Supporting Information

Water Soluble Ru(II)-arene Complexes of Antidiabetic Drug Metformin: DNA and Protein Binding, Molecular Docking, Cytotoxicity and Apoptosis-inducing Activity

Durairaj Gopalakrishnan, Mani Ganeshpandian, Rangasamy Loganathan, Nattamai S. P. Bhuvanesh, Xavier Janet Sabina and Karthikeyan J

aDepartment of Chemistry, SRM University, Kattankulathur, Chennai– 603 203, Tamil Nadu, India.
bDepartment of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States.
cX-ray Diffraction Lab, Department of Chemistry, Texas A&M University, College Station, TX 77842, USA.
dDepartment of Chemistry, Sathyabama University, Chennai – 600119, India

*To whom correspondence should be addressed, e-mail: ganeshpandian.m@ktr.srmuniv.ac.in and ganeshpandibdu@gmail.com
Experimental Methods
Data Reduction, Structure Solution, and Refinement

Integrated intensity information for each reflection was obtained by reduction of the data frames with the program APEX3. The integration method employed a three dimensional profiling algorithm and all data were corrected for Lorentz and polarization factors, as well as for crystal decay effects. Finally the data was merged and scaled to produce a suitable data set. The absorption correction program SADABS was employed to correct the data for absorption effects. Systematic reflection conditions and statistical tests of the data suggested the space group P-1. A solution was obtained readily using XT/XS in APEX3. A molecule of water was found hydrated. Hydrogen atoms were placed in idealized positions and were set riding on the respective parent atoms. All non-hydrogen atoms were refined with anisotropic thermal parameters. Absence of additional symmetry or void were confirmed using PLATON (ADDSYM). The structure was refined (weighted least squares refinement on $F^2$) to convergence. Olex2 was employed for the final data presentation and structure plots. The crystallographic data and details of data collection of 1 are given in Table 1.

References

1. APEX3 “Program for Data Collection on Area Detectors” BRUKER AXS Inc., 5465 East Cheryl Parkway, Madison, WI 53711-5373 USA

2. SADABS, G. M. Sheldrick, “Program for Absorption Correction of Area Detector Frames”, BRUKER AXS Inc., 5465 East Cheryl Parkway, Madison, WI 53711-5373 USA.


DNA Binding Experiments

Concentrated stock solutions of metal complexes were prepared and then diluting them suitably with 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 to required concentrations for all the experiments. For absorption and emission spectral experiments the DNA solutions were pretreated with the solutions of metal complexes to ensure no change in the concentration of the metal complexes. Absorption spectral titration experiments were performed by maintaining a constant concentration of the complex and varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the metal complex and DNA stock solutions while maintaining the total volume constant (1 ml). This results in a series of solutions with varying concentrations of DNA but with a constant concentration of the complex. The absorbance (A) of the complex was recorded after successive addition of CT DNA.

The tris buffer was used as a blank to make preliminary adjustments. The excitation wavelength was fixed and the emission range was adjusted before measurements. DNA was pretreated with ethidium bromide in the ratio [NP/EthBr] = 10 for 30 min at 27 °C. The metal complexes were then added to this mixture and their effect on the emission intensity was measured.

For viscosity measurements, a Brookfield automated viscometer was thermostated at 25 °C in a constant temperature bath. The concentration of DNA was 500 µM in NP and the flow times were noted from the digital timer attached with the viscometer (1/R = [Ru]/[DNA] = 0.5).

Gel Electrophoretic Mobility Studies

The electrophoretic mobility shift assay was carried out by using agarose gel electrophoresis technique. The super coiled pUC19 plasmid DNA (40 µM) was incubated with the complexes in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 in the absence activating agents. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (3 µL) was added and electrophoresis performed at 60 V for 3 h in Tris-Acetate-EDTA (TAE) buffer (40 mM Tris-base, 20 mM, acetic acid, 1 mM EDTA) using 1% agarose gel containing 1.0 µg/mL ethidium bromide. The gels were viewed in an GELSTAN 1312 gel documentation system and photographed using a CCD camera.
BSA Binding Experiments

The protein binding study was performed by tryptophan fluorescence quenching experiments using bovine serum albumin (BSA, 5 µM) as the substrate in Tris buffer (pH 7.1). Quenching of the emission intensity of tryptophan residues of BSA at 344 nm (excitation wavelength at 295 nm) was monitored using complexes 1 and 2 as quenchers with increasing complex concentration. The $F_0/F$ versus [complex] plot was constructed using the corrected fluorescence data taking into account the effect of dilution. The excitation and emission slit widths were 3 and 1.5 nm, respectively.
Table 1. Electronic absorption spectral properties of Ru(II) Complexes

<table>
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<th>Complex</th>
<th>$\lambda_{\text{max}}$, nm ($\varepsilon$, M$^{-1}$cm$^{-1}$)$^a$</th>
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<tr>
<td>[Ru($\eta^6$-p-cymene)(met)Cl]$^+$</td>
<td>1 378 (649)$^b$, 240$^c$</td>
</tr>
<tr>
<td>[Ru($\eta^6$-benzene)(met)Cl]$^+$</td>
<td>2 367 (603)$^b$, 236$^c$</td>
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$^a$In 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.1). $^b$Concentration, $0.5 \times 10^{-3}$ M. $^c$Shoulder, Concentration, $100 \times 10^{-6}$ M.
Figure S1. Absorption spectra of [Ru(η⁶-p-cymene)(met)Cl]⁺ (1) in water. [(a), 0.5 × 10⁻³ M, (b) 100 × 10⁻⁶ M]
Figure S2. Mass spectrum of [Ru(η⁶-p-cymene)(met)Cl]+ 1 in water
**Figure S3.** Mass spectrum of $[\text{Ru}(\eta^6\text{-benzene})(\text{met})\text{Cl}]^+$ in water

$[\text{Ru}(\eta^6\text{-benzene})(\text{met})\text{Cl}]^+$ calcd 344.02
Figure S4. $^1$H-NMR spectrum of [Ru($\eta^6$-p-cymene)(met)Cl]$^+$ (1) in D$_2$O.
Figure S5. $^1$H-NMR spectrum of [Ru($\eta^6$-benzene)(met)Cl]$^+$ (2) in D$_2$O.
Figure S6. $^{13}$C-NMR spectrum of $[\text{Ru}(\eta^6-p\text{-cymene})(\text{met})\text{Cl}]^+$ (1) in D$_2$O.
Figure S7. Infra-Red spectrum of [Ru(η⁶-benzene)(met)Cl]^+ (2) .
Figure S8. Cleavage of supercoiled pUC19 DNA (40 μM) by ruthenium(II) complexes 1 and 2 in absence of an external agent in 5 mM Tris HCl/50 mM NaCl buffer at 37 °C. Lane 1: DNA; Lane 2: DNA + RuCl₃·3H₂O (100 μM); Lane 3: DNA + [(η⁶-p-cymene)RuCl₂]₂ (100 μM); Lane 4: DNA + 1 (100 μM); Lane 5: DNA + 2 (100 μM); Incubation period: 1 h.
Figure S9. Changes in the electronic absorption spectra of BSA (5 μM) with increasing concentrations (0–60 μM) of complex 2.