Electronic Supplementary Material (ESI) for RSC Advances.

New copper(II) complexes of anti-inflammatory drug mefenamic acid: a concerted study including synthesis, physicochemical characterization and their biological evaluation

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S1. Interaction with CT DNA

The DNA-binding constant complexes **1–3** (K_b, in M^{-1}) may be obtained by monitoring the changes in the absorbance at the corresponding λ_{max} in the UV-Vis spectra with increasing concentrations of CT DNA. K_b is given by the ratio of slope to the y intercept in plots $\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)}$ versus [DNA], according to the Wolfe–Shimer equation [1]:

$$\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \qquad \text{(eq. S1)}$$

where [DNA] is the concentration of DNA in base pairs, $\varepsilon_A = A_{obsd}/[compound]$, ε_f = the extinction coefficient for the free compound and ε_b = the extinction coefficient for the compound in the fully bound form.

S2. Competitive studies with EB

The Stern–Volmer constant K_{SV} (in M^{-1}) is used to evaluate the quenching efficiency for each compound according to the Stern–Volmer equation [2]:

$$\frac{Io}{I} = 1 + K_{SV}[Q] \qquad \text{(eq. S2)}$$

where Io and I are the emission intensities in the absence and the presence of the quencher, respectively, [Q] is the concentration of the quencher (i.e. complexes **1–3**); K_{SV} is obtained from the Stern–Volmer plots by the slope of the diagram $\frac{Io}{I}$ versus [Q].

S3. Interaction with serum albumins

The extent of the inner-filter effect can be roughly estimated with the following formula:

$$I_{corr} = I_{meas} \times 10^{\frac{\varepsilon(\lambda_{exc})cd}{2}} \times 10^{\frac{\varepsilon(\lambda_{em})cd}{2}} \qquad \text{(eq. S3)}$$

where I_{corr} = corrected intensity, I_{meas} = the measured intensity, c = the concentration of the quencher, d = the cuvette (1 cm), $\varepsilon(\lambda_{exc})$ and $\varepsilon(\lambda_{em})$ = the ε of the quencher at the excitation and the emission wavelength, respectively, as calculated from the UV-vis spectra of the complexes [3].

The Stern–Volmer and Scatchard graphs are used in order to study the interaction of a quencher with serum albumins. According to Stern–Volmer quenching equation [2]:

$$\frac{Io}{I} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \qquad \text{(eq. S4),}$$

where Io = the initial tryptophan fluorescence intensity of SA, I = the tryptophan fluorescence intensity of SA after the addition of the quencher, k_q = the quenching rate constants of SA, K_{SV} =

the dynamic quenching constant, τ_o = the average lifetime of SA without the quencher, [Q] = the concentration of the quencher, the dynamic quenching constant (K_{SV}, M^{-1}) can be obtained by the slope of the diagram $\frac{Io}{I}$ versus [Q]. From the equation:

$$K_{SV} = k_q \tau_o \qquad \text{(eq. S5)}$$

and taking $\tau_o = 10^{-8}$ s as fluorescence lifetime of tryptophan in SA, the approximate quenching constant (k_q, $M^{-1}s^{-1}$) is calculated.

From the Scatchard equation [2]:

$$\frac{\Delta I / Io}{[Q]} = nK - K\frac{\Delta I}{Io} \qquad \text{(eq. S6)}$$

where n is the number of binding sites per albumin and K is the association binding constant, K (in M^{-1}) is calculated from the slope in plots $\frac{\Delta I / Io}{[Q]}$ versus $\frac{\Delta I}{Io}$ and n is given by the ratio of y intercept to the slope [2].

References

[1] A. Wolfe, G. Shimer and T. Meehan, *Biochemistry* 1987, **26**, 6392.

[2] Y. Wang, H. Zhang, G. Zhang, W. Tao and S. Tang, *J. Luminescence* 2007, **126**, 211.

[3] L. Stella, A.L. Capodilupo and M. Bietti, *Chem. Commun*. 2008, 4744.

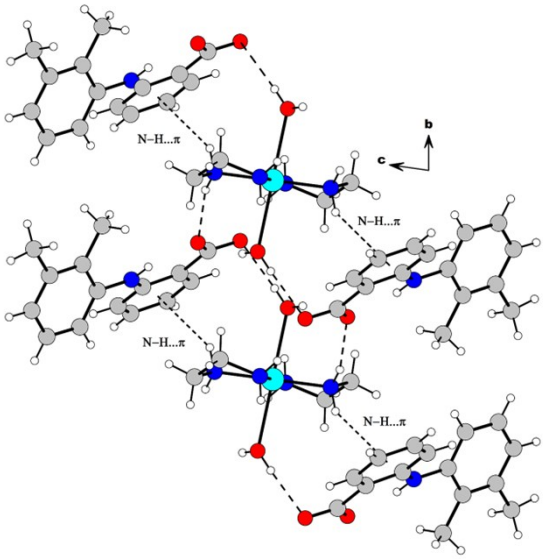

Figure S1. Packing diagram of complex **2**, stabilized by various non-covalent interactions such as N-H....π, O-H...O and N-H...O hydrogen bonding interactions.

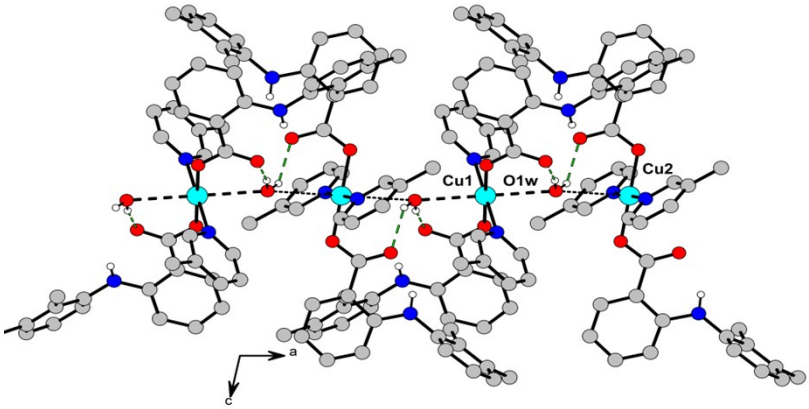

Figure S2. Chain of $[Cu(\beta\text{-pic})_2(mef)_2]$ in complex **3** running along the *a* axis (Cu..Ow contacts and Ow-H…O hydrogen bonds are shown as black and green broken bonds, respectively)

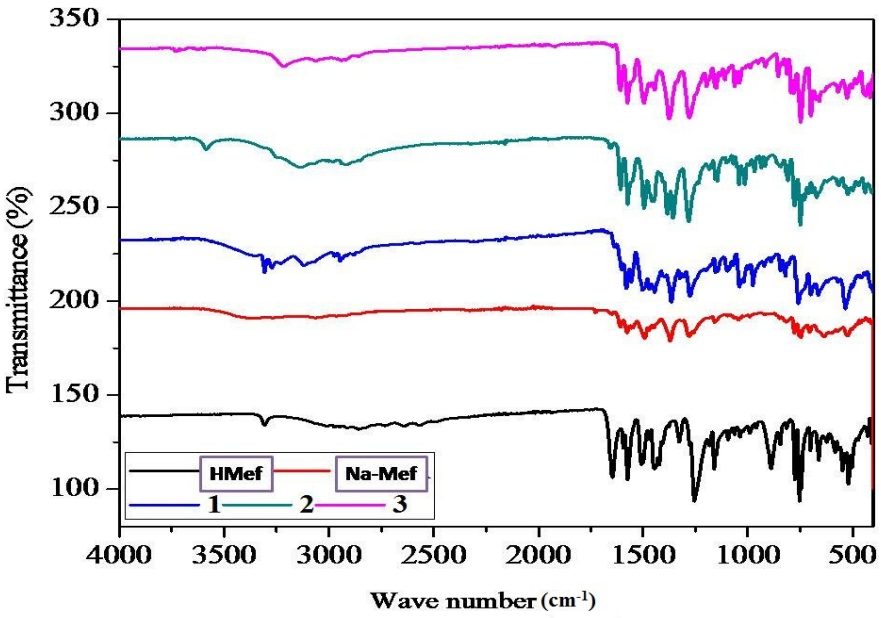

Figure S3. FT-IR spectra of complexes **1-3** in comparison with mefenamic acid and sodium salt of mefenamic acid.

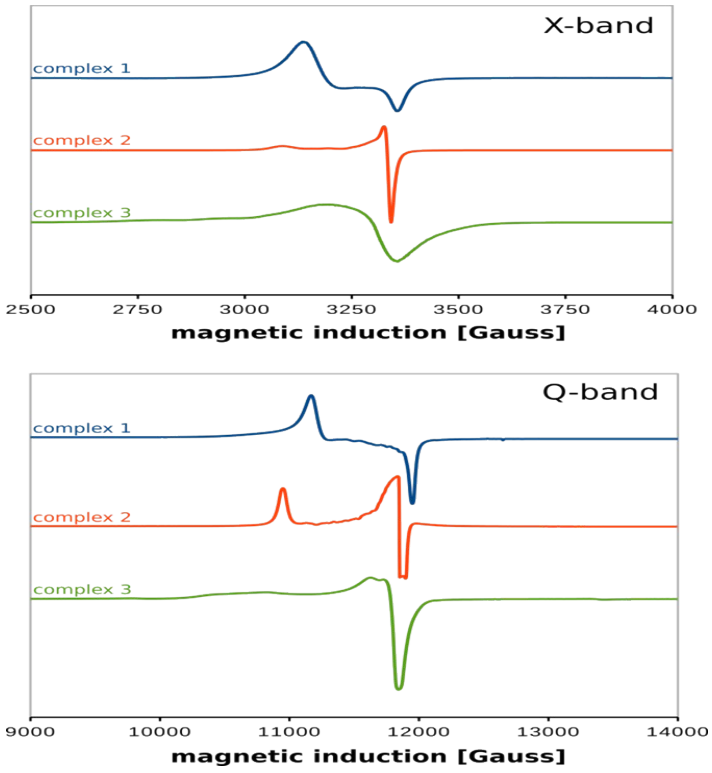

Figure S4. X- (9.6 GHz) and Q-band (34 GHz) EPR spectra of powdered complex **1**, **2** and **3** at 77 K (X-band) and 100 K (Q-band). The parameters are given in the text.

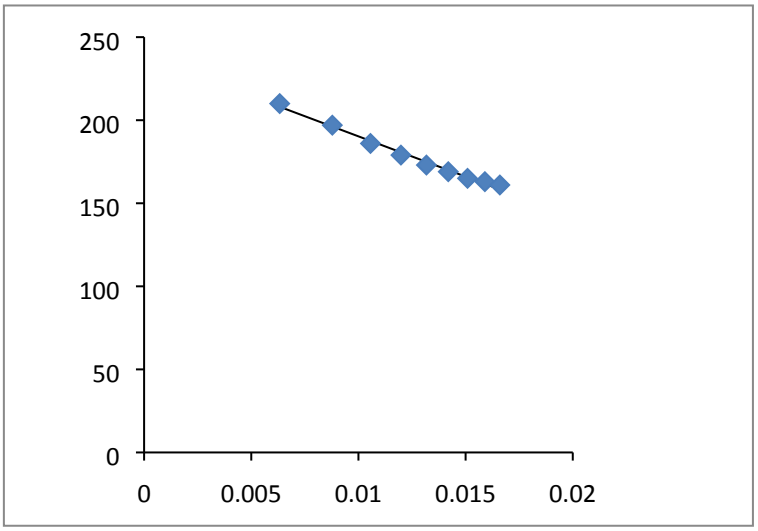

Figure S5. Plot of K (molar conductance) versus $C^{1/2}$ (square root of concentration) of complex **2.**

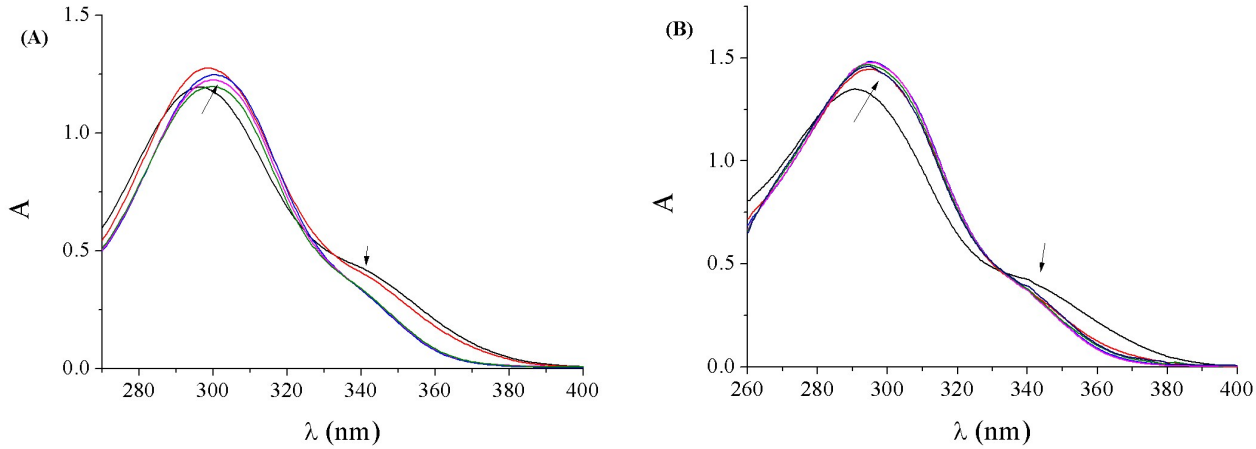

Figure S6. UV-Vis spectra of DMSO solution of complex (A) **1** (3×10^{-5} M) and (B) **2** (4×10^{-5} M) in the presence of increasing amounts of CT DNA (r' = [DNA]/[compound] = 0–0.8). The arrows show the changes upon increasing amounts of CT DNA.

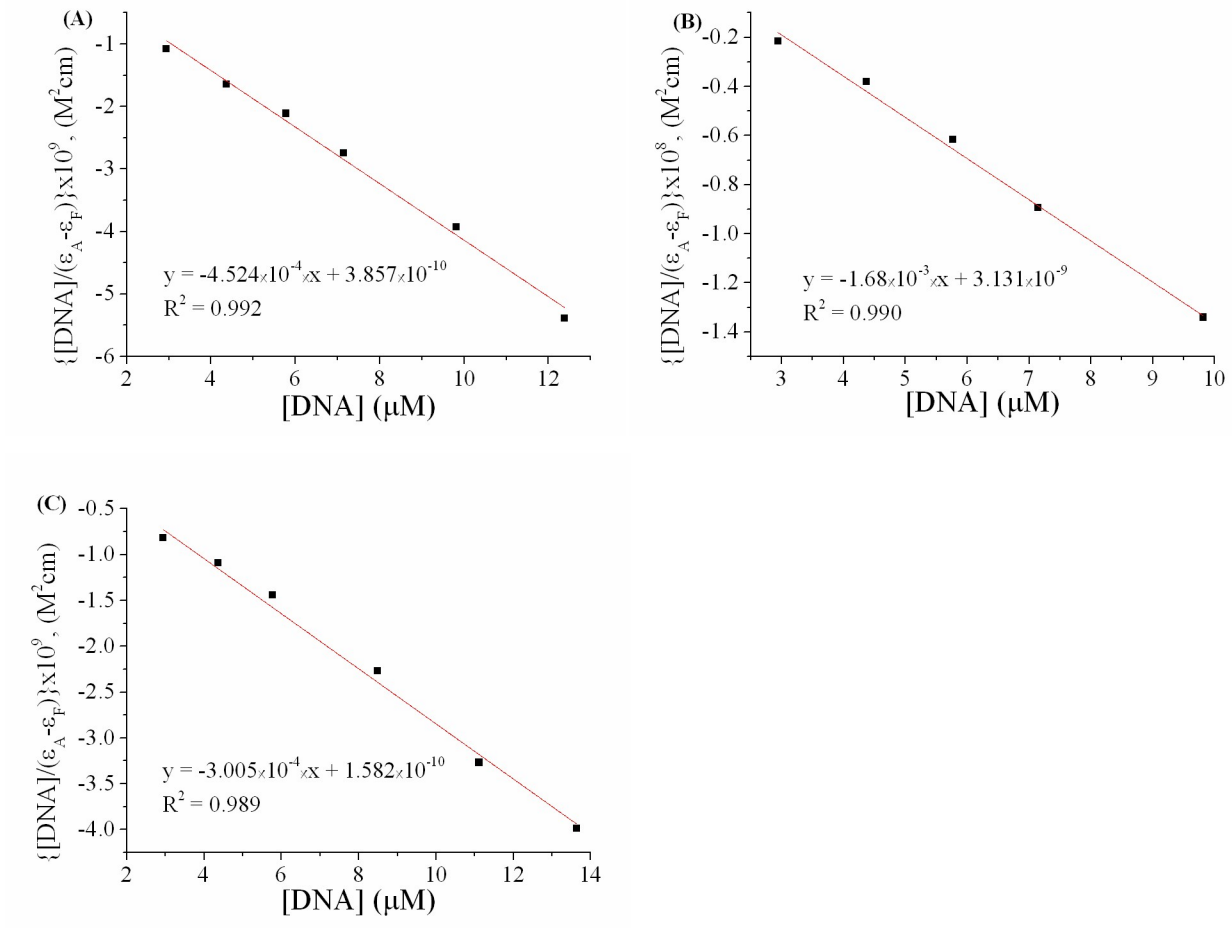

Figure S7. (A)–(C) Plot of $\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)}$ versus [DNA] for complexes **1–3**, respectively.

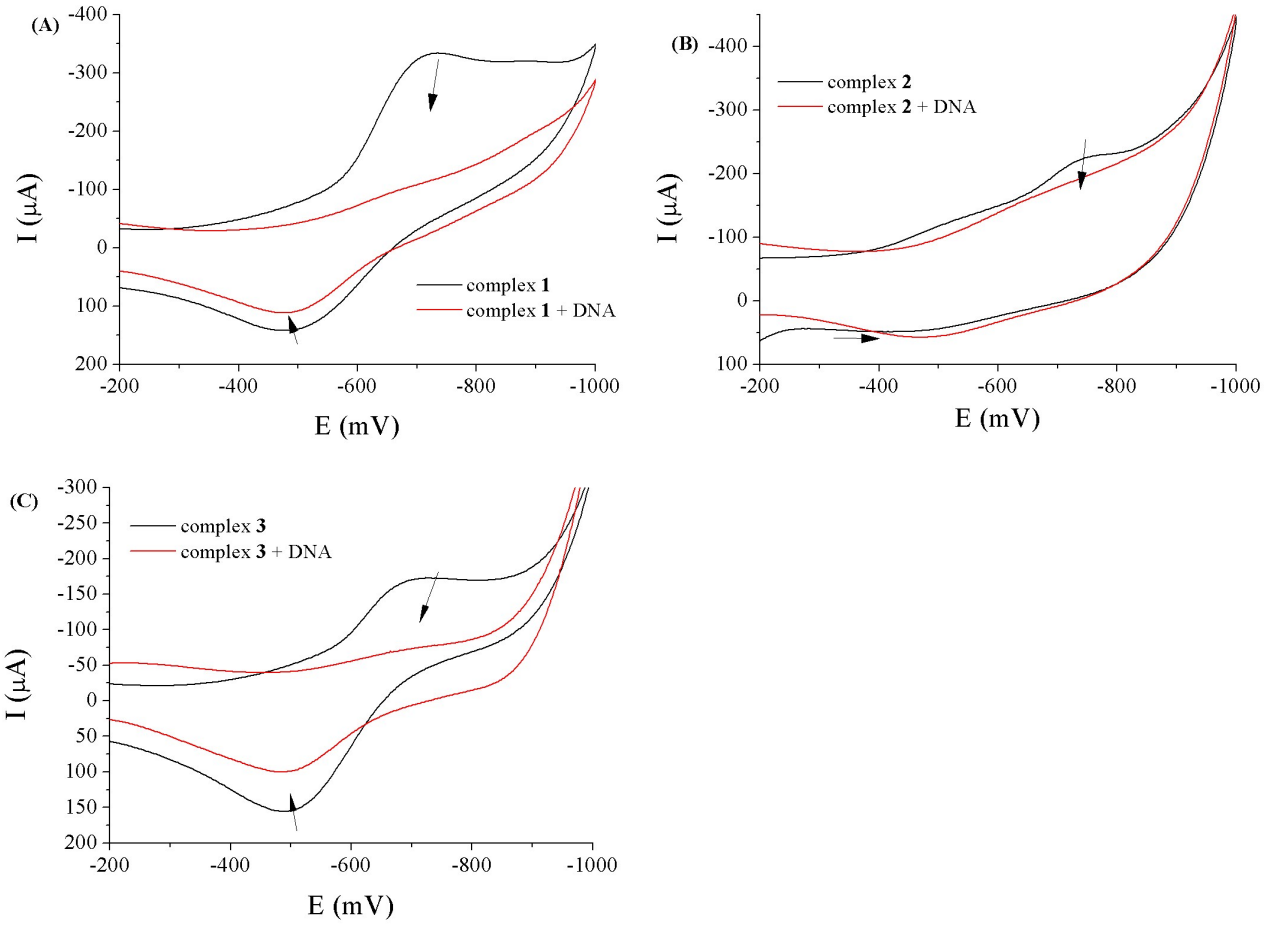

Figure S8. Cyclic voltammogram of 0.33 mM 1/2 DMSO/buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) solution of complex (A) **1**, (B) **2** and (C) **3** in the absence or presence of CT DNA. Scan rate = 100 mV s^{-1}. Supporting electrolyte = buffer solution.

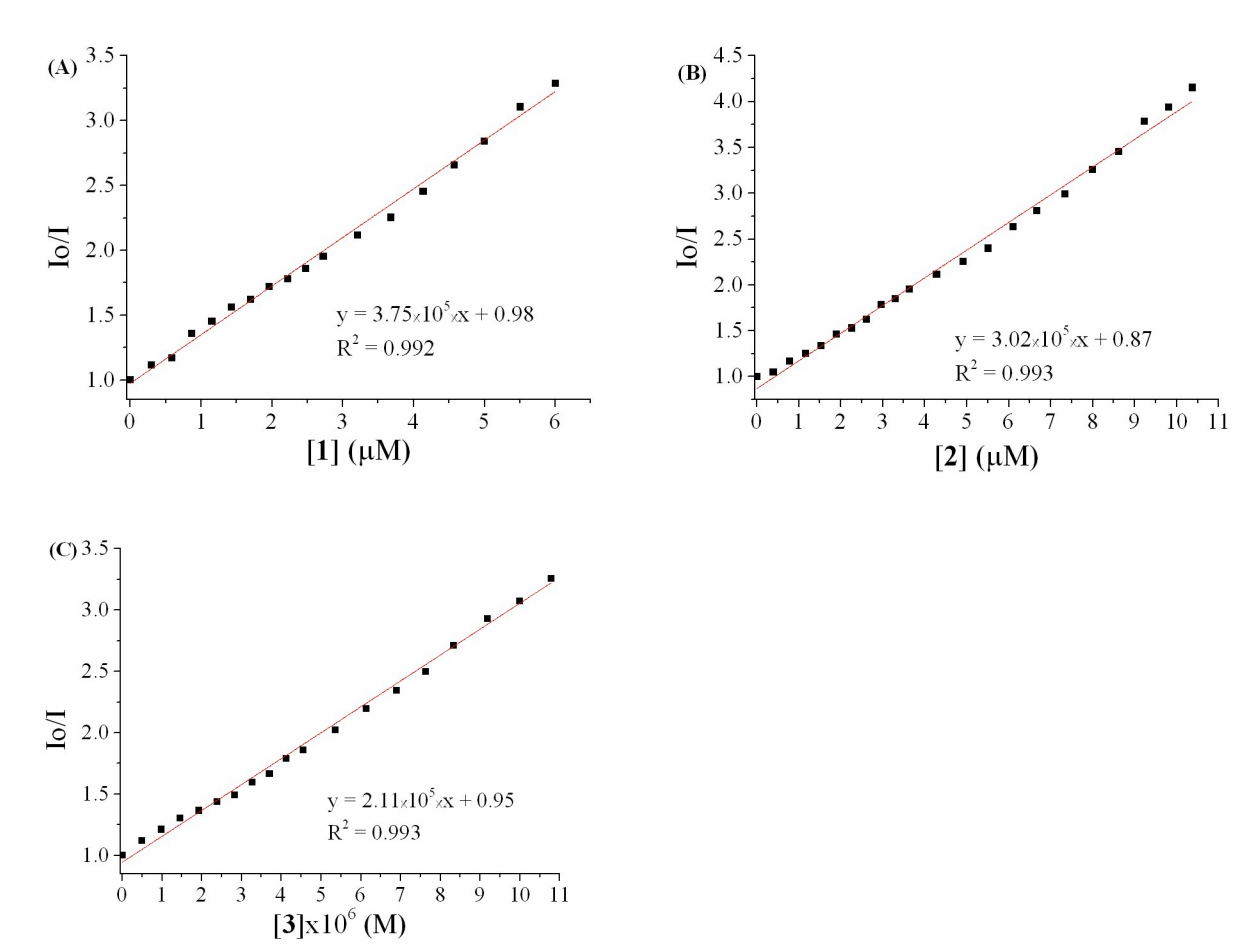

Figure S9. (A)–(C) Stern–Volmer quenching plot of EB bound to CT DNA for complexes **1–3**, respectively.

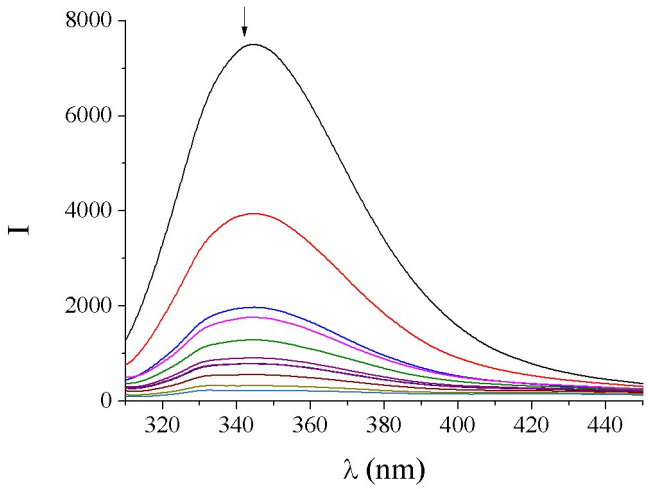

Figure S10. Fluorescence emission spectra (λ_{exc} = 295 nm) for BSA ([BSA] = 3 μM) in buffer solution in the absence and presence of increasing amounts of **2** (r = [**2**]/[BSA] = 0–7.3). The arrow shows the changes of intensity upon increasing amounts of the complex.

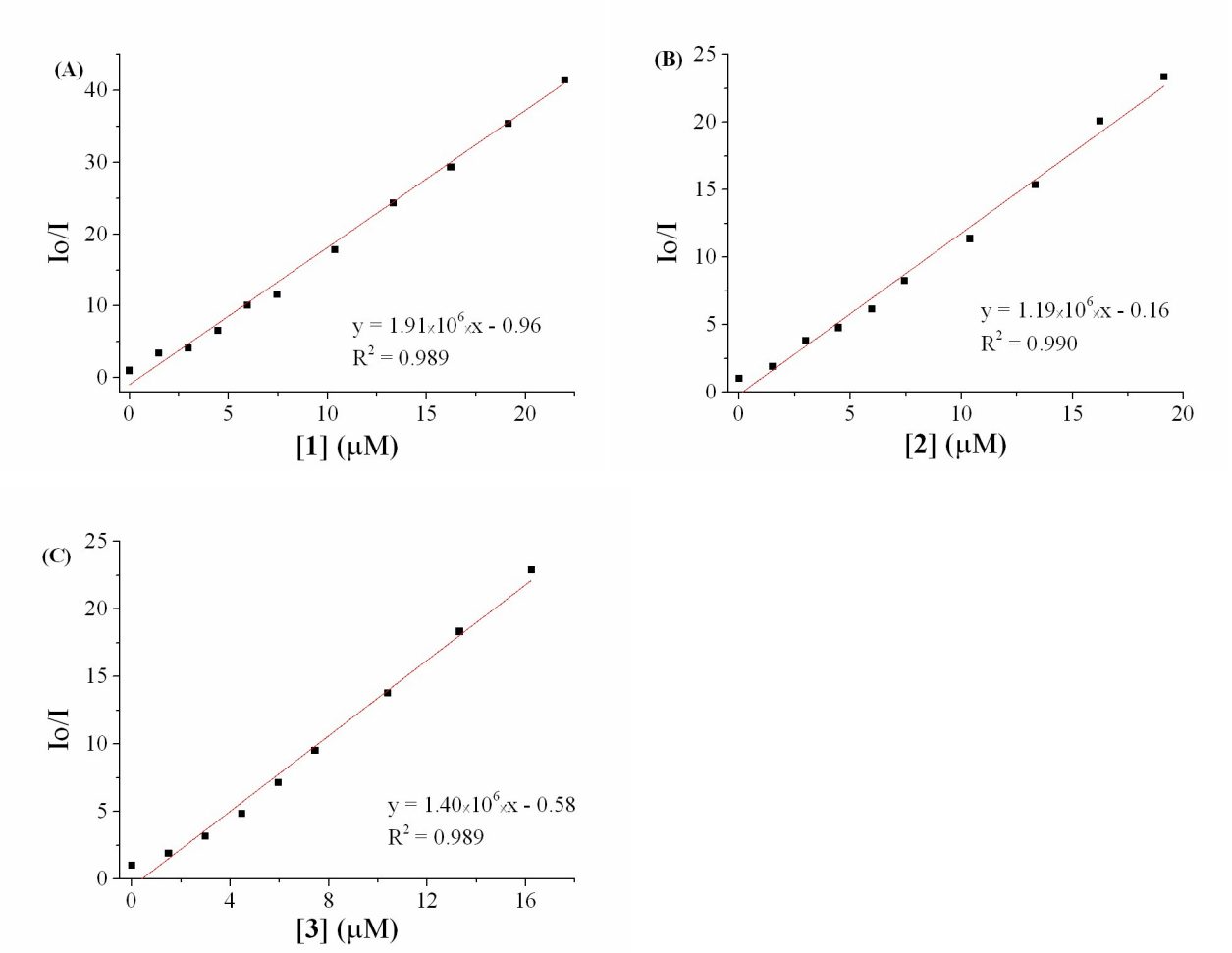

Figure S11. (A)–(C) Stern–Volmer quenching plot of BSA for complexes **1–3**, respectively.

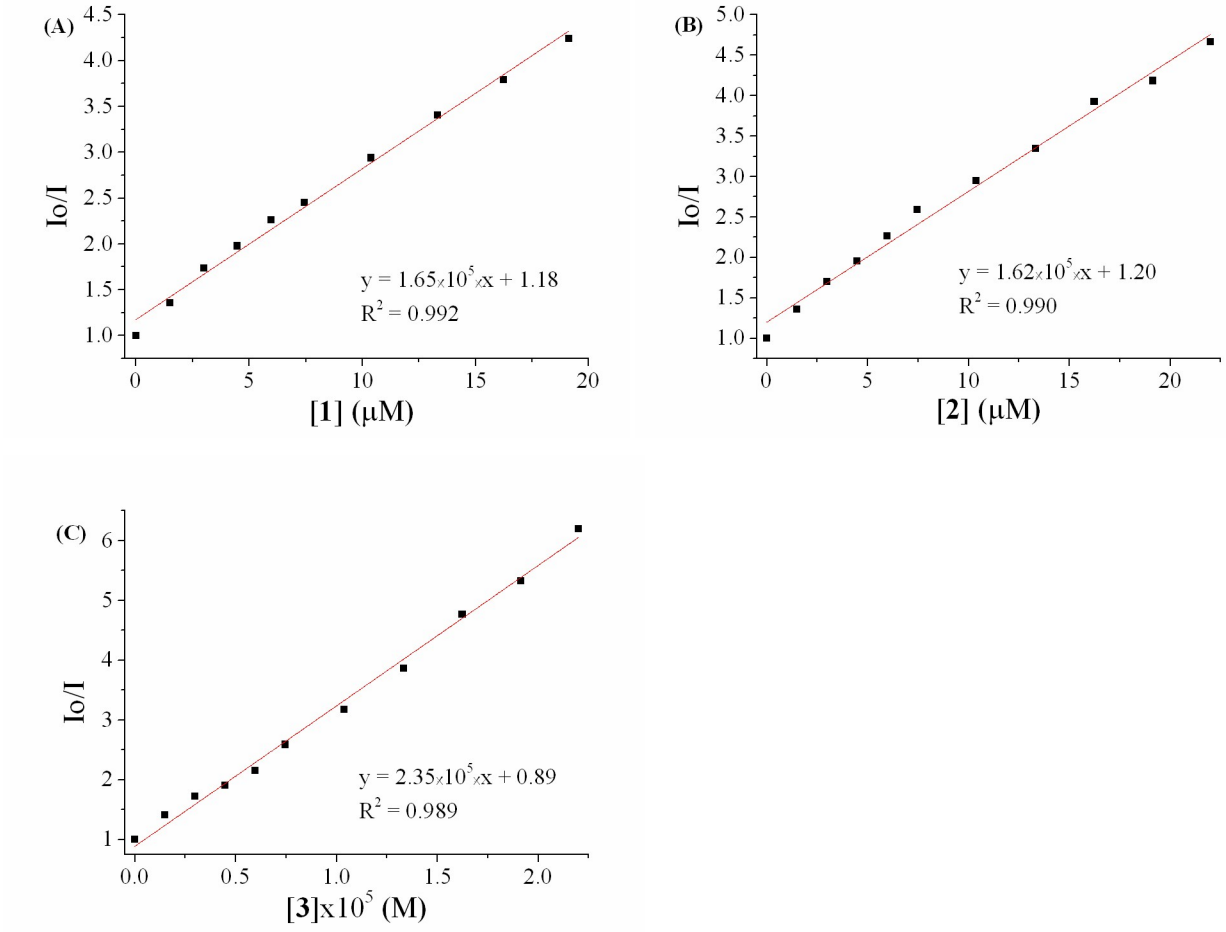

Figure S12. (A)–(C) Stern–Volmer quenching plot of HSA for complexes **1–3**, respectively.

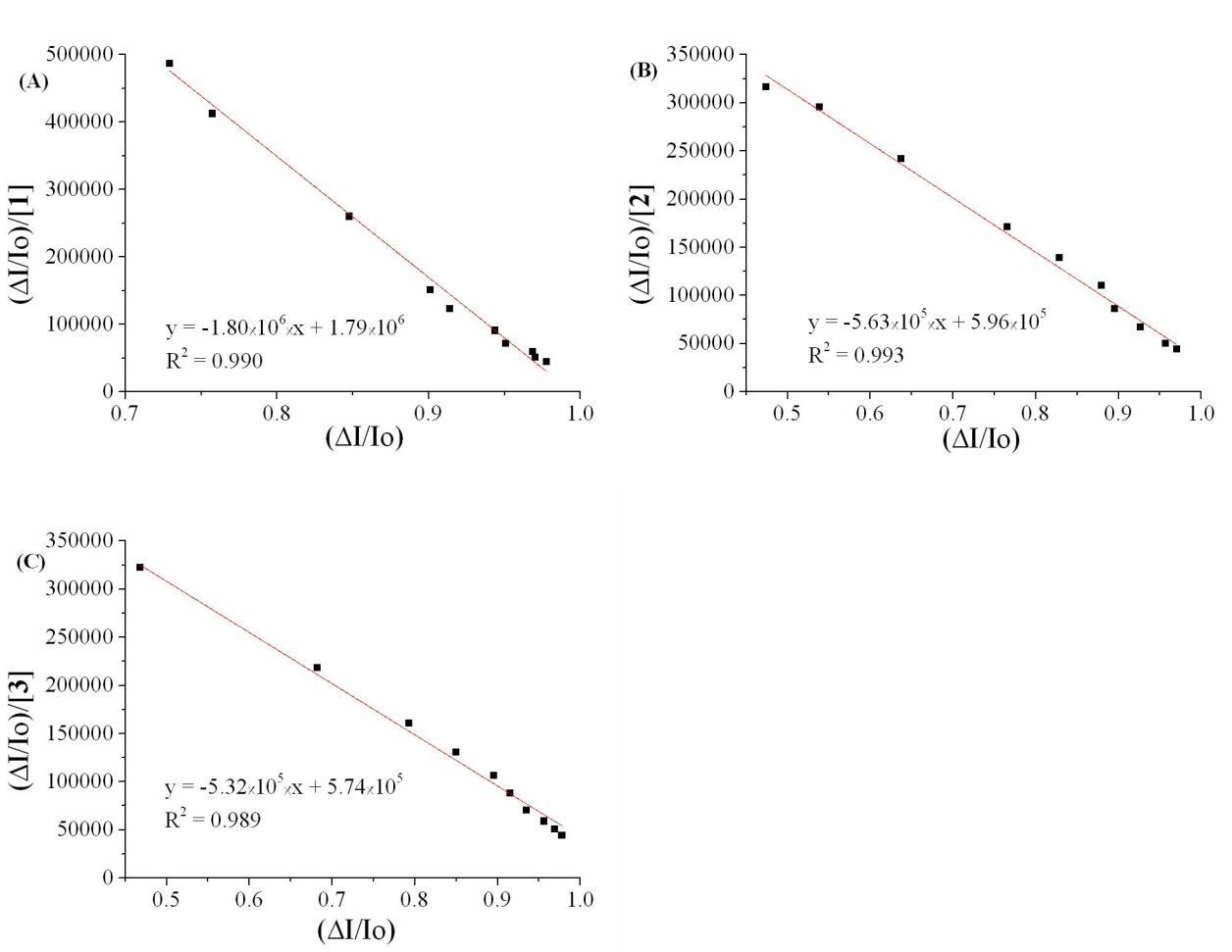

Figure S13. (A)–(C) Scatchard plot of BSA for complexes **1–3**, respectively.

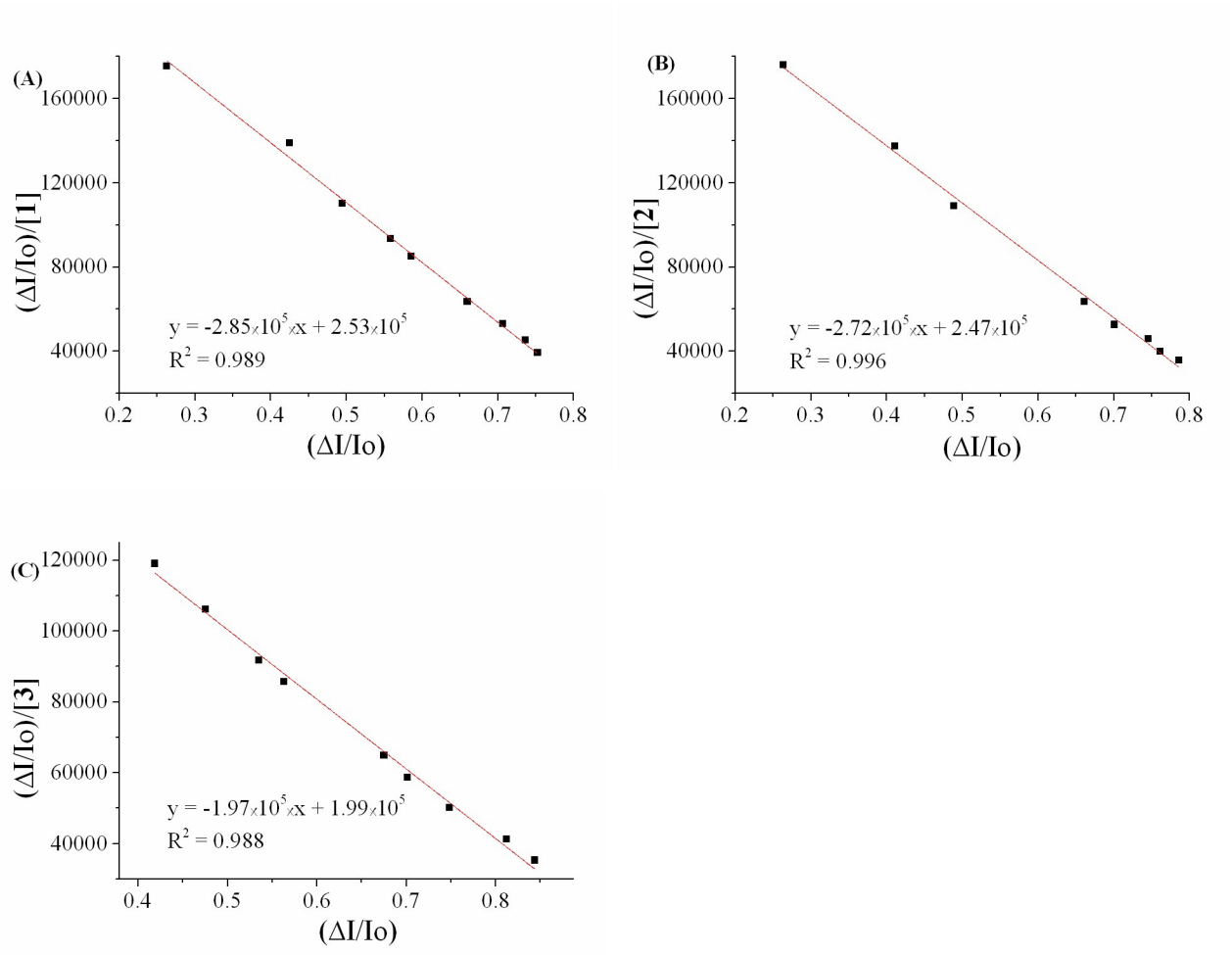

Figure S14. (A)–(C) Scatchard plot of HSA for complexes **1–3**, respectively.

Table S1. Hydrogen bonding parameters of complexes **2** and **3**; D= donor, A= acceptor.

D-H…A	**D-H (Å)**	**D…A (Å)**	**H…A (Å)**	**<D-H…A (°)**	**Equivalent positions**
Complex 2					
N1-H...O1	0.90	2.968(3)	2.10	161	
O1W-H...O1	0.76(3)	2.832(3)	2.09(3)	164(3)	
N3-H...O1	0.84(3)	2.611(2)	1.89(2)	143(2)	
N2-H...O2^{i}	0.90	2.980(3)	2.10	165	(i) -x,-y,1-z
N2-H...O2^{ii}	0.90	3.098(2)	2.30	146	(ii) x-1,y,z
O1W-H...O2^{iii}	0.80(3)	2.812(3)	2.01(3)	173(3)	(iii)-x,1-y,1-z
Complex 3					
N2-H...O	0.79(4)	2.651(6)	1.99(4)	140(4)	
O1W-H...O2	0.87(7)	2.726(6)	1.86(7)	171(6)	
O1W-H...O4	0.74(7)	2.752(6)	2.12(6)	142(6)	
N4-H...O4	0.80(7)	2.632(5)	1.91(6)	149(7)	