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De novo design of hydrolytic DNA cleavage agent, mono nitratobis(phen)cobalt(II) aqua nitrate complex

Surbhi Sharma[a], **Loic Toupet**[b], and **Farukh Arjmand**[a,*]

[a]*Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India.*
[b]*Institut de Physique de Rennes, UMR 625, Université de Rennes 1, Campus de Beaulieu Bat. 11 A, 263 av. Général Leclerc, 35042 Rennes Cedex, France.*
*E−mail: farukh_arjmand@yahoo.co.in. *Corresponding author. Tel.: +91 5712703893

Experimental

Materials and instrumentation

Commercially obtained chemicals and solvents were used without further purification.1, 10 phenanthroline, Ethidium bromide (EB) and tris–(hydroxymethyl)aminomethane (tris–buffer) (Sigma Aldrich), cobalt nitrate hexahydrate, (Merck), 6X loading dye (Fermentas Life Science), and supercoiled pBR322 plasmid DNA (Genei) were utilized as received. Disodium salt of ct–DNA was purchased from Sigma Chem. Co. and was stored at 4 °C.

Elemental analysis was carried out on Carlo Erba Analyser Model 1106. Fourier–transform infrared (FT–IR) spectra were recorded on Spectrum Two (Pelkin Elmer) FT–IR spectrometer. ESI–MS spectrum was recorded on Micromass Quattro II triple quadrupole mass spectrometer. Electronic spectra were recorded on Perkin–Elmer Lambda 25 using cuvettes of 1 cm path length and data were reported in λ_{max}/nm. All the experiments involving the interaction of the complex with CT–DNA were carried out in aerated buffer (5.0 mM Tris–HCl, 50.0 mM NaCl, pH = 7.3). The concentration per base pairs for DNA was determined spectrophotometrically by assuming ε_{260nm} values to be 6600. Cleavage experiments were performed with the help of Axygen electrophoresis supported by a Genei power supply with a potential range of 50–500 V, visualized and photographed by Vilber–INFINITY gel documentation system.

In vitro DNA binding and cleavage experiments

CT−DNA binding experiments were performed in Tris−HCl/NaCl (5:50 mM) buffer at pH 7.2 which included absorption spectral traces conformed to the standard methods and practices previously adopted by our laboratory.[1-3] While measuring the absorption spectra an equal amount of CT−DNA was added to both the compound solution and the reference solution to eliminate the absorbance of the CT−DNA itself, and absorbance of the Tris buffer was subtracted through base line correction.

The cleavage experiments of supercoiled pBR322 DNA (300 ng) by **1** in Tris–HCl/NaCl (5:50 mM) buffer at pH 7.2 was carried out using agarose gel electrophoresis. The samples were incubated for 45 min at 25°C and cleavage process was monitored using agarose gel electrophoresis in a concentration dependent manner. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol was added and electrophoresis was carried out at 50 V for 1h in Tris–HCl buffer using 1% agarose gel containing 1.0 mg/ml ethidium bromide.

Religation experiment with T4 DNA ligase enzyme

The DNA religation experiments were performed using T4 DNA ligase enzyme to support the hydrolytic mechanism of DNA cleavage by following the standard DNA religation protocol. Complex1 treated with pBR322 plasmid DNA, ligation buffer of 1.5 mL in 10 x, T4 DNA ligase, 1 mL (2 units) and 2.5 mL of H_2O were mixed and incubated at 4°C for 1 h. Subsequently, the samples were loaded on 1% agarose gel and visualized by staining with EB solution.

Molecular Docking

HEX 8.0 software was used for Molecular docking studies,[4] which is an interactive molecular graphics program for calculating and displaying feasible docking modes of an enzymes and DNA molecule. Structure of the **1** was converted it into PDB format from mol format while the PDB file of the B−DNA dodecamer $d(CGCGAATTCGCG)_2$ (PDB ID: 1BNA), was downloaded from the protein data bank (http://www.rcsb.org./pdb). Visualization of the docked pose has been done by using CHIMERA (www.cgl.ucsf.edu/chimera), PyMol (http://pymol.sourceforge.net/) and Discovery Studio molecular graphics program.

Synthesis of complex 1

To a methanol solution of 1,10-phenanthroline(2 mmol), a methanol solution of $Co(NO_3)_2 \cdot 6H_2O$ (1 mmol) was added, resulting deep red solution which was refluxed for 1 hour and then filtered. Crystalsof deep red colored were obtained after the slow evaporation of filtrate at room temperature.

The synthesizedcomplex was found to be stable towards air and soluble in organic solvents like MeOH, DMF and DMSO. The molecular structure of complexwas established by single X−ray diffraction studies which corroborated well with elemental analysis and other spectroscopic measurements. The synthesis was repeated several times and found to be reproducible.

1: Yield: (71%), M.p. 276 °C; Anal. Calc. for $C_{24}H_{18}CoN_6O_7$ (%):Calc. C, 53.05; H, 2.97, N, 15.47. Found: C, 52.97; H, 3.042, and N, 15.18. UV–vis (1 x 10^{-3} M, MeOH, λ_{max} nm): 272, 296, 510 nm. FTIR (KBr pellet, υ_{max}/cm^{-1}) 912, 858, 765, 535, 428.ESI-MS (DMSO) (+) (*m/z*,): 479 and 435. CCDC: 1532544.

X–ray crystal structure determination and refinements

Single-crystal X-ray data of **1** was collected at 296(2) K on a Bruker SMART APEX CCD diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The linear absorption coefficients, scattering factors for the atoms and the anomalous dispersion corrections were taken from the International Tables for X–ray Crystallography[5]. The data integration and reduction were processed with SAINT Software[6]. An empirical absorption correction was applied to the collected reflections with SADABS[7] and the space group was determined using XPREP[8]. The structures were solved by direct methods using SHELXL-2016[9] and refined on F^2 by full matrix least squares using the SIR-97 program package. All non-hydrogen atoms were refined with anisotropic displacement parameters.

Table S1. Crystal stucture refinement data for $C_{24}H_{18}CoN_6O_7$.

Parameters	**1**
CCDC	1532544
formula	$C_{24}H_{18}CoN_6O_7$
Fw (g mol^{-1})	561.37
crystal system	Monoclinic
space group	P21/n
a (Å)	13.6499(5)
b (Å)	10.6491(4)
c (Å)	16.0789(6)
α (deg)	90
β(deg)	106.365(1)
γ (deg)	90
U (Å^3)	2242.53(14)
Z	4
ρ_{calc} (g/cm^3)	1.663
ε (mm^{-1})	0.828
F(000)	1148.0
Temp (K)	296 K
measured reflns	26512
unique reflns	3436
θ Range (deg)/completeness (%)	2.306 to 25.050/99.9
GOF^a	1.073
R^b[I>2σ(I)]	0.0376(3436)
wR_2^b (all data)	0.1042(3973)
largest diff. peak/hole (e.Å^{-3})	0.994-0.707

[a] GoF is defined as $\{\Sigma[w(F_0^2–F_c^2)]/(n–P)\}^{1/2}$ where n is the number of data and p is the number of parameters. [b] $R = \{\Sigma||F_0|–|F_c||/\Sigma|F_0|$, $wR^2 = \{\Sigma\, w(F_0^2–F_c^2)^2 / \Sigma\, w(F_0^2)^2\}^{1/2}$.

Table S2. Selected bond lengths for complex **1a**, $C_{24}H_{18}CoN_6O_7$

Bond lengths	(Å)
Co(1)-O(1)	1.8936(19)
Co(1)-O(2)	1.8894(18)
Co(1)-N(1)	1.949(2)
Co(1)-N(4)	1.948(2)
Co(1)-N(3)	1.932(2)
Co(1)-N(2)	1.926(2)
N(5)-O(1)	1.323(4)
N(5)-O(2)	1.326(3)
N(5)-O(3)	1.226(3)

Table S3. Selected bond angles for complex **1a**,$C_{24}H_{18}CoN_6O_7$

Bond Angle	[deg]
O(2)-Co(1)-O(3)	69.97(8)
O(2)-Co(1)-N(1)	91.01(7)
O(3)-Co(1)-N(1)	87.74(7)
O(2)-Co(1)-N(4)	89.04(7)
O(3)-Co(1)-N(4)	91.70(7)
N(1)-Co(1)-N(4)	179.39(8)
O(2)-Co(1)-N(3)	91.04(7)
O(3)-Co(1)-N(3)	168.66(7)
N(1)-Co(1)-N(3)	96.72(8)
N(4)-Co(1)-N(3)	83.87(8)
O(2)-Co(1)-N(2)	92.11(7)
O(3)-Co(1)-N(2)	97.93(7)
N(1)-Co(1)-N(2)	84.00(8)

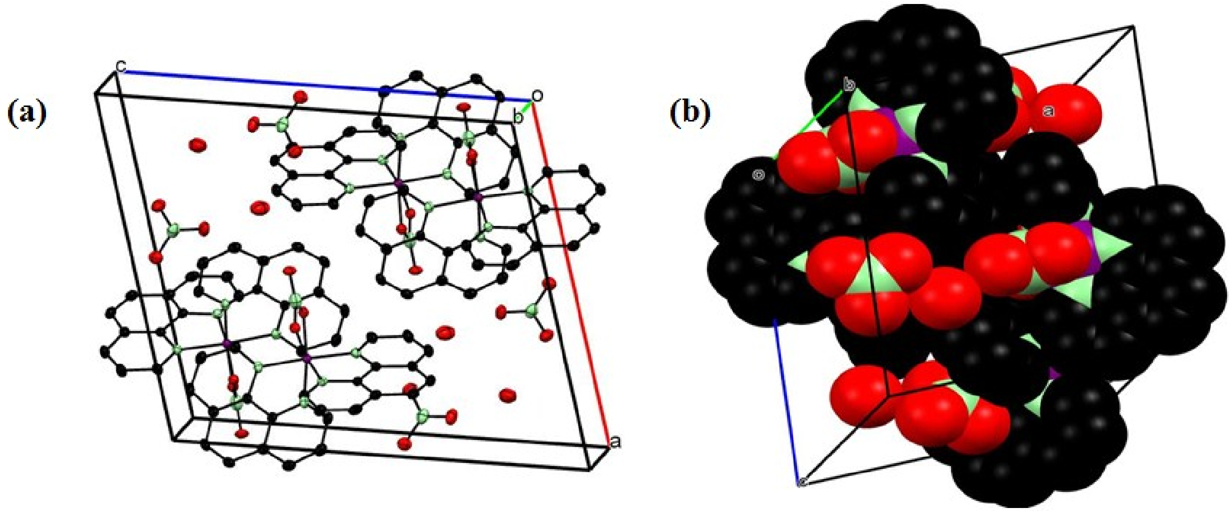

Fig.S1 Unit cell packing model projected down the b axis (a) ellipsoid and (b) space fill model of Complex **1**. Hydrogen atoms have been omitted for clarity.

The X-band EPR spectra of complex **1** have been measured to assess the oxidation state and geometry of complex. EPR spectra of **1**displayed isotropic signals indicative of the presence of Co(II) ions d^7 system, 'g_{iso}' values obtained forcomplex**1** was found to be 2.061, characteristic of octahedral cobalt(II) complexes[10] (Figure S2).

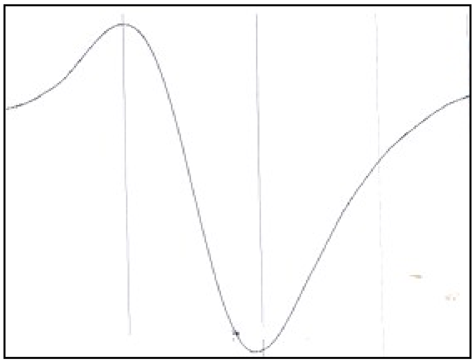

Fig. S2 EPR spectrum of complex **1**

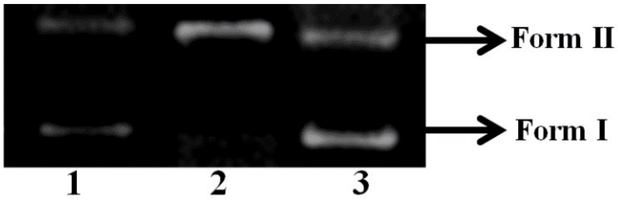

Fig. S3. Agarose gel electrophoresis for the ligation pBR322 plasmid DNA nicked by complex **1**. Lane 1: DNA control; Lane 2: pBR322 plasmid DNA cleaved by complex **1**; Lane 3: ligation of nicked pBR322 plasmid DNA by T4 DNA ligase in presence of **1**.

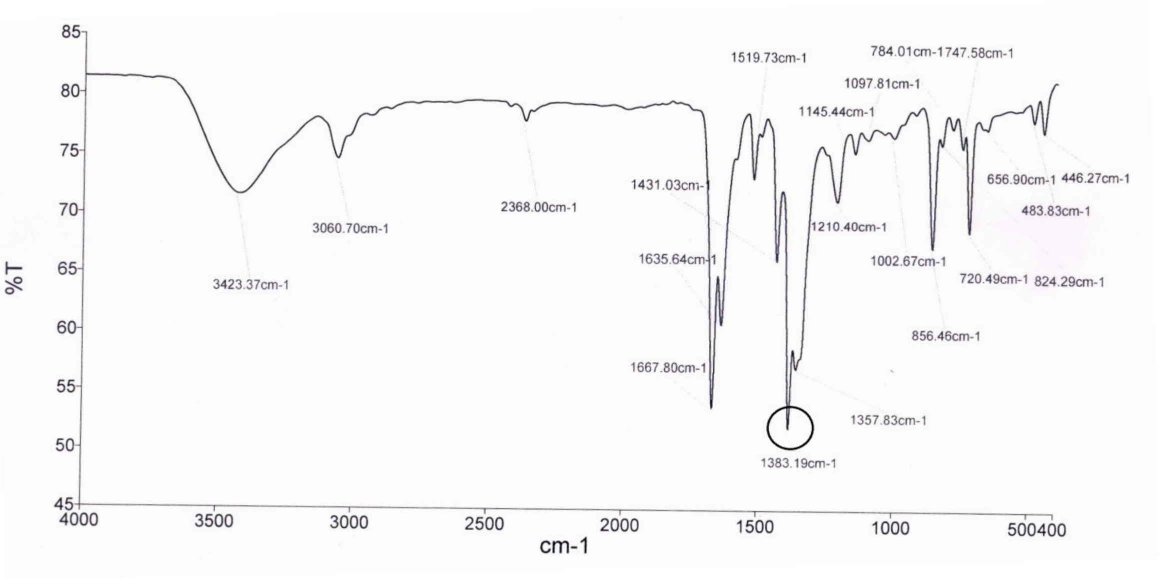

Fig. S4 IR spectrum of complex **1**

References

1. F. Arjmand and M. Muddassir, *Chirality*, 2011, **23**, 250–259; I. Yousuf, F. Arjmand, S. Tabassum, L. Toupet, R. A. Khan and M. A. Siddiqui, *Dalton Trans*., 2015, **44**, 10330–10342.

2. J. R. Lakowicz and G. Webber, *Biochemistry*, 1973, **12**, 4161-4170.

3. F. Arjmand, I. Yousuf, M. Afzal and L. Toupet, *Inorg. Chim. Acta*, 2014, **421**, 26–37; M. Chauhan, K. Banerjee and F. Arjmand, *Inorg. Chem*.,2007, **46**, 3072-3082.

4. D. Mustard and D. W. Ritchie, *Proteins: Struct. Funct. Bioinf.*, 2005, **60**, 269–274; G. Macindoe, L. Mavridis, V. Venkatraman, M.–D. Devignes and D. W. Ritchie, *Nucl. Acids Res.*, 2010, **38**, 445.

5. J. A. Ibers, W. C. Hamilton, International Tables for X-ray Crystallography, Kynoch Press, Birmingham, England, 1974, vol. IV.

6. SMART & SAINT Software Reference manuals, Version 6.45, Bruker Analytical X-raySystems, Inc., Madison, WI, 2003.

7. G. M. Sheldrick, SADABS, software for empirical absorption correction, Ver. 2.05, University of Göttingen, Göttingen, Germany, 2002.

8. XPREP, version 5.1, Siemens Industrial Automation Inc., Madison, WI, 1995.

9. Sheldrick, G. M. SHELXTL Version 2016/6. http://shelx.uni-ac.gwdg.de/SHELX/index.php.

10. A. Bravo and J. R. Anacona, *Transition Met. Chem.*, 2001, **26**,20–23.