Structure and biological perspectives of metal complexes of flumequine

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Supplementary material

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Interaction with CT DNA

The binding constant, K_b, can be obtained by monitoring the changes in the absorbance at the corresponding λ_{max} with increasing concentrations of CT DNA and it 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.

Competitive studies with EB

The Stern–Volmer constant K_{SV} is used to evaluate the quenching efficiency for each compound according to the Stern–Volmer equation:

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

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 **1–4**); K_{SV} is obtained from the Stern–Volmer plots by the slope of the diagram $\frac{Io}{I}$ vs [Q].

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.[2]

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:[3]

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

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 $\frac{Io}{I}$ vs [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:[3]

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

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.[3]

References

1 A. Wolfe, G. Shimer and T. Meehan, *Biochemistry*, 1987, **26**, 6392–6396.

2 L. Stella, A.L. Capodilupo and M. Bietti, *Chem. Commun.*, 2008, 4744–4746.

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Table S1. Selected bond distances and angles for [Co(flmq)$_2$(bipy)].

Bond	Distance (Å)	Bond	Distance (Å)
Co(1)–O(1)	2.0302(14)	C(1)–O(1)	1.253(2)
Co(1)–O(3)	2.0852(14)	C(1)–O(2)	1.232(2)
Co(1)–O(4)	2.0326(16)	C(4)–O(3)	1.259(2)
Co(1)–O(6)	2.0890(13)	C(15)–O(4)	1.265(3)
Co(1)–N(3)	2.1256(15)	C(15)–O(5)	1.247(3)
Co(1)–N(4)	2.1300(18)	C(18)–O(6)	1.260(2)

Bond angle	Angle (°)	Bond angle	Angle (°)
O(1)–Co(1)–O(4)	98.45(7)	O(3)–Co(1)–O(4)	92.15(7)
O(1)–Co(1)–O(3)	86.93(5)	O(3)–Co(1)–O(6)	174.66(5)
O(1)–Co(1)–O(6)	88.15(6)	O(3)–Co(1)–N(3)	85.78(6)
O(1)–Co(1)– N(3)	166.17(7)	O(3)–Co(1)–N(4)	99.33(7)
O(1)–Co(1)–N(4)	93.52(7)	O(4)–Co(1)–O(6)	86.52(6)
O(6)–Co(1)–N(3)	99.46(6)	O(4)–Co(1)–N(3)	93.55(6)
O(6)–Co(1)–N(4)	83.04(6)	O(4)–Co(1)–N(4)	163.85(7)
N(3)–Co(1)–N(4)	76.13(6)		

Table S2. Selected bond distances and angles for [Co(flmq)$_2$(phen)].

Bond	Distance (Å)	Bond	Distance (Å)
Co(1)–O(2)	2.028(2)	C(1)–O(1)	1.244(4)
Co(1)–O(3)	2.073(2)	C(1)–O(2)	1.254(3)
Co(1)–N(2)	2.152(2)	C(4)–O(3)	1.260(4)

Bond angle	Angle (°)	Bond angle	Angle (°)
O(2)–Co(1)–O(2)′	99.71(14)	O(3)–Co(1)–O(3)′	179.11(13)
O(2)–Co(1)–O(3)	86.63(9)	O(3)–Co(1)–N(2)′	94.55(9)
O(2)–Co(1)–O(3)′	92.79(9)	O(3)–Co(1)–N(2)	86.15(9)
O(2)–Co(1)–N(2)	166.43(9)	N(2)–Co(1)–N(2)′	77.04(13)
O(2)–Co(1)–N(2)′	92.11(9)		

Table S3. Selected bond distances and angles for [Co(flmq)$_2$(bipyam)].

Bond	Distance (Å)	Bond	Distance (Å)
Co(1)–O(1)	2.023(3)	C(1)–O(1)	1.260(5)
Co(1)–O(4)	2.052(2)	C(1)–O(2)	1.236(5)
Co(1)–O(3)	2.097(2)	C(4)–O(3)	1.266(4)
Co(1)–O(6)	2.136(2)	C(15)–O(4)	1.266(4)
Co(1)–N(1)	2.110(3)	C(15)–O(5)	1.237(4)
Co(1)–N(2)	2.112(3)	C(18)–O(6)	1.261(4)

Bond angle	Angle (°)	Bond angle	Angle (°)
O(1)–Co(1)–O(4)	173.55(10)	O(3)–Co(1)–O(4)	90.27(10)
O(1)–Co(1)–O(3)	86.61(10)	O(3)–Co(1)–O(6)	83.27(10)
O(1)–Co(1)–O(6)	88.47(11)	O(3)–Co(1)–N(1)	179.37(12)
O(1)–Co(1)–N(1)	94.02(12)	O(3)–Co(1)– N(2)	93.27(11)
O(1)–Co(1)–N(2)	93.67(12)	O(4)–Co(1)–O(6)	85.56(10)
O(6)–Co(1)–N(1)	96.79(11)	O(4)–Co(1)–N(1)	89.11(12)
O(6)–Co(1)–N(2)	175.83(11)	O(4)–Co(1)–N(2)	92.14(11)
N(1)–Co(1)–N(2)	86.63(12)		

Table S4. Hydrogen bonding interactions in **2**·2H_2O, **3**·1.6MeOH·0.4H_2O and **4**·H_2O.

D–H···A	D–H (Å)	H···A (Å)	D ···A (Å)	D–H···A (°)	Symmetry transformation for acceptors
2					
O(7)–H(1A)···O(2)	0.822(10)	1.928(10)	2.747(2)	175(3)	*x*, *y* , *z*
O(7)–H(1B)···O(5)	0.826(10)	1.956(15)	2.763(3)	165(4)	*x* – 1, *y*, *z*
O(8)–H(2A)···O(7)	0.830(10)	1.968(13)	2.792(4)	172(4)	*x* + 1, *y*, *z*
3					
O(4)–H(4)···O(1)	0.82	2.30	2.815(6)	121.5	–*x*, –*y* + 2, –*z* + 1
O(5)···O(1)			2.54(2)		–*x*, –*y* + 2, –*z* + 1
4					
N(3)–H(3A)···O(4)	0.86	2.14	2.980(4)	167.0	–*x* + 1, –*y*, –*z* + 2
N(3)–H(3A)···O(5)	0.86	2.63	3.297(4)	135.9	–*x* + 1, –*y*, –*z* + 2
O(7A)···O(2)			2.786(7)		–*x* + 2, –*y*, –*z* + 1
O(7B)···O(2)			2.79(2)		*x*, *y* , *z*

Table S5. The BSA and HSA binding constants and parameters (K_{sv}, k_q, K, n) derived for complexes **1–4**.

BSA				
Compound	**K_{sv}($M^{-1}s^{-1}$)**	**k_q($M^{-1}s^{-1}$)**	**K(M^{-1})**	**n**
Hflmq	$8.27(\pm0.36)\times10^{4}$	$8.26(\pm0.36)\times10^{12}$	6.67×10^{4}	0.66
[Co(flmq)$_2$(MeOH)$_2$], **1**	$5.22(\pm0.32)\times10^{4}$	$5.22(\pm0.32)\times10^{12}$	$1.57(\pm0.11)\times10^{5}$	0.69
[Co(flmq)$_2$(bipy)], **2**	$1.67(\pm0.03)\times10^{5}$	$1.67(\pm0.03)\times10^{13}$	$1.43(\pm0.07)\times10^{5}$	1.05
[Co(flmq)$_2$(phen)], **3**	$1.13(\pm0.03)\times10^{5}$	$1.13(\pm0.03)\times10^{13}$	$7.92(\pm0.12)\times10^{4}$	1.18
[Co(flmq)$_2$(bipyam)], **4**	$1.59(\pm0.09)\times10^{5}$	$1.59(\pm0.09)\times10^{13}$	$9.75(\pm0.32)\times10^{4}$	1.16

HSA				
Compound	**K_{sv}($M^{-1}s^{-1}$)**	**k_q($M^{-1}s^{-1}$)**	**K(M^{-1})**	**n**
Hflmq	$1.00(\pm0.17)\times10^{5}$	$1.00(\pm0.17)\times10^{13}$	2.37×10^{6}	0.67
[Co(flmq)$_2$(MeOH)$_2$], **1**	$3.64(\pm0.25)\times10^{4}$	$3.64(\pm0.25)\times10^{12}$	$5.04(\pm0.26)\times10^{5}$	0.57
[Co(flmq)$_2$(bipy)], **2**	$9.00(\pm0.31)\times10^{4}$	$9.00(\pm0.31)\times10^{12}$	$2.90(\pm0.27)\times10^{4}$	1.78
[Co(flmq)$_2$(phen)], **3**	$3.17(\pm0.14)\times10^{4}$	$3.17(\pm0.14)\times10^{12}$	$8.18(\pm0.35)\times10^{3}$	2.61
[Co(flmq)$_2$(bipyam)], **4**	$2.65(\pm0.11)\times10^{4}$	$2.65(\pm0.11)\times10^{12}$	$6.03(\pm0.33)\times10^{4}$	0.61

Table S6. Comparison of the DNA-, BSA- and HSA-binding constants for Hflmq and complexes **3**, **7**, **10** and **11**.

Compound	**K_b (M^{-1})**	**$K_{(BSA)}$ (M^{-1})**	**$K_{(HSA)}$ (M^{-1})**	**Reference**
Hflmq	$3.53(\pm0.45)\times10^5$	6.67×10^4	2.37×10^6	1
$[Co(flmq)_2(phen)]$, **3**	$1.41(\pm0.22)\times10^5$	$7.92(\pm0.12)\times10^4$	$8.18(\pm0.35)\times10^3$	2
[Cu(flmq)(phen)Cl], **7**	$2.39(\pm0.20)\times10^5$	$3.24(\pm0.26)\times10^5$	$1.28(\pm0.14)\times10^5$	3
[Zn(flmq)(phen)Cl], **10**	$1.22(\pm0.32)\times10^6$	1.68×10^4	6.51×10^4	4
$[Ni(flmq)_2(phen)]$, **11**	$5.38(\pm0.20)\times10^5$	8.85×10^4	1.29×10^5	1

1 K.C. Skyrianou, F. Perdih, I. Turel, D.P. Kessissoglou and G. Psomas, *J. Inorg. Biochem*., 2010, **104**, 740-749.

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3 E. Chalkidou, F. Perdih, I. Turel, D.P. Kessissoglou and G. Psomas, *J. Inorg. Biochem*., 2012, **113**, 55-65.

4 A. Tarushi, K. Lafazanis, J. Kljun, I. Turel, A.A. Pantazaki, G. Psomas and D.P. Kessissoglou, *J. Inorg. Biochem.*, 2013, **121**, 53–65.

(A)

(B)

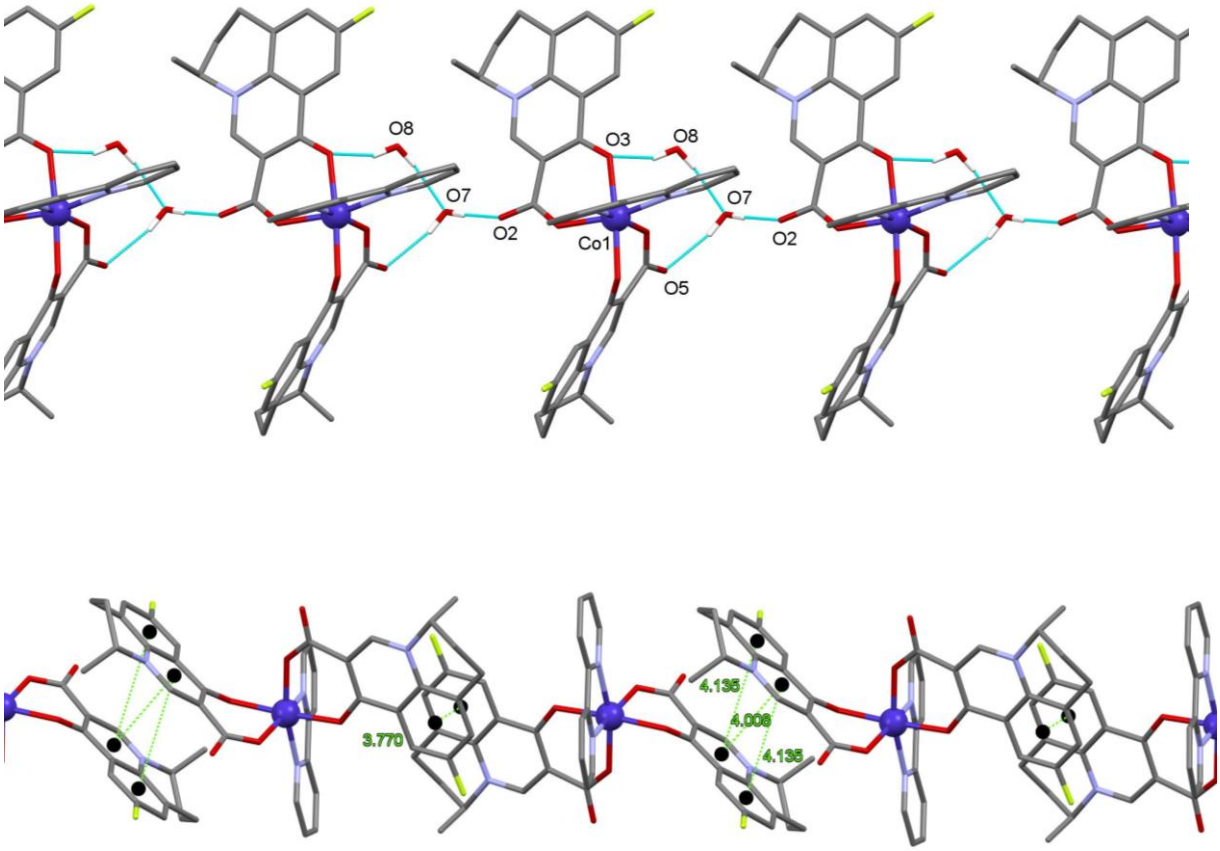


Figure S1. (A) Chain formation through hydrogen bonding and (B) π···π interactions in **2**·2H_2O. Hydrogen atoms not involved in the motif shown have been omitted for clarity.

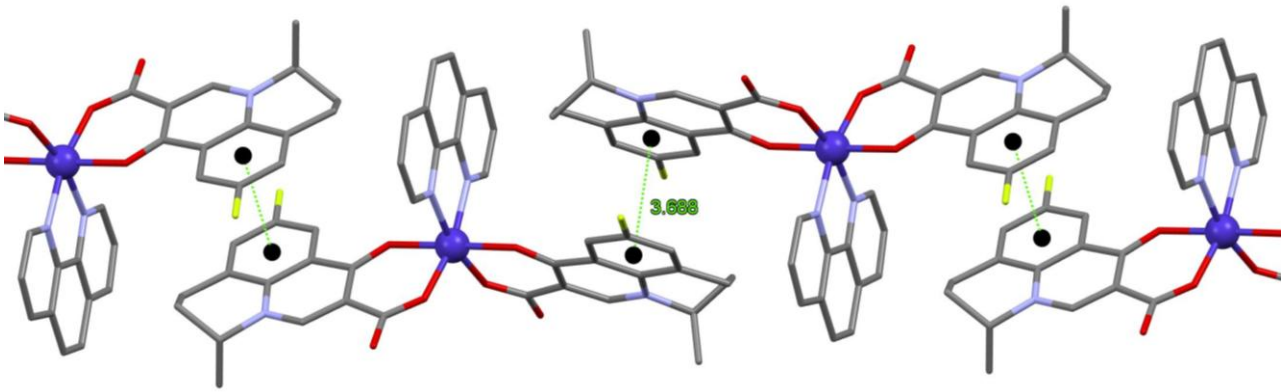
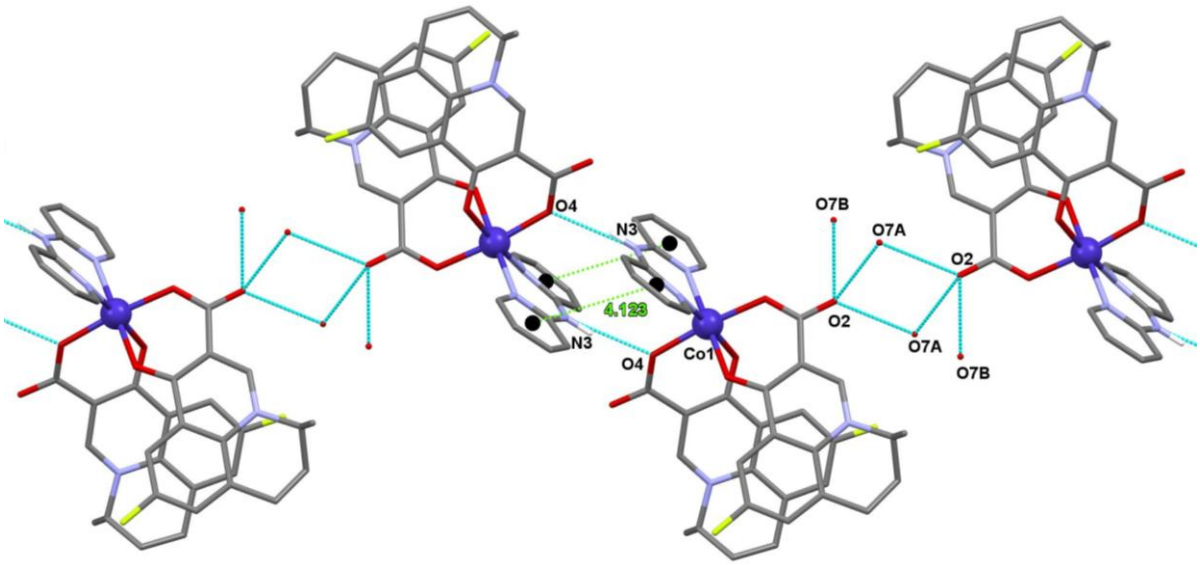


Figure S2. π···π interactions in **3**. Solvate molecules and hydrogen atoms have been omitted for clarity.

(A)



(B)

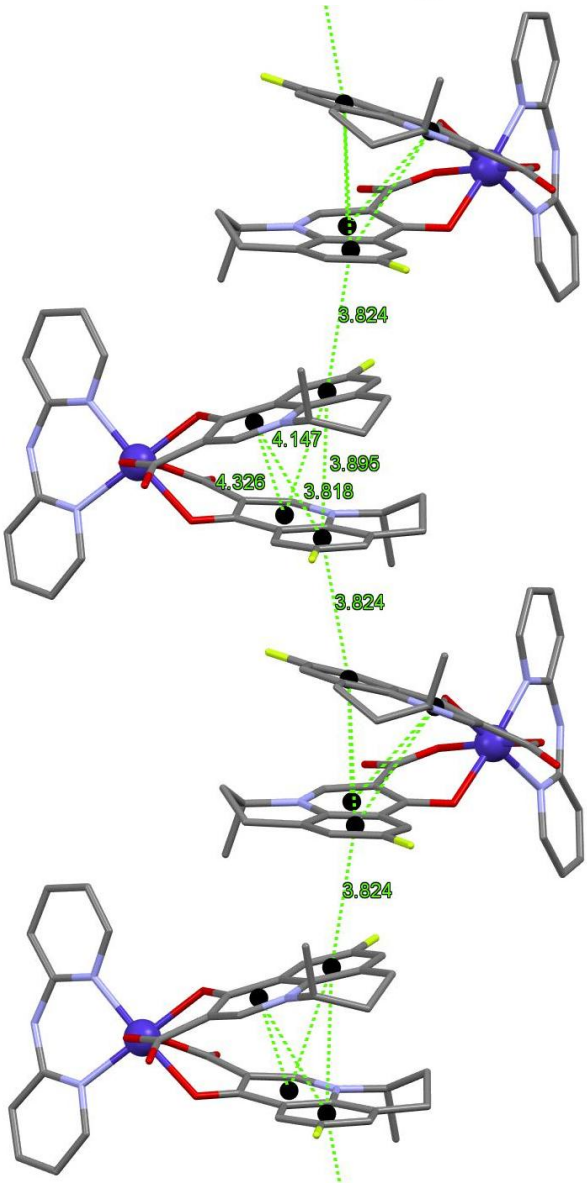


Figure S3. (A) Chain formation through hydrogen bonding enhanced by π···π interactions and (B) π···π stacking interactions in **4**·H_2O**.** Hydrogen atoms not involved in the motif shown have been omitted for clarity.

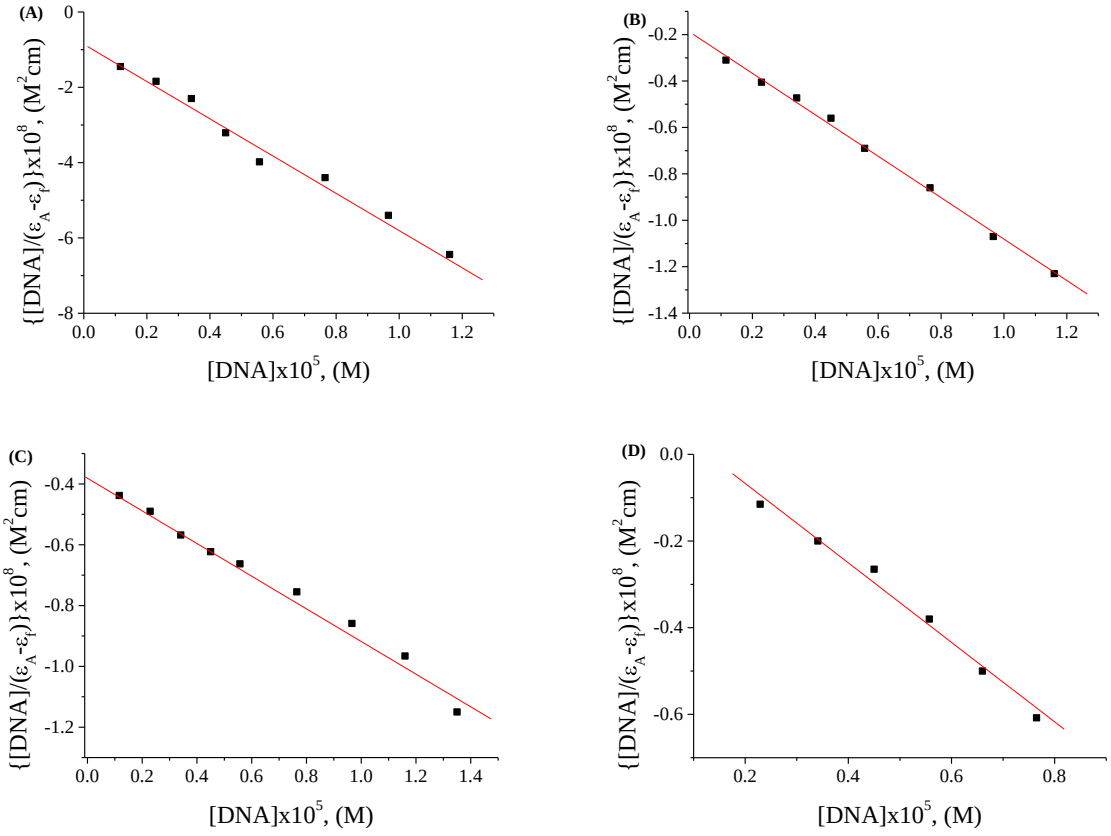


Figure S4. (A)–(D) Plot of $\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)}$ vs [DNA] for complexes **1–4**, respectively.

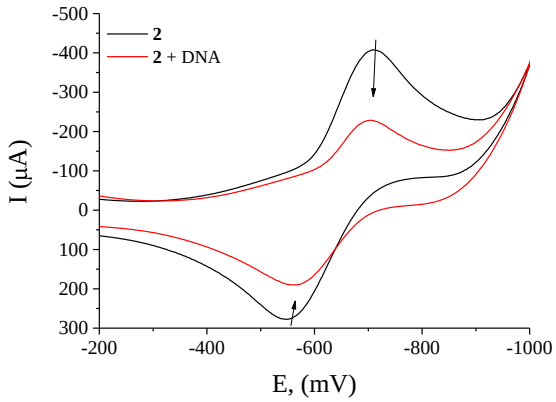


Figure S5. Cyclic voltammogram of 0.4 mM 1/2 dmso/buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) solution of $[Co(flmq)_2(bipy)]$, **2** in the absence or presence of CT DNA. Scan rate = 100 mV s^{-1}. Supporting electrolyte = buffer solution.

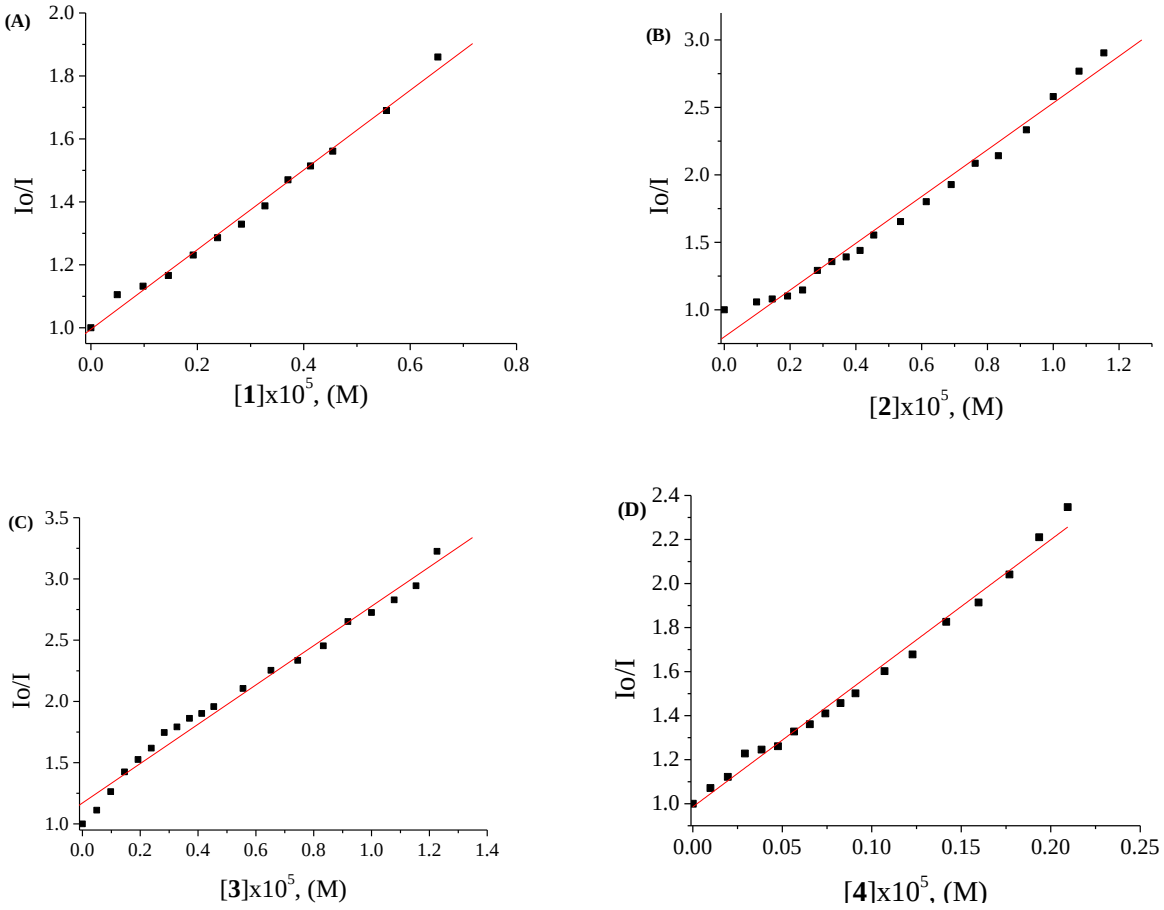


Figure S6. (A)–(D) Stern–Volmer quenching plot of EB bound to CT DNA for complexes **1–4**, respectively.

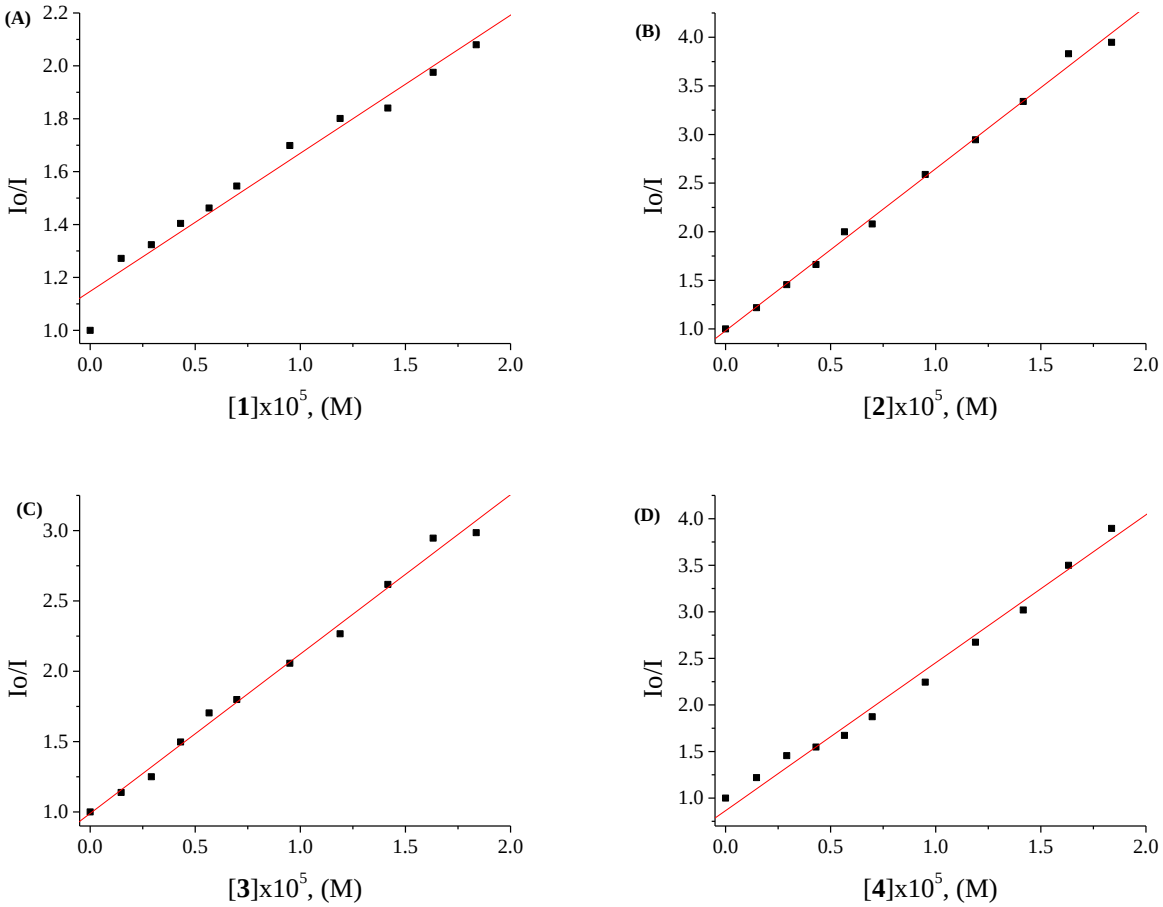


Figure S7. (A)–(D) Stern–Volmer quenching plot of BSA for complexes **1–4**, respectively.

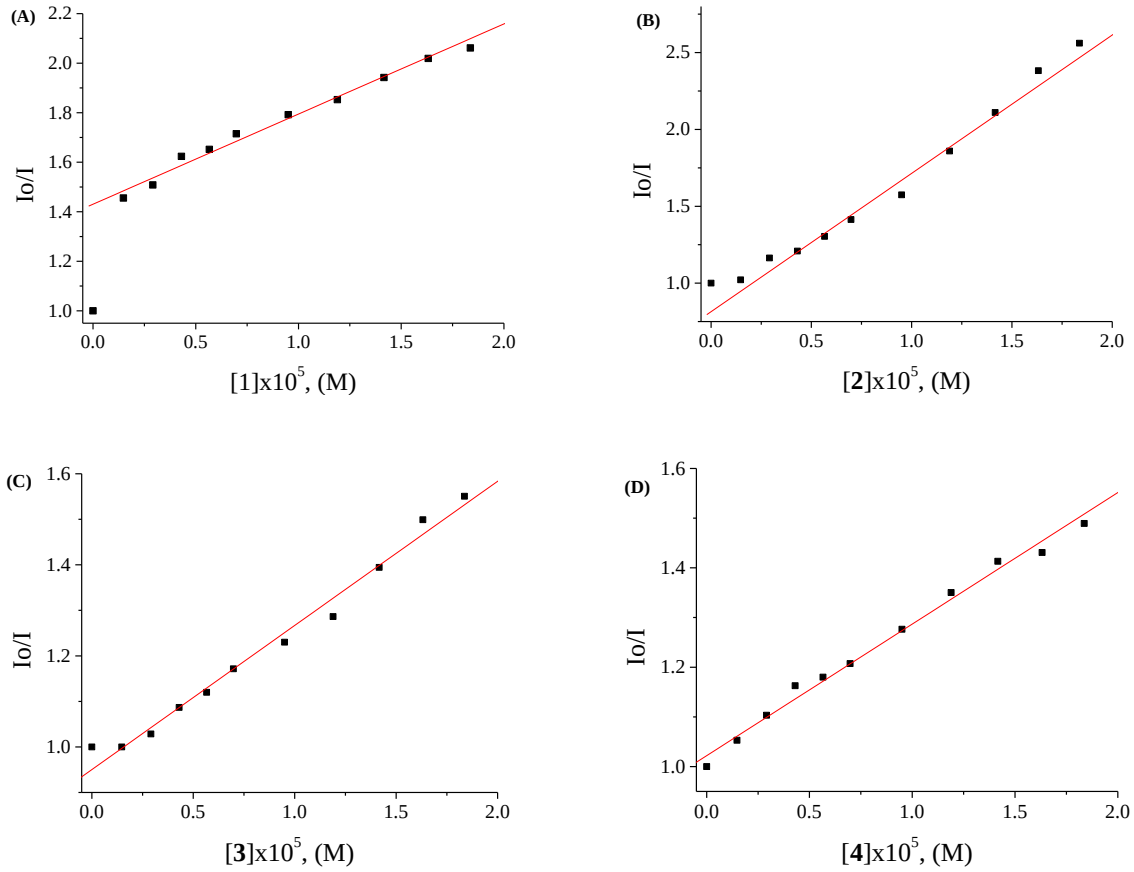


Figure S8. (A)–(D) Stern–Volmer quenching plot of HSA for complexes **1–4**, respectively.

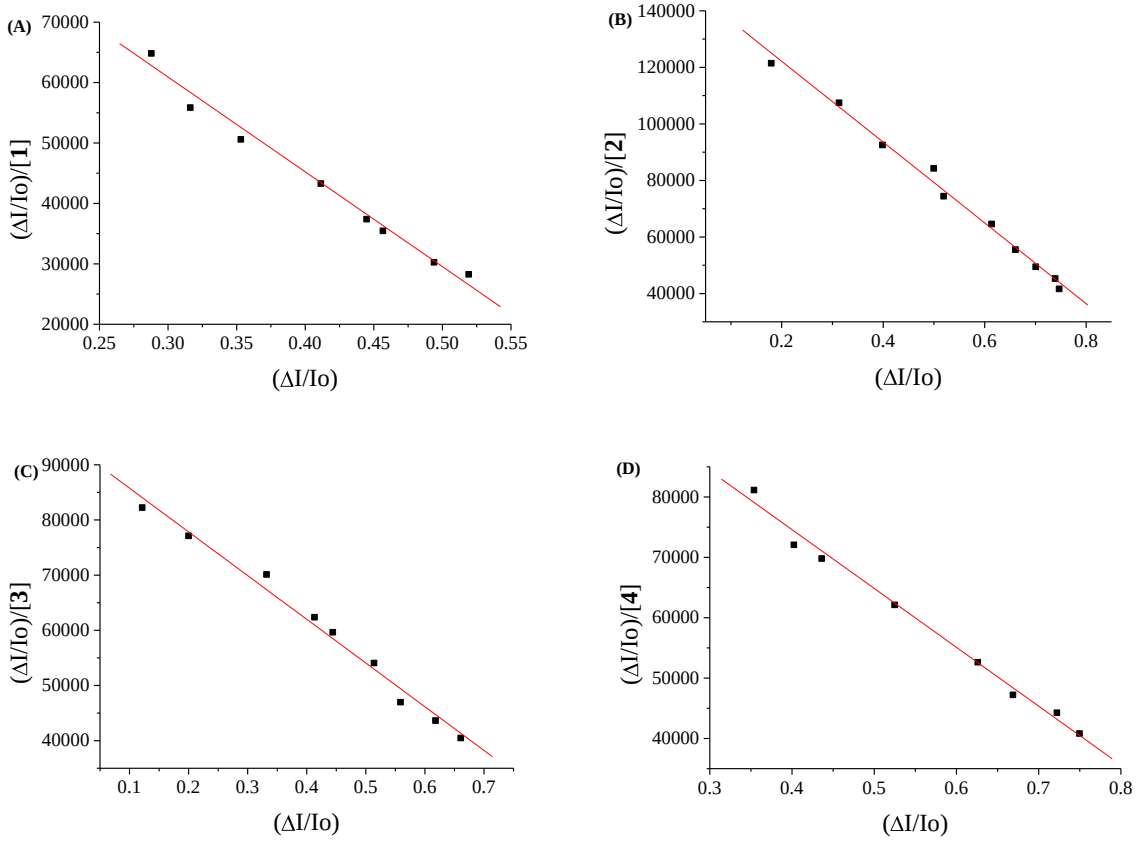


Figure S9. (A)–(D) Scatchard plot of BSA for complexes **1–4**, respectively.

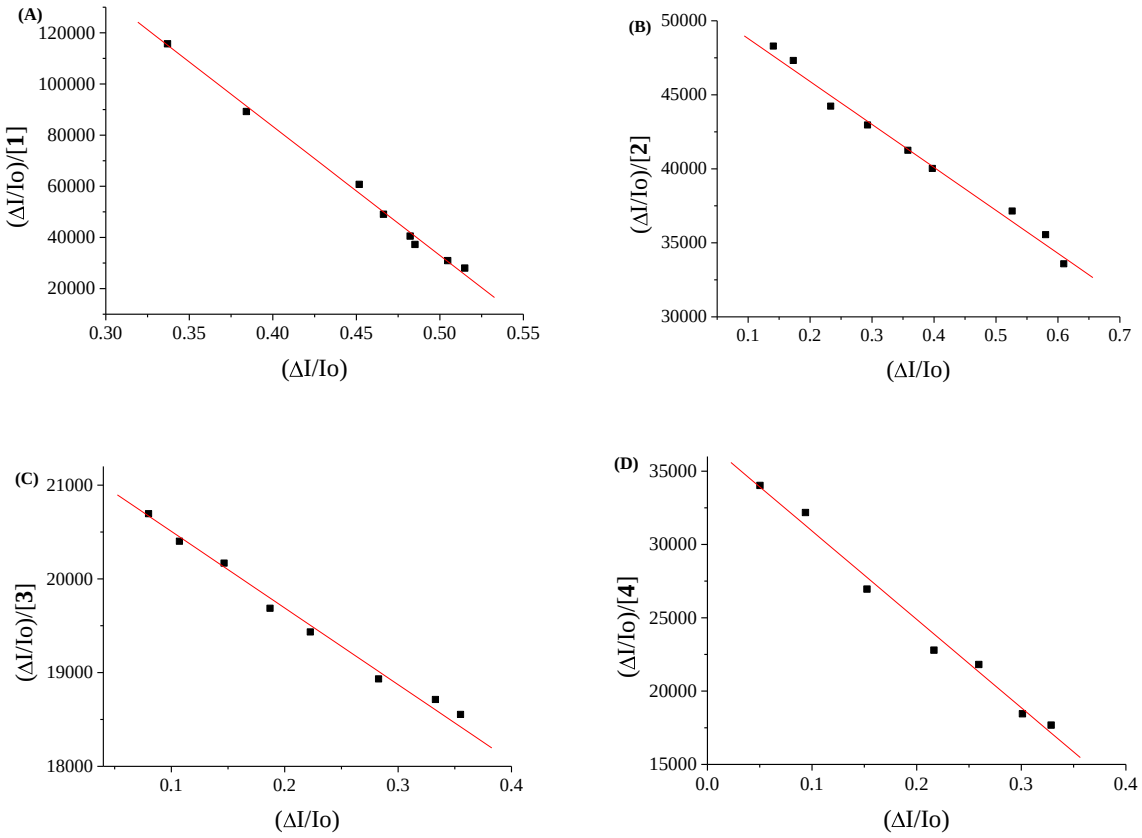


Figure S10. (A)–(D) Scatchard plot of HSA for complexes **1–4**, respectively.