A mononuclear zinc(II) complex and a tetranuclear copper(II) azametallacoronate with (*E*)−2−((2−(quinazolin−4−yl)hydrazono)methyl)phenol: Structure and biological activity

Chrisoula Kakoulidou,[a] Antonios G. Hatzidimitriou,[a] Konstantina C. Fylaktakidou,[b,*] George Psomas,[a,*]

[a] *Department of General and Inorganic Chemistry, Faculty of Chemistry, Aristotle University of Thessaloniki, GR–54124 Thessaloniki, GREECE.*

[b] *Laboratory of Organic Chemistry, Faculty of Chemistry, Aristotle University of Thessaloniki, GR−54124 Thessaloniki, GREECE.*

Supplementary material

[*] Corresponding author's e–mails:

kfylakta@chem.auth.gr (K.C. Fylaktakidou); gepsomas@chem.auth.gr (G. Psomas)

Content

EXPERIMENTAL PROTOCOLS

S1 Binding studies with CT DNA

In order to study the interaction of the complex with DNA, the compound was initially dissolved in DMSO (1 mM). Mixing of such solutions with the aqueous buffer solutions DNA or BSA used in the studies never exceeded 5% DMSO (v/v) in the final solution, which was needed due to low aqueous solubility of most compounds. In all experiments, the effect of DMSO on the data has been taken into consideration and the appropriate corrections have been performed. The interaction of the compound with CT DNA was monitored by UV−vis spectroscopy, and viscosity measurements, and *via* competitive studies with EB by fluorescence emission spectroscopy.

S1.1 Binding study with CT DNA by UV−vis spectroscopy

The interaction of the compound with CT DNA has been studied by UV−vis spectroscopy in order to investigate the possible binding mode to CT DNA and to calculate the DNA−binding constant (K_b).

The UV−vis spectra of a CT DNA (0.15−0.17 mM) solution in buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) were recorded in the presence of each compound at diverse [compound]/[DNA] mixing ratios (= *r*). Control experiments with DMSO were performed and no changes in the spectra of CT DNA were observed.

The K_b constant (in M^{-1}) of the compounds was determined by the Wolfe−Shimer equation (equation S1)[1] and the plots [DNA]/($\varepsilon_A-\varepsilon_f$) *versus* [DNA] using the UV−vis spectra of the compound (10 μM) recorded for a constant concentration with increasing amounts of CT DNA for diverse [compound]/[DNA] mixing ratios (= *r*). According to the Wolfe−Shimer equation (equation S1):

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

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. K_b is given by the ratio of slope to the y intercept in plots [DNA]/($\varepsilon_A-\varepsilon_f$) *versus* [DNA].

S1.2 CT DNA−binding studies by viscosity measurements

The interaction of compound with DNA was evaluated *via* the study of the CT DNA viscosity ([DNA] = 0.1 mM) in a buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the presence of increasing amounts of the compound (up to the value of r = 0.36). The obtained data are presented as $(\eta/\eta_0)^{1/3}$ *versus* r, where η is the viscosity of DNA in the presence of the compound, and η_0 is the viscosity of DNA alone in buffer solution.

S1.3 EB−displacement studies

The competition of the complex with EB was investigated by fluorescence emission spectroscopy to examine whether the compound can displace EB from its DNA−EB adduct. The CT DNA−EB complex was formed by pre−treating 40 μM EB and 46 μM CT DNA in buffer (150 mM NaCl and 15 mM trisodium citrate at pH 7.0). The possible displacement of EB by the compound and subsequently the intercalating effect was studied by the stepwise addition of a certain amount of the solution of each compound into the solution of the CT DNA−EB adduct. The solutions were excited

at 540 nm and the emission was monitored from 550−700 nm with λ_{max} = 592−594 nm and the effect of the addition of the compound to the CT−DNA EB solution was recorded. The compound did not display any fluorescence emission bands at room temperature in solution or in the presence of CT DNA or EB under the same experimental conditions ($\lambda_{excitation}$ = 540 nm); therefore, the observed quenching of the EB−DNA solution may be attributed to the displacement of EB from its EB−DNA adduct.

The Stern−Volmer constants (K_{SV}, in M^{-1}) were calculated according by the linear Stern−Volmer equation (equation S2)[2] and the respective plots Io/I *versus* [compound]:

$$\frac{Io}{I} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \qquad \text{(equation S2)}$$

where Io and I are the emission intensities of the EB−DNA solution in the absence and the presence of the compound, respectively, τ_o = the average lifetime of the emitting system without the quencher and k_q = the quenching constant. Taking τ_o = 23 ns as the fluorescence lifetime of the EB−DNA adduct,[3] the quenching constant (k_q, in $M^{-1}s^{-1}$) of the compound was calculated according to equation S3:[2]

$$K_{SV} = k_q\tau_o \qquad \text{(equation S3)}$$

S2 Plasmid DNA cleavage experiments

The reaction mixtures (20 μL) containing supercoiled circular pBR322 plasmid DNA stock solution (Form I, 50 μM/base pair, ~500 ng), compounds, and Tris buffer (25 μM, pH 6.8) in Pyrex vials were incubated for 30 min at 37 °C and centrifuged under aerobic conditions at room temperature.

After addition of the gel−loading buffer [6x Orange DNA Loading Dye 10 mM Tris−HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol, and 60 mM EDTA, by Fermentas], the reaction mixtures were loaded on a 1% agarose gel with EB staining. The electrophoresis tank was attached to a power supply at a constant current (75 V for 30 min). The gel was visualized by the Mupid−ONE LED Illuminator and photographed by a Nikon Digital Camera D3400. Quantification of DNA−cleaving activities was performed by integration of the optical density as a function of the band area using the program "Image J" available at the site http://rsb.info.nih.gov/ij/download.html.

The ss% and ds% damages were calculated according to the equations S4 and S5:

$$ss\% = \frac{FormII}{(FormI+FormII+FormIII)} \times 100 \qquad \text{(equation S4)}$$

$$ds\% = \frac{FormIII}{(FormI+FormII+FormIII)} \times 100 \qquad \text{(equation S5)}$$

where, as Form II we consider Form II of each series minus Form II of the irradiated control DNA and as Form I, we consider Form I of each series. The amount of supercoiled DNA was multiplied by factor of 1.43 to account for reduced EB intercalation into supercoiled DNA.[4]

S3 Antioxidant activity assay

The antioxidant activity of the compound was evaluated *via* the ability to scavenge *in vitro* free radicals such as DPPH and ABTS and to reduce H_2O_2. All the experiments were carried out at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

S3.1 Determination of the reducing activity of the radical DPPH

To an ethanolic solution of DPPH (0.1 mM) an equal volume solution of the compound (0.1 mM) in ethanol was added. Absolute ethanol was also used as control solution. The absorbance at 517 nm was recorded at room temperature after 30 and 60 min in order to examine the possible existence of a potential time−dependence of the DPPH radical scavenging activity.[5] The DPPH−scavenging activity of the compound was expressed as the percentage reduction of the absorbance values of the initial DPPH solution (DPPH%). NDGA and BHT were used as reference compounds.

S3.2 Assay of radical cation ABTS−scavenging activity

The ABTS assay was performed to determine the activity of the compound to scavenge the radical cation ABTS. Initially, a water solution of ABTS was prepared (2 mM). ABTS radical cation ($ABTS^{+\cdot}$) was produced by the reaction of ABTS stock solution with potassium persulfate (0.17 mM) and the mixture was stored in the dark at room temperature for 12−16 h before its use. The ABTS was oxidized incompletely because the stoichiometric reaction ratio of ABTS and potassium persulfate is 1:0.5. The absorbance became maximal and stable only after more than 6 h of reaction although the oxidation of the ABTS started immediately. The radical was stable in this form for more than 2 days when allowed to stand in the dark at room temperature. Afterwards, the $ABTS^{+\cdot}$ solution was diluted in ethanol to an absorbance of 0.70 at 734 nm and 10 μL of diluted compound or standards (0.1 mM) in DMSO were added. The absorbance was recorded out exactly 1 min after initial mixing.[5] The ABTS radical scavenging activity was expressed as the percentage inhibition of the absorbance of the initial ABTS solution (ABTS%). Trolox was used as an appropriate standard.

S3.3 Reduction of hydrogen peroxide

The ability of the compound to reduce hydrogen peroxide (H_2O_2) was estimated according to the method described in the literature [6]. The reaction mixture contained 20 μL of each of the tested compound (0.1 mM) and 5 μL H_2O_2 solution (40 mM) in phosphate buffer (50 mM, pH 7.4). The absorbance was measured at 230 nm after 10 min. The antioxidant activity (reduction of H_2O_2) of the compound was expressed as the percentage decrease of the initial H_2O_2 solution (H_2O_2%). L−ascorbic acid (or vitamin C) was used as a standard.

S4 Albumin−binding studies

S4.1 Interaction with BSA

In order to investigate if the compound can bind to carrier protein like serum albumins, we carried out albumin binding study by tryptophan fluorescence quenching experiments using BSA (3 μM) in buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0). The quenching of the emission intensity of tryptophan residues of BSA at 343 nm was monitored using the compound as quenchers with increasing concentration.[2] The fluorescence emission spectra of the compound were also recorded with λ_{ex} = 295 nm; in case that an additional emission band appeared the BSA−fluorescence emission spectra were corrected by subtracting the spectra of the compound. The influence of the inner−filter effect on the measurements was evaluated by equation S6:[7]

$$I_{corr} = I_{meas} \times 10^{\frac{\varepsilon(\lambda_{exc})cd}{2}} \times 10^{\frac{\varepsilon(\lambda_{em})cd}{2}} \qquad \text{(equation S6)}$$

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 compound.[7]

The Stern−Volmer and Scatchard graphs are used to study the interaction of the compound with BSA. According to Stern−Volmer quenching equation (equation S2), where Io = initial tryptophan fluorescence intensity of BSA, I = tryptophan fluorescence intensity of BSA after the addition of the quencher, k_q = quenching constant, K_{SV} = Stern−Volmer constant, τ_o = average lifetime of BSA without the quencher, and, taking as fluorescence lifetime (τ_o) of tryptophan in BSA at around 10^{-8} s,[2] K_{SV} (in M^{-1}) can be obtained by the slope of the diagram Io/I *versus* [compound] (Stern−Volmer plots), and subsequently the quenching constant (k_q, in $M^{-1}s^{-1}$) may be calculated from equation S3.

From the Scatchard equation (equation S6):

$$\frac{\Delta I / Io}{[Q]} = nK - K\frac{\Delta I}{Io} \qquad \text{(equation S7)}$$

where n is the number of binding sites per albumin and K is the BSA−binding constant (K, in M^{-1}) is calculated from the slope in plots (ΔI/Io)/[compound] *versus* (ΔI/Io) and n is given by the ratio of y intercept to the slope [8].

S4.2 Competitive BSA−fluorescence studies with warfarin and ibuprofen

The competitive studies with warfarin or ibuprofen (site−markers)[9] were performed by tryptophan fluorescence quenching experiments using a fixed concentration of BSA and site markers (3 μM) in buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0). The fluorescence emission spectra were recorded in the presence of increasing amounts of the compounds as quenchers with an excitation wavelength of 295 nm. The Scatchard equation (equation S7)[8] and plots were applied on the corrected BSA−fluorescence emission spectra to determine the BSA−binding constant of the compounds in the presence of warfarin or ibuprofen.

S5 References

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TABLES

Table S1. Experimental crystallographic details for the compounds.

	H_2L	Complex **1**	Complex **2**
Crystal data			
Chemical formula	$C_{15}H_{12}N_4O$	$C_{63}H_{58}N_{16}O_8Zn_2$	$C_{72}H_{84}Cu_4N_{20}O_{16}$
M_r	264.29	1298.02	1739.77
Crystal system	Orthorhombic	Monoclinic	Tetragonal
Space group	*Pbca*	$P2_1/n$	$I4_1/a$
Temperature (K)	295	295	295
a, *b*, *c* (Å)	8.2408(14), 13.887(3), 22.884(5)	20.8047(10), 11.9286(8), 26.7212(12)	19.931(3), 19.931(3), 20.830(4)
β (°)	90	111.086 (7)	90
V (Å^3)	2618.9 (9)	6187.4 (7)	8275 (3)
Z	8	4	4
Radiation type	Mo *K*α	Mo *K*α	Mo *K*α
μ (mm^{-1})	0.09	0.84	1.09
Crystal size (mm)	0.20 × 0.15 × 0.11	0.15 × 0.03 × 0.02	0.18 × 0.17 × 0.12
Data collection			
Diffractometer	Bruker Kappa Apex2		
Absorption correction	Numerical Analytical Absorption (De Meulenaer & Tompa, 1965)		
T_{min}, T_{max}	0.99, 0.99	0.98, 0.98	0.83, 0.88
No. of measured reflections	23389	65715	13533
No. of independent reflections	2494	11348	3963
No. of observed [$I > 2.0\sigma(I)$] reflections	1844	8368	2721
R_{int}	0.031	0.062	0.040
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.611	0.607	0.611
Refinement			
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.036, 0.057, 1.00	0.050, 0.068, 1.00	0.058, 0.097, 1.00
No. of reflections	1844	8368	2721
No. of parameters	181	802	250
No. of restraints	−		28
H−atom treatment	H−atom parameters constrained		
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.12, −0.11	0.43, −0.39	0.39, −0.49

Table S2. Selected bond lengths (Å) and angles (°) for H_2L.

Bond	**Length (Å)**	**Bond**	**Length (Å)**
O1—C11	1.3596 (17)	N2—C2	1.4031 (16)
N1—C1	1.3523 (16)	N3—N4	1.4046 (14)
N1—C8	1.3796 (15)	N3—C8	1.2882 (15)
N2—C1	1.2798 (16)	N4—C9	1.2847 (15)
Bonds	**Angle (°)**	**Bonds**	**Angle (°)**
C1—N1—C8	122.14 (11)	N2—C2—C7	122.46 (11)
C1—N2—C2	115.26 (11)	C7—C8—N1	114.00 (10)
N4—N3—C8	114.97 (10)	C7—C8—N3	120.50 (10)
N3—N4—C9	110.98 (10)	N1—C8—N3	125.49 (11)
N1—C1—N2	126.84 (12)	C10—C11—O1	121.63 (12)
N2—C2—C3	118.31 (11)	O1—C11—C12	118.13 (13)

Table S3. Hydrogen bonds (lengths in Å, angles in °) for the compounds.

D*—H···*A	***D*—H (Å)**	**H···*A* (Å)**	***D*···*A* (Å)**	***D*—H···*A* (º)**	**Symmetry code**
H_2L					
N1—H12···N2^{i}	0.90	2.10	2.8869 (19)	146	(i) *x*+1/2, −*y*+1/2, −*z*+1
O1—H11···N4	0.85	1.98	2.7185 (19)	144	
1					
N2—H21···O2^{i}	0.85	1.93	2.767 (5)	167	(i) −*x*+3/2, *y*+1/2, −*z*+3/2
O5—H51···O1	0.82	2.07	2.889 (5)	173	
O88—H881···O1	0.81	2.11	2.925 (5)	177	
O5—H52···O6	0.82	2.11	2.789 (5)	141	
N6—H61···O88^{ii}	0.84	2.10	2.923 (5)	167	(ii) −*x*+1, −*y*+1, −*z*+1
O8—H81···O88^{ii}	0.81	2.20	2.962 (5)	157	(ii) −*x*+1, −*y*+1, −*z*+1
N10—H101···O4^{iii}	0.85	1.90	2.732 (5)	165	(iii) −*x*+3/2, *y*−1/2, −*z*+1/2
O6—H884···O3	0.82	1.95	2.766 (5)	180	
N14—H885···O5^{iv}	0.85	1.92	2.761 (5)	173	(iv) −*x*+2, −*y*+1, −*z*+1
2					
O3—H32···O1	0.85	2.03	2.877 (15)	175	
O6—H61···O3	0.84	2.18	2.796 (15)	130	
O6—H62···O4	0.85	2.16	2.819 (15)	134	
O3—H215···N3^{iv}	0.86	2.18	2.993 (15)	157	(iv) *y*+1/4, −*x*+5/4, *z*+1/4

Table S4. Selected bond lengths (Å) and angles (°) for complexes **1A** and **1B**.

	1A		**1B**
Bond	**Length (Å)**	**Bond**	**Length (Å)**
Zn1—N1	2.271 (3)	Zn2—N9	2.262 (3)
Zn1—N4	2.101 (3)	Zn2—N12	2.115 (2)
Zn1—N5	2.235 (3)	Zn2—N13	2.193 (3)
Zn1—N8	2.123 (3)	Zn2—N16	2.143 (3)
Zn1—O1	2.114 (2)	Zn2—O3	2.098 (2)
Zn1—O2	2.105 (2)	Zn2—O4	2.085 (2)

Bonds	**Angle (°)**	**Bonds**	**Angle (°)**
N1—Zn1—N4	73.77 (10)	N9—Zn2—N12	73.18 (9)
N1—Zn1—N5	85.81 (10)	N9—Zn2—N13	93.20 (10)
N4—Zn1—N5	99.72 (10)	N12—Zn2—N13	103.80 (10)
N1—Zn1—N8	92.04 (10)	N9—Zn2—N16	91.45 (10)
N4—Zn1—N8	165.41 (10)	N12—Zn2—N16	164.29 (10)
N5—Zn1—N8	75.38 (11)	N13—Zn2—N16	73.18 (11)
N1—Zn1—O1	159.43 (9)	N9—Zn2—O3	160.53 (9)
N4—Zn1—O1	86.69 (9)	N12—Zn2—O3	88.08 (9)
N5—Zn1—O1	91.35 (9)	N13—Zn2—O3	86.08 (10)
N8—Zn1—O1	106.97 (9)	N16—Zn2—O3	106.87 (9)
N1—Zn1—O2	94.51 (9)	N9—Zn2—O4	93.92 (9)
N4—Zn1—O2	97.69 (10)	N12—Zn2—O4	97.60 (10)
N5—Zn1—O2	161.95 (10)	N13—Zn2—O4	158.58 (9)
N8—Zn1—O2	86.57 (10)	N16—Zn2—O4	86.46 (10)
O1—Zn1—O2	94.41 (9)	O3—Zn2—O4	93.73 (9)

Table S5. Selected bond lengths (Å) and angles (°) for complex **2**.

Bond	**Length (Å)**	**Bond**	**Length (Å)**
Cu1—N1	1.962 (7)	Cu1—O1	1.905 (6)
Cu1—N4	1.967 (7)	Cu1—O2	2.663 (11)
Cu1—N2^{i}	2.027 (7)		
Cu1…Cu1^{i}	5.797	Cu1…Cu1^{ii}	6.574

Bonds	**Angle (°)**	**Bonds**	**Angle (°)**
N2^{i}—Cu1—N1	96.9 (3)	N4—Cu1—O1	94.6 (3)
N2^{i}—Cu1—N4	175.9 (3)	N2^{i}—Cu1—O2	93.5 (3)
N1—Cu1—N4	79.0 (3)	N1—Cu1—O2	84.1 (4)
N2^{i}—Cu1—O1	89.5 (3)	N4—Cu1—O2	85.8 (3)
N1—Cu1—O1	169.9 (3)	O1—Cu1—O2	103.5 (4)
Cu1…Cu1^{i}…Cu1^{ii}	69.08		

Symmetry codes: (i) −*y*+5/4, *x*+1/4, −*z*+5/4; (ii) *y*−1/4, −*x*+5/4, −*z*+5/4.

FIGURES

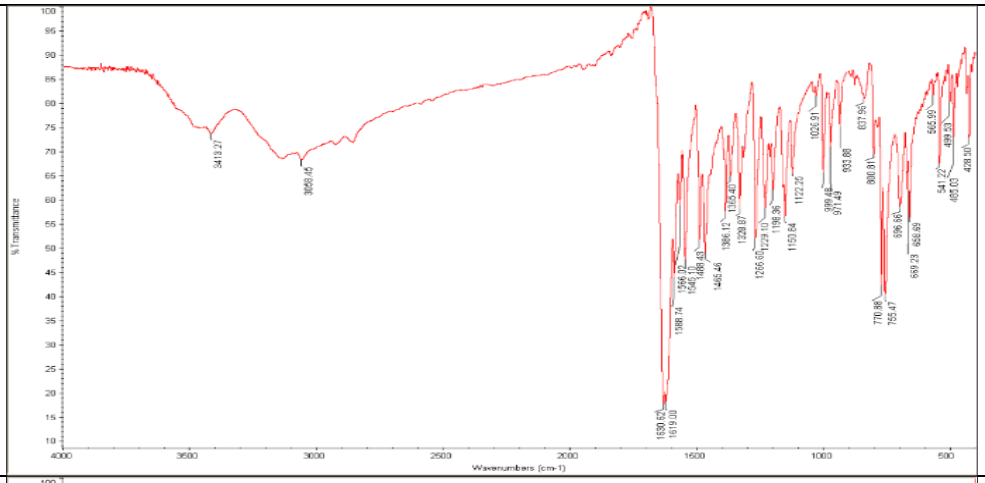
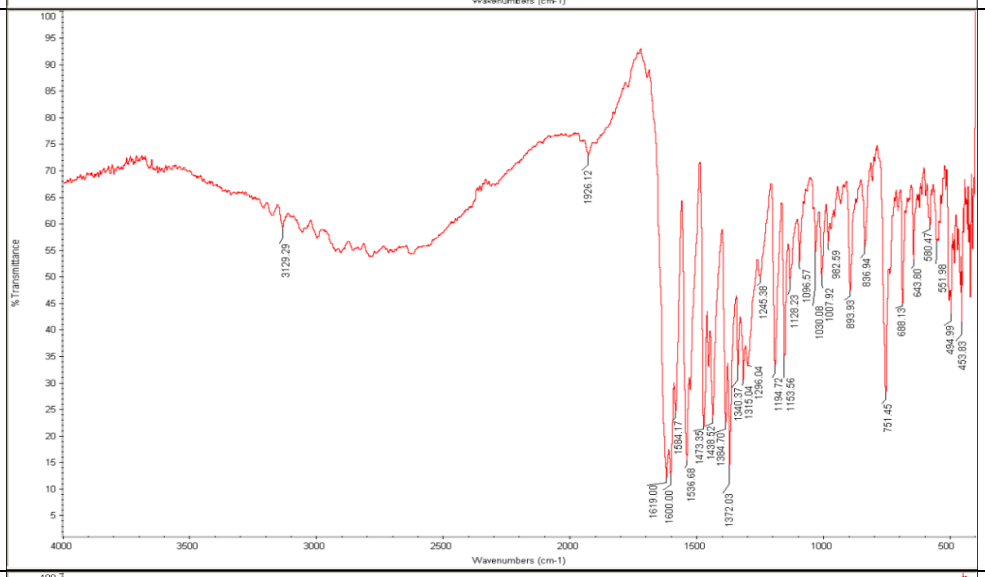
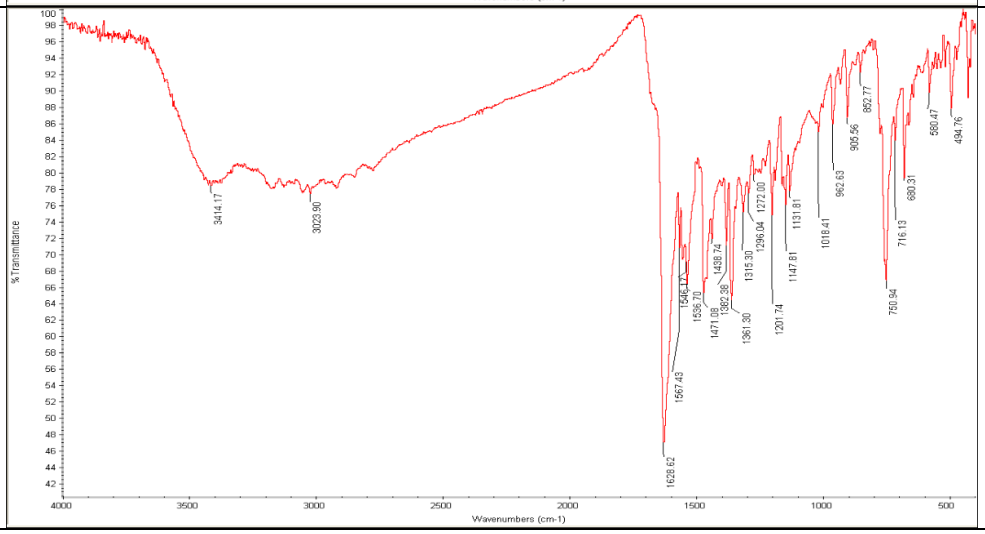
H_2L	
Complex **1**	
Complex **2**	

Figure S1. IR spectra (KBr pellets) of the compounds.

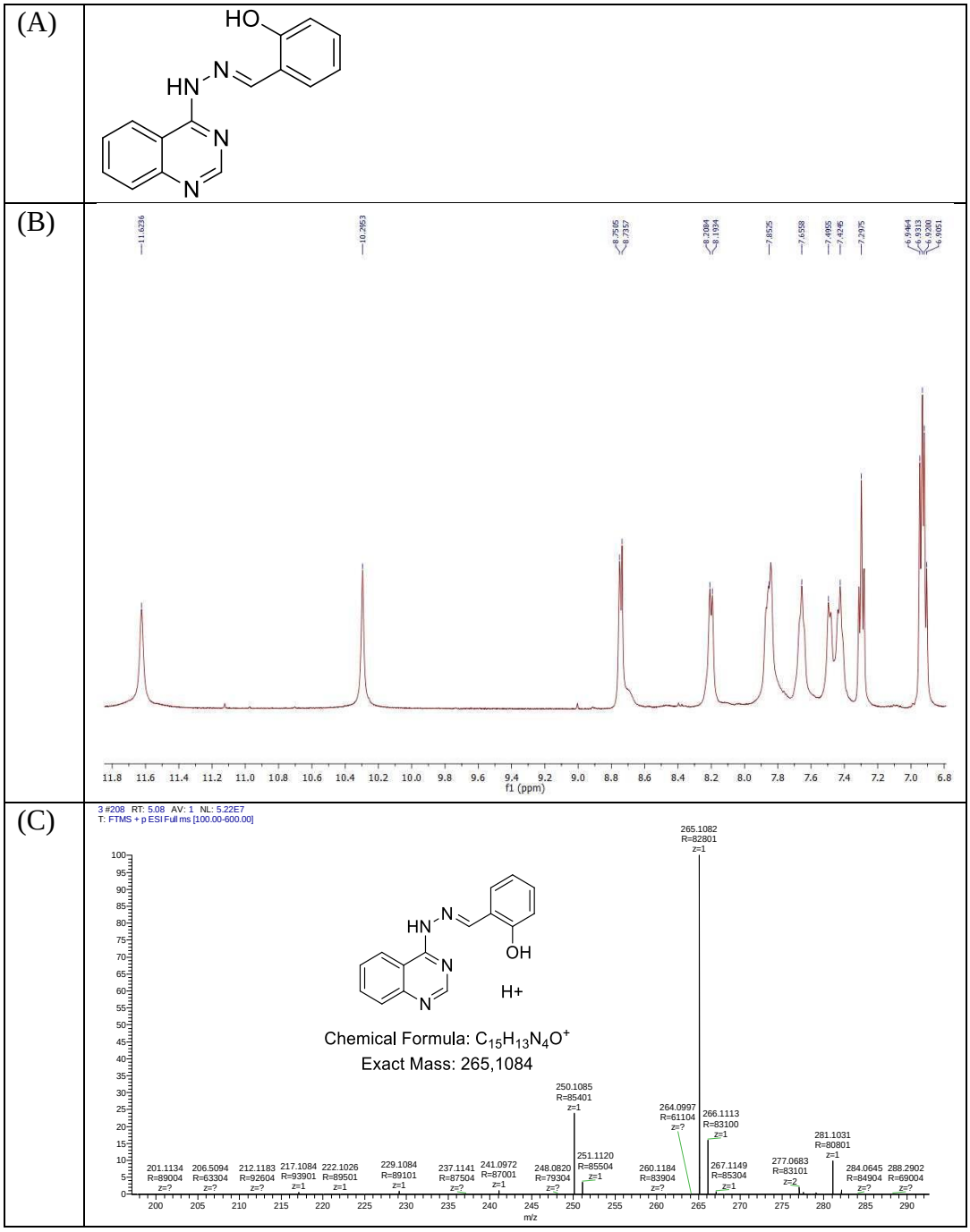
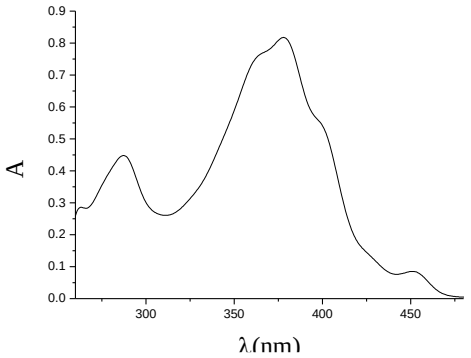
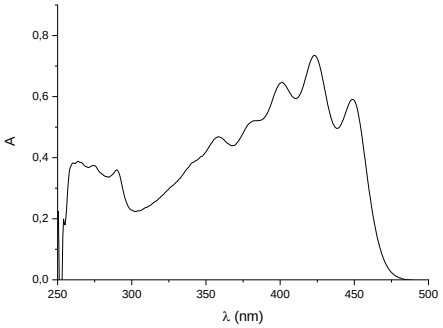


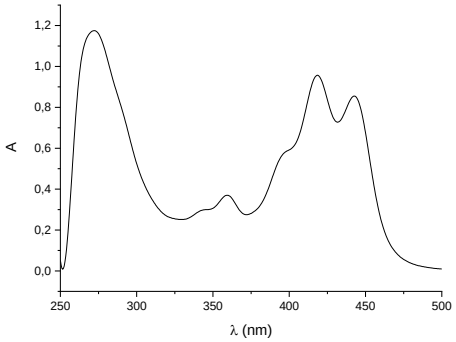
Figure S2. (A) Syntax formula of H_2L. (B) ^1H NMR spectrum of H_2L in DMSO−d_6. (C) HRMS of H_2L.



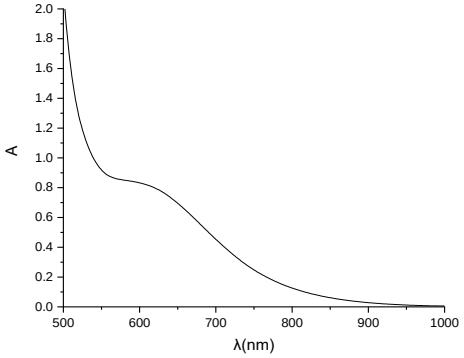
H_2L (10^{-5} M)



Complex **1** (0.05 mM)

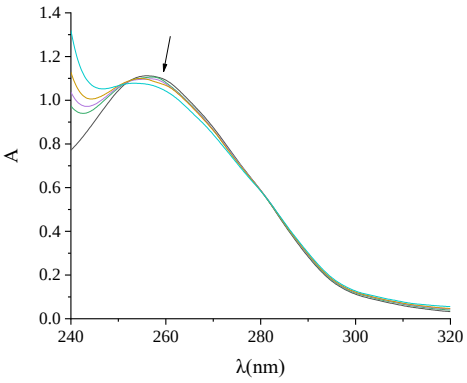


Complex **2** (0.01 mM)

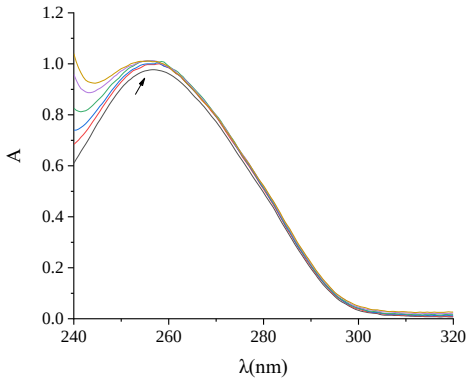


Complex **2** (5 mM)

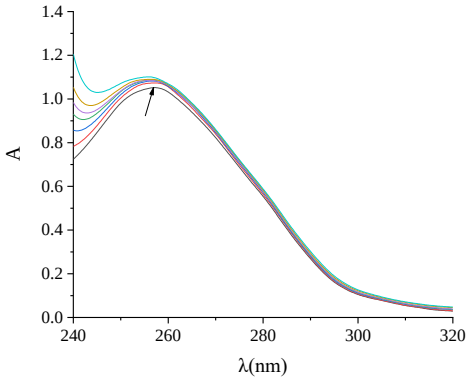
Figure S3. UV−vis spectra of the compounds in DMSO (the concentrations are given in parentheses).



H_2L ([DNA] = 0.17 mM)



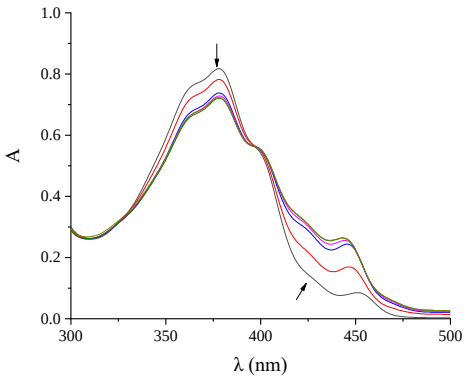
Complex **1** ([DNA] = 0.15 mM)



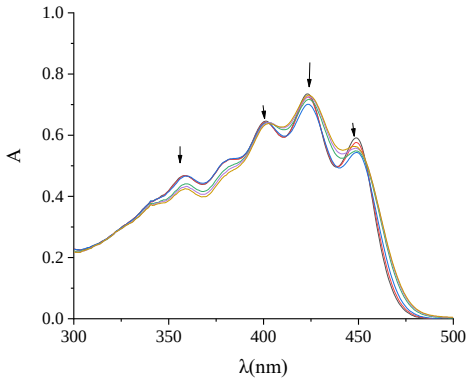
Complex **2** ([DNA] = 0.16 mM)

Figure S4. The UV−vis spectra of a CT DNA solution (its concentration is given in parentheses) in buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) recorded in the presence of increasing amounts of the compounds.

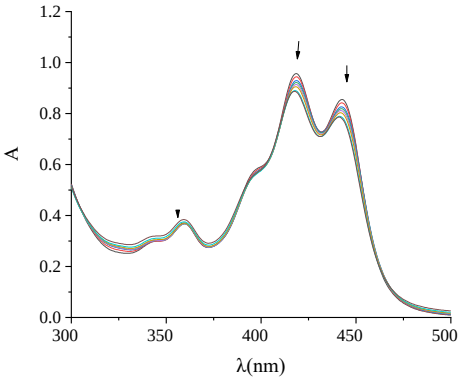
The arrow shows the changes upon increasing amounts of the compound.



H_2L (0.01 mM)



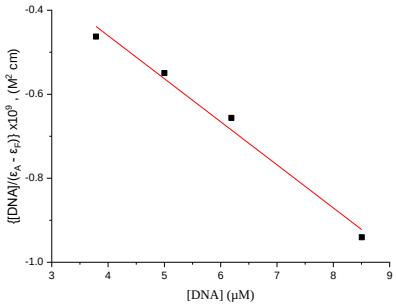
Complex **1** (0.05 mM)



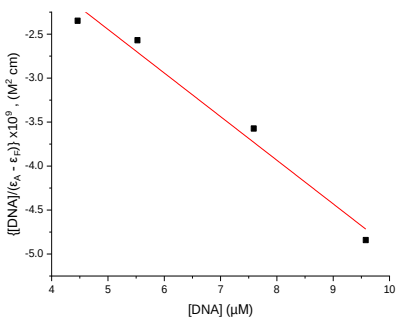
Complex **2** (0.01 mM)

Figure S5. UV−vis spectra of a DMSO solution of the compounds (the concentrations are given in parentheses) in the presence of increasing amounts of CT DNA.

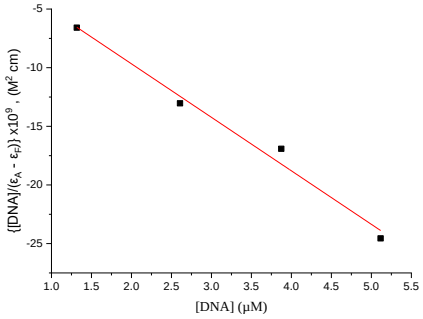
The arrows show the changes upon increasing amounts of CT DNA.



H_2L

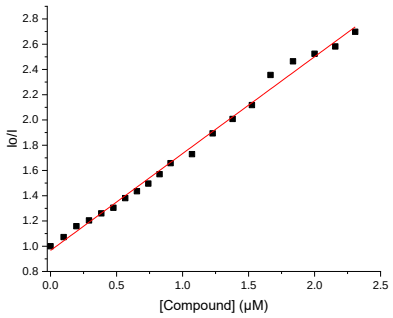


Complex **1**

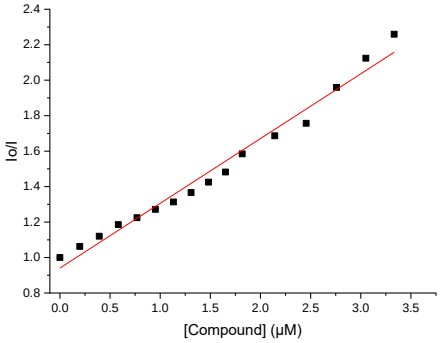


Complex **2**

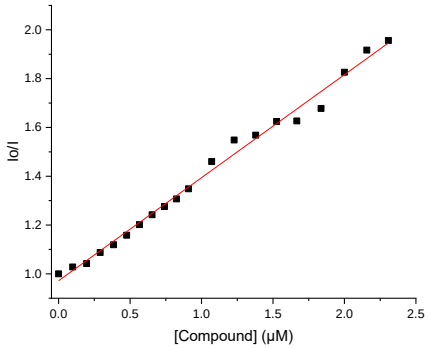
Figure S6. Plots of $\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)}$ *versus* [DNA] for the compounds.



H_2L



Complex **1**



Complex **2**

Figure S7. Stern−Volmer plots of the EB−DNA quenching experiments upon addition of the compounds.

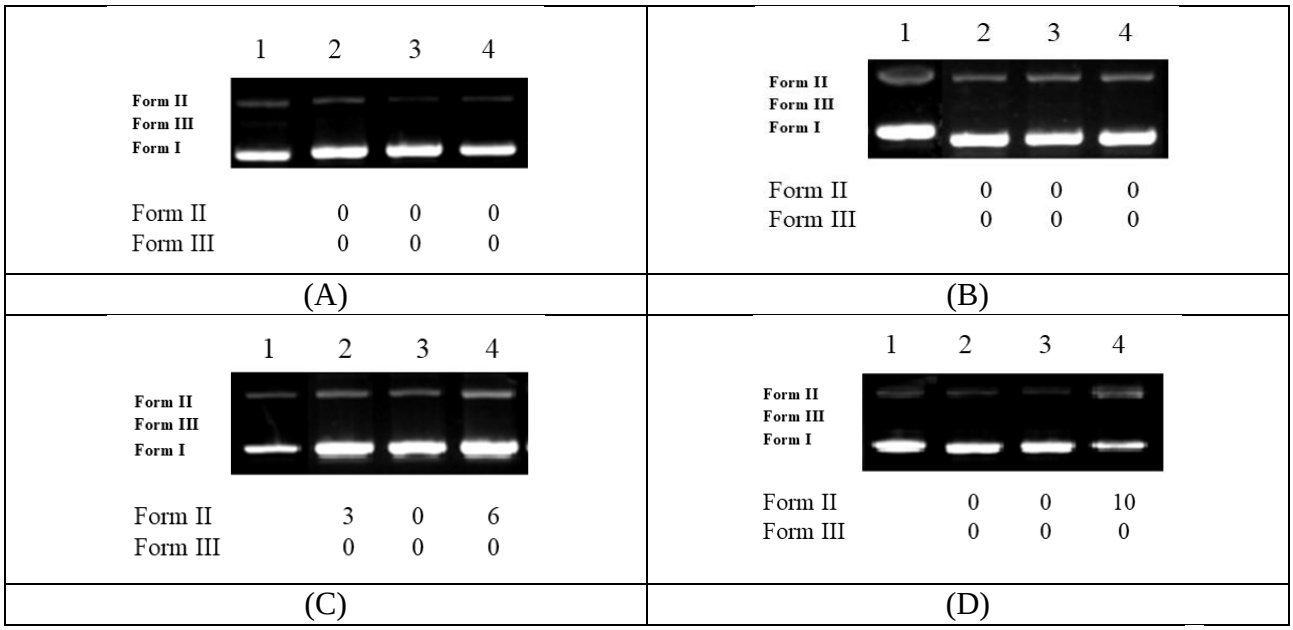
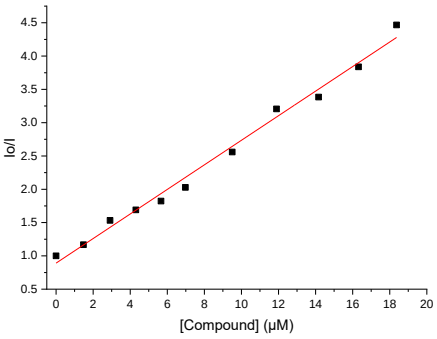


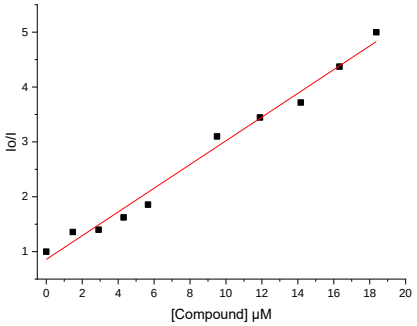
Figure S8. Agarose gel electrophoretic pattern of EB−stained plasmid DNA (pBR322 DNA) with H_2L and its complexes **1** and **2** at 500 μM after 60 min of electrophoresis.

Top: Gel electrophoreses pictures: (A) without irradiation, (B) upon irradiation at 312 nm (UVB), (C) upon irradiation at 365 nm (UVA), and (D) upon irradiation with visible light. Lanes 1−4: Lane 1: DNA; Lane 2: DNA + H_2L; Lane 3: DNA + complex **1**; Lane 4: DNA + complex **2**.

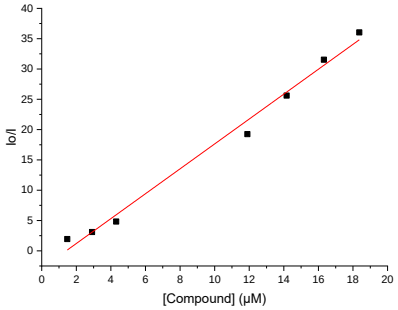
Bottom: Calculation of the % conversion to ss and ds damage. DNA forms: Form I = supercoiled, Form II = relaxed, Form III = linear plasmid DNA.



H_2L

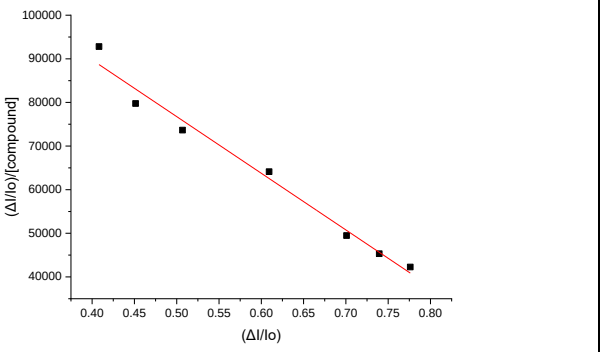


Complex **1**

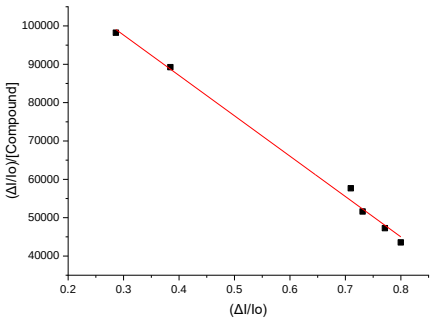


Complex **2**

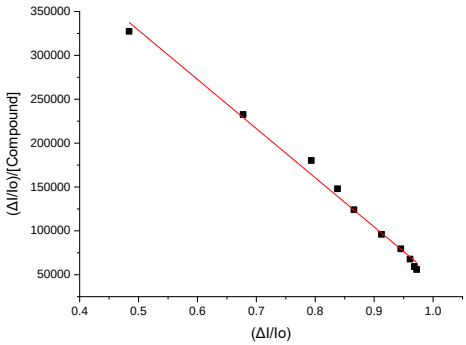
Figure S9. Stern−Volmer plots of the BSA quenching experiments upon addition of the compounds.



H_2L

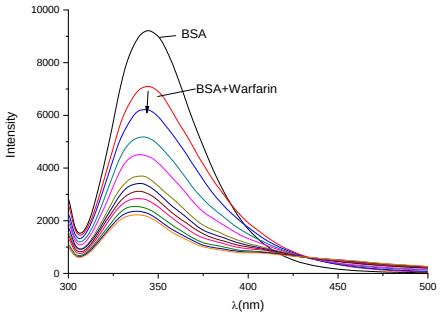


Complex **1**

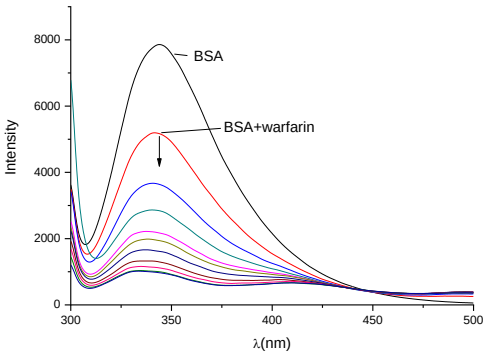


Complex **2**

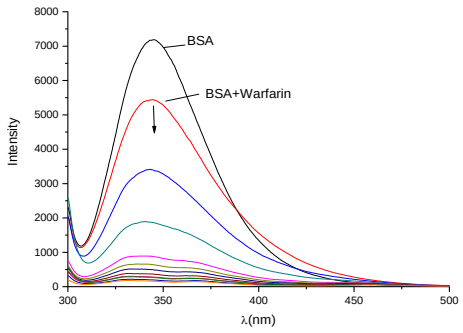
Figure S10. Scatchard plots of the BSA quenching experiments upon addition of the compounds.



H_2L

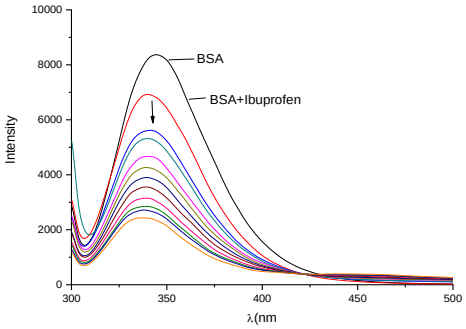


Complex **1**

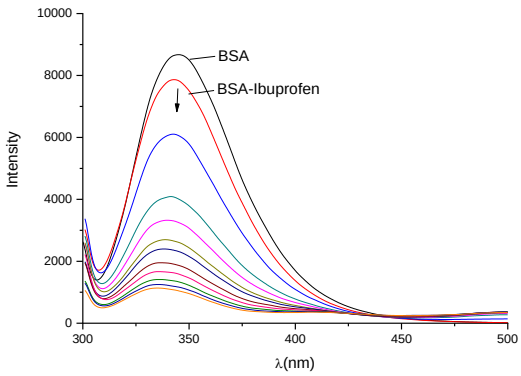


Complex **2**

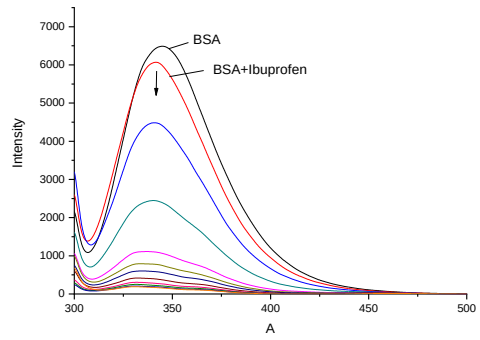
Figure S11. Fluorescence emission spectra ($\lambda_{excitation}$= 295 nm) for BSA ([BSA] = 3 μM) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the presence of warfarin (3 μM) upon addition of increasing amounts of the compounds.



H_2L

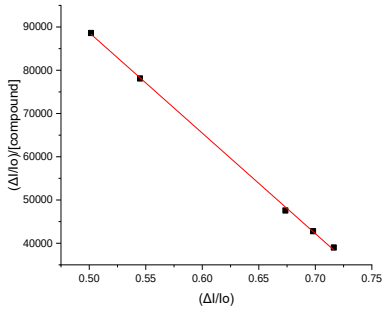


Complex **1**

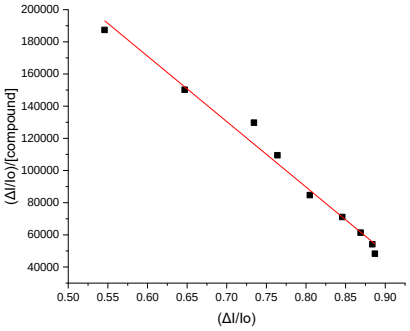


Complex **2**

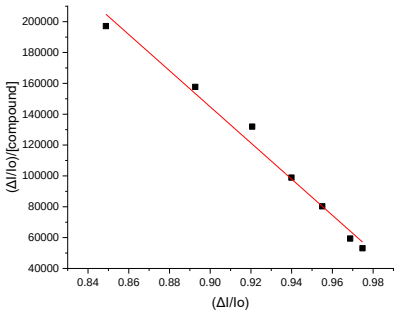
Figure S12. Fluorescence emission spectra ($\lambda_{excitation}$= 295 nm) for BSA ([BSA] = 3 μM) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the presence of ibuprofen (3 μM) upon addition of increasing amounts of the compounds.



H_2L

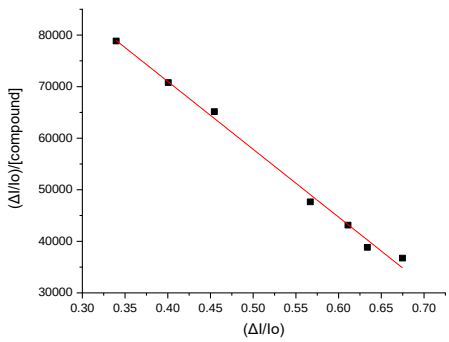


Complex **1**

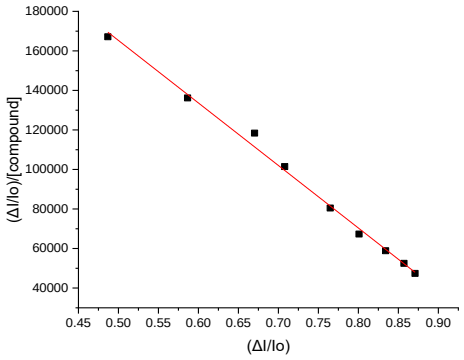


Complex **2**

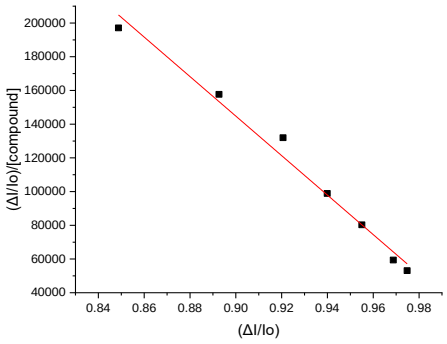
Figure S13. Scatchard plots of the BSA quenching experiments in the presence of warfarin upon addition of the compounds.



H_2L



Complex **1**



Complex **2**

Figure S14. Scatchard plots of the BSA quenching experiments in the presence of ibuprofen upon addition of the compounds.