Supporting Information

Ru(II)arene(N^N bpy/phen) based RAPTA complexes for \textit{in vitro} anti-tumor activity in human glioblastoma cancer cell lines and \textit{in vivo} toxicity study in zebrafish model†

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**Table S1** Physicochemical characterization of RAPTA complexes (4a-4h)

| Samples | stokes shift | OD<sup>a</sup> | ε (M<sup>-1</sup> cm<sup>-1</sup>)<sup>b</sup> | (φ)<sup>c</sup> | solubility (M)<sup>d</sup> | log P<sup>e</sup> | Conductivity (Λ<sub>M</sub>) (µS/cm)<sup>f</sup> |
|---------|-------------|---------|----------------|---------|----------------|---------|-----------------|----------------|
| 4a      | 44          | 0.03    | 3000               | 0.014   | 0.021             | 0.49±0.03 | 72              | 74              |
| 4b      | 42          | 0.04    | 4000               | 0.002   | 0.007             | 1.2±0.07 | 74              | 78              |
| 4c      | 41          | 0.07    | 7000               | 0.10    | 0.018             | 0.30±0.04 | 76              | 78              |
| 4d      | 74          | 0.08    | 8000               | 0.010   | 0.018             | 0.55±0.06 | 74              | 77              |
| 4e      | 65          | 0.04    | 4000               | 0.033   | 0.006             | 1.4±0.08 | 75              | 76              |
| 4f      | 63          | 0.04    | 4000               | 0.081   | 0.017             | 0.33±0.03 | 75              | 77              |
| 4g      | 40          | 0.1     | 10000              | 0.003   | 0.024             | 0.48±0.05 | 72              | 74              |
| 4h      | 38          | 0.02    | 2000               | 0.008   | 0.008             | 1.0±0.06 | 71              | 77              |
| Qnine Sulphate | 102        | 0.064   | 6400               | 0.546   | -                 | -       | -               | -               |

<sup>a</sup> optical density, <sup>b</sup> extinction coefficient, <sup>c</sup> quantum yield, <sup>d</sup>DMSO-10% DMEM medium (1:99 v/v, comparable to cell media), <sup>e</sup>Partition Coefficients in n-Octanol/Water, <sup>f</sup>conductance in DMSO and 10% DMSO (3 x 10<sup>-5</sup> M)
Fig. S2 (a) and (b) UV-Visible spectral responses of RAPTA complex, 4c (1x10^{-5} M) in 5 mM Tris-HCl-NaCl (pH, 7.2) with incremental accumulation of Ct-DNA (5x10^{-5} M); (c) and (d) Responses of fluorescence from EtBr bound DNA in the occurrence of complex 4c (pH 7.2)

Table S2 Binding parameters for interaction of complex 4c with Ct-DNA

| Complex | Change in absorbance | Δε (%)\textsuperscript{a} | K\textsubscript{b} (×10\textsuperscript{5} M\textsuperscript{-1})\textsuperscript{b} | K\textsubscript{sv} (×10\textsuperscript{4} M\textsuperscript{-1})\textsuperscript{c} | K\textsubscript{app} (×10\textsuperscript{6} M\textsuperscript{-1})\textsuperscript{d} |
|---------|---------------------|-----------------|-----------------|-----------------|-----------------|
| 4c      | Hyperchromism       | 50              | 1.38±0.28       | 0.018           | 1.38            |

\textsuperscript{a} % of hyperchromism/hypochromism, \textsuperscript{b} K\textsubscript{b}, intrinsic DNA binding constant from UV–visible absorption titration, \textsuperscript{c} K\textsubscript{sv}, Stern-Volmer quenching constant, \textsuperscript{d} K\textsubscript{app}, apparent DNA binding constant from competitive displacement from fluorescence spectroscopy.
Fig. S3 Effect of Increasing Amounts of complex 4c and 4d on the Viscosity of Ct-DNA at 298 K ([EtBr] = 1×10^{-6} M, [DNA] = 1×10^{-6} M, [complex] = 1×10^{-3} M)
**Fig. S4** Fluorescence Quenching of BSA on Addition of complex 4c and 4d in 5 mM TrisHCl/NaCl Buffer (pH 7.2)
Table S3. Binding Parameters of Ligand and Ru(II) Complexes with BSA

| Complex | $K_{BSA}$ (M$^{-1}$)$^a$ | $k_q$ (M$^{-1}$ s$^{-1}$)$^b$ | $K$ (M$^{-1}$)$^c$ | $n$$^d$ |
|---------|--------------------------|-----------------------------|------------------|-----|
| 4c      | 6.3x10$^6$               | 6.3x10$^{14}$               | 6.02x10$^4$      | 1   |
| 4d      | 1.8x10$^6$               | 1.8x10$^{14}$               | 5.25x10$^4$      | 1   |

$^aK_{BSA}$, Stern Volmer quenching constant; $^bK_q$, quenching rate constant (BSA); $^cK$, binding constant with BSA; $^d$n, number of binding sites (BSA). Concentrations of complexes = 0–60 μM in distilled water, BSA concentration was fixed at 5μM.

Fig. S5 RT-PCR study of complex 4c against U87MG
Characterization of the RAPTA complexes

$^1$H NMR of 4a

$^{13}$C NMR of 4a
19F NMR of 4a

31P NMR of 4a
$^1$H NMR of 4b

$^{13}$C NMR of 4b
$^{19}$F NMR of 4b

Signature SIF VIT VELLORE 4b

$^{31}$P NMR of 4b

Signature SIF VIT VELLORE 4b
\(^1\)H NMR of 4c

\[^{13}\]C NMR of 4c
19F NMR of 4c

Signature SIF VIT VELLORE

31P NMR of 4c

Signature SIF VIT VELLORE
1H NMR of 4d

Signature SIF VIT VELLORE
4d

Phenanthroline ring

H-1&6
H-2&5,3&4
H-7&8
H-1-6
H-7&8
H1
H6

13C NMR of 4d

Signature SIF VIT VELLORE
4d
$^{19}$F NMR of 4d

Signature SIF VIT VELLORE

$^{31}$P NMR of 4d

Signature SIF VIT VELLORE
$^{1}H$ NMR of 4e

$^{13}C$ NMR of 4e
$^{19}$F NMR of 4e

Signature SIF VIT VELLORE
4e

$^{31}$P NMR of 4e

Signature SIF VIT VELLORE
4e
\textbf{\textsuperscript{1}H NMR of 4f}

Signature SIF VIT VELLORE

\textbf{\textsuperscript{13}C NMR of 4f}

Signature SIF VIT VELLORE
19F NMR of 4f

Signature SIF VIT VELLORE

31P NMR of 4f

Signature SIF VIT VELLORE
$^1$H NMR of 4g

$^{13}$C NMR of 4g
$^{19}$F NMR of 4g

Signature SIF VIT VELLORE
4g

$^{31}$P NMR of 4g

Signature SIF VIT VELLORE
4g
FT-IR Spectra

Complex 4a

Complex 4b

Complex 4c
Complex 4h
Purity (UPLC)

Complex 4a

Complex 4b
Complex 4c

Complex 4d
Complex 4g

Complex 4h
ESI-MS spectra

Complex 4a

Complex 4b
Complex 4c

Complex 4d
Complex 4e

Complex 4g
Complex 4h
Experimental Section

DNA binding study

The binding capability of the complexes with calf-thymus DNA (Ct-DNA) was evaluated using electronic absorption spectroscopy, and the competitive binding assay was studied using ethidium bromide (EtBr) as a quencher by fluorescence spectroscopy.

UV–visible studies

The DNA binding experiment was performed in aqueous medium using RAPTA complex 4c in Tris-HCl buffer (5 mM Tris-HCl in water, pH 7.4). The concentration of Ct-DNA was determined using its absorbance at 260 nm and a known molar absorption coefficient of 6600 M\(^{-1}\)cm\(^{-1}\). In cuvettes, an equal amount of DNA was introduced to both the sample and the reference. The concentration of CT-DNA was increased as the titration progressed. The sample was equilibrated with CT-DNA for around 5 minutes before each measurement, and then the complex's absorbance was measured. The intrinsic DNA binding constant (\(K_b\)) was calculated using the equation (i):

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

Where [DNA] is the concentration of DNA in the base pairs, \(\varepsilon_a\) is the apparent extinction coefficient observed for the complex, \(\varepsilon_f\) corresponds to the extinction coefficient of the complex in its free form, and \(\varepsilon_b\) refers to the extinction coefficient of the complex when fully bound to DNA. The resultant data were plotted using Origin Lab, version 8.5 to obtain the [DNA]/(\(\varepsilon_a - \varepsilon_f\)) vs. [DNA] linear plot. The ratio of the slope to intercept from the linear fit gave the values of the intrinsic binding constants (\(K_b\)).

UV and Fluorescence study

All of these RAPTA complexes were studied in a 10% DMSO solution using UV and fluorescence. The fluorescence quantum yields (\(\Phi\)) were then estimated using the comparative William's approach, which entails utilising a well-characterized standard with a known quantum yield value and a 10% DMSO solution. Quinine sulphate was employed as a standard. Quantum yield was calculated according to the equation (ii):

\[
\varphi = \varphi_R \times \frac{I_S}{I_R} \times \frac{OD_R}{OD_S} \times \frac{\eta_S}{\eta_R} \ldots\ldots(ii)
\]
Where, $\varphi$ = quantum yield, $I$ = peak area, $OD$ = absorbance at $\lambda_{\text{max}}$, $\eta$ = refractive index of solvent (s) and reference (R). Here, we have used quinine sulphate as a standard for calculating the quantum yield.

**Ethidium bromide displacement assay**

To demonstrate the manner of binding between the complexes and DNA, the ethidium bromide (EtBr) displacement experiment was used. Using ethidium bromide (EtBr) as a spectral probe in 5 mM Tris-HCl buffer, the apparent binding constant ($K_{\text{app}}$) of the RAPTA complex 4c to Ct-DNA was determined at pH = 7.4. Because the fluorescence of EtBr was quenched by the solvent molecules, it was unable to show any fluorescence in its free state. However, in the presence of Ct-DNA, its fluorescence intensity began to increase, indicating that EtBr binds to DNA grooves in an intercalative manner. With increasing concentrations of the complexes, the intensity of the fluorescence was shown to diminish. As a result, the complexes displaced EtBr from CT-DNA grooves and were linked to the DNA base pairs itself. The values of the apparent binding constant ($K_{\text{app}}$) were obtained by using the equation (iii):

$$K_{\text{app}} \times [\text{Complex}]_{50} = k_{\text{EtBr}} \times [\text{EtBr}] \ldots \ldots \ldots (iii)$$

Where $K_{\text{EtBr}}$ is the EtBr binding constant ($K_{\text{EtBr}} = 1.0 \times 10^7 \text{ M}^{-1}$), and $[\text{EtBr}] = 8 \times 10^{-6} \text{ M}$. Stern-Volmer equation was followed for quantitative determination of the Stern-Volmer quenching constant ($K_{\text{SV}}$). Origin (8.5) software was used to plot the fluorescence data to obtain linear plot of $I_0/I$ vs. [complex]. The value of $K_{\text{SV}}$ was calculated from the following equation.

$$I_0/I = 1 + K_{\text{SV}} [Q] L \ldots \ldots (iv)$$

Where $I_0$ = fluorescence intensity in absence of complex and $I$ = fluorescence intensities in presence of complex of concentration [Q].

**Protein binding studies**

The main component is serum albumin proteins in drug transport and metabolism, as we all know. The interaction of the complex with human serum albumin (BSA) was examined using a tryptophan emission quenching experiment. The association of the RAPTA complexes 4c and 4d with the protein BSA was detected using a tryptophan emission quenching assay.
In a Tris-HCl/NaCl buffer, a BSA solution (2 x 10^{-6} M) was first made. Following that, the complex’s aqueous solutions were gradually added to the BSA solution, with increasing their concentrations gradually. The solutions were carefully agitated for 5 minutes after each addition before the fluorescence was measured at 295 nm (λex = 295 nm). When the concentration of complex was increased, a gradual drop in BSA fluorescence intensity at 340 nm was detected, confirming that the complex and BSA had interacted. To calculate the quenching constant, the Stern-Volmer equation was used (K_{BSA}). Origin Lab, version 8.5 was used to plot the emission spectral data to obtain linear plot of $I_0/I$ vs. [complex] using the equation (v) given below:

$$
\frac{I_0}{I} = 1 + K_{BSA} [Q] = 1 + k_q \tau_0 [Q] 
$$

Where $I_0$ is the fluorescence intensity of HSA in absence of complex and $I$ indicates the fluorescence intensity of HSA in presence of complex of concentration [Q], $\tau_0$ = lifetime of the tryptophan in HSA found as 1 x 10^{-8} and $k_q$ is the quenching constant. Scatchard equation (vi) gives the binding properties of the complexes.\(^6\) Where $K$ = binding constant and $n$ = number of binding sites.

$$
\log (I_0 - I/I) = \log K + n \log [Q] \quad (vi)
$$

**Conductivity measurement**\(^7\)

The conductivity of the RAPTA complexes was measured using a conductivity-TDS meter-307 (Systronics, India) and a cell constant of 1.0 cm\(^{-1}\) to verify the interaction of the complexes with DMSO and aqueous DMSO.

**n-Octanol–water partition coefficient (log P\(_{o/w}\))**\(^8\)

Using the previously reported shake flask approach, the log $P_{o/w}$ of the RAPTA complexes followed the previously described procedure. An orbital shaker was used to shake a known amount of each complexes in water (pre-saturated with n-octanol). The solution was centrifuged at 3000 rpm for 10 minutes to allow phase separation. Different ratios (0.5:1, 1:1, and 2:1) of saturated solutions were shaken for 20 minutes on an orbital shaker with pre-saturated n-octanol to get the partition coefficient. The absorbance of aliquots of the aqueous and octanol layers were measured with a UV-Vis spectrophotometer after adequate dilution. The concentrations of the complexes in each layer were estimated using the corresponding
molar extinction coefficients, and the partition coefficient ($\log P_{o/w}$) values were computed from the ratio.

**Viscosity measurement**

A hydrodynamic method such as a viscosity was performed using an Ostwald Viscometer to determine the binding manner of complexes using compound 4c, 4d, and EtBr treated DNA with respect to cisplatin.

**Notes and References**

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