Chapter 2: Development of Self-assembled Nanostructures from Pyrene-coupled Coumarin Derivatives: Application in Cell Imaging

Abstract

Three different pyrene-coupled coumarin derivatives with varying hydrophobic units (alkyl chains) have been synthesized and well characterized using NMR and mass spectral analysis. The gelation behavior and self-aggregation properties of these compounds were studied relative to the molecular structure and solvent affinity. Among these derivatives, the one which is not having any hydrophobic tail displays efficient gelation in higher alcohols such as decanol and dodecanol. However, the other derivatives having saturated and unsaturated hydrophobic tails form weak gel in different solvents. The morphology of gel was investigated by optical microscopy and high resolution transmission electron microscopy (HRTEM). The investigation of absorption and emission spectra of these compounds revealed that the photo-physical properties were significantly influenced by self-assembly process in different solvents. The concentration dependent emission and $^1$H NMR studies clearly suggest that the π-π stacking interaction and hydrogen bonding between carbonyl groups of pyrene-coupled coumarin with –OH group of solvent were the driving force for the process of gelation and self-aggregation. Rheological investigation clearly demonstrates the flow behavior and reversible nature of organogel under temperature and strain ramp up and ramp down experimental conditions. By getting clue from self-assembly mechanism in different solvents, nanoflakes were derived from pyrene-coupled coumarin derivatives and their potential applications in the field of cell imaging was explored. The size of the self-aggregated particles in the DMSO-water mixture has been identified using HRTEM and a zetasizer. The nanomaterials obtained via the self-assembly process
were used for fibroblast and PC3 prostate cancer cell imaging applications. Further investigation reveals that these compounds suppress the proliferation of PC3 cells.

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2.1 Introduction

Fabricating supramolecular architecture via molecular self-assembly of small molecules through non-covalent interactions has attracted substantial interest.\textsuperscript{1} Self-assembled materials hold vast applications in the field of drug delivery, gene therapy, tissue engineering, enzyme immobilization, wound healing, water purification, biosensors and construction of novel nano- or microscopic materials and devices.\textsuperscript{2} In this context low molecular weight organic gels (LMOGs) formed by the hierarchical assembly of gelators in a suitable solvent to structures such as fibrils, tapes, rods and tubes are known to be an important class of soft materials.\textsuperscript{3} In particular, LMOGs based on π-conjugated organic compounds have drawn significant interest due to their typical advantages such as diversity, flexibility, and promising applications in optoelectronics, light harvesting and energy materials.\textsuperscript{4}

Moreover, these fluorescent gel systems show a remarkable variation in emission property which might be due to the fact that the phase transition process render a valuable information at molecular level self-assembly. Most frequently information on molecular self-assembly renders opportunity to construct different nanostructures. In the recent past, a number of fluorescent organogels with potential applications has been reported and reviewed in detail by Ajayaghosh and co-workers.\textsuperscript{5} The self-assembled soft material \textit{i.e.} gels derived from π-gelators are called “π-gels”.\textsuperscript{5,6} Fluorescent supramolecular gels derived from biologically relevant molecules have received much attention because of their wide range of applications.

Coumarin, a class of naturally occurring benzopyrone derivatives has been used as anticancer, antibacterial, antifungal, anticoagulant and anti-HIV agent (Figure 2.1).\textsuperscript{7} For example, 7-hydroxycoumarin, 6-nitro-7-hydroxycoumarin and 3,6,8-trinitro-7-hydroxycoumarin were shown to have anti-proliferative activity in
human malignant cell lines *in vitro* and in xenograft models.\(^8\) 7-Methoxy-8-isopentenyl coumarin was found to suppress the lung cancer cell invasion and migration.\(^9\) Cancer cell growth inhibition followed by cell death was observed for coumarin 3-(N-aryl) sulfonamide.\(^{10}\)

**Figure 2.1.** Representative examples of coumarin derivatives.

Identification of improved methodology to detect and diagnosis cancer cells at the early stage is a major challenge faced in the cancer therapy. Currently, for clinical detection and diagnosis, the imaging techniques like magnetic resonance imaging (MRI), positron emission tomography (PET), computerized tomography (CT), X-ray, single photon emission computed tomography (SPECT), ultrasound imaging (US) are used which are either expensive with radioactive risks or lack of specificity and sensitivity.\(^{11}\) Optical imaging approaches emerge as non-invasive high resolution modalities for cancer detection by providing information on biological tissues.\(^{11b}\) Owing to the low auto fluorescence and absorption by tissues in NIR region (700-1400 nm), NIR fluorescence imaging has emerged as potential tool for cancer
detection and diagnosis. Cyanine dyes (e.g. Indocyanine Green (ICG)), fluorescent proteins and quantum dots (e.g. CdTe, CdSeTe and PbS) are used as fluorescent probes. Among these fluorescent probes, ICG is the only NIR fluorescent material approved for clinical diagnosis. The drawbacks such as decrease in fluorescence due to self-quenching, instability of ICG and rapid removal from the body limits its use for in vitro and in vivo fluorescence imaging. The high fluorescence quantum yield and sensitivity toward the small changes in micro environment are unique to the self-assembled pyrene derivatives, which enables their applications in biomedical and biological research. These prospects drive us through extensive synthetic efforts to obtain more diverse pyrene-coupled coumarin based π-gelator with various hydrophobic tails, which could be used as drug carrier under high concentration, cell imaging agent under low concentration and may exhibit therapeutic value too.

Herein, a new class of π-gelator derived from renewable plant-based resource, cashew nut shell liquid, which could self-assemble into gel form and nanoflakes form in different solvents was reported. The major component of CNSL being cardanol, a biobased non-isoprene lipid, comprising of rich mixture of phenolic lipids. The naturally occurring varying degree of cis-double bonds and an odd number of carbon chain with easily accessible saturated and unsaturated hydrocarbon chains are the unique features of cardanol.

2.2 Results and Discussion

2.2.1 Synthesis of Pyrene-coupled Coumarin Derivatives

Pyrene-coupled coumarin derivatives that were designed for self-assembly studies are shown in Scheme 2.1. By harnessing electrophilic aromatic substitution reaction on 3-alkylphenol 8b and 8c in acetonitrile with paraformaldehyde in the presence of anhydrous MgCl₂ and triethylamine,
2-hydroxy-4-alkylbenzaldehydes 9b and 9c were synthesized in good yield. The appearance of phenolic hydroxyl protons at $\delta = 11.05$ ppm and $-\text{CHO}$ proton at $\delta = 9.83$ ppm for compound 9b and 9c confirms the formation of the expected product. Knoevenagel reaction of compounds 9a-c in ethanol with ethyl acetoacetate in the presence of piperidine and glacial acetic acid under reflux condition led to the formation of desired 3-acetyl coumarins 10a-c in good yields. Two sharp singlets corresponding to $-\text{CH}$ attached to C-3 and C-7 were observed at around $\delta = 8.43$ and 7.10 ppm, respectively.

\[ \text{Scheme 2.1. Synthesis of pyrene-coupled coumarin derivatives 11a–c.} \]

\[ \pi\text{-Gelators 11a-c were obtained in good yields by performing aldol condensation of 3-acetyl coumarins 10a-c in n-butanol with 1-pyrenecarboxaldehyde in the presence of piperidine (Scheme 2.1). The appearance of doublet for } trans \text{ alkene } (-\text{CH}=\text{CH}-) \text{ at } \delta = 7.78 \text{ and } 8.89 \text{ ppm, 8.20 and 9.0 ppm, 8.20 and 9.07 ppm with coupling constant } J = 15.6 \text{ Hz for} \]
compounds **11a**, **11b** and **11c**, respectively, confirms the formation of the expected products.

All the synthesised compounds were completely characterized by $^1$H and $^{13}$C NMR and mass spectral techniques. After characterization, these compounds with varying hydrophobicity were utilized for self-assembly studies.

### 2.2.2 Gelation Studies

Supramolecular gelation is a process in which a pool of solvent molecules were immobilized within highly entangled fibrous network obtained by the self-assembly of gelator.\(^{15}\) Pyrene based low molecular weight organogelators (LMOG) have a tendency to gelate solvents by using weak bonding mechanism.\(^{16}\) Pyrene-coupled coumarin **11a** act as an excellent supramolecular gelator. Self-assembly of such an efficient gelator through non-covalent interactions into fibrillar aggregates could immobilize the solvent molecule by capillary force to form a gel. The gelation ability of the gelator in aromatic solvents, alcohols and vegetable oils is summarized in Table 2.1.

It is evident from Table 2.1 that π-gelators **11a** and **11b** exhibit excellent organogelation ability, showing critical gelation concentrations (CGCs) of 0.28 and 1.0% (w/v), respectively, in higher alcohols such as decanol and dodecanol, respectively. These gels melt upon heating and form gel upon cooling, hence they fall under the category of thermoreversible gel. In fact organogel formed by **11a** and **11b** experiences a gel to sol transition upon heating-cooling cycles ($T_{gel} = 65 \degree C$). $T_{gel}$ increases with increase in concentration of gelator until it reaches the saturation point. Compound **11c** did not form gel in any of the tested solvents because of its enhanced hydrophobicity.
Table 2.1. Gelation studies of compounds 11a-c in organic solvents and oils.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Solvent/Oils</th>
<th>11a</th>
<th>11b</th>
<th>11c</th>
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<tr>
<td>1</td>
<td>Ethanol</td>
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<td>2</td>
<td>n-Butanol</td>
<td>PG</td>
<td>PG</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>Octanol</td>
<td>G (1.3)</td>
<td>G (1.3)</td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td>Decanol</td>
<td>G (0.28)</td>
<td>G (1.3)</td>
<td>P</td>
</tr>
<tr>
<td>6</td>
<td>Dodecanol</td>
<td>G (0.4)</td>
<td>G (1.0)</td>
<td>G (4.0)</td>
</tr>
<tr>
<td>7</td>
<td>Toluene</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
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<td>Benzene</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>1,2-Dichlorobenzene</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>10</td>
<td>Chloroform</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>11</td>
<td>Hazelnut oil</td>
<td>S</td>
<td>G (1.3)</td>
<td>P</td>
</tr>
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<td>Olive oil</td>
<td>S</td>
<td>G (1.3)</td>
<td>P</td>
</tr>
<tr>
<td>13</td>
<td>Heavy paraffin oil</td>
<td>PG</td>
<td>S</td>
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<td>Light paraffin oil</td>
<td>S</td>
<td>S</td>
<td>PG</td>
</tr>
<tr>
<td>15</td>
<td>Sesame oil</td>
<td>S</td>
<td>S</td>
<td>PG</td>
</tr>
</tbody>
</table>

*Critical gelation concentration (CGC) is presented in parenthesis (% w/v). *aS = solution; P = precipitate; I = insoluble; G = gel; PG = partial gel.

A further detailed gelation test clearly shows that compounds 11a-c do not form gel in any of the aromatic solvents tested and form stable gel in long chain alcohols and vegetable oils. Increasing the lipophilicity of the pyrene-coupled coumarin derivatives by introducing unsaturated and saturated alkyl chain decreases the gelation ability.
2.2.3 Morphological Analysis

2.2.3.1 Organogel

The morphology of gels was examined by using optical microscopy and HRTEM. The optical microscopy image of organogel obtained from 11a in dodecanol is shown in Figure 2.2.

![Figure 2.2. Optical microscopy image of gel 11a in dodecanol (0.28% w/v) (a and b) under white light (the inset shows the formation of twisted fibers); (c and d) under fluorescence light. (e and f) HRTEM images of gel 11a in dodecanol. (g and h) Pictures of gel under day light and UV light, respectively [left-11a in decanol, middle-11b in hazelnut oil and right-11a in dodecanol].](image-url)
All the images clearly explain the formation of entangled thin fiber and twisted fiber-like structures with the dimension ranging between 100-200 nm. Figures 2.2a and 2.2b show the optical microscopy image of gel formed by compound 11a in dodecanol under white light. The inset in Figure 2.2b clearly depicts the formation of helical nanofibers. The fluorescence nature of self-assembled supramolecular structure has been identified by optical microscopy under fluorescence light. Figures 2.2c and 2.2d explain the fluorescence character of self-assembled fibers and twisted fibers.

In order to gain further insights into gel, HRTEM analysis was performed (Figures 2.2e and 2.2f). It should be remarked that the width of the fiber or twisted fiber is higher than the molecular dimension of gelator 11a. This result clearly depicts that several gelator molecules self-assemble to form supramolecular architecture. The morphology and properties of the π-gel resemble the self-assembly mechanism of π-conjugated molecule. Morphological studies reveal the formation of uniformly entangled fibrillar structure due to the hierarchical fashion of self-assembly of pyrene-coupled coumarin derivatives in highly cross-linked three dimensional supramolecular polymeric structures to form gel. Pictures of gel prepared in various solvents such as decanol, dodecanol and hazelnut oil under day light and UV light are shown in Figures 2.2g and 2.2h.

2.2.3.2. Self-assembled Nanoflakes

Since pyrene-coupled coumarin 11a, self-assembles to form gel in long chain alcohols, the self-assembly behavior of gelator 11a in water has created much interest. Self-assembled nanoflakes were prepared by refluxing the calculated amount of gelator 11a dissolved in 1:1 ratio of DMSO-water mixture
followed by cooling at room temperature. Upon heating gelator 11a got completely dissolved in the solvent mixture and self-assembly has been induced by keeping the mixture undisturbed at room temperature. This resultant solution has been considered as stock solution and could be further diluted for cell imaging application. HRTEM analysis clearly depicts the formation of nanoflakes. The self-assembly studies in DMSO-water were extended to other compounds 11b and 11c. The average size of nanoflakes formed by 11a-c in DMSO-water mixture (1x10^{-3} M solution) at lower concentration ranges between 10-100 nm (Figure 2.3).

![Figure 2.3.](image)

**Figure 2.3.** (a) Self-assembly of 11a in DMSO-water (1:1 ratio; 1x10^{-3} M solution); (b-d) HRTEM images of the self-assembly of 11a, 11b and 11c in DMSO-water mixture, respectively.

### 2.2.4 $^1$H NMR Studies

The supramolecular interaction of 11a has been inferred by NMR spectral analysis. From $^1$H NMR under the influence of different solvents, dilutions and at different temperatures could reveal both H-bonding and also the interaction between the aromatic moieties (π-π interaction). Solvent and or concentration dependent NMR studies for gelator 11a have been performed to probe the driving force for the self-assembly process (Figure 2.4).
The $^1$H NMR spectra of compound 11a clearly show concentration dependent features. The resonance signal corresponding to the aromatic protons displayed a downfield shift with the increase in the concentration of gelator solvent. In non-gelling solvent (DMSO-$d_6$) aromatic protons showed signals between 7.45-8.90 ppm. Upon increasing the concentration of gelling solvent, these protons experience a downfield shift and appear in between 7.85-9.60 ppm (Figure 2.4). It is a well known phenomenon that the existence of $\pi$-$\pi$ stacking in $\pi$-conjugated system shifts the aromatic protons more towards downfield. Thus the magnitude of $\pi$-$\pi$ stacking in 11a increases with increase in the concentration of gelling solvent.

**Figure 2.4.** $^1$H NMR spectra (aromatic region alone) of compound 11a (a) in DMSO-$d_6$; (b) in DMSO-$d_6$ + dodecanol (1:1 ratio); (c) in DMSO-$d_6$ + dodecanol (1:2 ratio).
2.2.5 X-ray Diffraction Studies

The X-ray diffraction (XRD) experiment was employed to acquire additional information about molecular packing of self-assembled 11a in the gel state. Figure 2.5 shows the small angle XRD pattern of gel prepared from decanol. Small angle X-ray diffraction (SAXD) of the gel provides a Bragg’s reflection at 2.3 nm obtained from the packing of decanol due to the van der Waals interactions in the gel network. The peak observed at 1.75 nm articulates the pyrene-coupled coumarin moiety. Bragg’s reflection at 1.27 nm and 1.06 nm attributed to hydrogen bonded decanol with the carbonyl groups of pyrene-coupled coumarin derivative 11a (Figure 2.5a). These reflections are approximately equal to the molecular length of 11a and hydrogen bonded decanol, which was confirmed by molecular modelling studies using energy minimized calculations.

![SAXD data for gel prepared by 11a in decanol; (b) proposed self-assembly mechanism.](image)
In addition, XRD spectra of this gel show a peak with position in the ratio of 1:2:3 suggest the existence of lamellar structure. Peaks at 0.37-0.48 nm are assignable to the (001) aspect of \(\pi-\pi\) stacking of aromatic units.\(^\text{17}\) Based on the results obtained by NMR studies and XRD, mechanism for the formation of self-assembled molecular gel was proposed (Figure 2.5b).

### 2.2.6 Absorbance and Emission Studies

As discussed previously, \(\pi\)-conjugated fluorescent gel has attracted much interest because of its potential applications especially in electronic and medical fields.\(^\text{18}\) The spectroscopic investigation on fluorescent organogel was performed to investigate their potential application in the field of medicine. The absorption spectrum of compound 11a in different solvents such as acetonitrile, DMSO and dodecanol is shown in Figure 2.6. Compound 11a in acetonitrile, shows three bands centred at 307, 392 and 427 nm, which are assigned to the un-aggregated form of coumarin and pyrene units. The peak observed at 307 nm experiences a red shift and appeared at 352 nm by changing the solvent to DMSO. This red shift may be due to the weak interaction of DMSO with the coumarin core of 11a. Absorption spectra of compound 11a in dodecanol, a gelling solvent show red shift in all these peaks centred at 324 and 448 nm, which is due to molecular aggregation involving the formation of hydrogen bonding between carbonyl oxygen of coumarin moiety and dodecanol, \(\pi-\pi\) stacking of pyrene (Figure 2.6).

Molecular self-assembly of 11a in higher alcohol has been clearly identified using UV-vis spectroscopy. Since gelator 11a forms nanoflakes in DMSO-water mixture, UV titration of compound 11a dissolved in DMSO (1x10\(^{-5}\) M) with PBS buffer solution was performed. The absorbance band for
$11a$ in DMSO solvent observed at 427 nm gradually decreases with sequential addition of 100 µL of PBS buffer solution. The addition of PBS buffer to $11a$ dissolved in DMSO resulted in the formation of self-assembled nanoflakes (Figure 2.6). The absorption spectral shift may take place either by solute-solvent interaction or solute-solute interaction induced by self-assembly or via chemical process such as charge transfer and proton transfer. π-Conjugated molecule $11a$ displays a higher molar extinction coefficient in the solvents tested clearly indicating the involvement of $\pi-\pi^*$ and $n-\pi^*$ transitions.

![Figure 2.6.](image)

**Figure 2.6.** (a) UV-vis spectra of $11a$ in different solvents ($1x10^{-5}$ M), dotted line represents the UV titration of $11a$ in DMSO with PBS buffer (The direction of arrow shows the response of absorption intensity with piecemeal addition of 100 µL of corresponding solvent).

Right after investigating the self-assembly features of $11a$ to form gel and nanoflakes using UV-vis spectroscopy, the fluorescence properties were evaluated. In the self-assembled state intense fluorescence was observed which stimulated to further explore the emission properties of compounds $11a$-$c$ under different experimental conditions. Emission spectra of compound $11a$-$c$
displayed substitution dependence emission behavior \( i.e. \) substitution on the coumarin moiety slightly influences the emission properties.

Compound 11a in dodecanol can self-assemble to form gel. In the self-assembled state, the emission spectrum of 11a in dodecanol shows three peaks centred at 389, 409 and 554 nm (Figure 2.7a). Dis-assembly has been induced by the gradual addition of dodecanol, the intensity of peak observed at 409 nm got increased and the peak centred at 554 nm showed a blue shift. The observed significant blue shift of fluorescence maxima is mainly due to the dis-assembly of self-assembled structure attained by titrating against dodecanol (Figures 2.7a and 2.7b).

![Figure 2.7.](image)

**Figure 2.7.** (a) Emission spectra of 11a in dodecanol and its response with respect to dilution; (b) plot of emission intensity \( \text{vs} \) concentration of 11a in dodecanol (The direction of arrow shows the response of emission intensity with piecemeal addition of 100 \( \mu \)L of corresponding solvent).

Fluorescence emission spectra of 11a were also measured in DMSO to look insight the effect of solvent. Compound 11a in DMSO shows three intense peaks at 397, 409 and 478 nm, which are different from the peaks observed for dodecanol. The molecular aggregation of 11a dissolved in DMSO was induced by piecemeal addition of 100 \( \mu \)L of PBS buffer. In aggregated state, the
emission spectrum covers a broad range of visible spectral range and exhibit vibronic coupling maximum at 414 and 576 nm. The formation of self-assembled nanoflakes-like structure has been identified by the drastic increase in emission intensity with a red shift (Figures 2.8a and 2.8b). The fluorescence quantum yields of 11a-c in different solvents (1x10^{-5} M) were determined. A very low fluorescence quantum yield ranging from 9-12% has been observed for compounds 11a-c in DMSO-PBS buffer (1:1 ratio), which could be due to the self-aggregation process. Quantum yield of 73% were observed in DMSO solvent alone. Similarly self-assembly of 11b and 11c was also identified by using UV and fluorescence studies (Figures 2.9a and 2.9b).

![Figure 2.8](image)

*Figure 2.8* (a) Fluorescence titration of 11a in DMSO with PBS buffer; (b) plot of wavelength vs concentration (The direction of arrow shows the response of emission intensity with piecemeal addition of 100 µL of corresponding solvent).
Figure 2.9. (a and b) Emission spectra of 11b and 11c in dodecanol respectively and their response with respect to dilution (The direction of arrow shows the response of emission intensity with piecemeal addition of 100 µL of corresponding solvent. 2 mL of solution (1x10^{-5} M) was taken).

Fluorescence of self-assembled system was not quenched even in extreme pH conditions (pH = 4.0 and pH = 10.0), and thus this system can be applied for cell imaging application under physiologically important conditions at various pH values (Figure 2.10).

Figure 2.10. Emission spectra of 11a in DMSO under the influence of different pH (1x10^{-5} M) [λ_{ex} = 325 nm].
From these results, it is clear that at higher concentration 11a and 11b self-assemble to form gel in decanol and dodecanol, and at lower concentration compounds 11a-c self-assemble to form nanostructures such as nano-sheet and nanoflakes in 1:1 DMSO-water mixture. Since bioapplications of organogel were recently researched in the literature, the possible application of self-assembled material *i.e.* nanoflakes in cell imaging applications were explored.

### 2.2.7 Rheological Studies

The elastic behavior and flow characteristics of an organogel were determined by measuring rheology. In frequency sweep experiments the variation of storage modulus \(G'\) and loss modulus \(G''\) was monitored as a function of applied frequency at a constant strain of 0.1% at room temperature by using organogel prepared from compound 11a in dodecanol at a concentration of 0.5% w/v. Constant strain has been fixed by performing amplitude sweep. \(G'\) represents the ability of deformed gel to restore its original geometry and \(G''\) represents tendency of gel to flow. For non-viscous liquid, \(G' = 0\) and for solids \(G'' = 0\). In viscoelastic material like gels, throughout the entire range of frequency sweep, the value of \(G'\) was found to be more than that of \(G''\) (Figure 2.11a). \(G'\) and \(G''\) exhibited a very little frequency dependency with an increase in applied angular frequency. Thus visco-elastic deportment of gel was independent of frequency sweep, and this result suggests that the gel possess good tolerance to external forces (Figure 2.11a). For organogel, at an angular frequency 0.1 rad/s, \(G'\) is in the order of magnitude 11 times greater than \(G''\). The rheological behavior of a viscoelastic soft material is independent of strain up to a critical strain level \(\gamma_c\) and beyond \(\gamma_c\), \(G'\) start decline and the material behaves in a non-linear fashion. Figure 2.11b shows a strain sweep of organogel prepared by compound 11a in dodecanol. With a gradual increase in strain, \(G'\) and \(G''\) remains
constant and at a certain point a gradual drop was observed and a cross-over occurs between $G'$ and $G''$, the point at which the cross-over occurs is considered as the critical strain ($\gamma_c$) of a gel. The $\gamma_c$ for organogel was found to be 2.73% ($G' = G'' = 490.7 \text{ Pa}$) (Figure 2.11b).

![Figure 2.11.](image)

Figure 2.11. (a and b) Angular frequency and strain amplitude dependence of $G'$ and $G''$ of organogel 11a.

Below $\gamma_c$ ($G' > G''$) gel behaves like a rigid solid and this result clearly depicts the formation of highly structured material, which could be disturbed by increasing the strain above $\gamma_c$, where it eventually become fluid-like. Continuous temperature ramp up and ramp down experiments clearly depict the stability of gel and hold back both structural and mechanical properties even at elevated temperatures for more than three cycles (Figure 2.12a).

The strain experiment demonstrates the exceptional mechanical behavior of these gels which has been identified by simultaneously applying high and low magnitude of strain such as 100% and 0.1%, respectively. Under 100% strain, both $G'$ and $G''$ values were apparently decreased because of the broken network structure and recovery of $G'$ and $G''$ was observed within 1-5 seconds by decline in strain to 0.1%.
This result clearly argues the reversible nature of gel and fast recovery of the mechanical properties (Figure 2.12b).

![Figure 2.12](image)

**Figure 2.12.** (a and b) Response of storage ($G'$) and loss modulus ($G''$) of organogel, 11a (0.5 w/v% in dodecanol) with respect to (a) temperature; (b) step strain condition. 100% strain (4.5 min) and 0.1% strain (4.5 min) were applied alternatively.

### 2.2.8 Cell Imaging Studies

The study on the effect of solvent in self-assembly process indicates that pyrene-coupled coumarin derivatives can slowly assemble into fluorescent gel and nanoflakes in different solvents. The fluorescent nanoflakes thus derived have been potentially used for cell imaging applications. Yao *et al.* in his review discussed the significance of combination of fluorescence and nanomaterials. The recent development and innovation of fluorescent nanoparticles with unique optical properties establish a new map for fluorescence imaging and sensing applications both *in vitro* and *in vivo*. The use of organic fluorescent molecules possesses more advantage of being much brighter and more stable than the fluorescent proteins. In addition, the position of organic fluorescent molecules could be determined in precision than the fluorescent proteins. Most of the applications such as *in vitro* and *in vivo*...
labelling in cells, tissues, and organisms, proteomic and genomic studies, disease diagnostics, pharmaceutical screening, drug delivery, assembled molecular control, protein purification, biological therapeutics and medical imaging, sensing in cancer research, and selective tumor targeting rely on fluorescence spectroscopy. Fluorescence imaging is one of the sensitive and most informative analytical techniques in modern research.

Self-assembled nanoflakes of 11a-c formed in DMSO-water mixture could be potentially used for live cell imaging application. These nanoparticles of size between 10-100 nm are in the same range of dimension as that of proteins, antibodies, membrane receptors, etc. These interesting characteristics, linked with their high surface to volume ratio impressed us to proceed further in this field. For these studies, fibroblast L929 cells and PC3 human prostate cancer cells were chosen. Medium has been prepared by dissolving 250 µg of compound 11a-c in 1000 µL of solvent (0.1% DMSO-water mixture). The concentration of medium is as follows: 11a: 0.6x10^{-3} M, 11b: 0.4x10^{-3} M and 11c: 0.4x10^{-3} M.

At first, the time dependent internalization of self-assembled nanoflake formed by 11a was studied at different time intervals such as 1 min, 5 min, 10 min and 2 h, respectively (Figure 2.13). Figure 2.13 explains that the intensity of green fluorescence, due to self-fluorescent π-conjugated nanoflakes derived from 11a increases with respect to time.
Figure 2.13. LCSM images of fibroblast L929 incubated with nanoflake derived from 11a. (a-e) Internalization of nanoparticles at 0-120 min respectively; (f) 3D view of internalization of nanoparticles at 1 min. (a-e) (top left): blue color from Hoechst stain used to differentiate the nucleus; (top middle): green fluorescence from the cytoplasm and perinuclear region of the cells by fluorescent nanoparticles; (bottom middle): combined view of blue and green color fluorescence from nucleus, and the cytoplasm and perinuclear region of the cells.

For further detailed investigation, cells were incubated with medium containing 11a-c for 24 h. After the incubation period, cellular localization was traced using laser confocal scanning microscopy (LCSM). Green fluorescence arising from self-assembled π-conjugated derivatives 11a-c was clearly observed in the cytoplasm and perinuclear region of the cells. In compound 11a-c, substituent at the 7th position of coumarin moiety directly influences the fluorescence intensity. Compound 11a not having any substituent at the coumarin core possesses more fluorescence character, whereas the other derivatives 11b and 11c have alkyl substituents with comparatively less fluorescence property (Figure 2.14). It is interesting to note that among 11b and 11c, 11b shows comparatively more fluorescence character. This result indicate that the fluorescence intensity decreases with increase in hydrophobicity of π-conjugated systems (Figure 2.14). Nanoparticles/nanoflakes prepared by the
self-assembly process from 11a-c were uniformly located in the cytoplasm of the cells. The increase in incubation time enhances the uptake of fluorescent nanoflakes. Endocytosis is the internalization of extracellular material via membranous vesicles.\textsuperscript{22} The endocytic mechanism regulates how cells interact with their environment and it involves four different mechanisms such as clathrin-mediated endocytosis, caveolae mediated endocytosis, macropinocytosis and phagocytosis. Inhibitors such as sucrose and chlorpromazine, blocking agents of clathrin-coated pit formation, had no significant effect on cellular uptake. Filipin, an inhibitor of caveolae-associated endocytosis also had no significant inhibition effect on the nanoparticle uptake. Nocodazole, an inhibitor of macro-pinocytosis inhibited the uptake of nanoparticles up to 60%. These results suggested that macro-pinocytosis and phagocytosis are the prominent cell uptake pathway for self-assembled nanoparticles.\textsuperscript{23} Any cell damage in fibroblast L929 upon treatment with nanoflakes were not observed, which entails the low cytotoxicity of nanoflakes in fibroblast.

Cytotoxicity assay on both fibroblast and PC3 cells also supports the results obtained from LCSM. Treatment of PC3 cells with self-assembled nanoflakes heads to the cell death in PC3 cells by inhibiting the Wnt/\(\beta\)-catenin pathway, which has been identified based on cell membrane rupture and the overflow of cytoplasm (Figure 2.14d-f). A coumarin based anti-cancer drug, decursin, inhibits Wnt/\(\beta\)-catenin pathway and cellular proliferation.\textsuperscript{24}
In order to check the toxicity of compounds 11a-c, cell viability were examined towards fibroblast and PC3 cells. Fibroblast incubated with different concentrations of 11a-c experience low cytotoxicity. The incubation of PC3 cells with different concentrations of 11a-c shows considerable cytotoxic effects (Figure 2.15). Thus, these self-fluorescent probes can be potentially used for optical diagnosis and treatment for prostate cancer.
Figure 2.15. Graphical representation of cell viability of (a) fibroblast and PC3 cells when exposed to 250 µg/1000 µL of compounds 11a-c; (b) PC3 cells when exposed to increasing concentration of 11a-c (20-80 µg/1000 µL). In fibroblast % of cell viability was around 95-98%.

2.3 Experimental Section

2.3.1 Materials and Methods

All chemicals used for the synthesis of coumarin derivatives 10a-c and fluorescent probes 11a-c were purchased from Sigma-Aldrich, Merck, Alfa Aesar and Avra chemicals and were used without further purification. All solvents were dried and freshly distilled before use. Solvents used for gelation studies are of AR grade. Double distilled water was used for preparing self-assembled nanostructures. Column chromatography was performed on silica gel (100-200 meshes) purchased from Avra chemicals, India. Fibroblast L929 cell lines were purchased from Sigma-Aldrich and PC3 cells were obtained from national centre for cell science (NCCS), Pune, India.

2.3.2 Characterization Methods

$^1$H and $^{13}$C NMR spectra were recorded in either CDCl$_3$ or CDCl$_3$ with a few drops of DMSO-$d_6$ at room temperature on Bruker 300 MHz instrument. $^1$H and $^{13}$C NMR chemical shifts were reported relative to TMS. Coupling constants ($J$) are denoted in Hz and chemical shifts ($\delta$) are reported in parts per million (ppm). Proton multiplicity is assigned using the following
abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). High resolution MS analysis were performed on an Agilent 6520 Q-TOF instrument by dissolving the solid sample in methanol.

2.3.3 Gelation Experiments

2.3.3.1 Gelation Method

A known quantity of gelator (2 mg) was mixed with an appropriate amount of solvent (200 μL) in a sealed glass vial and the system was heated to 90-120 °C until the solid was dissolved. The resulting solution was slowly allowed to cool to room temperature, and gelation was visually observed by inverting the glass vial. A gel sample that exhibited no gravitational flow in an inverted vial was obtained and denoted as “G”. Instead of forming gel it remains as solution at the end of the tests which is referred to as “S” (solution) and when it remains as a precipitate, the system was denoted as “P” (precipitation). The system, in which the gelator is not soluble even at the boiling point of the solvent, was called an insoluble system (I).

2.3.3.2 Critical Gelation Concentration

To a gel sample (2 mg of gelator in 200 μL of solvent) from the previous experiments, 100 μL of solvent was added. The mixture was heated, cooled and analyzed for stability by inverting the vial. The steps of solvent addition and gelation were repeated until the contents of the vial begin to flow on inversion of the vial. The preceding gelator concentration, ie. the lowest gelator concentration required to form a stable gel, was considered as the CGC value for the gelator in that particular solvent.
2.3.3.3 Gel Melting Temperature ($T_{\text{gel}}$)

Gel melting temperature was determined by flow of gel using the test tube inversion method. All gels obtained are thermally reversible. Above their gelation temperature, the gel phase becomes solution phase, but could be returned to their original gel state upon cooling. Freshly prepared gel was taken in a 5 mL glass vial and slowly heated by immersing the vial ‘upside down’ in the oil-bath. The temperature at which the gel melted down to a solution was recorded as gel melting temperature ($T_{\text{gel}}$).

2.3.4 UV-vis and Fluorescence Measurements

UV-vis spectra were recorded on an Evolution 220 UV/visible spectrophotometer (Thermo Scientific). The spectra were recorded in the continuous mode between 200 and 700 nm, with a wavelength increment of 1 nm and a bandwidth of 1 nm. Emission spectra were measured on a JASCO spectrofluorometer FP-8200, by fixing the excitation value at 325 nm for dodecanol and DMSO. The selection of excitation values is based on the absorbance maximum of probes in different solvents. Samples for absorption and emission measurements were contained in 1 cm x 1 cm quartz cuvette.

2.3.5 Morphological Analysis

Morphological analysis of gel formed by gelator was studied using Carl Zeiss AXIO ScopeA1 fluorescent/phase contrast microscope. A glass slide containing a small portion of gel was mounted on Phase Contrast Microscope and the morphology of gel was identified. Morphology of self-assembled structure such as gel and nanoflakes was studied using JEOL JEM 2100 F FETEM.
2.3.6 Molecular Modelling Studies

The MM2 energy minimized diagram was performed using ChemBio 3D Ultra 13. The Red color dotted line shows the possible hydrogen bond formation.

2.3.7 X-ray Diffraction Studies

A small portion of a wet gel sample formed by gelator in decanol was transferred in a sample holder and coated like a thin film. The XRD measurement was performed on XPert-PRO Diffractometer system.

2.3.8 Rheological Measurements

The mechanical properties of gel were investigated using a stress controlled rheometer (Anton Paar 302 rheometer) equipped with a steel-coated parallel-plate geometry (25 mm diameter). The gap between two plates was 1 mm. The measurements were carried out at 23 °C. Firstly, the amplitude sweep measurement was conducted, which provides the information about linear viscoelastic range, which is directly proportional to the mechanical strength of the gel sample. Secondly, the storage modulus, $G'$ and the loss modulus, $G''$ were monitored as functions of frequency sweep from 0.1 to 300 rad.s$^{-1}$.

2.3.9 Cell Proliferation Assay

The anti-proliferation activity was tested by MTS assay on fibroblast L929 and PC3 cell lines using the fluorescent dye, yellow tetrazolium dye, which when treated with cells, forms a purple colored formazan product. The quantity of formazan is presumably directly proportional to the number of viable cells and is measured using a spectrophotometer (1420-040 Victor 3 Multilabel Counter, PerkinElmer, USA) at 490 nm dissolved in PBS buffer.
Viable cells with active metabolism convert MTT into a purple colored formazan product. When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of only the viable cells. Cells were seeded into a 96-well plate for 48 h prior treatment. They were then exposed to 40 µL of different concentrations (20, 40, 80 and 250 µg/1000 µL) of compounds 11a-c in 0.1% DMSO-water mixture and to control (0.1% of DMSO-water mixture). The relative viability was expressed as a percentage of the control well that was treated with the solvent 0.1% of DMSO-water mixture only. Cell viability (%) was estimated as a ratio of the absorbance of treated cell (N_t) to absorbance of untreated cells (solvent) (N_u) multiplied by 100.

\[
\text{Cell viability (\%)} = \left(\frac{N_t}{N_u}\right) \times 100
\]

2.3.10 Cell Imaging/Cell Uptake Studies

The Cell imaging/Cell uptake study of our fluorescent compounds 11a-c were tested on Fibroblast L929 and PC3 cell lines. The cells were seeded into a 6-well plate for 24 h prior treatment. They were then exposed to 40 µL of 11a-c dissolved in 0.1% DMSO-PBS buffer mixture with the concentration of 250 µg/1000 µL. After 4 h of exposure, the media were drained and the cells were washed with PBS buffer for more than 2 times. In order to differentiate the nucleus the Hoechst stain was then added and incubated for 15 min. After 15 min incubation, the stain was drained and the cells were again washed with PBS and subjected for imaging. The cells were imaged using confocal microscope.
2.3.11 Distillation of Cardanol

CNSL was distilled at a temperature between 210 and 280 °C, under a pressure of 2 to 8 mm Hg to get cardanol. Cardanol was obtained as pale yellow liquid which darkens on further storage. After a second distillation, mixture of cardanol mono-, di- and tri-ene was obtained. The synthetic procedure for 3-pentadecyl phenol (hydrogenated cardanol) 8c is as follows: to the solution of cardanol (10 mL) dissolved in dry methanol, 5% Pd/C was added slowly and the entire mixture was stirred in the presence of H₂ gas (1 atm) for 5 h. After completion of reaction as identified using TLC, the solution was filtered through a celite bed to obtain crude 3-pentadecyl phenol. The pure product was obtained by recrystallization process using hexane as solvent.

2.3.12 Synthetic Procedure and Characterization Data

2.3.12.1 General Procedure for the o-Formylation of Substituted Phenols

Dry paraformaldehyde (35 mmol) was added to a mixture of 3-alkyl phenol 8 (4 mmol), anhydrous MgCl₂ (6 mmol) and triethylamine (15 mmol) in acetonitrile (25 mL) and the mixture was heated under reflux for about 12-15 h. After the completion of the reaction as identified by using TLC, the reaction mixture was cooled to room temperature and 5% aq. HCl was added. The crude product was extracted with ethylacetate, dried under Na₂SO₄ and purified using column chromatography.
Compound 9b

Appearance : Yellow liquid

Yield : 88%

$^1$H NMR (CDCl$_3$, 300 MHz) $\delta = 11.05$ (s, 1H), 9.83 (s, 1H), 7.44 (d, $J = 8.1$ Hz, 1H), 6.83 (d, $J = 7.8$ Hz, 1H), 6.80 (s, 1H), 5.39-5.33 (m, 2H), 2.61 (t, $J = 7.5$ Hz, 2H), 2.05-1.93 (m, 2H), 1.64-1.59 (m, 4H), 1.37-1.24 (m, 16H), 0.88 (t, $J = 6.9$ Hz, 3H)

$^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta = 195.80, 161.80, 153.82, 133.58, 130.00, 129.74, 120.50, 118.85, 117.07, 36.44, 32.62, 31.80, 30.66, 29.74, 29.72, 29.67, 29.63, 29.44, 29.34, 29.25, 29.21, 29.16, 29.00, 27.24, 27.17, 22.67, 14.12.$

Compound 9c

Appearance : White solid

Yield : 92%

Mp : 52-54 °C

$^1$H NMR (CDCl$_3$, 300 MHz) $\delta = 11.05$ (s, 1H), 9.83 (s, 1H), 7.45 (d, $J = 7.8$ Hz, 1H), 6.83 (dd, $J = 7.8, 1.5$ Hz, 1H), 6.80 (s, 1H), 2.61 (t, $J = 7.8$ Hz, 2H), 1.66-1.57 (m, 2H), 1.34-1.24 (m, 24H), 0.88 (t, $J = 6.9$ Hz, 3H)

$^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta = 195.78, 161.79, 153.82, 133.57, 120.49, 118.84, 117.07, 36.45, 31.94, 30.67, 29.71, 29.67, 29.65, 29.54, 29.44, 29.38, 29.25, 22.71, 14.13.$
2.3.12.2 General Method for the Synthesis of 3-Acetyl-7-alkyl-2H-chromen-2-one 10a-c

To a solution of 2-hydroxy-4-alkylbenzaldehyde 9a-c (1 mmol) dissolved in ethanol (15 mL), ethylacetoacetate (1.3 mmol), 0.3 mL of piperidine and 2-3 drops of glacial acetic acid were added. The mixture was refluxed for 4 h. After the completion of the reaction as identified by TLC, the reaction mixture was cooled to room temperature and 20 mL of ice cold water was added. The crude product was extracted with chloroform and dried over anhydrous Na$_2$SO$_4$ and purified using column chromatography using silica gel. Compound 10a is synthesized by following the procedure reported in the literature.$^{14}$

**Compound 10b**

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Yellow liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>82%</td>
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</table>

$^1$H NMR (CDCl$_3$, 300 MHz)

$\delta$ = 8.43 (s, 1H), 7.47 (d, $J = 8.1$ Hz, 1H), 7.1 (s, 1H), 7.09 (d, $J = 7.5$ Hz, 1H), 5.33-5.25 (m, 1H), 4.94-4.88 (m, 1H), 2.65 (s, 3H), 2.65 (t, $J = 8.4$ Hz, 2H), 1.97-1.86 (m, 2H), 1.60-1.53 (m, 4H), 1.25-1.18 (m, 16H), 0.80 (t, $J = 6.3$ Hz, 3H)

$^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ = 194.52, 158.50, 154.56, 150.42, 146.52, 128.95, 124.63, 122.20, 115.08, 35.29, 30.90, 29.80, 29.55, 28.68, 28.44, 28.34, 28.27, 28.11, 28.04, 27.96, 27.81, 27.28, 26.19, 26.11, 21.67, 21.63, 13.10.
**Compound 10c**

**Appearance**: White solid

**Yield**: 89%

**Mp**: 98-100 °C

**1H NMR** (CDCl₃, 300 MHz) δ = 8.50 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.17 (s, 1H), 7.16 (d, J = 8.1 Hz, 1H), 2.72 (t, J = 6.9 Hz, 2H), 2.72 (s, 3H), 1.67-1.59 (m, 2H), 1.32-1.24 (m, 24H), 0.85 (t, J = 7.2 Hz, 3H)

**13C NMR** (CDCl₃, 75 MHz) δ = 194.97, 158.94, 154.98, 150.87, 146.95, 129.33, 125.04, 122.61, 115.51, 35.70, 31.29, 30.21, 29.96, 29.06, 29.02, 28.99, 28.89, 28.77, 28.73, 28.55, 22.06, 13.49.

**2.3.12.3 General Procedure for the Synthesis of Pyrene-coupled Coumarin**

**Derivatives 11a-c**

To a solution of 3-acetyl coumarin **10a-c** (1 mmol) dissolved in n-butanol (10 mL), 1-pyrenecarboxyaldehyde (1.3 mmol), 3 drops of glacial acetic acid and 0.3 mL of piperidine were added and the contents were refluxed at 120 °C for 12 h. After completion of reaction as identified by TLC, the solvent was removed under vacuum. The residue thus obtained was triturated with 10 mL of ethanol until the formation of fine precipitate. The precipitate was then filtered and crystallized from methanol.

**Compound 11a**

**Appearance**: Yellow solid

**Yield**: 77%

**Mp**: 212-214 °C
$^1$H NMR  (CDCl$_3$, 300 MHz)  $\delta =$  
8.88 (d, $J = 15.6$ Hz, 1H),  
8.79 (s, 1H), 8.67 (d, $J = 9.6$ Hz, 1H), 8.59 (d, $J = 8.1$ Hz, 1H), 8.41-8.24 (m, 6H),  
8.15 (t, $J = 7.8$ Hz, 1H), 7.99 (d, $J = 15.6$ Hz, 1H), 7.79 (t, $J = 7.8$ Hz, 1H), 7.54 (d, $J = 8.7$ Hz, 1H), 7.47 (t, $J = 7.8$ Hz, 1H) 

HRMS (ESI+)  $m/z$ calculated for C$_{28}$H$_{16}$O$_3$ [M-H+2Li]$^+$: 413.1320.  
Found: 413.2647.  

**Compound 11b**  

**Appearance**  :  Yellow solid  

**Yield**  :  74%  

**Mp**  :  126-128 °C  

$^1$H NMR  (CDCl$_3$, 300 MHz)  $\delta =$  
8.99 (d, $J = 15.3$ Hz, 1H), 8.59 (s, 1H), 8.54 (d, $J = 9.3$ Hz, 1H), 8.45 (d, $J = 8.1$ Hz, 1H),  
8.21 (d, $J = 15.6$ Hz, 1H), 8.18-7.94 (m, 7H), 7.52 (d, $J = 7.8$ Hz, 1H), 7.15 (s, 1H), 7.11 (d, $J = 7.8$ Hz, 1H), 5.32-5.27 (m, 2H), 2.67 (t, $J = 7.5$ Hz, 2H), 1.96-1.910 (m, 2H), 1.61-1.50 (m, 4H), 1.26-1.19 (m, 16H), 0.82 (t, $J = 5.4$ Hz, 3H)  

$^{13}$C NMR  (CDCl$_3$, 75 MHz)  $\delta =$  
184.81, 158.40, 154.14, 149.86, 146.91, 139.65, 131.76, 129.91, 129.32, 129.20, 128.40, 127.40, 127.31, 127.27, 126.05, 124.88, 124.72, 124.56,
124.38, 124.21, 123.81, 123.52, 123.42, 123.20, 122.63, 121.23, 115.09, 114.70, 34.99, 31.29, 30.49, 29.50, 28.43, 28.02, 27.91, 27.86, 27.69, 25.94, 25.86, 21.36, 12.82

**HRMS (ESI+)**  
$m/z$ calculated for C$_{43}$H$_{45}$O$_3$ [M+Na]$^+$: 632.3266. Found: 632.2545.

**Compound 11c**

**Appearance** : Yellow solid

**Yield** : 82%

**Mp** : 158-160 °C

**$^1$H NMR** (CDCl$_3$, 300 MHz) $\delta = 9.07$ (d, $J = 15.6$ Hz, 1H), 8.67 (s, 1H), 8.62 (d, $J = 9.3$ Hz, 1H), 8.53 (d, $J = 8.1$ Hz, 1H), 8.29 (d, $J = 15.6$ Hz, 1H), 8.26-8.19(m, 4H), 8.15-8.02 (m, 3H), 7.60 (d, $J = 7.8$ Hz, 1H), 7.23 (s, 1H), 7.19 (d, $J = 8.1$ Hz, 1H), 2.74 (t, $J = 7.8$ Hz, 2H), 1.68-1.59 (m, 2H), 1.50-1.25 (m, 24H), 0.88 (t, $J = 6.3$ Hz, 3H).

**$^{13}$C NMR** (CDCl$_3$, 75 MHz) $\delta = 184.71$, 158.23, 153.98, 149.75, 146.77, 139.53, 131.59, 129.73, 129.15, 129.04, 128.23, 127.22, 127.13, 127.10, 125.86, 124.70, 124.53, 124.36, 124.23, 124.09, 123.62, 123.37, 123.25, 123.05, 122.52, 121.06, 114.93, 115.58, 34.81, 30.39, 29.32, 28.17, 28.13, 28.11, 28.01, 27.90, 27.83, 27.68, 21.17, 12.60.

**HRMS (ESI+)**  
$m/z$ calculated for C$_{43}$H$_{47}$O$_3$ [M+Na]$^+$: 634.3439. Found: 634.5325.
2.4 Conclusion

By utilizing aldol condensation as key step, a new class of pyrene-coupled coumarin derivatives were successfully synthesized. Molecular structure of these compounds were completely characterized using NMR and mass spectral analysis. The formation of transparent fluorescent organogel with CGC of 0.28% w/v via supramolecular self-assembly through hydrogen bonding and π-π stacking of pyrene units were reported. The morphology of organogel was investigated by optical microscopy and HRTEM. The concentration dependent absorbance and emission studies and 1H NMR analysis reveal that hydrogen bonding between the carbonyl groups of 11a and long chain alcohols, and π-π stacking interactions was the driving force for the process of self-aggregation and gel formation. Optical property of π-conjugated derivatives have been strongly influenced by aggregation in different solvents, which resulted in red shift and increase in emission intensity. By getting clue from different modes of self-assembly in different solvents, nanoflakes in DMSO-water mixture were prepared. At lower concentration, compounds 11a-c in DMSO-water form nanoparticles (nanoflakes) and at higher concentration form gel in long chain alcohols and vegetable oils. The development of fluorescent organic functional nanoparticles has progressed exponentially over the past two decades because of its vast research in nanotechnology and considerable interest as feasible biomedical materials. In vitro fluorescence imaging visualized the fluorescent emission from π-conjugated molecules in fibroblast L929 and PC3 prostate cancer cells. Although small molecular dyes were used for imaging applications, the development of fluorescent multifunctional organic nanoparticle for in vitro fluorescence imaging offers powerful tool for many exciting applications. The
continuous evolution of these multifunctional self-assembled soft materials provides a promising platform for materials and medical applications.

2.5 References


