Supplementary information

for

Synthesizing a Cu^{II} complex of tinidazole to tune the generation of the nitro radical anion in order to strike a balance between efficacy and toxic side effects

Crystallographic Data Collection and Refinement

A suitable single crystal of the complex was used for data collection using a 'Bruker SMART APEX II' diffractometer equipped with graphite-monochromated Mo K_α radiation (λ = 0.71073 Å) at 273K. The molecular structure was solved using SHELX-97 package.[1] Non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were placed in their geometrically idealized positions and constrained to ride on their parent atoms. Multi-scan empirical absorption corrections were applied to the data using SADABS.[2] The CCDC reference number for the structure of the complex is 1446192. A summary of the crystallographic data is given in Table 1. Selected geometric parameters for the complex are provided in Table 2. Figures were prepared using DIAMOND.[3]

Table 1: Crystallographic and structural refinement parameters for $[Cu(tnz)_2Cl_2]$

Formula	$C_{16}H_{26}Cl_2CuN_6O_8S_2$
Formula Weight	629.02
Crystal Size [mm]	0.20 x 0.20 x 0.30
Temperature (K)	273
Crystal system	Monoclinic
Space group	*C2/c*
a(Å)	13.9357(3)
b(Å)	6.6873(1)
c(Å)	27.1213(4)
β(deg)	92.325(1)
Z	4
d_{calc} (g cm^{-3})	1.654
μ(mm^{-1})	1.295
F(000)	1292
Total Reflections	20108
Unique Reflections	2890
Observed data [I > 2 σ (I)]	2542
No. of parameters	161
R(int)	0.030
R1, wR2 (all data)	0.0356,0.0866
R1, wR2 [I > 2 σ (I)]	0.0306,0.0829

Table 2: Selected bond lengths (Å) and angles (°) for $[Cu(tnz)_2Cl_2]$

Bond lengths (Å)	
Cu(1)-Cl(1)	2.2265(8)
Cu(1)-N(1)	1.9992(15)
Bond angles (°)	
Cl(1)-Cu(1)-N(1)	143.33(5)
Cl(1)-Cu(1)-Cl(1)*	99.60(3)
Cl(1)-Cu(1)-N(1)*	94.56(5)
N(1)-Cu(1)-N(1)*	93.86(6)

Characterization of the prepared complex by different spectroscopy and magnetic susceptibility measurements:

UV-VIS Spectra

Electronic spectrum of $Cu(tnz)_2Cl_2$ in ethanol was measured. An intense band at 318 nm was attributed to intra-ligand charge transfer (ε = 18000 $M^{-1}cm^{-1}$). A weak band for d-d transition was observed at 710 nm (ε = 80$M^{-1}cm^{-1}$).

IR spectra

The IR spectrum of $Cu(tnz)_2Cl_2$ (Fig. S1) showed a shift to higher frequencies (1560 cm^{-1}) for ν(C=N) with respect to that for tnz (1522 cm^{-1}) indicating co-ordination of imidazole nitrogen to Cu(II).[4, 5] Splitting of the two NO_2 stretching vibrations for the complex, ν_{as} 1450 cm^{-1} and 1368 cm^{-1} being similar to tnz indicates -NO_2 does not participate in coordinating Cu(II). The two bands at ν_{as} 1305 cm^{-1} and 1132 cm^{-1} for splitting of two SO_2 stretching vibrations are similar as that in tnz indicating SO_2 groups too do not participate in bonding.[4, 5]

Fig. S1:

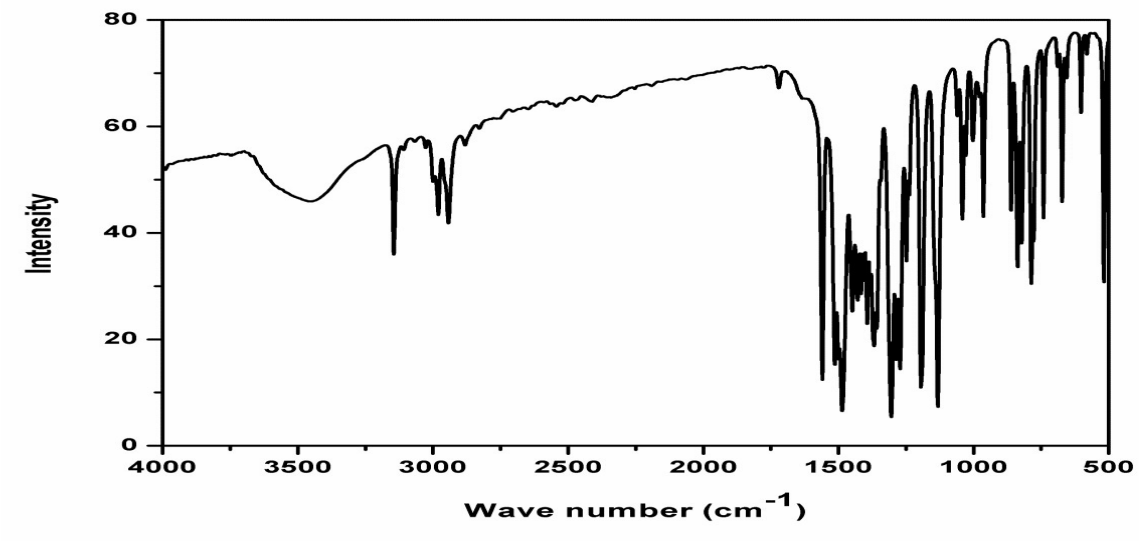

Fig. S1: IR spectrum of $[Cu(tnz)_2Cl_2]$

EPR spectrum and the magnetic property of $Cu(tnz)_2Cl_2$

Magnetic moment was recorded at 300K using Gouy method. The value was 1.71 BM. EPR spectrum (Fig. S2) recorded at room temperature at X-band frequency of the powdered sample showed resonance signal at 329 mT with a g value of 2.08.

Fig. S2:

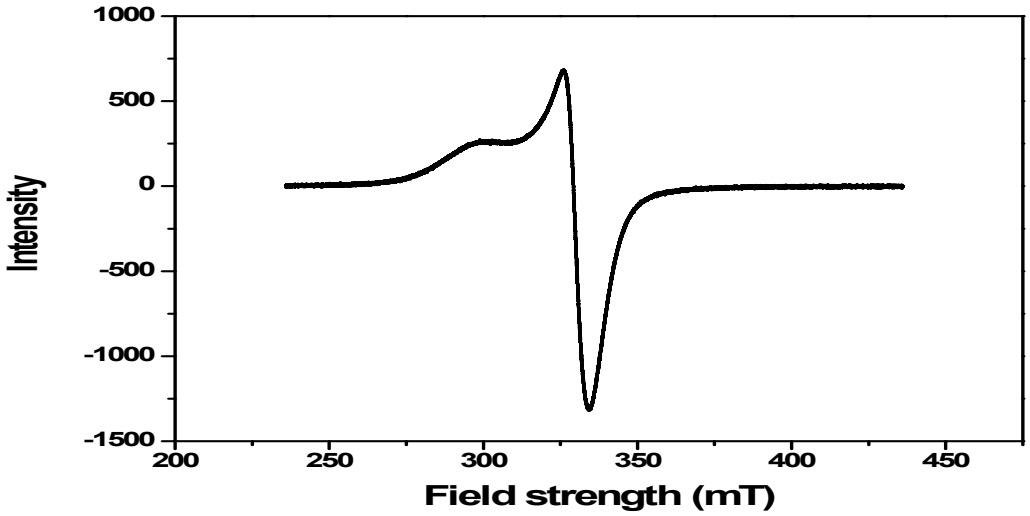

Fig. S2: Room temperature EPR spectrum of $[Cu(Tnz)_2Cl_2]$

The cyclic voltammetry data for the interaction of $Cu(tnz)_2Cl_2$ with calf thymus DNA was analyzed with the help of different equations given below.

The complex–DNA equilibrium is shown in Equation S1.[6-9]

$$L + D = L\text{-}D \qquad (S1)$$

If we write Equation S1 in the reverse direction we get Equation S2, then K_d (Equation S3) indicates the dissociation constant of the process. The apparent binding constant (K_{app}) is the inverse of K_d. K_{app} being the binding of a compound to an isolated site. Equation S2 is often considered than Equation S1 as it makes mathematical calculations easier.

$$L\text{-}D = L + D \qquad (S2)$$

$$K_d = \frac{C_L C_D}{C_{LD}} \qquad (S3)$$

Here C_L is the concentration of $Cu(tnz)_2Cl_2$ while C_D and C_{LD} are the concentrations of calf thymus DNA and $Cu(tnz)_2Cl_2$–DNA adduct respectively at equilibrium. Since for $Cu(tnz)_2Cl_2$ the cathodic peak current (I_{pc}) is linearly proportional to its concentration therefore an increase or decrease of it may be used to create binding isotherms. Using the linear relationship between cathodic peak current and concentration of either the free form of the complex or its bound form Equations S4, S5 & S6 may be derived and used.

$$K_d = \frac{\left[C_0 - \left(\frac{\Delta I}{\Delta I_{max}}\right)C_0\right]\left[C_D - \left(\frac{\Delta I}{\Delta I_{max}}\right)C_0\right]}{\left(\frac{\Delta I}{\Delta I_{max}}\right)C_0} \qquad (S4)$$

$$C_0\left(\frac{\Delta I}{\Delta I_{max}}\right)^2 - (C_0 + C_D + K_d)\left(\frac{\Delta I}{\Delta I_{max}}\right) + C_D = 0 \qquad (S5)$$

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{K_d}{\Delta I_{max}(C_D - C_0)} \qquad (S6)$$

ΔI is the change in cathodic peak current (I_{pc}) for $Cu(tnz)_2Cl_2$ for each point of the titration curve. ΔI_{max} is the same parameter that provides the maximum change in ΔI when $Cu(tnz)_2Cl_2$ is totally bound to DNA. C_0 is the initial concentration of the complex. The double reciprocal plot [Equation S6] provides a value for ΔI_{max} as the inverse of the intercept while the slope of the plot provides an estimate of K_d. Using ΔI_{max} from Equation S6, K_d was evaluated using both Equations S5 & S6. Knowing values for K_d from the double reciprocal plot and non-linear curve fit analysis apparent binding constant of $Cu(tnz)_2Cl_2$ bound to calf thymus DNA was evaluated. Since K_{app} provides only the binding constant of a molecule binding to an isolated site, in order to calculate the overall binding constant (K^*), K_{app} was multiplied with site size n_b; n_b denoting the number of nucleotide bases bound to $Cu(tnz)_2Cl_2$ during an interaction with calf thymus DNA.

Considering the interaction of $Cu(tnz)_2Cl_2$ with calf thymus DNA to be non-specific and non-cooperative, the relation between the ratio, r, of the concentration of each bound compound C_b [represented in Equations S1 & S2 as C_{LD}] to the total concentration of calf thymus DNA i.e. C_D is $r = \frac{C_b}{C_D}$. C_f represents the concentration of the free complex. Data for the titration was also fitted to the Scatchard equation [Equation S7].[10]

$$\frac{r}{C_f} = K(1-nr)\left[\frac{1-nr}{(1-(n-1)r)}\right]^{n-1} \qquad \text{(S7)}$$

C_f was obtained from I_{pc} at the cathodic peak potential for the complex with "n" indicating binding site size in nucleotide bases for each bound molecule interacting with double stranded calf thymus DNA. The advantage of using Equation S7 over the ones described

earlier is that both overall binding constant and site size of interaction are obtained directly.

Fig. S3:

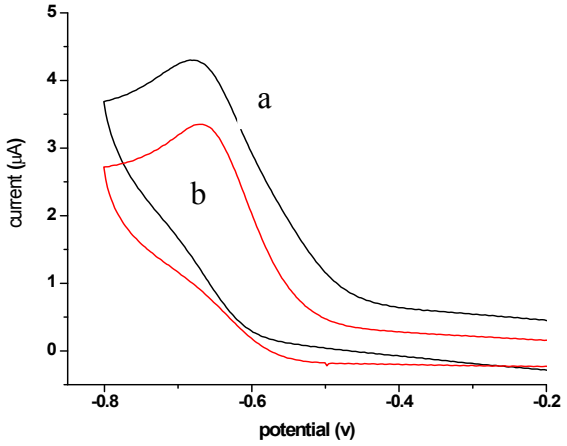

Fig. S3: Cyclic voltammogram of 1mM $Cu(tnz)_2Cl_2$ (curve a) and 1mM tnz (curve b) in pure ethanol, scan rate = 0.1 V/s; T = 25°C.

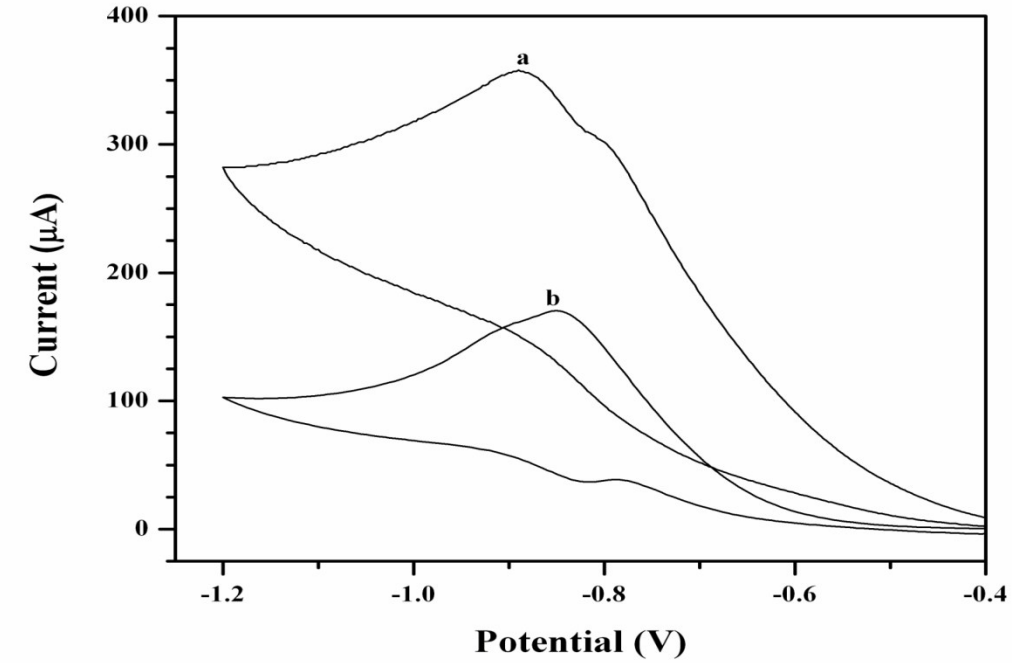

Fig. S4: Cyclic voltammogram of 1mM $[Cu_2(OAc)_4(tnz)_2]$ (curve a) and 1mM tnz (curve b) in pure methanol, scan rate = 0.1 V/s; T = 25°C.

References:

1. G. M. Sheldrick, SHELXS–97 and SHELXL–97, University of Göttingen, Germany, 1997.
2. G. M. Sheldrick, SADABS: Software for Empirical Absorption Correction, University of Gottingen, Institute fur Anorganische Chemieder Universitat, Gottingen, Germany, 1999-2003.
3. H. Putz, K. Brandenburg, Diamond-Crystal and Molecular Structure Visualization; Crystal Impact Kreuzherrenstr 102, 53227 Bonn, Germany,. http://www.crystalimpact.com/diamond.
4. R. C. Santra, K. Sengupta, R. Dey, T. Shireen, P. Das, P. S. Guin, K. Mukhopadhyay, S. Das, *J. Coord. Chem*., **2014**, **67**, 265-285.
5. N. Galván-Tejada, S. Bernès, S. E. Castillo-Blum, H. Nöth, R. Vicente, N. Barba-Behrens, *J. Inorg. Biochem*. 2002, **91**, 339-348.
6. P.S. Guin, S. Das, P.C. Mandal. *J. Phys. Org. Chem*., **23**, 477 (2010).
7. P. Das, P.S. Guin, P.C. Mandal, M. Paul, S. Paul, S. Das. *J. Phys. Org. Chem*., **24**, 774 (2011).
8. P.S. Guin, S. Das, P.C. Mandal. *J. Inorg. Biochem*. **103**, 1702 (2009).
9. P.S. Guin, P. Das, S. Das, P.C. Mandal. *Int J Electrochem*., Article ID 183745 10 pages (2012), doi:10.1155/2012/183745.
10. G. Scatchard, *Ann. N. Y. Acad. Sci*. **51**, 660 (1949).