ELECTRONIC SUPPLEMENTARY INFORMATION (ESI†)

An oxo(corrolato)chromium(V) complex selectively kills cancer cells by inducing DNA damage

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Experimental Section

Materials

The precursor's pyrrole, DDQ, chromium hexacarbonyl, tetrabutyl ammonium perchlorate (TBAP) were purchased from Aldrich, USA. Methylated Control DNA [(Fully methylated Jurkat DNA) {5-methyl-cytidine (5mC) content determined by mass spectrometry}] was purchased from Aldrich, USA. 3-bromobenzaldehyde was purchased from Merck, India. Other chemicals were of reagent grade. Hexane and CH_2Cl_2 were distilled from KOH and CaH_2 respectively. The synthetic methodologies and full spectroscopic characterization of **1** are provided by us in the previous literature.[1]

Physical Measurements

UV−Vis spectral studies were performed on a Perkin–Elmer *LAMBDA*-750 spectrophotometer. Emission spectral studies were performed on a Perkin Elmer, LS 55 spectrophotometer using optical cell of 1 cm path length. The elemental analyses were carried out with a Perkin−Elmer 240C elemental analyzer. FT−IR spectra were recorded on a Perkin–Elmer spectrophotometer with samples prepared as KBr pellets. The NMR measurements were carried out using a Bruker 700 MHz NMR spectrometer. Chemical shifts are expressed in parts per million (ppm) relative to residual chloroform (δ= 7.26). Electrospray mass spectra were recorded on a Bruker Micro TOF─QII mass spectrometer.

Cell culture and DNA isolation

Jurkat (acute T cell leukemia), Daudi (Burkitt's lymphoma) and EL4 (mouse T cell leukemia) cells were procured from American Type Culture Collection (ATCC). Cells were grown in T25 culture flask (Nunc, Denmark) in RPMI (Rosewell Park Memorial Institute, Invitrogen, USA) or IMDM (Iscove's Modified Dulbecco's Medium, Invitrogen) supplemented with 10% heat inactivated FBS (Fetal Bovine Serum, Lonza, USA), 1% glutamine (HiMedia, India) and 1% penicillin and streptomycin (HiMedia). All cells were cultured in a CO_2 incubator at 37°C with 5% CO_2 and 90% humidity.

Isolation of human PBMC

All experiments for human PBMCs were performed according to the guidelines set by the Institutional Human Ethics Committee of National Institute of Science Education and Research (Bhubaneswar). PBMC from human blood (hPBMC) was separated following the protocol

published elsewhere[2,3] and were cultured in IMDM supplemented with 10% FBS, 1% glutamine and 1% penicillin and streptomycin.

Isolation of mouse splenocytes

All the experiments performed for the study were according to the Institutional guidelines for animal care and approved by Institutional Animal Ethics Committee (IAEC) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals), Govt. of India. Male C57BL/6 mice (B6) aged 6-8 weeks were procured from the breeding facility of Imgenex India Pvt. Ltd., Bhubaneswar, India.

Mouse splenocytes (from C57BL/6 mouse) were isolated according to the procedure described earlier (Chattopadhyay et al., 2008). In brief, cells were isolated and centrifuged at 1500 rpm for 10 minutes and washed with PBS. Splenocytes were kept in 2 ml of RBC lysis buffer (Imgenex, USA) for 5 minutes to remove RBCs. After neutralizing and washing with PBS, cells were resuspended in IMDM supplemented with 10% FBS, 1% glutamine and 1% penicillin and streptomycin. PBMC from human blood was separated following the protocol published elsewhere.[2,3] Cells were grown in T25 culture flask (Nunc) in IMDM supplemented with 10% heat inactivated FBS, 1% glutamine and 1% penicillin and streptomycin.

Cell viability assay

To detect the effect of oxo(corrolato)chromium(V) complex, **1** on cell viability following treatment, a WST-1 assay (Roche) was performed. In brief, About 1 million cells (Jurkat, PBMC, EL4 and mouse splenocytes) in IMDM media were seeded in a 96 well plate (Nunc). For dose kinetics study, the cells were incubated for a period of 24 hours in the absence (0μM) and presence of increasing concentration of oxo(corrolato)chromium(V), **1** (0.25 μM, 0.5μM and 1μM). Similarly, for time kinetics purpose, the cells were incubated for different time periods (0 hr- 2 hr) with 1μM concentration of oxo(corrolato)chromium(V) complex, **1**. After incubation, cells were treated with 10 μL WST-1 each, and then incubated for 4 h before absorption readout at 450 nm using a reference wavelength of 600 nm in an ELISA plate reader. All experiments were performed in triplicate and the experiment was repeated twice.

DNA isolation and cleavage studies

All the tumor cell lines (Jurkat, Daudi and EL4) and above two primary cell lines (Human PBMC and Mouse splenocytes) were subjected for genomic DNA isolation according to the standard method of DNA isolation kit (QIAamp DNA Mini Kit – Qiagen, cat# 51304).

Initially the reactivity of oxo(corrolato)chromium(V) complex, **1** with DNA was studied by agarose gel electrophoresis. Each DNA samples(0.5-1µg/ml of TE buffer) were treated with 5µl of chromium(V)-oxo corrole, **1** (stock solution of 1mM prepared with 10% DMSO and 90% media), Tris –HCl buffer(20mM, pH-7.4) and 1mM DTT (reductant) to a final volume of 15µl with distilled water. The mixtures were incubated for 90 min, and then 5µl loading buffer was added to all reaction mixtures (24% glycerol and 0.1% bromophenol blue) and electrophoresed in 1% agarose at 50V for 90 min to 2 hr. The gels pre-stained with ethidium bromide were photographed under ultraviolet trans illumination. Dose and time kinetics of the oxo(corrolato)chromium(V) complex, **1** was also performed using increasing concentration of oxo(corrolato)chromium(V) complex, **1** and at various time intervals respectively.

DNA isolation from drug treated cells (after treatment with oxo(corrolato)chromium(V) complex, 1) and cleavage studies

Raji cells were grown in 10% RPMI at 37°C, in humidified CO_2 incubator. Cells were harvested and for each dose application 5 million cells were taken. Oxo(corrolato)chromium(V) complex, **1** (stock solution of 1mM prepared with 10% DMSO and 90% media) was added in the media at 750µM, 500µM, 250µM, and 125µM conc. The cells were then incubated and, harvested after 24h for DNA extraction. Tumor cell line (Raji cells) were subjected for genomic DNA isolation according to the standard method of DNA isolation kit (QIAamp DNA Mini Kit – Qiagen, cat# 51304).

DNA cleavage studies of oxo(corrolato)chromium(V) complex, 1 with fully methylated control DNA

Methylated Control DNA [Fully methylated Jurkat DNA; 5-methyl-cytidine (5mC) content determined by mass spectrometry] was purchased from Aldrich, USA. The concentration of the stock solution was 50 ng/µL. The reactivity of oxo(corrolato)chromium(V) complex, **1** with DNA was studied by agarose gel electrophoresis. 350 ng of fully methylated control DNA was incubated with oxo(corrolato)chromium(V) complex, **1** (1 µM and 10 µM doses) in tris-HCl buffer (pH 7.4)

in 20 µl reaction mixture at 37°C for half an hour. The mixtures were loaded onto 1% agarose gel with loading buffer (Sigma) and was electrophoresed at 50V for 30 mins. The gels pre-stained with ethidium bromide were photographed under ultraviolet transillumination. As the oxo(corrolato)chromium(V) complex is not soluble in water except the aid of DMSO, thus DNA was also tested with equivalent amount of DMSO and used as a control.

References

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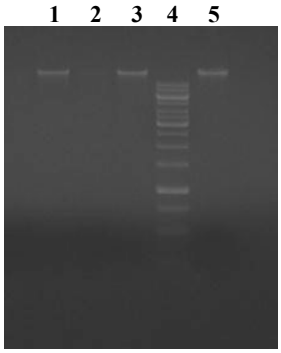

Fig. S1 Agarose gel electrophoresis analysis of fully methylated Jurkat DNA (50 ng/μL), 7μl of fully methylated Jurkat DNA is incubated with oxo(corrolato)chromium(V) complex, **1** (1 μM and 10 μM doses) in Tris-HCl buffer (pH7.4) in 20ml reaction mixture at 37°C for half an hour; The whole mixture is loaded on gel. Lane 1: 1 μM oxo(corrolato)chromium(V) complex, **1** treated fully methylated Jurkat DNA; Lane 2: 10 μM oxo(corrolato)chromium(V) complex, **1** treated fully methylated Jurkat DNA; Lane 3: 5μl DMSO treated fully methylated Jurkat DNA; Lane 4: 1Kb DNA Ladder; Lane 5: fully methylated Jurkat DNA.