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Host-guest complexes of baicalein and silybin with C-hexylpyrogallol[4]arene: Determination of the stoichiometry and the mode of binding

Chandrasekaran Sowrirajan[1], Israel V. M. V. Enoch[1]* and Murugesh Easwaran[2]

[1]*Department of Chemistry, School of Science and Humanities, Karunya University, Coimbatore, Tamilnadu, India*
[2]*Department of Bio-Informatics, Bharathiar University, Coimbatore, Tamilnadu, India*

ABSTRACT

Apart from the trapping of metal ions by the various calixarenes, C-Hexylpyrogallol[4]arenes are studied for their host–guest interactions with the organic guest molecules. In this article, we report the host–guest association of a flavone, baicalein and a chromanone, silybin with C-Hexylpyrogallol[4]arene. The stoichiometry for the inclusion of these guest molecules with C-Hexylpyrogallol[4]arene is determined as 1:1 ratio by Benesi–Hildebrand equation. The strength and mode of binding of baicalein and silybin with is supported by time-resolved fluorescence, nuclear magnetic resonance correlation spectroscopic techniques and molecular docking studies. The structure of the inclusion complex is proposed.

Keywords: Baicalein; Silybin; C-Hexylpyrogallol[4]arene; host–guest complex; molecular docking

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INTRODUCTION

Molecular capsules have been of interest to many because they offer unique chemical environments for molecules through encapsulation. This confinement of chemical space remains less explored [1–6]. C-Hexylpyrogallol[4]arene (C-HPA) assembles into a multi-component hydrogen-bonded nanocapsule encapsulating guest molecules [7–10]. These molecules are flexible compared to the relatively rigid cyclodextrin molecules. Host-guest association in the nanocapsule of C-HPA is an active area of research [7]. The modes of binding of guest molecules to these host molecules C-HPA cannot depend only on the size of the cavity and different factors which decide the mode and the strength of binding of guests need to be explored. These capsules are able to isolate the encapsulated guests from the bulk, which may allow stabilization of the reactive intermediates [11, 12] and the catalysis of reactions [13]. Therefore, understanding the factors that govern the affinity and the tendency of guests toward the host molecule, C-HPA is essential.

The IUPAC name of baicalein is 5,6,7-trihydroxyflavone. Baicalein (2-phenyl-4*H*-chromene-4,5,6,7-tetrol) inhibits the in vitro growth of human cancer cells and induces apoptosis. This takes place due to the inhibition of CDC2-surviving pathway [14]. Baicalein inhibits the enzyme CYP2C9, of the cytochrome P450 system, which does the metabolism of drugs in the body [15]. The chemically silybin is known as (2R,3R)-3,5,7-trihydroxy-2-[(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl]chrom-an-4-one.

Silybin is used as a hepatoprotectant, an anticancer agent and a cancer-protecting compound. Hence, it is applied to the diseases of the prostate gland, lungs, CNS, kidneys, and pancreas. New functions of silybin are discovered based on its specific interaction with the receptor [16]. Since the host-guest complexation could modify the ligand-receptor interactions [17, 18], we are interested in exploring the mode of association of flavones and chromanones, which are important pharmacophores [19, 20], with C-HPA. In this paper, we report the stoichiometry, the binding strength, and the mode of binding of baicalein and silybin with C-HPA.

EXPERIMENTAL SECTION

Chemicals

Pyrogallol, heptanal, baicalein and silybin were purchased from Sigma–Aldrich, Bangalore, India and used without further purification. Phosphoric acid and sodium hydroxide from Qualigens were used to adjust the various pH solutions. All the solvents and reagents used from Merck were of spectral grade, which were used as received without further purification. Double distilled water was used throughout the experiments.

Synthesis of C-hexylpyrogallol[4]arene (C-HPA)

The synthesis of C-hexylpyrogallol[4]arene is reported [21]. An alcoholic solution of pyrogallol (10 mmol) was acidified with concentrated hydrochloric acid (1.7 ml) at 0 to 5° C. Then heptanal (10 mmol) was added to the reaction mixture and the cold, clear solution was brought to room temperature slowly then refluxed for 8 hours. The C-HPA formed as a brown solid was filtered and dried. The product was re-crystallized with hot methanol.

Preparation of Baicalein/C-HPA complex

A solution of baicalein (0.2 g, 0.74 mmol) and an equimolar amount of C-HPA (0.66 g) were taken in 5 ml of methanol separately. Baicalein was added slowly to the solution of C-HPA at room temperature. The solution was sonicated for 30 min and then warmed at 50° C for 10 min. It was then kept at room temperature for the formation of solid product.

Preparation of Silybin/C-HPA complex

An equal molar (0.52 mmol) proportions of silybin (0.25 g) and C-HPA (0.46 g) were dissolved in 5 ml of methanol separately. A solution of silybin was added slowly to a solution of C-HPA at room temperature. The mixed solution was sonicated in an ultra sonicator for 30 min. and warmed to 50° C for 10 min. Then the mixture was kept at room temperature for two days. The solid obtained was collected and analyzed.

Preparation of test solutions

Test solutions were prepared with double distilled water by appropriate dilution of stock solutions of baicalein (1.48×10^{-5} mol dm^{-3}), silybin (8.29×10^{-5} mol dm^{-3}), and C-HPA (2×10^{-5} mol dm^{-3}) in methanol due to their less solubility in water. The test solutions were having the concentration of methanol as 3 %. The absorption and the fluorescence spectra were recorded for homogeneous test solutions against appropriate blank solutions. The measurement of H_0 and pH value below the pH 2 from a modified Hammett's acidity scale [22]. Various concentrations of sulfuric acid (0.5, 1.0, 2.0, 3.0, and 4.0 mol dm^{-3}) were used to vary the acid strength and H_0 values were interpreted from a modified Hammett's acidity scale. All experiments were carried out at ambient temperature of 25 ± 2° C.

Instrumentation

A Jasco V-630, double beam UV–Visible spectrophotometer used to measure the absorbance by using 1cm path length quartz cells. The fluorescence measurements carried out in a Perkin-Elmer spectrofluorimeter (Model: LS55) equipped with a Xenon lamp (120 W) for excitation. Both the excitation and the emission band widths were set up at 4 nm. Time–resolved fluorescence measurements were done on a time-correlated single photon counting HORIBA spectrofluorimeter using an LED source. ^{1}H NMR and two dimensional Rotating-frame overhauser effect spectroscopy (2D ROESY) spectra were recorded on a Bruker AV III instrument operating at 500 MHz with $CDCl_3$ as solvent for baicalein/C-HPA and silybin/C-HPA complexes. The chemical shift values are reported in ppm. The 2D ROESY experiments were performed on the prepared solid complexes of baicalein/C-HPA and silybin/C-HPA. The mixing time for ROSEY spectra was 200 ms under the spin lock condition. Tetramethylsilane (TMS) was used as an internal standard. The chemical shift values were obtained downfield from TMS in part per million (ppm). Ultra-sonicator PCI 9L 250H, India was used for sonication. Elico LI 120 pH meter, India was used to adjust various pH solutions.

The three dimensional structure of baicalein, silybin and C-HPA was optimized and the length, breadth of the molecules are measured by using a software RasWin molecular graphics, a product of Rasmol [23] version 2.7.5.2 [Figs. SI 1, SI 2 and SI 3 respectively]. The molecular docking of the guest molecules, baicalein and silybin with the host C-HPA were studied by the Schrödinger suite 2013, update 2, Glide 5.5. In the inclusion complexation study, the molecular docking suggests that the orientation of the guest to the host. The molecular docking study support the inclusion complexation with the glide score (G-score) value, hydrogen bonding value and the hydrophobicity of the host exerts on the guest. The Glide (Grid-based Ligand Docking with Energetics) algorithm [24] approximates a systematic search of active binding sites of the guest with the host binding site using a series of hierarchical filters [25].

RESULTS AND DISCUSSION

The chemical structures of the guest molecules baicalein, silybin and the host molecule C-hexylpyrogallol[4]arene are given in the Figs. 1 (a), (b) and (c) respectively . The host-guest interactions of the selected system are discussed in the following sections.

Host–guest interaction of Baicalein with C-HPA

The absorption spectra of baicalein in water and increase in concentrations of C-HPA solution are shown in Fig. 2 (a). The absorption spectrum of baicalein in the absence of C-HPA (0 mol dm^{-3}) showed that the n→π* transition band at 321 nm. The hyperchromic shift is observed in the absorption band with the increasing concentration of C-HPA. Also the absorption band is significantly shifted to lower wavelength (2 nm) with the addition of C-HPA. There is enhancement of the absorption band with the blue shift occurred due to the host-guest interactions of baicalein– C-HPA molecules resulted by the inclusion complexation. The absorption and the fluorescence spectral data are given in Table 1. In the fluorescence analysis, there is increase of the two fluorescence bands at 364 and 411 nm observed for baicalein with the addition of C-HPA (0 to 6.54×10^{-6} mol dm^{-3}). The fluorescence spectrum is shown in Fig. 2 (b). This increase in fluorescence intensity indicates that the encapsulation of baicalein by C-HPA molecules due to the π electron interactions. The binding constant and the stoichiometry for the baicalein–C-HPA complex are determined by using the Benesi – Hildebrand equation as given in equation (1),

$$\frac{1}{I-I_0} = \frac{1}{I'-I_0} + \frac{1}{I'-I_0}\frac{1}{K[\text{C-HPA}]} \qquad (1)$$

where I_0 is the intensity of fluorescence of baicalein in water, I is the intensity at each concentration of C-HPA, and I' is the intensity of fluorescence at the highest concentration of C-HPA. K is the binding constant. The stoichiometry for the formed baicalein–C-HPA complex is determined as 1:1 ratio from the linearity in the plot of $1/(I-I_0)$ vs. 1/[C-HPA]. The slope for the straight line obtained is 2.73×10^{-8} with the correlation coefficient (R) = 0.99. The binding constant K is calculated as 6.71×10^{4} mol^{-1} dm^{3}.

In order to confirm the inclusion complexation of baicalein with C-HPA are studied by the time–resolved fluorescence spectroscopy. Fig. SI 4 shows that the time-resolved spectrum of baicalein in water, low and high concentrations of C-HPA. The fluorescence decay profiles are compiled in Table 2. The bi-exponential decay is observed in water with the life span of 1.48 and 6.39 ns (Relative amplitude of each state 86.26 and 13.74 respectively). The relative amplitudes of the two state in the presence of C-HPA changed to tri-exponential from that in water. Apparently there is a decrease in relative amplitude of the shorter lifetime state and an increase in the longer lifetime states. These observations suggested that the inclusion complexation of baicalein with C-HPA indeed occurred. The complete encapsulation of guest molecule and the shift of the equilibrium

$$H + G \rightleftharpoons HG \qquad (2)$$

where H, G, and HG refer to the host, guest and the host-guest complex respectively. The complexation shifts the equilibrium towards the right side would result in the relative amplitude getting closer to 100.

Decrease of absorbance of baicalein at 251 nm from the decrease pH 4.6 to the acidic range up to H_0 –1.85 of C-HPA solution results with a corresponding increase at around 315 nm. There is an isosbestic point at 290 nm due to the possible formation of cationic equilibrium of baicalein in C-HPA. All the spectra did not pass through the isosbestic point, specifically below the pH 1.4. This explains that the baicalein molecule is shielded by the host C-HPA and the formation of cationic equilibrium got disturbed at higher acidic condition. Hence this neutral – monocationic form of baicalein is influenced by the C-HPA encapsulation. The ground state pK_a value is calculated as below,

$$C_1 = \frac{A(\lambda_1)\varepsilon_2(\lambda_2) - A(\lambda_2)\varepsilon_2(\lambda_1)}{\varepsilon_2(\lambda_1)\varepsilon_2(\lambda_2) - \varepsilon_1(\lambda_2)\varepsilon_2(\lambda_1)} \qquad (3)$$

$$C_2 = C_T - C_1 \qquad (4)$$

where C_T is the total concentration of the compound in both forms and $\varepsilon_1(\lambda_1)$, $\varepsilon_2(\lambda_2)$, $\varepsilon_2(\lambda_1)$, $\varepsilon_2(\lambda_2)$ are the molar extinction coefficients of the protonated and neutral forms at wavelengths λ_1 and λ_2 respectively.

$$pK_a = pH + \log C_1/C_2 \qquad (5)$$

The calculated pK_a value is 0.14.

Table 1: Absorption and fluorescence spectral data of baicalein in various concentrations of C-hexylpyrgallol[4]arene (C-HPA)

Conc. of C-HPA, (mol dm^{-3})	Absorption maximum (nm)	Absorbance	Fluorescence maximum (nm)	Fluorescence signal
0	321.0	0.019	364.0	1195.22
1.48×10^{-7}	321.0	0.035	364.0	1236.35
9.48×10^{-7}	320.0	0.041	364.0	1309.81
4.14×10^{-6}	320.0	0.053	362.0	1400.17
4.94×10^{-6}	320.0	0.059	360.0	1503.79
5.74×10^{-6}	319.0	0.071	359.0	1596.37
6.54×10^{-6}	319.0	0.079	356.0	1691.01

Table 2: Time–resolved fluorescence spectral data of baicalein in water and C-hexylpyrgallol[4]arene (C-HPA)

Conc. of C-HPA (mol dm^{-3})	Lifetime (s)	Relative Amplitude (%)	$\chi 2$	Standard deviation (s)
0	1.48×10^{-9}	86.26	1.15	5.89×10^{-11}
	6.40×10^{-9}	13.74		6.07×10^{-11}
1.60×10^{-7}	2.16×10^{-9}	60.86	1.07	3.73×10^{-11}
	7.51×10^{-10}	14.83		2.55×10^{-11}
	1.16×10^{-8}	24.31		7.70×10^{-11}
6.60×10^{-6}	3.29×10^{-9}	37.17	1.10	1.48×10^{-10}
	1.14×10^{-9}	32.65		1.50×10^{-11}
	1.23×10^{-8}	30.18		8.77×10^{-11}

Table 3: ^{1}H NMR spectral data of C-hexylpyrgallol[4]arene (C-HPA), baicalein and silybin complexes with C-hexylpyrgallol[4]arene

C-HPA		Baicalein–C-HPA complex		Silybin–C-HPA complex	
Position of protons	Chemical shift, δ (ppm)	Position of protons	Chemical shift, δ (ppm)	Position of protons	Chemical shift, δ (ppm)
C- Hexyl chain:		Benzopyran ring:		A –Ring:	
Methyl protons	0.93	3-CH	6.92	3-Hydroxyl	5.79
Methylene protons	1.28 – 1.45	8-CH	6.68	5-Hydroxyl	12.08
CH – linked in pyrogallol rings	2.21 – 2.28	5-OH	12.66	7-Hydroxyl	10.90
		6-OH	8.84	2-CH	5.03
		7-OH	10.62	3-CH	4.60
				6-CH	5.89
				8-CH	5.91
Pyrogallol unit:		Aromatic protons:		B –Ring:	
Aromatic protons	7.49	13, 15 & 14	7.49 – 7.58	2'-CH	4.91
Hydroxyl protons	6.86, 6.90 and 8.80	12 & 16	7.86 -7.92	3'-CH	4.20
				3'-substituted CH_2	3.83
				3'-substituted OH	1.92
				C –Ring:	
				Methoxy proton	3.80
				4"-Hydroxyl	9.10 – 9.30
				Aromatic protons	6.71 – 7.10

Table 4: Absorption and fluorescence spectral data of silybin in various concentrations of C-hexylpyrgallol[4]arene (C-HPA)

Conc. of C-HPA (mol dm^{-3})	Absorption maximum (nm)	Absorbance	Fluorescence maximum (nm)	Fluorescence signal
0	325.0	0.110	425.0	58.08
2.50×10^{-6}	323.0	0.150	423.0	62.16
6.50×10^{-6}	322.0	0.222	423.0	67.18
1.00×10^{-5}	322.0	0.267	423.0	69.76
1.40×10^{-5}	322.0	0.346	423.0	70.31
1.80×10^{-5}	322.0	0.396	423.0	70.49
2.20×10^{-5}	322.0	0.444	423.0	70.86
3.00×10^{-5}	322.0	0.543		

Table 5: Time–resolved fluorescence spectral data of silybin in water and C-hexylpyrgallol[4]arene (C-HPA)

Conc. of C-HPA (mol dm^{-3})	Lifetime (s)	Relative Amplitude (%)	$\chi 2$	Standard deviation (s)
0	1.78×10^{-9}	58.98	1.11	1.07×10^{-11}
	7.62×10^{-9}	41.02		5.65×10^{-11}
2.5×10^{-6}	1.74×10^{-9}	51.66	1.03	1.42×10^{-11}
	8.85×10^{-9}	48.34		3.56×10^{-11}
3.0×10^{-5}	1.53×10^{-9}	51.54	1.13	1.41×10^{-11}
	1.03×10^{-8}	48.46		3.55×10^{-11}

Fig. 1
(a). Structural representation of baicalein
(b). Structural representation of silybin
(c). Structural representation of C-Hexylpyrogallol[4]arene

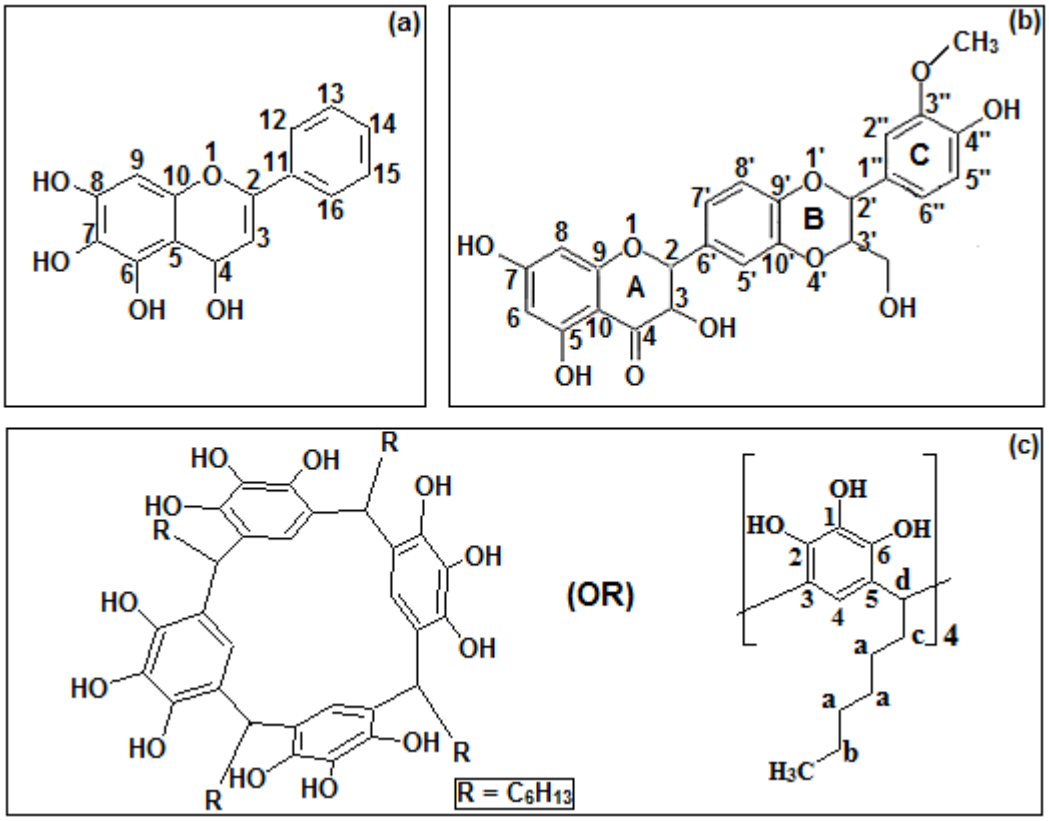
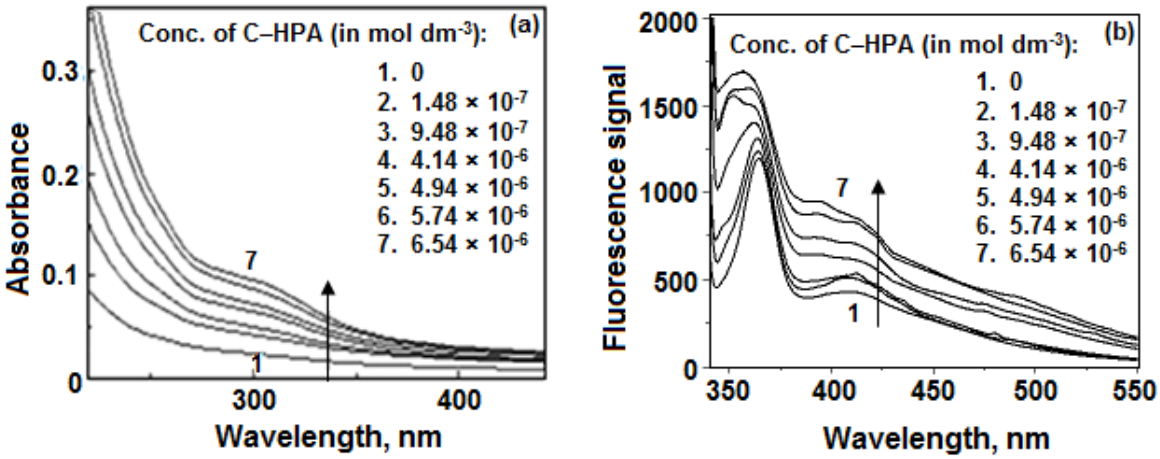


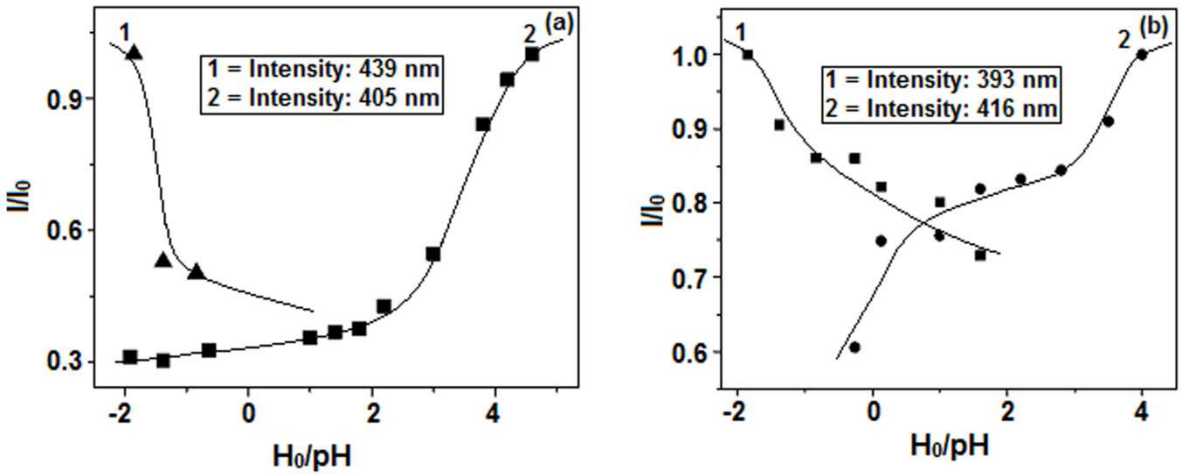
Fig. 2
(a). Absorption spectra of baicalein at various concentrations of C-Hexylpyrogallol[4]arene
(b). Fluorescence spectra of baicalein at various concentrations of C-Hexylpyrogallol[4]arene



The effect of acid strength on the fluorescence emission of baicalein in the presence of C-HPA is studied. The addition of protons to baicalein causes a quenching of fluorescence at the wavelength 410 nm. This may be a case of proton–induced fluorescence quenching of baicalein in C-HPA. At a longer wavelength, there is formation of a new band at 445 nm which becomes more distinct below H_0 –0.84. This is due to the formation of cationic species of baicalein and it should be exists in an equilibrium. But the equilibrium shift happens at higher acid concentration (H_0 –0.84) and did not meet at an isosbestic point. This is due to the influence of C-HPA host molecule on the acidification of baicalein and this is evident that the baicalein involved in the host-guest interactions with C-HPA. The excited state pK_a is calculated as –1.5 from the sigmoidal fluorimetric titration curve and the plot of I/I_0 vs. H_0/pH is given in Fig. 3 (a).

NMR spectroscopic technique is utilized to substantiate the host–guest interactions of baicalein with C-HPA and the mode of encapsulation. The 2D ROESY spectrum is shown in Fig. 4. The ^{1}H NMR spectral chemical shift values of C-HPA, baicalein–C-HPA and silybin–C-HPA complexes are compiled in the Table 3. In the 2D ROESY spectra, off-diagonal peaks were observed for the spatial interactions of inter and intra molecular interactions of baicalein and C-HPA. The host–guest interactions of baicalein–C-HPA complex resulted with a cross peak at the chemical shift of 8.84 ppm in the abscissa and the 4.37 ppm in the ordinate axes are marked by circle symbol in Fig. 4. This signal obtained by the close proximity between the hydroxyl group (at position 6) of baicalein and the CH (labeled 'd') proton of C-HPA which is the linker for the pyrogallol units. Such a CH protons of C-HPA are exists in the inner rim of the C-HPA, they were cross correlated to the hydroxyl proton of baicalein molecule.

Fig. 3.
(a). I/I_0 vs. H_0/pH plot of baicalein in C-Hexylpyrogallol[4]arene solution
(b) I/I_0 vs. H_0/pH plot of silybin in C-Hexylpyrogallol[4]arene solution



The mode of binding of baicalein to C-HPA concluded by the ROESY NMR correlation results is evidenced by the molecular docking studies. The molecular docking poses for the hydrogen bonding and the hydrophobic interactions are shown in Fig. SI 5. The molecular docking of baicalein with C-HPA study resulted with good correlations results viz., the G-score value is –4.7 kcal mol^{-1} and hydrogen bonding interaction score is –2.37 kcal mol^{-1}. The hydroxyl groups of baicalein involved in the hydrogen bonding interactions with the hydroxyls of C-HPA. The docking results support the orientation of the inclusion of baicalein is occurred through the chromenetetrol moiety to the host, C-HPA.

From the NMR results discussed in the preceding paragraph, the hydroxyl groups of baicalein are involved in the inclusion complex formation. Hence, the host molecule should encapsulate baicalein from the chromenetetrol moiety. Again, the hydrogen bonding is possible if only the host structure approaches baicalein from the chromenetetrol part. With the detailed discussions of the obtained results suggested that the structure for the baicalein–C-HPA complex and it is proposed as in Fig. 5 (a).

Host–guest interaction of Silybin with C-HPA

The absorption and the fluorescence spectral data are given in Table 4. The absorption spectrum of silybin with various amounts of C-HPA is shown Fig. 6 (a). There are two distinct absorption maxima observed in the spectrum of silybin, viz., 284 and 325 nm are corresponding to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. There is a continuous enhancement (hyperchromic shift) observed at both the absorption maxima of silybin while increase in concentration of C-HPA. The enhancement of the absorbance is resulted by the host–guest complex formation of silybin–C-HPA. The longer wavelength band got shifted significantly by 3 nm towards the blue region with the addition of C-HPA. This bathochromic shift is characteristic of the light absorbing chromophore dislodging from a polar solvent environment to a less-polar C-HPA microenvironment. The apolar nature of C-HPA cavity is raised owing to a rich π electron density. The fluorescence spectrum of silybin showed an increase in the intensity upon the addition of C-HPA in increasing concentrations and it is displayed in Fig. 6 (b). Two fluorescence bands of silybin are observed at 366 and 415 nm in water. The addition of C-HPA to silybin the fluorescence intensity is enhanced in the both bands. A significant (2 nm) blue shift of longer wave length band is observed while the addition of C-HPA. This is due to the dislodging of the silybin molecule from the polar water cage to the apolar microenvironment of the host system. A linearity in the Benesi – Hildebrand plot with the slope value is 7.96×10^{-7} and the correlation co-

efficient (R) = 0.99 resulted to the 1:1 stoichiometry for the silybin–C-HPA complex. The binding constant (K) calculated is 4.99×10^4 mol^{-1} dm^{-3}.

Fig. 4. 2D ROESY spectra of baicalein–C-Hexylpyrogallol[4]arene complex

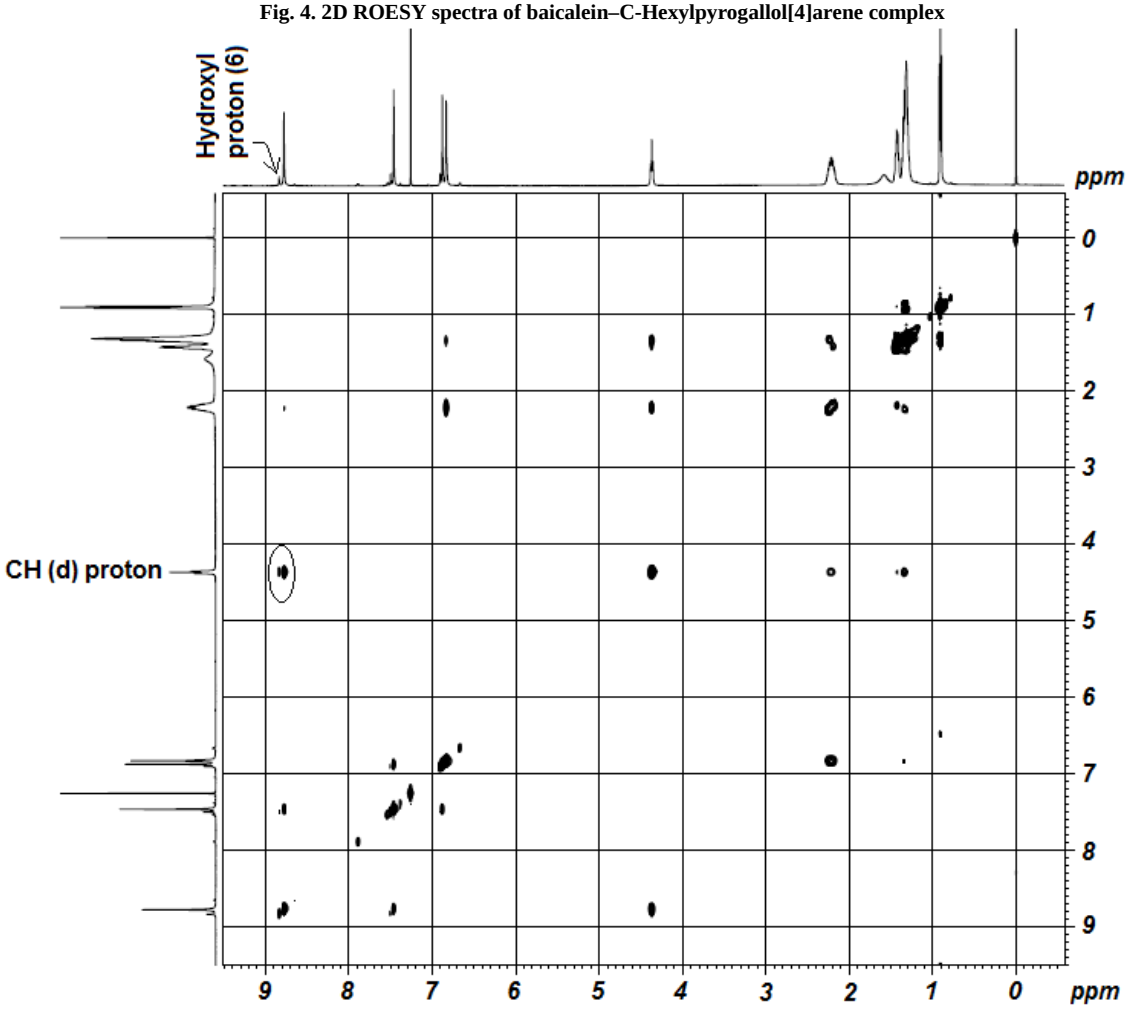


Fig. 5
(a). Schematic representation of baicalein–C-Hexylpyrogallol[4]arene complex
(b). Schematic representation of silybin–C-Hexylpyrogallol[4]arene complex

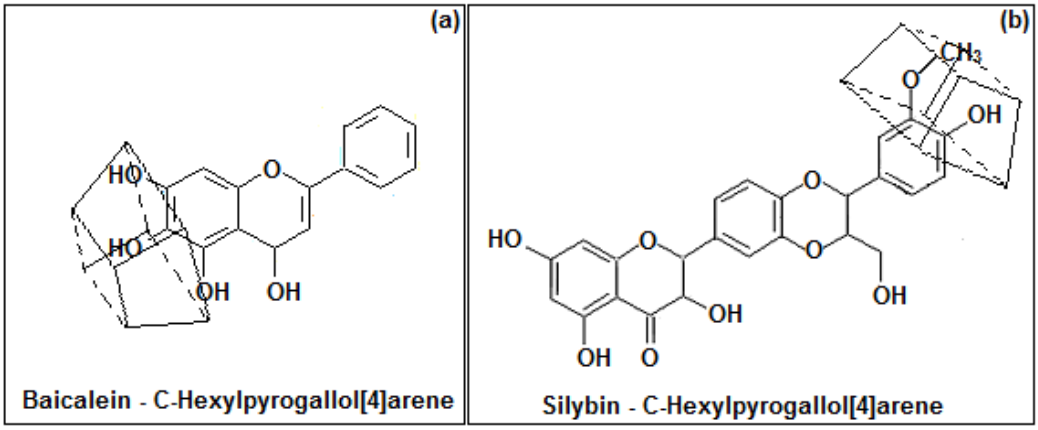


Figure SI 6 shows the time-resolved fluorescence spectra of silybin in water and increase in concentration of C-HPA. The lifetime, relative amplitude, $\chi 2$ and standard deviation spectral data of silybin–C-HPA complex are compiled in Table 5. In water silybin shows bi-exponential decay with the lifetimes of 1.77 and 7.62 ns with the relative amplitudes of 58.98 and 41.02 respectively. The silybin decay profile showed a decrease in the shorter lifetime species and an increase in the longer one by the added C-HPA. The increased lifetime and the

corresponding change in the relative amplitudes of the newly formed species which is due to the complex formation of silybin with C-HPA.

Fig. 6
(a). Absorption spectra of silybin at various concentrations of C-Hexylpyrogallol[4]arene
(b). Fluorescence spectra of silybin at various concentrations of C-Hexylpyrogallol[4]arene

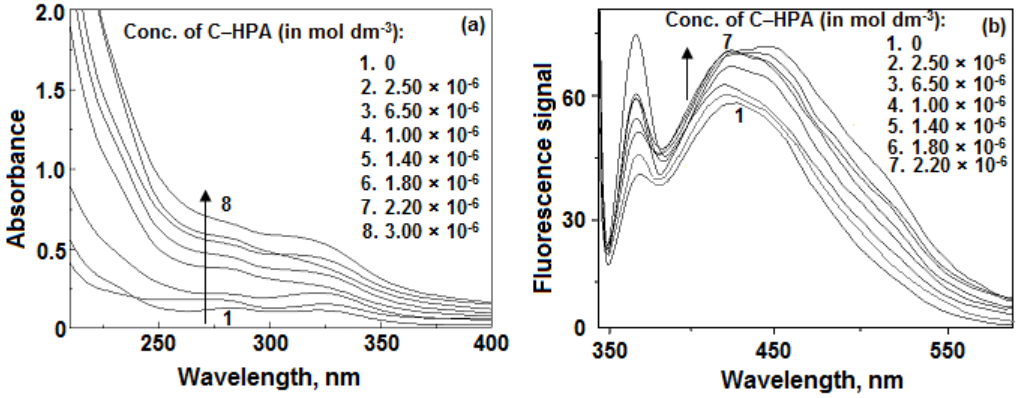
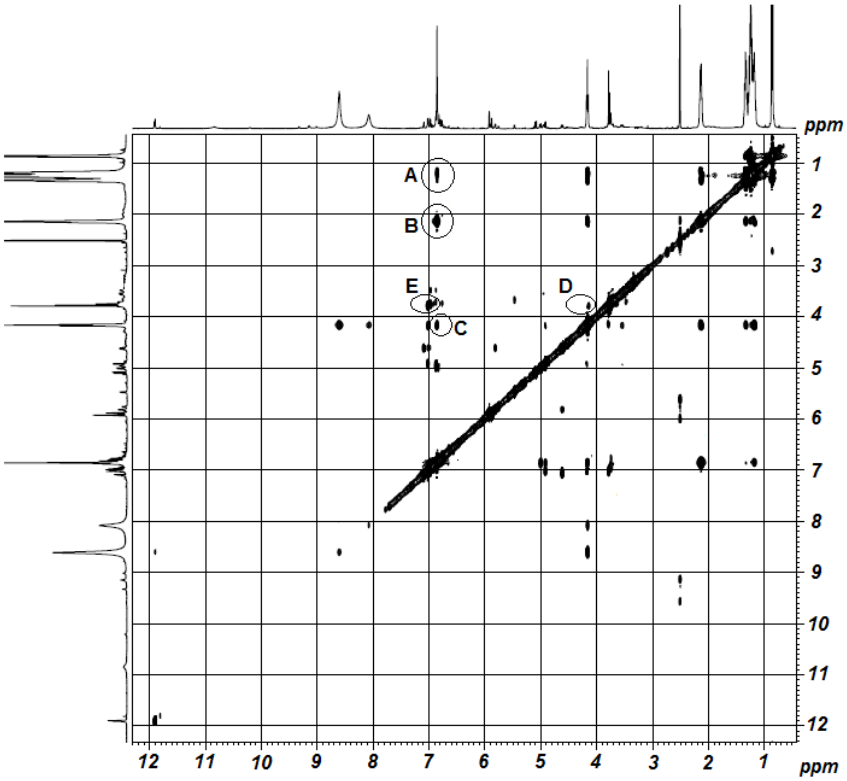


Fig. 7. 2D ROESY spectra of silybin–C-Hexylpyrogallol[4]arene complex



The absorption spectrum of silybin in the presence of C-HPA at various H_0/pH values is recorded. Decrease of pH from 4 resulted in a decrease of absorbance at the wavelength 282 nm. The absorbance band quenches continuously on the acidification and there was no formation of a monocation band observed. It is due to the silybin molecule shielded by C-HPA host molecule and restricts to the silybin molecule to protonate in the ground state. But this was occurred in the excited energy state and it was monitored by fluorescence spectroscopy.

The quenching of fluorescence of silybin with C-HPA was observed at 417 nm by the decrease of pH from 4. The fluorescence quenching occurred owing to the proton–induced and then there was formation of the monocation fluorescence band at 394 nm from the acidic range H_0 –0.26. The blue shift of fluorescence band was observed due to the equilibrium between the neutral–monocationic forms of the silybin in C-HPA complex. The excited state pK_a of this equilibrium was obtained from the fluorimetric curve as –1.3 and it is displayed in Fig. 3 (b). In this

fluorimetric titration plot, the quenching of fluorescence occurred completely to the neutral form and then the formation of monocation species.

Fig. SI 1. Length and breadth of the baicalein molecule measured using RasMol

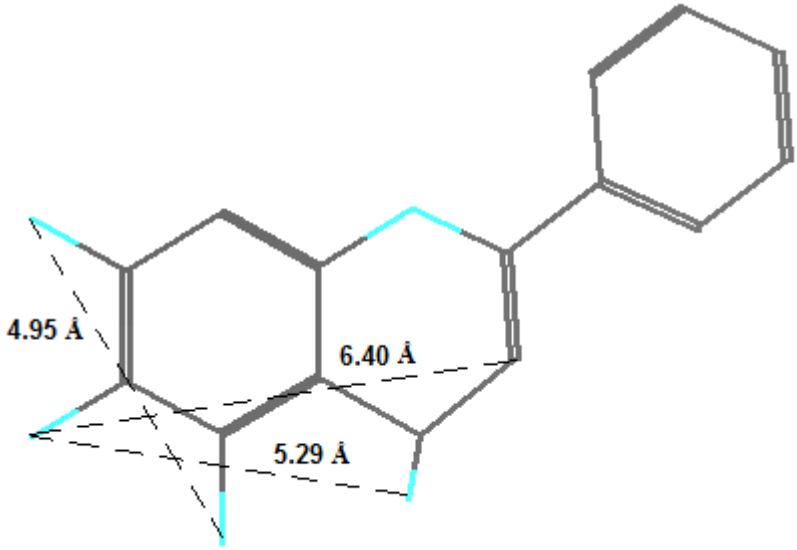


Fig. SI 2. Length and breadth of the silybin molecule measured using RasMol

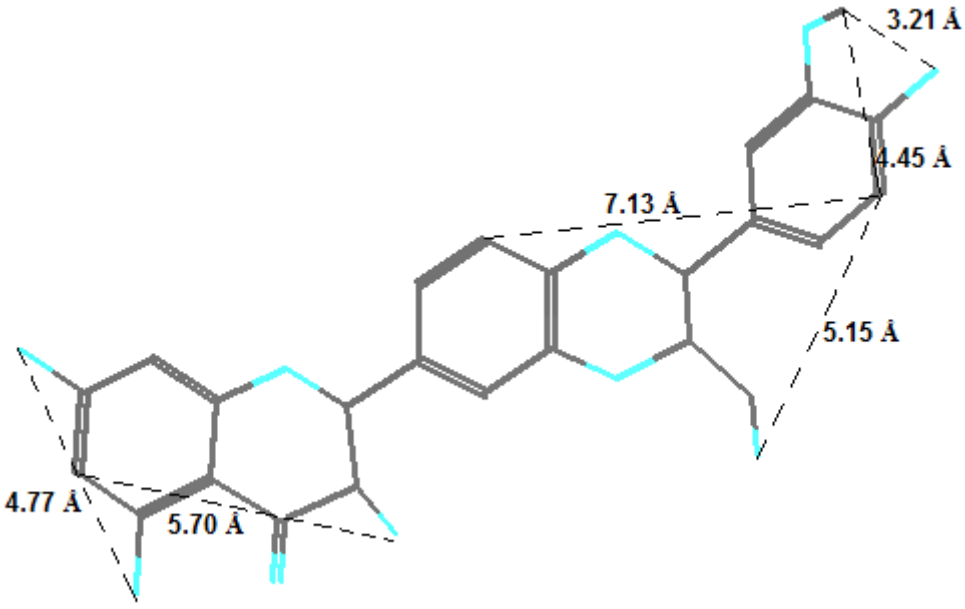


Figure 7 shows the 2D ROESY spectrum for the inclusion complex of silybin in C-HPA. The diagonal and off-diagonal peaks were obtained for the correlations of silybin and C-HPA protons. There were cross peaks found for the cross correlations of the aromatic protons (chemical shift, δ 6.71 to 6.90 ppm) of silybin molecule with the protons of hexyl chain in C-HPA viz., (i) the methylene protons of 'c' (δ = 2.16 ppm), (ii) the methylene protons of 'a', 'b' (δ = 1.15 to 1.39 ppm) and (iii) the CH protons of 'd' (δ = 4.18 ppm). These cross peaks are labeled as A, B and C respectively in Fig. 7. Since the C-HPA molecule having four hexyl chains at their lower rim of the cavity, these methylene and methyne protons are being closeness to the aromatic protons of silybin while it is encapsulated by C-HPA. The methylene protons substituted in the dioxo ring (position 3', δ = 3.83 ppm) and the methoxy protons (substituted in the 4-hydroxyphenyl, δ = 3.80 ppm) of silybin gave the cross peaks with (i) the CH (position 'd') protons of C-HPA which is exist at lower rim of C-HPA (ii) the hydroxyl protons of 2 & 3 of pyrogallol units (δ = 6.89 to 7.02 ppm) which are located at upper rim of C-HPA. These cross peaks are resonated due to the close proximity of these groups by the inclusion of silybin through the 4-hydroxy-3-methoxy phenyl group (Ring C) substituted in the dioxo ring in to the cavity of C-HPA.

Fig. SI 3. Length and breadth of the C-Hexylpyrogallol[4]arene molecule measured using RasMol

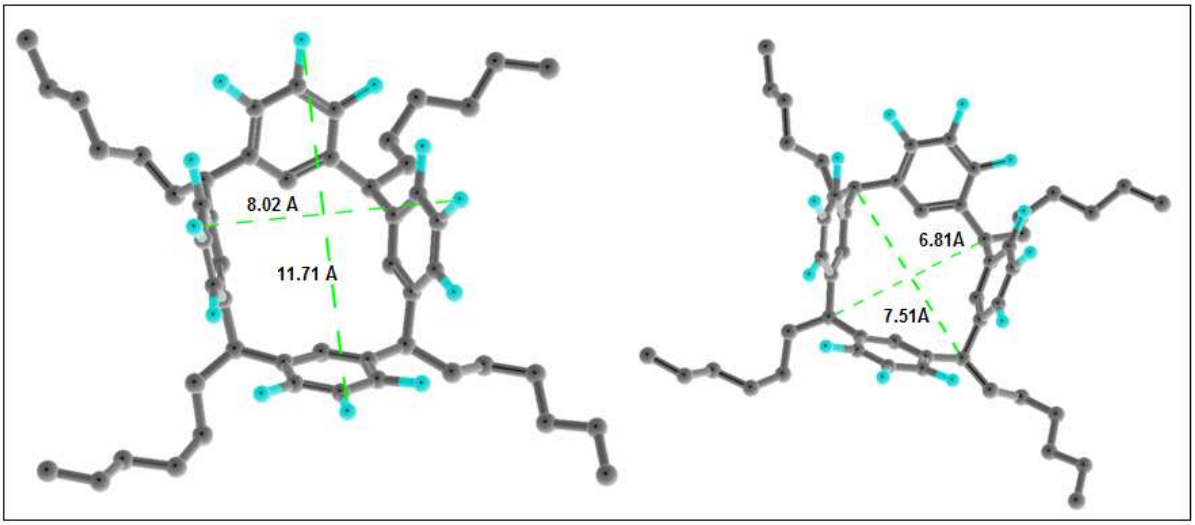


Fig. SI 4. Time-resolved fluorescence spectra of baicalein with various concentrations of C-Hexylpyrogallol[4]arene

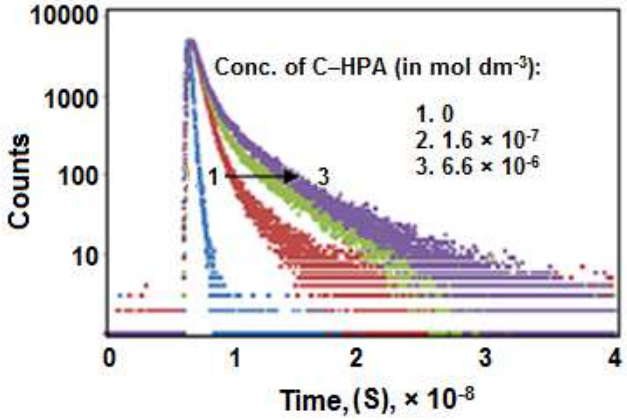


Fig. SI 5. Molecular docking poses of baicalein–C-Hexylpyrogallol[4]arene (a). Hydrogen bonding interactions (b). hydrophobic interactions

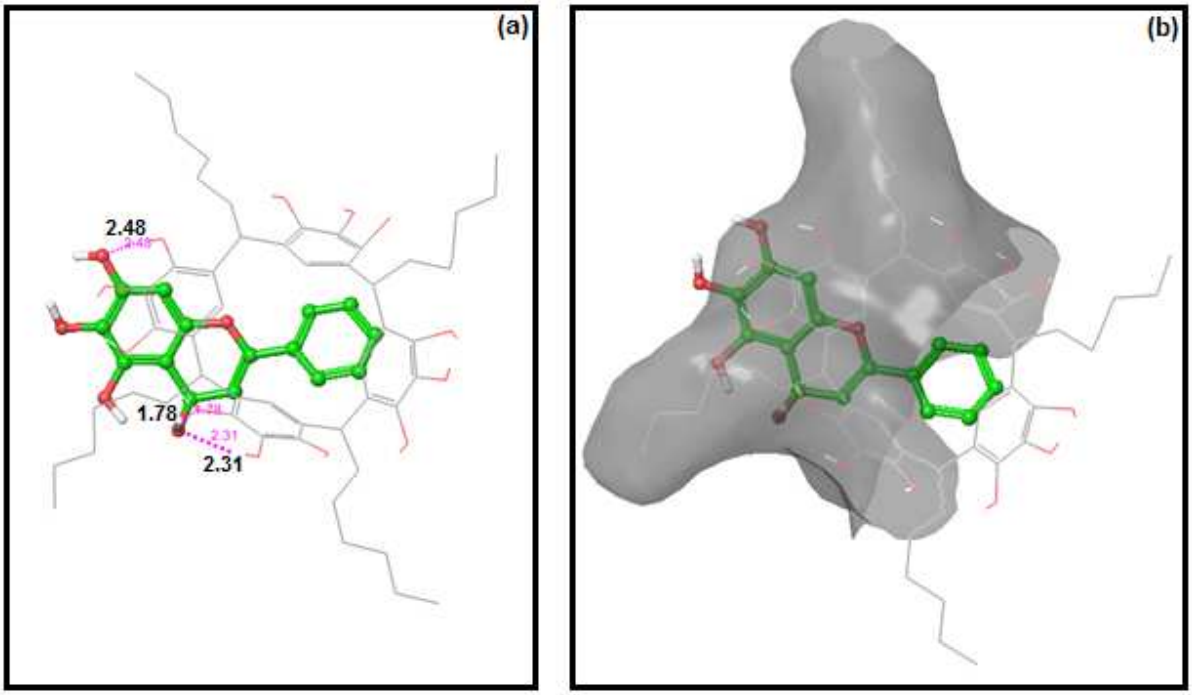


Fig. SI 6. Time-resolved fluorescence spectra of silybin with various concentrations of C-Hexylpyrogallol[4]arene

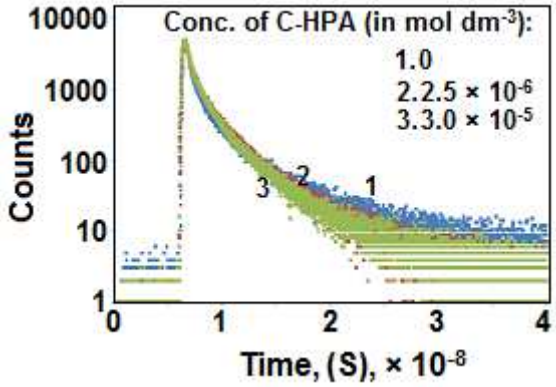
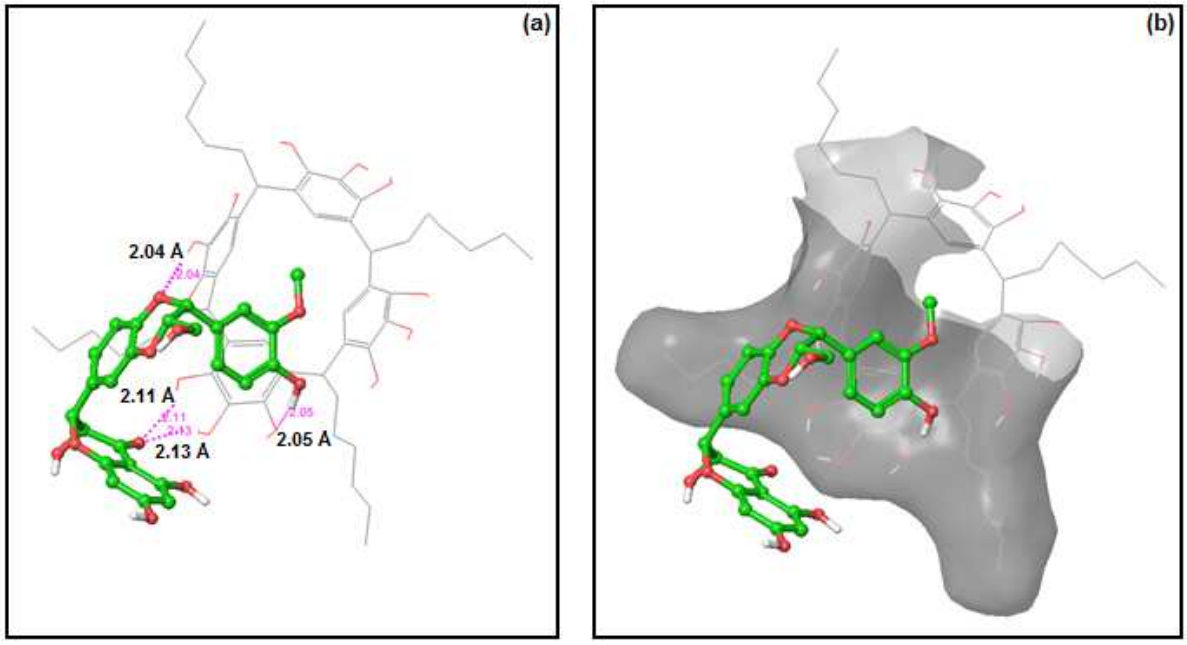


Fig. SI 7. Molecular docking poses of silybin–C-Hexylpyrogallol[4]arene (a). Hydrogen bonding interactions (b). hydrophobic interactions



The discussed mode of inclusion of silybin by the C-HPA is supported with molecular docking study. The molecular docking study between silybin and C-HPA showed the G-score value is –4.75 kcalmol^{-1} and the hydrogen bonding score is –2.85 kcalmol^{-1}. The interaction of silybin through the 4-hydroxy-3-methoxy phenyl group (i.e., Ring C substituted in the dioxo ring) in to the cavity of C-HPA by the hydrogen bonding and the hydrophobic interactions. The hydrogen bonding and the hydrophobic interaction docking poses of silybin with C-HPA is given in Figs. SI 7 (a) and (b) respectively. Hence we concluded the mode inclusion of silybin–C-HPA and the structure of the complex is proposed as shown in Fig. 5 (b).

CONCLUSION

Host–guest association of the baicalein and silybin with the host C-HPA resulted with the formation of inclusion complexes with the stoichiometric ratio of 1:1 individually. The binding strength of baicalein/silybin–C-HPA complex is determined from the Benesi–Hildebrand equation and the binding constant values are 6.71 × 10^4 mol^{-1} dm^3 and 4.99 × 10^4 mol^{-1} dm^{-3} respectively. This is evidenced by the time-resolved fluorescence study. There is a decrease in the shorter lifetime species of guests (baicalein/silybin) in water and an increase in the longer one by the added C-HPA which is due to the complex formation. The change in the pK_a values in the absence and the presence of C-HPA also supports the host-guest association occurred between the baicalein/silybin–C-HPA complexes. The structures of the inclusion complexes are proposed by the correlation peaks obtained from 2D ROESY NMR spectroscopy and the orientations the guest molecules to the host by molecular docking study.

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