

Stimuli-responsive supramolecular gels from fatty acid amides for theophylline delivery and anticancer applications[☆]

Meenakshi^a, Manisha^a, Yangala Sudheer Babu^b, Mettle Brahma^b, Mulaka Maruthi^b, Deepak Kumar^c, Azaj Ansari^{a,*}, Manoj K. Gupta^{a,**}

^a Department of Chemistry, School of Basic Sciences, Central University of Haryana, Mahendergarh, 123031, Haryana, India

^b Department of Biochemistry, School of Interdisciplinary and Applied Sciences, Central University of Haryana, Mahendergarh, 123031, Haryana, India

^c Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh, 173229, India

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ABSTRACT

Supramolecular gels derived from low-molecular-weight gelators (LMWGs) uniquely combine features of both homogeneous and heterogeneous systems, making them highly valuable in synthetic and medicinal chemistry. Their rising prominence in cancer therapy is attributed to their excellent biocompatibility, stimuli-responsive behavior, and ability to deliver anticancer drugs directly to tumor sites-reducing systemic side effects and improving treatment efficacy. In this study, a series of fatty acid amide-based gelators (**3a-3h**) were synthesized through a one-step amidation of 4-aminopyridine, with alkyl chain lengths varying from C₆ to C₂₂. These gelators formed stable organo/hydrogels in DMSO/water at low minimum gelation concentrations (1 % w/v), qualifying as supergelators. Longer alkyl chains enhanced gelation and thermal stability due to synergistic interactions between amide groups and hydrophobic segments. Specifically, gelators **3e** and **3h** demonstrated effective encapsulation of the hydrophilic drug theophylline, facilitating controlled, pH-responsive release at physiological temperature without compromising drug integrity. Cytotoxicity assays of these gelators demonstrated promising anticancer activity against A549 lung cancer cells, along with low toxicity in normal Vero cells. Furthermore, the synergistic anticancer efficacy of the theophylline-loaded gel formulations (**3e** + **Th** and **3h** + **Th**) showed enhanced potency, with IC₅₀ values of 12.89 ± 0.4 and 21.50 ± 2.8 µg/mL, respectively, highlighting their potential as advanced drug delivery systems for cancer therapy.

1. Introduction

Gels derived from low-molecular-weight gelators (LMWGs) represent a rapidly growing area of research in materials science due to their remarkable versatility and responsiveness to external stimuli [1]. These self-assembling soft materials can be finely tuned, making them suitable for a wide range of applications including drug delivery, bone repair, wound healing, tissue engineering, cosmetics, sensors, and imaging [2]. LMWGs, typically with molecular weights below 3000 Da, act as the fundamental building blocks that immobilize solvents through supramolecular gelation. This process is mediated by weak, non-covalent interactions such as hydrogen bonding, van der Waals forces, hydrophobic-hydrophilic interactions, ion-dipole and dipole-dipole interactions, as well as π - π stacking [3–6]. These properties make LMWGs

superior to conventional polymers, particularly in biomedical applications. One key advantage of LMWG-based gels is their reversibility, i.e. the gels can transition between solid and liquid states under environmental changes such as pH, temperature, or ionic strength [7–9]. This dynamic behavior enhances their suitability for applications like controlled drug delivery, sensing, and water purification [10,11]. Their adaptability enables precise localization and release of active pharmaceutical ingredients (APIs), particularly in response to physiological stimuli such as pH changes, which is valuable in drug delivery and tissue regeneration [12,13]. Supramolecular gels, formed by non-covalent self-assembly, offer unique characteristics compared to traditional polymeric gels [14]. LMWG-based supramolecular gels are especially promising for delivering APIs due to their tunable physical properties and responsive behavior [15,16]. Injectable organogels are being

[☆] Dedicated to Dr. J. S. Yadav on his 75th birthday.

^{*} Corresponding author.

^{**} Corresponding author.

E-mail addresses: ajaz.alam2@gmail.com (A. Ansari), mk Gupta@cuha.ac.in (M.K. Gupta).

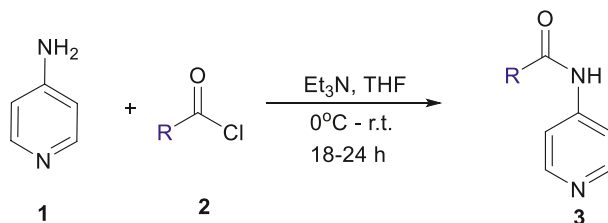
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Table 1
Synthesis of 4-aminopyridine derived fatty acid amide gelators.



Entry	R	Time (h)	Yield (%) ^a
3a		18	78
3b		18	80
3c		22	76
3d		22	82
3e		24	82
3f		24	80
3g		24	70
3h		24	72

^a Isolated yields.

actively investigated for their potential to provide sustained drug release, while aqueous organo/hydrogels are attractive for their compatibility with biological environments and ability to modulate API release based on pH [17–19]. In many cases, gel disassembly is required for API release, which can be triggered by chemical or physical stimuli. Therefore, detailed characterization of new LMWGs is critical to understanding their interactions with APIs and optimizing their drug delivery performance [20,21]. The growing interest in gel-based drug delivery systems is fueled by several advantages: biodegradability, mechanical robustness, high surface area, biocompatibility, non-toxicity, renewability, and hydrophilicity, along with the ability to modify surface functional groups [22,23]. Our research is aligned with these goals, with a focus on developing LMWGs for efficient drug delivery systems [24]. Specifically, our group is dedicated to synthesizing novel LMWGs that can spontaneously self-assemble in suitable solvents [25–31]. This strategy provides a cost-effective and scalable alternative to expensive polymer gelators, making it attractive for industrial applications. A significant challenge in drug delivery is the poor aqueous solubility of many pharmaceutical compounds, which limits their oral bioavailability. Organo/hydrogels offer an effective solution due to their moisture-insensitive nature, skin penetration enhancement, and microbial resistance. Additionally, the gelation and drug encapsulation processes are both simple and efficient [32–34]. Their biocompatibility, biodegradability, and non-immunogenicity further reinforce their suitability for pharmaceutical applications [35]. To address these opportunities, we have designed LMWGs using 4-aminopyridine as the core scaffold. By functionalizing the amine group with various fatty acids (C₆–C₂₂), we developed a series of gelators capable of forming organogels and organo/hydrogels. These gels were used to encapsulate theophylline, and its release was studied at physiological pH values (5.5 and 7.4). To the best of our knowledge, this is the first report on the gelation behavior of 4-aminopyridine-based fatty acid amides. Our

study demonstrates that the chain length of the fatty acid significantly influences gel formation and drug release properties. Furthermore, we assessed the biological potential of these compounds by evaluating their anticancer activity against A549 lung adenocarcinoma and Vero cell lines. These investigations revealed a correlation between alkyl chain length and biological efficacy. The drug-loaded gels also displayed significant anticancer properties, suggesting potential utility as pharmacophores in medicinal chemistry. Gel-based drug delivery platforms are increasingly being recognized in the pharmaceutical industry due to their affordability, ease of preparation, and long-term stability. Our findings contribute to the growing field of supramolecular gelators and support their application in developing advanced, responsive drug delivery systems.

2. Experimental methods

2.1. Materials

Commercially available 4-aminopyridine, caproic acid, decanoic acid, lauric acid, myristic acid, palmitoyl chloride, stearic acid, oleic acid, behenic acid, *n*-pentane, oxalyl chloride and triethylamine were used as purchased (Central drug house). Theophylline was procured from Sigma Aldrich. For purification purposes, the solvents were distilled prior to use.

2.2. Methods

¹H and ¹³C NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz and 101 MHz, respectively. Additionally, compound 3b was analyzed using a Magritek 80 MHz benchtop NMR spectrometer. Samples were dissolved in CDCl₃ and chemical shifts were reported relative to the internal standard tetramethylsilane (TMS) at $\delta =$

0.00 ppm for ^1H NMR and the CDCl_3 triplet at $\delta = 77.00$ ppm for ^{13}C NMR. High-resolution mass spectrometry was used to determine the molecular weights of the synthesized compounds. IR spectroscopy conducted on FT-IR Bruker Instrument within a scanning range of 4000 to 600 cm^{-1} , was used to analyze the structural features of the compounds. A FE-SEM 7610F Plus JEOL model was used for morphological analysis of lyophilized xerogels. Rheological experiments were conducted at 25°C using an Anton Paar MCR 302e modular compact rheometer. A 1 mm gap and 20 mm parallel plates were used, with temperature precisely controlled by a thermostatic water bath. Silica gel column chromatography (100–120 mesh, Central Drug House) with specific solvent systems was employed for compound separation and purification. Reaction progress was monitored by thin-layer chromatography (TLC) with visualization under UV light and iodine stain for detection.

2.3. General procedure

To synthesize a small library of gelators, 4-aminopyridine (1 mmol) was coupled with corresponding acyl chloride (1.2 mmol) in presence of triethylamine (2.5 mmol) using anhydrous tetrahydrofuran (10 mL) as solvent. The reaction mixture was stirred continuously at 25°C for 20–24 h and reaction progress was monitored by thin-layer chromatography (TLC). After completion of the reaction as indicated by the TLC, the solvent was removed under reduced pressure to obtain the crude product. Ethyl acetate was added to the crude product and the resulting organic layer was washed three to four times with saturated sodium bicarbonate solution to remove any unreacted acid from the product. Following this, the organic layer was further washed with brine solution. The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4) and then the solvent was evaporated under vacuum. The concentrated product was initially purified by washing it with *n*-pentane and then drying the solid. If further impurities were present, the concentrated extract was subjected to additional purification using column chromatography with ethyl acetate/hexane (7:3) as the eluent system. Table 1 shows the chemical synthesis pathway for fatty acid amide gelators, highlighting the diverse set of precursor molecules utilized during the reaction. The purity of the synthesized compounds was evaluated using spectral analysis.

2.3.1. *N*-(Pyridin-4-yl)hexanamide (3a) [38]

Yield: 78 %; Off white solid; m.p. $62\text{--}64^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3240, 2932, 2863, 1698, 1592, 1510, 1414, 1292, 1171\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 9.88 (s, 1H), 8.33 (d, $J = 6.0$ Hz, 2H), 7.56 (d, $J = 5.0$ Hz, 2H), 2.32 (t, $J = 8.0$ Hz, 2H), 1.70–1.52 (m, 2H), 1.20 (s, 4H), 0.77 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 149.5, 146.7, 114.0, 37.5, 31.3, 25.1, 22.3, 13.9; HRMS calcd for $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 193.1335; found, 193.1333.

2.3.2. *N*-(Pyridin-4-yl)decanamide (3b) [36]

Yield: 80 %; Off white solid; m.p. $68\text{--}70^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3288, 2916, 2852, 1686, 1594, 1510, 1415, 1290, 1253, 1161\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 9.18 (s, 1H), 8.16 (d, $J = 6.1$ Hz, 2H), 7.36 (d, $J = 5.9$ Hz, 2H), 2.14 (t, $J = 7.0$ Hz, 2H), 1.44 (s, 2H), 1.01 (bs, 12H), 0.62 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.3, 149.0, 146.8, 113.9, 37.6, 31.8, 29.5, 29.4, 29.2, 25.3, 22.6, 14.1; HRMS calcd for $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 249.1961; found, 249.1964.

2.3.3. *N*-(Pyridin-4-yl)dodecanamide (3c) [37]

Yield: 76 %; Off white solid; m.p. $72\text{--}74^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3292, 2914, 2850, 1684, 1596, 1510, 1413, 1327, 1293, 1159\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 8.62 (s, 1H), 8.35 (d, $J = 6.2$ Hz, 2H), 7.48 (d, $J = 6.4$ Hz, 2H), 2.35–2.20 (m, 2H), 1.68–1.54 (m, 2H), 1.17 (bs, 16H), 0.80 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.8, 149.4, 146.2, 113.8, 37.7, 31.9, 29.5, 29.3, 29.2, 25.3, 22.7, 14.1; HRMS calcd for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 277.2274; found, 277.2273.

2.3.4. *N*-(Pyridin-4-yl)tetradecanamide (3d) [38]

Yield: 82 %; Off white solid; m.p. $73\text{--}75^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3304, 2917, 2848, 1688, 1597, 1513, 1416, 1381, 1294, 1206\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 9.16 (s, 1H), 8.35 (d, $J = 5.9$ Hz, 2H), 7.52 (d, $J = 6.3$ Hz, 2H), 2.32 (t, $J = 7.5$ Hz, 2H), 1.69–1.53 (m, 2H), 1.16 (bs, 20H), 0.80 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.1, 149.6, 146.4, 113.9, 37.6, 31.9, 29.6, 29.5, 29.2, 25.4, 22.7, 14.1; HRMS calcd for $\text{C}_{19}\text{H}_{32}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 305.2587; found, 305.2582.

2.3.5. *N*-(Pyridin-4-yl)palmitamide (3e) [39]

Yield: 82 %; Off white solid; m.p. $75\text{--}77^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3295, 2912, 2850, 1689, 1598, 1514, 1469, 1415, 1293\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 9.44 (s, 1H), 8.33 (d, $J = 6.1$ Hz, 2H), 7.54 (d, $J = 6.4$ Hz, 2H), 2.32 (t, $J = 7.5$ Hz, 2H), 1.68–1.53 (m, 2H), 1.16 (bs, 24H), 0.79 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.4, 149.4, 146.6, 113.9, 37.6, 31.9, 29.6, 29.5, 29.3, 29.2, 25.4, 22.7, 14.1; HRMS calcd for $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 333.2900; found, 333.2904.

2.3.6. *N*-(Pyridin-4-yl)stearamide (3f)

Yield: 80 %; Off white solid; m.p. $79\text{--}80^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3298, 2912, 2846, 1686, 1598, 1514, 1469, 1416, 1297\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 9.02 (s, 1H), 8.35 (d, $J = 6.4$ Hz, 2H), 7.51 (d, $J = 4.9$ Hz, 2H), 2.32 (t, $J = 7.5$ Hz, 2H), 1.71–1.54 (m, 2H), 1.17 (bs, 28H), 0.80 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.0, 149.7, 146.2, 113.8, 37.7, 31.9, 29.7, 29.5, 29.4, 29.3, 25.4, 22.7, 14.1; HRMS calcd for $\text{C}_{23}\text{H}_{40}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 361.3213; found, 361.3209.

2.3.7. *N*-(Pyridin-4-yl)oleamide (3g)

Yield: 70 %; Pale yellow viscous liquid; IR (neat): $\nu_{\text{max}} = 3289, 3167, 3079, 2925, 2856, 1705, 1596, 1518, 1462, 1414, 1295, 1169\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 8.78 (s, 1H), 8.36 (d, $J = 3.5$ Hz, 2H), 7.50 (d, $J = 5.3$ Hz, 2H), 5.43–5.05 (m, 2H), 2.32 (t, $J = 7.5$ Hz, 2H), 2.13–1.73 (m, 4H), 1.69–1.55 (m, 2H), 1.30–1.15 (m, 20H), 0.80 (t, $J = 6.5$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.9, 150.0, 146.1, 130.0, 129.6, 113.8, 37.6, 31.9, 29.7, 29.5, 29.3, 29.1, 27.2, 27.1, 25.3, 22.7, 14.1; HRMS calcd for $\text{C}_{23}\text{H}_{38}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 359.3057; found, 359.3058.

2.3.8. *N*-(Pyridin-4-yl)docosanamide (3h)

Yield: 72 %; Off white solid; m.p. $84\text{--}86^\circ\text{C}$; IR (neat): $\nu_{\text{max}} = 3300, 2914, 2847, 1688, 1598, 1514, 1470, 1416, 1329, 1210, 1166\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ 8.92 (s, 1H), 8.32 (d, $J = 5.3$ Hz, 2H), 7.49 (d, $J = 6.2$ Hz, 2H), 2.31 (t, $J = 7.6$ Hz, 2H), 1.88–1.40 (m, 2H), 1.18 (bs, 36H), 0.80 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.1, 150.0, 145.9, 113.6, 37.6, 31.9, 29.6, 29.5, 29.4, 29.2, 25.3, 22.7, 14.1; HRMS calcd for $\text{C}_{27}\text{H}_{48}\text{N}_2\text{O}$ [$\text{M} + \text{H}^+$], 417.3839; found, 417.3834.

2.4. Gelation studies

2.4.1. Preparation of organo/hydrogels

A known mass of the gelator was added to the required amount of an organic solvent in a glass vial. The vial was then heated slowly until the complete dissolution of the gelator. After complete dissolution of the gelator in the organic solvent, the required amount of water was added, and the resulting mixture was heated until it became a clear solution. This solution was then left to cool undisturbed at room temperature for the necessary duration. The gel formation was evaluated by turning the glass sample vial upside down.

2.4.2. Determination of minimum gelation concentration (MGC)

To determine the minimum gelation concentration of the gelator, a known amount of gelator was dissolved in 1 mL of solvent and heating it until it was completely dissolved [40]. After the dissolution, the resulting solution was placed in a thermostat water bath at 25°C . The solution was checked for gelation after 15–20 min. If no gel formed, then another 1 mg of gelator was added and the process was repeated until gelation occurred. The minimum amount of the gelator (in mg/mL) that

results in gel formation is known as the minimum gelation concentration (MGC).

2.4.3. T_{gel} : Gel-sol phase transition temperature determination

The tube inversion technique was employed to find and to calculate the gel-to-sol phase transition temperature. A portion of the gel was placed in a transparent glass vial, which was subsequently placed in an oil bath maintained at a constant temperature of 20 °C. The oil bath temperature was increased at a rate of 1 °C per minute. The temperature at which the viscous gel structure breaks down or integrates corresponds to its melting point, known as the gel-sol phase transition temperature.

2.5. Scanning electron microscopy (SEM)

The compound and drug entrapped organo/hydrogels were subjected to freeze-drying via lyophilization and their morphology was subsequently examined using an FE-SEM 7610F Plus JEOL model.

2.6. Rheological measurements

Rheological experiments were performed at 25 °C using an Anton Paar MCR 302e modular compact rheometer with a 1 mm gap and 20 mm parallel plates. A water circulating bath with thermostatic control was used to regulate the temperature. Strain sweep experiments were conducted from strain range 0.1–100 % ($\omega = 1$ Hz). To investigate the materials response to varying frequencies, a frequency sweep test was performed, maintaining a constant strain amplitude of 1 % across the frequency range of 0.1–100 Hz. To ensure reproducibility, each experiment was conducted at least three times.

2.7. Drug encapsulation

In accordance with the established procedure in the literature [41] theophylline (Th) was incorporated into the gel matrix. At the MGC of the gelator, the required amount of gelator was dissolved in 0.8 mL of DMSO in a sealed 15 mL vial by heating it until a clear solution formed. Following the formation of the clear gelator solution, a specific amount of theophylline was introduced and the resulting mixture was heated at a temperature of 70 °C. Once the clear solution was obtained, 0.2 mL of water was then introduced to it. After the complete mixing, the solution was allowed to stand and cool at 25 °C for a period of 2 h. To achieve the formation of a stable gel the amounts of theophylline were gradually increased upto 80 mg as with more than 80 mg, the gel was not stable. Following the stable gel formation, confirmed by vial inversion, the process continued until reproducible gel formation were obtained to calculate maximum drug loading. The drug loading content was determined using the following equation [42].

$$\text{Drug loading content\%} = \frac{\text{Weight of the drug in the gel}}{\text{Weight of the gel}} \times 100$$

2.8. Drug release study

The pH-responsive release of theophylline from the drug composite gel was studied at pH 5.5, 6.5 and 7.4 respectively. Prior to the introduction and thoroughly mixing of the buffer, the drug-loaded gel was equilibrated at room temperature for 2 h. UV-vis spectroscopy was employed to calculate the total amount of drug released. Through UV-vis spectroscopy, the UV absorption wavelength of theophylline (Th) was determined to be 272 nm. This wavelength was specifically chosen for analysis, as the gelator showed no absorption in this region. In a typical drug release experiment, by following the literature, 12 mL of buffer solution is placed on top of the drug-loaded gel in a glass vial at 37 °C. During the drug release experiments, after regular intervals, 200 μ L samples were periodically withdrawn from the 12 mL buffer solution, which was further diluted to 3.0 mL. The amount of drug in these

samples was determined using UV-vis spectroscopy. To maintain a constant buffer volume of 12 mL, an equal volume (200 μ L) of fresh buffer solution was added back after each sampling. To study about the degradation pattern of gel, change in the absorbance at 272 nm was monitored in the kinetic absorption analysis.

2.9. Kinetics evaluation and release behavior of drug

The kinetics of theophylline release from the gel matrix were thoroughly investigated at both pH 7.4 and pH 5.5 and assessed by fitting release data to several models, including zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models. The data were plotted as the log of the cumulative drug release percentage over time. The correlation coefficient (R^2) is used in understanding the release behavior.

2.10. Biological assessment

2.10.1. Cytotoxicity assay

2.10.1.1. Culture of animal cell line. A549 cells (Human lung adenocarcinoma cells), Vero cells (Immortalized, non-cancerous African green monkey kidney epithelial cells), were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS:Gibco) and Penicillin-Streptomycin with continuous supply of 5 % CO₂ at 37 °C i.e. in CO₂ incubator [43].

2.10.1.2. Preparation of compounds. Prior to addition of compounds into cell culture, compounds were prepared in 90 % DMSO and treated into cells with final concentration of 0.5 % DMSO at highest concentration treated cells. Whereas, hydrogels were dissolved in 90 % DMSO and pre-heated at 70–90°C for 30 min with continuous stirring. After the dissolution, hydrogels were added into the culture plate and maintained 0.5 % DMSO as the final concentration at highest concentration treated cells.

2.10.1.3. Cell viability assay. Cell viability was investigated with MTT assay using yellow 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) salt. After the culture reached sufficient confluency, cells were harvested with 0.25 % trypsin-EDTA. Cell counting was done with hemocytometer. In 96 well plate 1×10^4 cells/well seeded and incubated for 24 h in CO₂ incubator for cell attachment and growth. After 24 h, test compounds (gelators and the drug loaded gel formulations) with varying concentrations were added to the culture and incubated for 48 h. The viability of treated cell culture with test compounds were assessed after 48 h of incubation. The wells were gently washed with 1x phosphate buffer saline (PBS) to remove the test compounds and added MTT salt solution (5 mg mL⁻¹ in 1X PBS) then incubated at 37 °C. After 3 h of incubation, DMSO was used to solubilize the formazan crystal and incubated for 30 min. The 96 well plates were then read for absorbance in Synergy H1 microplate reader at 540 nm. IC₅₀ values were calculated for each test compounds in GraphPad Prism 8.0.1 by fitting the experimental results to sigmoidal equation.

3. Results and discussion

3.1. Synthetic pathway

Drawing inspiration from our previously published work on therapeutic compounds, we aimed to develop compounds with enhanced activities. In this study, we have synthesized a small library of 4-aminopyridine derived fatty acid amides low molecular weight gelators, by varying the alkyl chain lengths. Further self-assembling properties of the synthesized gelators was studied in various organic solvents and also in organic and aqueous solvent mixtures. To further explore the biological

Table 2Organo/hydrogel study of gelator **3e** in different ratios of DMSO-H₂O mixtures.

Entry	Gelator (mg/mL)	DMSO (mL)	Water (mL)	Description	Gel	Gelation time (min.)
1	10	0.1	0.9	Clear solution	No	–
2	10	0.2	0.8	Clear solution	No	–
3	10	0.3	0.7	Clear solution	No	–
4	10	0.4	0.6	Clear solution	No	–
5	10	0.5	0.5	Turbid suspension	No	–
6	10	0.6	0.4	Turbid suspension	No	–
7	10	0.7	0.3	Unstable gel with excess solvent	No	–
8	10	0.8	0.2	Opaque gel	Yes	10
9	10	0.9	0.1	Opaque gel	Yes	10
10	10	1.0	0.0	Clear solution	No	–

potential of the synthesized compounds we have studied the anticancer activity of the different alkyl chain length compounds. Compounds **3a-3h** (Table 1) were prepared in a single step, utilizing acyl chlorides of various chain lengths coupled with 4-aminopyridine using THF as a solvent. The products were obtained in good yields and characterized using ¹H NMR, ¹³C NMR spectroscopy and high-resolution mass spectrometry (HRMS).

3.2. Gelation studies

The gelation behavior of the synthesized gelators in different solvent systems was investigated using a heating-cooling approach and the gelation capability of compounds **3a-3h** in various organic solvents was assessed via the vial inversion technique [44]. A specific amount of gelator was dissolved in 1 mL of the selected solvent in a vial until a homogeneous solution formed. The solution was then stirred and allowed to stand undisturbed at room temperature. Using vial inversion test gel formation was evaluated [45,46]. To achieve stable gel formation proper dissolution and precipitation of the gelator molecule in a specific solvent is required. We initially tried to induce gel formation in water, however, the gelator remained undissolved despite repeated efforts. Following this, gelation was attempted in various organic solvents, compounds **3a-3h** failed to form gels in any of the single organic solvents tested, with the exception of compound **3h**, which formed gel in DMSO. Furthermore, we attempted to dissolve other compounds in DMSO, which produced clear solutions at ambient temperature, however, the subsequent application of heating and cooling did not induce

the formation of organogels in these solutions. After this, our next focus was co-solvent system and for initial screening, we employed a 0.9 mL DMSO and 0.1 mL water mixture (10 mg mL⁻¹). Upon heating to 70 °C, this resulted in a homogeneous and transparent clear solution. Further this sample solution was allowed to cool undisturbed at room temperature and subsequently compound **3e** afforded organo/hydrogel. Following these results, we adjusted the ratio to 0.8:0.2 mL (DMSO: water) and on heating this new mixture to 70 °C, we observed further formation of stable organo/hydrogel. So, this adjustment successfully yielded a stable gel at room temperature. To further explore gelation behavior under different DMSO-water ratios and concentrations, we focused on compound **3e**, and the outcomes are detailed in (Table 2). These results clearly showed that a DMSO-water mixture is well-suited for the self-assembly and aggregation of fatty acid amides. After determination of the optimal gelation conditions, we next sought to understand the impact of alkyl chain length on gelation behavior.

To explore this, we systematically reduced and increased the number of carbon atoms in the alkyl chain, starting from sixteen carbon chain (**3e**) down to ten carbon chain (**3b**) and up to twenty two carbon chain (**3h**). However, this modification did not led to favorable outcomes with (**3b**) and organo/hydrogel formation failed to initiate even after the vial was cooled to room temperature for 24 h. However, increasing the length of the fatty acid amides to twenty-two carbons (**3h**) led to rapid organo/hydrogel formation, even more rapid than sixteen carbon chain compound (**3e**), indicates the direct correlation between the stability of organo/hydrogels and the strength of van der Waals interactions.

In our earlier evaluation, we observed that compound **3e** forms organo/hydrogel at 1 % w/v concentration. Subsequently, the stability of the gels from all the gelators was assessed using a test tube inversion method as shown in (Fig. 1), the gels formed exhibited thermoreversible behavior. Furthermore, the organo/hydrogel demonstrated long-term stability, remaining intact at 25 °C for over six months. The gel-to-sol transition temperature (*T_{gel}*) for each gel was determined at its minimum gelation concentration and the results showed that as the length of the aliphatic side chain in the gelator molecule increased, the resulting gels exhibited higher thermal stability [47] (Table 3). This enhanced stability is attributed to the stronger van der Waals forces arising from the increased surface area and interactions between the longer aliphatic chains. From this observation, we suggests that the ability of the solvent molecules to effectively integrate within the hydrophobic regions of the gelator molecules plays a crucial role in reinforcing the overall stability of the three-dimensional gel network. By positioning themselves favorably among the hydrophobic chains, the solvent molecules can enhance the intermolecular interactions, thereby strengthening the gel structure.

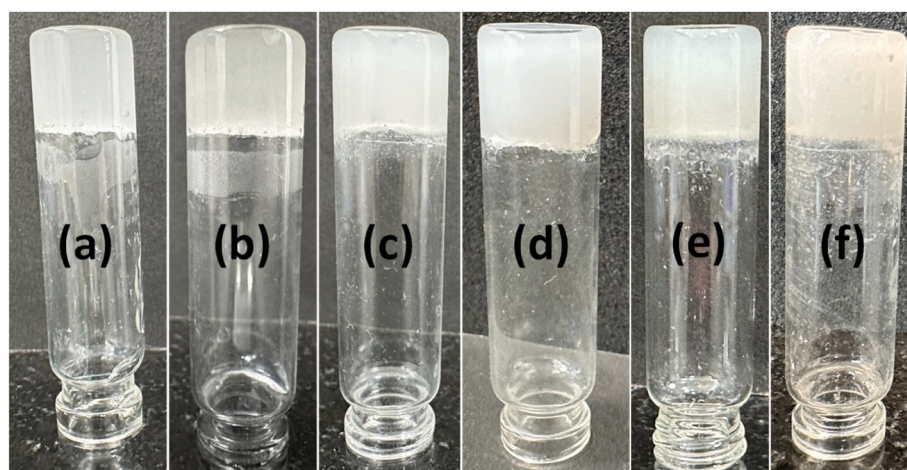


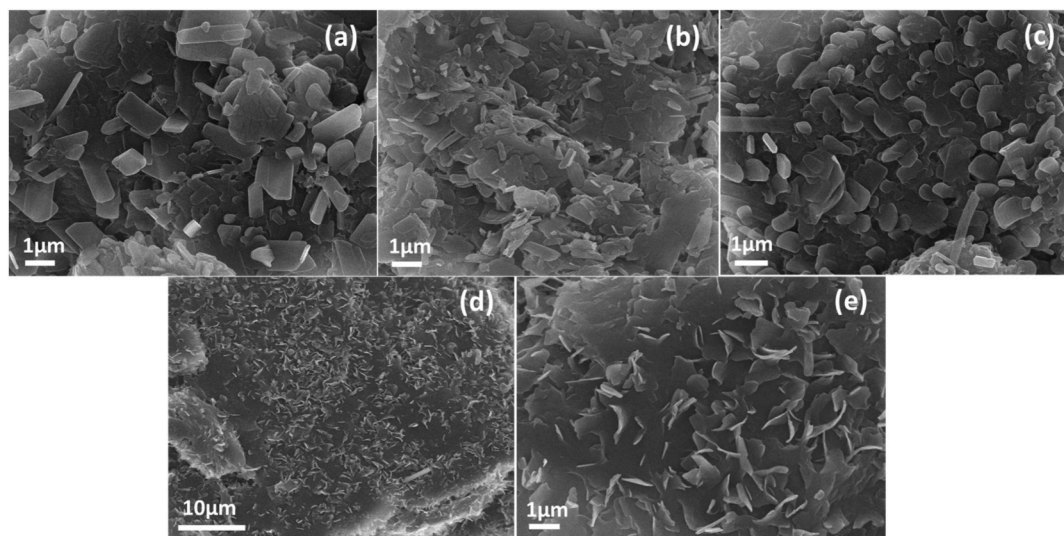
Fig. 1. Photographs of vials showing gelation of fatty acid amide gelators; (a to e) gelation of compound **3c**, **3d**, **3e**, **3f** and **3h** in DMSO:H₂O (0.8:0.2 ml) (f) gelation of compound **3e** in Methanol:H₂O (0.8:0.2 ml).

Table 3

Organo/hydrogelation study of gelators in various solvent systems.

Compd.	H ₂ O	MeOH	MeOH:H ₂ O (0.8:0.2 mL)	DMSO	DMSO:H ₂ O (0.8:0.2 mL)	CH ₃ CN	CH ₃ CN:H ₂ O (0.8:0.2 mL)	DMF	DMF:H ₂ O (0.8:0.2 mL)	Hexane
3a	I	S	S	S	S	S	S	S	S	I
3b	I	S	S	S	S	S	S	S	S	I
3c	I	S	G 15 mg [53°C] (20 min)	S	G 15 mg [60°C] (20 min)	S	S	S	S	I
3d	I	P	G 10 mg [60°C] (20 min)	S	G 10 mg [65°C] (15 min)	S	S	S	S	I
3e	I	P	G 10 mg [62°C] (15 min)	S	G 10 mg [68°C] (10 min)	S	G 10 mg [62°C] (30 min)	S	G 10 mg [66°C] (20 min.)	I
3f	I	P	G 10 mg [66°C] (15 min.)	S	G 10 mg [72°C] (10 min)	S	G 10 mg [65°C] (30 min)	S	G 10 mg [68°C] (20 min)	I
3g	I	P	S	S	G 15 mg [60°C] (60 min)	S	S	S	S	I
3h	I	P	G 10 mg [72°C] (10 min)	G 10 mg [70°C] (20 min)	G 10 mg [76°C] (10 min)	S	G 10 mg [70°C] (20 min)	S	G 10 mg [72°C] (20 min)	I

I: Insoluble; P: Precipitation; S: Soluble; G: Gel at 25°C, the numbers denotes the corresponding minimum gelation concentrations (MGCs) in mg/mL⁻¹, [] denotes T_{gel}, () denotes time required for the gelation.

**Fig. 2.** SEM images of xerogels: (a) **3e** in DMSO:H₂O (b) **3e** in Methanol:H₂O (c) **3d** in DMSO:H₂O (d and e) **3h** in DMSO:H₂O.

3.3. Morphological analysis of organo/hydrogels

To gain insights into the microstructure of the organo/hydrogels, field emission scanning electron microscopy (FE-SEM) images of the prepared gels were acquired. Xerogels were prepared from the organo/hydrogels by freeze-drying (lyophilization) by the complete removal of the solvent. Fig. 2 illustrates that compound **3e** shows a rod-shaped morphology in a DMSO-water mixture (1 % w/v) and sheet like morphology in MeOH-water mixture. Compound **3h** exhibits a sheet-like morphology in DMSO-water mixture whereas compound **3d** shows plate like morphology in DMSO-water mixture. The synthesized compounds contain an aromatic ring, suggesting that π - π interactions between these aromatic parts likely drive the self-aggregation process that leads to gel formation. In addition to π - π interactions, van der Waals interactions between the long-chain groups also contribute significantly to the rigidity and overall gel formation process.

3.4. Rheological analysis

Rheological evaluation is an essential step in characterizing the mechanical strength of gels. This process is crucial for understanding the material's structural integrity and its response to applied forces. Each gel underwent two tests: a strain amplitude sweep and a frequency sweep. The gel's resistance to mechanical disturbances was measured by its storage elastic modulus (G'), which indicates the gel strength and the gels' ability to flow under stress was measured using the loss viscous modulus (G''). During rheological evaluation, we assessed the storage modulus (G') and loss modulus (G'') at different frequencies and strains. The rheological study clearly shows that the storage modulus (G') and loss modulus (G'') behave similarly (in parallel manner) in both gelators **3e** and **3h** as well as in the theophylline-loaded gels, **3e** + **Th** and **3h** + **Th**. The rheological data showed that G' was always greater than G'' throughout the entire frequency range. This indicates that the gels elastic properties dominate over their viscous properties, showing their viscoelasticity and their ability to withstand external forces.

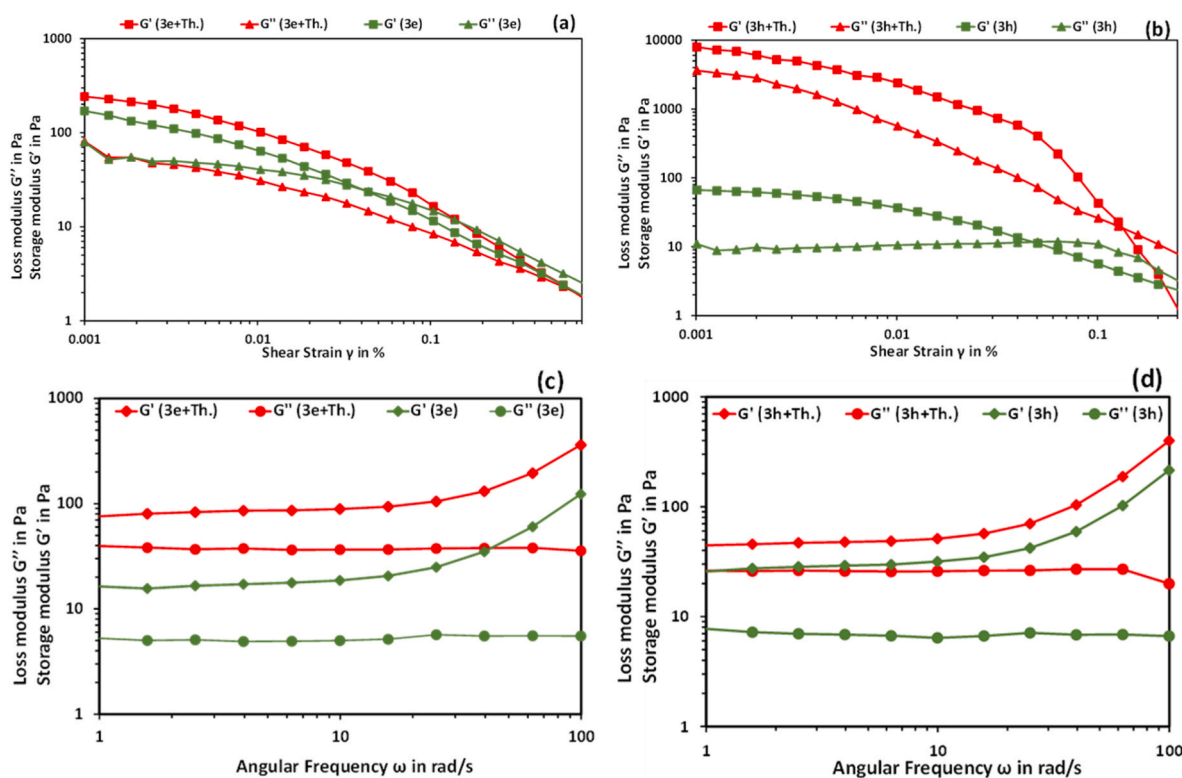


Fig. 3. (a,b) Strain amplitude sweep rheological experiment for **3e**, **3e + Th**, **3h** and **3h + Th** in DMSO/H₂O at a constant frequency of 1 Hz; (c,d) Frequency sweep rheological experiment for **3e**, **3e + Th**, **3h** and **3h + Th** in DMSO/H₂O at a constant strain of 1 %.

In Fig. 3 graphs show how the storage modulus (G') and loss modulus (G'') of organo/hydrogels (**3e**, **3e + Th**, **3h** and **3h + Th**) change as applied strain (%) increases while maintaining a constant frequency of 1 Hz. As shear stress increases, the gel's storage modulus (G') decreases, indicating the deformation of the gel. However, at a specific threshold stress, the storage modulus (G') of the gel becomes greater than its loss modulus (G'') indicating that the 3D network of the gel is breaking down, leading to the disruption of its structure. After this threshold point, the gel shift from viscoelastic solid to fluid-like behavior. This transition enables us for direct comparison of the mechanical strength among various gelators that form gels in the same solvent. The gels of **3e + Th** and **3h + Th** experience structural breakdown at their yield stresses (10.81 % and 19.85 %), respectively, which is when the gel starts to flow. In conclusion, organo/hydrogels containing longer chain-length theophylline show greater elastic strength because of the stronger non-covalent cross-links and increased entanglements compared to gels with shorter chain-length theophylline. Overall, the rheological experiments shows that the **3e**, **3e + Th**, **3h** and **3h + Th** organo/hydrogels act as soft viscoelastic solids.

3.5. Drug incorporation and pH sensitive release

Over the past few decades, the increasing variety of delivery systems designed for carriers has led to the development of novel strategies for administering poorly soluble drugs [48]. Designing effective ways to dissolve and deliver water insoluble (hydrophobic) drugs presents a significant challenge in pharmaceutical research. In this approach, organogels are attracting significant interest as potential drug delivery systems because they exhibit unique "smart" material properties, allowing them to respond to various external signals and trigger the release of incorporated drugs [49]. 4-Aminopyridine derivatives, known for their ability to interact with ligands via hydrogen bonds, presents a unique and promising avenue for the development as gel-based drug delivery vehicles. This suggests that the organo/hydrogel can entrap drugs using a hydrogen bond network, which is contrasts with methods employing minor chemical changes, where the drug's inherent activity could be compromised during the chemical modification of the gelator. In this study, we introduce a simple method for synthesizing organo/-hydrogelators which acts as a pH-sensitive drug delivery vehicle for the

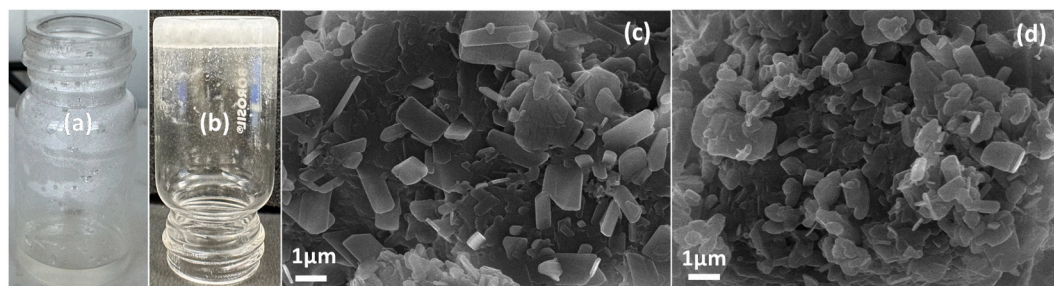


Fig. 4. Photographs of (a) the gelator **3e** and the drug in liquid state (b) the drug loaded gel (c) SEM image of xerogel (Compound **3e**) organo/hydrogel in DMSO/H₂O without theophylline; (d) SEM image of xerogel (**3e**) with theophylline.

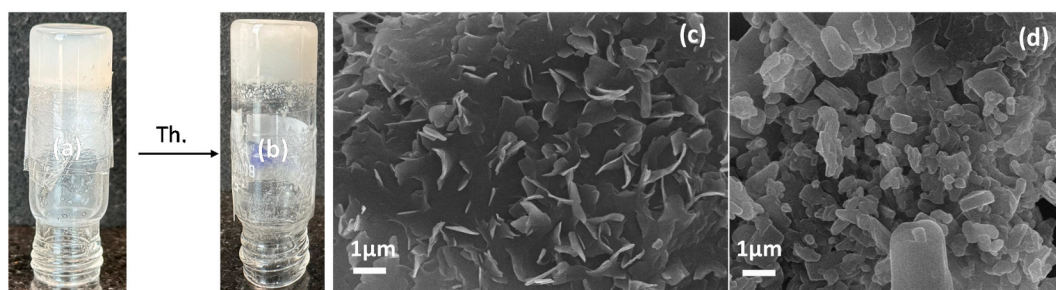


Fig. 5. Photographs of (a) the gel of **3h** in DMSO/H₂O (b) the drug loaded gel of compound **3h** (c) SEM image of xerogel (Compound **3h**) organo/hydrogel in DMSO/H₂O without theophylline; (d) SEM image of xerogel (**3h**) with theophylline.

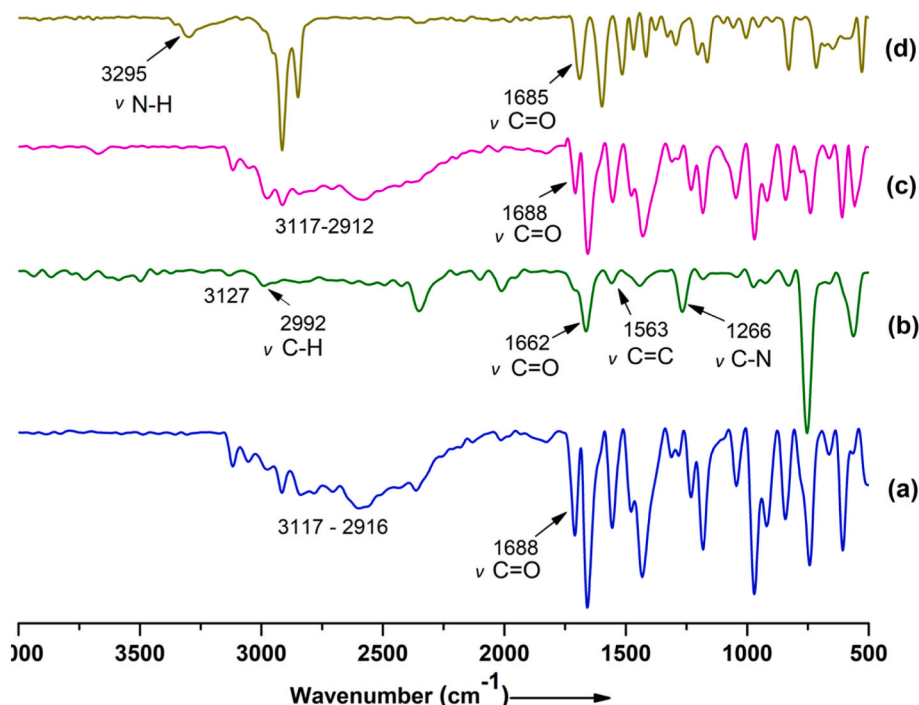


Fig. 6. FTIR spectra of (a) the theophylline drug composite xerogel of compound **3e**; (b) the drug theophylline; (c) the gel state of the gelator **3e**; (d) the gelator **3e**.

effective release of water insoluble drugs like theophylline. In this study, we have chosen theophylline as the model drug, which is a purine derivative, 1,3-dimethylxanthine or 1,3-dimethyl-7H-purine-2,6-dione derivative having two methyl groups at 1 and 3 positions on the purine ring [50].

Next, we investigated the release of theophylline, which we used as a representative drug, from the organo/hydrogel systems incorporating compounds **3e** and **3h**. Compounds **3e** and **3h** were chosen for this study because they both possess an aromatic ring, tends to exhibits π - π interactions. Furthermore, the amide bonds present in these compounds were crucial for enabling hydrogen-bonding interactions. The presence of long-chain contributes hydrophobic and van der Waals forces, which strengthens the gel formation. Moreover, the variations in the lengths of these chains significantly influence the release rate of theophylline at different pH levels.

The hybrid gel containing the drug was opaque, with the quantity of encapsulated theophylline in **3e** and **3h** organo/hydrogel measuring 7.07 and 8.28 % respectively, in 1 % w/v gelator solution indicating the high degree of encapsulation. Figs. 4 and 5 shows the surface structure of the theophylline-containing xerogels which shows a sheet-like gel network which is extensively interconnected with spherical structures throughout the scaffold.

3.6. FTIR analysis

Fig. 6 displays the FTIR analysis results for the gelator (**3e**), the gel state of the gelator, theophylline drug, and the xerogel containing the drug. Theophylline comprises characteristics peaks at 3127 and 2992 cm^{-1} for N-H and C-H stretching respectively, peaks at 1662 and 1563 cm^{-1} corresponds to C=O and C=C functionalities and peak at 1266 cm^{-1} corresponds to C-N stretching respectively.

The FTIR analysis during gel formation in DMSO/water Fig. 6(c) showed a broad signal between 3117 and 2912 cm^{-1} rather than a characteristics peak which suggests hydrogen bonding during gel formation. A peak at 1688 cm^{-1} corresponds to the stretching vibration of carbonyl functionality. Fig. 6(a) the xerogel corresponds to drug and gelator shows broad signal at 3117-2916 cm^{-1} shows hydrogen bonding between the drug and the gelator, respectively. The shift from 1685 to 1688 cm^{-1} corresponds to the C=O stretching of the amide. This pattern indicates that -NH and -C=O groups play a vital role in the self-assembly process, driven by strong interactions between molecules through hydrogen bonding. Furthermore, the rest of the spectral pattern shows no significant changes, suggesting that the gelator's functional groups remains intact even after the drug was incorporated.

Similar results are obtained with compound **3h**, (Fig. 7), xerogel of drug and gelator shows a broad signal at 3117-2838 cm^{-1} because of

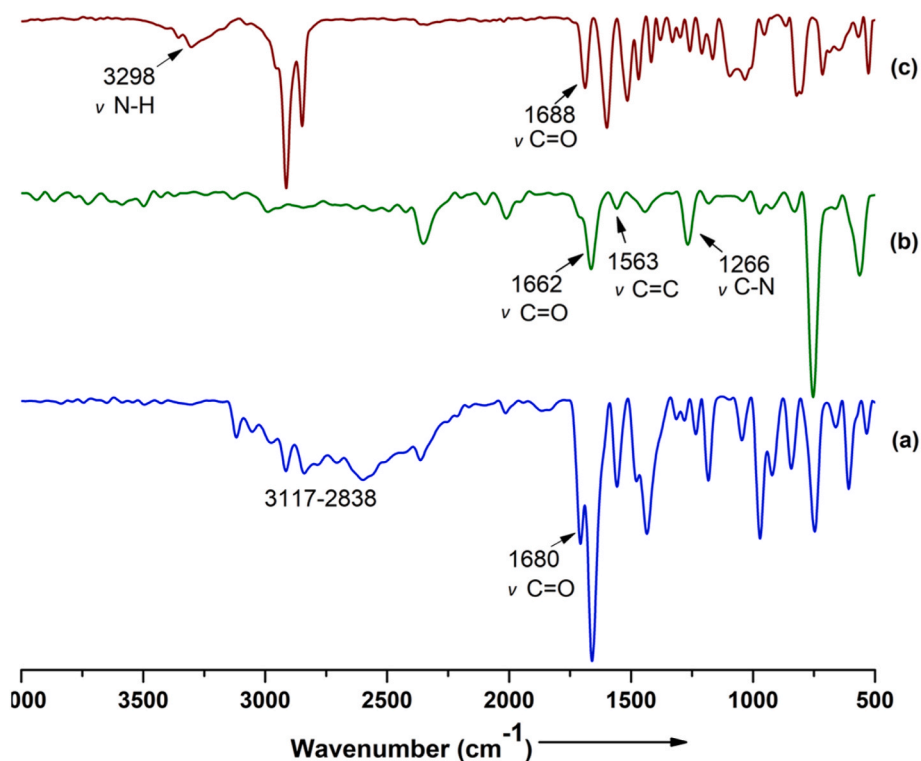


Fig. 7. FTIR spectra of (a) the theophylline drug composite xerogel of compound **3h**; (b) the drug theophylline; (c) the gelator **3h**.

hydrogen bonding during gel formation and the shift from 1688 cm^{-1} – 1680 cm^{-1} corresponds to the C=O stretching of the amide during gel formation.

3.7. Comparative study of pH-responsive release of drug theophylline from organo/hydrogel

In recent decades, topical administration has gained prominence because it avoids first-pass metabolism, offering several benefits. To avoid the risks and drawbacks associated with intravenous treatments and to optimize absorption across various physiological conditions, adjusting pH in topical formulations is crucial [51]. Based on this, our initial study focused on understanding how theophylline is released from an organo/hydrogel by using pH changes as a trigger. As the gelator is sensitive to pH, altering the medium's pH causes the gel structure to break down. This enables the encapsulation of the drug within the organo/hydrogel and its controlled release by simply adjusting the pH [52]. By using the calibration equation derived from UV–vis absorbance data, we have calculated the cumulative release of theophylline (Fig. S17). Theophylline shows a maximum UV absorption peak at 272 nm, where the gelator does not show any absorption (Fig. S18). To study the theophylline release, we first performed experiments at different pH levels using a 0.1 M phosphate buffer solution. These experiments were performed in a temperature-controlled bath at 37°C , using the minimum gelation concentration of the gelator. For the release study, 12 mL of phosphate buffer solution was carefully added to the theophylline encapsulated gel. From this solution, we collected 200 μL samples of the supernatant at specific time intervals. These samples were then diluted to 3 mL and filtered before their UV spectra were obtained. We then measured each sample's absorbance at 272 nm to determine the theophylline released from the organo/hydrogel. All calculations were observed using the UV–vis data (Fig. S19–21). To maintain the original volume to 12 mL of the buffered solution, fresh buffer (3 mL) was subsequently added. After that, we used UV–vis spectroscopy to study how the drug-encapsulated gel degraded in response to pH changes.

Fig. 8 displays the release curves, comparing the theophylline release from both **3e** + **Th** and **3h** + **Th** gels under different pH conditions. In case of **3e** + **Th** gel, at pH 7.4, the theophylline shows release of 15 % in 2 h, 43 % in 12 h, 59 % in 20 h, 72 % in 30 h, 82 % in 32 h and 86 % in 45 h. On the other hand, the theophylline release from the **3h** + **Th** under the same pH conditions follows the release order as 9 % in 2 h, 34 % in 12 h, 47 % in 20 h, 60 % in 30 h and 65 % in 40 h. This difference is probably due to the increased stability of the gel, resulting from stronger van der Waals interactions due to increase in length of fatty acids alkyl chains.

Additionally, we observed that lowering the buffer solution pH to 5.5 slowed theophylline release from both **3e** + **Th** and **3h** + **Th** gels. Specifically, at pH 5.5, from **3e** + **Th** gel 75 % of theophylline released over 45 h, while in case of **3h** + **Th** only 50 % release within 40 h, with no further release afterward. We conducted additional experiments at pH 6.5 to further examine the drug release behavior of **3e** + **Th**. As illustrated in Fig. 8(c), the release rate was higher compared to pH 5.5, with 82 % of the drug released over 45 h. In contrast, free theophylline, when not encapsulated within the gel, exhibited a rapid and complete release, as shown in Fig. 8(d). These results clearly demonstrate that our gelator effectively enables a controlled and sustained release of theophylline.

The results were replicated three times to ensure their consistency and reliability. The cumulative release of theophylline from the drug-encapsulated organo/hydrogel clearly showed a pH-dependent pattern. This suggests that pH is crucial in disrupting the gel's self-assembly, ultimately causing its degradation. This disruption primarily occurs because of the reduction in hydrogen bonding that interferes with the self-assembly process, which is essential for the gel's structure. Here, drug release happens because of the disintegration of the composite gel as the pH changes. Based on these experimental results, we conclude that these unique organo/hydrogels could be valuable for medication administration and serve as drug carriers in the pharmaceutical industry.

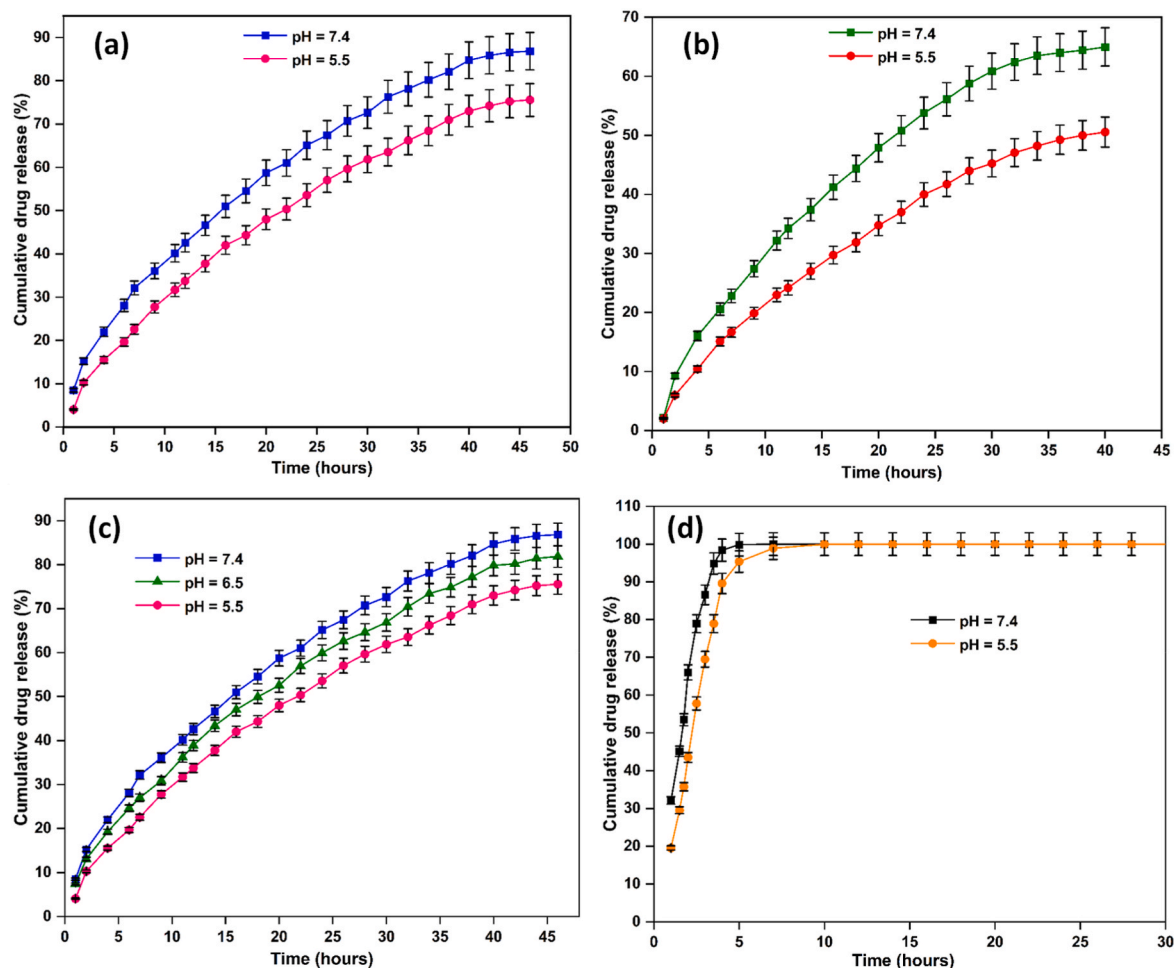


Fig. 8. Comparative study of pH sensitive cumulative release of theophylline loaded organo/hydrogels; (a) 3e at pH 5.5 and 7.4; (b) 3h at pH 5.5 and 7.4; (c) 3e at pH 5.5, 6.5 and pH 7.4; (d) Theophylline drug organo/hydrogel at pH 5.5 and 7.4.

3.8. Kinetics of the release of theophylline

To understand the rate and pattern of drug release, it becomes crucial to study its kinetic release profile. To deeply understand how theophylline released from the gel at both pH 7.4 and pH 5.5, we have analyzed the data obtained from the drug release using several common kinetic models, as zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models [53]. We have evaluated the accuracy of each model by observing its correlation coefficient (R^2) value. For both pH levels, we plotted cumulative drug release against time. The R^2 value for zero order kinetics at pH 5.5 was 0.9839, while for first order kinetics was found to be 0.9918. Similarly, at pH 7.4 the R^2 value for zero order kinetics was 0.9644, while for the first order kinetics was found to be 0.9835 (Fig. S22–24 and Fig. S25). This implies that theophylline release from the gel follows a first-order kinetic pattern, rather than zero-order kinetics indicating that the release rate depends on the drug concentration. Initially, the gel was stable, but its degradation over time led to an increased release rate. We observed this breakdown of the gel phase throughout the drug release study. We used the Higuchi model to specifically examine the diffusion-related factors and to get more precise details about the release rate. The value of R^2 in Higuchi plot was 0.9685 at pH 5.5, and 0.9887 at pH 7.4 respectively, which confirms that release occurs via diffusion from the organo/hydrogels. The Hixson-Crowell model also showed a strong correlation ($R^2 > 0.95$) for drug release from the organo/hydrogels at both pH levels.

To better understand the drug release process, the release data was fitted to the Korsmeyer-Peppas equation to determine the diffusion

Table 4

Drug release kinetics of drug loaded organo/hydrogels showing R^2 values.

Kinetic Models	3e		3h	
	pH 5.5	pH 7.4	pH 5.5	pH 7.4
Zero order	0.9839	0.9644	0.9863	0.9769
First order	0.9918	0.9835	0.9902	0.9860
Higuchi	0.9685	0.9887	0.9430	0.9519
Hixson-Crowell	0.9895	0.9778	0.9890	0.9833
Korsmeyer	0.9601	0.9505	0.9552	0.9767
Diffusion coefficient(n) ^a	0.97	0.98	0.99	0.99

^a By applying linear regression in Korsmeyer-Peppas equation of $\log (M_t/M_\infty)$ versus $\log t$; n is the diffusion exponent, which is indicative of the drug release mechanism.

exponent (n value). The n value was calculated as 0.98 at both pH levels which was >0.89 , these results suggest the super Case II type of release, which signifies a non-Fickian diffusion mechanism [54]. This confirms that the drug is released from the gel through a combination of diffusion and swelling and the release is time-dependent. The values of the correlation coefficient (R^2) assess the accuracy of individual models in approximating the data (Table 4).

3.9. Cell viability assessment

The compounds (gelators) were assessed for their anticancer activity against A549 (human lung adenocarcinoma) cells. The results for the

Table 5IC₅₀ values of compounds in A549 and Vero cells.

Compounds (Gelators)	A549 cells IC ₅₀ values (μM ± SEM)	Vero cells IC ₅₀ values (μM ± SEM)
3a	201.50 ± 1.3	496.45 ± 5.95
3b	131.70 ± 3.2	100.1 ± 2.0
3c	106.43 ± 5.3	267.5 ± 9.4
3d	87.60 ± 2.9	604.2 ± 28.4
3e	40.71 ± 4.7	139 ± 0.5
3f	77.16 ± 5.6	292.4 ± 2.4
3g	87.92 ± 5.1	339.9 ± 7.1
3h	68.31 ± 3.8	468.2 ± 22.7
Carboplatin	65.38 ± 2.7	30.845 ± 0.4

cytotoxicity and anticancer properties of test compounds against the A549 human lung adenocarcinoma cells and Vero (normal) cells are shown in Table 5. Carboplatin taken as positive control and only cells plus media with no drugs were taken as negative control. All the tested compounds possess antiproliferative efficacy against the A549 cells when compared with the negative control. All the compounds possess negligible toxicity levels in Vero cells when compared with the control based on their obtained IC₅₀ values (Fig. 9). Among all the compounds 3e, 3h, 3f, 3d and 3g were having higher efficiency as anti-proliferative drugs for therapeutics with their respective IC₅₀ values. Compounds 3c, 3b and 3a revealed moderate effect as anti-proliferative compounds when compared with the negative control. Further, it is revealed that, all the compounds possess no toxic effects on Vero cells based on their IC₅₀ values (Fig. 10).

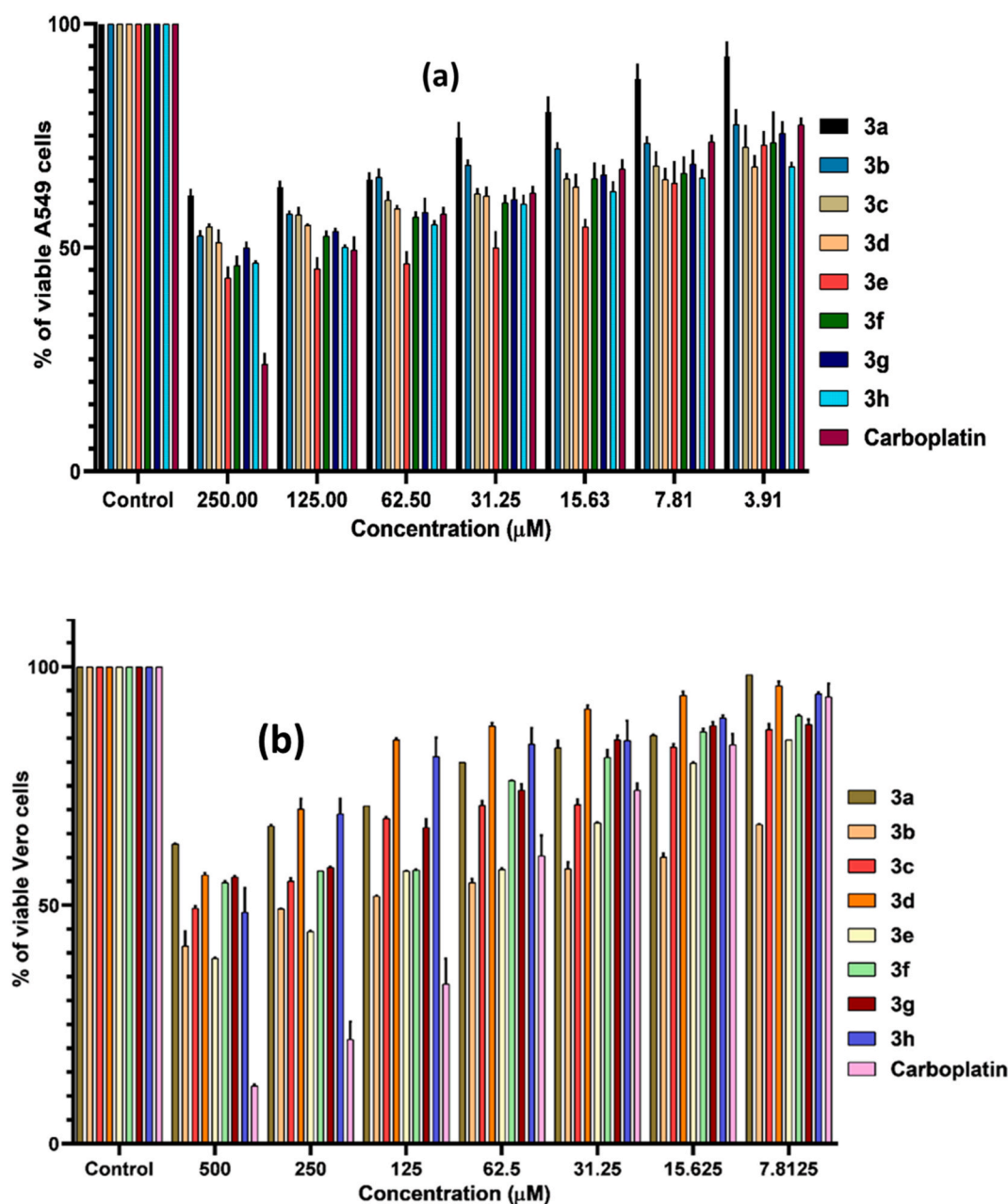


Fig. 9. Cytotoxicity assays of test compounds and carboplatin on A549 and Vero cells. The effect of different concentrations (X-axis) of the test compounds on the percentage of viability (Y-axis) of the cells representing (a) on A549 cells; and (b) on Vero cells. Statistical analysis performed in one way ANOVA. Obtained p value for (a) 0.5993, p value summary non-significant (ns); for (b) p = 0.0927, p value summary non-significant (ns).

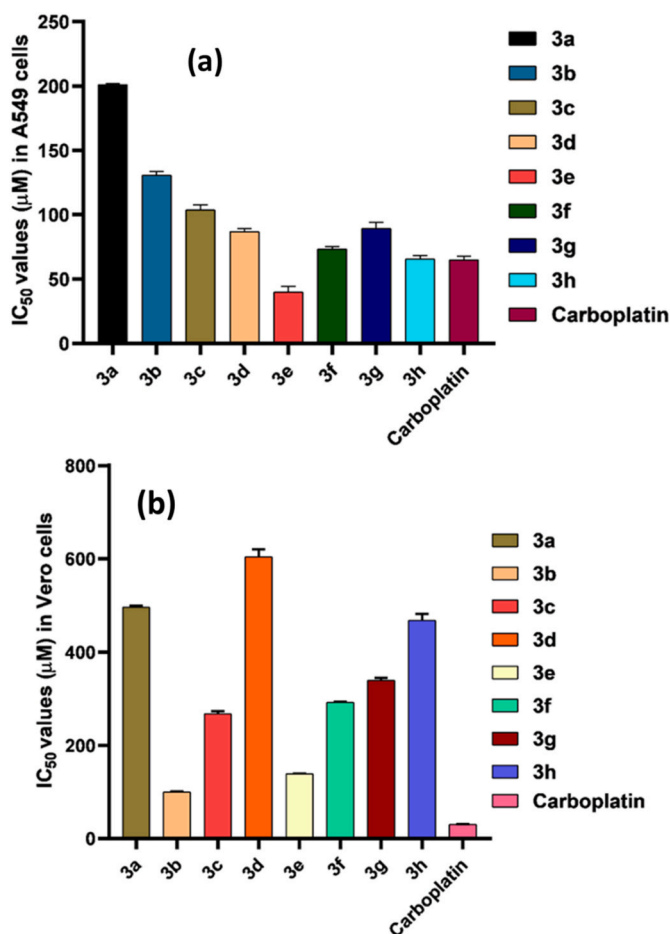


Fig. 10. IC₅₀ values of the test compounds (gelators) with which a) A549 cells; and b) Vero cells.

Table 6

IC₅₀ values of gelators and drug loaded gel formulations in A549 cells.

Gelators/Gel Formulations	A549 cells IC ₅₀ values (μg ± SEM)
Theophylline	79.75 ± 8.3
Gelator (3e)	22.15 ± 1.1
Drug Loaded Gel (3e + Th)	12.89 ± 0.4
Gelator (3h)	29.05 ± 2.0
Drug Loaded Gel (3h + Th)	21.50 ± 2.8

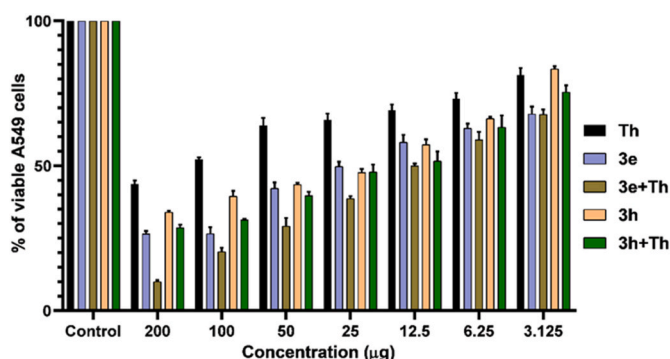


Fig. 11. Cell viability assay: Cytotoxicity assays of gelators and drug loaded gel formulations on A549 cells. Statistical analysis performed using one way ANOVA and obtained p value is 0.5993, p value summary non-significant(ns).

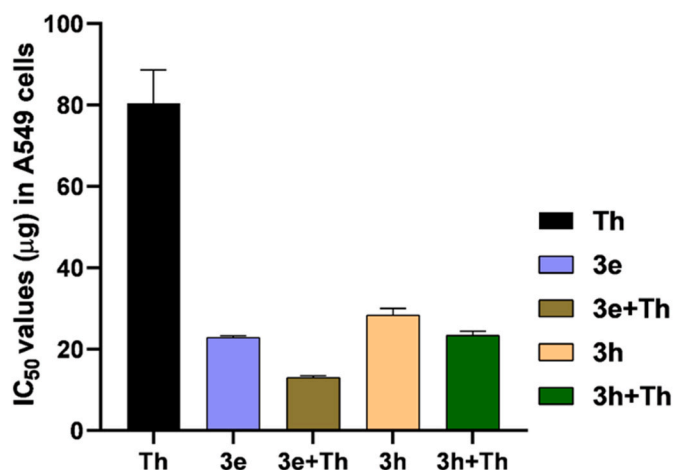


Fig. 12. IC₅₀ values of tested gelator and drug loaded gel formulations.

3.10. Synergistic combinational effect of gelator and organo/hydrogel

The gelators **3e**, **3h** and drug loaded gels formulations **3e + Th** and **3h + Th** were tested for their anticancer properties against A549 and Vero cells (Table 6) (Fig. S26 and 27). We initially formed organo/hydrogels of compounds **3e** and **3h** in DMSO/water. Subsequently, theophylline (**Th**) was added during gelation to yield gel formulations **3e + Th** and **3h + Th**. The drug release from the gels has been discussed previously. We further investigated the anticancer potential of the drug-loaded gels, and the results clearly indicates an increase in the anticancer activity of compounds **3e** and **3h** when conjugated with theophylline. Among gelators **3e** and **3h**, a greater enhancement in activity was observed for compound **3e** than for compound **3h** when conjugated with theophylline (Figs. 11 and 12).

4. Conclusion

In this study, we synthesized a small library of low molecular weight gelators derived from 4-aminopyridine and fatty acid amides. The gelation behavior of these compounds was evaluated in various solvent systems. Among the eight synthesized gelators, six formed stable, thermoreversible organogels and organo/hydrogels. Notably, gels formed with longer-chain fatty acids exhibited enhanced mechanical strength compared to those with shorter chains. The minimum gelation concentration (MGC) for the organo/hydrogels ranged from 1 to 2 % w/v. Rheological analyses confirmed that both the blank and drug-loaded gels possessed good mechanical integrity and elasticity. FE-SEM images revealed that the xerogels displayed diverse morphologies, including rod, sheet, and plate-like structures. The hydrophilic drug theophylline was successfully encapsulated within the gel matrices of compounds **3e** and **3h**, enhancing their gel strength and demonstrating their potential for drug delivery. Drug release studies in buffer solutions at pH 5.5 and 7.4 (37 °C) showed a faster release at higher pH, as confirmed by UV-vis spectroscopy. The anticancer activity of the compounds was tested against A549 (cancer) and Vero (normal) cell lines. Compounds **3d-3h**, bearing longer acid chains, showed higher cytotoxicity against A549 cells, while **3a-3c** exhibited moderate activity. Importantly, both unloaded and theophylline-loaded gels (**3e** and **3h**) displayed significant anticancer potential with minimal cytotoxicity toward Vero cells. Overall, the findings suggest that these organo/hydrogels are promising candidates for use as drug delivery systems, particularly in cancer therapy, due to their selective cytotoxicity and favorable physicochemical properties. However, the current study limited to *in vitro* analysis with target compounds in determining antiproliferative and cytotoxicity properties on both A549 and Vero cells. Further, *in vivo* investigations are essential to validate therapeutic

efficacy and safety measures of compounds.

CRedit authorship contribution statement

Meenakshi: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Manisha:** Writing – review & editing, Investigation. **Yangala Sudheer Babu:** Writing – review & editing, Investigation. **Mettile Brahma:** Writing – review & editing, Investigation. **Mulaka Maruthi:** Writing – review & editing. **Deepak Kumar:** Writing – review & editing. **Azaj Ansari:** Writing – review & editing. **Manoj K. Gupta:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Meenakshi reports financial support was provided by Haryana State Council for Science Innovation and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jddst.2025.107630>.

Data availability

The data associated with this work are included in the main manuscript and the electronic supplementary information.

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