Supporting Information

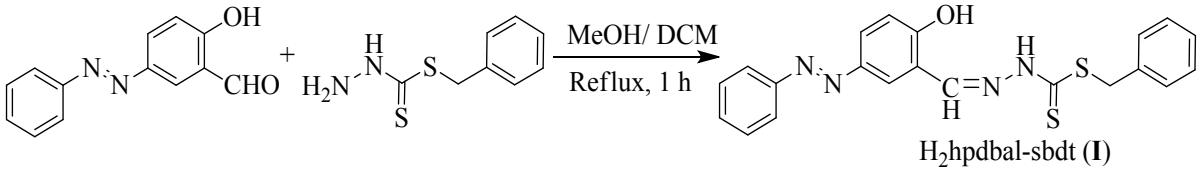
ONS donor entwined iron(III) and cobalt(III) complexes with exemplary safety profile as potent anticancer and glucose uptake agents

Manasa Kongot[a], Dinesh Reddy[a], Vishal Singh[b], Rajan Patel[c], Nitin Kumar Singhal[b], Amit Kumar[a]*

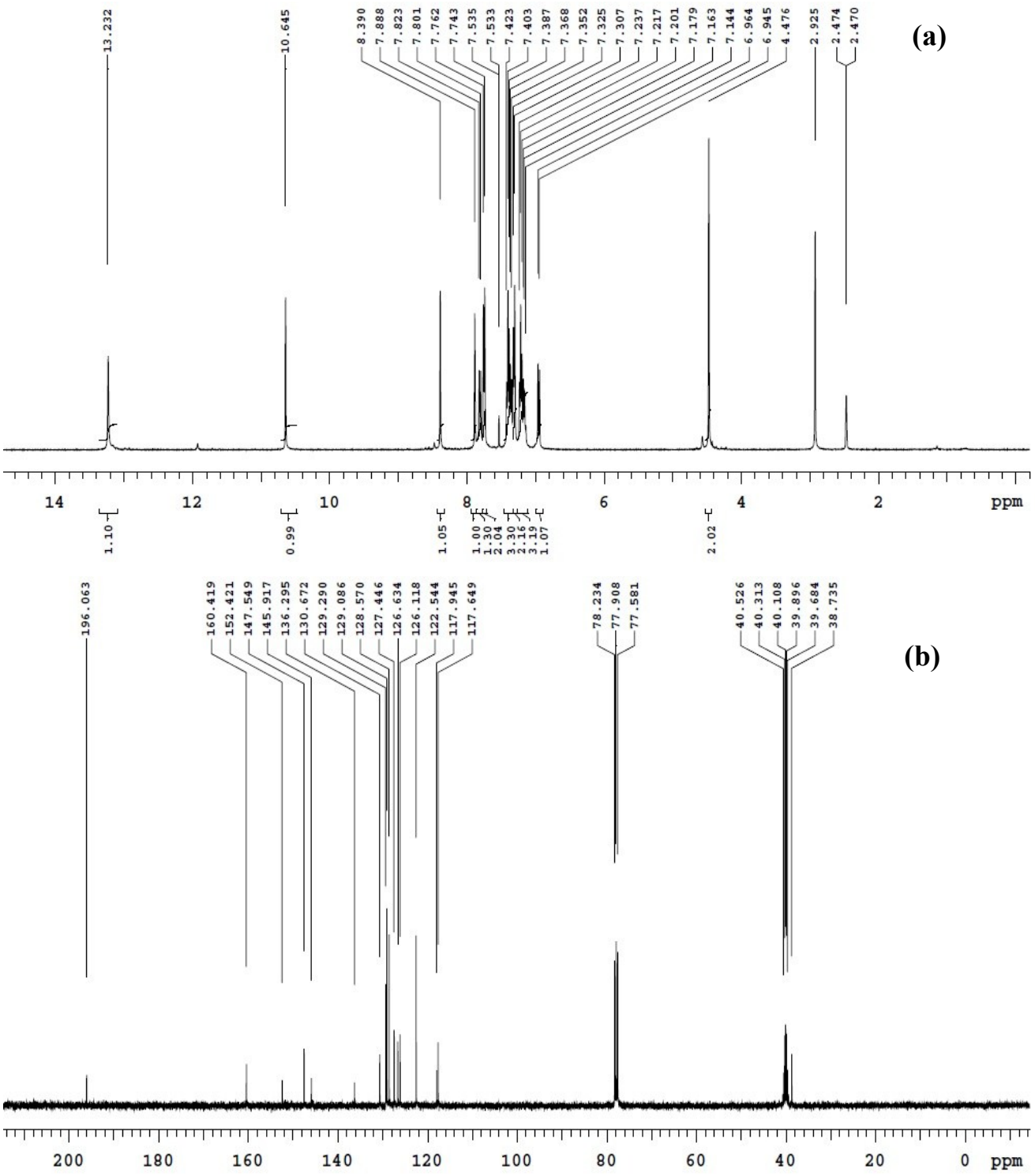
[a]Centre for Nano and Material Sciences, JAIN (Deemed-to-be University), Jain Global Campus, Bengaluru, 562112, Karnataka, India.
[b]NationalAgri Food Biotechnology Institute, Mohali, 140306, India
[c]Biophysical Chemistry Laboratory, Centre for Interdisciplinary Research in Basic Sciences, JamiaMilliaIslamia (A Central University), New Delhi-110025, India.
*To whom correspondence should be addressed.
E-mail: amit.kumar@jainuniversity.ac.in, Phone: +91-80-27577251, Fax: +91-80-2757 7211

SI 1. Synthesis of benzyl 2-(2-hydroxy-5-(phenyldiazenyl)benzylidene)hydrazine carbodithioate [H_2hpdbal-sbdt] (I)

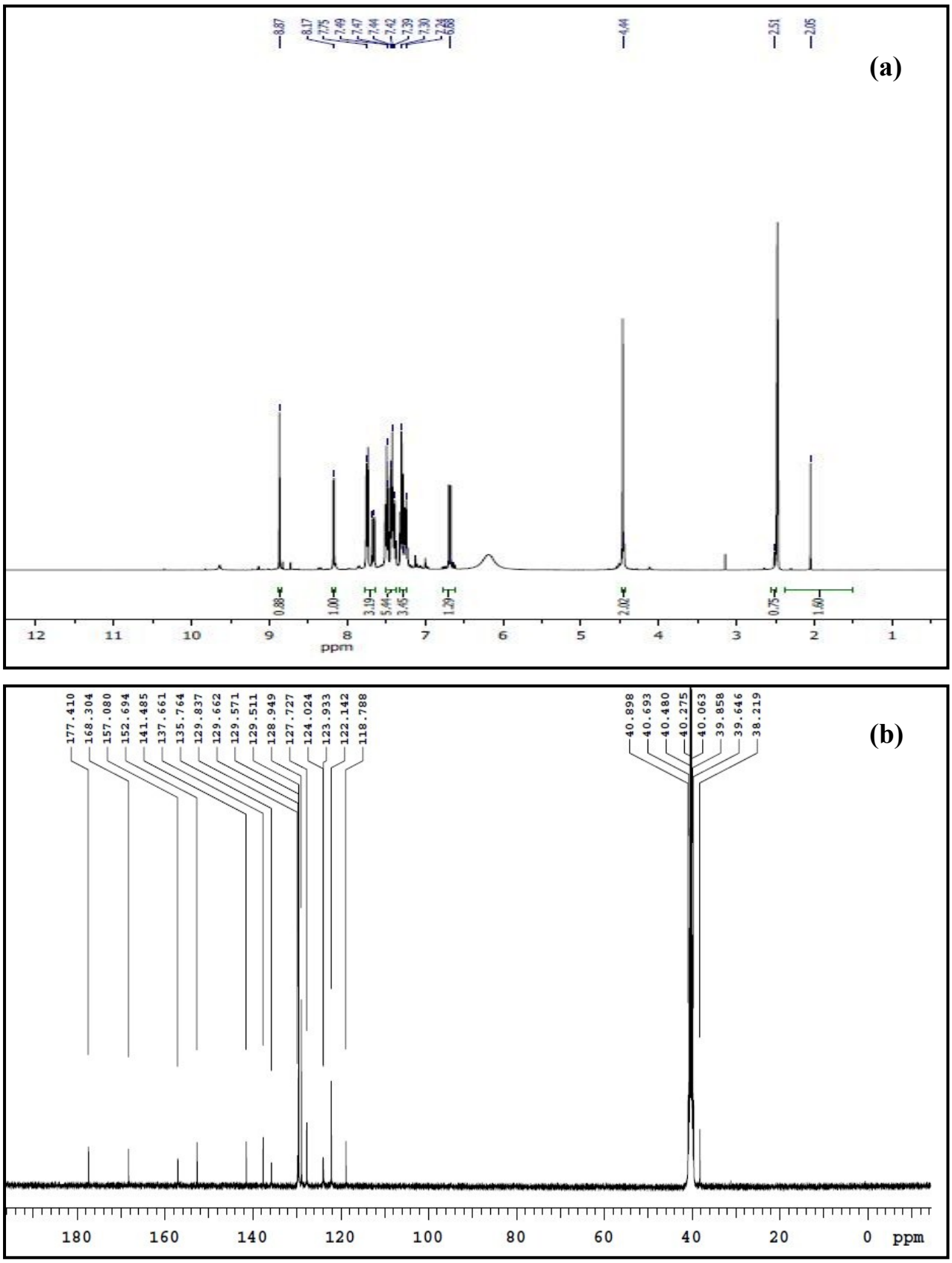
A solution of 2-hydroxy-5-(phenyldiazenyl)benzaldehyde (10 mmol, 2.26 g) in 20 mL of dichloromethane (DCM) was added to a solution of S-benzyldithiocarbazate (10 mmol, 1.98 g) in 20 mL of methanol and this mixture was refluxed on a water bath for 1 h. Upon evaporation of the solvent and cooling of the reaction mixture, a yellowish-orange solid of **I** precipitated, which was filtered off, washed with methanol, and dried. Finally, it was recrystallized from dichloromethane to give yellowish-orange solid. Yield: 91%; m.p: 198^{0}C; Anal. Calcd. for $C_{21}H_{18}N_4OS_2$ (MW: 406.52): C, 62.04; H, 4.46; N, 13.78; S, 15.78. Found: C, 62.26; H, 4.60; N, 13.94; S, 15.58; Selected ATIR data (ν_{max}/cm^{-1}): 1607 (C=N), 1032 (C=S), 771 (C–S), 1320 (C–N), 1112 (C–O), 1518, 1483 (N=N), 2964–3095 (aromatic C–H); ^{1}H–NMR (400 MHz,DMSO-d_6): δ 13.23 (s, 1H, aromatic O–H), 10.65 (s, 1H, N–H/S-H), 8.39 (s, 1H, N=CH), 7.89–6.94 (m, 13H, aromatic H), 4.48 (s, 2H, S–CH_2) , ^{13}C–NMR (400 MHz,DMSO-d_6): δ117.95(C-1), 160.42(C-2), 117.65(C-3), 126.12(C-4), 145.29(C-5), 122.54(C-6, C-8, C-12), 152.42(C-7), 129.09(C-9, C-11), 130.67(C-10), 147.55(C-13), 196.06(C-14), 40.53(C-15), 136.29(C-16), 127.45(C-17, C-21), 128.57(C-18, C-20), 126.63(C-19).



SI Scheme 1. Synthesis of the Schiff base ligand H_2hpdbal-sbdt (**I**).



SI Figure 1. (a) ^{1}H-NMR spectrum and **(b)** ^{13}C-NMR spectrum of H_2hpdbal-sbdt (**I**).



SI Figure 2. (a) ^{1}H-NMR spectrum and **(b)** ^{13}C-NMR spectrum of cobalt complex, $[Co^{III}(OH_2)(Cl)(Hhpdbal\text{-}sbdt)_2]$ (**2**) which confirmed the complex formation, coordination modes and supported oxidation state of metal centre.

Protein interaction study of bioactive complexes

1. Intrinsic, synchronous and 3-dimensional fluorescence studies

BSA (5μM in phosphate buffer of pH 7.4) fluorescence emission spectra were obtained with titration of ten different concentrations (1 μM to 10 μM each) of compounds **1** and **2** (in DMSO)at 298 K by fixing the wavelength of excitation at 280 nm.

The type of quenching induced by the compounds was preliminarily attempted to be known by Stern-Volmer (SV) plot drawn using the SV equation (SI eq. 1)

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \quad \textbf{(1)}$$

Herein, F_0 is the fluorescence peak intensity BSA alone and F is the fluorescence peak intensity given by BSA after the addition of various concentrations of compounds **1** and **2**. k_q is a constant which defines the rate of quenching of BSA and $K_{sv}$is the SV quenching constant. [Q] and τ_0 are concentration of compounds and the lifetime average of BSA ($\approx 10^{-9}$ s) respectively.

As the SV plot did not give any conclusive evidence on the type of quenching, modified SV plot was drawn to decide on the quenching type using modified SV equation (SI eq. 2)

$$\frac{F_0}{F} = (1 + K_D[Q])(1 + K_S[Q]) \quad \textbf{(2)}$$

Herein, K_D and K_S are constants which define dynamic and static quenching respectively.

Double log graph was drawn to estimate the number of binding sites (n) using the double log equation (SI eq. 3), where K_a is the binding constant.

$$log\left(\frac{F_0 - F}{F}\right) = logK_a + nlog[Q] \quad \textbf{(3)}$$

Fluorescence of BSA is majorly contributed by two amino acids, tyrosine (Tyr) and tryptophan (Trp) and they are measured simultaneously in synchronous measurements. For this, a wavelength interval of $\Delta\lambda$=15 nm and $\Delta\lambda$=60 nm for Tyr and Trp respectively were set in the instrument with the scan set at λ_{ex}= 200–350 nm and λ_{em}= 290–500 nm.

In 3-dimensional fluorescence study, three optimal concentrations (1 μM, 5 μM and10 μM) of the compounds **1** and **2**were titrated with BSA in the previously mentioned manner. In the instrument, the excitation wavelength was set between 200 to 360 nm and emission wavelength between 200 to 650 nm at a scan rate and increment of 600 nm/min and 2 nm respectively after which the resultant spectra were recorded.

2. Secondary structure conformational study using deconvoluted IR spectroscopy

The IR spectrum of BSA protein exhibits a very characteristicamide I and amide II bands due to carbonyl group stretching and amine group bending associated with the amide bonds present in the polypeptide backbone. IR spectra were recorded for BSA solutions (100μM) titrated with three optimal concentrations of **1** and **2 (**20 μM, 100 μM and 180 μM) as the amide bands in BSA are sensitive to their environment. The IR spectrum of pure BSA showed Amide I, amide II and shoulder bands at 1649 cm^{-1}, 1539 $cm^{-1}$and 1468cm^{-1}respectively. The observed bands confirmed the α-helical conformational dominancy of the structure of native BSA.

3. Temperature controlled UV-Vis studies

BSA (5μM in phosphate buffer of pH 7.4) absorbance spectra were obtained by titration of ten different concentrations (1 μM to 10 μM each) of compounds**1** and **2** (in DMSO) at 298 K. The binding/association constant, K_a at four different temperatures were calculated using the double reciprocal plot ($A_0/(A–A_0)$ vs 1/c). The thermodynamically acting forces between BSA and compounds were found out using the Van't Hoff's plot using eq. 4.

$$lnK_a = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad \textbf{(4)}$$

The free energy relation given by eq. 5 was used to calculate Gibbs free energy change ΔG at different temperatures.

$$\Delta G = \Delta H - T\Delta S \qquad \textbf{(5)}$$

4. Energy transfer study between bioactive complexes and BSA using FRET theory

In this study, the fluorophore protein is considered as a donor and the quencher molecule (**1** and **2**) is regarded as an acceptor.

The energy transferred between BSA and compounds was calculated using the FRET theory (using eq. 6).

$$E = 1 - \frac{F_0}{F} = \frac{R_0^6}{R_0^6 + r^6} \qquad \textbf{(6)}$$

Herein, E,r and R_0 define the efficiency of energy transfer, binding site proximity between fluorophore and quencher molecules and the Forster critical energy transfer distance at which there is 50% efficiency of energy transfer respectively. R_0 was calculated using eq. 7.

$$R_0^6 = 8.79 \times 10^{-25} K^2 N^{-4} \Phi J \qquad \textbf{(7)}$$

Herein, K^2 is the spatial orientation factor of BSA whose value is 2/3, N is the average refractive index of the medium which is equal to 1.336, ϕis the fluorescence quantum yield equalling a value of 0.15, J is the integral of overlap between the absorption spectra of compounds and fluorescence emission spectrum of BSA. J was obtained using eq. 8.

$$J = \frac{\int_0^\infty F(\lambda)\epsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty F(\lambda)d\lambda} \quad \textbf{(8)}$$

Herein, F(λ) is the corrected emission intensity of BSA over a wavelength range of λ to (λ+dλ). ε(λ) is the molar extinction coefficient of the quencher compounds at wavelength λ.