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Supplementary Material

Therapeutic potency of substituted chromones as Alzheimer's drug: Elucidation of acetylcholinesterase inhibitory activity through spectroscopic and molecular modelling investigation

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ST1. Kinetic characterization of AChE enzyme

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 considered for calculating the initial velocity of the reaction, V_0 by using Eq. 1.

$$\text{Initial rate, } V_0 \text{ (in moles per litre per second)} = \frac{\text{Slope}}{\epsilon \times l} \qquad (1)$$

Where ε represents the molar absorptivity of the yellow anion, $l = 0.442$ cm^1 is the path length and the slope is in the units of absorbance per second.

Michaelis-Menten (MM) constant, K_m and maximum hydrolysis rate, V_{max} which are the standard measures for enzyme catalysis were obtained by non-linear regression analysis of the velocity (V_0) data against [S_0]. The relations corresponding to normal and inhibition cases given by Eq. 2a and 2b, respectively. [2]

$$V_0 = \frac{V_{max}[S_0]}{K_m + [S_0]} \quad (2a)$$

$$V_0 = \frac{V_{max}'[S_0]}{K_m' + [S_0]} \quad (2b)$$

Where the various terms are defined as follows:

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

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

The IC_{50} values of all the inhibitors both in aqueous buffer and serum matrix were calculated by non-linear regression analysis using modified Hill relation (Eq. 3).[3-5]

$$\frac{\Delta V}{\Delta V_{max}} = \frac{[I]^{n_H}}{K_{0.5}^{n_H} + [I]^{n_H}} \quad (3)$$

Where, ΔV is the decrease in initial velocity in the presence of certain inhibitor concentration [I], and ΔV_{max} represents the maximum decrease in initial velocity, $K_{0.5}$ denotes the concentration of the inhibitor that gives half-maximal initial velocity change (equivalent to IC_{50}) and n_H is the Hill coefficient.

ST2. Fluorescence measurements

Fluorescence experiments were carried out in Quanta Master (QM-40) steady state apparatus obtained from Photon Technology International (PTI). All solutions were prepared afresh in 0.1 M phosphate buffer of pH 8.0 and incubated for sufficient time before the measurements. All spectra were corrected for the instrument response function. The excitation wavelength for monitoring ThT emission was 412 nm. Quartz cell of 1 cm path length was used for recording the fluorescence data. The results were averaged from three independent experiments

and analysed. The fluorescence spectra were corrected for any possible inner filter effect using the following relation.[6]

$$F^{Corr}(\lambda_E, \lambda_F) = F_{Obs}(\lambda_E, \lambda_F) \times \frac{A(\lambda_E)}{A_{tot}(\lambda_E)} \quad (4)$$

Where A implies the absorbance of the free ThT, and A_{tot} represents the total absorbance of the solution at the excitation wavelength (λ_E).

ST3. Statistical parameters for the IC_{50} value with Student's t-test

The standard student t-test which was used as a measure of statistical hypothesis was based on the below equation

$$t - value = |IC_{50}^{a} - IC_{50}^{b}| / \sqrt{\frac{S_a^2}{n_a} + S_b^2/n_b} \quad (5)$$

Where, a and b represent the two inhibitory systems respectively, and S is the standard error of IC_{50} value, n denotes the number of data points for the systems. In all calculations, the system with lower IC_{50} value has been taken as a. The results have been depicted in Table 4. For triplicate replication, the degrees of freedom for testing the significance of t-value was calculated using following equation[7,8]

$$df = \{[(n_a * 3) - 1] + [(n_b * 3) - 1]\} \quad (6)$$

Where n holds the same meaning as in the earlier application.

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Table S1: Student t-test for comparison of IC_{50} values amongst the inhibitory systems

	CyC in buffer	AMC in buffer	CyC in HSA
AMC in buffer	1.45		
CyC in HSA	12.21	3.75	
AMC in HSA	2.31	0.89	0.13

Table S2: Binding affinities of the docked poses of ThT with AChE in decreasing stability.[a]

Mode	ThT		
	Affinity ((kJ/ mol)	Distance from r.m.s.d lb (Å)	Distance from r.m.s.d ub (Å)
1	-31.31	0.00	0.00
2	-30.07	2.28	3.03
3	-28.84	1.26	1.61
4	-28.42	2.22	8.13
5	-27.60	16.234	17.17

[a] r.m.s.d is the root mean square deviation from lb (upper bound) and ub (upper bound)

Table S3: Binding affinities of the docked poses of inhibitors in decreasing stability[a]

Mode	CyC			AMC		
	Affinity ((kJ/ mol)	Distance from r.m.s.d lb (Å)	Distance from r.m.s.d ub (Å)	Affinity ((kJ/ mol)	Distance from r.m.s.d lb (Å)	Distance from r.m.s.d ub (Å)
Docked with AChE						
2	-32.14	3.37	5.12	-32.54	2.61	4.57
3	-32.14	3.26	4.78	-32.14	2.51	4.10
4	-31.31	2.81	4.06	-31.72	2.61	4.84
5	-30.07	2.53	5.04	-28.01	3.17	5.45
Docked with HSA						
2	-34.60	2.12	2.74	-31.72	1.03	2.76
3	-31.72	1.59	2.23	-31.31	2.71	4.29
4	-31.72	1.81	2.34	-30.48	1.97	2.66
5	-30.90	1.84	2.97	-28.01	40.06	41.03

[a] r.m.s.d is the root mean square deviation from lb (upper bound) and ub (upper bound)

Supplementary Figures

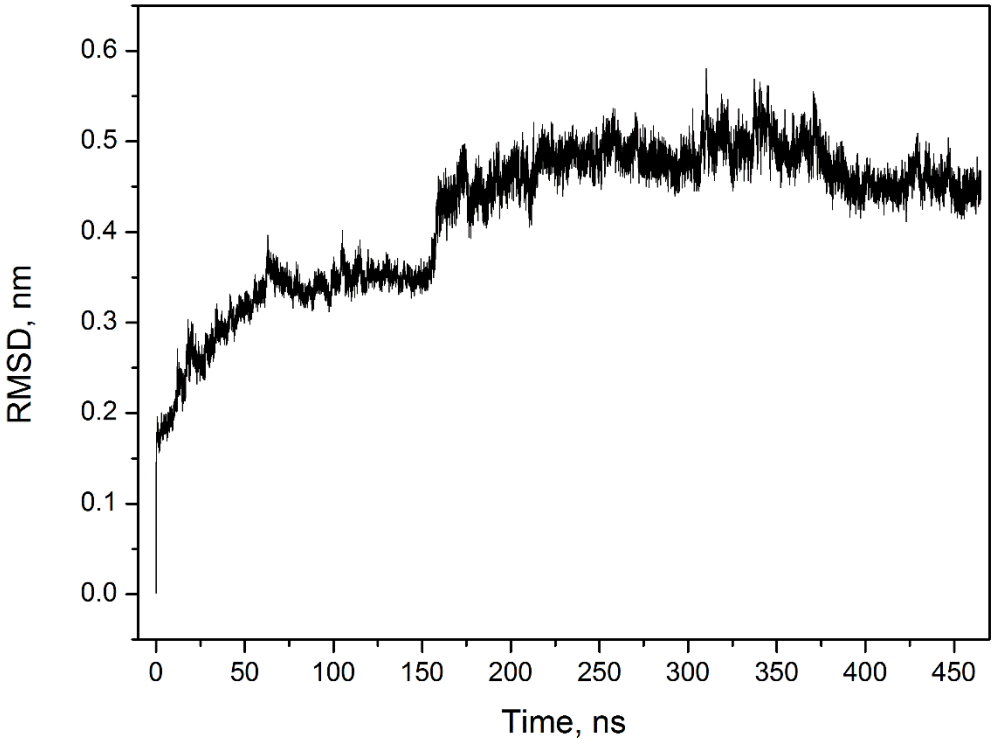

Fig S1. Root mean square deviations of the AChE protein during MD simulations.

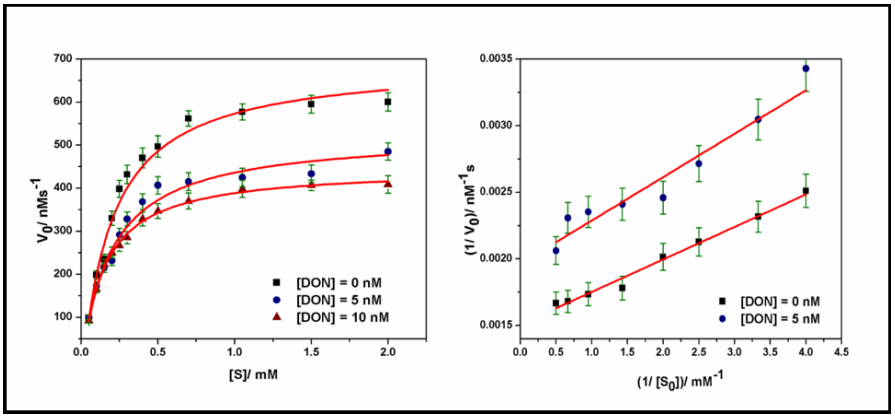

Fig. S2. Hydrolysis curve (left) and Lineweaver-Burke (right) plot representing AChE activity and its inhibition in presence of different concentrations of DON in phosphate buffer solution of pH = 8.0. [AChE] = 0.079 u/ml.

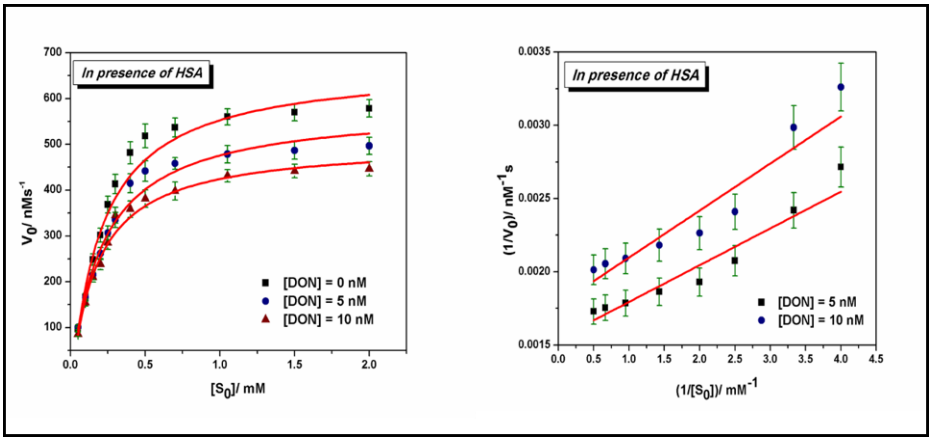

Fig. S3. Hydrolysis curve (left) and Lineweaver-Burke (right) plot for AChE activity and its inhibition in presence of different concentrations of DON in HSA of pH = 8.0. [AChE] = 0.079 u/ml.

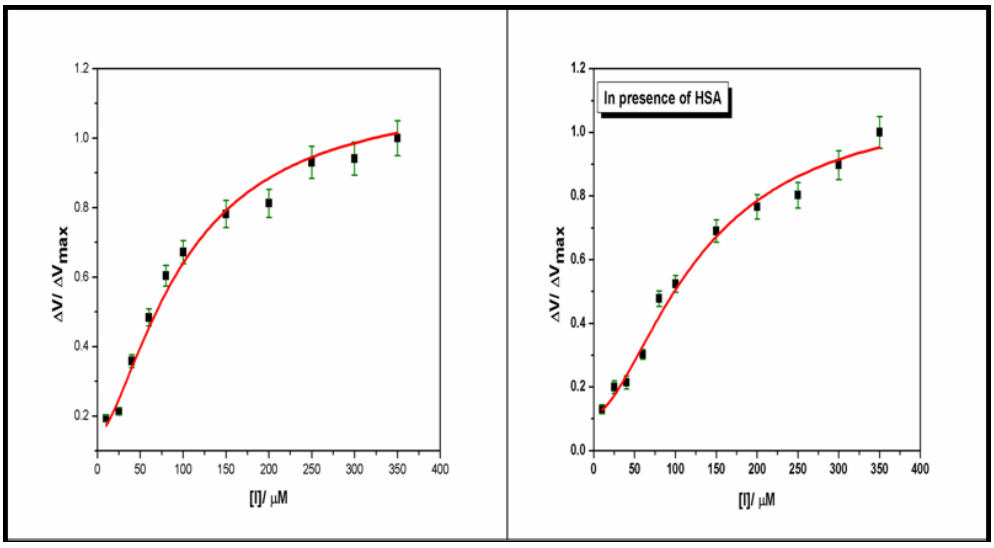

Fig. S4. Modified Hill analysis for DON induced AChE inhibition in aqueous buffer (left) and HSA matrix (right).

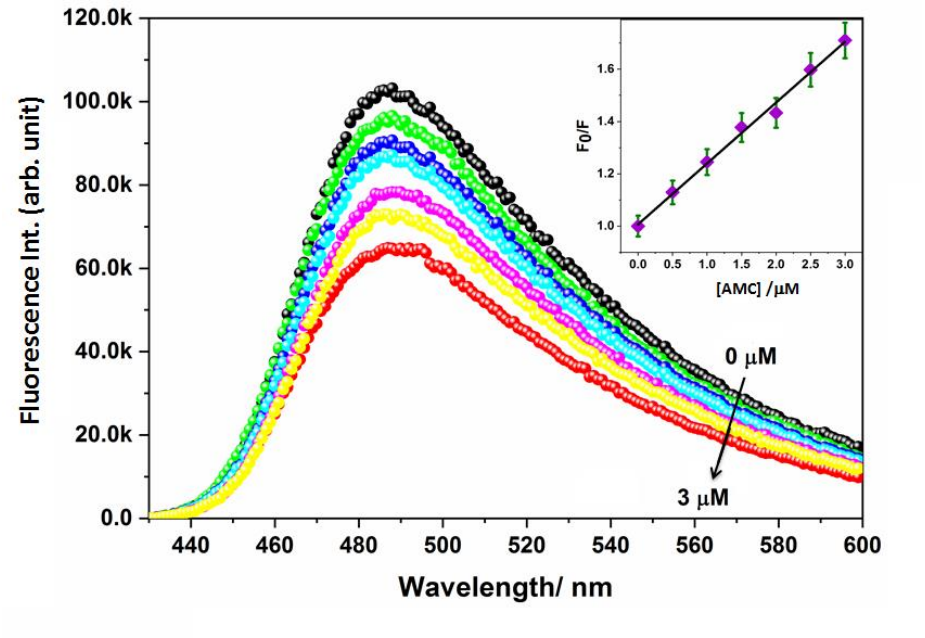

Fig. S5. Left: Quenching of emission intensity of ThT-AChE binary system in presence of increasing concentrations of AMC. Right: Stern Volmer (SV) analysis for the fluorescence quenching data.

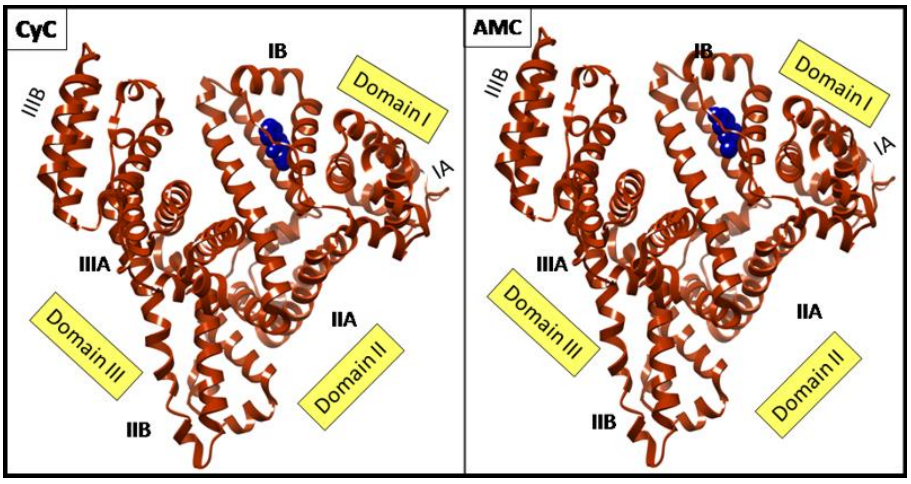

Fig. S6. Docked poses of CyC and AMC with HSA. The binding site has been magnified for clarity.

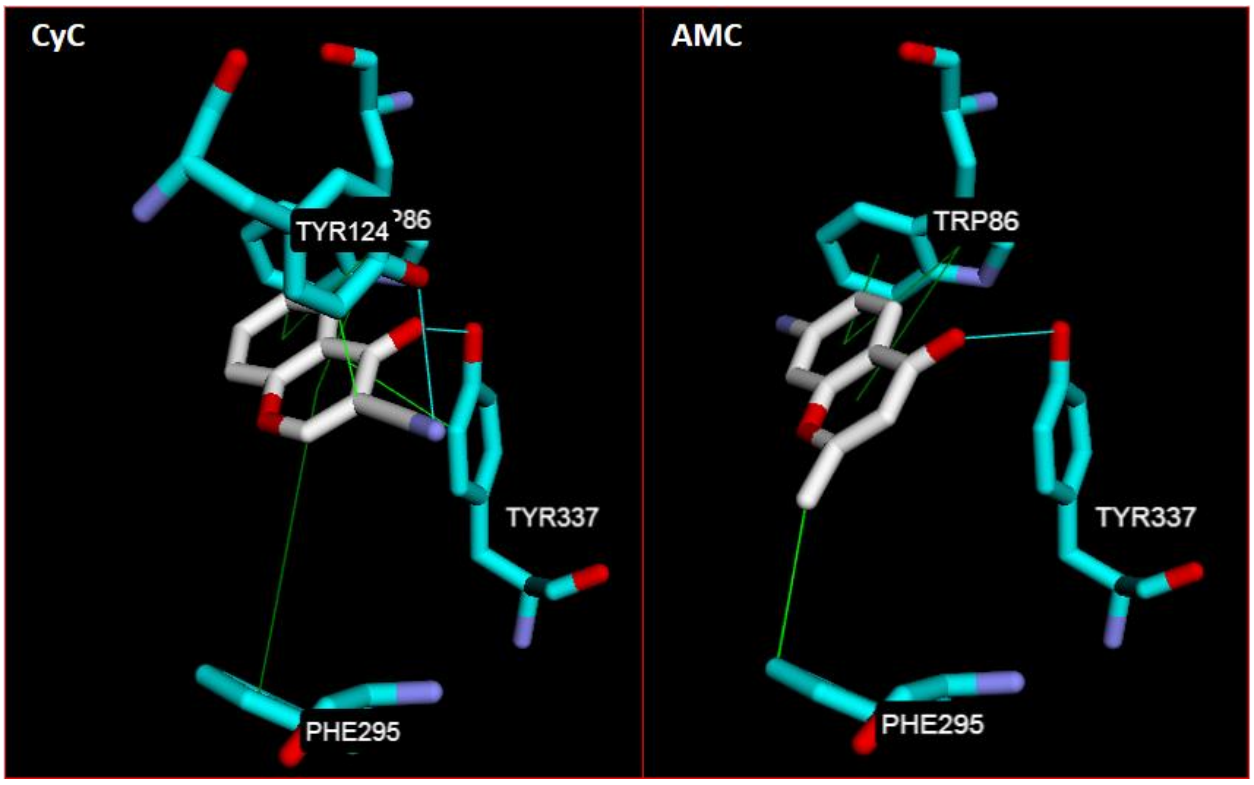

Fig. S7. The interactions of CyC and AMC with closest contact residues. The dark green lines represent the stacking interactions.