Electronic supplementary information

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Fluorescence solvatochromism and modulated anticholinergic activity of novel coumarin compounds sequestered in human serum albumin nanocavities

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Contents

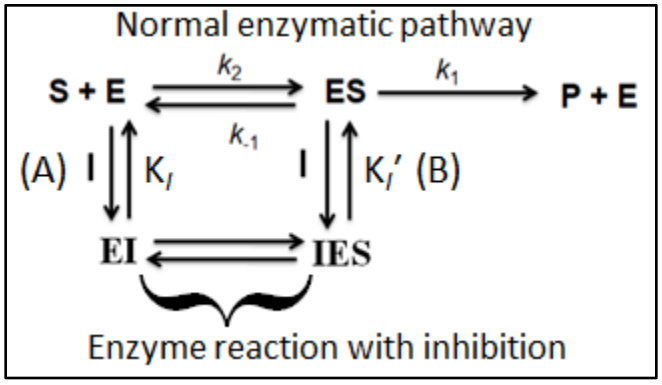
Table 1S: List of solvents used in the present study along with the characteristic parameters listed in terms of different scales.

No	Solvent	$\Delta f(\varepsilon,n)^a$	$E_T(30)^b$	α^c	β^c	π^c	SA^d	SB^d	SdP^d	SP^d
1	Cyclohexane	0.00	49.7	0.00	0.00	0.00	0.000	0.073	0.000	0.683
2	Toluene	0.02	33.9	0.00	0.11	0.54	0.000	0.128	0.284	0.782
3	1,4-dioxane	0.03	36.0	0.00	0.37	0.55	0.000	0.444	0.312	0.737
4	Acetonitrile	0.3	45.6	0.19	0.40	0.75	0.044	0.286	0.974	0.645
5	Methanol	0.31	55.4	0.98	0.66	0.60	0.044	0.286	0.974	0.645
6	Tetrahydrofuran	0.21	37.4	0.00	0.55	0.58	0.000	0.590	0.634	0.714
7	Dimethyl sulfoxide	0.26	45.1	0.00	0.76	0.10	0.072	0.647	1.000	0.830
8	Water	0.32	63.1	1.17	0.47	1.09	1.062	0.025	0.997	0.681
9	Ethyl acetate	0.19	38.1	0.00	0.45	0.55	0.000	0.542	0.603	0.656
10	n-Butanol	0.26	49.7	0.84	0.84	0.47	0.341	0.809	0.655	0.674

[a]Polarity parameter ($=(\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$), where the solvent dielectric constant and refractive indices are represented by ε and n, respectively. [b]Reichardt solvent parameter. [c]Kamlet-Taft solvent parameters; [d] Catalán solvent parameter

ST1: Estimation of anticholinergic activity of the investigated systems.

Both the investigated systems act as acetylcholinesterase (AChE) inhibitor, modelled on the cholinergic hypothesis of Alzheimer's disease (AD). This hypothesis states that decline in levels of acetylcholine (ACh) in the brain, whose breakdown is the primary function of the enzyme AChE, is the principal reason for the cause and progression of AD [1]. Therefore, cholinesterase inhibitors impede the activity of AChE and consequently results in an increase in ACh levels in forebrain regions, thus facilitating neurotransmission. Hence, AChE inhibitors have been utilized as therapeutic avenues for AD.



Scheme ST1: Mechanistic representation of normal and inhibited enzymatic activity of AChE.

Modified version of Ellman method [2] was used for assaying the activity of AChE in phosphate buffer of pH = 8.0 (0.1 M) at 298 K and its kinetic characterization [3,4]. The basis of the enzyme kinetics data interpretation is shown in Scheme ST1. Data analysis and evaluation of the kinetic parameters was done using the Microcal Origin 2017 software. The non-enzymatic hydrolysis curve was subtracted off from the corresponding enzymatic hydrolysis curve in each case and the initial linear portion of the progress curve was considered for calculating the initial velocity (v_0) of the reaction (Eq. ST1).

$$\nu_0 \text{ (moles } l^{-1} s^{-1}) = \frac{\text{Slope (absorbance } s^{-1})}{\epsilon \times l} \quad \text{(ST1)}$$

Here ε represents the absorption coefficient of the yellow anion, l is the path length (= 0.442 cm).

Non-linear regression analysis of (ν_0) against [S_0] gave the values of the Michaelis-Menten (MM) constant, K_m and maximum hydrolysis rate, V_{max} in the normal and enzyme inhibition cases using Eq. ST2a and ST2b, respectively.

$$\nu_0 = \frac{V_{max}[S_0]}{K_m + [S_0]} \quad \text{(ST2a)}$$

$$\nu_0 = \frac{V_{max}'[S_0]}{K_m' + [S_0]} \quad \text{(ST2b)}$$

Here, $V_{max}' = \frac{V_{max}}{\alpha'}$, $K_m' = \frac{\alpha}{\alpha'} \times K_m$, or, $K_m' = \alpha \times K_m$ (representing only inhibition path A);

$\alpha = 1 + \frac{[EI]}{[E]}$ and $\alpha' = 1 + \frac{[IES]}{[ES]}$ (representing both paths A and B).

The characteristic IC_{50} parameter for inhibition in both the media were obtained from the modified Hill relation (Eq. ST3).

$$\frac{\Delta V}{\Delta V_{max}} = \frac{[I]^{n_H}}{K_{0.5}^{n_H} + [I]^{n_H}} \quad \text{(ST3)}$$

Here ΔV is the initial rate decrease observed in presence of a definite concentration of inhibitor [I], ΔV_{max} represents maximal initial velocity decrease, $K_{0.5}$, which is pharmacologically equivalent to IC_{50}, i.e., the inhibitor concentration to induce half-maximal change in the initial velocity. The term n_H represents the Hill coefficient.

References:

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2. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone,*Biochem. Pharmacol*. **1961**,7, 88–95.
3. M. M. Islam, A. B. Gurung, A. Bhatacharjee, K. Aguan, S. Mitra, *Chem. Biol. Interact*.**2016**, *249*, 1 – 9
4. P. Baruah, G. Basumatary, S. O. Yesylevskyy, K. Aguan, G. Bez, S. Mitra, *J. Biomol Struct Dyn*. **2019**, *37*, 1750 - 1765.

Fig. 1S: Steady state absorption spectra of MHC (a) and MHCB (b) in different solvents. Solvent abbreviations: 1,4-dioxane (DIO), ethyl acetate (EAC), dimethyl sulfoxide (DMSO), acetonitrile (ACN), methyl alcohol (MeOH).

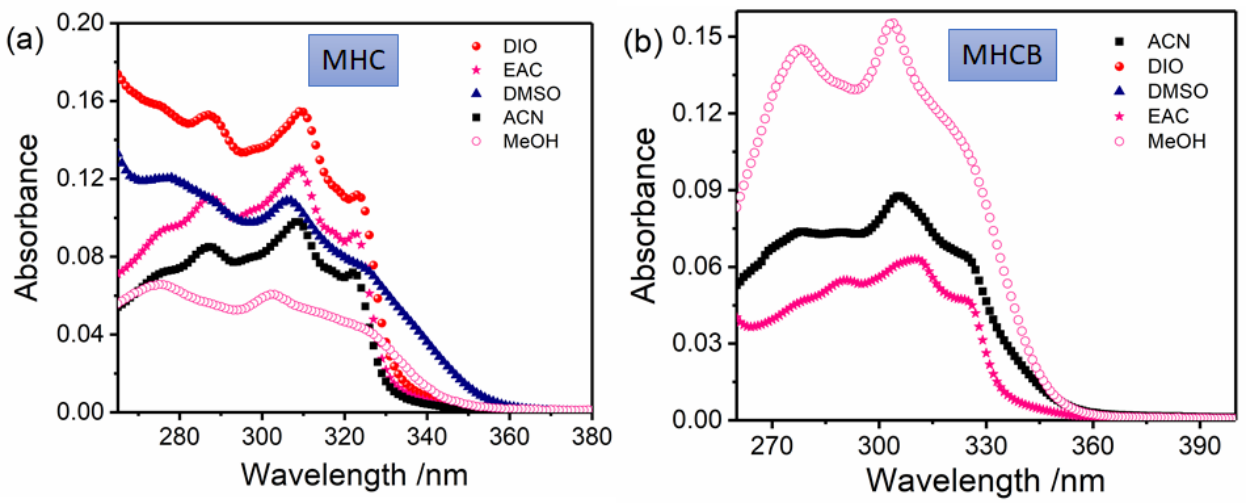

Fig. 2S: Time-resolved fluorescence decay traces of MHC (a) and MHCB (b) in some selected solvents collected by exciting the sample with 295 nm LED. The emission was monitored at the maximum of the respective fluorescence emission spectrum.

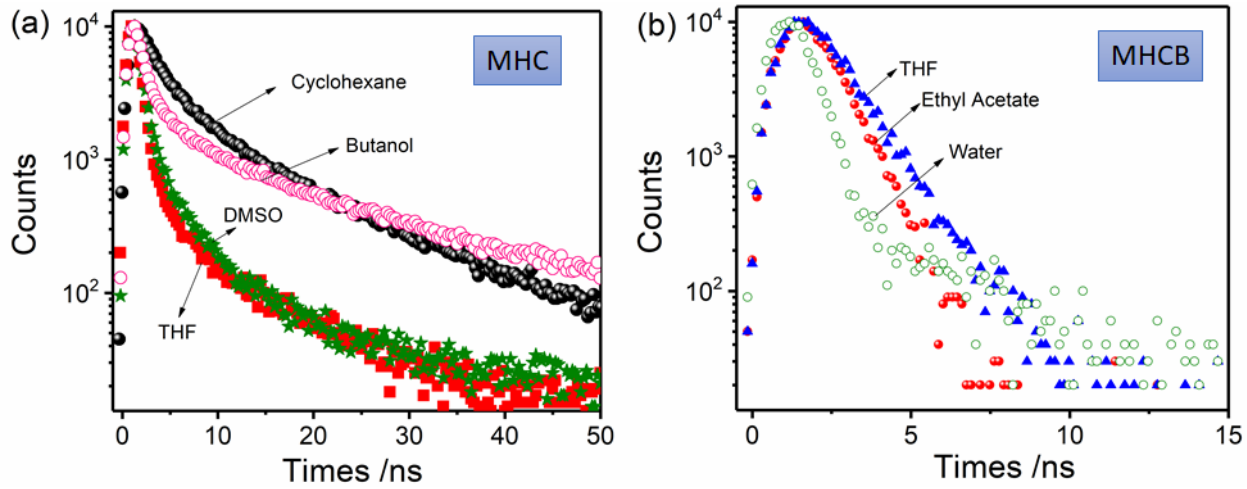

Table 2S: Time-resolved decay parameters for MHC in different solvents. The values of reduced chi-square (χ_R^2) and Durbin-Watson parameter (DW) for each decay fitting are also listed.

Solvent	a_1 (%)	τ_1 / ns	a_2 (%)	τ_2 / ns	a_3 (%)	τ_3 / ns	χ_R^2	DW
For MHC								
Cyclohexane	64.15	1.83	25.17	5.49	10.68	16.11	1.24	2.22
Toluene	49.73	0.97	26.99	4.439	23.27	12.43	1.07	2.30
1,4-dioxane	100	0.39	-	-	-	-	1.13	2.31
Acetonitrile	74.13	0.94	25.87	3.449	-	-	1.33	2.36
Methanol	79.64	0.904	20.36	3.85	-	-	1.14	2.88
Tetrahydrofuran	100	0.64	-	-	-	-	1.17	2.31
DMSO	93.43	0.54	6.57	3.23	-	-	1.17	2.01
Water	100	0.84	-	-	-	-	1.86	1.51
Ethyl acetate	100	0.51	-	-	-	-	1.05	1.86
n-Butanol	83.32	0.664	16.68	4.213	-	-	1.08	2.01
For MHCB								
Cyclohexane	74.48	2.61	25.16	13.50	-	-	1.17	2.00
Toluene	70.33	1.19	29.67	9.60	-	-	1.16	2.01
1,4-dioxane	100	0.72	-	-	-	-	1.09	1.51
Acetonitrile	100	0.51	-	-	-	-	1.02	1.59
Methanol	100	1.34	-	-	-	-	1.06	1.83
Tetrahydrofuran	100	1.19	-	-	-	-	1.03	1.82
DMSO	100	0.57	-	-	-	-	1.01	1.87
Water	100	0.47	-	-	-	-	1.28	2.27
Ethyl acetate	100	0.91	-	-	-	-	1.07	1.98
n-Butanol	100	0.62	-	-	-	-	1.27	2.13

Fig. 3S: Modulation in MHC emission spectral behavior in DMSO/Methanol (a) and acetonitrile/water (b) mixtures. Inset shows then variation in fluorescence intensity at 385 and 404 nm in (a) and (b), respectively. Time-resolved fluorescence decay traces of MHC (c) and MHCB (d) with varying water content in DMSO/water mixtures.

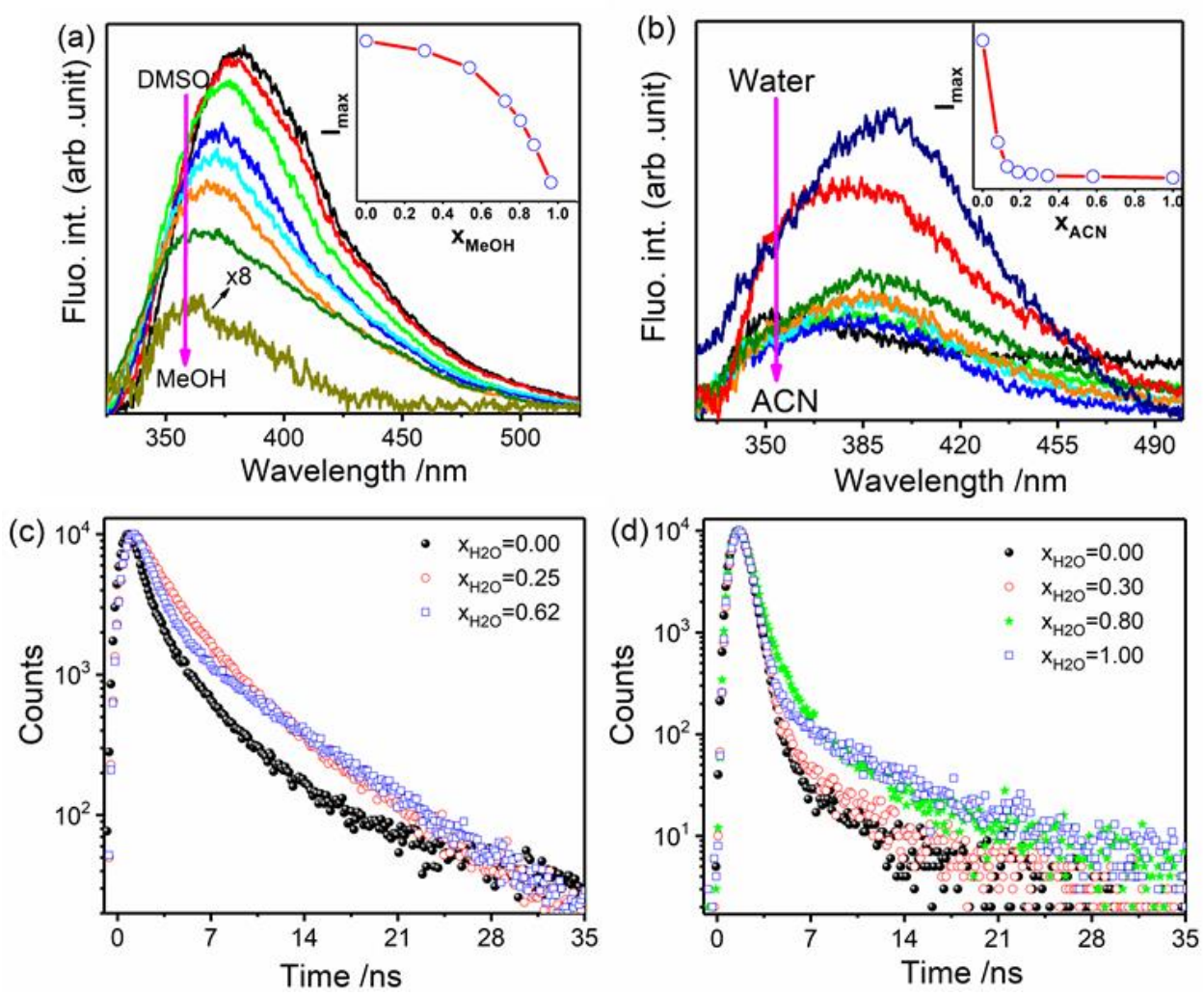

Table 3S: Time-resolved decay parameters for MHC and MHCB in DMSO-water (variant) binary mixtures.

Sl. No	x_{water}	a_1 (%)	τ_1 / ns	a_2 (%)	τ_2 / ns	τ_{av} /ns	χ_R^2	DW
MHC								
1	0	93.43	0.5	6.57	3.2	1.34	1.17	2.01
2	0.17	71.40	1.1	28.60	3.7	2.59	1.19	2.65
3	0.35	65.52	1.4	34.48	4.0	2.94	1.27	2.67
4	0.57	82.06	2.3	17.94	6.7	4.02	1.21	2.22
5	0.80	80.76	1.8	19.24	6.9	4.22	1.28	1.89
6	0.87	82.95	1.3	17.05	6.9	4.18	1.05	1.71
7	0.92	82.70	1.0	17.30	6.6	4.32	1.10	2.24
8	0.98	81.06	0.8	18.94	5.9	4.10	1.00	1.69
9	1.00	100	0.84	-	-	1.50	1.86	1.51
MHCB: Emission monitored at 385 nm								
1	0	87.74	0.87	12.25	4.77	9.69	1.17	2.15
2	0.17	83.53	0.89	16.46	4.85	10.94	1.15	2.0
3	0.30	75.55	0.89	24.45	4.50	8.91	1.2	2.01
4	0.57	58.07	1.39	41.92	4.09	7.23	1.16	2.27
5	0.80	85.53	0.83	14.47	2.03	1.82	1.14	2.41
6	0.92	99.67	0.50	0.33	6.43	0.74	1.29	2.21
7	1.00	99.91	0.40	0.09	6.09	0.48	1.04	3.2
MHCB: Emission monitored at 485 nm								
1	0	100	0.57	-	-	0.57	1.25	2.04
2	0.17	99.76	0.45	0.24	5.16	0.57	1.09	2.19
3	0.30	99.66	0.46	0.34	5.16	0.63	1.01	2.00
4	0.57	98.87	0.45	1.13	4.19	0.81	1.06	2.89
5	0.80	98.9	0.77	1.09	4.98	1.05	1.34	1.71
6	0.92	99.14	0.64	0.86	7.45	1.26	1.07	2.18
7	1.00	100	0.47	-	-	0.47	1.32	2.62

Table 4S: Estimated KT parameters for solvatochromism of MHC and MHCB.[a]

Sl.No	P^0	a	b	S	% of a	% of b	% of s
For MHC							
ν_{abs}	34838.59	605.96	-1084.73	-1651.04	18.13	-32.46	-49.41
ν_{em}	29316.24	-116.07	-1645.61	-1870.91	-3.19	-45.30	-51.51
$\Delta\nu_{SS}$	5522.35	722.02	560.88	219.86	48.05	37.32	14.63
ϕ_f	0.01	0.02	-0.03	0.02	35.29	-41.22	23.49
τ_{av}	9.84	5.49	-3.80	-9.19	29.71	-20.57	-49.72
κ_r	6.83×10^{-4}	4.00×10^{-3}	-0.014	0.028	8.52	-30.15	61.33
$\Sigma\kappa_{nr}$	0.13	-0.66	0.17	0.95	-36.85	9.73	53.41
For MHCB							
ν_{abs}	30627.30	1086.02	-1597.94	-395.64	35.26	-51.88	-12.85
ν_{em}	17471.22	447.48	2086.46	4441.31	6.42	29.91	63.67
$\Delta\nu_{SS}$	13156.08	638.55	-3684.41	-4836.94	6.97	-40.22	-52.80
ϕ_f	5.15×10^{-4}	-8.36×10^{-4}	0.002	2.99×10^{-6}	-31.68	68.21	0.11
τ_{av}	10.18	3.09	-11.55	-6.48	14.67	-54.67	-30.65
κ_r	1.83×10^{-4}	-1.41×10^{-3}	3.81×10^{-3}	-2.72×10^{-4}	-25.67	69.37	-4.95
$\Sigma\kappa_{nr}$	-0.06018	-0.65349	1.95756	0.81	-19.09	57.18	23.73

[a] Absorption/emission maxima (denoted by ν_{abs} and ν_{em}, respectively) and Stokes-shift ($\Delta\nu_{SS}$) are expressed in cm^{-1}; average lifetime (τ_{av}) in ns; radiative (κ_r) and total non-radiative ($\Sigma\kappa_{nr}$) decay rate constants are in ns^{-1}.

Table 5S: Estimated Catalán parameters for solvatochromism of MHC and MHCB.[a]

Sl.No	P^0	SA	SB	SdP	SP	% of SA	% of SB	% of Sdp	% of SP
For MHC									
ν_{abs}	283.09	0.01	-0.13	7.36	13.22	0.06	-0.65	35.49	63.80
ν_{em}	276.11	32.43	-4.22	23.01	98.52	20.50	-2.67	14.55	62.28
$\Delta\nu_{SS}$	1315.62	2216.09	-210.72	893.65	5632.9	24.75	-2.35	9.98	62.91
ϕ_f	-0.01	0.06	-0.02	-0.009	0.04	46.35	-12.97	-6.98	33.70
τ_{av}	13.99	1.34	3.36	-5.049	-10.25	6.69	16.82	-25.24	-51.24
κ_r	-0.02	0.04	-0.004	-0.01	0.05	35.92	-4.56	-12.17	47.34
$\Sigma\kappa_{nr}$	-0.51	0.33	0.35	-0.57	1.69	11.23	11.89	-19.51	57.37
For MHCB									
ν_{abs}	333.60	-8.43	12.45	2.91063	-8.26	-26.29	38.84	9.08	-25.79
ν_{em}	450.16	-95.25	-46.59	-29.89	120.18	-32.63	-15.96	-10.24	41.17
$\Delta\nu_{SS}$	8203.26	-4110.66	-2527.54	-1390.47	4871.1	-31.87	-19.59	-10.78	37.76
ϕ_f	-0.003	7.26×10^{-4}	0.002	-3.42×10^{-4}	0.01	9.19	23.14	-4.32	63.35
τ_{av}	3.81	-1.58	-6.71	-5.2054	6.81	-7.79	-33.02	-25.62	33.57
κ_r	-0.01	7.95×10^{-4}	0.003	3.43×10^{-4}	0.01	5.79	22.14	2.50	69.56
$\Sigma\kappa_{nr}$	-0.04747	0.30647	1.23983	0.80972	0.0869	12.55	50.75	33.15	3.56

[a]Absorption/emission maxima (denoted by ν_{abs} and ν_{em}, respectively) and Stokes-shift ($\Delta\nu_{SS}$) are expressed in cm^{-1}; average lifetime (τ_{av}) in ns; radiative (κ_r) and total non-radiative ($\Sigma\kappa_{nr}$) decay rate constants are in ns^{-1}.

Table 6S: Time-resolved fluorescence decay parameters of MHC and MHCB with increasing concentration of β-cyclodextrin (β-CD_x).

[β-CD_x] /mM	a_1 (%)	τ_1 / ns	a_2 (%)	τ_2 / ns	τ_{av} /ns	χ_R^2	DW
MHC: Emission monitored at 440 nm							
0.00	100.00	0.84	-	-	0.84	1.18	2.11
7.20	68.52	1.06	31.47	4.64	3.45	1.25	2.14
13.70	72.25	1.12	27.76	4.87	3.46	1.15	2.26
19.60	74.25	1.00	25.76	4.98	3.52	1.04	2.45
24.40	75.26	1.11	24.74	5.19	3.58	1.17	2.20
29.10	74.65	1.12	25.13	5.17	3.58	1.09	2.01
33.60	75.93	1.06	24.07	5.12	3.52	1.16	2.24
MHCB: Emission monitored at 440 nm							
1.40	67.20	6.99	32.80	2.16	6.36	1.35	2.09
2.00	77.46	7.17	22.57	2.99	6.72	1.26	2.00
2.72	76.24	7.27	23.76	3.14	6.78	1.26	2.4
3.56	77.60	7.34	22.37	2.48	6.91	1.28	2.02
4.76	74.28	7.78	25.72	2.94	7.03	1.19	2.28

Fig. 4S: Fluorescence quenching of HSA in presence of MHC (a) and MHCB (b) at some representative temperatures.

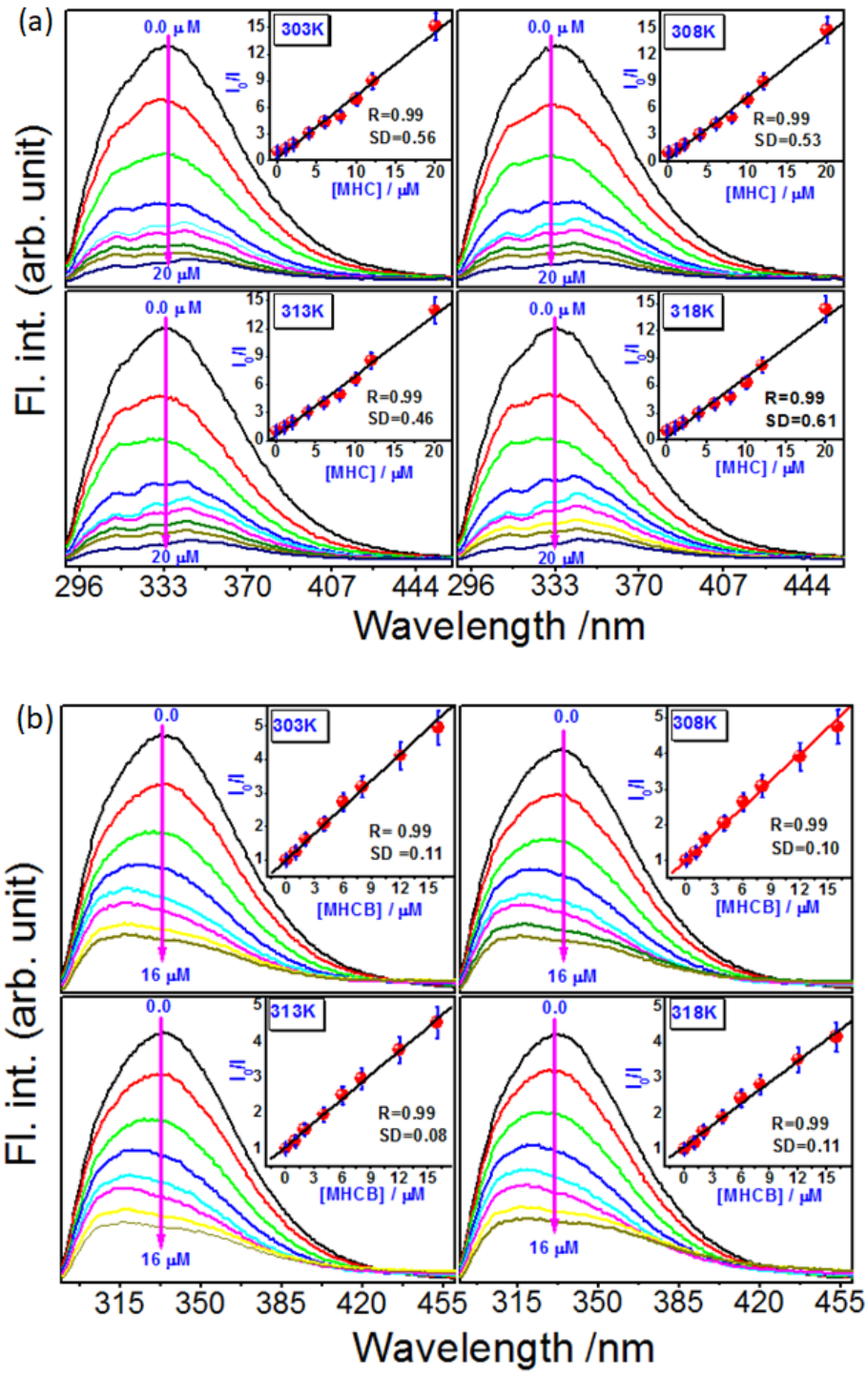

Fig. 5S: Double log plots of MHC with HSA at some representative temperatures.