Supplementary Information

In vitro Analysis of the Interaction between Human Serum Albumin and Semi-Synthetic Clerodanes

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Inner filter correction analysis

In order to compensate the inner filter effect, fluorescence intensity values were corrected for the absorption of each ligand at the excitation and emission wavelength using equation S1:

$$F_{cor} = F_{obs} 10^{\left[(A_{ex} + A_{em})/2\right]} \quad \text{(S1)}$$

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensity values, respectively; A_{ex} and A_{em} represent the experimental absorbance values at the excitation wavelength (280 nm; $\varepsilon = 2535\ M^{-1}\ cm^{-1}$ for MHDCTN and $\varepsilon = 1887\ M^{-1}\ cm^{-1}$ for PHDCTN) and emission wavelength (340 nm; $\varepsilon = 720\ M^{-1}\ cm^{-1}$ for MHDCTN and $\varepsilon = 707\ M^{-1}\ cm^{-1}$ for PHDCTN), respectively.

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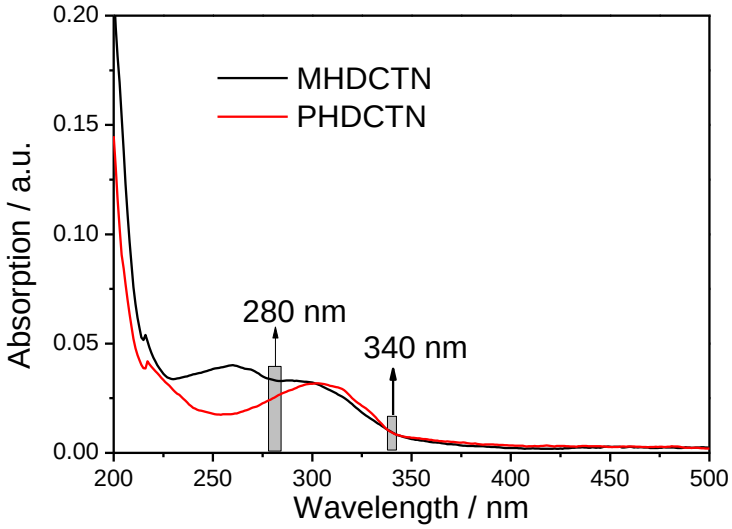

Figure S1. UV-Vis spectra for MHDCTN and PHDCTN at pH = 7.4 and 310 K. [MHDCTN] = [PHDCTN] = 1.32×10^{-5} M.

Table S1. Binding constant values (K_{SV}, k_q, and K_a) for the interaction HSA:MHDCTN and HSA:PHDCTN at 296, 303 and 310 K. The values were calculated upon inner filter correction

Ligand	T / K	K_{SV} ($\times 10^3$) / M^{-1}	k_q ($\times 10^{11}$) / ($M^{-1}s^{-1}$)	K_a ($\times 10^5$) / M^{-1}
MHDCTN	296	4.00 ± 0.19	6.78	3.05 ± 0.26
	303	3.87 ± 0.23	6.56	2.67 ± 0.26
	310	3.47 ± 0.14	5.87	2.16 ± 0.26
PHDCTN	296	8.22 ± 0.31	13.9	1.54 ± 0.26
	303	8.78 ± 0.28	14.9	1.99 ± 0.26
	310	8.95 ± 0.21	15.2	2.53 ± 0.26

K_{sv}: Stern-Volmer quenching constant; k_q: bimolecular quenching rate constant; K_a: modified Stern-Volmer binding constant. Note: r^2 for K_{SV} and k_q: 0.9659-0.9853; r^2 for n: 0.9816-0.9969; r^2 for K_a 0.9834-0.9995.

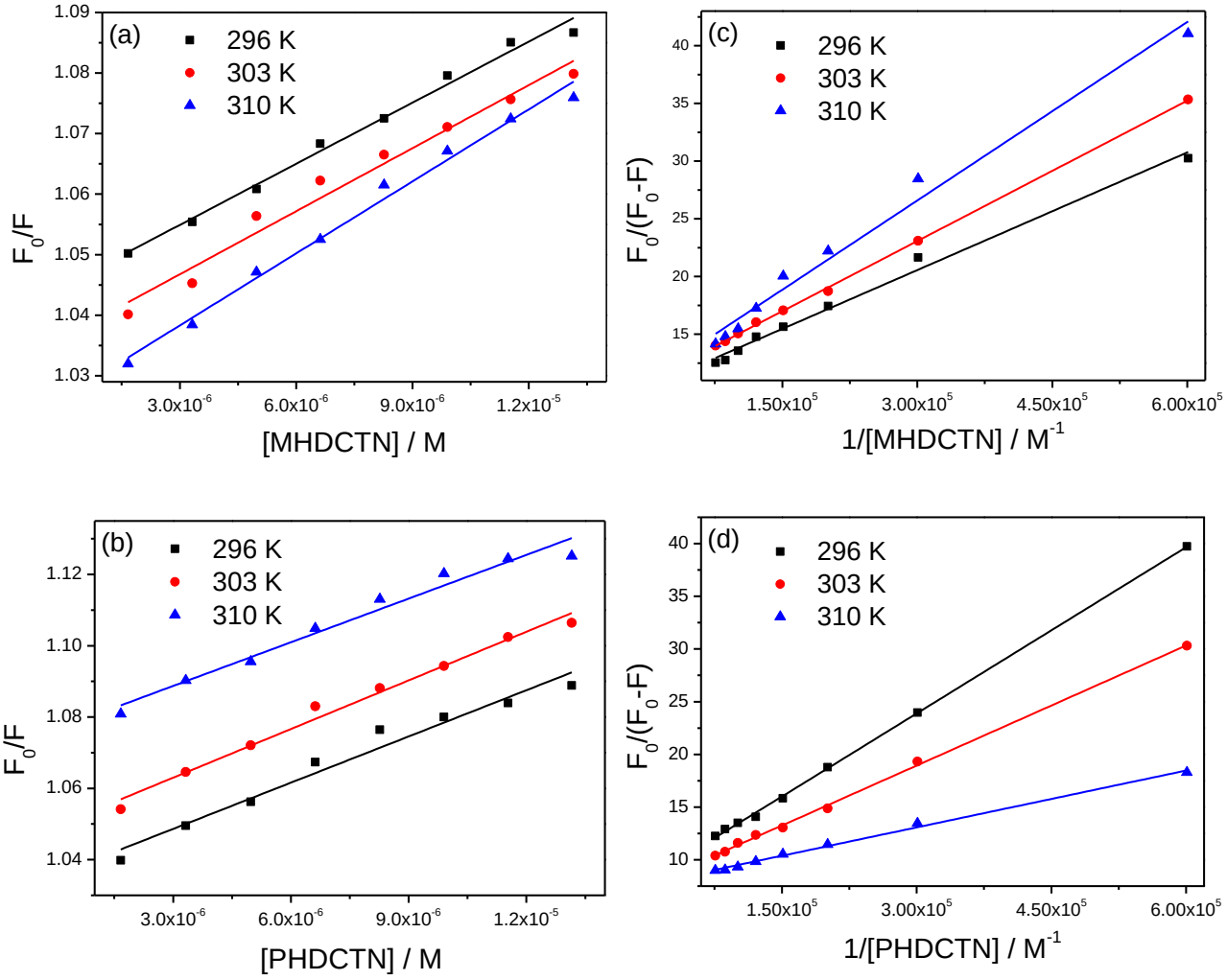

Figure S2. Stern-Volmer plots for the interaction between HSA and MHDCTN (a) and PHDCTN (b) at 296, 303 and 310 K. Modified Stern-Volmer plots for the interaction HSA:MHDCTN (c) and HSA:PHDCTN (d) at pH = 7.4 and three different temperatures. All plots were obtained upon inner filter correction. [HSA] = 1.00 × 10^{-5} M and [MHDCTN] = [PHDCTN] = 0.17; 0.33; 0.50; 0.66; 0.83; 0.99; 1.15 and 1.32 × 10^{-5} M.

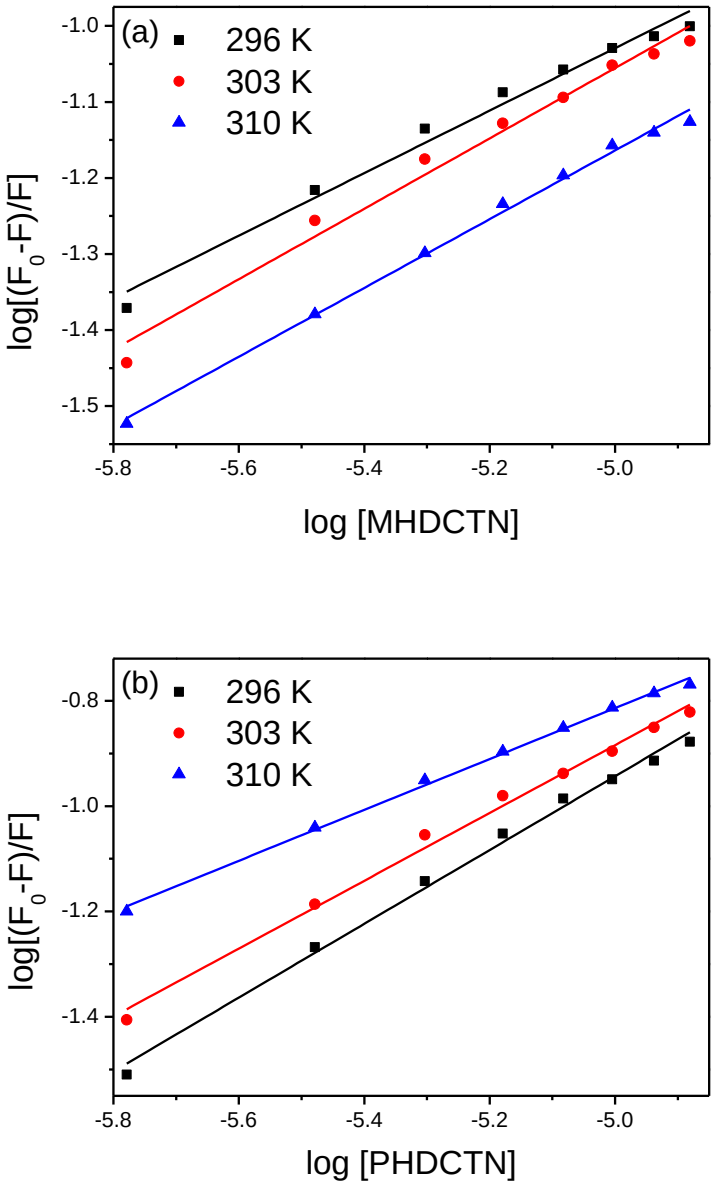

Figure S3. Double logarithmic plot for the interaction HSA:MHDCTN (a) and HSA:PHDCTN (b) at pH = 7.4 and 296, 303 and 310 K. [HSA] = 1.00 × 10^{-5} M and [MHDCTN] = [PHDCTN] = 0.17; 0.33; 0.50; 0.66; 0.83; 0.99; 1.15 and 1.32 × 10^{-5} M.

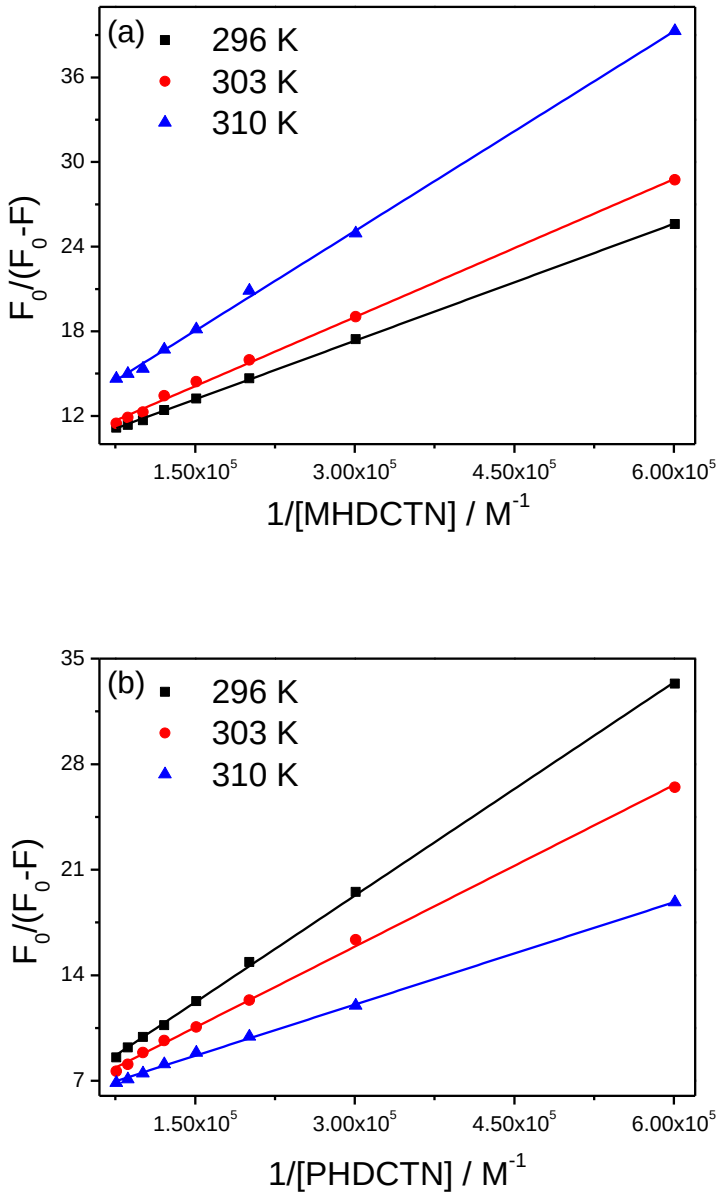

Figure S4. Modified Stern-Volmer plot for the interaction HSA:MHDCTN (a) and HSA:PHDCTN (b) at pH = 7.4 and 310 K. [HSA] = 1.00 × 10^{-5} M and [MHDCTN] = [PHDCTN] = 0.17; 0.33; 0.50; 0.66; 0.83; 0.99; 1.15 and 1.32 × 10^{-5} M.

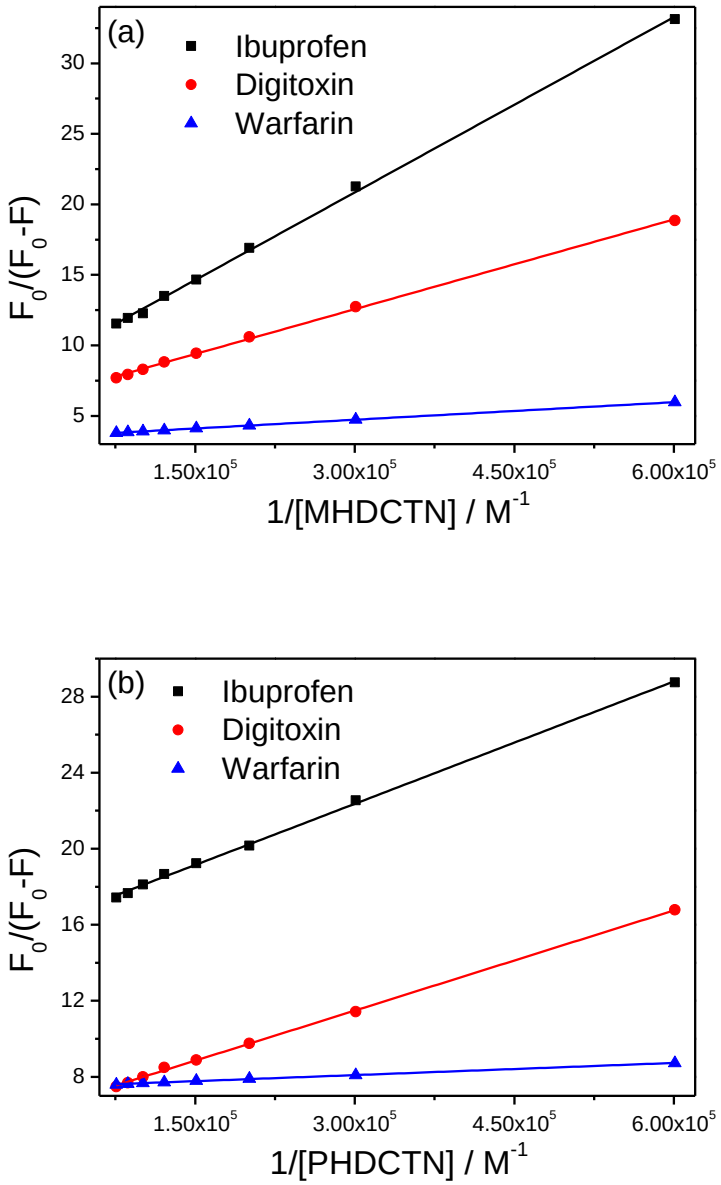

Figure S5. Modified Stern-Volmer plot for the interaction HSA:MHDCTN (a) and HSA:PHDCTN (b) in the presence of site markers ibuprofen, digitoxin and warfarin, at pH = 7.4 and 310 K. [HSA] = 1.00×10^{-5} M and [MHDCTN] = [PHDCTN] = 0.17; 0.33; 0.50; 0.66; 0.83; 0.99; 1.15 and 1.32×10^{-5} M.