Cobalt(II) complexes with quinolone antimicrobial drug oxolinic acid: Structure and biological perspectives

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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 (complexes **1**-**6**); 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.

3 Y. Wang, H. Zhang, G. Zhang, W. Tao and S. Tang, *J. Luminescence*, 2007, **126**, 211–218.

Table S1. Crystallographic data for complex **2**·3CH_3OH.

	2·3CH_3OH
Empirical formula	$C_{39}H_{40}CoN_4O_{13}$
M_w	831.68
T, K	150(2)
Crystal system	triclinic
Space group	*P–1*
a (Å)	10.247(5)
b (Å)	13.803(5)
c (Å)	14.185(5)
α (°)	105.164(5)
β (°)	101.176(5)
γ (°)	96.925(5)
V (Å^3)	1868.4(13)
Z	2
D_{calc} (g/cm^3)	1.478
μ (mm^{-1})	0.534
F(000)	866
Data collected / unique / [I>2σ(I)]	14270 / 7830 / 5181
R_{int}	0.0593
Restraints / parameters	1 / 524
S	1.063
R_1, wR_2 [I>2σ(I)]	0.0786 / 0.2027
R_1, wR_2 (all data)	0.1233 / 0.2285
Largest diff. peak / hole (e Å^{-3})	1.553 / –0.583

Table S2. The BSA and HSA binding constants and parameters (K_{sv}, k_q, K, n) derived for Hoxo and complexes **1–6**.

HSA				
Complex	**K_{sv} (M^{-1})**	**k_q (M^{-1} s^{-1})**	**K (M^{-1})**	**n**
Hoxo	$6.39(\pm0.26)\times10^4$	$6.39(\pm0.29)\times10^{12}$	$1.13(\pm0.20)\times10^5$	0.60
$[Co(oxo)_2(MeOH)_2]$, **1**	$2.13(\pm0.19)\times10^4$	$2.13(\pm0.19)\times10^{12}$	$1.92(\pm0.10)\times10^5$	0.29
$[Co(oxo)_2(bipy)]$, **2**	$2.97(\pm0.34)\times10^4$	$2.97(\pm0.34)\times10^{12}$	$1.81(\pm0.17)\times10^5$	0.47
$[Co(oxo)_2(bipyam)]$, **3**	$2.21(\pm0.10)\times10^4$	$2.21(\pm0.10)\times10^{12}$	$3.87(\pm0.31)\times10^4$	0.68
$[Co(oxo)_2(phen)]$, **4**	$4.11(\pm0.24)\times10^4$	$4.11(\pm0.24)\times10^{12}$	$2.46(\pm0.20)\times10^4$	1.35
$[Co(oxo)_2(py)_2]$, **5**	$2.18(\pm0.12)\times10^5$	$2.18(\pm0.12)\times10^{13}$	$1.46(\pm0.10)\times10^5$	1.10
$[Co(oxo)_2(4bzpy)_2]$, **6**	$1.85(\pm0.14)\times10^4$	$1.85(\pm0.14)\times10^{12}$	$2.10(\pm0.10)\times10^5$	0.29
BSA				
Complex	**K_{sv} (M^{-1})**	**k_q (M^{-1} s^{-1})**	**K (M^{-1})**	**n**
Hoxo	$5.01(\pm0.22)\times10^4$	$5.01(\pm0.22)\times10^{12}$	$1.09(\pm0.09)\times10^5$	0.75
$[Co(oxo)_2(MeOH)_2]$, **1**	$1.34(\pm0.08)\times10^4$	$1.34(\pm0.08)\times10^{12}$	$1.73(\pm0.14)\times10^5$	0.30
$[Co(oxo)_2(bipy)]$, **2**	$1.05(\pm0.09)\times10^5$	$1.05(\pm0.09)\times10^{13}$	$3.56(\pm0.25)\times10^5$	0.78
$[Co(oxo)_2(bipyam)]$, **3**	$9.69(\pm0.33)\times10^4$	$9.69(\pm0.33)\times10^{12}$	$1.55(\pm0.12)\times10^4$	1.02
$[Co(oxo)_2(phen)]$, **4**	$7.20(\pm0.33)\times10^4$	$7.20(\pm0.33)\times10^{12}$	$3.70(\pm0.31)\times10^4$	1.38
$[Co(oxo)_2(py)_2]$, **5**	$7.09(\pm0.24)\times10^5$	$7.09(\pm0.24)\times10^{13}$	$4.38(\pm0.19)\times10^5$	1.06
$[Co(oxo)_2(4bzpy)_2]$, **6**	$2.25(\pm0.31)\times10^4$	$2.25(\pm0.31)\times10^{12}$	$2.05(\pm0.19)\times10^5$	0.38

Table S3. Binding interactions of [Co(oxo)$_2$(bipy)], **2,** with CT DNA (Atom numbering and bond lengths are derived from PyMol software).

[Co(oxo)$_2$(bipy)] (2)	**CT DNA**		
ligand	**Pyrimidine/ Purine**	**Bond length (Å)**	**Type**
Oxo-O37 (carboxylic)	O3/DC15/B	3.5	Polar
Oxo-O34 (carboxylic)	O3/DC15/B	3.3	Polar
Oxo-O34 (carboxylic)	HN2/DG14/B	3.1	H bond
Oxo-O35(carboxylic)	O4/DG16/B	2.7	Polar
Oxo-O24 (etheric)	HN2/DG12/A	2.5	H bond
Oxo-O24 (etheric)	N3/DG12/A	2.9	Polar
Oxo-O22 (etheric)	N9/DG12/A	2.8	Polar
Oxo-O22 (etheric)	O4/DG12/A	3.8	Polar
Oxo-O8 (etheric)	OP1/DG12/A	2.4	Polar
Oxo-O8 (etheric)	O3/DC11/A	3.3	Polar
Oxo-O6 (etheric)	OP1/DG12/A	2.2	Polar
Oxo-O35(carboxylic)	C4/DG16/B	2.7	Polar
Oxo-C15	C4/DA17/B	3.2	Hydrophobic
Oxo-C4	C5/DG12/A	4.0	Hydrophobic
Bipy-C42	C4/DG16/B	3.1	Hydrophobic
Bipy-C41	C3/DG16/B	3.9	Hydrophobic

Table S4. Binding interactions of [Co(oxo)$_2$(bipy)], **2**, with HSA (Atom numbering and bond lengths are derived from PyMol software).

[Co(oxo)$_2$(bipy)] (2)	**HSA**		
ligand	**Amino acid residue**	**Bond length (Å)**	**Type**
Bipy-N4	NH2/Arg222	2.6	hydrogen bond
Oxo-O42 (etheric)	O/His288	3.3	polar
Oxo-O46 (carbonylic)	NZ/Lys195	3.8	polar
Oxo-O44 (etheric)	NZ/Lys195	3.1	polar
Oxo-O45 (etheric)	C/Asp451	3.2	polar
Oxo-C15	OG/Ser192	3.4	polar
Oxo-C23	N/Tyr452	4.0	polar
Oxo-C8	CE1/His288	3.0	hydrophobic
Oxo-C16	CG/Glu153	3.5	hydrophobic
Oxo-C29	CD/Lys195	3.5	hydrophobic
Oxo-C23	CD1/Tyr452	4.0	hydrophobic
Oxo-C20	CB/Asp451	4.0	hydrophobic
Oxo-C20	CG2/Val455	3.9	hydrophobic
Oxo-C24	CZ2/Trp214	3.0	hydrophobic
Oxo-C30	CH2/Trp214	2.3	hydrophobic
Oxo-C37	CZ/Arg218	2.3	hydrophobic
Oxo-C30	CE/Lys199	2.0	hydrophobic
Bipy-C32	CA/Ala291	3.5	hydrophobic
Bipy-C31	CZ/Arg222	3.8	hydrophobic
Bipy-C36	CZ/Arg222	2.9	hydrophobic
Bipy-C39	CG/Arg218	3.4	hydrophobic
Bipy-C38	CD/Arg218	2.7	hydrophobic

Table S5. Binding interactions of [Co(oxo)$_2$(bipy)], **2,** with BSA (Atom numbering and bond lengths are derived from PyMol software).

[Co(oxo)$_2$(bipy)] (2)	**BSA**		
ligand	**Amino acid residue**	**Bond length (Å)**	**Type**
Oxo-O35 (carboxylic)	NH1/Arg198	1.9	hydrogen bond
Oxo-O35 (carboxylic)	NH2/Arg198	2.4	hydrogen bond
Oxo-N9	NE1/Trp213	2.7	hydrogen bond
Bipy-N43	NH2/Arg198	3.9	hydrogen bond
Oxo-O35 (carboxylic)	HO/Tyr149	3.4	hydrogen bond
Oxo-O6 (etheric)	NH1/Arg194	3.6	hydrogen bond
Oxo-O6 (etheric)	NH2/Arg194	3.8	hydrogen bond
Oxo-O37 (carboxylic)	NH2/Arg256	2.8	hydrogen bond
Oxo-O37 (carboxylic)	HO/Water745	2.9	hydrogen bond
Oxo-O37 (carboxylic)	HO/Water746	3.1	hydrogen bond
Oxo-O8 (etheric)	OD1/Asp450	3.2	polar
Bipy-C47	O/Ala290	3.4	polar
Oxo-O37 (carboxylic)	C/Ser191	3.6	polar
Bipy-C47	CB/Ala290	2.5	hydrophobic
Bipy-C46	CB/Ala290	2.7	hydrophobic
Bipy-C50	CD/Glu291	3.7	hydrophobic
Bipy-C50	CG/Glu291	3.0	hydrophobic
Bipy-C49	CB/Glu291	3.1	hydrophobic
Bipy-C40	CB/Ser191	3.1	hydrophobic
Bipy-C41	C/Ser191	3.6	hydrophobic
Bipy-C41	CD/Arg194	3.0	hydrophobic
Bipy-C41	CB/Arg194	3.5	hydrophobic
Oxo-C1	CZ/Arg194	2.7	hydrophobic
Oxo-C0	CZ/Arg194	3.0	hydrophobic
Oxo-C5	CDArg194	3.6	hydrophobic
Oxo-C13	CD/Arg194	3.9	hydrophobic
Oxo-C36	CE2/Tyr149	3.4	hydrophobic
Oxo-C14	CD1/Trp213	3.1	hydrophobic
Oxo-C35	CZ/Arg198	2.2	hydrophobic
Oxo-C11	CH2/Trp213	3.3	hydrophobic
Oxo-C10	CZ2/Trp213	2.0	hydrophobic
Oxo-C14	CE2/Trp213	2.6	hydrophobic

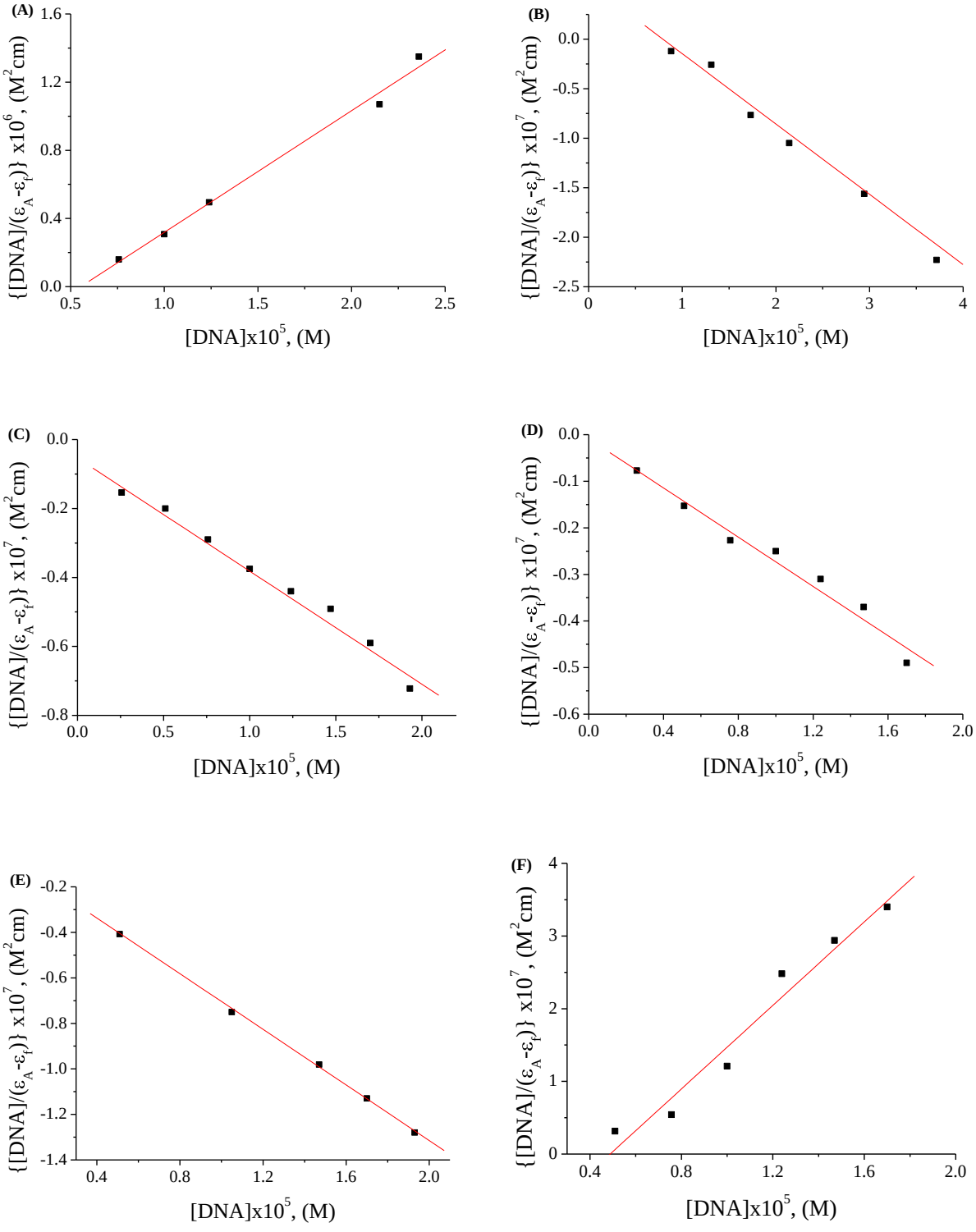

Figure S1. (A)-(F) Plot of $\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)}$ vs [DNA] for complexes **1-6**, respectively.

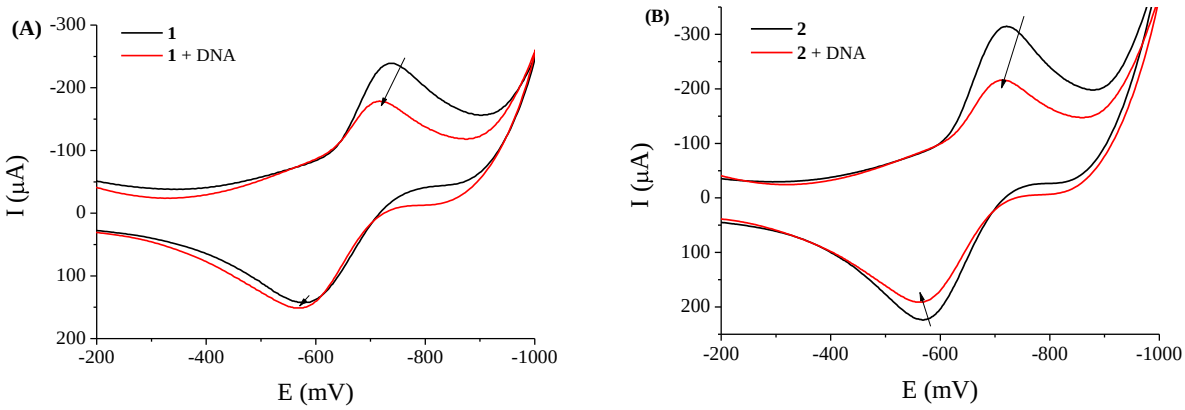

Figure S2. Cyclic voltammogram of 0.4 mM 1/2 dmso/buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH = 7.0) solution of (A) $[Co(oxo)_2(H_2O)_2]$, **1** and (B) $[Co(oxo)_2(bipy)]$, **2** in the absence (black line) or presence (red line) of CT DNA. The arrows show the changes upon addition of CT DNA. Scan rate = 100 mV s^{-1}. Supporting electrolyte = buffer solution.

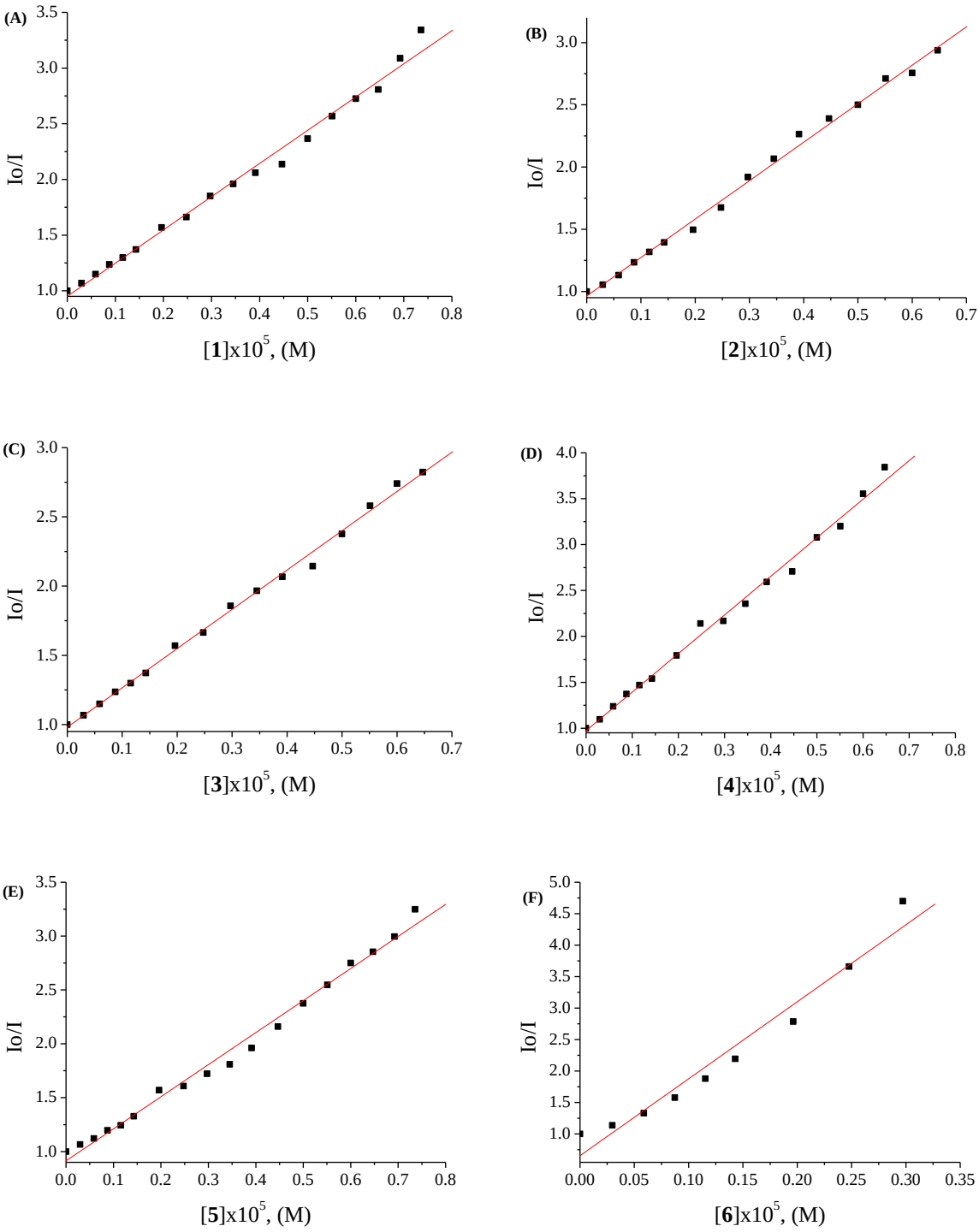

Figure S3. (A)-(F) Stern-Volmer quenching plot of EB bound to CT DNA for complexes **1-6**, respectively.

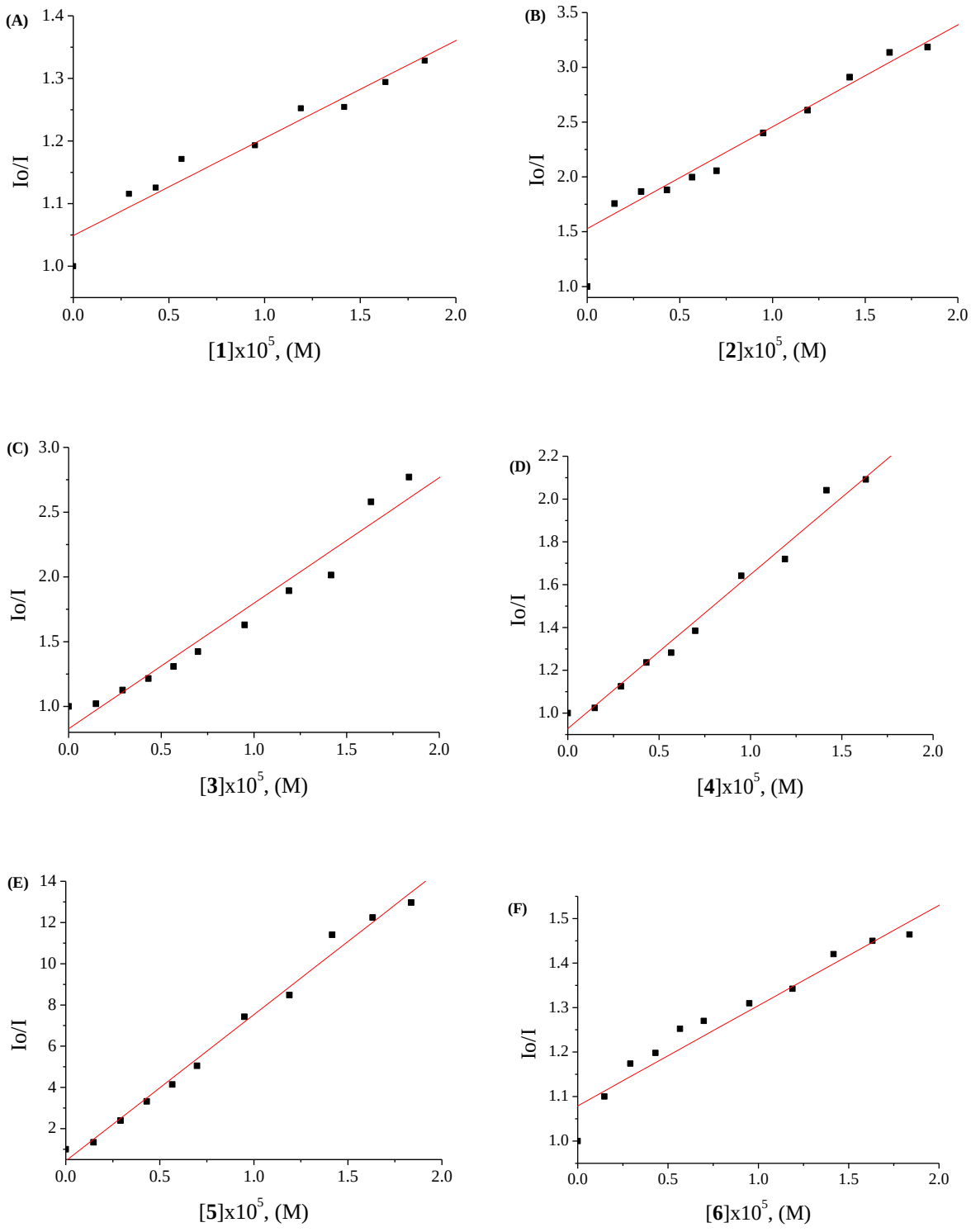

Figure S4. (A)-(F) Stern-Volmer quenching plot of BSA for complexes **1-6**, respectively.

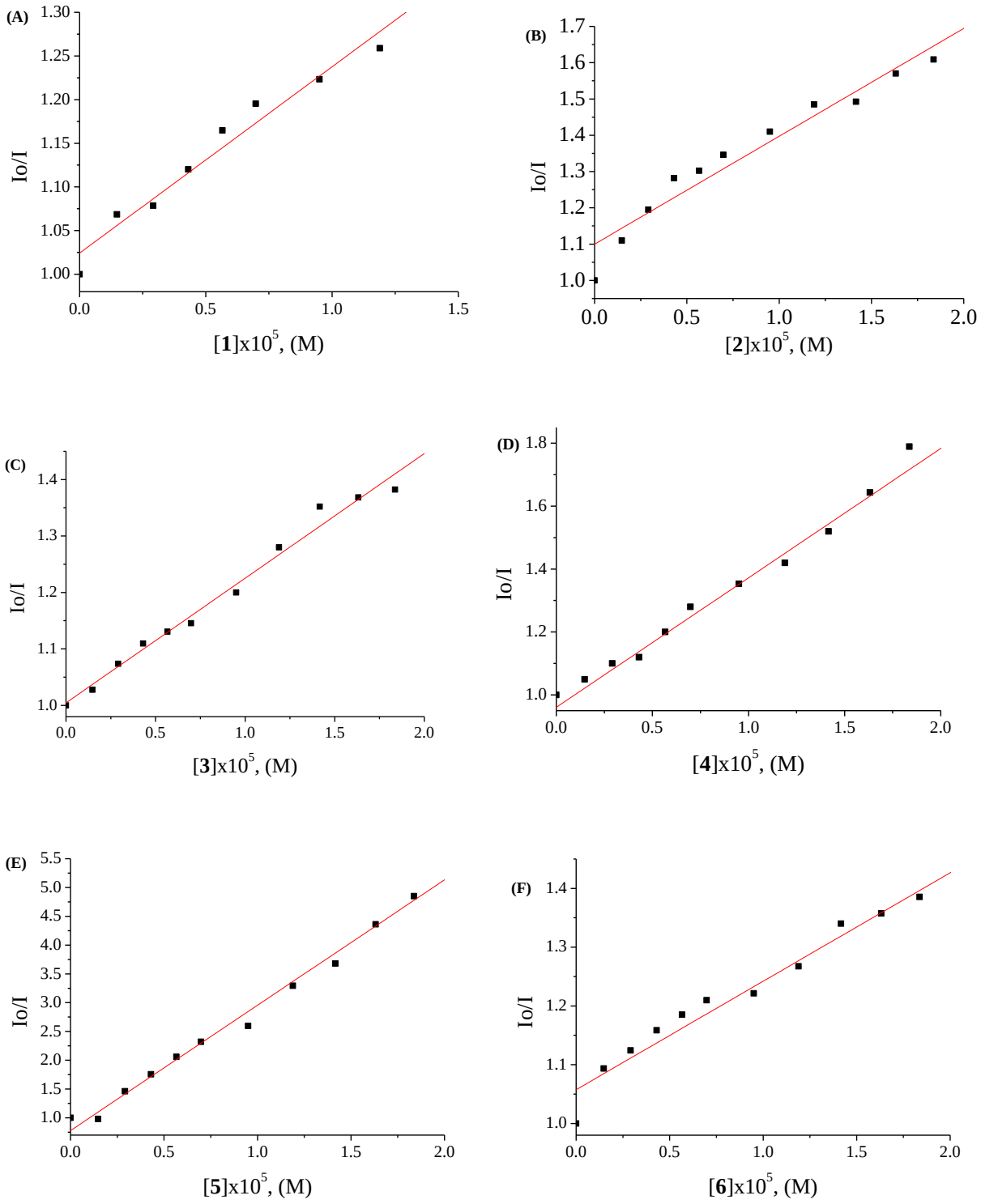

Figure S5. (A)-(F) Stern-Volmer quenching plot of HSA for complexes **1-6**, respectively.

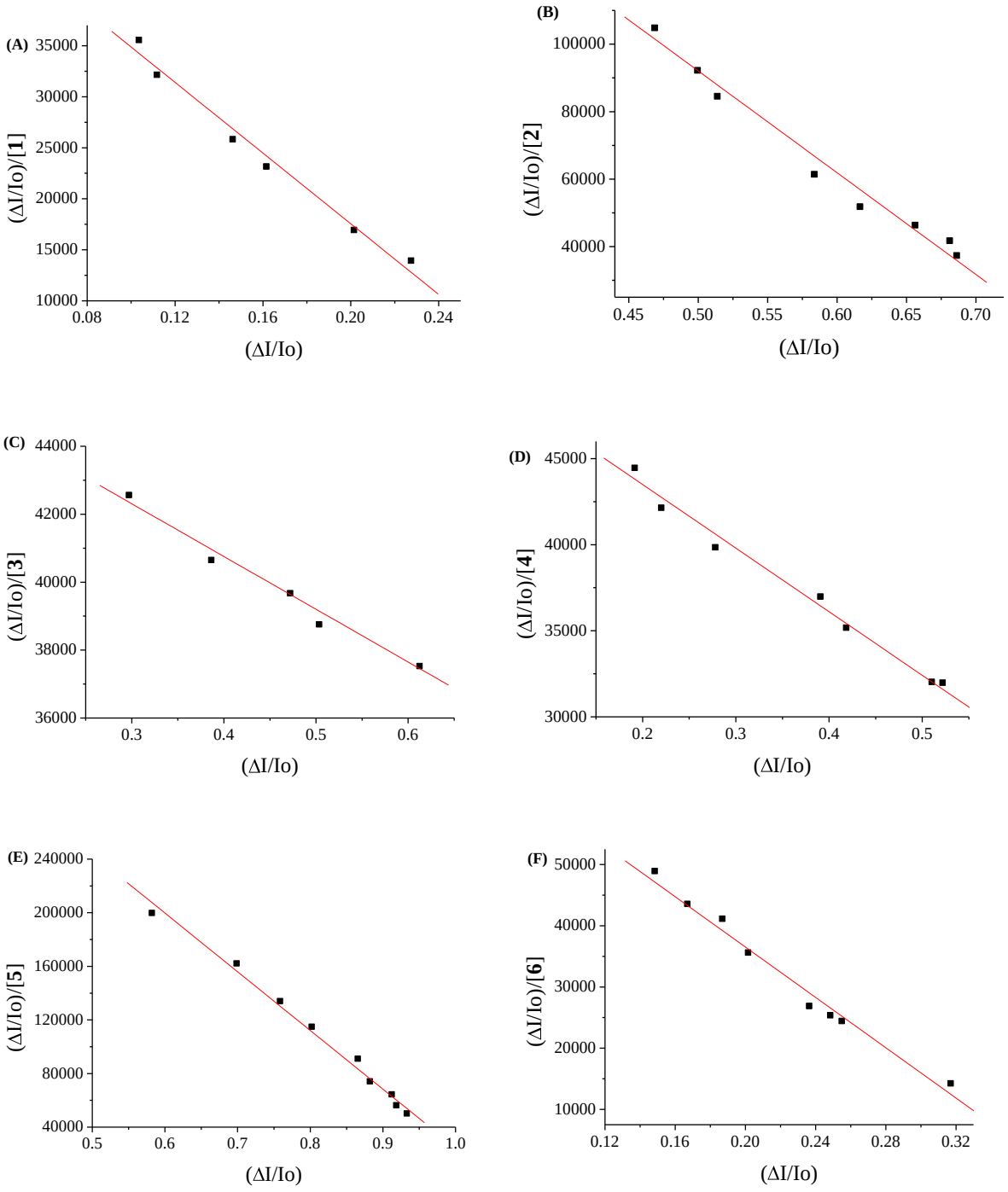

Figure S6. (A)-(F) Scatchard plot of BSA for complexes **1-6**, respectively.

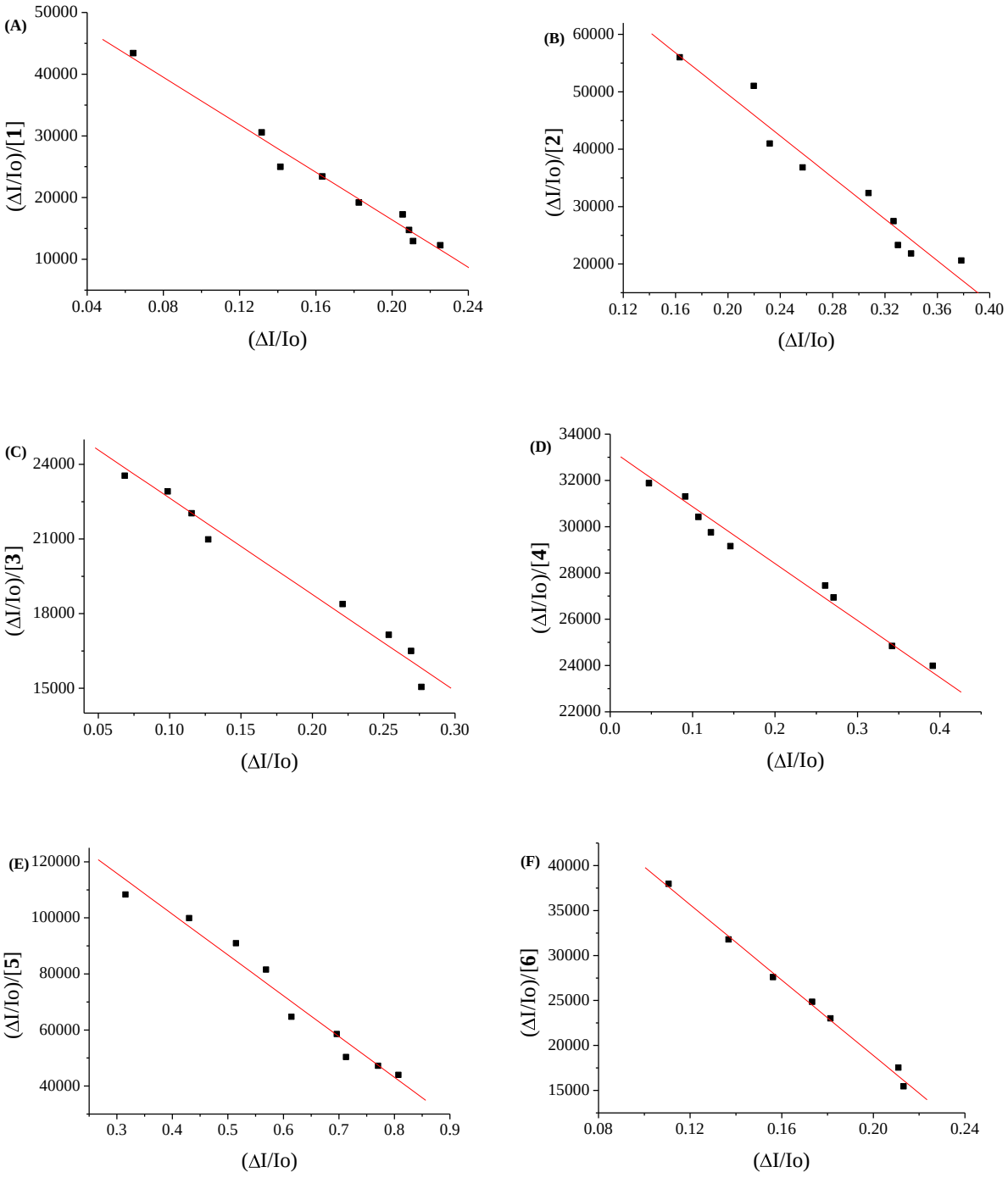

Figure S7. (A)-(F) Scatchard plot of HSA for complexes **1-6**, respectively.

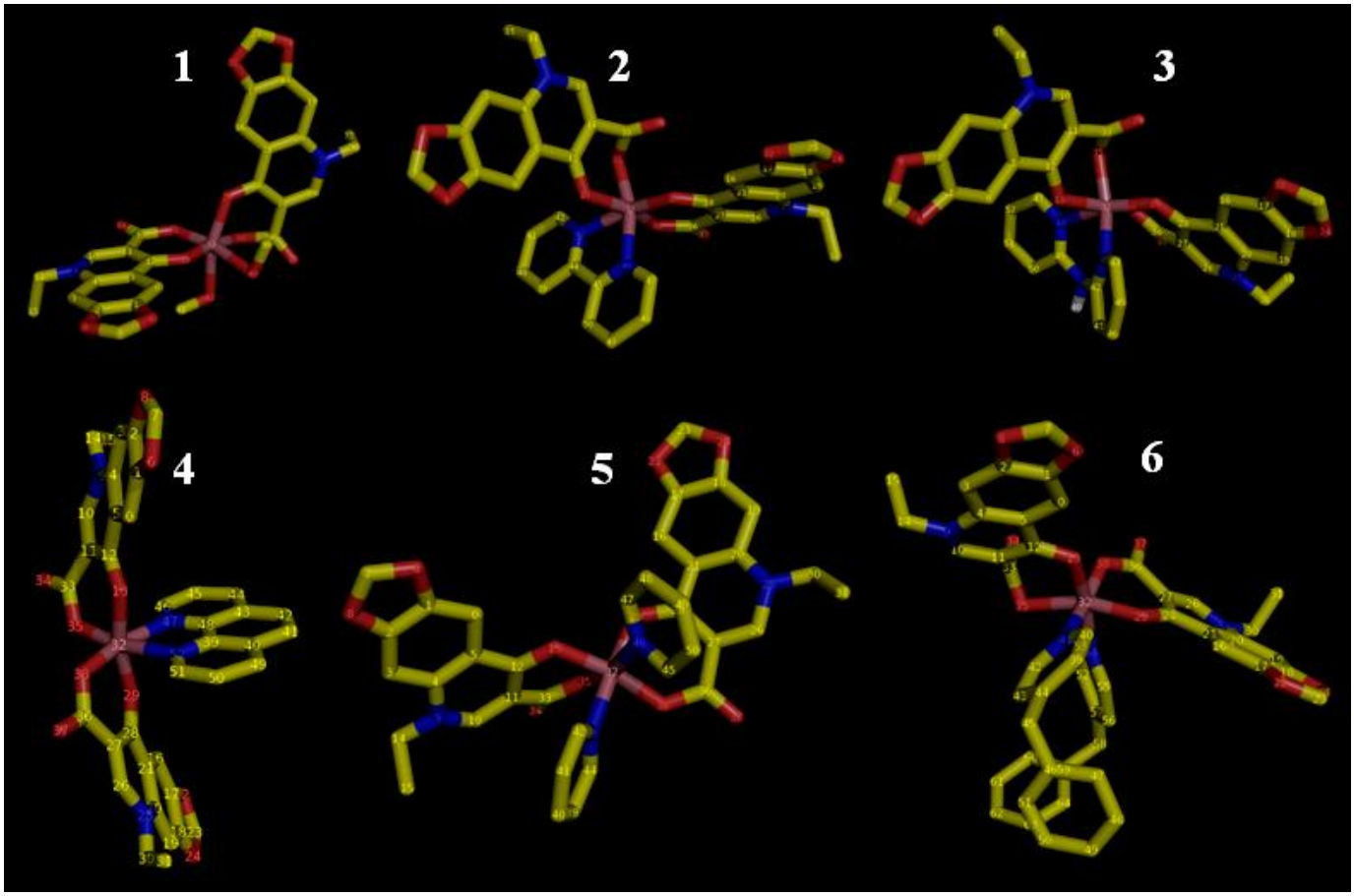

Figure S8. Molecular structure of complexes **1–6** after geometry optimization by Hartree–Fock model with 6-31G* basis set, depicted as semitransparent stick colored by atom type (C, O, N, and Co atoms; yellow, red, blue, and light pink, respectively). Hydrogen atoms are omitted for clarity. Atoms are numbered according to PyMol software.

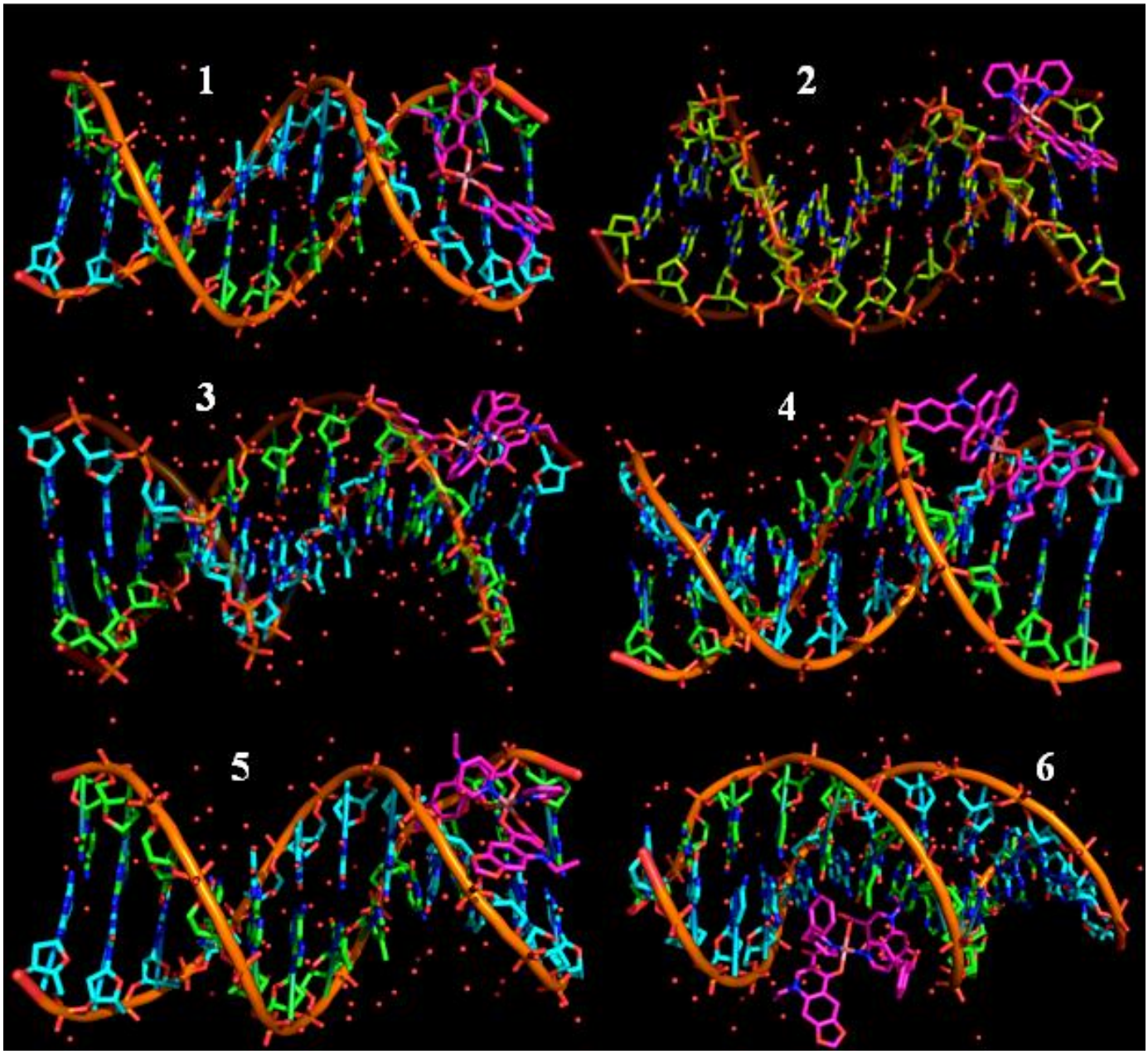

Figure S9. Molecular docking of complexes **1**–**6** in the crystal structure of CT DNA (PDB ID 1BNA). DNA structure is illustrated as cartoon color-coded according to chain (green and light blue C atoms), while superimposed docked molecules are represented in stick model and colored according to atom type (hot pink C atoms). Hydrogen atoms are omitted from all molecules for clarity. The final structure was ray-traced.

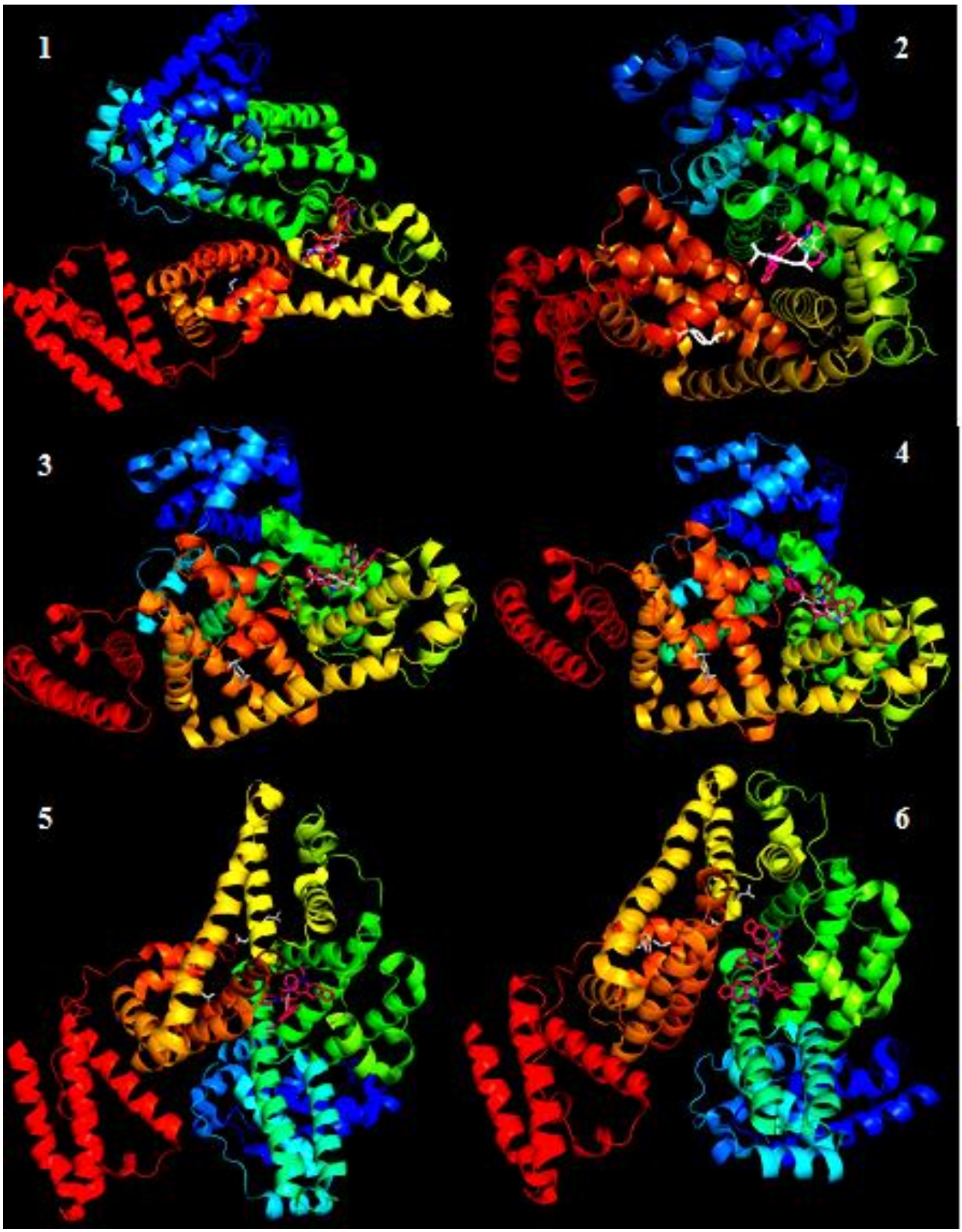

Figure S10. Molecular docking of complexes **1–6** and the co-crystallized drug ibuprofen (IBP) in the crystal structure of HSA target protein (chain A) (PDB ID 2BXG). Target protein is illustrated as cartoon with sub-domains color-coded according to chainbow, while superimposed docked molecules are represented in stick model and colored according to atom type: Complexes **1-6** (hot pink C atoms) and IBP (white C atoms). Dockings of both ligands were performed individually. Hydrogen atoms are omitted from all molecules for clarity.

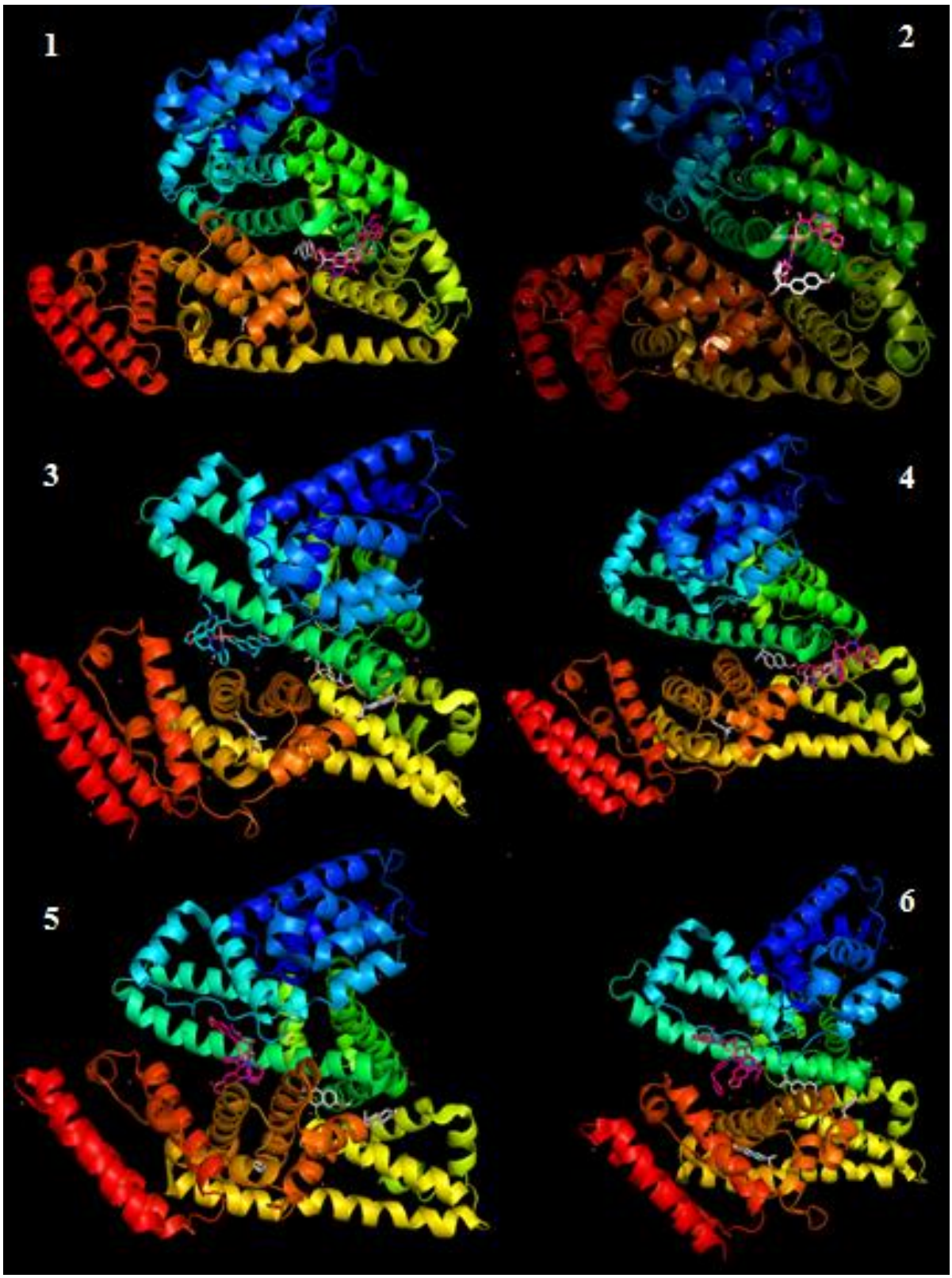

Figure S11. Molecular docking of complexes **1–6** and the co-crystallized drug naproxen (NPS) in the crystal structure of BSA target protein (chain A) (PDB ID 4OR0). Target protein is illustrated as cartoon with sub-domains color-coded according to chainbow, while superimposed docked molecules are represented in stick model and colored according to atom type: Complexes **1–6** (hot pink C atoms) and NPS (white C atoms). Dockings of both ligands were performed individually. Hydrogen atoms are omitted from all molecules for clarity.

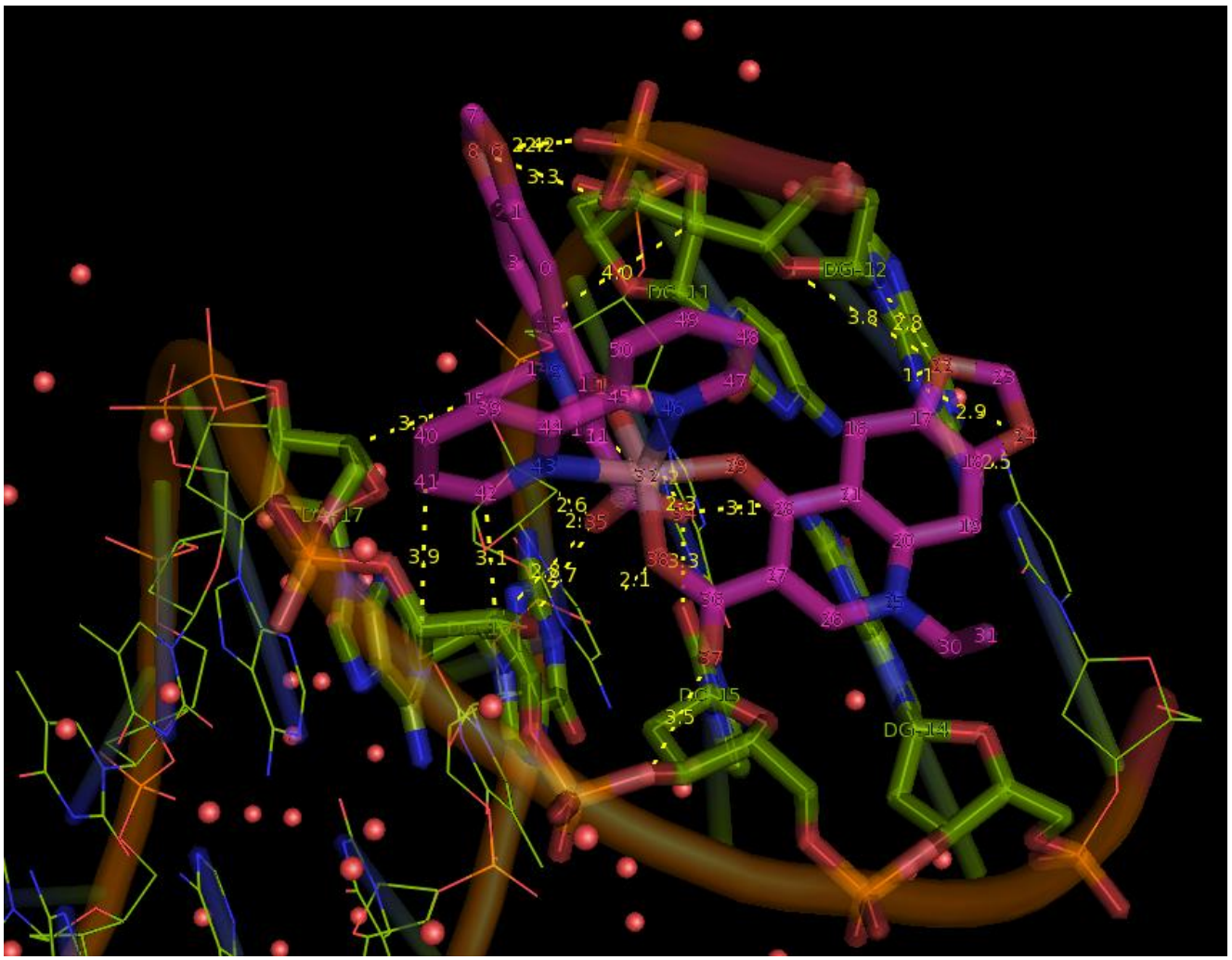

Figure S12. Ligand binding site interactions of [Co(oxo)$_2$(bipy)] **2**, in the crystal structure of CT DNA (PDB ID 1BNA). Yellow dotted lines indicate hydrogen bond, polar and hydrophobic interactions between the docked molecule and the nucleotides of the binding pocket in the minor groove of DNA. Complex **2** is represented in semitransparent stick model colored according to atom type in hot pink and C atoms, while nucleotide molecules rendered in semitransparent stick and line model are colored in green. Hydrogen atoms are omitted from all molecules for clarity. Atoms are numbered according to PyMol software. The final structure was ray-traced.

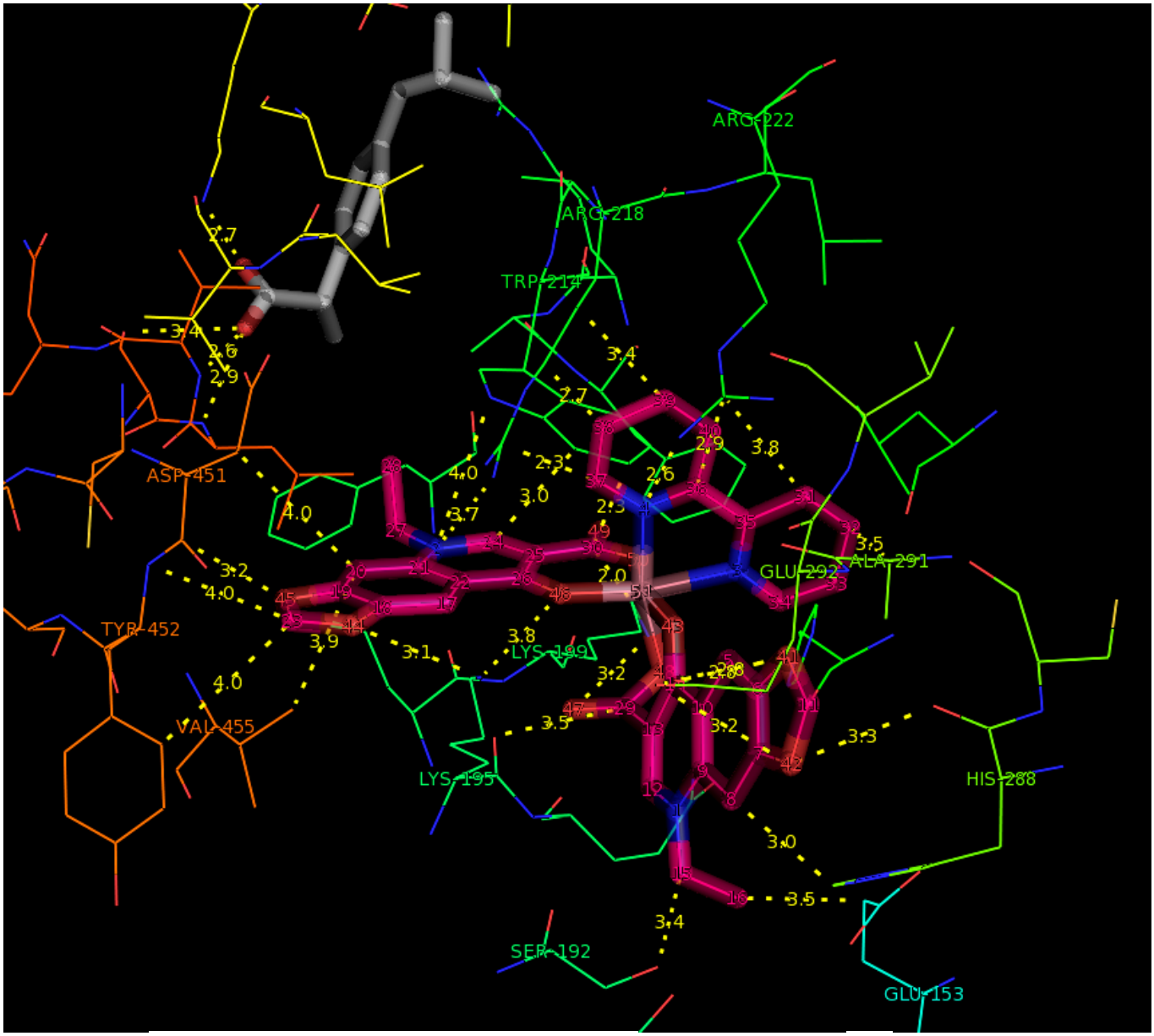

Figure S13. Ligand binding site interactions of complex [Co(oxo)$_2$(bipy)] **2**, and co-crystallized drug ibuprofen (IBP) docked onto HSA target protein (PDB ID 2BXG) (chain A). Both molecules are represented in semitransparent stick model and colored according to atom type in hot pink and white C atoms, respectively. Yellow dotted lines indicate hydrogen bond, polar and hydrophobic interactions between the docked molecules and the amino acid residues (rendered in line model and colored according to chainbow) of the binding pocket. Docking of both ligands was performed individually. Hydrogen atoms are omitted from both molecules for clarity. Atoms are numbered according to PyMol software.

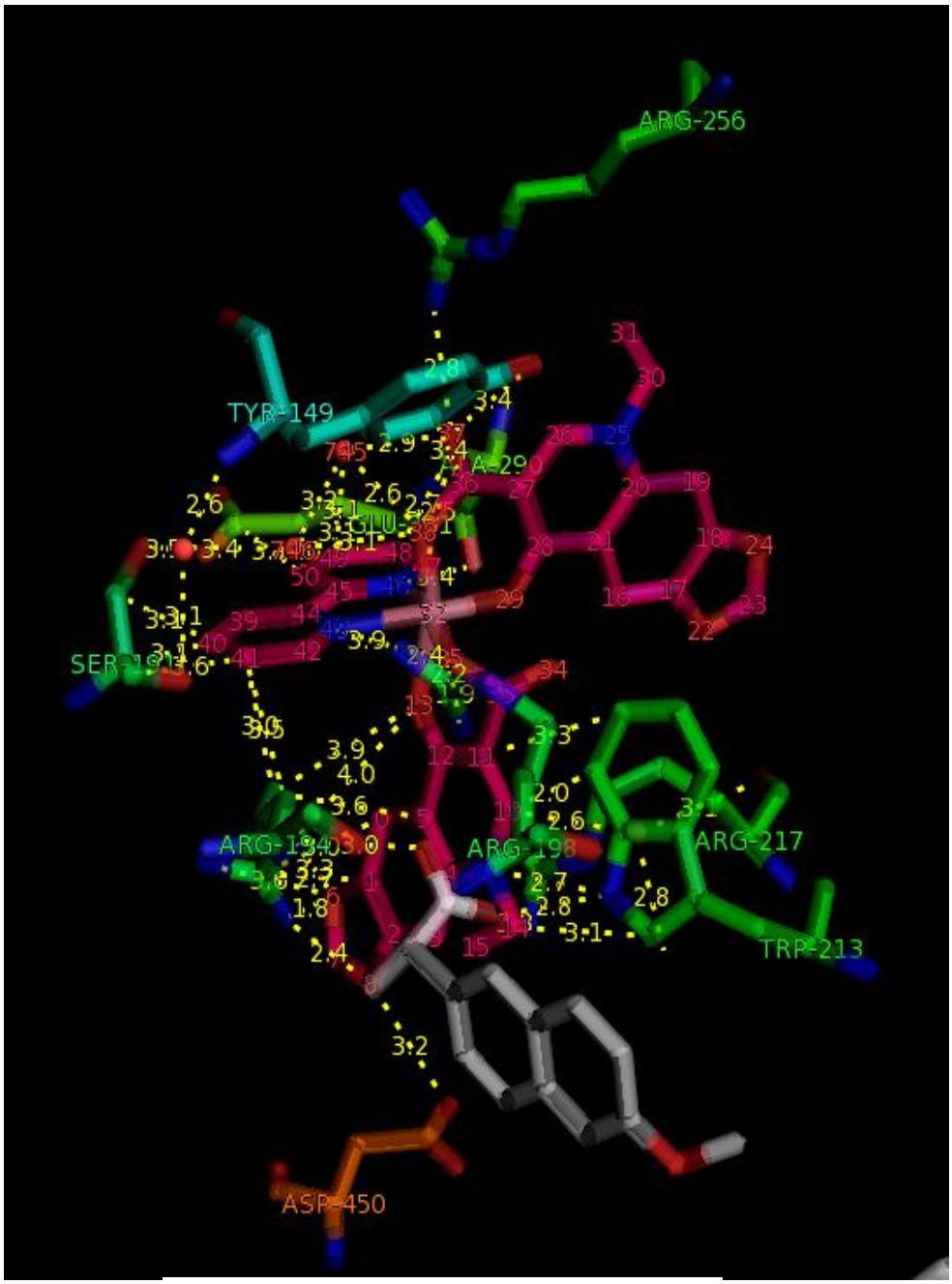

Figure S14. Ligand binding site interactions of complex [Co(oxo)$_2$(bipy)] **2,** and co-crystallized drug naproxen (NPS) in the crystal structure of BSA target protein (PDB ID 4OR0) (chain A). Both molecules (**2** and NPS**)** are represented in semitransparent stick model and colored according to atom type in hot pink and white C atoms, respectively. Yellow dotted lines indicate hydrogen bond, polar and hydrophobic interactions between the docked molecules and the amino acid residues (rendered in line model and colored according to chainbow) of the binding pocket. Docking of both ligands was performed individually. Hydrogen atoms are omitted from both molecules for clarity. Atoms are numbered according to PyMol software.