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1. Physical Measurements and Instrumentation.

Materials and Methods. All chemicals and solvents were purchased from well-known commercial sources. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Bruker-400 Advance NMR spectrometer. FT-IR studies were performed in a Shimadzu FT-IR instrument using the ATR technique. Mass spectrometry of individual compounds was performed using a MicroMass ESI-TOF MS instrument. The UV-Vis spectra of specified solutions/suspensions were recorded on the Shimadzu model 2100 spectrophotometer.

Determination of CGC value. If a gel was formed, it was evaluated quantitatively by determining the critical gelator concentration (CGC), which is the minimum amount of gelator required to immobilize 1 mL of solvent. The above measurement was carried out three times, and the average CGC value has been reported.

Water retention properties of the drug-loaded hydrogel. An aqueous solution of streptomycin was added to a slightly hot (50-50 °C) clear solution of 2ClCA-EA and then cooled to room temperature (~25 °C) to prepare a streptomycin-loaded robust hydrogel where the concentration of streptomycin and gelator was 10, and 26.1 mM respectively. Water retention properties of the drug-loaded hydrogel, a crucial parameter for wound healing applications, were evaluated. A vial cap (171 mg) was filled with the drug-loaded hydrogel (W_1 = 119.2 mg) and kept at open atmosphere (~ 31 °C, humidity ~76%) (Fig Sx). The weight of the hydrogels was recorded at different time intervals (W_t). After reaching a constant weight after ~40 h, the residual water was removed under high vacuum. The weight of the dry gelator and drug was found to be 0.7 mg (W_{dry}), and water retention properties were evaluated using equation S1 as shown below.

$$W_{r\%} = \frac{W_t - W_{dry}}{W_1 - W_{dry}} \times 100$$

Circular Dichroism (CD) Spectroscopy. All the CD spectra of specified solutions/suspensions were recorded in the JASCO J-815 CD machine with a 0.2 cm path length cuvette and a 50 nm/min scan speed with two accumulations of a scan. The CD data were recorded after baseline subtraction in a water solution.

Scanning Electron Microscopy (SEM). The gels were carefully drop-cast on top of the freshly cleaned glass surface and allowed to air-dry overnight. The samples were then coated with gold vapor and analyzed on a ZEISS instrument operated at 10-15 kV.

Rheological Studies. All the rheology experiments were performed upon fixing the gap distance between the cone and the plate at 0.5 mm. The gels were scooped onto the plate of the rheometer. An oscillatory strain amplitude sweep experiment was performed at a constant oscillation frequency of 1 Hz for the applied strain range 0.001-1000 % at 25 °C. Oscillatory frequency sweep experiments were performed in the linear viscoelastic region (strain 0.1%) to ensure that calculated parameters correspond to intact network structures. The thixotropy test was performed by fixing the oscillatory frequency at 1 Hz.

X-ray Diffraction (XRD). The gels were carefully drop-cast on top of the freshly cleaned glass slide and allowed to air-dry overnight. Henceforth, the samples were dried under a vacuum for the corresponding XRD measurement. The X-ray beam generated with a rotating Cu anode at the wavelength of KR beam at 1.5418 Å was directed toward the film edge, and scanning was done up to a 2θ value of 30°. Data were analyzed and interpreted using the Bragg equation.

Computational Details. In the present work, all density functional theory (DFT) calculations were performed using Gaussian 16 program suite.[1a] The conformation of the ground state monomer of 2ClCA-EA and 4ClCA-EA was obtained using the B3LYP D3/6-31+G(d) level of theory. Previous studies described that non-covalent interaction energies like hydrogen bonding, π–π stacking are accurately described by the hybrid B3LYP D3/6-31+G(d) level of theory.[1b] At the time of geometry optimization, solvent effects (water) were introduced by applying the Polarizable Continuum Model (PCM)[1c] using the integral equation formalism variant. Furthermore, Vibration frequency analysis was performed with the same level of

theory to confirm that the optimized geometries resemble to global minima on the potential energy surfaces.[1d,e]

Loading and release of streptomycin from the hydrogels. An aqueous solution of streptomycin was added to a slightly hot (50-50 ºC) clear solution of 2ClCA-EA and then cooled to room temperature (~25 ºC) to prepare streptomycin-loaded robust hydrogel, where the concentration of streptomycin and gelator was 10, and 26.1 mM respectively.
In order to determine the percentage of drug release, 1 mL of water was added cautiously on top of the hydrogel and the same was removed for analysis at given time intervals and replaced with 1 mL of fresh water. We have attempted to quantify the cumulative amount of streptomycin released into the aqueous medium. However, it could not be studied because the drug and a significant amount of the gelator leached from the drug-loaded hydrogel when fresh water was carefully added on top of the hydrogel and kept for hours. In addition, the molar extinction coefficient of the gelator was much larger than the drug streptomycin, which creates another difficulty in finding out the accurate concentration of the drug release at different time intervals by UV/Vis spectroscopy.

Analysis of Cell viability via MTT assay. The cytotoxic effect of the gelator against WI38 cell line was measured by using 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[1].[2] WI38 cell line was purchased from NCCS Pune, India, and was plated at a density of $5x10^4$ cells/ well in 100µl media (DMEM, Himedia) and incubated overnight at 37°C. Then, the gelator was added to the respective wells at different concentrations (12.5, 25, 50, and 100µM) and incubated for 24 hours. After the incubation, MTT reagent was added to the wells (0.5mg/ml) and incubated for 2 hours. When these purple-coloured formazan crystals were formed, the media was aspirated out from the wells, and 100 µl solubilization buffer was added to the wells and incubated for 15 minutes. The absorbance of solubilized formazan crystals was measured at 540 nm by an ELISA plate reader.[3]

Cell attachment assay by phase contrast microscope and confocal microscope imaging. To assess the property of the 2ClCA-EA hydrogel as a cell attachment media, the hydrogel was added to cover slips at concentrations of 20.8 mM (concentrated) and 10.4 (diluted) and incubated for 30 minutes at room temperature. Then, WI38 cells (105/well) were plated on the cover slips within a 6 well cell culture plate, and 1 ml media was added and incubated for 4 and 8 hours at 37°C in a 5% CO_2 incubator. Live cell images were taken using Phase Contrast Microscope. After the incubation period, the media was aspirated from the culture plates and

washed with 1X PBS. Then the cells were fixed with 1X fixation buffer for 15 minutes. Further, 1X permeabilization buffer was added to each well for 1 hour, and washed the cells with 1X PBST. Followed by the permeabilization buffer, PE-conjugated Phalloidin was added to each well and incubated for 15 minutes, and then the wells were washed with 1X PBST twice. Then, mounting media with DAPI was applied to each wells, and each coverslip was mounted on separate slides.[4] Images were taken to check the cell attachment with the help of a confocal laser scanning microscope (DMi8, Leica Microsystems, Germany).

Antibacterial activity of the streptomycin-loaded hydrogel nanocomposite. At the initial stage, the antibacterial property of streptomycin-loaded nanocomposite hydrogel and the native hydrogel was explored. Gram-negative *Klebsiella pneumoniae* (KP) (ATCC BAA 1705) and Gram-positive Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 700699) were utilized to predict the anti-bacterial properties of the compound. Fresh overnight cultures (200 µL) of both organisms were separately transferred. Culture Petri plates were prepared with nutrient agar with an added supplement of both 1% glucose and NaCl. After transferring the fresh overnight cultures to the surface of the plates, the cultures were spread evenly using a sterile, cooled glass spreader to ensure uniform distribution over the agar surface. The plates were then placed inside a refrigerator at a temperature of 4°C for half an hour. A loopful of drug loaded in the hydrogel and the native hydrogel was separately applied to both MRSA and KP culture plates to evaluate antibacterial efficacy. The prepared plates were left for 24 hours at 37°C. Finally, after incubation, a clear zone was observed, indicating antibacterial activity.[5,8]

Minimum inhibitory concentration (MIC) of streptomycin-loaded gelator nanocomposite solution. The Minimum Inhibitory Concentration (MIC) can be defined as the lowest concentration of any antimicrobial agent that can inhibit the growth of any bacterium throughout incubation for 24 hours at a definite temperature. The standard broth microdilution method was implemented to determine the MIC in both MRSA and KP cases. The overnight-grown bacterial cell broth was diluted in a ratio of 1:100. From the newly created stock, 2 µL was taken and added to each well containing 200 µL of nutrient broth in a 96-well microtiter plate. In this experiment, streptomycin-loaded gelator nanocomposite in liquid form was utilized in different concentrations, ranging from 312.50 to 2.44 µM of streptomycin. After preparing the samples in the 96-well microtiter plate, the plate was placed inside an incubator at 150 rpm and 37°C. The plate was then incubated for 24 hours. Then, utilizing a microplate

reader, the optical density (OD) was measured at 595 nm. Each step of the experiment was performed twice. Finally, a graph was generated to show OD values concerning the concentration of the streptomycin in the streptomycin-loaded gelator nanocomposite solution and to determine the exact MIC concentration.[7]

Microscopic Evidence (SEM) of Antibacterial Activity of the Streptomycin-loaded Nanocomposite Hydrogel. Overnight-grown MRSA and KP were both treated with a sub-MIC concentration of streptomycin-loaded nanocomposite in liquid form and incubated at 160 rpm and 37°C for 24 hours. Next day, the broth (containing treated organisms) was transferred into microcentrifuge tubes and centrifuged at 10,000 rpm for 5 minutes. Then, the pellets were washed three times with the help of 1XPBS. After the process of washing, all the samples were dissolved in 100 µL 1XPBS. In the next step, only 5 µL of the cell suspension was applied to the surface of a coverslip and allowed to dry. To fix the sample on the coverslip, 2.5% glutaraldehyde was prepared, and 10 µL of it was added. Afterwards, the coverslips were stored overnight at 4°C in a dark environment. Then, the coverslips were led to a process of washing by ethanol of different concentrations ranging from 30% to 100%, and for complete drying, the samples were placed into a desiccator. A similar process was followed, where no treatment was applied.[6,7]

Hemolysis assay using streptomycin-loaded gelator nanocomposite solution. Goat red blood cells (RBCs) were utilized to assay the hemolytic activity of the nanocomposite solution. Initially, fresh goat blood was stabilized utilizing heparin. At room temperature, the blood sample was centrifuged at 5,000 rpm for 10 minutes the erythrocytes were harvested. Afterward, all the samples were washed utilizing phosphate-buffered saline (PBS, pH 7.4). Next, washed pellets were given into the phosphate-buffered saline to yield a 10 % (v/v) erythrocytes/PBS suspension. Followed by a 10% suspension being diluted to 1:10 in PBS. Next, different concentrations of nanocomposite solution (conc. of streptomycin 78.13 µM, 39.06 µM, and 19.53 µM) were prepared using the diluted erythrocyte suspension. At 37°C for 60 minutes, each sample was incubated, followed by a centrifugation at 10,000 rpm for 5 minutes. For the study, Triton X was set as the positive control, while PBS served as the negative control. Each sample was incubated at 37°C for 60 minutes, followed by centrifugation at 10,000 rpm for 5 minutes. Finally, the hemolysis was evaluated by comparing the results with both control samples.[7]

Streptomycin-loaded nanocomposite hydrogel mediated *in vivo* wound healing assay. With the approval of the Institutional Ethics Committee, University of Kalyani, the entire *in vivo* experiment was conducted. In this experiment, male Swiss albino mice, which are aged between 6 to 10 weeks were chosen. All the animals were taken from the State Centre for Laboratory Animal Breeding, located in Kalyani, Nadia, West Bengal, India. During the experiment, different parameters were set, such as (a) temperature: 25±1°C, (b) humidity: 15%-25%, and (c) a standard 12-hour light-to-dark cycle was maintained over time. The animals were separated into different cages, and fresh water along with a balanced diet was provided. For experimental procedures, the mice were divided into different groups and treated accordingly based on the protocol. For pre-surgery preparations, their body hair was removed and cleaned with 70% alcohol. After anesthetizing the animals using a ketamine-xylazine mixture, the skin was removed with a 4 mm biopsy punch kit. The wound area was then washed using a PBS solution. All the animals were kept under observation until they fully recovered from anaesthesia.

The experiment was designed with a total of five groups as follows:

Group 1 (control group): only a wound was created.

Group 2 (only KP): wounds contaminated with *Klebsiella pneumoniae* (KP).

Group 3 (KP and streptomycin-loaded nanocomposite hydrogel): wounds infected by KP and treated with streptomycin-loaded nanocomposite hydrogel.

Group 4 (only MRSA): wounds contaminated with MRSA.

Group 5 (MRSA and streptomycin-loaded nanocomposite hydrogel): wounds infected by MRSA and treated with streptomycin-loaded nanocomposite hydrogel. [2ClCA-EA] = 26.1 mM, [Streptomycin] = 10 mM.

Post-treatment, the mice were monitored regularly, and photographs were taken at regular intervals for documentation purposes.[5]

Hematoxylin and eosin Staining: The skin tissue samples were cut from the wound and fixed in 10% neutral buffered formalin (NBF). The samples were embedded in paraffin to prepare a histopathological slide. Afterward, hematoxylin and eosin (H&E) staining was performed on the tissue samples, and all samples were analysed using a brightfield microscope.[7,9]

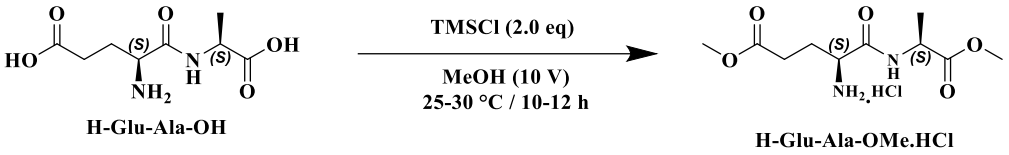
In silico toxicity analysis of native hydrogel: In silico method is being implemented to understand the toxicity profile of native hydrogel. For this study, pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) is being used. The pkCSM platform runs based on a machine learning system to predict toxicity properties. Various parameters under toxicity profile were evaluated, among them the key parameters are AMES toxicity, skin sensitisation etc.[10]

Biofilm assay: Since biofilms typically show more resistance than free-living bacteria, antibiofilm assays are used to evaluate the efficacy of antimicrobial drugs against biofilms. The crystal violet (CV) assay was used in this work to assess biofilm inhibition. By evaluating the added CV dye's ability to attach to extracellular polymeric materials and both living and dead bacterial cells, this technique measures the overall mass of the biofilm. The investigation was conducted for both KP and MRSA. In a 96-well microtiter plate, 1×10^6 number of cells were added to each well. Then, in each well with drug-loaded hydrogel (in liquid form) were treated, where streptomycin was used at different concentrations (0.08, 0.16, 0.31, 0.62, 1.25, 2.5, and 5 mM). Afterwards, the 96-well plate was incubated for 72 hours at 37 °C. The biofilms were again washed with PBS to remove the extra treatment solution. The biofilms were then stained for 20 minutes at room temperature after 100 μL of 1% (w/v) CV solution was applied to each well. The biofilms were then washed three times with PBS to get rid of the extra colour. After adding 150 μL of 70% ethanol to each well, a microplate reader was used to detect the destaining solution's absorbance at 550 nm.[11]

2. Syntheses of 2ClCA-EA and 4ClCA-EA.

Preparation of methyl (S)-4-amino-5-(((S)-1-methoxy-1-oxopropan-2-yl)amino)-5-oxopentanoate hydrochloride: To a cleaned and well dried 500 mL 2-neck RBF, methanol (500.0 mL; 10.0V) was charged followed by H-Glu-Ala-OH (50.0 g; 0.229 mol) at 25-30 ℃ under nitrogen atmosphere. The mixture was stirred for 10 min to get slurry, and it was cooled to 0-5 °C. Then the trimethylsilyl chloride (TMSCl) (58.12 mL; 0.458 mol) was added slowly keeping the reaction mass temperature below 15 °C. After the addition of trimethylsilyl chloride the reaction mass was allowed to 25-30 °C and stirred for 10.0-12.0 h at 25-30 °C. Afterward, the mixture was concentrated under vacuum at 45-55 ℃ and co-distilled with toluene (100.0 mL; 2.0V) to remove the traces of methanol. Finally, the obtained mass was degassed for 1.0 h at 50 °C under vacuum and then cooled it to 25-30 °C to afford titled compound. (52.7g; yield = 92.5%; 1.05 w/w).

Reaction scheme:



H-Glu-Ala-OH

H-Glu-Ala-OMe.HCl

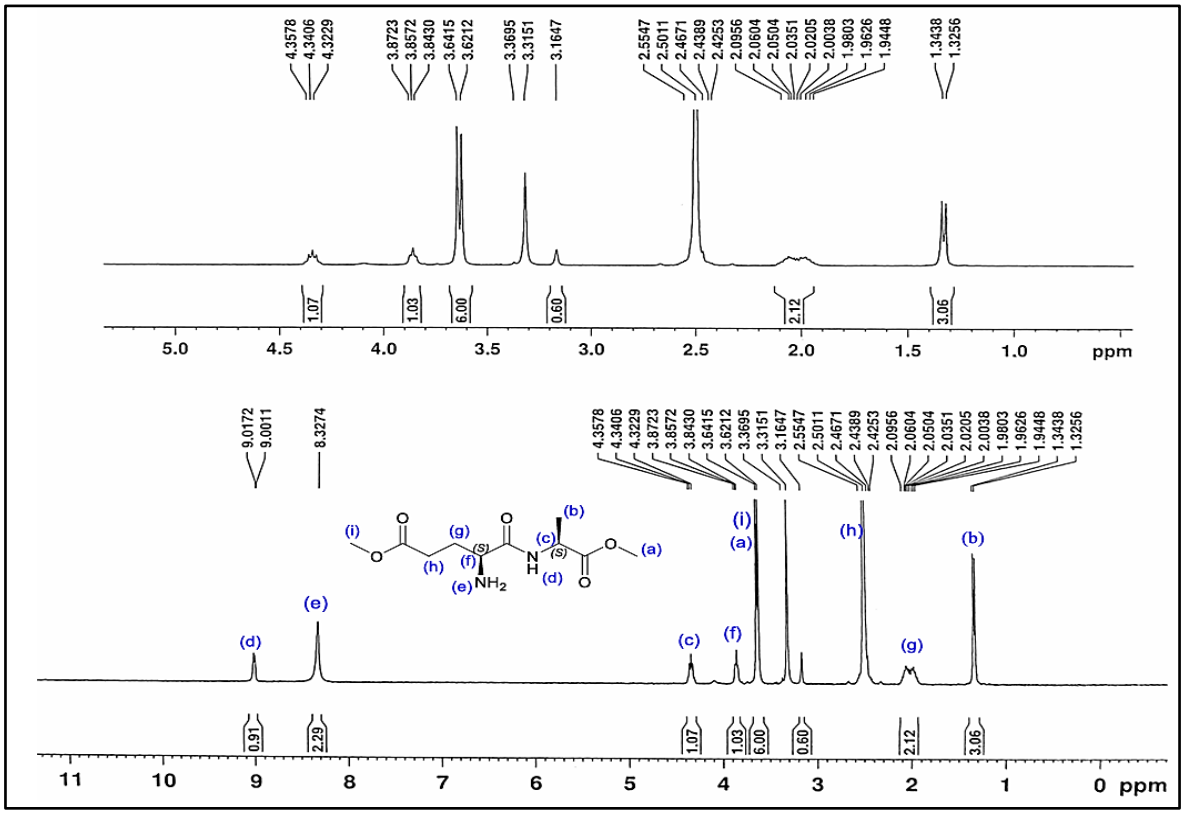
^{1}H-NMR in DMSO (400 MHz): 9.01 (d, 6.4 Hz, 1 H), 8.32 (s, 1 H), 4.34 (t, 6.8 Hz, 1 H), 3.85 (t, 6.0 Hz, 1 H), 3.63 (d, 8.1 Hz, 6 H), 2.09-1.94 (m, 2 H), 1.33 (d, 7.3 Hz, 3 H) ppm

^{1}H-NMR in D_2O (400 MHz): 4.53 (q, 7.28 Hz, 1 H), 4.13 (6.84 Hz, 1 H), 3.80 – 3.76 (6 H), 2.69 – 2.63 (m, 1 H), 2.29 – 2.22 (m, 1 H), 1.49 (dd, 3.56 & 3.84 Hz, 3 H) ppm.

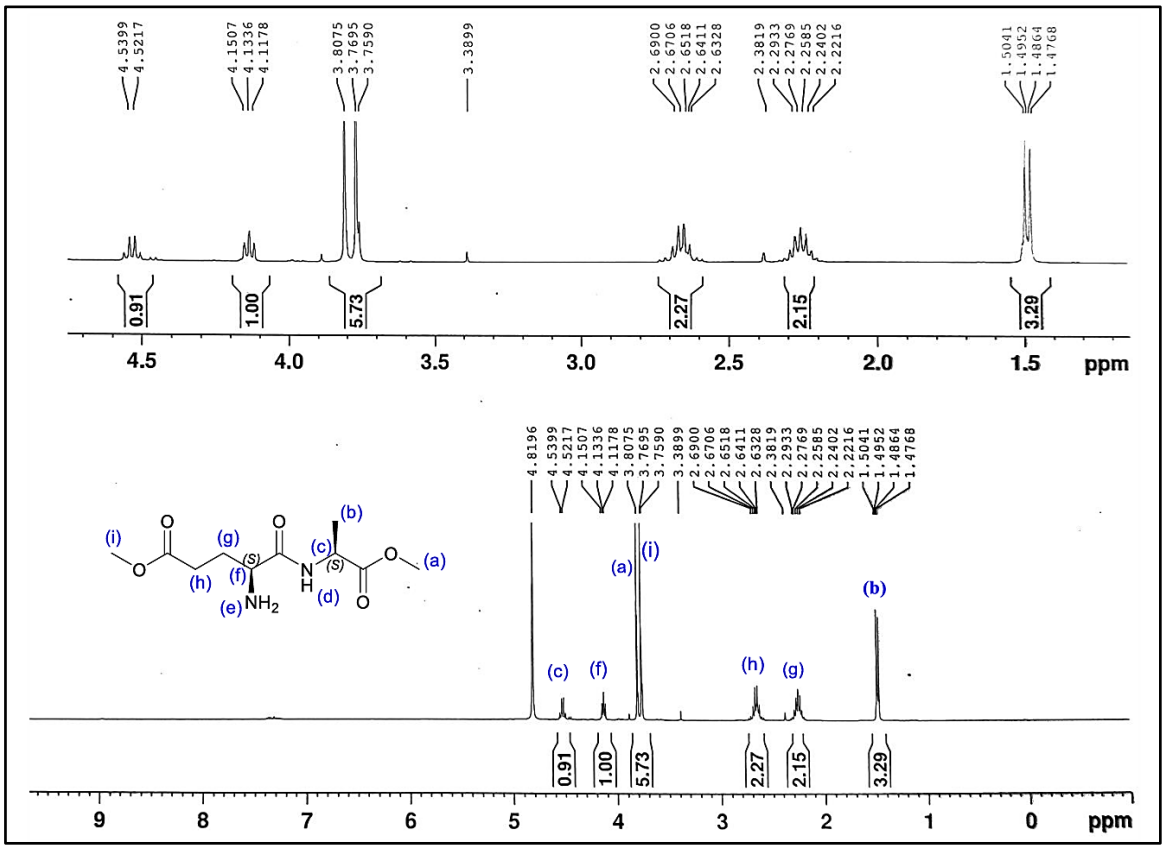
^{13}C-NMR in D_2O (100 MHz): 174.76 (C=O), 174.41 (C=O), 52.96-52.07 (CH), 48.82 (CH), 28.71 (CH2), 25.77 (CH2), 15.75 (CH3) ppm.

LCMS: M+H = 247

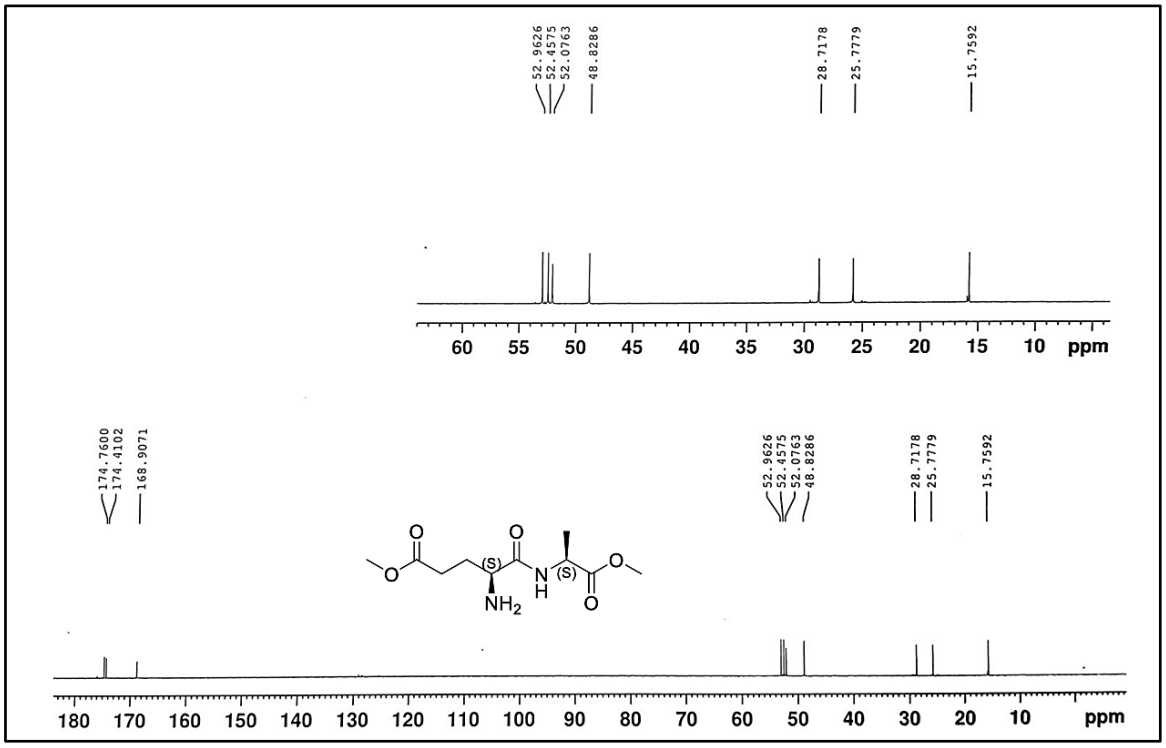
^{1}H-NMR in DMSO (400 MHz):



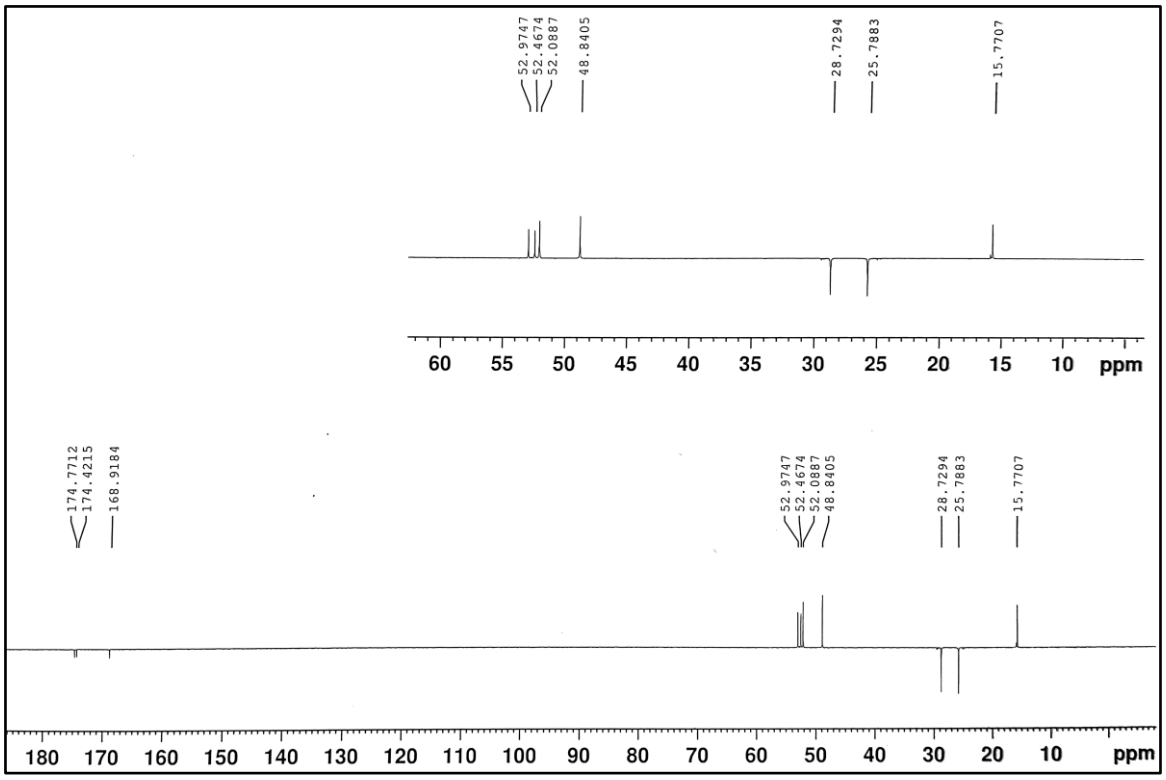
^{1}H-NMR in D_2O (400 MHz):



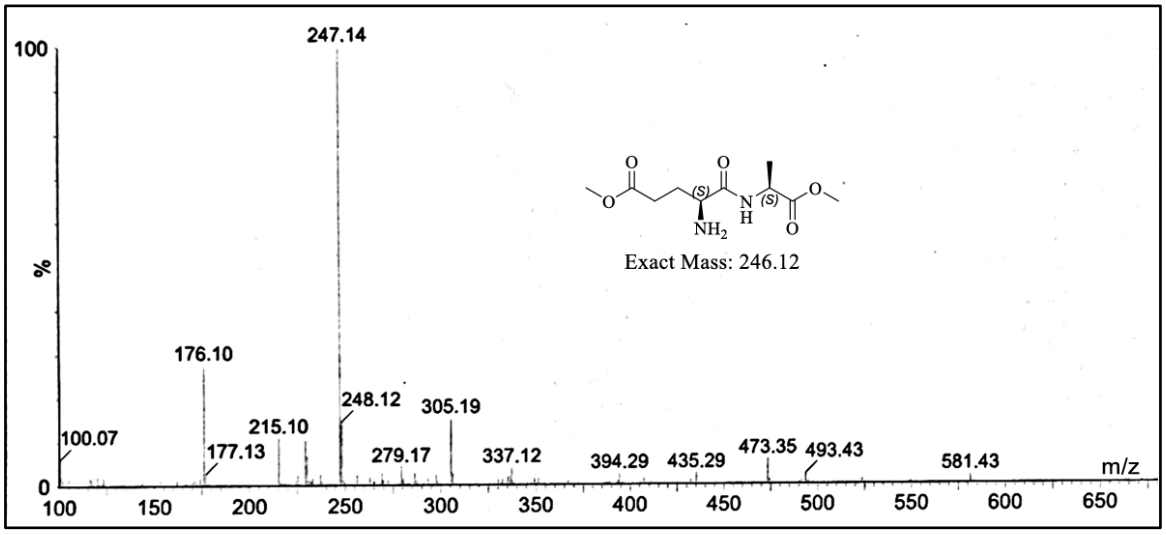
^{13}C-NMR in D_2O (100 MHz):



APT in D_2O (100 MHz):

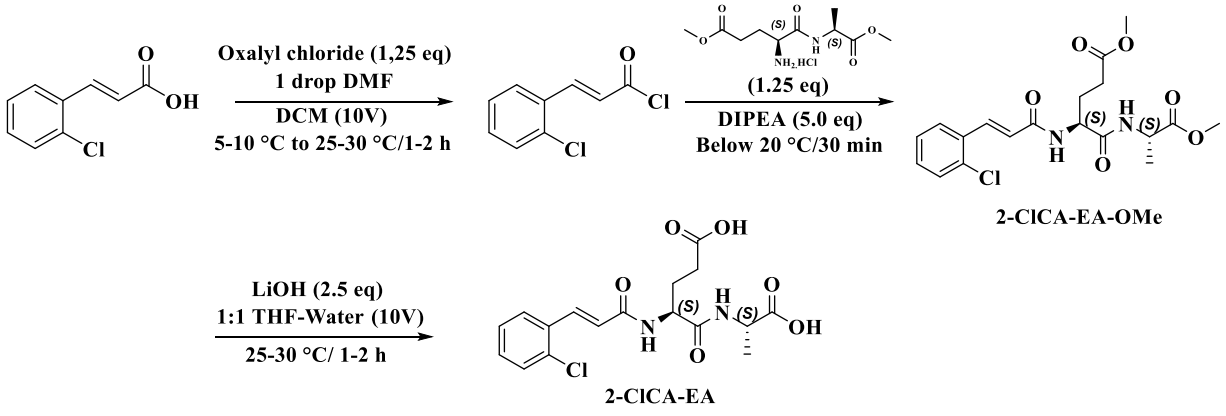


LCMS:


247.14
100
%
0
176.10
248.12
305.19
215.10
100.07
177.13
279.17
337.12
394.29
435.29
473.35
493.43
581.43
m/z
100
150
200
250
300
350
400
450
500
550
600
650
Exact Mass: 246.12

Synthesis of (S)-5-(((S)-1-carboxyethyl)amino)-4-((E)-3-(2-chlorophenyl)acrylamido)-5-oxopentanoic acid (2-ClCA-EA-OMe): (E)-3-(2-chlorophenyl)acrylic acid (3.0 g; 16.43 mmol) was charged into a reaction vessel in dichloromethane (DCM) (30.0 mL; 10.0 V) under nitrogen at 25-30 °C and stirred for 5-10 min at 25-30 °C. The obtained slurry was cooled at 5-10 °C and the oxalyl chloride (1.75 g; 20.54 mmol) was added to the reaction mixture, maintaining the internal temperature of reaction vessel 5-10 °C. After complete addition of oxalyl chloride, 1-2 drops dimethylformamide (DMF) was added to the reaction mixture. Then the reaction mixture was allowed to 25-30 °C and stirred for 1.0-2.0 h. After complete consumption of 2-chlorocinnamic acid, the solvent was removed under vacuum at below 50 °C to get the (E)-3-(2-chlorophenyl)acryloyl chloride (3.2 g; 1.06 w/w). To another reaction vessel methyl(S)-4-amino-5-(((S)-1-methoxy-1-oxopropan-2-yl)amino)-5-oxopentanoate hydrochloride (5.80 g; 20.53 mmol) was dissolved in the mixture of dichloromethane (30. mL; 10.0 V) and diisopropylethylamine (DIPEA) (10.62 g; 82.15 mmol) at 25-30 °C under nitrogen. The solution was cooled at below 20 °C, and the (E)-3-(2-chlorophenyl)acryloyl chloride in dichloromethane (15.0 mL; 5.0 V) was added to it at below 20 °C. The mixture was stirred for 30 min at below 20 °C. Afterward, the water (30.0 mL; 10.0 V) was charged to the reaction mass and stirred for 15 min. The layers were separated, and washed the organic layer with 5.0 (N) aqueous hydrochloric acid (30.0 mL; 10.0 V) twice followed by water (15.0 mL; 5.0 V). Then the organic layer was washed with 5.0% Aq. sodium bicarbonate solution (15.0 mL; 5.0 V) followed by water (15.0 mL; 5.0 V) and 20.0% Aq. sodium chloride solution (15.0 mL; 5.0 V). The organic layer was dried over anhydrous sodium sulphate (7.5 g; 2.5 w/w) and concentrated under reduced pressure at below 50 °C to afford the titled compound (4.8 g; 71.11 % molar yield; 1.60 w/w).

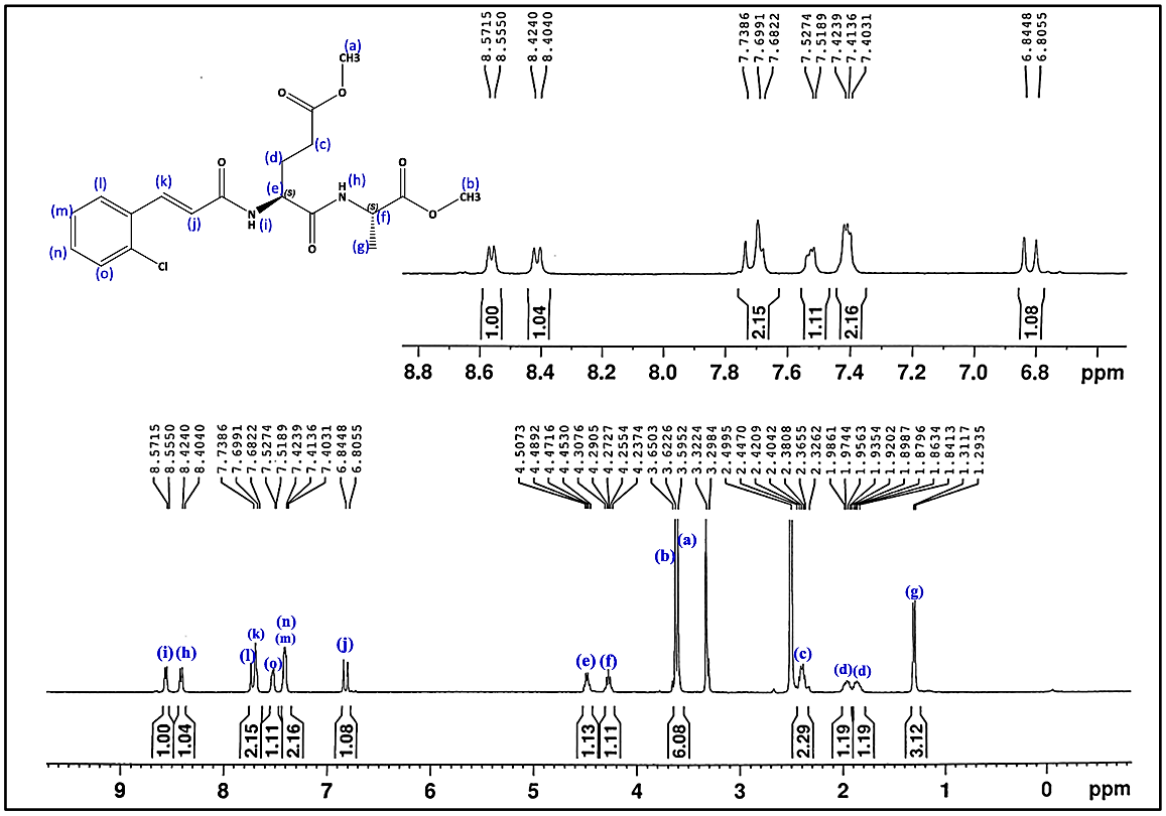
Reaction scheme:



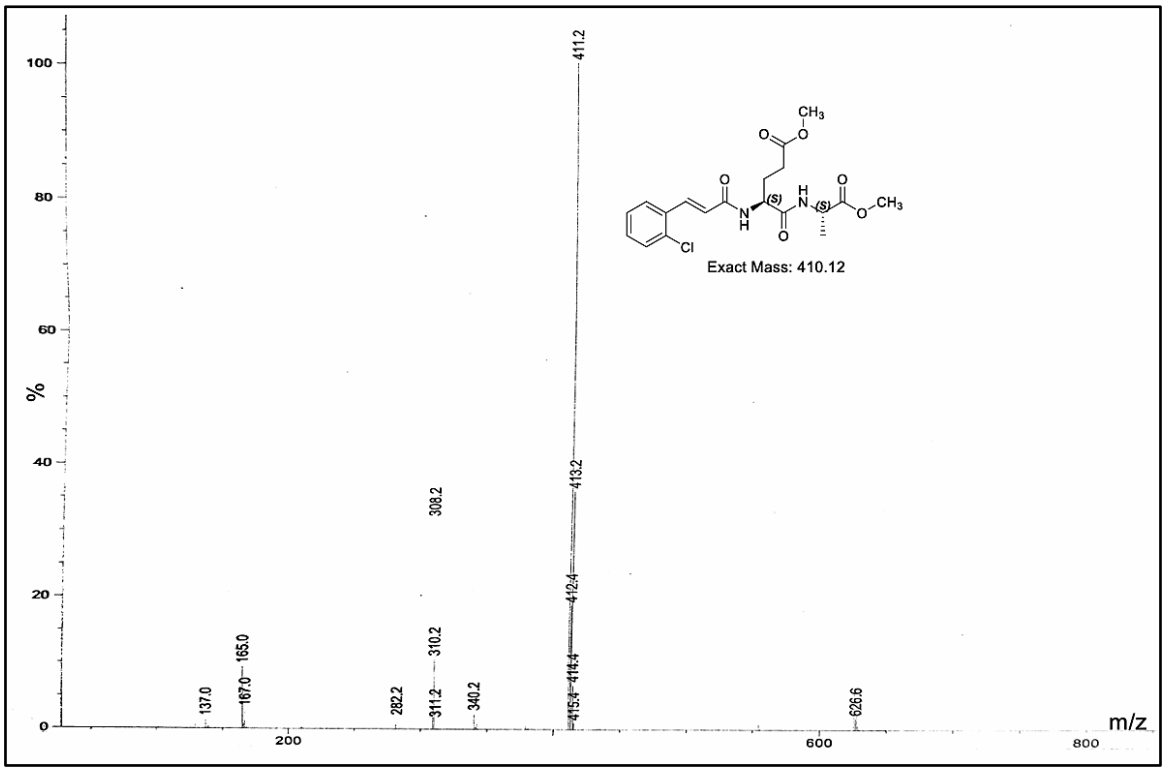
^{1}H-NMR in DMSO (400 MHz): 8.56 (d, 6.6 Hz, 1 H), 8.41 (d, 8.0 Hz, 1 H), 7.68 – 7.74 (s, d, 6.8 Hz, 2 H), 7.52 (d, 3.4 Hz, 1 H), 7.41 (t, 2.1 Hz, 2 H), 6.82 (d, 15.7 Hz, 1 H), 4.47 (q, 7.2 Hz, 1 H), 4.27 (p, 6.9 Hz, 1 H), 3.59 – 3.65 (6 H), 2.40 (m, 2 H), 1.84 – 1.98 (m, 2 H), 1.30 (d, 7.3 Hz, 3 H) ppm.

LCMS: (M+H) = 411.

^{1}H-NMR in DMSO (400 MHz):



Mass:



Preparation of (S)-5-(((S)-1-carboxyethyl)amino)-4-((E)-3-(2-chlorophenyl)acrylamido)-5-oxopentanoic acid (2-ClCA-EA): To a cleaned and well dried RBF, methyl (S)-4-((E)-3-(2-chlorophenyl)acrylamido)-5-(((S)-1-methoxy-1-oxopropan-2-yl)amino)-5-oxopentanoate (2.5 g; 6.085 mmol) was charged in the mixture of water (12.5 mL; 5.0 V) and tetrahydrofuran (THF) (12.5 mmol; 5.0 V) at 25-30 °C. The mixture was stirred for 5-10 min at 25-30 °C, and then lithium hydroxide monohydrate ($LiOH.H_2O$) (0.64 g; 15.212 mmol) was charged into the RBF at same temperature. The reaction mixture was stirred for 1.0 – 2.0 h at 25-30 °C. After completion of the reaction, the dichloromethane (DCM) (25.0 mL; 10.0 V) was charged to the reaction mixture and stirred for 10-15 min at 25-30 °C. Afterward, the mixture was transferred into a separatory funnel and settled for 30 min. The layers were separated, and the aqueous layer was washed with dichloromethane (12.5 mL; 5.0 V) at 25-30 °C. Now the aqueous layer was cooled at below 20 °C and the pH = 1 – 2 was adjusted with 5(N) aqueous hydrochloric acid solution at below 20 °C. Then dichloromethane (25.0 mL; 10.0 V) was charged to the obtained white thick viscous mass, and the mixture was stirred for 15-20 min at 25-30 °C. The mixture was transferred into a separatory funnel and settled for 30 min, and separated the layers. The product from the aqueous layer was extracted with dichloromethane (25.0 mL; 10.0

V). The total combined organic layer was washed with water (25.0 mL; 10.0 V) followed by 20% aqueous sodium chloride solution (12.5 mL; 5.0 V). The organic layer was dried over anhydrous sodium sulphate (5.0 g; 2.0 w/w) and concentrated under vacuum at 40 – 45 °C keeping the internal volume 2.0 – 3.0 vol (5.0 mL – 7.5 mL). Afterward, the methyl *tert*-butyl ether (MTBE) (15.0 mL; 6.0 V) was added to the obtained residue at 25-30 °C. Then the obtained slurry was stirred for 30 min at 25-30 °C and then 1.0 h at 10 – 15 °C. The solid mass was filtered at 10 – 15 °C and washed with MTBE (12.5 mL; 5.0 V). The wet cake was suck dried for 30 min and then dried under reduced pressure at 45 – 50 °C for 6 – 8 h to get the titled compound (2.1 g; 0.84 w/w; 90.15% molar yield).

^{1}H-NMR in DMSO (400 MHz): 12.29 – 12.39 (b, 2H), 8.38 (t, 8.1 & 3.6 Hz, 2 H),7,70 – 7.74 (s, s, 2 H), 7.53 (t, 4.9 & 4.1 Hz, 1 H), 7.41 (t, 4.3 & 2.6 Hz, 2 H), 6.84 (d, 15.7 Hz, 1 H), 4.46 (q, 8.1 Hz, 1 H), 4.19 (p, 7.2 Hz, 1 H), 2.30 (m, 2 H), 1.95 (m, 1 H), 1.81 (m, 1 H), 1.27 (d, 7.3 Hz, 3H) ppm.

D_2O-Ex NMR in DMSO (400 MHz): 7.72 (q, 6.3 Hz, 2 H), 7.51 (q, 3.4 Hz, 1 H), 7.40 (q, 3.5 Hz, 2 H), 6.79 (d, 15.7 Hz, 1 H), 4.43 (t, 8.0 & 5.6 Hz, 1 H), 4.17 (q, 7.2 Hz, 1 H), 2.30 (d, 7.2 Hz, 2 H), 1.94 (m, 1 H),1.79 (m, 1 H), 1.28 (d,7.3 Hz, 3 H) ppm.

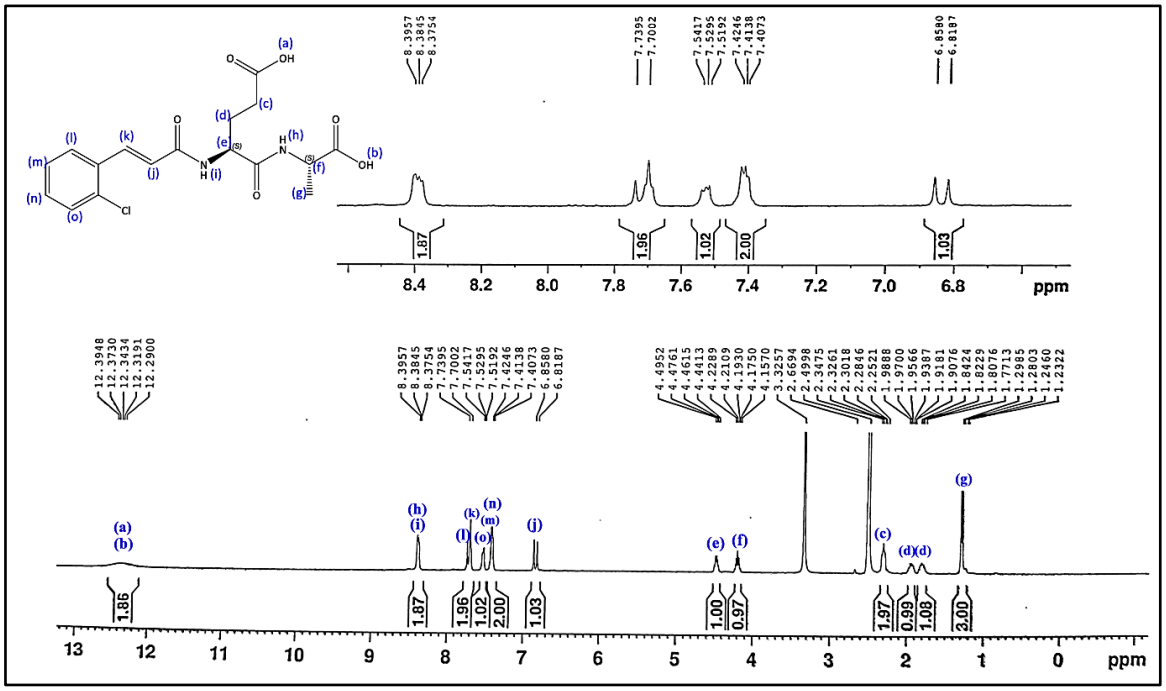
^{13}C-NMR in DMSO (100 MHz): 15.86, 27.82(-CH_2), 29.96 (-CH_2), 47.48, 51.59, 124.99, 127.45 – 127.72, 129.92, 130.87, 132.64 (C), 133.26 (C), 134.23, 164.19 (C=O), 170.78 (C=O), 173.89 (C=O) ppm.

FT-IR (cm^{-1}): 3328, 3304, 3179, 2972, 2938, 1739, 1709, 1644, 1574, 1553, 1451, 1406, 1362, 1325, 1299, 1276, 1208, 1179, 1138

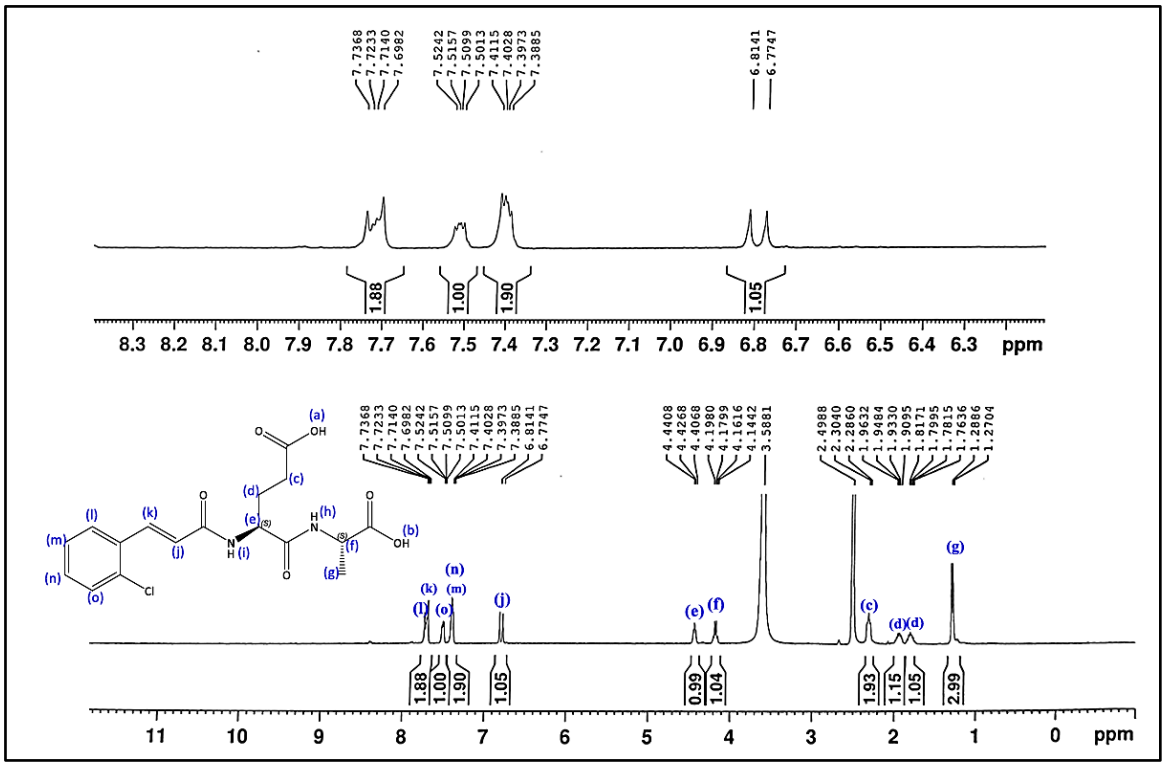
Thermal Study (DSC): The synthesized molecule has shown the melting point 183.3 °C and it does not decompose up to 380 °C.

LCMS: M+H = 383.

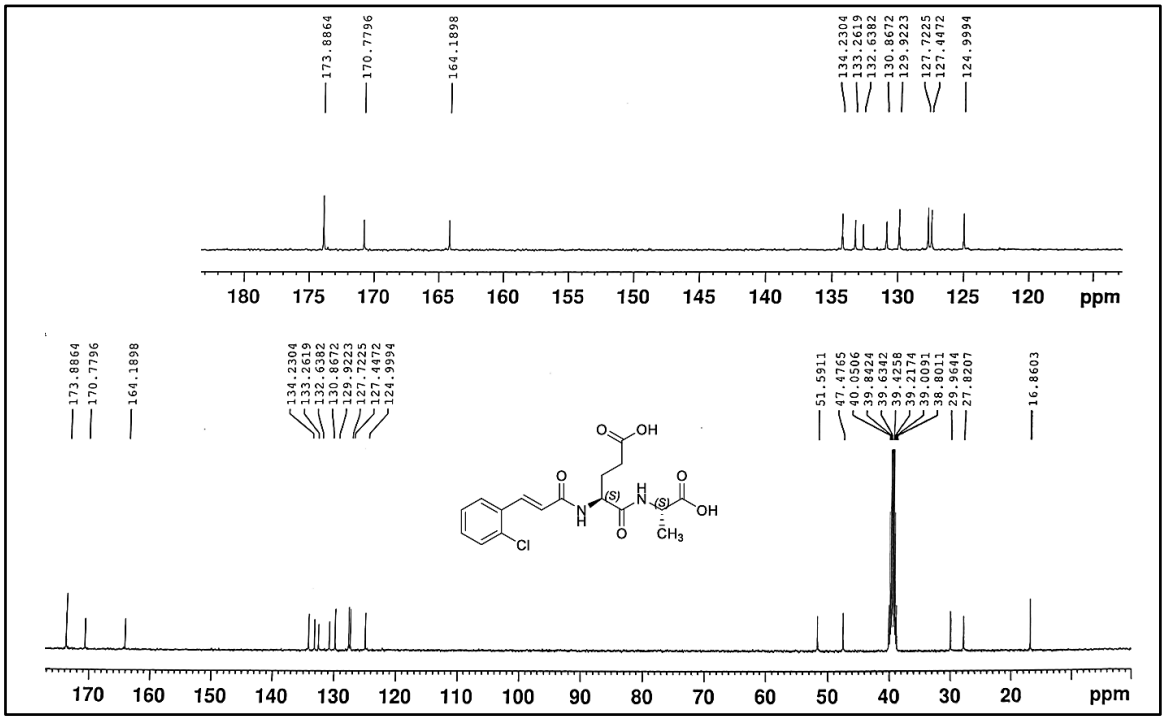
^{1}H-NMR in DMSO (400 MHz):



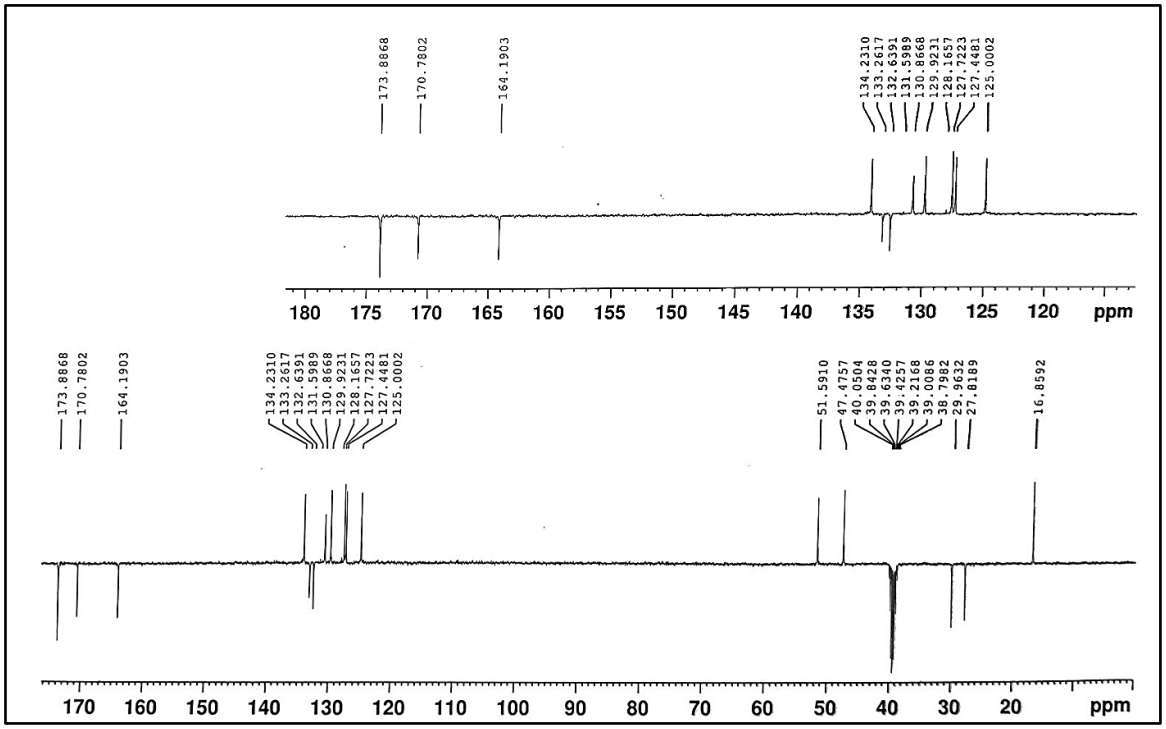
D_2O-Ex in DMSO (400 MHz):



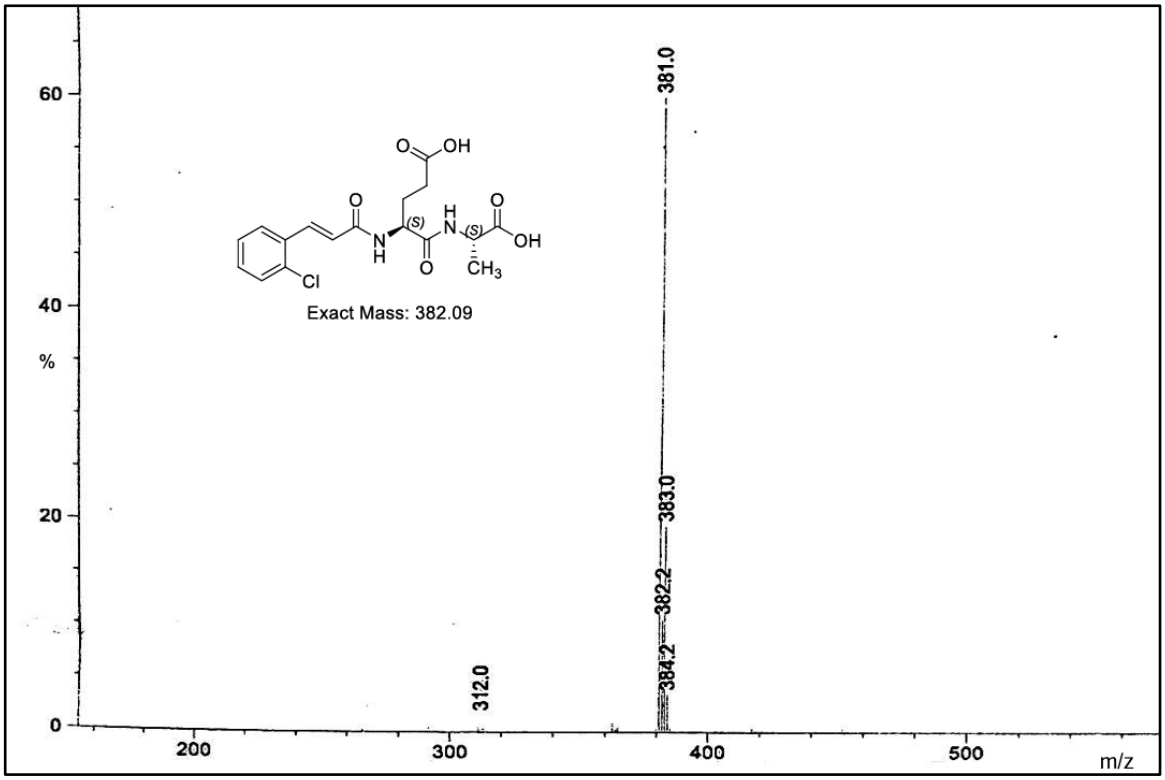
^{13}C-NMR in DMSO (100 MHz):



APT NMR in DMSO (100 MHz):

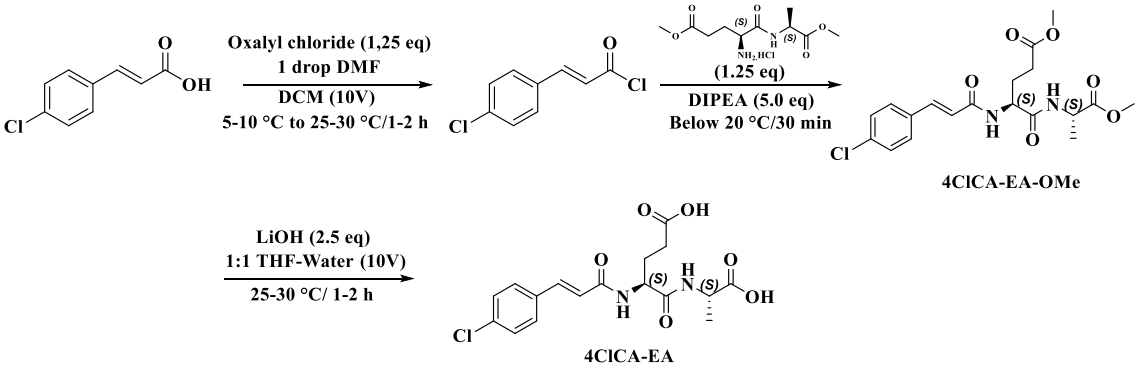


LCMS:



Synthesis of Methyl (S)-4-((E)-3-(4-chlorophenyl)acrylamido)-5-(((S)-1-methoxy-1-oxopropan-2-yl)amino)-5-oxopentanoate (4-ClCA-EA-OMe): Synthesis of 4-ClCA-EA-OMe was performed following the same protocol of 2-ClCA-EA-OMe.

Reaction scheme:

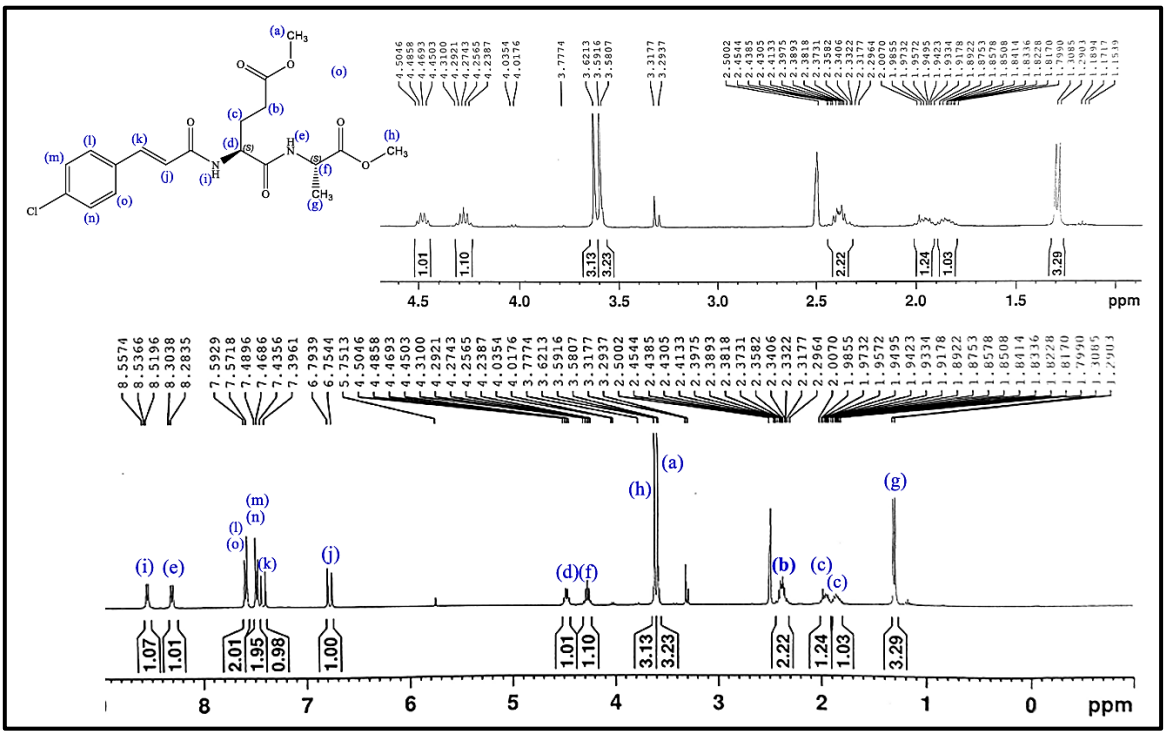


^{1}H-NMR in DMSO (400 MHz): 1.29 (d, 7.28 Hz, 3 H), 1.9770 – 1.8922 (m, 1H), 1.9178 – 2.007 (m, 1H), 2.2964 – 2.4544 (m, 2 H), 3.5916 (s, 3 H), 3.6213 (s, 3 H), 4.2743 (p, 7.12 Hz, 1 H), 4.47 (q,

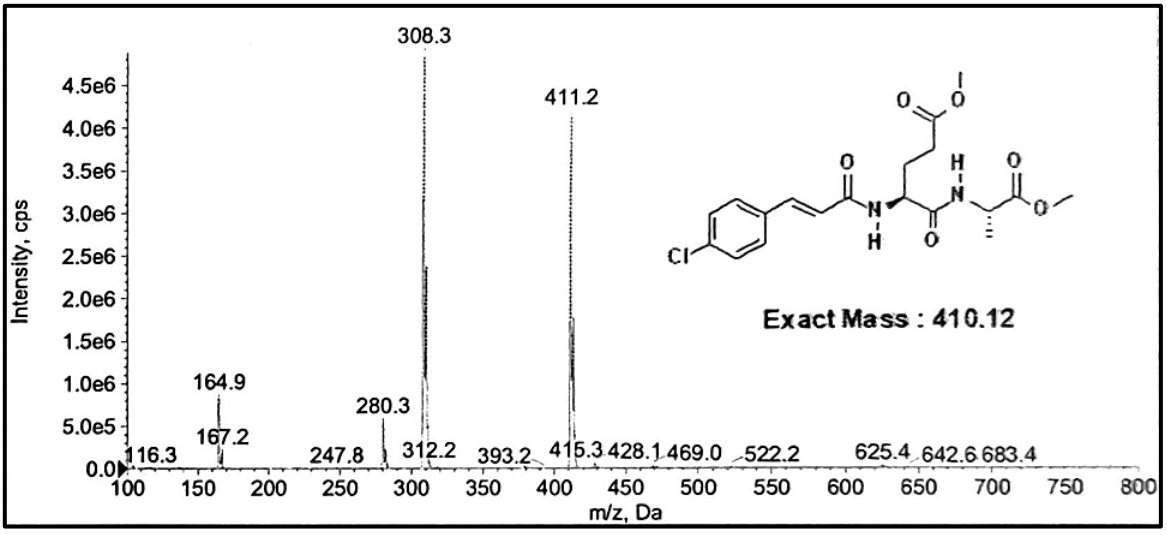
6.6 Hz, 1 H), 6.77 (d, 15.8 Hz, 1 H), 7.22 (d, 15.8 Hz, 1 H), 7.48 (d, 8.4 Hz, 2 H), 7.58 (d, 8.44 Hz, 2 H), 8.29 (d, 8.12 Hz, 1 H), 8.52 (d, 8.32 Hz, 1 H) ppm.

LCMS: (M+H) = 411.

^{1}H-NMR in DMSO (400 MHz):



LCMS:



Preparation of (S)-5-(((S)-1-carboxyethyl)amino)-4-((E)-3-(4-chlorophenyl)acrylamido)-5-oxopentanoic acid (2-ClCA-EA): Synthesis of 4-ClCA-EA was performed following the same protocol of 2-ClCA-EA.

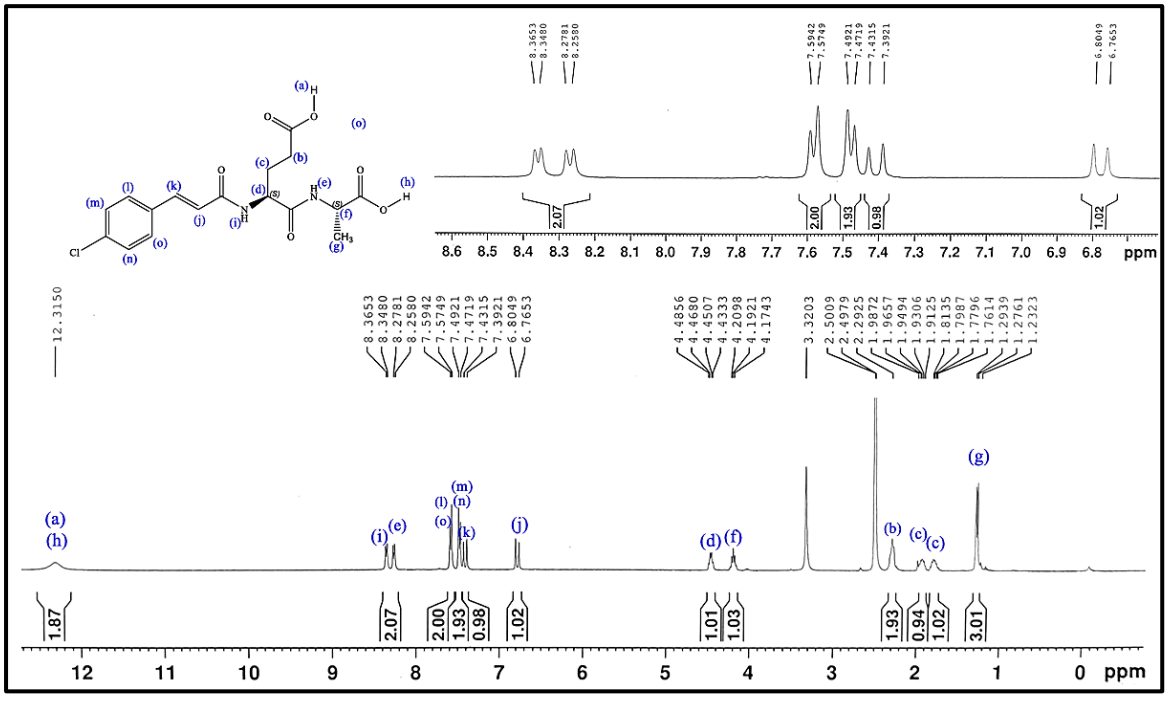
^{1}H-NMR in DMSO (400 MHz): 1.28(d, 7.1 Hz, 3 H), 1.76 – 1.81 (m, 1 H), 1.91 – 1.99 (m, 1 H), 2.29 (s, 2 H), 4.19 (t, 7.1 Hz, 1 H), 4.46 (q, 6.9 Hz, 1 H), 6.68 (d, 15.8 Hz, 1 H), 7.41 (d, 15.8 Hz, 1 H), 7.48 (d, 8.0 Hz, 2 H), 7.58 (d, 7.7 Hz, 2 H), 8.27 (d, 8.0 Hz, 1 H), 8.36 (d, 6.9 Hz, 1 H), 12.32 (b, 2 H) ppm.

^{13}C-NMR in DMSO (100 MHz): 16.88 (-CH_3), 27.86 (-CH_2), 29.98 (-CH_2), 47.48 (-CH), 51.55 (-CH), 122.77 (=CH), 128.94 – 129.87 (-CH), 133.84 (C), 137.63 (=CH), 164.53 (C=O), 170.87 (C=O), 173.92 (C=O) ppm.

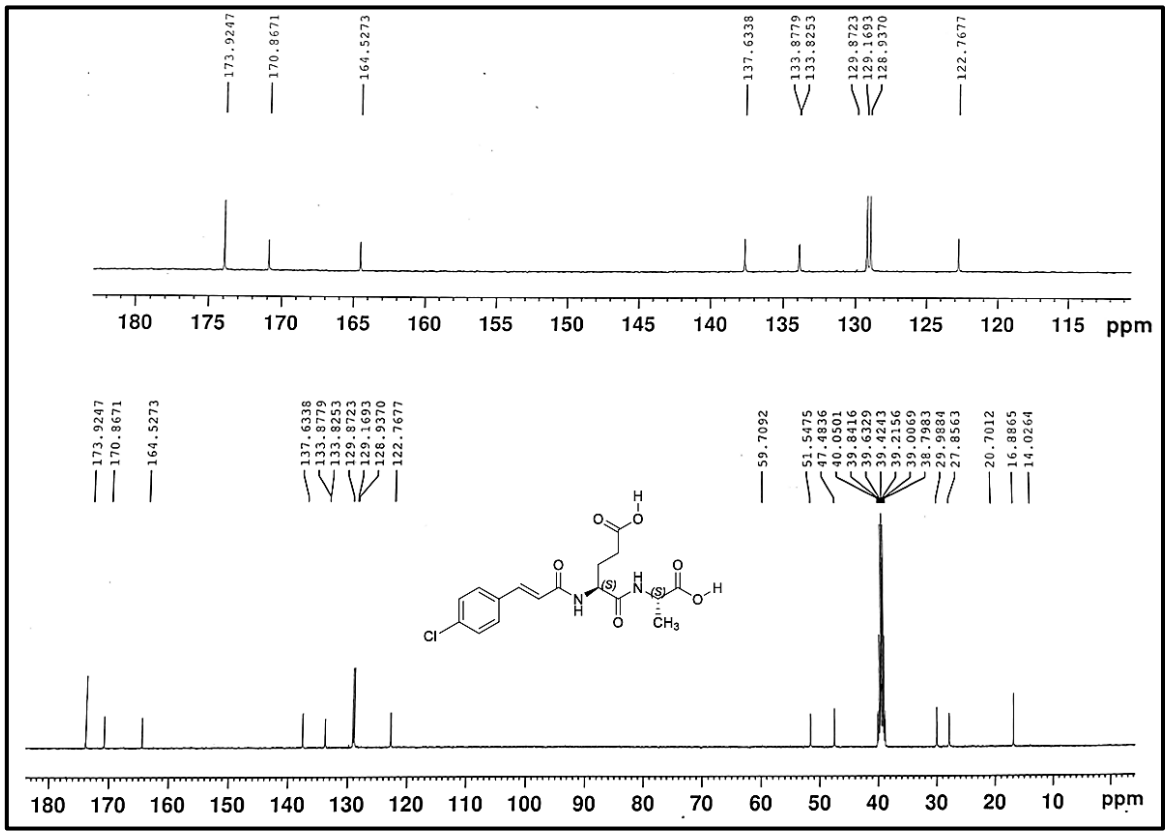
FT-IR (in KBr) (cm^{-1}): 3284.61, 3065.03, 1727.58, 1646.99, 1615.07, 1593.34, 1549.02, 1455.26, 1423.19, 1388.64, 1277.23, 1213.81, 1177.60, 1146.21, 1131.19, 1095.67, 1013.46, 1052.82, 996.80, 972.93, 944.24, 822.56.

LCMS: M+H = 383.

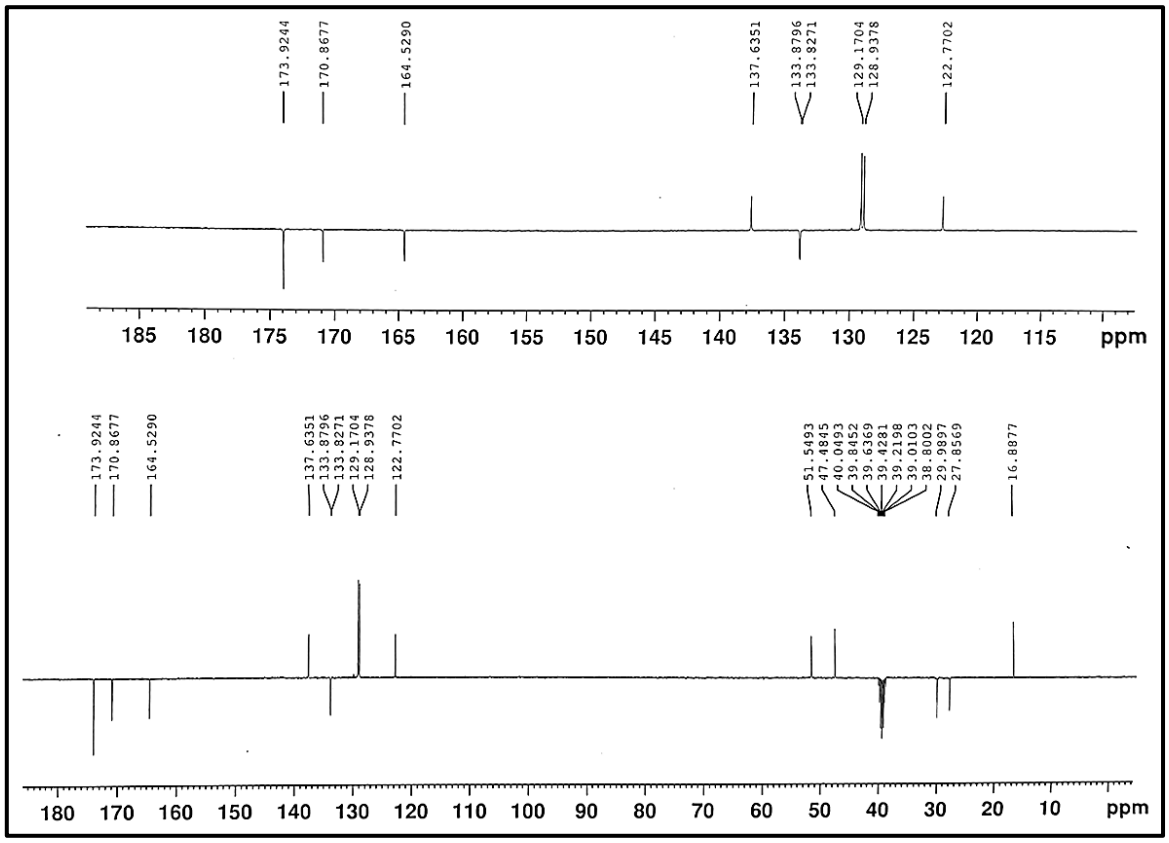
^{1}H-NMR in DMSO (400 MHz):



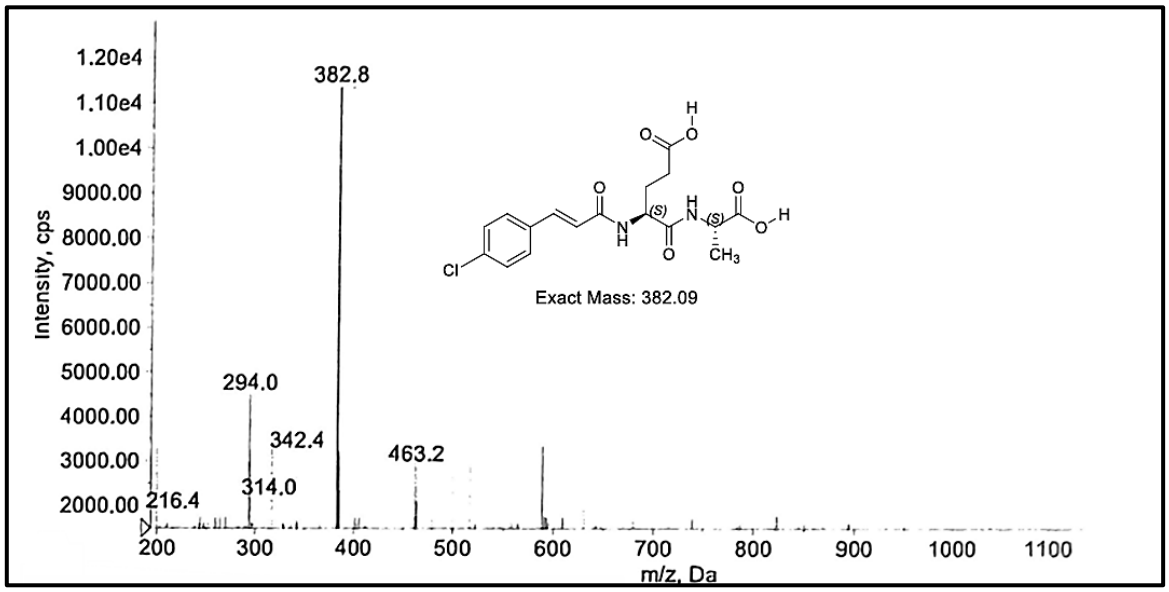
^{13}C-NMR in DMSO (100 MHz):



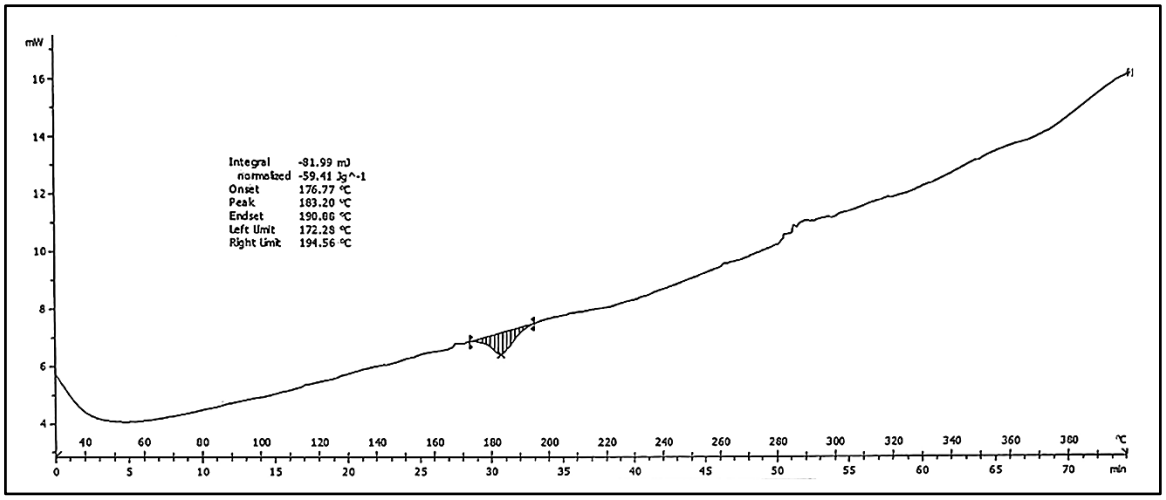
APT-NMR:



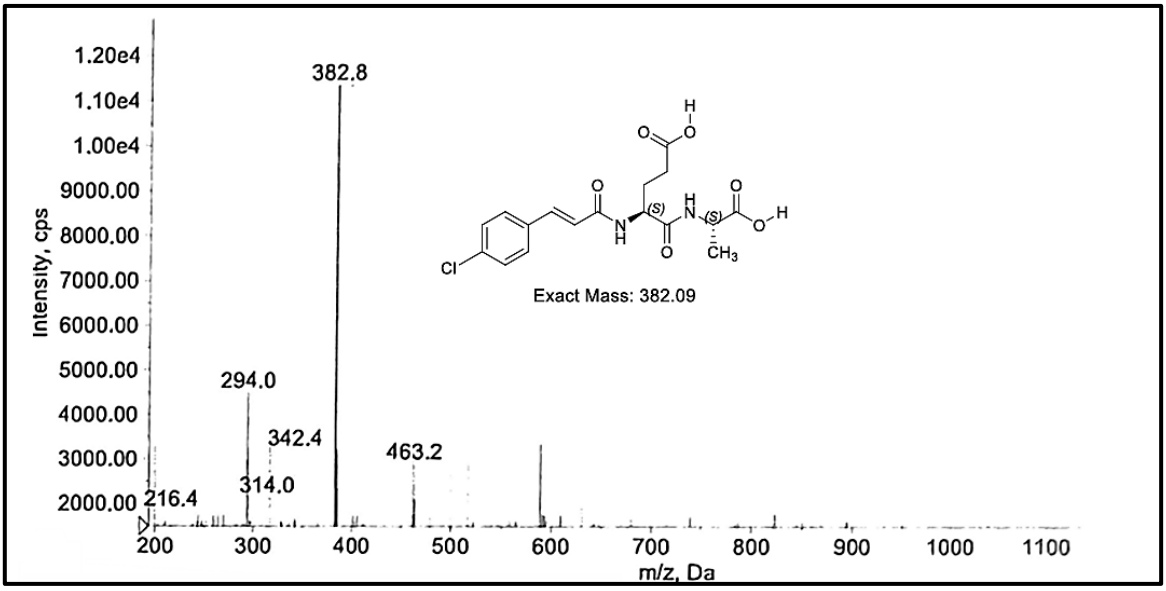
LCMS:



Thermal Study (DSC):



Mass:


1.20e4
1.10e4
1.00e4
9000.00
8000.00
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
Intensity, cps
382.8
294.0
342.4
463.2
314.0
216.4
200
300
400
500
600
700
800
900
1000
1100
m/z, Da
Exact Mass: 382.09

3. Table S1-S2.

Table S1. Optimized energy of the gelators in water at B3LYP/6-31+g(d) level of theory.

Gelators	Optimized Energy (kJ/mole)
2ClCA-EA	-1680.432007
4ClCA-EA	-1680.437443

Table S2. The predicted toxicity values of different parameters of native hydrogel using the pkCSM server.

Model Name	Predicted Value	Unit
AMES toxicity	**No**	Categorical (Yes/No)
Max. tolerated dose (human)	**0.094**	Numeric (log mg/kg/day)
hERG I inhibitor	**No**	Categorical (Yes/No)
hERG II inhibitor	**No**	Categorical (Yes/No)
Oral Rat Acute Toxicity (LD50)	**2.415**	Numeric (mol/kg)
Oral Rat Chronic Toxicity (LOAEL)	**2.404**	Numeric (log mg/kg_bw/day)
Hepatotoxicity	**Yes**	Categorical (Yes/No)
Skin Sensitisation	**No**	Categorical (Yes/No)
T.Pyriformis toxicity	**0.285**	Numeric (log ug/L)
Minnow toxicity	**1.086**	Numeric (log mM)

4. Figure S1-S14.

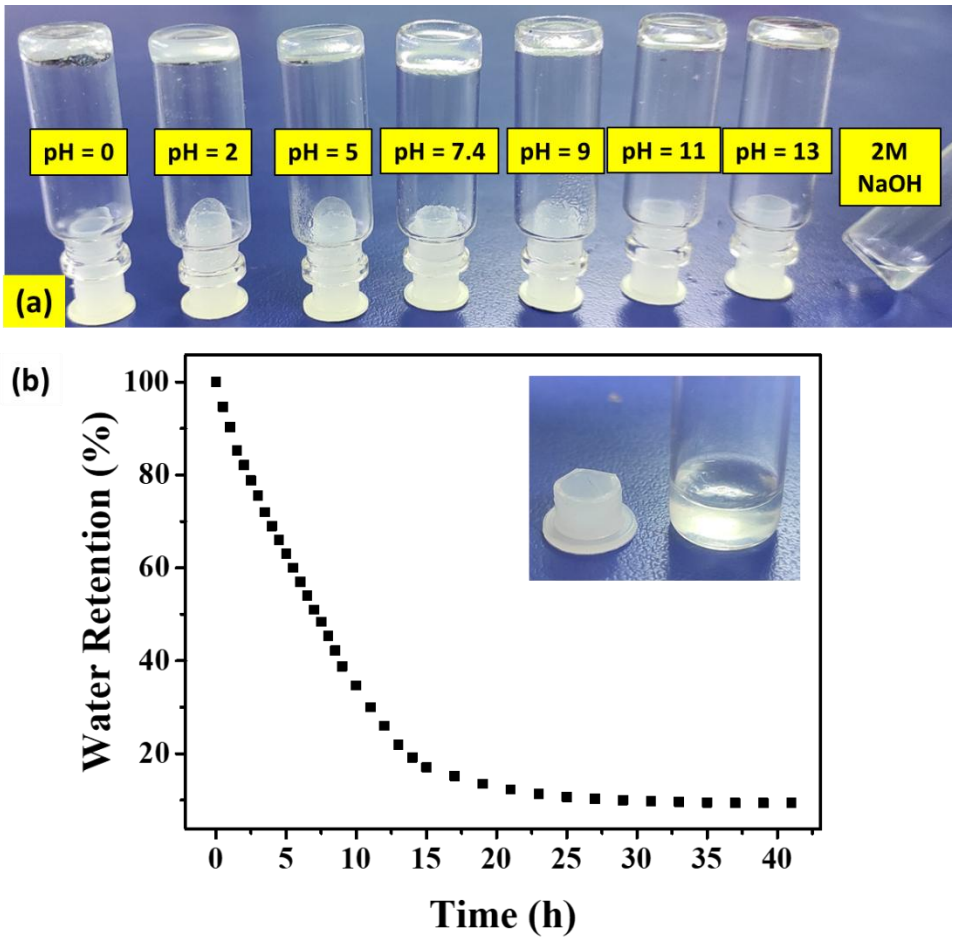


Figure S1. (a) The stability of the hydrogel was checked in the wide pH range of 0-13 and in presence of 2 M NaOH solution. (b) Water retention properties of the drug-loaded hydrogel over different time intervals. The inset in Fig. S1b shows the image of a vial cap where the drug-loaded hydrogel (shown in inset) was filled for the experiment; [2ClCA-EA] = 26.1 mM, [streptomycin] = 10 mM.

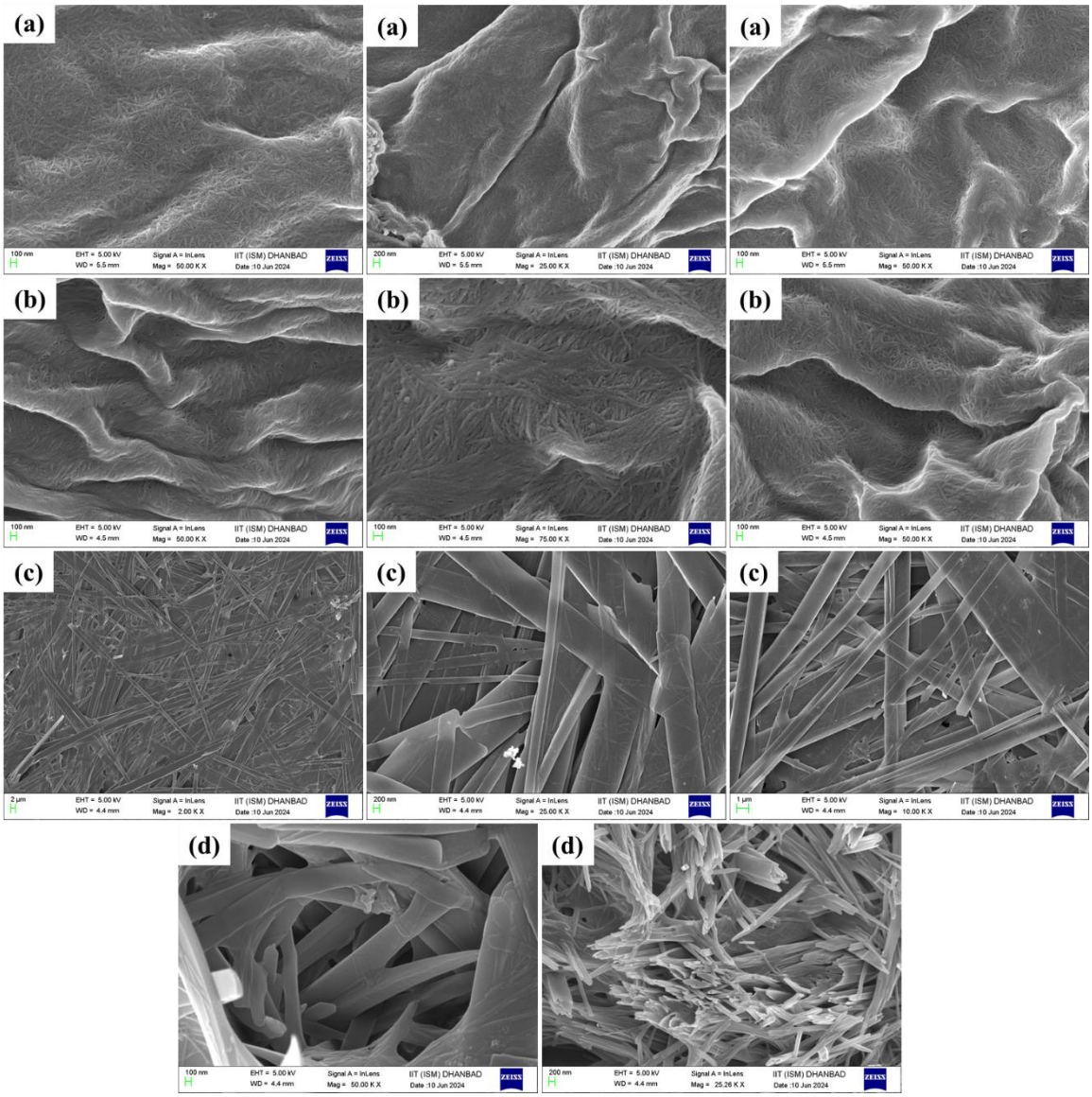


Figure S2. SEM images of the xerogels of (a) native 2ClCA-EA, and (b) streptomycin-loaded 2ClCA-EA hydrogels respectively, [2ClCA-EA] = 26.1 mM, [streptomycin] = 10 mM. (c,d) SEM images of the xerogel of native 4ClCA-EA at different magnifications, [4ClCA-EA] = 26.1 mM.

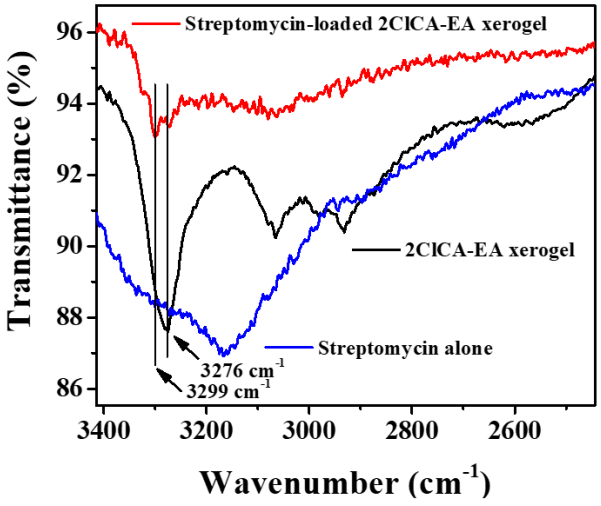


Figure S3. FT-IR spectra of the streptomycin alone (neat) and xerogels of 2ClCA-EA (26.1 mM) with and without streptomycin (10 mM).

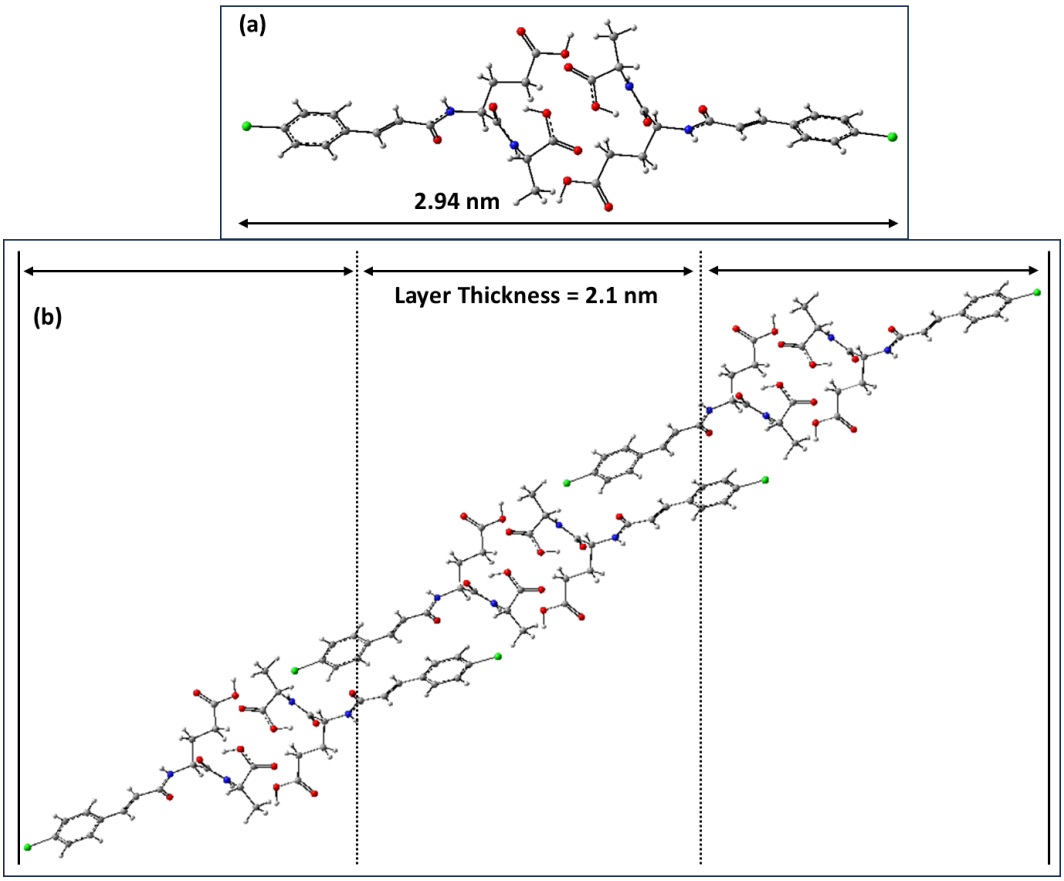


Figure S4. A schematic illustration of the formation of (a) a dimer of 4ClCA-EA, and (b) Tilted lamellar structure formation with a layer thickness of ~2.1 nm was proposed. Color code: C = gray, O = red, N = blue, H = white.

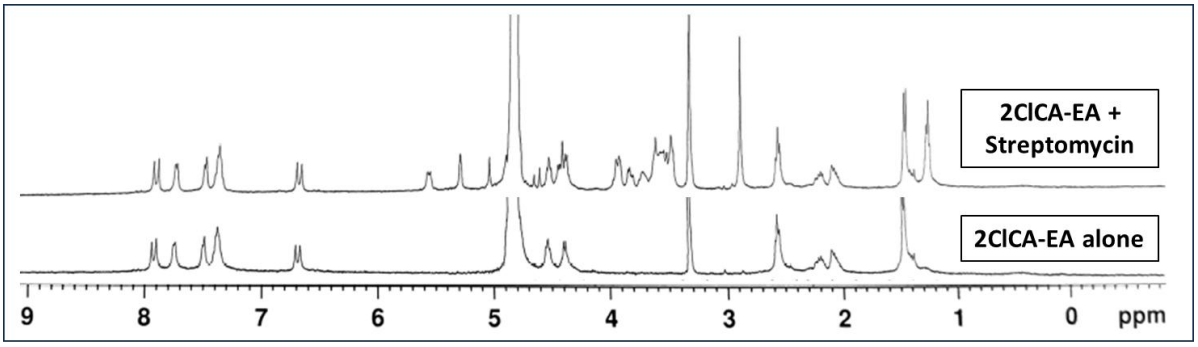


Figure S5. ^{1}H-NMR of 2ClCA-EA with and without streptomycin in D_2O:CD_3OD = 9:1 (v/v) in the gel form; [2ClCA-EA] = 26.1 mM, [streptomycin]= 10 mM. The peak position of the ^{1}H-NMR proton signals of different protons of 2ClCA-EA remained unchanged before and after the addition of the drug, streptomycin.

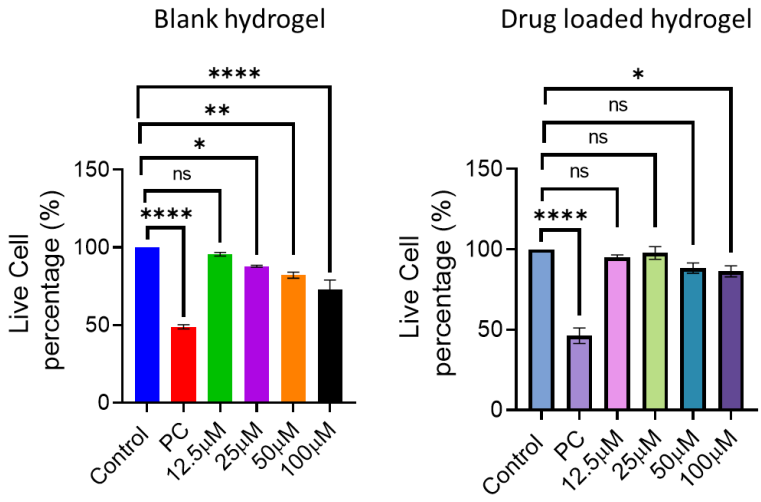


Figure S6. PBMCs were exposed to different concentrations (12.5, 25, 50, and 100µM) of the blank hydrogels and drug-loaded hydrogels and incubated at standard culture conditions for 24 hours. Application of different concentrations of blank hydrogels showed slightly higher cytotoxicity at 100 µM dose compared to the untreated cells, whereas the drug-loaded hydrogels showed negligible changes in the live cell percentage at higher doses compared to the untreated cells.

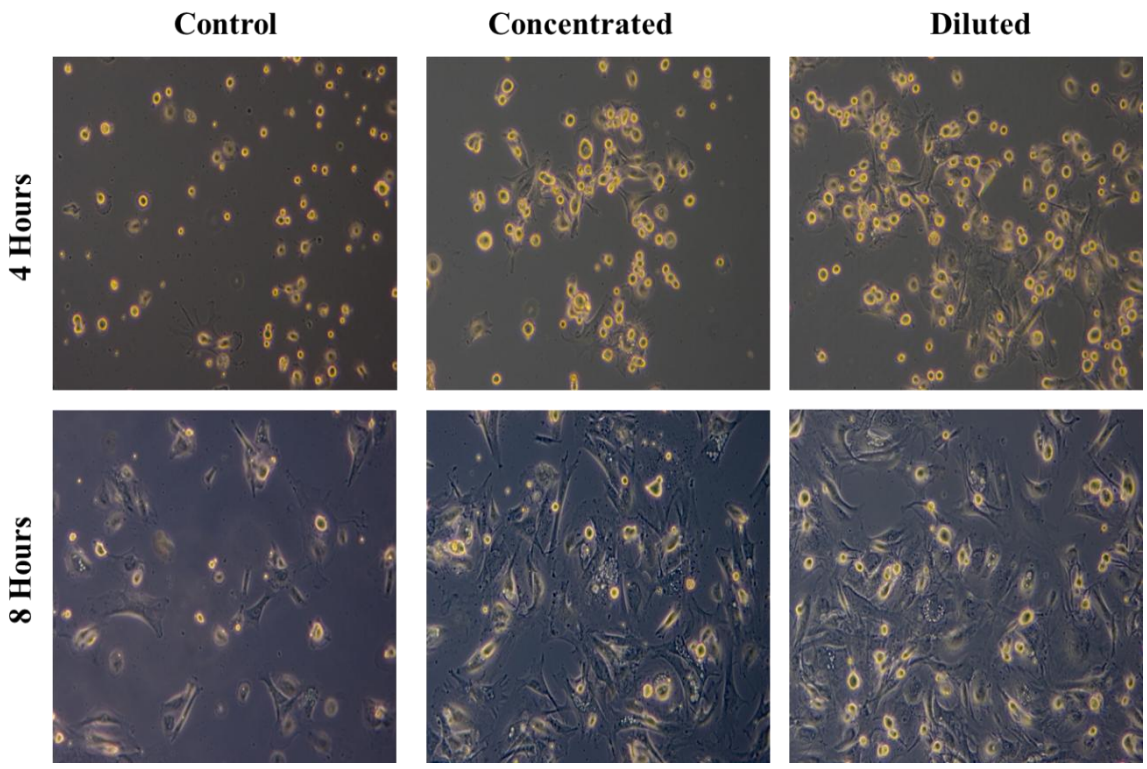


Figure S7. Two different concentrations of the hydrogel {20.8 mM (concentrated) and 10.4 mM (diluted)} were applied on coverslips and left for 30 minutes. After that, 10^4 cells were seeded on the coverslips placed in 6 wells plate and incubated at standard culture conditions for 4 and 8 hours. After 4 and 8 hours of incubation, images were captured by Phase Contrast Microscope. Figure showing that after 4 hours, more cells adhered on drug-treated coverslips compared to the non-treated ones, and at 8 hours, the same trend was shown.

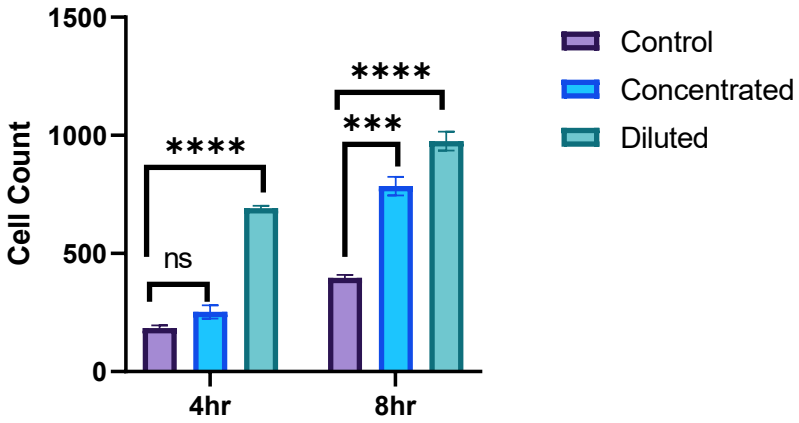


Figure S8. The number of cells adhered to the plate (determined by flat morphology and phalloidin stain)/field was counted using ImageJ software. The graph represents the average adhered cells/field from 3 independent fields.

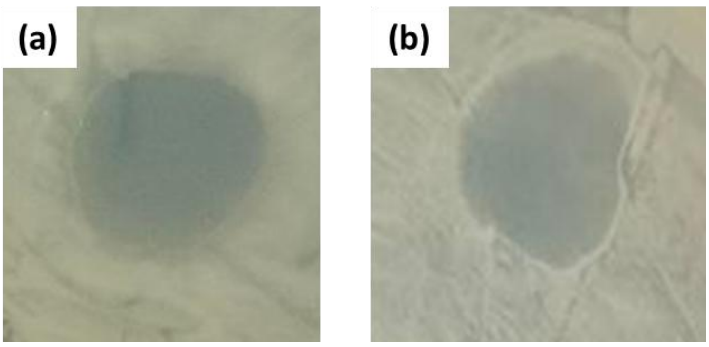


Figure S9. Antibacterial activity of streptomycin-loaded nanocomposite hydrogel after aging the hydrogel for 6 months at ambient conditions against both (a) MRSA and (b) KP; [2ClCA-EA] = 26.1 mM, [streptomycin] = 10 mM.

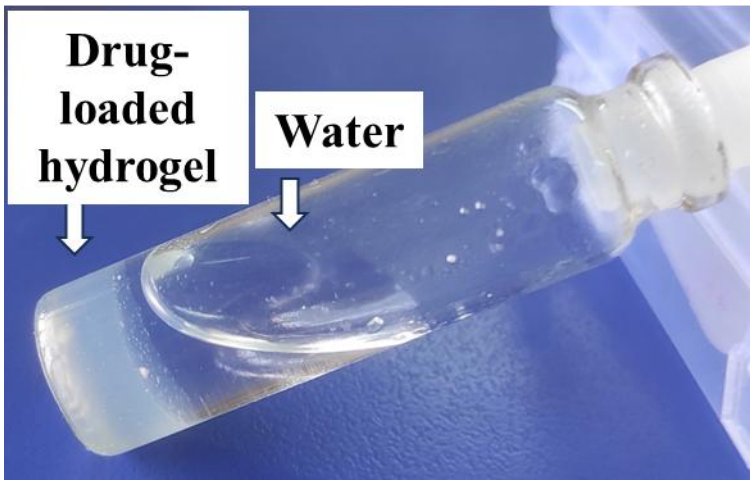
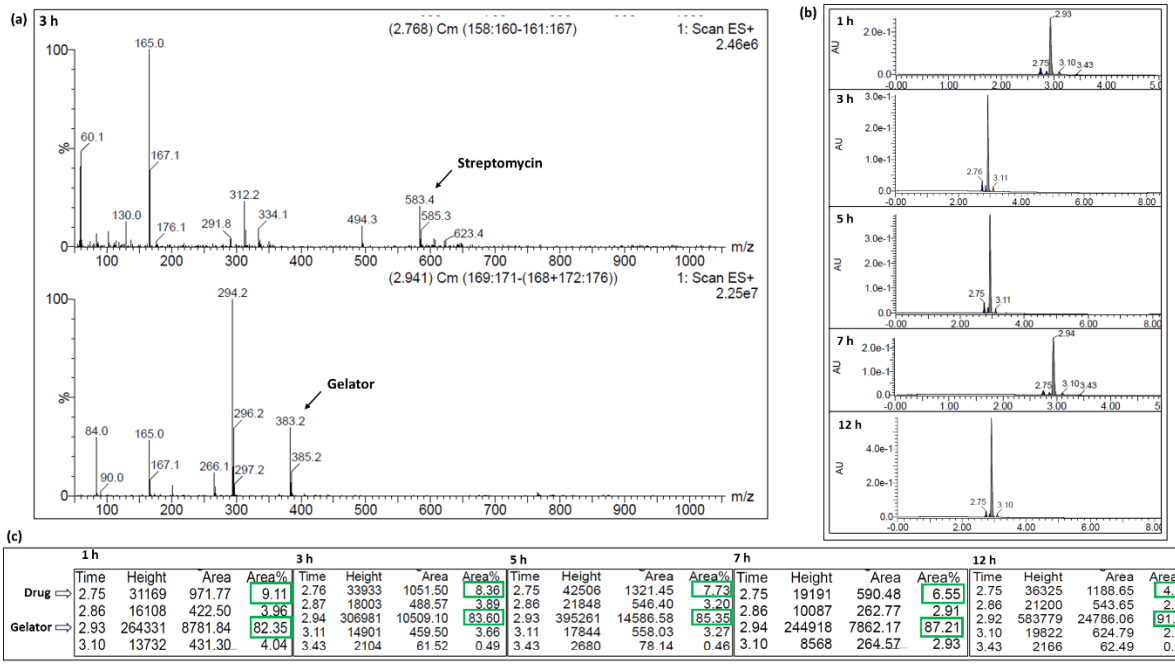


Figure S10. Photographs of the streptomycin-loaded 2ClCA-EA hydrogel, [2ClCA-EA] = 26.1 mM, [streptomycin] = 10 mM. We have attempted to explore the sustained release profile of the drug from the hydrogel matrix by UV/Vis spectroscopy. However, it could not be studied because the drug and a significant amount of the gelator leached from the drug-loaded hydrogel when fresh water was carefully added on top of the hydrogel and kept for hours. In addition, the molar extinction coefficient of the gelator was much larger than the drug streptomycin, which creates another difficulty in finding out the accurate concentration of the drug release at different time intervals by UV/Vis spectroscopy.



(c)

	1 h				3 h				5 h				7 h				12 h			
	Time	Height	Area	Area%	Time	Height	Area	Area%	Time	Height	Area	Area%	Time	Height	Area	Area%	Time	Height	Area	Area%
Drug ⇨	2.75	31169	971.77	9.11	2.76	33933	1051.50	8.36	2.75	42506	1321.45	7.73	2.75	19191	590.48	6.55	2.75	36325	1188.65	4.37
	2.86	16108	422.50	3.96	2.87	18003	488.57	3.89	2.86	21848	546.40	3.20	2.86	10087	262.77	2.91	2.86	21200	543.65	2.00
Gelator ⇨	2.93	264331	8781.84	82.35	2.94	306981	10509.10	83.60	2.93	395261	14586.58	85.35	2.94	244918	7862.17	87.21	2.92	583779	24786.06	91.11
	3.10	13732	431.30	4.04	3.11	14901	459.50	3.66	3.11	17844	558.03	3.27	3.10	8568	264.57	2.93	3.10	19822	624.79	2.30
					3.43	2104	61.52	0.49	3.43	2680	78.14	0.46					3.43	2166	62.49	0.23

Figure S11. The sustained release profile of the drug (10 mM) from the hydrogel matrix (gelator concentration 26.1 mM) was studied by adding fresh water on top of the drug-loaded hydrogel on each occasion and kept for hours. (a) LC-MS spectra of the aqueous solution taken out from the top of the drug-loaded hydrogel after 3 h interval. (b) The LC-MS spectra showed the retention time of the gelator and drug at different time (1, 3, 5, 7, and 12 hours) intervals. (c) The area % of the gelator and drug detected by LC-MS at different time intervals.

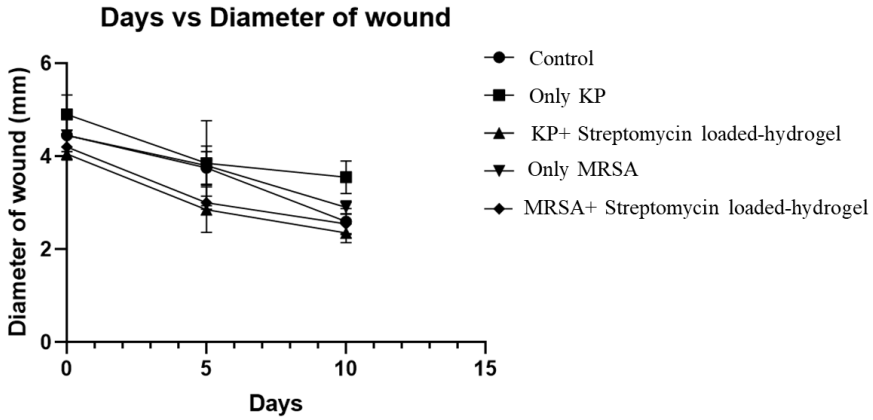


Figure S12. Graphical representation of the streptomycin-loaded hydrogel-mediated *in vivo* wound healing assay, plotting the diameter of the wound over different days. The 'Control' represents wounds where no organism or treatment was applied. 'Only KP' refers to wounds infected with KP, while 'KP + streptomycin-loaded hydrogel' indicates wounds infected with KP and subsequently treated with streptomycin-loaded hydrogel. Similarly, 'Only MRSA' represents wounds infected with MRSA, and 'MRSA + streptomycin-loaded hydrogel' denotes wounds infected with MRSA and subsequently treated with streptomycin-loaded hydrogel.

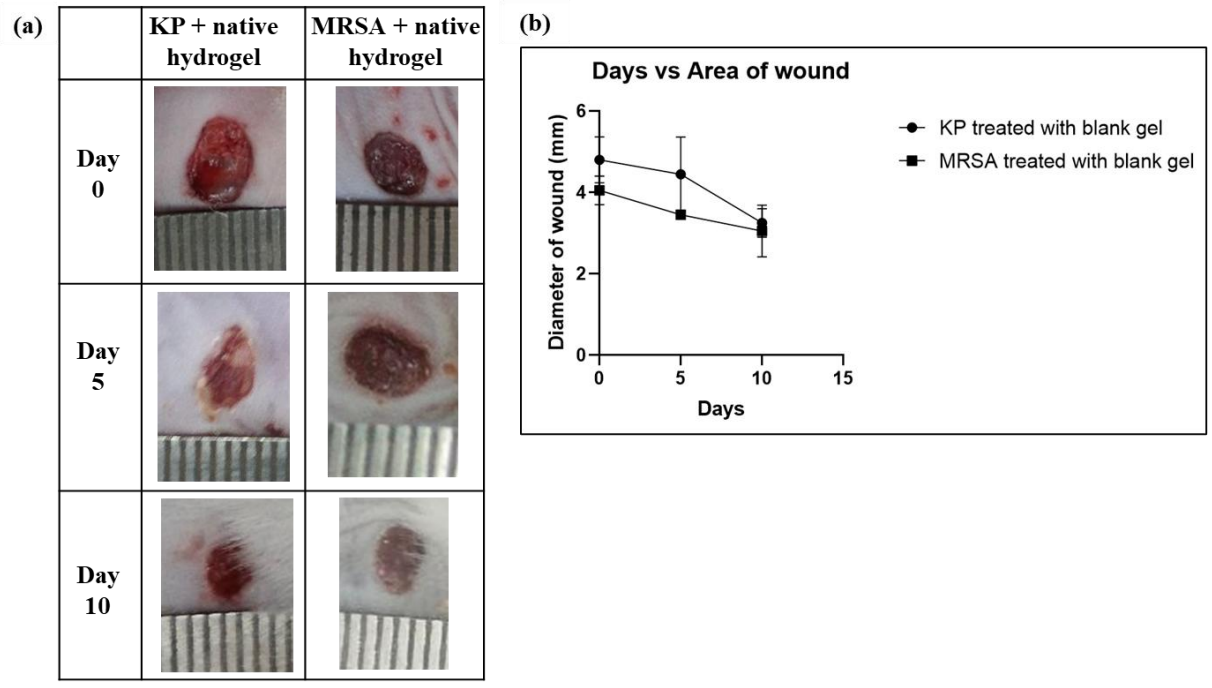


Figure S13. (a) Photographs of wounds infected by KP and MRSA over 0, 5, and 10 days, treated with native hydrogel. (b) Graph of wound's diameter over 0, 5, and 10 days, treated with native hydrogel; [2ClCA-EA] = 26.1 mM.

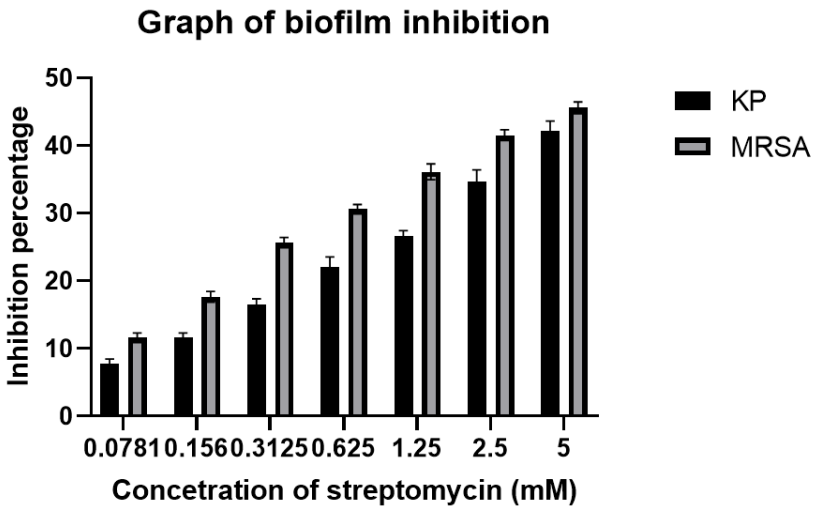


Figure S14. Bar graph showing the compound applied at different concentrations of streptomycin in the streptomycin-loaded gelator solution with respect to the percentage of biofilm inhibition in both MRSA and KP. In both MRSA and KP, the percentage of biofilm inhibition was around 42-45% at 5 mM concentration of the drug in the drug-loaded hydrogel.

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