</td>
<td>2.49</td>
<td>63.89</td>
<td>11.80</td>
<td>22.22</td>
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<tr>
<td>3 (1.0µM)</td>
<td>1.1</td>
<td>70.51</td>
<td>11.11</td>
<td>17.34</td>
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**Fig. S46** EFGR kinase domain (PDB: 1M17) interaction of the clinical EGFR inhibitor Erlotinib (A) vs. the PAIDH ligand (B) using Schrödinger. (C) A representative diagram of both Erlotinib and PAIDH at the active sight cavity of the 1M17 protein showing both of them occupy almost the same site but the PAIDH shows a pi-pi stacking with phenylalanine (Phe 699) from the active. Yellow: PAIDH, Red: Erlotinib.
References: