ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Heterodinuclear Ru–Pt Complexes Bridged with 2,3-Bis(pyridyl)pyrazinyl Ligands: Studies on Kinetics, Deoxyribonucleic Acid/Bovine Serum Albumin Binding and Cleavage, In Vitro Cytotoxicity, and In Vivo Toxicity on Zebrafish Embryo Activities

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Figure S1. UV-Vis spectral changes for the reaction between 50 μM of C₂ (a) / C₃ (b) and Tu (40-fold excess); Inset is a typical kinetic trace of absorbance versus time at λ = 524 nm, 5 mM Tris-HCl/50 mM NaCl (pH = 7.2) and T = 35 °C.

Figure S2. Plots of $k_{obs}$ on [L-Met] for the first step for the reaction between Ru-Pt complexes with L-Met: [C₁/C₂/C₃] = 50 μM and pH = 7.2 (5.0 mM Tris-HCl and 50 mM NaCl).

Figure S3. Plots of $k_{obs}$ on [5-GMP] for both first and second steps for the reaction between Ru-Pt complexes with 5-GMP: [C₁/C₂/C₃] = 50 μM and pH = 7.2 (5.0 mM Tris-HCl and 50 mM NaCl).
Figure S4. Plots of $k_{obs}$ versus [Nu] for both first and second steps for the reaction between complex C$_2$ with Nu: [C$_2$] = 50 μM and pH = 7.2 (5.0 mM Tris-HCl and 50 mM NaCl).

Figure S5. Plots of $k_{obs}$ versus [Nu] for both first and second steps for the reaction between complex C$_3$ with Nu: [C$_3$] = 50 μM and pH = 7.2 (5.0 mM Tris-HCl and 50 mM NaCl).
Figure S6. Eyring plots for the first step for the reaction between Ru-Pt complexes with Tu (a) and L-Met (b) and 5'-GMP (c).

Figure S7. Eyring plots for the second step for the reaction between Ru-Pt complexes with Tu (a) and L-Met (b) and 5'-GMP (c).

Figure S8. Iso-kinetic plot for the first step of the reactions of all the three Ru-Pt complexes with Nus.
Figure S9. ORTEP view and atom numbering scheme of the Pt(Tu)$_4$·2NH$_2$(CH$_3$)$_2$ClO$_4$.Cl complex with displacement ellipsoid at 50% probability.

Figure S10. Absorption spectra of 14 μM C$_2$ (a) and C$_3$ (b) in Tris-HCl/50 mM NaCl buffer at pH 7.2) upon addition of CT-DNA (0-90 μM). The arrow shows the change in absorbance upon increasing the CT-DNA concentration. Inset: Wolfe-Shimmer plot.

Figure S11. (a) Fluorescence emission spectra of EtBr bounded to CT-DNA in the presence of C$_2$: [EtBr] = 20.0 μM, [CT-DNA] = 20.0 μM and [C$_2$] = 0-150 μM. The arrow shows the intensity changes upon increasing the C$_2$ concentration. (b) Stern-Volmer plot of $I_0/I$ versus [Q] and (c) Scatchard plot of log([I$_0$–I]/I) versus log[Q].
**Figure S12.** (a) Fluorescence emission spectra of EtBr bounded to CT-DNA in the presence of C₃: [EtBr] = 20.0 μM, [CT-DNA] = 20.0 μM and [C₃] = 0-150 μM. The arrow shows the intensity changes upon increasing the C₃ concentration. (b) Stern-Volmer plot of Iₒ/I versus [Q] and (c) Scatchard plot of log([Iₒ–I]/I) versus log[Q].

**Figure S13.** Effect of increasing amounts of C₁, C₂, C₃ and EtBr on the relative viscosities of CT-DNA in 5 mM Tris-HCl/50 mM NaCl, pH 7.2.

**Figure S14.** Absorption spectra of 10 μM BSA with and without 5 μM of each Ru-Pt complex.
Figure S15. (a) Fluorescence emission spectra of BSA in the absence and presence of C₂: [BSA] = 1.08 μM and [C₂] = 0 - 20 μM. The arrow shows the intensity changes upon increasing the C₂ concentration. (b) Stern-Volmer plot of $I_o/I$ versus [Q] and (c) Scatchard plot of log([I₀−I]/I) versus log[Q].

Figure S16. (a) Fluorescence emission spectra of BSA in the absence and presence of C₃: [BSA] = 1.08 μM and [C₃] = 0 - 20 μM. The arrow shows the intensity changes upon increasing the C₃ concentration. (b) Stern-Volmer plot of $I_o/I$ versus [Q] and (c) Scatchard plot of log([I₀−I]/I) versus log[Q].
**Figure S17.** SDS-PAGE profile of concentration dependent photo-induced cleavage of BSA (4 μM) in UV light of 350 nm (80 W) for 30 min by complex, (a) C₂ and (b) C₃ Lane 1, Molecular marker; Lane 2, BSA + complex (1 μM); Lane 3, BSA + complex (5 μM); Lane 4, BSA + complex (10 μM); Lane 5, BSA + complex (25 μM); Lane 6, BSA + complex (50 μM); Lane 7, BSA+complex (100 μM); Lane 8, BSA+complex (250 μM); Lane 9, BSA+complex (500 μM); Lane 10, BSA alone.

**Figure S18.** The lowest binding free energy conformers of C₁ (a) and C₃ (b) with BSA.
Table S1. Summary of the second order rate constants, $k_2$ for the first steps of the Ru-Pt complexes with Nu at 25, 45 and 55 ºC.

| Complex | Nu     | $k_{21}^{1\text{st}} \times 10^1$, M$^{-1}$ s$^{-1}$ | 25 ºC | 45 ºC | 55 ºC |
|---------|--------|--------------------------------------------------|-------|-------|-------|
| C$_1$   | Tu     | 0.91 ± 0.12                                      | 2.26 ± 0.19 | 3.39 ± 0.35 |
|         | L-Met  | 0.49 ± 0.10                                      | 1.15 ± 0.16 | 1.75 ± 0.22 |
|         | 5'-GMP | 0.20 ± 0.07                                      | 0.65 ± 0.11 | 1.07 ± 0.15 |
| C$_2$   | Tu     | 2.96 ± 0.21                                      | 6.15 ± 0.33 | 8.67 ± 0.40 |
|         | L-Met  | 1.21 ± 0.13                                      | 2.52 ± 0.27 | 3.47 ± 0.36 |
|         | 5'-GMP | 0.62 ± 0.10                                      | 1.51 ± 0.19 | 2.27 ± 0.20 |
| C$_3$   | Tu     | 5.56 ± 0.28                                      | 10.24 ± 0.48 | 13.69 ± 0.53 |
|         | L-Met  | 2.17 ± 0.20                                      | 4.45 ± 0.41 | 6.19 ± 0.40 |
|         | 5'-GMP | 0.90 ± 0.13                                      | 2.17 ± 0.20 | 3.27 ± 0.34 |

Table S2. Summary of the rate constants for the second steps of the Ru-Pt complexes with Nu at 25, 45 and 55 ºC (* $k_{12}^{2\text{nd}} \times 10^4$, s$^{-1}$).

| Complex | Nu     | $k_{22}^{2\text{nd}} \times 10^2$, M$^{-1}$ s$^{-1}$ | 25 ºC | 45 ºC | 55 ºC | $k_{-12}^{2\text{nd}} \times 10^5$, s$^{-1}$ | 25 ºC | 45 ºC | 55 ºC |
|---------|--------|--------------------------------------------------|-------|-------|-------|--------------------------------------------------|-------|-------|-------|
| C$_1$   | Tu     | 1.63 ± 0.09                                      | 4.65 ± 0.18 | 7.51 ± 0.31 | 1.13 ± 0.02 | 0.98 ± 0.04                                      | 1.24 ± 0.06 |
|         | L-Met  | 0.19 ± 0.06                                      | 0.63 ± 0.13 | 1.04 ± 0.45 | - | - | - | - |
|         | 5'-GMP | 0.40 ± 0.03                                      | 1.31 ± 0.08 | 2.27 ± 0.11 | 0.21 ± 0.01 | 0.17 ± 0.01                                      | 0.20 ± 0.02 |
| C$_2$   | Tu     | 5.26 ± 0.19                                      | 13.86 ± 0.36 | 21.51 ± 0.50 | 5.07 ± 0.05 | 4.68 ± 0.08                                      | 5.50 ± 0.12 |
|         | L-Met  | 0.57 ± 0.21                                      | 1.89 ± 0.48 | 3.32 ± 0.94 | - | - | - | - |
|         | 5'-GMP | 0.75 ± 0.08                                      | 3.05 ± 0.14 | 5.77 ± 0.23 | 1.00 ± 0.02 | 1.01 ± 0.03                                      | 0.92 ± 0.05 |
| C$_3$   | Tu     | 8.45 ± 0.23                                      | 24.08 ± 0.41 | 39.17 ± 0.63 | 13.02 ± 0.09 | 10.18 ± 0.13                                      | 12.11 ± 0.19 |
|         | L-Met  | 1.42 ± 0.44                                      | 2.97 ± 0.85 | 4.25 ± 1.27 | - | - | - | - |
|         | 5'-GMP | 1.09 ± 0.10                                      | 4.62 ± 0.17 | 8.73 ± 0.33 | 2.63 ± 0.03 | 3.10 ± 0.03                                      | 2.18 ± 0.06 |
Table S3. Activation parameters for the both first and second steps of the reaction between Ru-Pt and Nus.

| Complex | Nu   | First step | Second step |
|---------|------|------------|-------------|
|         |      | $\Delta H^\circ$/kJ mol$^{-1}$ | $-\Delta S^\circ$/J mol$^{-1}$ K$^{-1}$ | $\Delta G^\circ_{35^\circ C}$/kJ mol$^{-1}$ | $\Delta H^\circ$/kJ mol$^{-1}$ | $-\Delta S^\circ$/J mol$^{-1}$ K$^{-1}$ | $\Delta G^\circ_{35^\circ C}$/kJ mol$^{-1}$ |
| $C_1$   | Tu   | 33 ± 2     | 155 ± 6     | 81 ± 4       | 39 ± 3       | 147 ± 8       | 85 ± 5       |
|         | L-Met| 32 ± 2     | 163 ± 7     | 82 ± 5       | 42 ± 4       | 194 ± 16      | 102 ± 7      |
|         | 5'-GMP | 44 ± 3     | 131 ± 9     | 84 ± 3       | 44 ± 4       | 143 ± 9       | 88 ± 2       |
| $C_2$   | Tu   | 27 ± 1     | 166 ± 4     | 78 ± 2       | 36 ± 2       | 150 ± 8       | 82 ± 2       |
|         | L-Met| 26 ± 2     | 175 ± 6     | 80 ± 3       | 45 ± 3       | 174 ± 12      | 99 ± 6       |
|         | 5'-GMP | 33 ± 2     | 159 ± 9     | 82 ± 4       | 53 ± 5       | 108 ± 7       | 86 ± 4       |
| $C_3$   | Tu   | 22 ± 1     | 177 ± 3     | 76 ± 3       | 39 ± 3       | 135 ± 7       | 81 ± 3       |
|         | L-Met| 26 ± 2     | 171 ± 5     | 79 ± 3       | 27 ± 2       | 168 ± 11      | 79 ± 4       |
|         | 5'-GMP | 32 ± 4     | 157 ± 10    | 81 ± 4       | 54 ± 4       | 101 ± 4       | 85 ± 5       |
Table S4. Crystallographic data and structure refinement details for complex Pt(Tu)₄

| Parameters                        | Data                                      |
|-----------------------------------|-------------------------------------------|
| Empirical formula                 | C₆H₂₄Cl₂N₆O₄Pt₀.₅S₂                       |
| Formula weight                    | 476.88                                    |
| Temperature/K                     | 100.04                                    |
| Crystal system                    | Monoclinic                                |
| Crystal size/mm³                  | 0.235 × 0.035 × 0.025                     |
| Space group                       | P2₁/n                                     |
| 2Θ range for data collection/°    | 7.226 to 144.492                          |
| Radiation                         | CuKα (λ = 1.54178)                        |
| Z                                 | 4                                         |
| Unit Cell dimensions              | a/Å = 14.0830(3); α° = 90                 |
|                                    | b/Å = 9.1616(2); β° = 112.9140(10)        |
|                                    | c/Å = 15.3986(3); γ° = 90                 |
| Volume/Å³                         | 1829.99(7)                                |
| Density(ρcalc)g/cm³               | 1.731                                     |
| Absorption coefficient(μ)/mm⁻¹    | 12.444                                    |
| Index ranges                      | -16 ≤ h ≤ 17, -10 ≤ k ≤ 9, -18 ≤ l ≤ 18  |
| Reflections collected             | 33252                                     |
| Independent reflections           | 3540 [Rint = 0.0358, Rsigma = 0.0176]     |
| Data/restraints/parameters        | 3540/0/191                                |
| Goodness-of-fit on F²             | 1.092                                     |
| Final R indexes [I>=2σ (I)]       | R₁ = 0.0279, wR₂ = 0.0825                 |
| Final R indexes [all data]        | R₁ = 0.0308, wR₂ = 0.0849                 |
| Largest diff. peak/hole / e Å⁻³   | 2.20/-1.54                               |
Table S5. UV-Vis titration data between Ru-Pt complexes (C₁ – C₃) with CT-DNA.

| S.No. | [CT-DNA], μM. | [εₐ-ε₇] x 10⁴ | [CT-DNA], μM. | [εₐ-ε₇] x 10⁴ | [CT-DNA], μM. | [εₐ-ε₇] x 10⁴ |
|-------|---------------|----------------|---------------|----------------|---------------|----------------|
| 1.    | 17.23         | -2.90          | 1.40          | -3.79          | 1.40          | -3.37          |
| 2.    | 25.71         | -2.78          | 6.87          | -3.78          | 2.79          | -3.37          |
| 3.    | 36.95         | -2.52          | 13.43         | -3.75          | 5.53          | -3.36          |
| 4.    | 47.30         | -2.37          | 22.14         | -3.74          | 10.85         | -3.36          |
| 5.    | 56.81         | -2.29          | 31.45         | -3.71          | 20.93         | -3.33          |
| 6.    | 65.69         | -2.24          | 40.14         | -3.66          | 30.32         | -3.32          |
| 7.    | 73.90         | -2.21          | 47.30         | -3.65          | 39.09         | -3.29          |
| 8.    | 81.55         | -2.18          | 56.85         | -3.68          | 47.30         | -3.27          |
| 9.    | 88.68         | -2.14          | 65.71         | -3.54          | 56.81         | -3.32          |
| 10.   |               |                | 73.90         | -3.52          | 65.69         | -3.20          |
| 11.   |               |                | 81.55         | -3.50          | 73.90         | -3.20          |
| 12.   |               |                | 88.68         | -3.47          | 81.55         | -3.19          |
| 13.   |               |                | 88.68         | -3.17          |               |                |
| 14.   |               |                | 95.14         | -3.13          |               |                |

[ε₇] = 3.63 x 10⁴
| S.No. | $[C_1]$, μM. | Emission intensity | $[C_2]$, μM. | Emission intensity | $[C_3]$, μM. | Emission intensity |
|-------|---------------|--------------------|---------------|--------------------|---------------|--------------------|
| 1.    | 0.0           | 809.99             | 0.0           | 779.78             | 0.0           | 835.92             |
| 2.    | 1.20          | 787.98             | 0.40          | 772.23             | 0.40          | 825.57             |
| 3.    | 3.58          | 754.77             | 1.19          | 742.30             | 1.19          | 798.50             |
| 4.    | 5.94          | 729.74             | 1.98          | 723.82             | 1.98          | 776.21             |
| 5.    | 8.28          | 691.97             | 3.15          | 701.65             | 3.15          | 744.08             |
| 6.    | 12.92         | 652.50             | 4.69          | 670.84             | 4.69          | 709.48             |
| 7.    | 17.48         | 621.79             | 6.95          | 637.46             | 6.58          | 667.42             |
| 8.    | 24.21         | 580.53             | 9.16          | 607.36             | 8.80          | 625.81             |
| 9.    | 30.73         | 537.53             | 11.68         | 581.18             | 10.96         | 590.09             |
| 10.   | 37.15         | 505.96             | 14.47         | 547.92             | 13.43         | 558.09             |
| 11.   | 44.44         | 467.41             | 17.85         | 508.19             | 16.18         | 521.86             |
| 12.   | 52.55         | 424.09             | 21.75         | 469.49             | 19.17         | 482.69             |
| 13.   | 60.43         | 390.74             | 26.08         | 424.07             | 22.38         | 442.70             |
| 14.   | 69.03         | 351.43             | 30.80         | 380.75             | 25.48         | 415.97             |
| 15.   | 77.35         | 341.84             | 35.80         | 338.72             | 28.67         | 383.71             |
| 16.   |               |                    | 41.02         | 303.00             | 32.22         | 368.51             |
| 17.   |               |                    | 45.92         | 272.31             | 35.80         | 354.55             |
| 18.   |               |                    | 50.52         | 247.22             |              |                    |
| 19.   |               |                    | 54.86         | 225.92             |              |                    |
| 20.   |               |                    | 58.96         | 206.99             |              |                    |

$[\text{EtBr}]$ and $[\text{CT-DNA}] = 20.0$ μM
Table S7. The percentage of 0.1 μM pcDNA cleavage at concentration gradient of Ru-Pt complexes $C_1 - C_3$

| Complex | Lane | Reaction | % of SC | % of NC |
|---------|------|----------|---------|---------|
| $C_1$   | 0    | 0 μM $C_1$ + DNA | 89.3    | 10.7    |
|         | 1    | 10 μM $C_1$ + DNA | 81.7    | 18.3    |
|         | 2    | 20 μM $C_1$ + DNA | 77.4    | 22.6    |
|         | 3    | 50 μM $C_1$ + DNA | 79.4    | 20.6    |
|         | 4    | 100 μM $C_1$ + DNA | 76.3   | 23.7    |
|         | 5    | 250 μM $C_1$ + DNA | 76.1    | 23.9    |
| $C_2$   | 0    | 0 μM $C_1$ + DNA | 97.4    | 2.6     |
|         | 1    | 10 μM $C_1$ + DNA | 92.6    | 7.4     |
|         | 2    | 20 μM $C_1$ + DNA | 88.3    | 11.7    |
|         | 3    | 50 μM $C_1$ + DNA | 84.5    | 15.5    |
|         | 4    | 100 μM $C_1$ + DNA | 80.5    | 19.5    |
|         | 5    | 250 μM $C_1$ + DNA | 82.0    | 18.0    |
| $C_3$   | 0    | 0 μM $C_1$ + DNA | 94.4    | 5.6     |
|         | 1    | 10 μM $C_1$ + DNA | 91.5    | 8.5     |
|         | 2    | 20 μM $C_1$ + DNA | 87.4    | 12.6    |
|         | 3    | 50 μM $C_1$ + DNA | 86.0    | 14.0    |
|         | 4    | 100 μM $C_1$ + DNA | 84.4    | 15.6    |
|         | 5    | 250 μM $C_1$ + DNA | 82.9    | 17.1    |
Table S8. Survival rates of zebrafish embryos assessments after the exposure of cis-platin and each Ru-Pt complex using zebrafish embryos. Data collected from three replicates of three independent experiments.

| Conc. (μM) | % of survival rate |
|------------|--------------------|
|            | 0 h  | 12 h | 24 h | 48 h | 72 h | 96 h |
| **Cis-Platin** |      |      |      |      |      |      |
| 0, Control  | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 | 96.7 ± 1.9 | 96.7 ± 0.9 |
| 15          | 100 ± 0 | 100 ± 0 | 96.7 ± 1.9 | 96.7 ± 2.2 | 93.3 ± 2.1 | 90.0 ± 2.6 |
| 30          | 100 ± 0 | 96.7 ± 2.4 | 93.3 ± 2.2 | 90.0 ± 2.4 | 86.7 ± 2.5 | 83.3 ± 2.4 |
| 45          | 100 ± 0 | 96.7 ± 2.1 | 93.3 ± 2.7 | 86.7 ± 4.0 | 83.3 ± 3.3 | 80.0 ± 2.1 |
| 60          | 100 ± 0 | 93.3 ± 3.6 | 86.7 ± 3.1 | 83.3 ± 2.4 | 76.7 ± 3.0 | 73.3 ± 3.0 |
| 75          | 100 ± 0 | 86.7 ± 2.9 | 83.3 ± 2.4 | 80.0 ± 1.1 | 73.3 ± 4.2 | 70.0 ± 3.9 |
| 90          | 100 ± 0 | 83.3 ± 3.4 | 76.7 ± 2.7 | 73.3 ± 3.1 | 66.7 ± 2.8 | 60.0 ± 2.0 |
| **C1**      |      |      |      |      |      |      |
| 0, Control  | 100 ± 0 | 100 ± 0 | 100 ± 0 | 96.7 ± 1.7 | 96.7 ± 1.6 | 93.3 ± 1.1 |
| 15          | 100 ± 0 | 93.3 ± 2.1 | 90.0 ± 2.4 | 90.0 ± 1.9 | 83.3 ± 2.2 | 83.3 ± 1.7 |
| 30          | 100 ± 0 | 90.0 ± 3.0 | 86.7 ± 1.9 | 83.3 ± 2.5 | 76.7 ± 2.6 | 73.3 ± 2.2 |
| 45          | 100 ± 0 | 83.3 ± 2.1 | 76.7 ± 3.6 | 73.3 ± 2.8 | 70.0 ± 1.6 | 70.0 ± 2.8 |
| 60          | 100 ± 0 | 76.7 ± 2.4 | 66.7 ± 2.5 | 60.0 ± 3.1 | 56.7 ± 3.7 | 53.3 ± 3.0 |
| 75          | 100 ± 0 | 63.3 ± 3.3 | 53.3 ± 2.5 | 50.0 ± 2.4 | 46.7 ± 2.4 | 46.7 ± 2.4 |
| 90          | 100 ± 0 | 46.7 ± 1.8 | 40.0 ± 4.1 | 36.7 ± 3.2 | 36.7 ± 2.0 | 33.3 ± 2.6 |
| **C2**      |      |      |      |      |      |      |
| 0, Control  | 100 ± 0 | 100 ± 0 | 100 ± 0 | 96.7 ± 1.1 | 96.7 ± 1.4 | 93.3 ± 2.2 |
| 15          | 100 ± 0 | 93.3 ± 2.1 | 90.0 ± 2.1 | 90.0 ± 2.7 | 86.7 ± 2.4 | 86.7 ± 1.9 |
| 30          | 100 ± 0 | 93.3 ± 2.4 | 86.7 ± 3.4 | 83.3 ± 3.4 | 83.3 ± 3.1 | 80.0 ± 1.6 |
| 45          | 100 ± 0 | 86.7 ± 3.3 | 83.3 ± 1.7 | 80.0 ± 3.0 | 76.7 ± 4.7 | 73.3 ± 2.4 |
| 60          | 100 ± 0 | 83.3 ± 4.6 | 76.7 ± 2.1 | 70.0 ± 4.1 | 66.7 ± 2.4 | 63.3 ± 3.4 |
| 75          | 100 ± 0 | 73.3 ± 2.2 | 66.7 ± 3.0 | 63.3 ± 4.4 | 56.7 ± 3.4 | 56.7 ± 3.3 |
| 90          | 100 ± 0 | 60.0 ± 3.0 | 53.3 ± 2.6 | 50.0 ± 2.6 | 50.0 ± 2.0 | 46.7 ± 2.0 |
| **C3**      |      |      |      |      |      |      |
| 0, Control  | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 | 96.7 ± 2.1 | 93.3 ± 3.0 |
| 15          | 100 ± 0 | 100 ± 0 | 96.7 ± 2.2 | 96.7 ± 2.9 | 93.3 ± 2.7 | 86.7 ± 2.4 |
| 30          | 100 ± 0 | 93.3 ± 3.0 | 90.0 ± 3.9 | 86.7 ± 1.4 | 86.7 ± 3.1 | 83.3 ± 2.6 |
| 45          | 100 ± 0 | 93.3 ± 4.4 | 86.7 ± 4.1 | 83.3 ± 2.3 | 80.0 ± 4.0 | 76.7 ± 3.5 |
| 60          | 100 ± 0 | 90.0 ± 2.8 | 86.7 ± 3.1 | 80.0 ± 3.5 | 73.3 ± 1.9 | 70.0 ± 2.0 |
| 75          | 100 ± 0 | 86.7 ± 3.2 | 80.0 ± 4.7 | 76.7 ± 3.7 | 70.0 ± 2.8 | 63.3 ± 3.8 |
| 90          | 100 ± 0 | 76.7 ± 3.1 | 70.0 ± 5.1 | 66.7 ± 4.1 | 60.0 ± 2.8 | 56.7 ± 2.8 |
Table S9. Hatching rates of zebrafish embryos assessments after the exposure of cis-platin and each Ru-Pt complex using zebrafish embryos. Data was collected from three replicates of three independent experiments.

| Compound | Conc. (μM) | % of hatching rate* |
|----------|------------|---------------------|
|          |            | 48 h | 72 h | 96 h |
| Cis-Platin | 0, Control | 80.0 ± 4.5 | 90.0 ± 5.3 | 96.7 ± 3.1 |
|           | 15         | 56.7 ± 3.7 | 70.0 ± 2.9 | 76.7 ± 6.1 |
|           | 30         | 40.0 ± 2.4 | 50.0 ± 3.7 | 53.3 ± 5.5 |
|           | 45         | 26.7 ± 4.1 | 36.7 ± 4.1 | 40.0 ± 3.6 |
|           | 60         | 13.3 ± 3.9 | 20.0 ± 3.3 | 30.0 ± 3.1 |
|           | 75         | 6.67 ± 4.1 | 10.0 ± 5.7 | 13.3 ± 3.9 |
|           | 90         | 0.0 ± 0 | 3.33 ± 1.7 | 6.67 ± 4.1 |
| C1        | 0, Control | 83.3 ± 5.1 | 90.0 ± 3.7 | 93.3 ± 5.5 |
|           | 15         | 40.0 ± 4.1 | 56.7 ± 4.4 | 63.3 ± 4.2 |
|           | 30         | 23.0 ± 2.8 | 36.7 ± 6.3 | 40.0 ± 6.1 |
|           | 45         | 13.0 ± 6.2 | 23.3 ± 2.4 | 30.0 ± 3.7 |
|           | 60         | 6.67 ± 3.5 | 16.7 ± 4.9 | 20.0 ± 4.6 |
|           | 75         | 0.0 ± 0 | 6.67 ± 5.7 | 6.67 ± 4.0 |
|           | 90         | 0.0 ± 0 | 0.0 ± 0 | 3.33 ± 3.1 |
| C2        | 0, Control | 76.7 ± 3.6 | 86.7 ± 7.7 | 96.7 ± 7.0 |
|           | 15         | 46.7 ± 3.2 | 63.3 ± 5.1 | 66.7 ± 6.2 |
|           | 30         | 33.3 ± 5.5 | 43.3 ± 7.5 | 50.0 ± 5.4 |
|           | 45         | 23.3 ± 4.6 | 30.0 ± 5.2 | 36.7 ± 2.1 |
|           | 60         | 13.3 ± 6.1 | 20.0 ± 4.4 | 23.3 ± 3.9 |
|           | 75         | 3.33 ± 3.7 | 6.67 ± 3.9 | 10.0 ± 2.4 |
|           | 90         | 0.0 ± 0 | 3.33 ± 1.1 | 3.33 ± 2.7 |
| C3        | 0, Control | 76.7 ± 3.4 | 86.7 ± 6.6 | 96.7 ± 5.1 |
|           | 15         | 50.0 ± 6.0 | 66.7 ± 2.8 | 70.0 ± 7.1 |
|           | 30         | 36.7 ± 5.8 | 46.7 ± 4.6 | 50.0 ± 5.7 |
|           | 45         | 30.0 ± 2.1 | 33.3 ± 6.1 | 40.0 ± 3.8 |
|           | 60         | 13.3 ± 4.6 | 20.0 ± 3.7 | 26.7 ± 3.5 |
|           | 75         | 3.33 ± 3.7 | 10.0 ± 5.2 | 16.7 ± 2.2 |
|           | 90         | 0.0 ± 0 | 3.33 ± 2.9 | 6.67 ± 3.7 |

*hatching noticed after 24 h.

Table S10. Morphological characteristics evaluated as measures of the Ru-Pt complexes at the designated time points

| Developmental endpoints to be evaluated | Time point for observation of normal development |
|----------------------------------------|-----------------------------------------------|
|                                        | 12 h | 24 h | 48 h | 72 h | 96 h |
| Egg coagulation                        | x    | X    | x    | X    | x    |
| Somites                                | X    | x    | X    | x    |     |
| Tail detachment                        | X    | x    | X    | x    |     |
| Hatching                               | x    | X    | x    |     |     |
| Hart beat                              | x    | X    | x    |     |     |
| Blood circulation                      | x    | X    | x    |     |     |
Preparation of Ru-Pt complexes and nucleophiles for kinetic analysis

A stock solution of 5 mM of each Ru-Pt complex (C₁, C₂ and C₃) was dissolved at requisite quantities in minimum amounts of DMF (ca. 2.0 % of final volume) to enhance solubility before diluting to a final volume with 5.0 mM tris(hydroxymethyl)aminomethane, Tris-HCl/50 mM NaCl buffer (pH = 7.2), aqueous medium. NaCl was added to suppress the spontaneous hydrolysis of the complex. Concentrations of nucleophiles were prepared in at least 20-fold excess over that of the complex at 20; 40; 60; 80; 100 and 120 times higher concentrations than the complexes. This afforded a ten-fold excess of the nucleophile concentration for each of the two coordinated leaving groups. This was to ensure that pseudo-first-order conditions were maintained at all times as a function of concentration and temperature (within 25 to 55 ºC at 10 ºC intervals).

Kinetic procedure

All the kinetic runs were monitored using the scanning kinetics model of the UV-Visible spectrophotometer, which records continuous spectral changes of the Ru-Pt complexes after mixing with nucleophiles, over a period of time. Kinetics was performed at four different temperatures at 10 ºC increment (25, 35, 45 and 55 ºC) under pseudo-first-order conditions. All the kinetic runs were performed in triplicate and the rate constants were reproducible within ± 3 %. The pseudo-first-order rate constants, \( k_{\text{obs}} \) were obtained by fitting the kinetic trace of absorbance growth/reduction to a non-linear double-exponential function using OriginPro 9.1 graphical analysis software.
DNA binding studies

Absorption spectral studies

The CT-DNA binding experiments were performed at room temperature in 5 mM tris(hydroxymethyl)aminomethane, Tris-HCl/50 mM NaCl buffer (pH = 7.2), stored at 4 °C in dark and used within 4 days. A stock solution of 5 mM of each Ru-Pt complex was prepared in 5 % DMSO and diluted to fixed concentrations of the complexes prior to treating them with the CT-DNA. The concentration of CT-DNA was determined from a Beer-Lambert plot by measuring the absorption intensity at 260 nm, where the molar absorptivity of CT-DNA is about 6600 M⁻¹ cm⁻¹. The absorbance ratio at 260 and 280 nm (A₂₆₀/A₂₈₀) was measured and found to be in the range of 1.8 to 1.9, indicating that the DNA was sufficiently free of protein.³

A fixed 20 μM concentration of each dichloro Ru-Pt complex (C₁/C₂/C₃) was titrated spectrophotometrically with increasing CT-DNA concentration (0 - 20 μM). The absorption spectra were obtained by adding the requisite amount of CT-DNA to both reference and sample solutions to eliminate the absorbance of CT-DNA. The Ru-Pt complex-DNA solutions were allowed to incubate for 10 minutes in a cuvette before the absorption spectra were recorded. The absorption changes were monitored as a function of increasing the concentration of CT-DNA. The binding affinities of the Ru-Pt complexes were calculated using the Wolfe-Shimmer equation (1).⁴

\[
\frac{[\text{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\text{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}
\]

(1)

where [DNA] is the concentration of CT-DNA, \(\varepsilon_a\), \(\varepsilon_f\) and \(\varepsilon_b\) are the molar absorptivities of the titrated mixture (\(A_{obs}/[\text{complex}]\)), unbound Ru-Pt complex and the Ru-Pt/CT-DNA complex, respectively. \(K_b\) is calculated from the ratio of the slope to intercept in the plot of \([\text{DNA}]/(\varepsilon_a - \varepsilon_f)\) versus [DNA]. The standard Gibbs free (\(\Delta G\)) of Ru-Pt complexes bound to CT-DNA was obtained using the Van’t Hoff equation (2).

\[
\Delta G = -RT \ln K_b
\]

(2)
Fluorescence spectral studies

The fluorescence quenching experiments were performed using 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide (EtBr) to probe the competitive binding abilities of the Ru-Pt complexes on the CT-DNA. Fixed concentration of CT-DNA-EtBr (10 μM each of CT-DNA and EtBr) was prepared in 5 mM Tris-HCl/50 mM NaCl buffer (pH = 7.2). This solution was stored for 4 hours at 4 °C. The competitive binding effects of the complexes C₁, C₂ and C₃ on the DNA-EtBr complex were monitored by adding aliquot amounts of stock Ru-Pt (5 mM) complexes solutions in incremental amounts to the CT-DNA+EtBr solutions. The decrease in the fluorescence emission was recorded within the wavelength range of 520 to 700 nm after the excitation of the solutions at 500 nm. Before recording the spectra, the solutions were thoroughly mixed and incubated for 10 minutes at room temperature. The quenching efficiency of the complexes was analysed using the Stern-Volmer equation (3).

\[
\frac{I_o}{I} = 1 + K_{sv}[Q] = 1 + k_q\tau_0[Q] \tag{3}
\]

where \(I_o\) and \(I\) are the emission intensities of CT-DNA+EtBr complex in the absence and each addition of complex, respectively and \([Q]\) is the concentration of quencher (dichloro Ru-Pt complex). The Stern-Volmer (quenching) constant, \(K_{sv}\) was determined from the slope of the linear plot of \(I_o/I\) versus \([Q]\). To have an insight into the kinetics of the competitive binding process, the bimolecular quenching rate constant, \(k_q\) values were also computed using the Stern-Volmer equation (4).

\[
K_{sv} = k_q\tau_0 \tag{4}
\]

where \(\tau_0\) is the average fluorescence lifetime of the CT-DNA+EtBr complex in the absence of the quencher and its value is 23 nanoseconds at room temperature. The apparent binding constant, \(K_{app}\) was computed from the equation (5)

\[
K_{EtBr}[EtBr] = K_{app}[Q] \tag{5}
\]

where \([Q]\) is the concentration of quencher causing 50 % reduction in fluorescence intensity of CT-DNA+EtBr complex, \(K_{EtBr} = 1.0 \times 10^7 \text{ M}^{-1}\) and \([EtBr]\) was taken as 60, 30 and 25 μM for C₁, C₂
and $C_3$, respectively. Scatchard plots also gave the binding constant $K_F$ as determined from the fluorescence titration using Scatchard equation (6).

$$\log(I_o - I)/I = \log K_F + n \log [Q]$$

(6)

where $n$ is the number of binding sites per nucleotide.

**BSA binding studies**

**Fluorescence spectral studies**

Quenching of fluorescence emission of BSA by the complexes ($C_1$, $C_2$ and $C_3$) was performed in order to determine their binding constants on the protein. The concentration of BSA was measured spectrophotometrically using the Beer-Lambert formulation where an $\varepsilon_{\text{max}}$ value of $4.4 \times 10^4$ M$^{-1}$ cm$^{-1}$ is assumed at 278 nm ($\lambda_{\text{max}}$). Stock concentration of BSA (10.4 μM) was prepared in 5 mM Tris-HCl / 50 mM NaCl buffer at pH = 7.2. The emission spectra of equilibrated 30 μM of the buffered BSA solutions in the absence and presence of Ru-Pt complexes of concentration ranging from 0 to 130 μM were acquired at room temperature. The emission changes were recorded in the $\lambda_{\text{em}}$ range of 250 to 450 nm, with the excitation wavelength set at 278 nm for all binding interactions with the complexes. Each spectrum was recorded after an incubation time of 10 minutes. The quenching efficiency of the Ru-Pt complexes was calculated using the Stern-Volmer equation (3) as discussed above. The Stern-Volmer (quenching) constant, $K_{sv}$ was determined from the slope of the linear plot of $I_o/I$ versus [Q]. To have an insight into the kinetics of the competitive binding process, the bimolecular quenching rate constant, $k_q$ values were also computed using the same Stern-Volmer equation (4), where $\tau_0$ is the average fluorescence lifetime of the BSA alone is 10 nanoseconds. Scatchard plots also gave the binding constant $K_F$ as determined from the fluorescence titration using Scatchard equation (6), results are tabulated.
Cleavage studies

DNA Cleavage studies

Samples of 0.1 \( \mu M \) of pcDNA were treated with different concentrations (from 10 to 500 \( \mu M \) containing 1 \% DMF) of each Ru-Pt complex to yield a total volume of 8.0 \( \mu L \) in 1.0X Tris-acetic acid-disodium EDTA, TAE buffer solution (40 mM Tris acetate/1 mM EDTA, pH = 8.3). The samples were incubated at 37 \( ^{\circ}C \) for 30 min under a UV-A lamp of 365 nm (80W), and then 2.0 \( \mu L \) of loading buffer (consisting of 0.25 \% xylene cyanol FF, 0.25% bromophenol blue and 30 \% glycerol in water) was added. The resulting solutions were loaded into the wells of 1 \% agarose gel, and electrophoresis was run for 1hr at 80 mV in 1.0X TAE buffer. After cleavage, the gel was stained with 1 \( \mu g/mL \) EB for 30 min followed by soaking it in deionised water for 5 to 10 minutes to remove excess EB. The bands were examined under UV light and the gel images were captured. The extent of cleavage of the supercoiled DNA was determined by measuring the intensities of the bands.

BSA Cleavage studies

A 24 \( \mu L \) of the protein solutions in Tris-HCl buffer medium containing complexes \( C_1-C_3 \) having concentrations ranging from 1 - 500 \( \mu M \), 4 \( \mu M \) BSA and 10 \( \mu L \) loading buffer which contains sodium dodecyl sulfate, SDS (4 \% w/v), glycerol (20 \% v/v), Tris-HCl buffer (125 mM, pH 6.8) and 2-mercaptopethanol (10 \% v/v) in Eppendorf vials. The sample solutions and then denatured on heating to boiling for 5 min and then incubated at 27 \( ^{\circ}C \) for 30 min prior to the photo exposure to UV-A light at 365 nm (100 W). The 20 \( \mu L \) samples were then loaded on a stacking gel\(^{11}\) which contains 0.94 mL of acrylamide (30 \% (W/V) acrylamide + 2.7 \% (W/V) bis-acrylamide), 1.75 mL of Tris-HCl buffer (500 mM, pH 6.8), 70 \( \mu L \) of 10 \% (W/V) SDS, 30 \( \mu L \) of TEMED, 35 \( \mu L \) of 10 \% (W/V) ammonium persulphate and 4.3 mL of millipore water. The separating gel was prepared by dissolving 6.25 mL of acrylamide (30 \% (W/V) acrylamide + 2.7 \% (W/V) bis-acrylamide), 3.75 mL of Tris-HCl buffer (1.5 M, pH 8.8), 0.15 mL of 10 \% (W/V) SDS, 15 \( \mu L \) of TEMED, 75 \( \mu L \) of 10 \% (W/V) ammonium persulphate and 4.75 mL of millipore water. Gel electrophoresis was done
at 300 V and 20 mA for about 2.0 h. Staining was done with Coomassie brilliant blue R-250, CBR-250 solution and destaining was done with distilled water for 4 h. The gels after destaining were scanned and the images were photographed/scanned with an HP Scanjet G3010 scanner and the images were further processed using the ImageJ software package. Molecular weight markers were used in each gel to calibrate the molecular weight of the protein.

In silico docking simulations

Structures of the Ru-Pt complexes in PDB format were obtained by converting CHK files of DFT optimised structures using Gaussian 09W software. The initial crystallized 3D structure of the target receptor structures of DNA (PDB ID: 1F8N with sequenced (ACCGACGTCGGT)2 and BSA (PDB ID: 3VO3) were taken from the Protein Data Bank (http://www.rcsb.org/pdb) with the resolution of 1.60 and 2.47 Å, and the r-value of 0.206 and 0.259 for DNA and BSA, respectively. Receptor molecules were prepared by amending the crystal structures of B-DNA whereby water molecules of crystallization were omitted, while the BSA structure was further altered by eliminating its calcium ions and second co-crystallized BSA molecule. Afterward, the preliminary structures of the B-DNA and BSA receptor molecules were edited by adding all hydrogens followed by the merging of non-polar hydrogens and computations of the Gasteiger charges. A binding site was chosen suitable enough for BSA or DNA to allow the ligands to rotate freely. To analyse the mode of binding docked confirmation at the lowest binding energy was selected. After each simulation, the data was refined using FireDock. Out of 10 different conformers, the lowest binding energy conformer was selected for analysis. Docked poses were visualized using the CHIMERA (http://www.cgl.ucsf.edu/chimera) molecular graphics program. This software package also aided in generating perspective views of the individual biomolecule-metal complex adducts.
In Vitro cytotoxicity studies

Protocol for the MTT cytotoxicity assay

The three different Ru-Pt complexes were tested for their in vitro cytotoxicity property using Vero and MCF-7 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 100 µL of both Vero and MCF-7 cells (1×10^4 cells/mL) were seeded in 96-well microplates and the plates were incubated at 37 ºC for 24 h in a humidified atmosphere of 5 % CO₂. After 24 hours of incubation, the cells were observed for 90 % confluence using a phase contrast microscope. Then each cell type was treated separately with 100 µL of 5, 10, 15, 20 and 25 µg/mL of each of the Ru-Pt complex (C₁, C₂ and C₃) and allowed to incubate at 37ºC in a humidified atmosphere of 5 % CO₂ for 24 h. A control blank, where no metal complexes were added was included along with the treated wells. At the end of the incubation, 20 µL of MTT solution (5 mg/mL in phosphate buffer saline, PBS) was added to each well and the plates were incubated for another 2 to 4 h. The excess medium in the wells was removed and the purple formazan crystals in each well were dissolved by adding 100 µL of dimethyl sulfoxide (DMSO). The contents of the plates were mixed well for 5 min and the absorbance of each well was measured at 560 nm using a microtitre plate reader. The percentage of cell viability was calculated using the formula, Cell viability (%) = (A₅₆₀ of treated cells / A₄₅₀ of control cells) × 100, where, A₄₅₀ is the absorbance at 450 nm of the control (cells treated with 1 % DMSO) and A₅₆₀ is the absorbance of cells treated with different test Ru-Pt complexes. The corresponding IC₅₀ values were determined after 24 h by nonlinear regression analysis of the % cell viability versus concentration data using OriginPro 9.1.

Nucleus morphological investigation by AO-EB assay

The morphological changes caused by the Ru-Pt complexes in MCF-7 cells were further investigated by using the AO-EB staining procedure. This inferred whether the inhibition is due to apoptotic induction or non-specific necrosis. AO intercalates into the DNA of viable cells and gives a green fluorescence and thus the viable cells appear as green nuclei while early apoptotic cells appear as condensed or fragmented nuclei. EB intercalates into the DNA of non-viable cells,
giving bright orange nuclei to the dead cells after AO staining. Briefly, MCF-7 cells were seeded in six well plates (4 × 10^5 cells per well) and incubated at 37 °C for 24 h in a humidified atmosphere of 5 % CO₂. The cells were treated with 25 μg/mL of the respective Ru-Pt complex and allowed to incubate for 24 h at 37 °C as described in the previous section. At the end of the incubation, the adhered cells were washed 3 times with 100 μL of PBS. 2 μL of EB and AO (1 mg/mL AO and 1 mg/mL EB in PBS) were added to each well and fluorescence was immediately observed under the fluorescence microscope. The stained MCF-7 cells from each treatment were mounted on a slide, and their images were observed under a fluorescent microscope with a green filter after excitation at 350 nm and observing their emission at 460 nm. The stained cells visual images were captured for their morphological changes using the FLoi cell imaging station (Life Technologies).

**In Vivo toxicity assessment using Zebrafish embryos**

Zebrafish experiments were carried out in the School of Life Science, University of KwaZulu-Natal, South Africa, using Singapore wild-type zebrafish (SGWT). Singapore wild-type (SGWT) zebrafish were housed in 3.5 L tanks and monitored at least once a day. Fish were provided with food (live and powder) three times daily during the week and twice daily on weekends. Regular system checks were carried out daily to ensure water quality and parameters are maintained. Light cycle: 14 hours day, 10 hours night. Fish were mated once a week using 2 pairs per 1 L breeding tank. Pairs were set up the previous evening. Males and females were separated by a divider. The divider was removed around 9 am (dawn). Embryos were collected and placed into petri dishes with fresh egg water.
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