Electronic Supplementary Information

Syntheses and structural investigation of some alkali metal ion-mediated $\text{LV}^\text{V} \text{O}_2^-$ (L$_2^-$ = Tridentate ONO ligands) species: DNA binding, photo-induced DNA cleavage and cytotoxic activities

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Fig. S12 (a) Gel diagram showing concentration dependent DNA cleavage by 1–8; 300 ng of SC pUC19 DNA at different concentrations of the complexes [1–500 µM in 10 mM Tris-HCl buffer (pH 8.0) containing 1% DMF] was photo-irradiated with UVA at 350 nm for 3 h. Lanes 1–9: 1, 2.5, 5.0, 7.5, 10, 50, 75, 100 and 500 µM of 1–8. (b) Concentration dependent DNA cleavage by 1–8; 300 ng of SC pUC19 DNA at different concentration of the complexes [1–500 µM in 10 mM Tris HCl buffer (pH 8.0) containing 1% DMF] was photo-irradiated with UVA at 350 nm for 3 h. The net DNA cleavage percent was calculated using Eq.2. Inset shows a bar diagram representation of the net DNA cleavage of different complexes at 10 and 100 µM.
Fig. S13 (a) Gel diagram showing concentration dependent DNA cleavage by 1–8; 300 ng of SC pUC19 DNA at different concentrations of the complexes [1–500 µM in 10 mM phosphate buffer (pH 7.8) containing 1% DMF] was photo-irradiated with UVA at 350 nm for 3 h. Lanes 1–9: 1, 2.5, 5.0, 7.5, 10, 50, 75, 100 and 500 µM of 1–8. (b) Concentration dependent DNA cleavage by 1–8; 300 ng of SC pUC19 DNA at different concentration of the complexes [1–500 µM in 10 mM phosphate buffer (pH 7.8) containing 1% DMF] was photo-irradiated with UVA at 350 nm for 3 h. Lanes 1–9: 1, 2.5, 5.0, 7.5, 10, 50, 75, 100 and 500 µM of 1–8. (b) Concentration dependent DNA cleavage by 1–8; 300 ng of SC pUC19 DNA at different concentration of the complexes [1–500 µM in 10 mM phosphate buffer (pH 7.8) containing 1% DMF] was photo-irradiated with UVA at 350 nm for 3 h. Lanes 1–9: 1, 2.5, 5.0, 7.5, 10, 50, 75, 100 and 500 µM of 1–8.
phosphate buffer (pH 7.8) containing 1% DMF] was photo-irradiated with UVA at 350 nm for 3 h. The net DNA cleavage percent was calculated using Eq.2. Inset shows a bar diagram representation of the net DNA cleavage of different complexes at 10 and 100 µM.

**Fig. S14** Effect of DMF (10%) and ligands on the photo-induced cleavage of SC pUC19 DNA. 300 ng SC pUC19 DNA was photo-irradiated in presence of 10% DMF and various ligands (100 µM) with UVA at 350 nm for 3 h. Lane 1, DNA in presence of 10% DMF; Lane 2, DNA + H₂L¹; Lane 3, DNA + H₂L²; Lane 4, DNA + H₂L³; Lane 5, DNA + H₂L⁴; Lane 6, DNA + H₂L⁵; Lane 7, DNA + H₂L⁶.

**Fig. S15** Gel diagram depicting cleavage of SC pUC19 DNA by 1–8 in presence of various additives in 50 mM Tris-HCl buffer (pH 8.0) containing 10% DMF. SC pUC19 DNA (300 ng) in the presence of various additives was photo-irradiated at 350 nm for 3 h with 1-8 (100 µM). The additive concentrations were: sodium azide (0.5 mM), L-histidine (0.5 mM), KI (0.5 mM) and D-mannitol (0.5 mM). Lane 1, DNA + complex; Lane 2, DNA + complex + sodium azide; Lane 3, DNA + complex + L-histidine; Lane 4, DNA + complex + KI; Lane 5, DNA + complex + D-mannitol.

**Fig. S16** Cleavage of SC pUC19 DNA by 1–4 (a) and 5–8 (b) in presence of various additives in 50mM Tris-HCl buffer (pH 8.0) containing 10% DMF. SC pUC19 DNA (300 ng) in the presence of various additives was photo-irradiated at 350 nm for 3 h with 1-8 (100 µM). The additive concentrations were: sodium azide (0.5 mM), L-histidine (0.5 mM), KI (0.5 mM) and D-mannitol (0.5 mM).

**Fig. S17** The plot represents the linear fit of log [(F₀-F)/F] vs log [Q] for 6 (a) and 7 (b) and the binding constant (K_{BSA}) was estimated using Eq.4. Here, [Q] stands for [quencher (complexes)].

**Fig. S18** SDS-PAGE profile of cleavage of BSA in presence of complexes 1-8 (100 µM) in (a) UVA light of 350 nm (84 W) and (b) dark. Lane 1, Molecular marker; Lane 2, BSA only; Lane 3, BSA + 1 (100 µM); Lane 4, BSA + 2 (100 µM); Lane 5, BSA + 3 (100 µM); Lane 6, BSA + 4 (100 µM); Lane 7, BSA + 5 (100 µM); Lane 8, BSA + 6 (100 µM); Lane 9, BSA + 7 (100 µM); Lane 10, BSA + 8 (100 µM).

**Fig. S19** Effect of ligand (H₂L³) on cell viability and growth: HeLa cells were treated with different concentrations of the test compound for 72 h and then cell viability was measured by MTT assay. Data reported as the mean ± S.D. for n = 6 and compared against 10% (v/v) DMF treated control by using a Student’s t-test. (*significant compared control).

**Fig. S20** Study of apoptosis by morphological changes in nuclei of HeLa cells: HeLa cells, from control and treated groups, were fixed with 3.7% formaldehyde for 15 min, permeabilized with 0.1% Triton X-100 and stained with 1 µg/ml DAPI for 5 min at 37 °C. The cells were then washed with PBS and examined by fluorescence microscopy (Olympus IX 71) (200×). HeLa cells were treated with 225 µM of ligand (H₂L³). Arrows showing the morphological changes in nuclei of HeLa cells were observed on applying ligand (H₂L³) in comparison to the control group treated with 10% (v/v) DMF.

**Fig. S21** ¹H NMR spectra of 1 (a), 5 (b), 6 (c) and 7 (d) in DMSO-d₆.

**Fig. S22** Electronic absorption spectra of 7 (2.5 x 10⁻⁵ M) in DMF.

**Fig. S23** FTIR spectra of complex 6.
Table S1: Summary of intermolecular interactions (A–H…B; Å, °) operating in the crystal structures of 3, 5-7.a

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<td>2.567(2)</td>
<td>146</td>
<td>x, y, z</td>
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<td>H6O</td>
<td>N3</td>
<td>0.84</td>
<td>1.83</td>
<td>2.571(2)</td>
<td>146</td>
<td>x, y, z</td>
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<td>O1W</td>
<td>H1W1</td>
<td>O9</td>
<td>0.80</td>
<td>1.93</td>
<td>2.7126(19)</td>
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<td>x, y, z</td>
</tr>
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<td>O2W</td>
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<td>O8W</td>
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<td>H4W1</td>
<td>O8</td>
<td>0.80</td>
<td>2.06</td>
<td>2.831(2)</td>
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<td>-1+x, y, z</td>
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<td>2.39</td>
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<td>H6W2</td>
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<td>0.80</td>
<td>2.36</td>
<td>3.0187(19)</td>
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<td>0.80</td>
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<td>H7W2</td>
<td>O6W</td>
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<td>2.27</td>
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<td>x, y, z</td>
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<td>H8W2</td>
<td>O7W</td>
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<td>1.97</td>
<td>2.760(2)</td>
<td>169</td>
<td>x, y, z</td>
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<td>H9W1</td>
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<td>178</td>
<td>x, y, z</td>
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<td>H9W2</td>
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<td>1.99</td>
<td>2.785(2)</td>
<td>171</td>
<td>1+x, y, z</td>
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C8   H8   O6   0.95   2.35   3.274(2)   165   1+x, y, z
C22  H22  O1   0.95   2.30   3.240(2)   168   x, y, z

5
O1W  H1W1 O2   0.84(2)   2.43(3)   3.038(3)   130(2)   x, y, z
O1W  H1W1 O4   0.84(2)   2.48(2)   3.274(3)   159(2)   x, y, z
O1W  H1W2 O4   0.84(3)   1.89(2)   2.723(3)   170(3)   1-x, 1-y, 1-z
O2W  H2W1 O6   0.85(3)   1.96(3)   2.776(3)   160(3)   -x, -1+y, 1-z
O2W  H2W2 O7   0.85(3)   2.15(3)   2.986(3)   171(2)   x, -1+y, z

6
O1W  H1W1 O1   0.85(3)   2.11(4)   2.931(3)   162(3)   ½-x, ½+y, z
O1W  H1W2 N1   0.837(18)   2.01(2)   2.815(3)   162(4)   1-x, -y, -z
C5   H5   O4   0.93   2.54   3.439(3)   164   1-x, -y, -z
C7'  H72  Cg1  0.96   2.56   3.477(3)   159   1½-x, -½+y, z
O2W  H2W2 O3   0.863(14)   2.44(5)   2.852(4)   110(4)   ½-x, ½+y, z

The O2W-water molecule atom lies in a pocket comprising the O3 atom, see above, and the O1W (separation 3.313(2) Å),
O3 (3.261(2) Å) and O4 (3.364(2) Å) atoms.

Cg1 is the centroid of the (C8-C13) ring

7
O1W  H1W1 O1   0.82(3)   2.17(3)   2.975(2)   165(2)   x, y, z
O1W  H1W2 O4W  0.83(3)   1.97(3)   2.787(2)   166(3)   1-x, -y, 2-z
O2W  H2W1 O3   0.84(3)   2.08(3)   2.885(2)   162(3)   -1+x, y, z
O2W  H2W2 O4W  0.83(3)   1.98(3)   2.809(2)   173(3)   x, y, z
O3W  H3W1 O2W  0.83(3)   2.24(3)   3.015(3)   156(3)   -x, -y, 2-z
O3W  H3W2 N2   0.83(3)   2.26(3)   2.991(3)   147(3)   1-x, 1-y, 2-z
O4W  H4W1 N1   0.834(13)   1.982(13)  2.808(2)   171(2)   -1+x, -1+y, -1+z
O4W  H4W2 O2   0.84(2)   2.06(2)   2.876(2)   166(2)   x, y, z

5
Table S2 Binding Constant ($K_b$) values for the interaction of CT-DNA with ligands

<table>
<thead>
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<th>Complex</th>
<th>Binding Constant ($K_b$) *$^\dagger$ (M$^{-1}$)</th>
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<td>$H_2L^1$</td>
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<td>$H_2L^3$</td>
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<td>$H_2L^4$</td>
<td>$3.82 \times 10^3$</td>
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<tr>
<td>$H_2L^5$</td>
<td>$1.09 \times 10^3$</td>
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<td>$H_2L^6$</td>
<td>$4.57 \times 10^3$</td>
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*DNA binding constant by UV-vis spectral method.*
Fig. S2
Fig. S3
Fig. S4
Fig. S5
Fig. S6
Fig. S7
Absorbance (a.u) vs. Wavelength (nm)

Inset: 

$\frac{[DNA]}{(\epsilon_a - \epsilon_f) \times 10^{-9} M}$ vs. $[DNA] \times 10^{-6} M$

Absorbance (a.u) vs. Wavelength (nm)
Fig. S8
Absorbance (a.u)

Wavelength (nm)

\[ \text{[DNA]} / (\varepsilon_a - \varepsilon_f) \times 10^{-9} \text{ M} \]

\[ \text{[DNA]} \times 10^{-6} \text{ M} \]
Fig. S10
Fig. S11
Fig. S12
Fig. S13

(a) Gel electrophoresis images showing Net DNA Cleavage (%).

(b) Graph depicting Net DNA Cleavage (%) against Complex (µM).

- Complex 1: Black squares
- Complex 2: Red circles
- Complex 3: Blue triangles
- Complex 4: Magenta inverted triangles
- Complex 5: Green diamonds
- Complex 6: Purple diamonds
- Complex 7: Magenta circles
- Complex 8: Purple circles
Fig. S14
Fig. S15
Fig. S16
Fig. S17
Fig. S18
**Fig. S19**

IC$_{50}$: 231.5 ± 5.4

[Graph showing cell viability vs. concentration with data points marked as * for significance]
Fig. S20
Fig. S21