Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

An Easy Access to Topical Gels of an Anti-cancer Prodrug (5-Flurouracil Acetic Acid) for Self-drug-delivery Applications

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Materials, Methods and Synthesis

Materials

All chemicals were commercially available and were used without any further purification. All solvents were of laboratory reagent (LR) grade and were used without any distillation. All the cell lines under study were purchased from the National Centre for Cell Science, (NCCS), Pune, India.

Methods

The mass spectra was collected with a QTOF Micro YA263 instrument. FTIR spectra were recorded by a Perkin Elmer FTIR spectrometer (spectrometer two) instrument. Both ^{1}H and ^{13}C NMR spectra were recorded with 400 and 500 MHz spectrometers (BrukerUltrashield Plus-500). TEM images were captured with a JEOL JEM 2100F (for FEG-TEM) and JEOL JEM 2010/11 (for High Resolution Transmission Electron Microscopy (HR-TEM)) instrument using 300 mesh carbon coated copper TEM grids. Rheology studies were carried out with the Anton Paar Modular Compact Rheometer MCR 102. MTT assay was conducted using a multi-plate ELISA reader (Varioskan Flash Elisa Reader, Thermo Fisher).

Synthesis of 5-FuA

5-Fluorouracilacetic acid (**5-FuA**) was synthesized using the reported procedure.[1] Aqueous solution (0.6 mL)of chloroacetic acid (3 mmol, 0.283 g) was added using a dropping funnel to an aqueous KOH (4 mmol, 0.224 g) solution (1.0 mL) of 5-fluorouracil (2 mmol, 0.260 gm) under stirring condition at room temperature. After complete addition of the chloroacetic acid solution, the reaction mixture was heated to 50 ^{0}C in an oil-bath under stirring condition for 8 H maintaining pH 10 of the reaction mixture by adding KOH solution (10 M) as and when required. The reaction mixture was finally acidified by conc. HCl to get the product as a precipitate (0.300 gm, ~80% yield) (Figure S1).

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1. M. Li, Z. Liang, X. Sun, T. Gong, Z. Zhang, *PLOS ONE***2014**, *9*, 1-13.

Synthesis of the Salts

The PAM salts (Scheme 2) were synthesized by reacting **5-FuA** with the corresponding primary amines in 1:1 molar ratio in MeOH at room temperature. In a typical experiment, calculated amount of the reactants was taken in a beaker (25 ml). MeOH (~10 ml) was added to it followed by half an hour sonication to make the solution homogeneous. The beaker was then kept overnight in open air at room temperature to obtain the salt.

Physicochemical Data

5-FuA: White Solid; ^{1}H NMR (400 MHz, DMSO-D_6) δ 11.92 (s, 1H), 8.06-8.05 (d, *J* = 6.6 Hz, 1H), 4.35 (s, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.3, 157.7, 149.7, 140.5, 138.2, 130.4, 48.7 ppm.(Figure S3); HRMS, ESI (CH_3OH) *m/z* (100%): Calculated for $[(C_6H_5FN_2O_4)][M+H]^+$: 189.03; found: 189.03.

FuA-3: White Solid; Elemental analysis calculated (%) for $C_9H_{14}FN_3O_4$: C 43.72, H 5. 71, N 17.00; found : C 43.35, H 5.80, N 16.97; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.96-7.94 (d, *J* = 6.9 Hz, 1H), 4.07 (s, 2H), 2.73 – 2.70 (m, 2H), 1.58 – 1.50 (m, 2H), 0.91-0.88 (t, *J* = 7.5 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.5, 157.6, 149.6, 140.1, 137.8, 131.3, 49.8, 40.3, 20.4,10.8 ppm.(Figure S4).

FuA-4: White Solid; Elemental analysis calculated (%) for $C_{10}H_{16}FN_3O_4$: C 45.97, H 6.17, N 16.08; found : C 45.58, H 5.74, N 15.73; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.91-7.90 (d, *J* = 6.9 Hz, 1H), 3.97 (s, 2H), 2.76 – 2.73 (m, 2H), 1.53 – 1.47 (m, 2H), 1.35-1.27 (m, 2H), 0.89-0.86 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.4, 157.6, 149.6, 139.9, 137.6, 131.8, 50.6, 38.2, 29.2, 19.1,13.4 ppm.(Figure S5).

FuA-5: White Solid; Elemental analysis calculated (%) for $C_{11}H_{18}FN_3O_4$: C 47.99, H 6.59, N 15.26; found : C 47.54, H 6.32, N 15.63; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.89-7.86 (dd, *J* = 6.7, 4.5 Hz, 1H), 3.92-3.91 (d, *J* = 7.1 Hz, 2H), 2.74-2.71 (t, *J* = 7.3 Hz, 2H), 1.54 – 1.49 (m, 2H), 1.28 – 1.27 (m, 4H), 0.88-0.85 (t, *J* = 6.9 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.5, 157.4, 149.7, 139.9, 137.6, 131.5, 50.7, 38.6, 28.0, 26.8, 21.6,13.6 ppm. (Figure S6).

FuA-6: White Solid; Elemental analysis calculated (%) for $C_{12}H_{20}FN_3O_4$: C 49.82, H 6.97, N 14.52; found : C 49.48, H 6.58, N 14.81; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.89-7.87 (d, *J* = 7.0

Hz, 1H), 3.93 (s, 2H), 2.75-2.72 (t, J = 7.3 Hz, 2H), 1.52-1.48 (m, 2H), 1.28 – 1.26 (m, 6H), 0.88-0.85 (t, J = 6.7 Hz, 3H).^{13}C NMR (100 MHz, DMSO-D_6) δ 169.7, 157.6, 149.7, 139.9, 137.6, 131.6, 50.8, 30.7, 27.4, 27.2, 25.5, 21.8,13.8 ppm.(Figure S7).

FuA-7: White Solid; Elemental analysis calculated (%) for $C_{13}H_{22}FN_3O_4$: C 51.48, H 7.31, N 13.85; found : C 51.83, H 7.72, N 14.00; ^{1}H NMR (500 MHz, DMSO-D_6) δ 8.97 (s, 1H), 7.90-7.89 (d, J = 7.0 Hz, 1H), 3.94 (s, 2H), 2.73 – 2.70 (m, 2H), 1.52-1.48 (m, 2H), 1.28 – 1.26 (m, 8H), 0.87-0.85 (t, J = 6.9 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-D_6) δ 169.1, 157.3, 149.6, 139.6, 137.8, 131.5, 50.6, 38.7, 30.9, 28.1, 27.2, 25.7, 21.8,13.8 ppm.(Figure S8).

FuA-8: White Solid; Elemental analysis calculated (%) for $C_{14}H_{24}FN_3O_4$: C 52.98, H 7.62, N 13.24; found : C 52.53, H 7.66, N 13.28; ^{1}H NMR (500 MHz, DMSO-D_6) δ 9.01 (s, 1H), 7.90-7.89 (d, J = 7.0 Hz, 1H), 3.94 (s, 2H), 2.74 – 2.71 (m, 2H), 1.54-1.48 (m, 2H), 1.29 – 1.25 (m, 10H), 0.87-0.85 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-D_6) δ 169.0, 157.5, 149.6, 139.6, 137.8, 131.8, 50.6, 40.1, 38.6, 31.0, 28.4, 27.2, 25.8, 21.9,13.8 ppm.(Figure S9).

FuA-9: White Solid; Elemental analysis calculated (%) for $C_{15}H_{26}FN_3O_4$: C 54.37, H 7.91, N 12.68; found : C 54.04, H 7.39, N 12.51; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.88-7.87 (d, J = 6.8 Hz, 1H), 3.91 (s, 2H), 2.74 – 2.71 (m, 2H), 1.52-1.47 (m, 2H), 1.29 – 1.25 (m, 12H), 0.87-0.84 (t, J = 6.9 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.2, 157.3, 149.6, 140.0, 137.7, 131.3, 50.3, 38.6, 31.2, 28.7, 28.5, 28.5, 27.0, 25.8, 22.0,13.9 ppm. (Figure S10).

FuA-10: White Solid; Elemental analysis calculated (%) for $C_{16}H_{28}FN_3O_4$: C 55.64, H 8.17, N 12.17; found : C 55.34, H 8.57, N 12.48; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.87-7.86 (d, J = 7.0 Hz, 1H), 3.90 (s, 2H), 2.73 – 2.70 (m, 2H), 1.51-1.47 (m, 2H), 1.29-1.24 (d, J = 20.5 Hz, 14H), 0.87-0.84 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.4, 157.7, 149.8, 139.9, 137.7, 131.9, 50.9, 31.3, 28.9, 28.8, 28.6, 28.6, 27.8, 27.7, 25.9, 22.1, 13.9ppm.(S11).

FuA-11: White Solid; Elemental analysis calculated (%) for $C_{17}H_{30}FN_3O_4$: C 56.81, H 8.41, N 11.69; found : C 56.53, H 8.31, N 11.25; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.88-7.86 (d, J = 7.0 Hz, 1H), 3.91 (s, 2H), 2.74 – 2.71 (m, 2H), 1.51-1.47 (m, 2H), 1.27-1.24 (d, J = 13.4 Hz, 16H), 0.87-0.84 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 169.4, 157.6, 149.7, 139.9, 137.6, 131.8, 50.7, 38.6, 31.2, 28.9, 28.9, 28.8, 28.6, 28.5, 27.2, 25.8, 22.0,13.8 ppm.(S12).

FuA-12: White Solid; Elemental analysis calculated (%) for $C_{18}H_{32}FN_3O_4$: C 57.89, H 8.64, N 11.25; found : C 57.49, H 9.0, N 10.84; ^{1}H NMR (500 MHz, DMSO-D_6) δ 9.01 (s, 1H), 7.90-7.89 (d, *J* = 6.9 Hz, 1H), 3.95 (s, 2H), 2.73-2.70 (t, *J* = 7.5 Hz, 2H), 1.52-1.48 (m, 2H), 1.24 (s, 18H), 0.87-0.84 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-D_6) δ 169.0, 157.3, 149.6, 139.6, 137.8, 131.8, 50.6, 38.6, 31.2, 28.9, 28.9, 28.8, 28.7, 28.6, 28.4, 27.2, 25.8, 21.9, 13.8 ppm.(Figure S13).

FuA-14: White Solid; Elemental analysis calculated (%) for $C_{20}H_{36}FN_3O_4$: C 59.83, H 9.04, N 10.47; found : C 60.01, H 8.83, N 10.64; ^{1}H NMR (400 MHz, DMSO-D_6) δ 7.87-7.85 (m, 1H), 3.91-3.90 (d, *J* = 3.8 Hz, 2H), 2.74-2.71 (t, *J* = 7.4 Hz, 2H), 1.51-1.46 (m, 2H), 1.28-1.24 (m, 22H), 0.87-0.83 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-D_6) δ 169.2, 157.7, 149.7, 139.7, 137.9, 131.9, 50.7, 38.7, 31.3, 29.0, 28.9, 28.9, 28.7, 28.6, 27.1, 25.9, 22.1, 14.0 ppm.(S14).

FuA-15: White Solid; Elemental analysis calculated (%) for $C_{21}H_{38}FN_3O_4$: C 60.70, H 9.22, N 10.11; found : C 60.29, H 9.59, N 9.82; ^{1}H NMR (400 MHz, DMSO-D_6) δ 7.87-7.85 (d, *J* = 7.0 Hz, 1H), 3.89 (s, 2H), 2.74 – 2.70 (m, 2H), 1.53 – 1.46 (m, 2H), 1.23 (s, 24H), 0.87-0.83 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 168.6, 157.4, 149.7, 139.8, 137.6, 131.8, 50.9, 31.3, 29.0, 29.0, 28.9, 28.8, 28.7, 28.6, 27.4, 25.9, 22.1,14.0 ppm. (Figure S15).

FuA-16: White Solid; Elemental analysis calculated (%) for $C_{22}H_{40}FN_3O_4$: C 61.51, H 9.39, N 9.78; found : C 61.24, H 9.31, N 9.58; ^{1}H NMR (400 MHz, DMSO-D_6) δ 7.87-7.85 (d, *J* = 7.0 Hz, 1H), 3.90 (s, 2H), 2.75 – 2.71 (m, 2H), 1.51 – 1.46 (m, 2H), 1.23 (s, 26H), 0.87-0.83 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-D_6) δ 168.7, 157.5, 149.7, 139.9, 137.6, 132.1, 50.9, 31.3, 29.0, 29.0, 28.9, 28.8, 28.7, 28.6, 27.3, 25.8, 22.1, 14.0 ppm. (Figure S16).

FuA-18: White Solid; Elemental analysis calculated (%) for $C_{24}H_{44}FN_3O_4$: C 62.99, H 9.69, N 9.18; found : C 62.67, H 9.47, N 8.97; ^{1}H NMR (400 MHz, DMSO-D_6) δ 7.91-7.89 (d, *J* = 7.0 Hz, 1H), 3.97 (s, 2H), 2.74 – 2.70 (m, 2H), 1.52-1.47 (m, 2H), 1.23 (s, 30H), 0.87-0.83 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-D_6) δ 169.0, 162.4, 157.4, 149.7, 139.9, 131.4, 72.3, 49.9, 49.8, 40.1, 38.8, 35.8, 31.3, 30.8, 29.0, 29.0, 28.9, 28.8, 28.7, 28.5, 27.0, 25.8, 22.1, 13.9 ppm.(Supporting Information, Figure S17).

Gelation and T_{gel} experiments

The hydrogels as well as the organogels were prepared by dissolving the gelator salts in pure water (hydrogel) or in the corresponding organic solvent (organogel) by heating and then keeping it at room temperature. Most of the gels were formed within a few minutes. The gel formation was confirmed by tube inversion method wherein the gel was found to withstand its own weight against gravity. Minimum gelator concentration (MGC) was determined by gradually diluting a 4 wt % w/v gel till gel formation was ceased. Dropping ball method was employed to determine T_{gel} (gel to sol dissociation temperature). In a typical experiment, a glass ball (weighing 216.4 mg) was carefully placed on a gel bed (1 ml, at MGC) prepared in a test tube (internal diameter –11mm) and immersed in an oil bath fitted with a thermometer. The oil bath was gradually heated and the temperature at which the ball touched the bottom of the test tube was recorded as the T_{gel}.

Microscopy

A small amount of freshly prepared gel (at MGC) was scooped and carefully smeared on carbon-coated Cu grids (300 mesh) and dried overnight under ambient condition. The images were recorded at an accelerating voltage of 200 kV without staining.

Rheology studies

A small amount of freshly prepared gel (4 wt %, w/v) was scooped and placed on the stationary plate of the rheometer and parallel plate geometry (diameter - 25 mm, 1 mm gap) was employed to carry out the rheological experiments.

X-ray Diffraction

X-ray quality single crystals of the salts (**FuA-9**, **FuA-10**, **FuA-11**, **FuA-12**, **FuA-14**,**FuA-15**) were grown at room temperature by slow evaporation technique from various solvent systems (Table S2). Single crystal X-ray diffraction data were collected using various diffractometer (Bruker APEX II, CCD area detector, Mo K_{α}, λ = 0.7107 Å, Bruker APEX III D8 Venture, PHOTON II detector, Cu K_{α}, λ = 1.54184 Å). Data collection, data reduction, structure solution and refinement were carried out using the software packages of the corresponding diffractometer. All the structures were solved by direct methods and refined in a routine manner.

Hydrogen atoms were geometrically fixed. All the non-hydrogen atoms were treated anisotropically. CCDC-numbers 1868726, 1868727, 1868722, 1868723, 1868725, 1868724 contain the crystallographic data for **FuA-9, FuA-10, FuA-11, FuA-12, FuA-14** and **FuA-15** respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data request/cif.

MTT assay

The cells were grown in high-glucose DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in a humidified incubator at 37^0 C and under 5% CO_2 atmosphere. For MTT assay, the cells were then seeded in a 96-well plate for each experiment at a density of approximately 0.5×10^4 cells per well. After incubating it for 24 h in a humidified incubator, various concentrations of the gelator salts or DMEM alone (control experiment) were applied to the cells and the mixtures were kept at 37^0 C under 5% CO_2 atmosphere for 72 h (for 36 h in case of B16F10 cell line). Then the culture medium of each well of the 96-well plate was replaced by the MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 100 μl) containing media and it was left for one and half hour under incubation followed by replacing the media by DMSO (100 μl) with gentle shaking in order to dissolve the formazan produced by mitochondrial reductase of the live cell. The colourintensity of formazan (purple) attributing to the live cell concentration (cell viability) was measured by using a multi-plate ELISA reader at 570 nm. The percentage of cells, alive in presence of the gelator, was calculated by considering the DMEM-treated sample (control) as 100%. All these experiments were done in triplicate.

Cell Migration Experiment

For B16F10 and Hep G2 cell lines, cells were seeded in a 6-well plate and kept for 1 day until the plates become almost confluent. A narrow path in the middle of the plate was created by scratching uniformly with a 200 μL sterile pipette tip. The IC_{50} concentration of the gelator compounds were added to the cells. For the control experiment, no gelator salt was added. Still images were captured under an optical microscope (OLYMPUS CKX31) after different time intervals for16 h (B16F10) and 72 h (HepG2) to measure migration speed for each case.

Hydrogel Leaching Experiment

2.5 wt% of **FuA-15** hydrogel (3 ml) was incubated with 3 ml of 1X PBS solution (3 ml) over the gel surface. The release was observed up to 30 hours for time intervals at 3, 6, 9, 12, 24 and 30 hour by taking an aliquot of 200 μl (from the PBS solution) each time and recording the UV spectrophotometry after appropriate dilution.

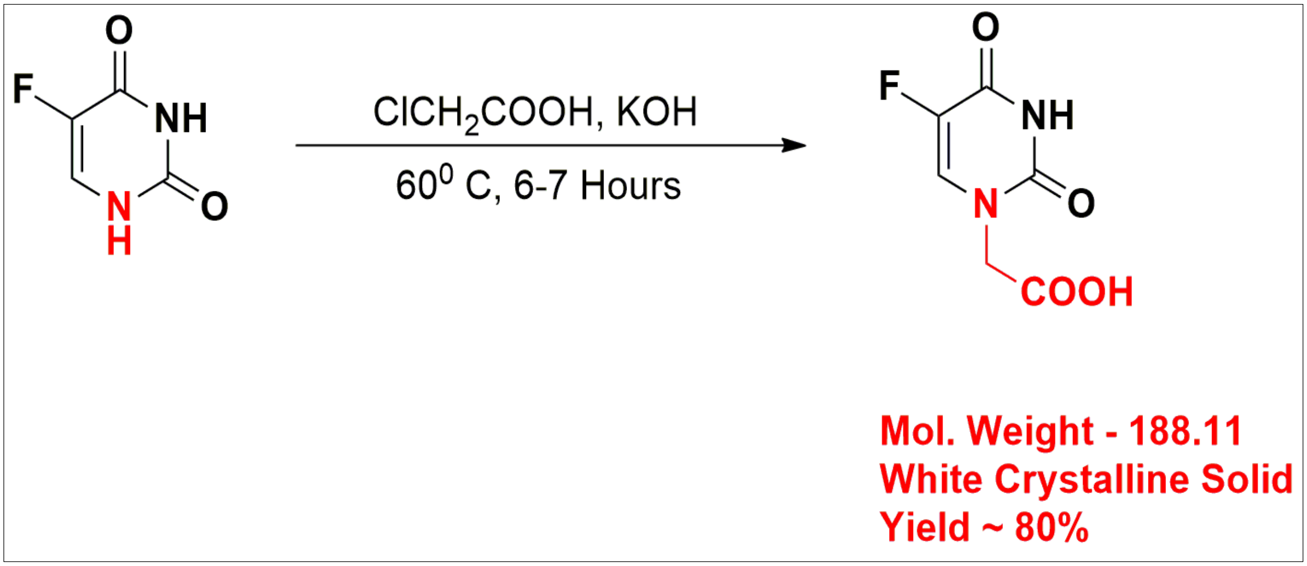


Figure S1: Synthesis Scheme of **5-FuA** (Acid).

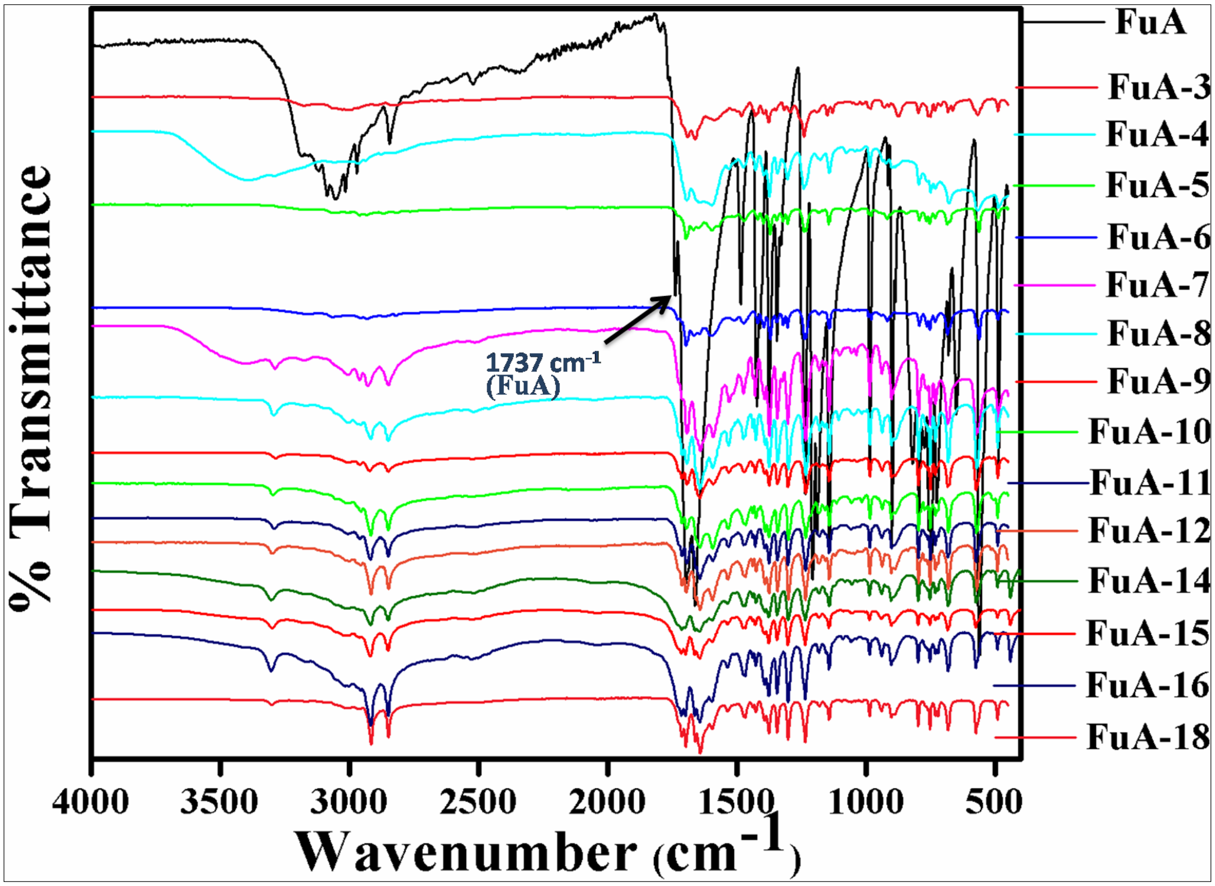
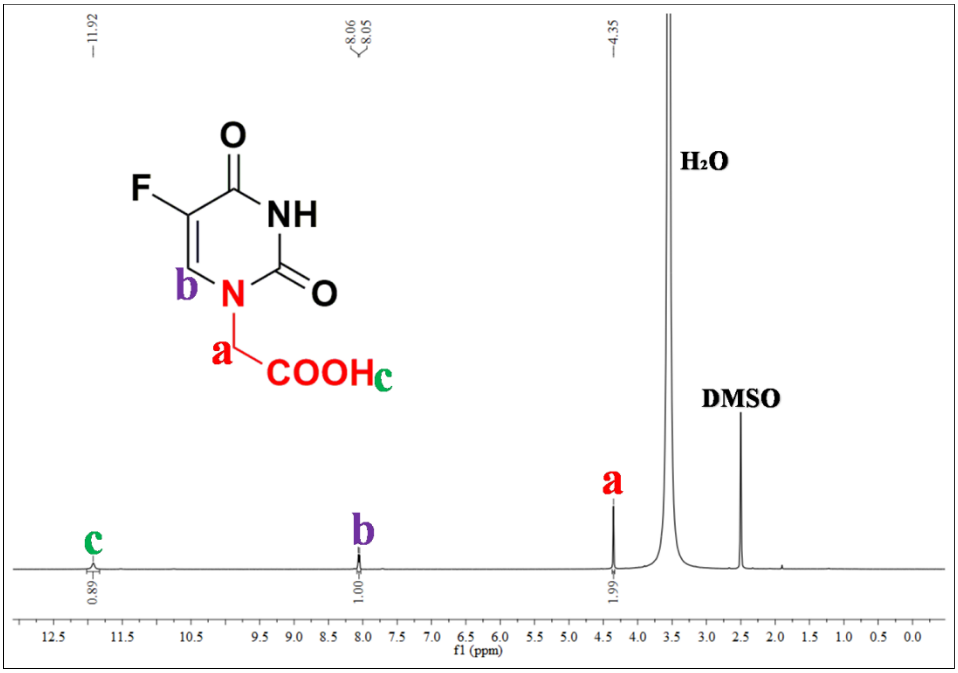


Figure S2: IR data comparison between the acid (**5-FuA**) and the salts (**FuA-3**to**FuA-18**) proving the formation of salts.



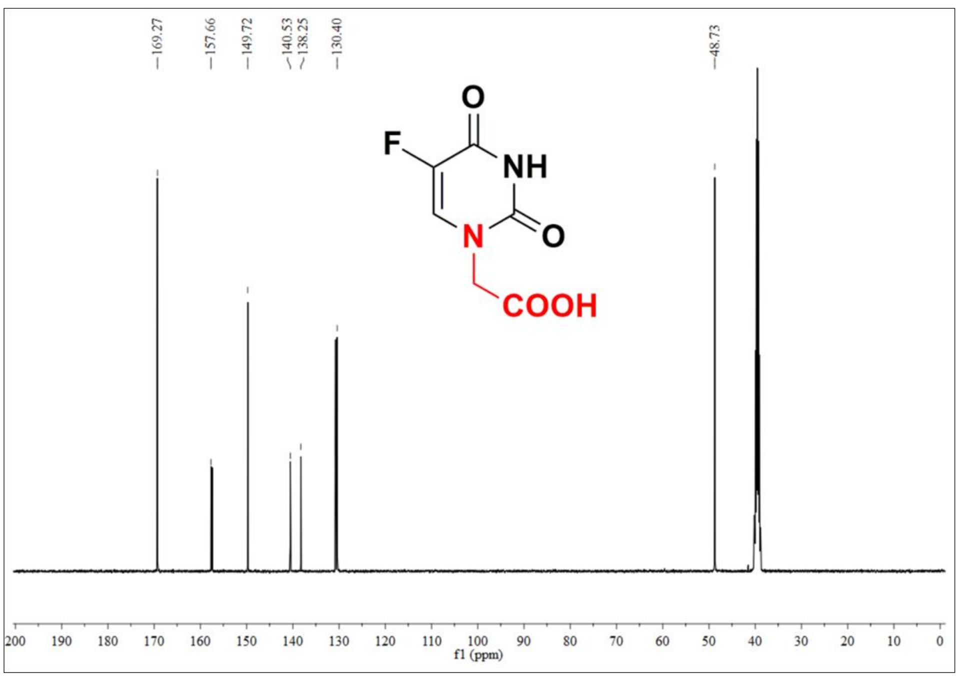
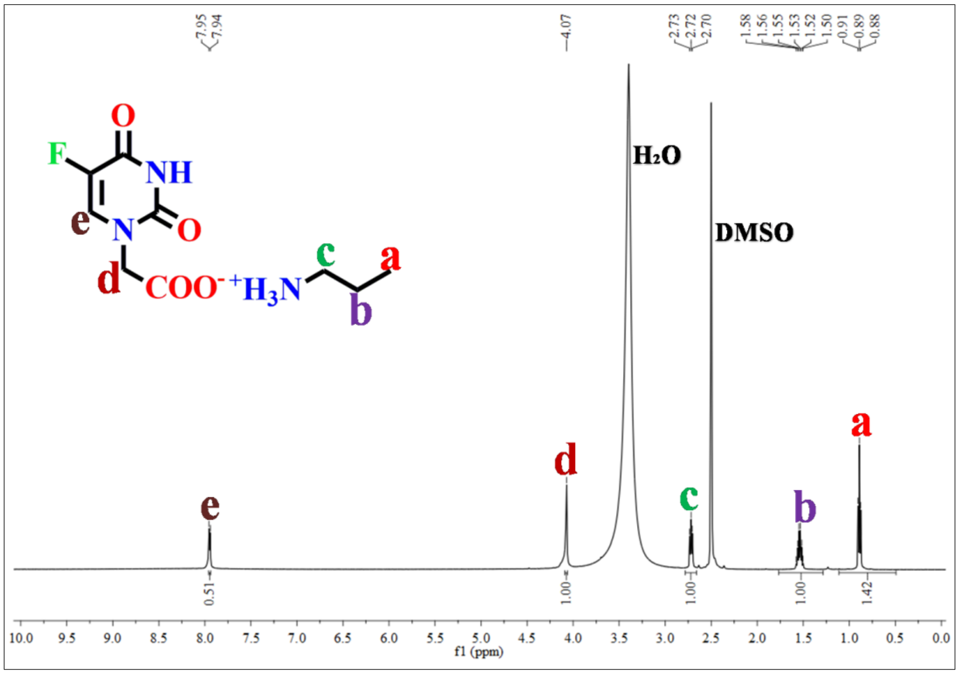


Figure S3: ^{1}H NMR and ^{13}C NMR spectra of **FuA** in DMSO-d_6.



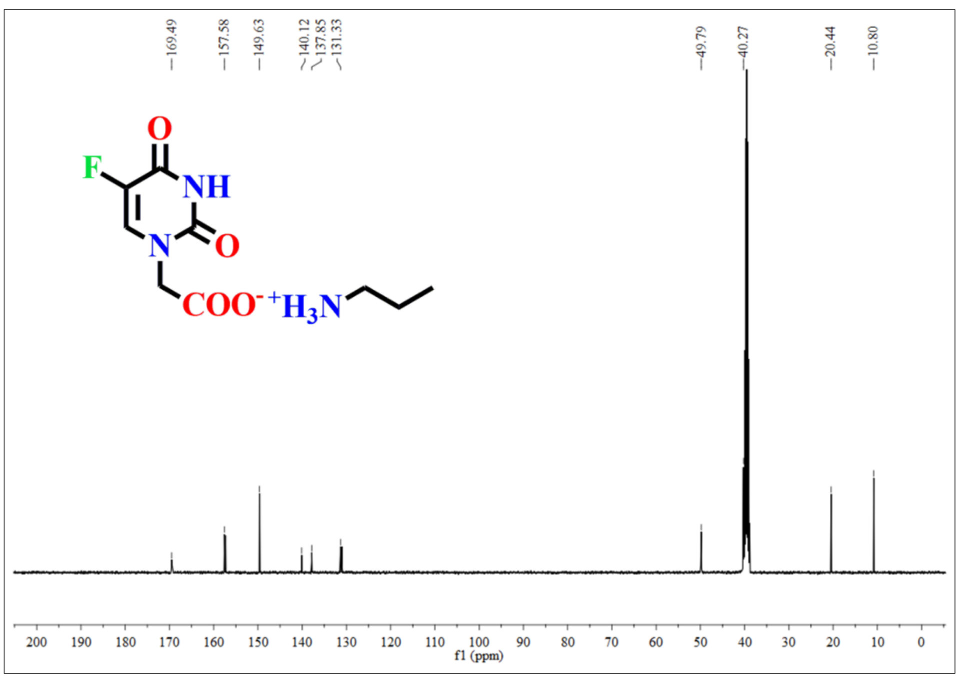
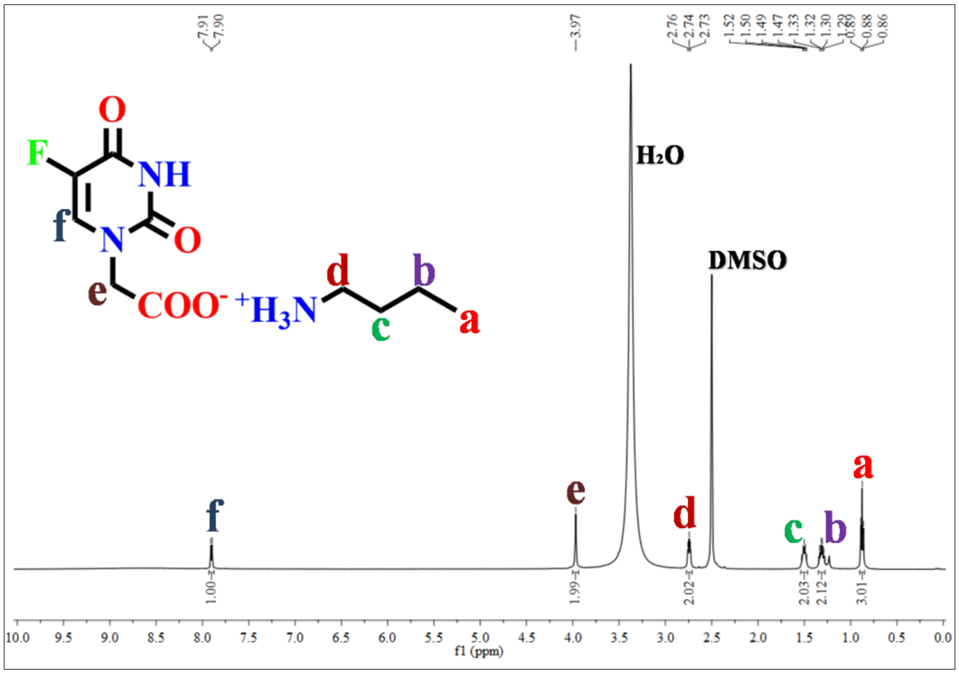


Figure S4: ^{1}H NMR and ^{13}C NMR spectra of **FuA-3** in DMSO-d_6.



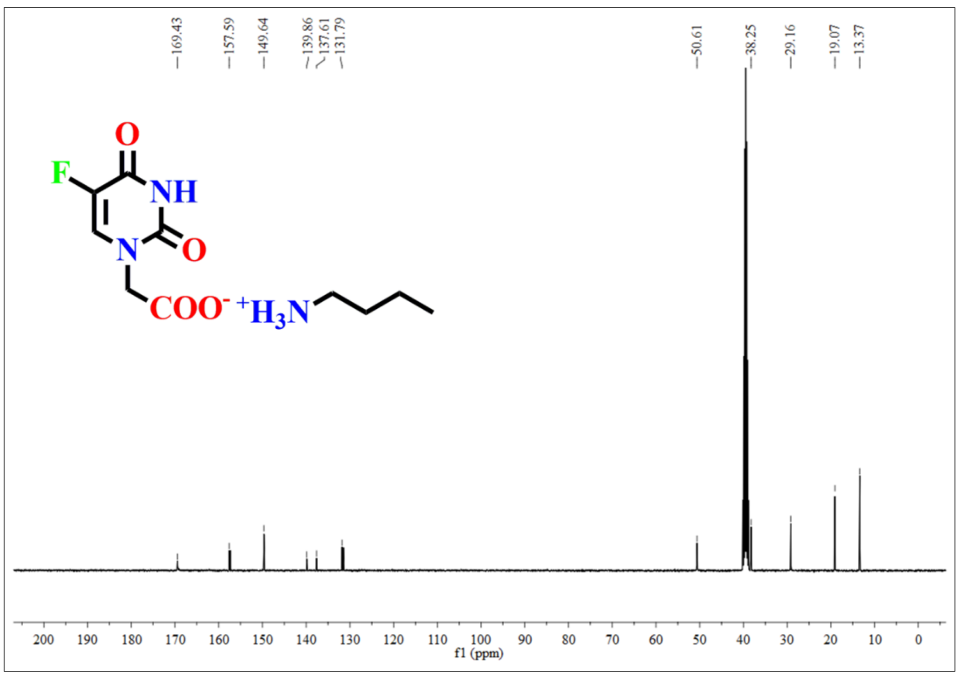
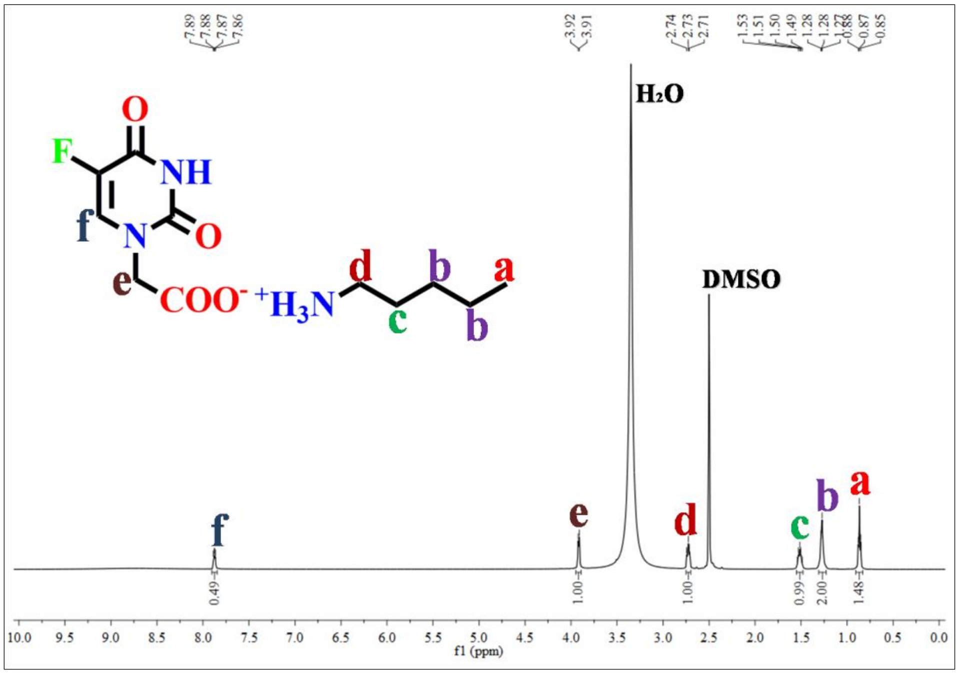


Figure S5: ^{1}H NMR and ^{13}C NMR spectra of **FuA-4** in DMSO-d_6.



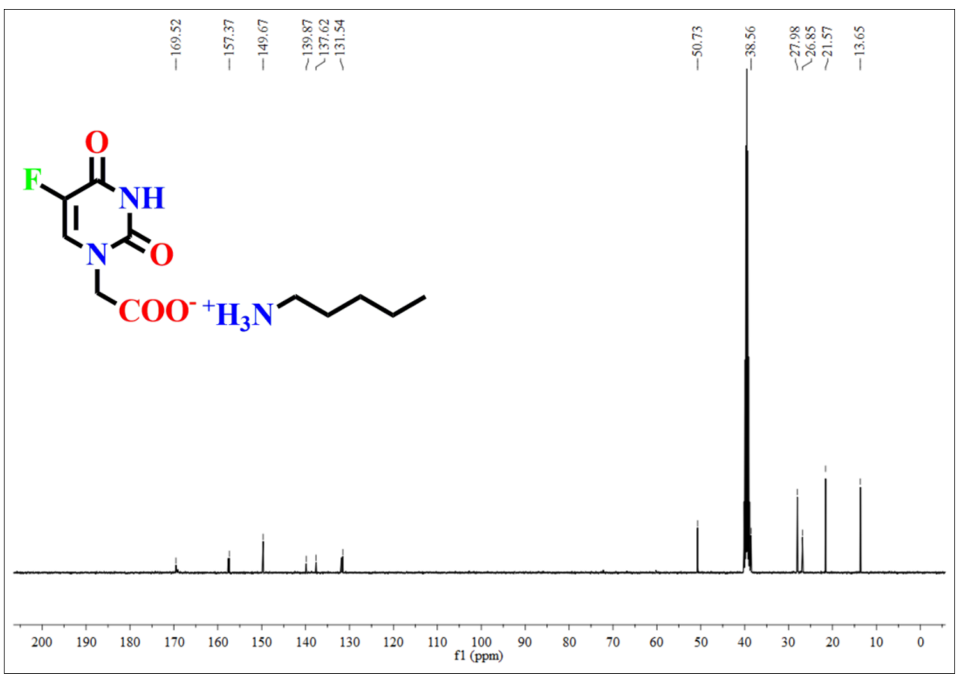
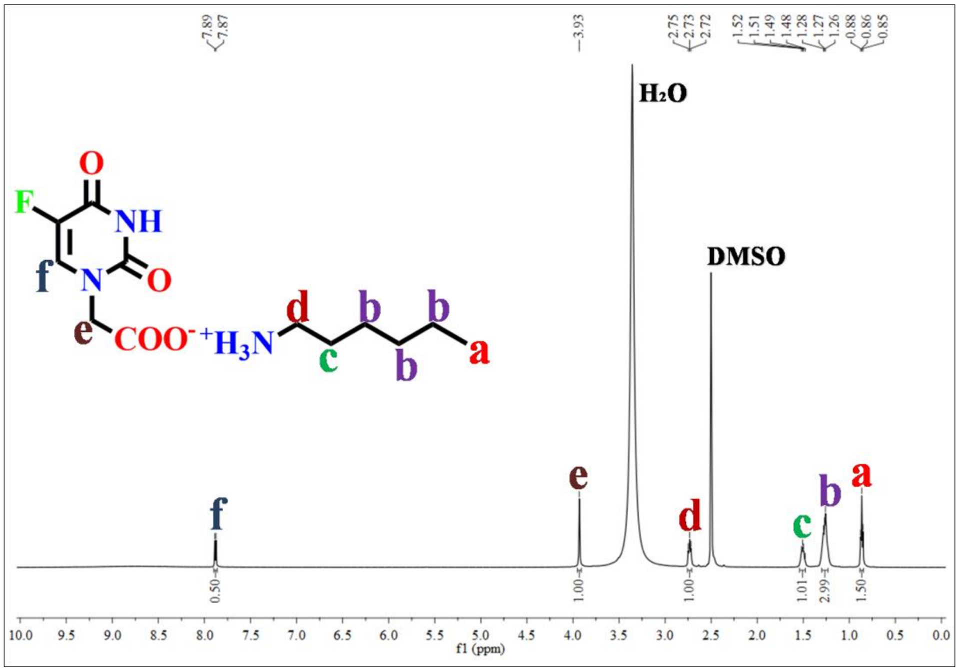


Figure S6: ^{1}H NMR and ^{13}C NMR spectra of **FuA-5** in DMSO-d_6.



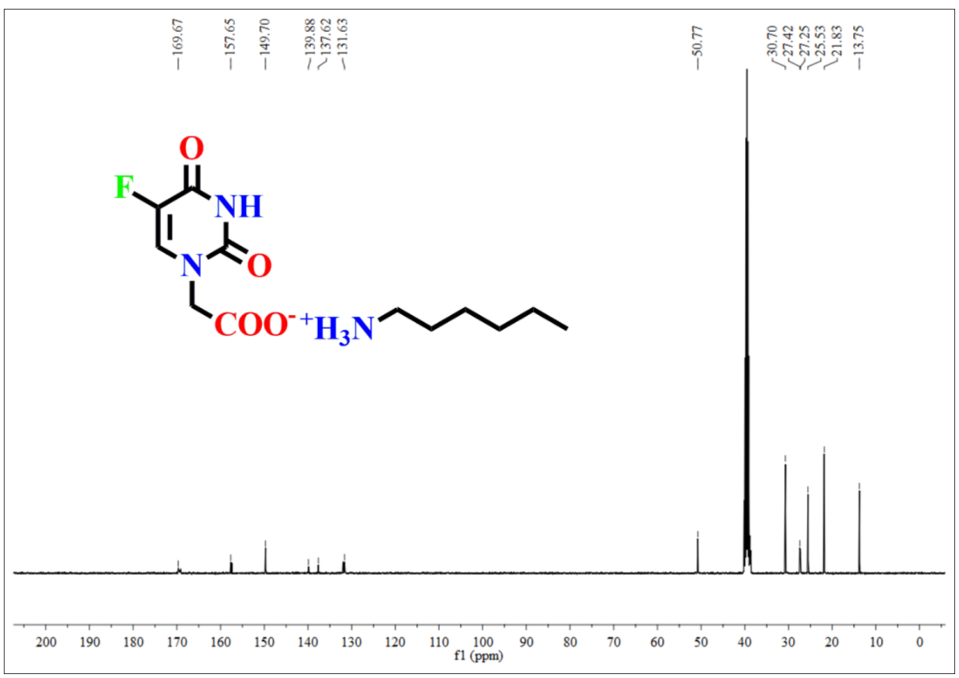
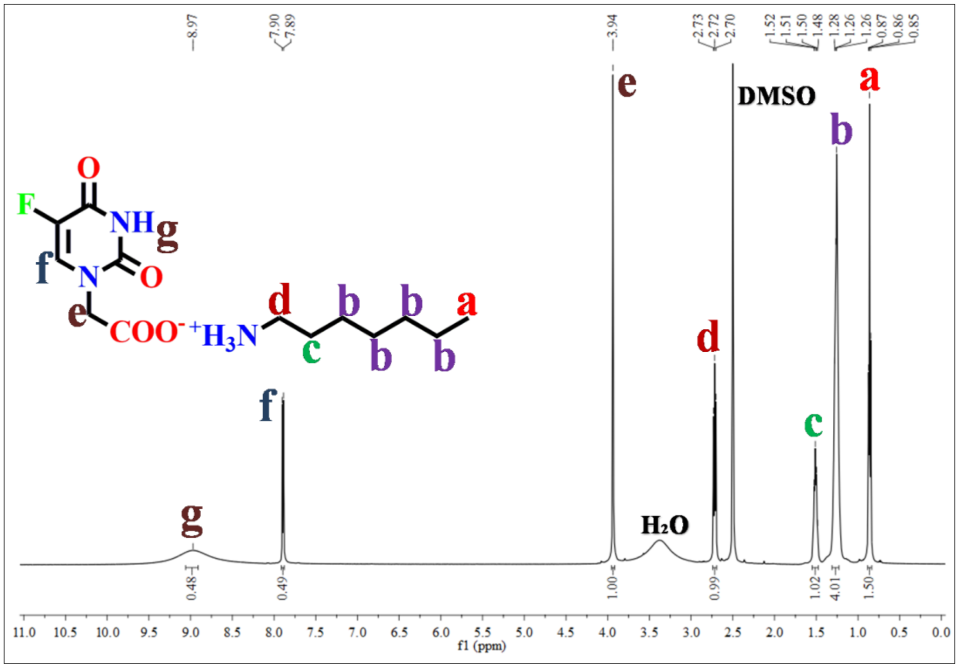


Figure S7: ^{1}H NMR and ^{13}C NMR spectra of **FuA-6** in DMSO-d_6.



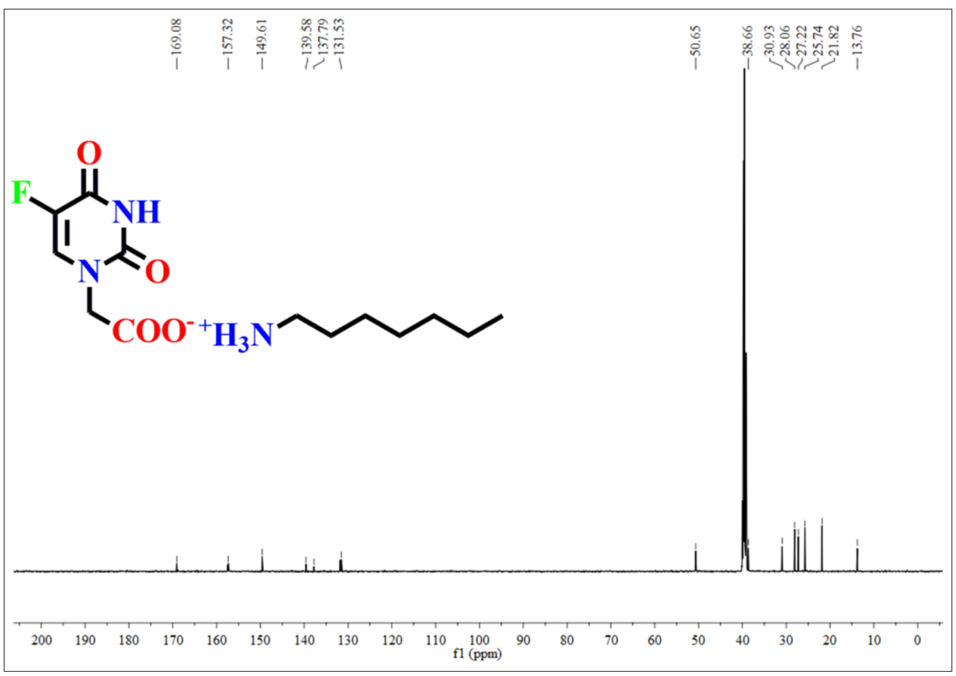
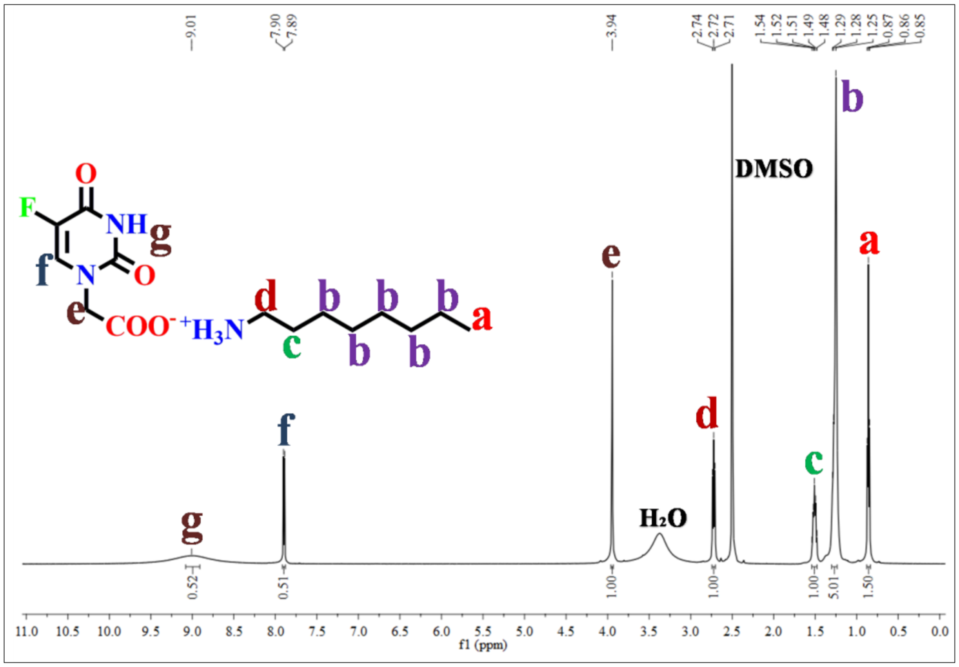


Figure S8: ^{1}H NMR and ^{13}C NMR spectra of **FuA-7** in DMSO-d_6.



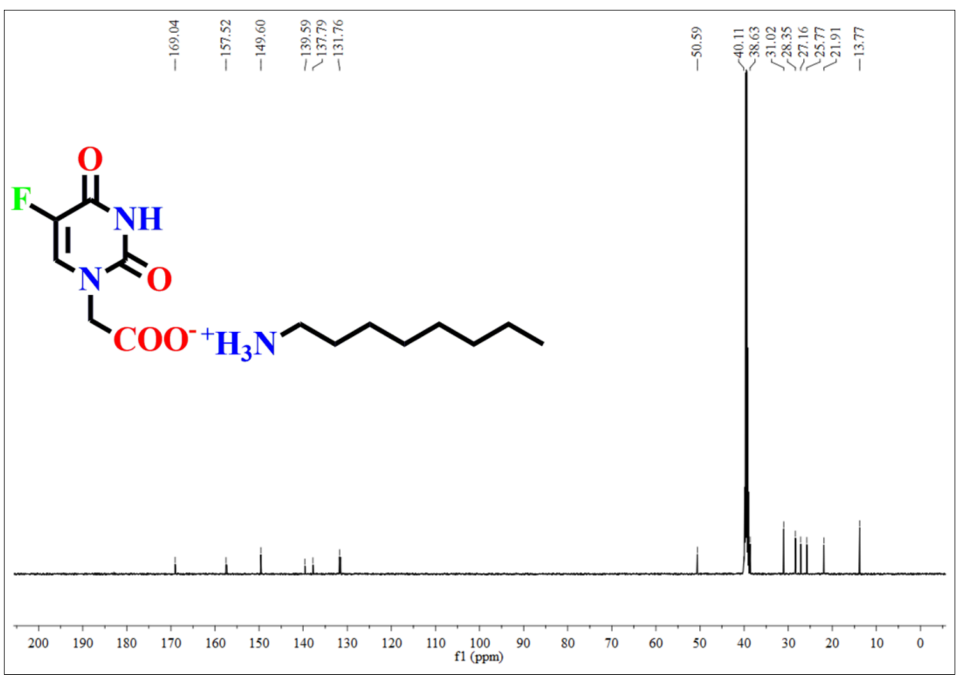
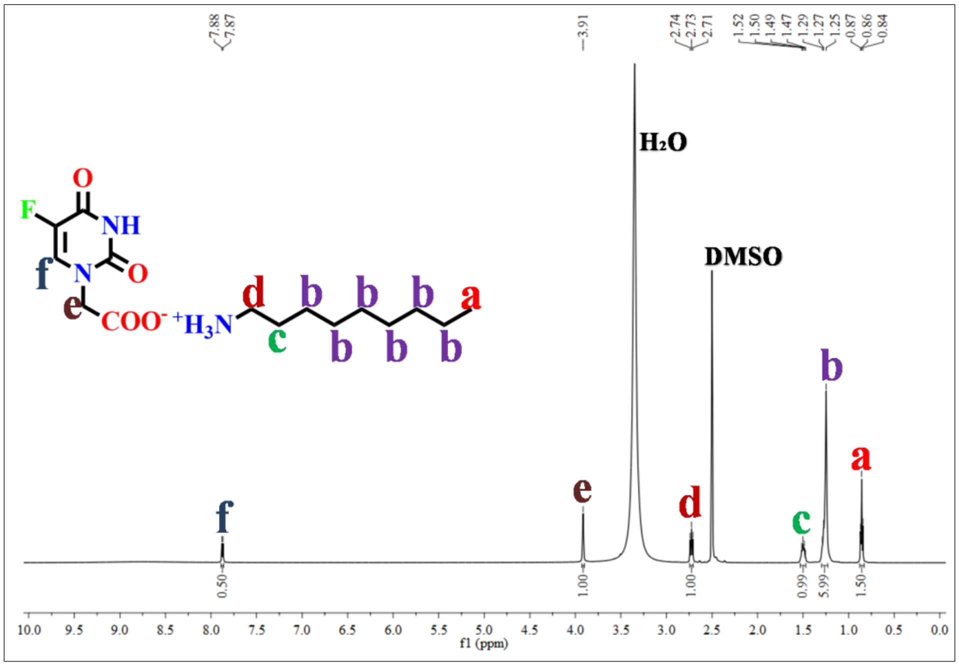


Figure S9: ^{1}H NMR and ^{13}C NMR spectra of **FuA-8** in DMSO-d$_6$.



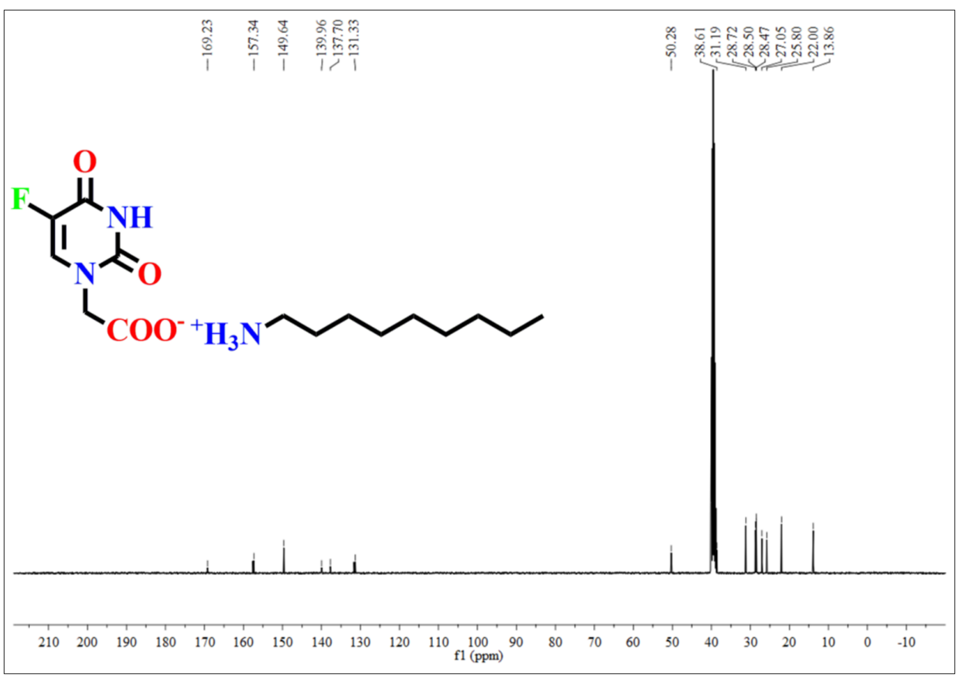
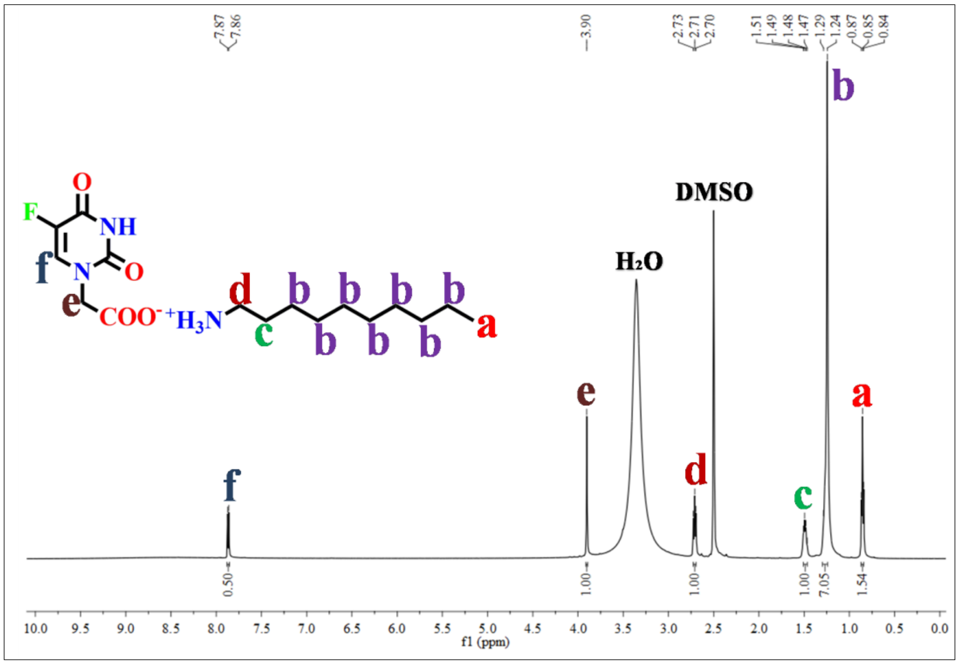


Figure S10: ^{1}H NMR and ^{13}C NMR spectra of **FuA-9** in DMSO-d_6.



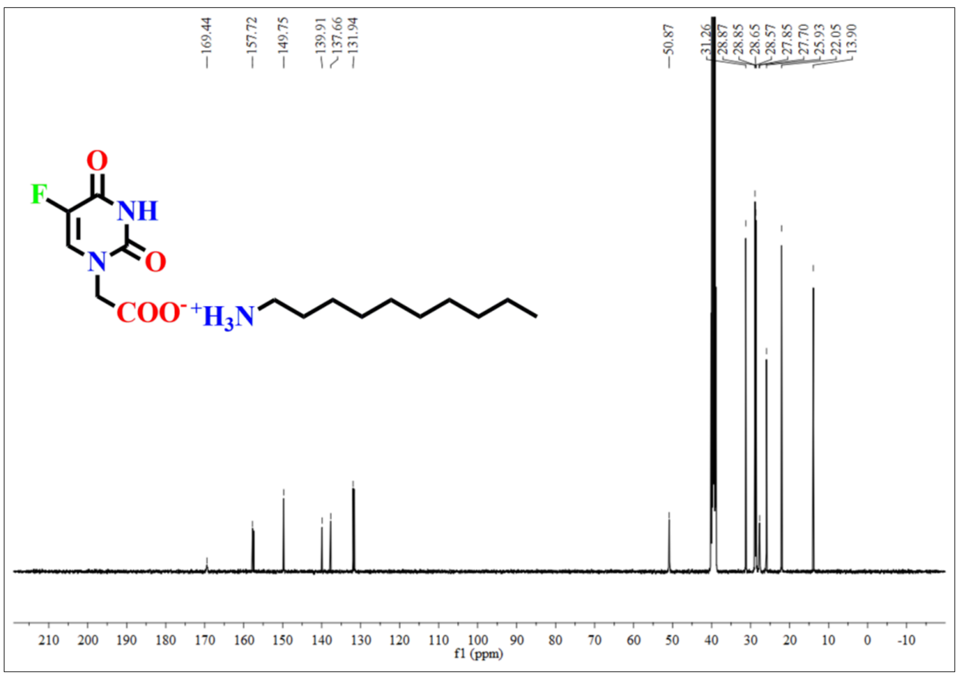
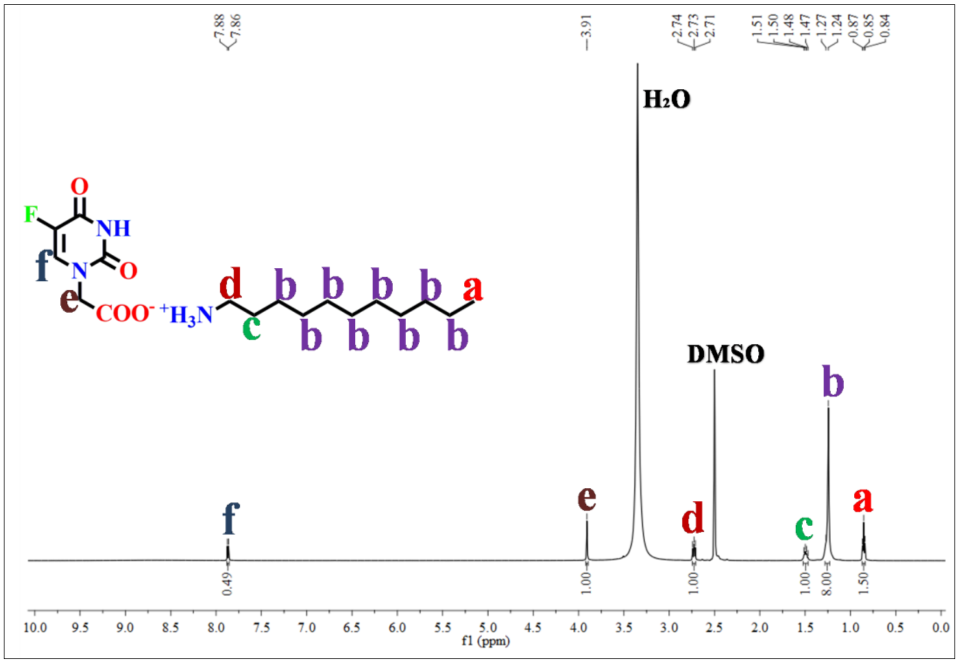


Figure S11: ^{1}H NMR and ^{13}C NMR spectra of **FuA-10** in DMSO-d_6.



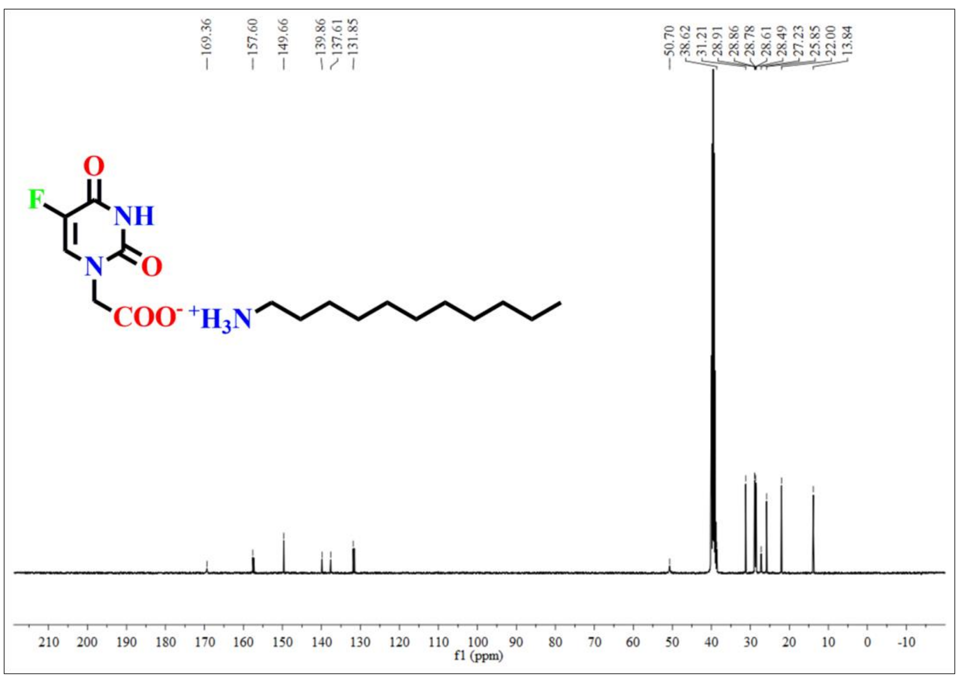
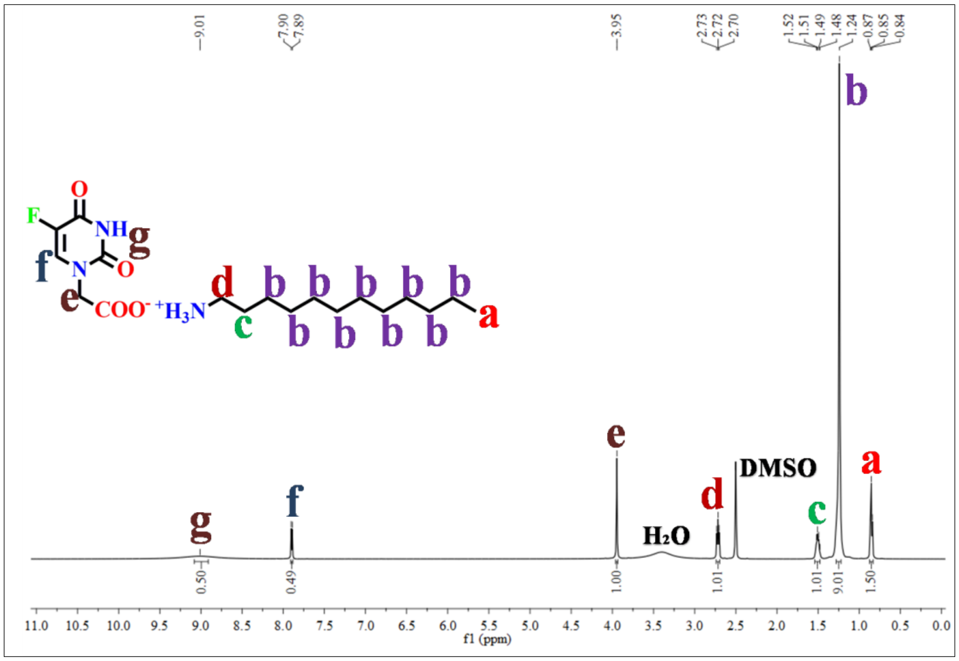


Figure S12: ^{1}H NMR and ^{13}C NMR spectra of **FuA-11** in DMSO-d_6.



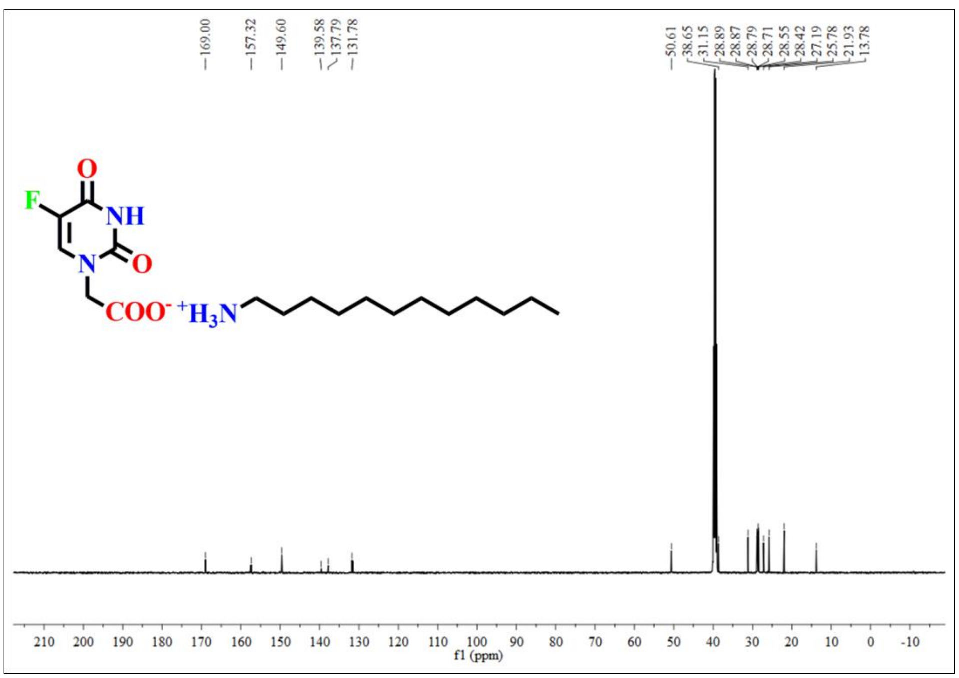
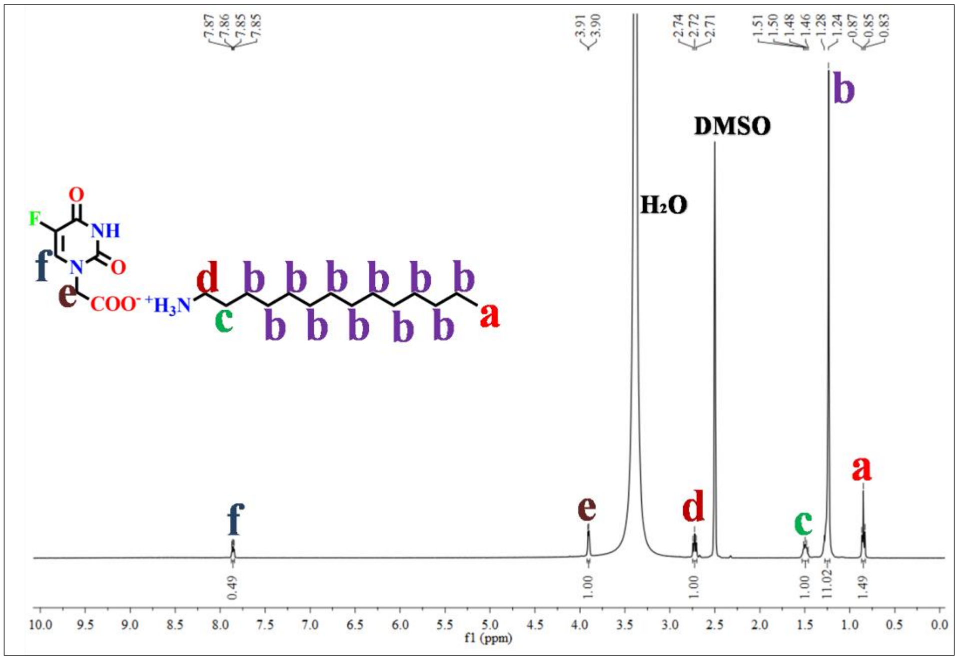


Figure S13: ^{1}H NMR and ^{13}C NMR spectra of **FuA-12** in DMSO-d_6.



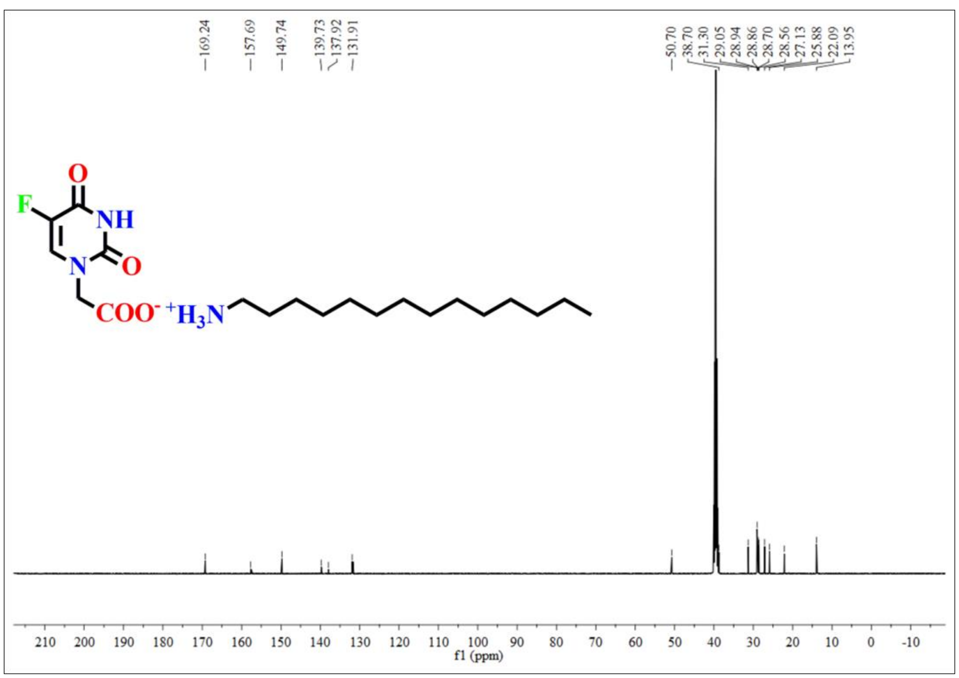
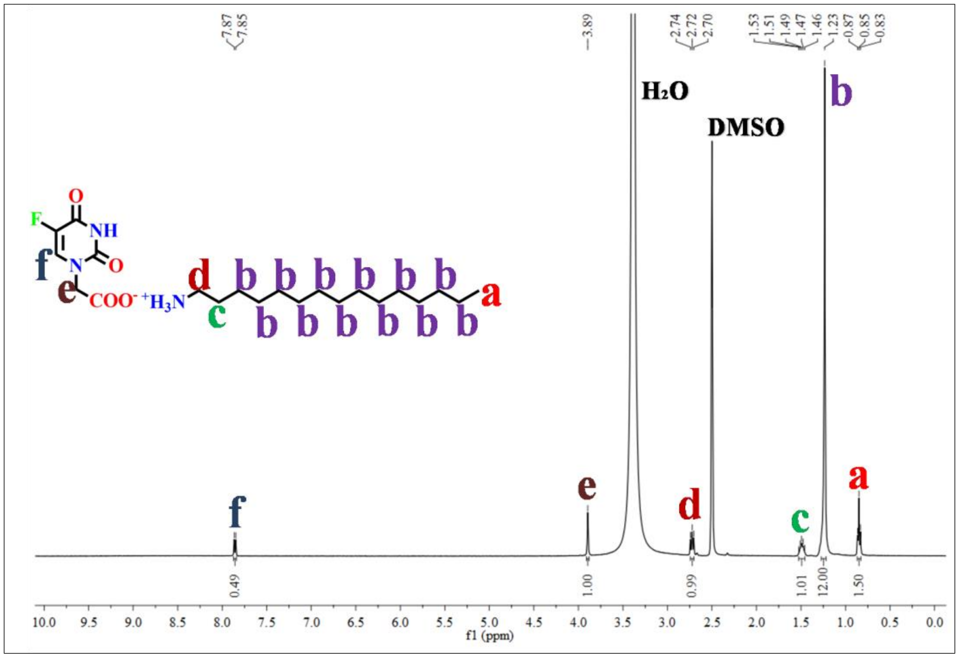


Figure S14: ^{1}H NMR and ^{13}C NMR spectra of **FuA-14** in DMSO-d_6.



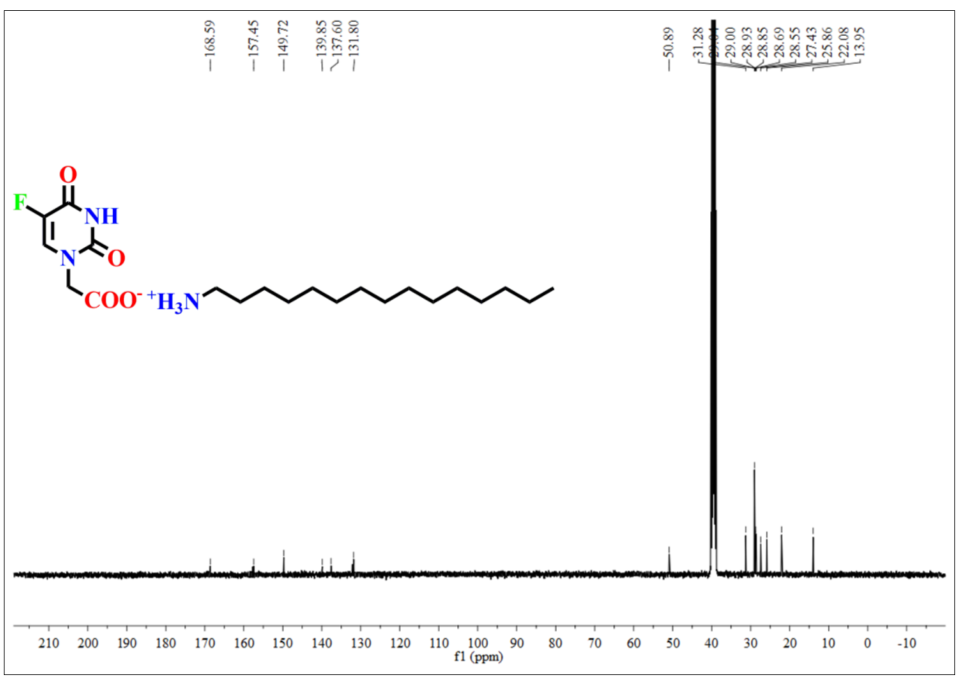


Figure S15: ^{1}H NMR and ^{13}C NMR spectra of **FuA-15** in DMSO-d_6.

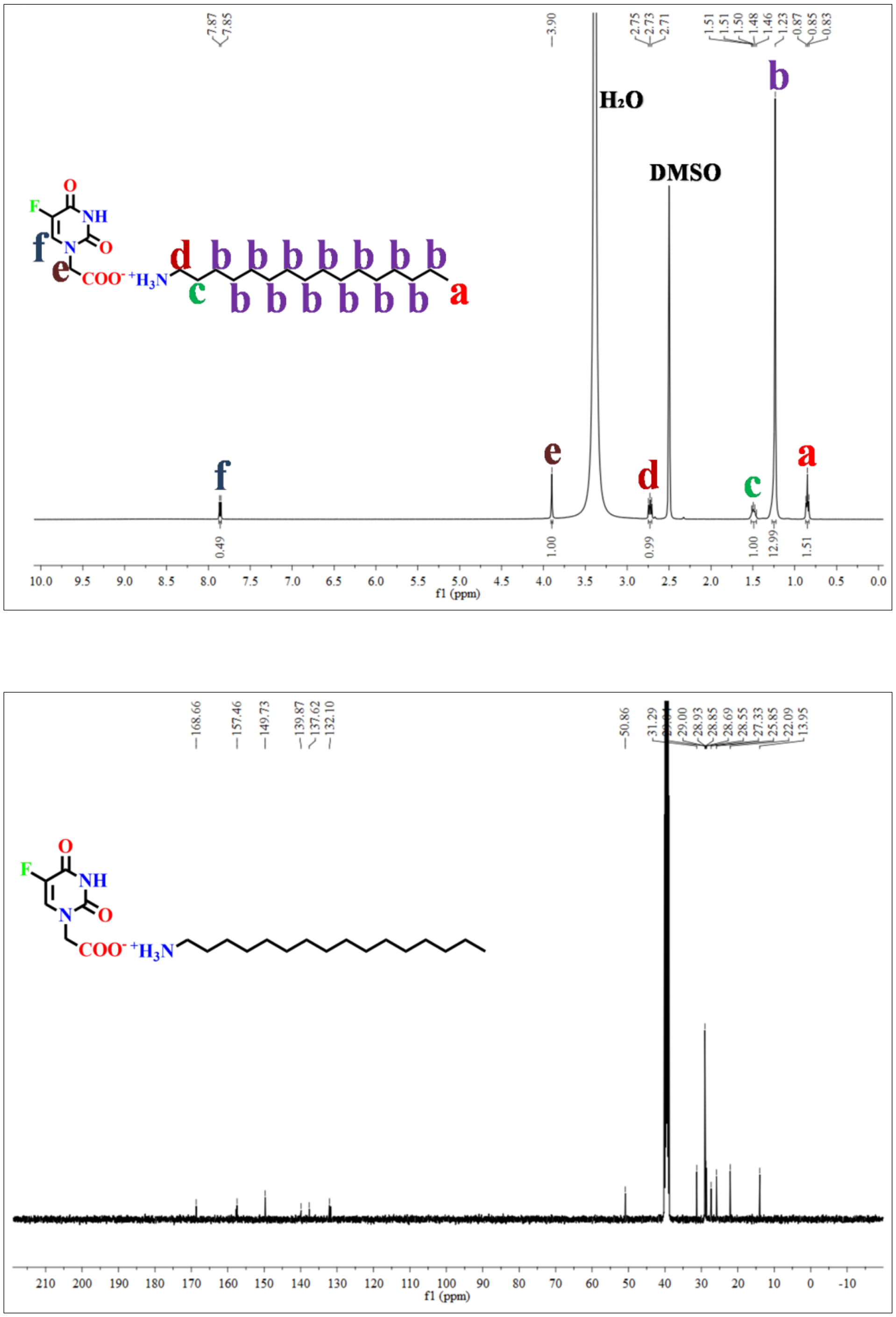


Figure S16: ^{1}H NMR and ^{13}C NMR spectra of **FuA-16** in DMSO-d$_6$.

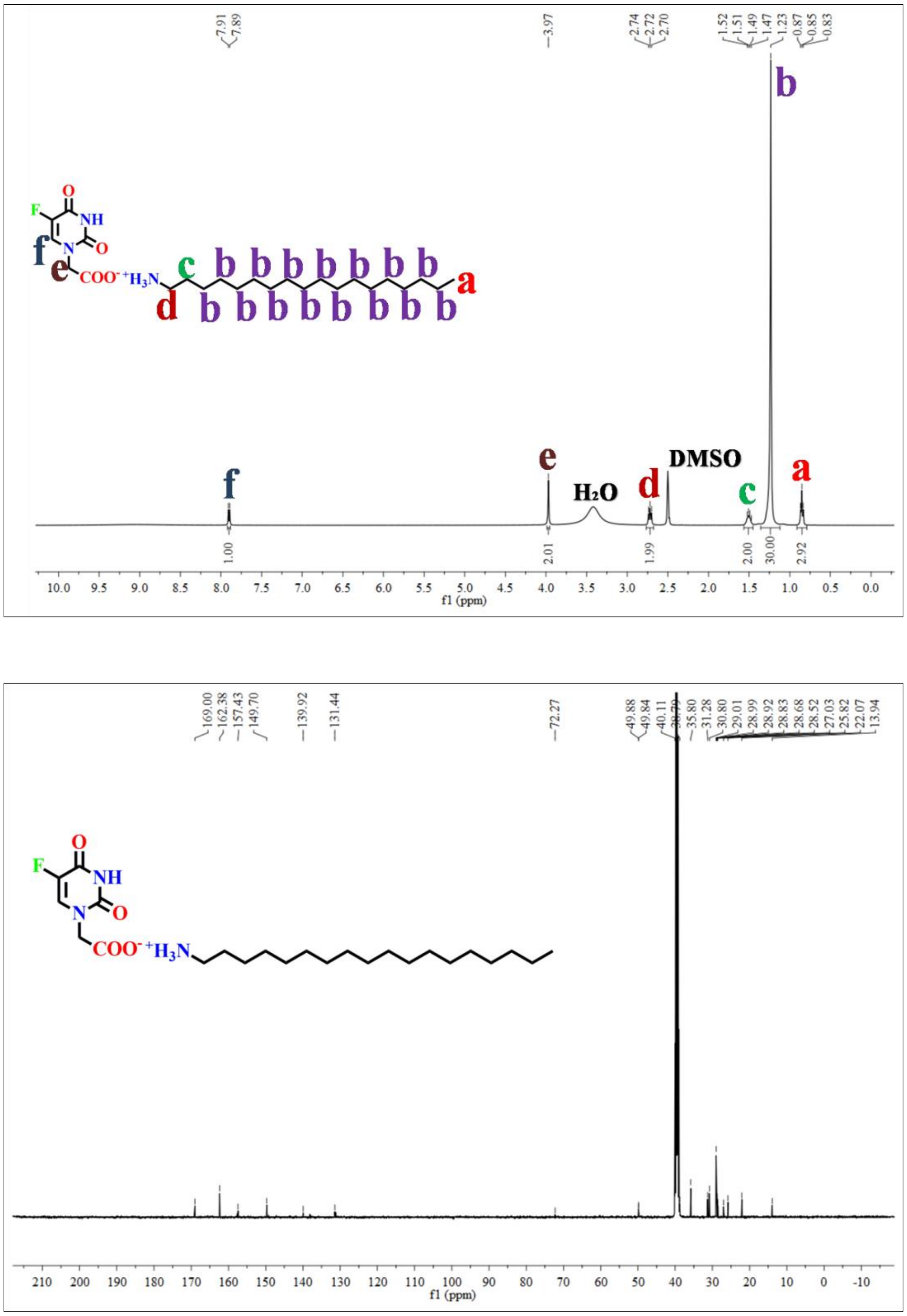


Figure S17: ^{1}H NMR and ^{13}C NMR spectra of **FuA-18** in DMSO-d_6.

Table S1: Gelation Table including Minimum Gelling Concentration and Gel Dissociation Temperature

Solvent	FuA-3	FuA-4	FuA-5	FuA-6	FuA-7	FuA-8	FuA-9	FuA-10	FuA-11	FuA-12	FuA-14	FuA-15	FuA-16	FuA-18
H_2O	S	S	S	S	S	S	S	${}^{4}G_{58}$	${}^{1.8}G_{55}$	${}^{4}G_{71}$	${}^{1}G_{71}$	${}^{1}G_{70}$	${}^{1.5}G_{60}$	${}^{3.3}G_{50}$
MS	S	INS	GP	INS	INS	GP	GP	${}^{2.5}G_{85}$	${}^{1.8}G_{88}$	${}^{2.5}G_{80}$	${}^{1}G_{76}$	${}^{1.5}G_{74}$	${}^{1.4}G_{89}$	${}^{2.2}G_{90}$
$PhNO_2$	S	INS	GP	${}^{4}G_{80}$	${}^{4}G_{94}$	${}^{4}G_{88}$	${}^{4}G_{92}$	${}^{2.5}G_{81}$	${}^{4}G_{109}$	${}^{2.5}G_{76}$	${}^{1.4}G_{98}$	${}^{1.5}G_{81}$	${}^{2}G_{91}$	${}^{2.5}G_{96}$
PhCl	INS	INS	INS	INS	INS	GP	GP	GP	${}^{4}G_{71}$	${}^{1.3}G_{93}$	${}^{1.2}G_{94}$	${}^{1.2}G_{91}$	${}^{1.2}G_{74}$	${}^{1.5}G_{78}$
PhBr	INS	INS	INS	INS	INS	GP	GP	${}^{1.2}G_{94}$	${}^{4}G_{145}$	${}^{1.2}G_{86}$	${}^{1.2}G_{91}$	${}^{1.3}G_{103}$	${}^{1.2}G_{69}$	${}^{2}G_{82}$
DMSO	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMF	S	S	S	S	S	S	S	S(C)	S	S(C)	S	S	S	S
DMA	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Toluene	INS	INS	INS	INS	INS	INS	INS	INS	INS	INS	GP	GP	GP	P
o-Xylene	INS	INS	INS	INS	INS	INS	INS	GP	INS	G_{149}	${}^{4}G_{138}$	${}^{4}G_{140}$	${}^{4}G_{142}$	${}^{4}G_{145}$
m-Xylene	INS	INS	INS	INS	INS	INS	INS	P	INS	GP	${}^{4}G_{120}$	${}^{4}G_{130}$	${}^{4}G_{134}$	${}^{2.9}G_{121}$
p-Xylene	INS	INS	INS	INS	INS	INS	INS	INS	INS	GP	${}^{4}G_{108}$	${}^{4}G_{120}$	${}^{4}G_{120}$	${}^{2.2}G_{110}$
Mesitylene	INS	INS	INS	INS	INS	INS	INS	INS	INS	INS	${}^{1.2}G_{103}$	${}^{1.2}G_{100}$	${}^{1.2}G_{101}$	${}^{2.9}G_{105}$
Dioxane	GP	${}^{4}G_{76}$	GP	${}^{4}G_{86}$	INS	${}^{4}G_{70}$	INS	GP	${}^{4}G_{62}$	${}^{4}G_{90}$	${}^{1.3}G_{95}$	${}^{1.5}G_{79}$	${}^{1.4}G_{80}$	${}^{3.3}G_{88}$
EG	S	S	S	S	S	S	S	S	S	S	S	S	S	S

${}^{MGC}G_{Tgel}$ - ${}^{\text{Minimum Gelling Concentration}}\text{Gel}_{\text{Gel dissociation temperature}}$, **S – Soluble, INS – Insoluble, GP – Gelatinous precipitate, C - Crystal**

Table S2: Crystallography Information Table

Identification Code	FuA_9	FuA_10	FuA_11	FuA_12	FuA_14	FuA_15
Crystallizing solvent	MeOH+DCM+Water +EtOAc	DMF	MeOH+DCM+ EtOAc	DMF	MeOH+DMF	MeOH+DMF
CCDC No.	1868726	1868727	1868722	1868723	1868725	1868724
Empirical Formula	$C_{20}H_{30}N_4O_5F$	$C_{20}H_{26}N_3O_4F$	$C_{20}H_{28}FN_3O_3$	$C_{20}H_{30}FN_4O_4$	$C_{30}H_{40}FN_3O_4$	$C_{30}H_{36}FN_2O_4$
Formula Weight	425.48	391.44	377.45	409.48	525.65	507.61
Temperature/K	296.15	296.15	100.09	99.98	115.14	105.94
Crystal System	triclinic	triclinic	triclinic	triclinic	triclinic	triclinic
Crystal Size/mm^3	0.24 × 0.2 × 0.12	0.32 × 0.24 × 0.18	0.23 × 0.18 × 0.12	0.3 × 0.28 × 0.26	0.24 × 0.12 × 0.08	0.2 × 0.1 × 0.07
Space Group	P-1	P-1	P-1	P-1	P1	P-1
a/Å	4.6735(7)	8.8655(11)	4.6593(2)	8.8667(6)	4.7844(2)	4.7775(4)
b/Å	10.5865(15)	9.0075(11)	10.5631(3)	9.0125(6)	10.4899(3)	10.4402(9)
c/Å	17.880(3)	18.138(2)	19.8391(7)	20.1479(11)	21.3123(7)	45.660(3)
$\alpha/^0$	100.883(2)	87.057(2)	97.467(2)	89.377(2)	82.522(2)	88.427(3)
$\beta/^0$	96.135(2)	76.644(2)	92.052(3)	80.718(2)	87.079(2)	88.793(2)
$\gamma/^0$	91.952(2)	88.318(2)	91.741(2)	88.288(2)	88.450(2)	88.556(3)
Volume/ $Å^3$	862.4(2)	1407.1(3)	966.91(6)	1588.22(17)	1058.92(6)	2275.4(3)
Density (ρ_{calc}g/cm^3)	1.638	1.848	1.296	1.712	1.649	1.482
μ/mm^{-1}	0.125	0.138	0.775	0.128	0.936	0.103
Z	2	4	2	4	2	4
F(000)	454.0	832.0	404.0	876.0	564.0	1084.0
Radiation	MoKα (λ = 0.71073)	MoKα (λ =0.71073)	CuKα (λ = 1.54178)	MoKα (λ = 0.71073)	CuKα (λ = 1.54178)	MoKα (λ = 0.71073)
2θ range for data collection/°	3.924 to 52.228	4.528 to 49.692	8.448 to 137.098	4.522 to 60.056	8.378 to 118.102	4.462 to 49.086
Index ranges	$-5 \le h \le 5$, $-13 \le k \le 13$, $-22 \le l \le 21$	$-10 \le h \le 10$, $-10 \le k \le 10$, $-21 \le l \le 21$	$-5 \le h \le 5$, $-12 \le k \le 12$, $-23 \le l \le 23$	$-12 \le h \le 12$, $-12 \le k \le 11$, $-28 \le l \le 27$	$-5 \le h \le 5$, $-11 \le k \le 10$, $-22 \le l \le 23$	$-5 \le h \le 5$, $-12 \le k \le 12$, $-53 \le l \le 52$
Reflections collected	22159	33446	6661	20939	6122	19877
Independent reflections	3370 [R_{int} = 0.0822, R_{sigma} = 0.0593]	4881 [R_{int} = 0.0464, R_{sigma} = 0.0312]	3367 [R_{int} = 0.0593, R_{sigma} = 0.0855]	9083 [R_{int} = 0.0563, R_{sigma} = 0.0745]	4231 [R_{int} = 0.0385, R_{sigma} = 0.0641]	7568 [R_{int} = 0.0563, R_{sigma} = 0.0818]
Data/restraints/parameters	3370/0/215	4881/0/321	3367/0/229	9083/0/357	4231/3/510	7568/0/238
Goodness of fit on F^2	1.023	1.054	1.036	1.041	1.035	1.091
Final R indexes[I>=2σ(I)]	R_1 = 0.0498, wR_2 = 0.0951	R_1 = 0.0376, wR_2 = 0.0795	R_1 = 0.0525, wR_2 = 0.1276	R_1 = 0.0573, wR_2 = 0.1429	R_1 = 0.0468, wR_2 = 0.1125	R_1 = 0.0910, wR_2 = 0.1886
Final R indexes [all data]	R_1 = 0.1002, wR_2 = 0.1179	R_1 = 0.0636, wR_2 = 0.0925	R_1 = 0.0828, wR_2 = 0.1482	R_1 = 0.0980, wR_2 = 0.1765	R_1 = 0.0601, wR_2 = 0.1260	R_1 = 0.1512, wR_2 = 0.2123
Largest diff. peak/hole/e $Å^{-3}$	0.28/-0.26	0.21/-0.22	0.23/-0.28	0.39/-0.35	0.26/-0.23	0.63/-0.48
Flack parameter	-	-	-	-	0.4(3)	-

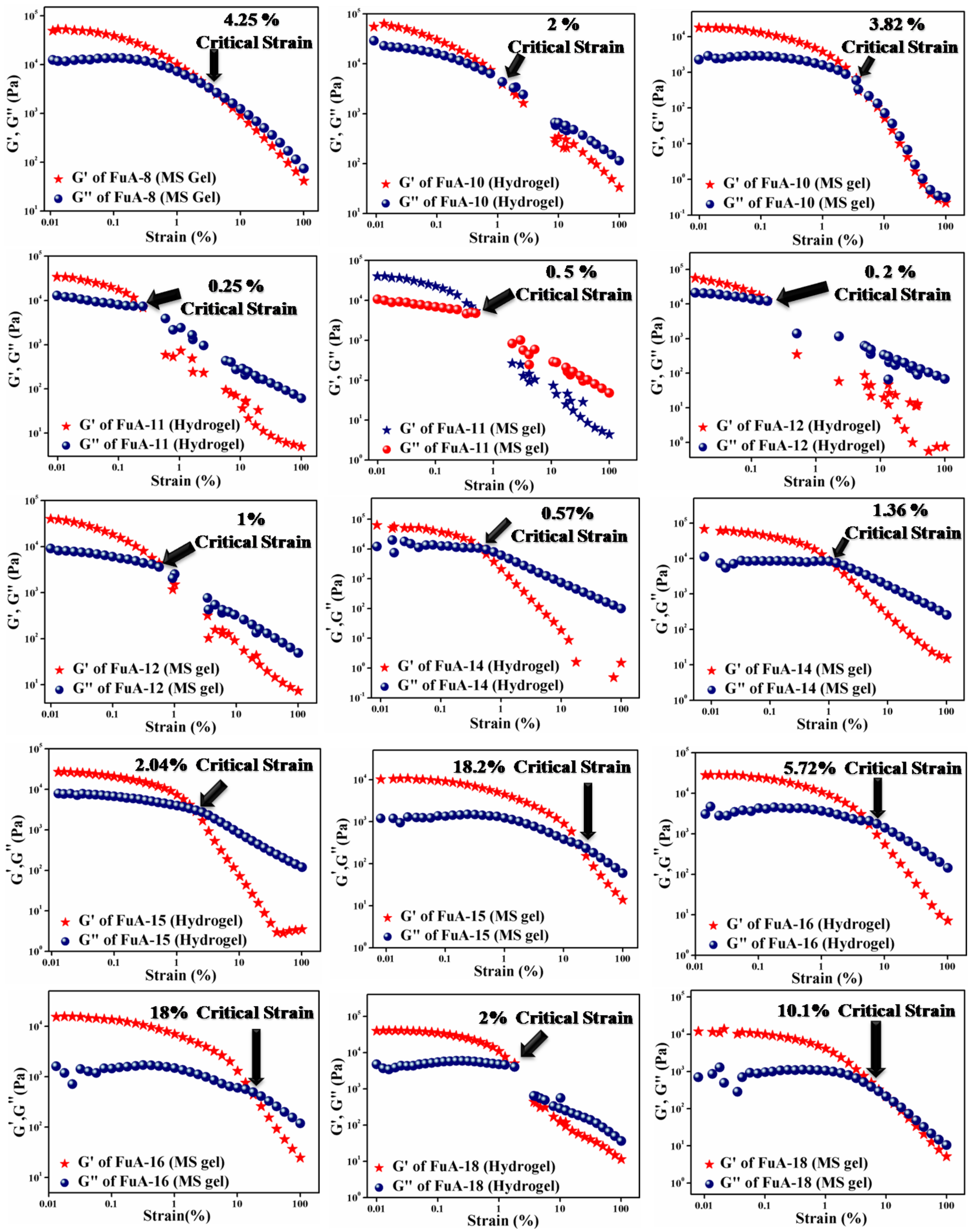


Figure S18: Amplitude Sweep Rheology Experiment of Some Selected Hydro and MS Gels.

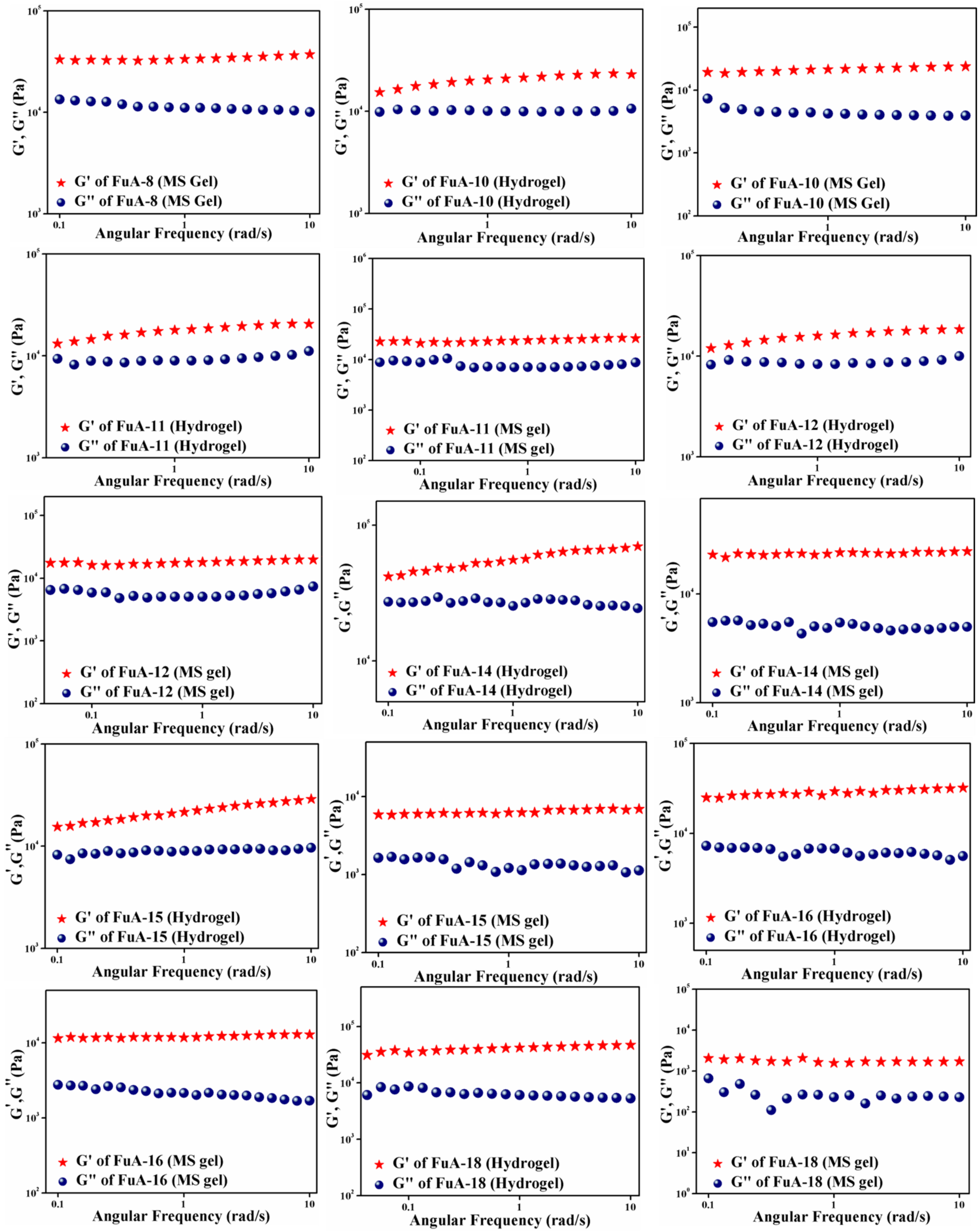


Figure S19: Frequency Sweep Rheology Experiment of Some Selected Hydro and MS Gels.

Table S3: Average **G'**, **G"** and **tan δ** values of the gels obtained from rheology

	Average G' (kPa)	Average G" (kPa)	G"/G' (tan δ)
FuA-10 (MS)	21.157	4.890	0.231165
FuA-10 (HG)	19.791	10.264	0.518641
FuA-11 (MS)	23.336	9.113	0.390536
FuA-11 (HG)	17.277	9.384	0.543162
FuA-12 (MS)	18.465	6.820	0.369382
FuA-12 (HG)	15.544	8.837	0.568564
FuA-14 (MS)	23.538	5.058	0.21491
FuA-14 (HG)	56.176	27.004	0.480715
FuA-15 (MS)	6.371	1.362	0.213885
FuA-15 (HG)	21.952	8.941	0.40731
FuA-16 (MS)	12.166	2.186	0.179687
FuA-16 (HG)	28.580	6.262	0.21911
FuA-18 (MS)	1.763	0.271	0.153769
FuA-18 (HG)	38.745	7.436	0.191939

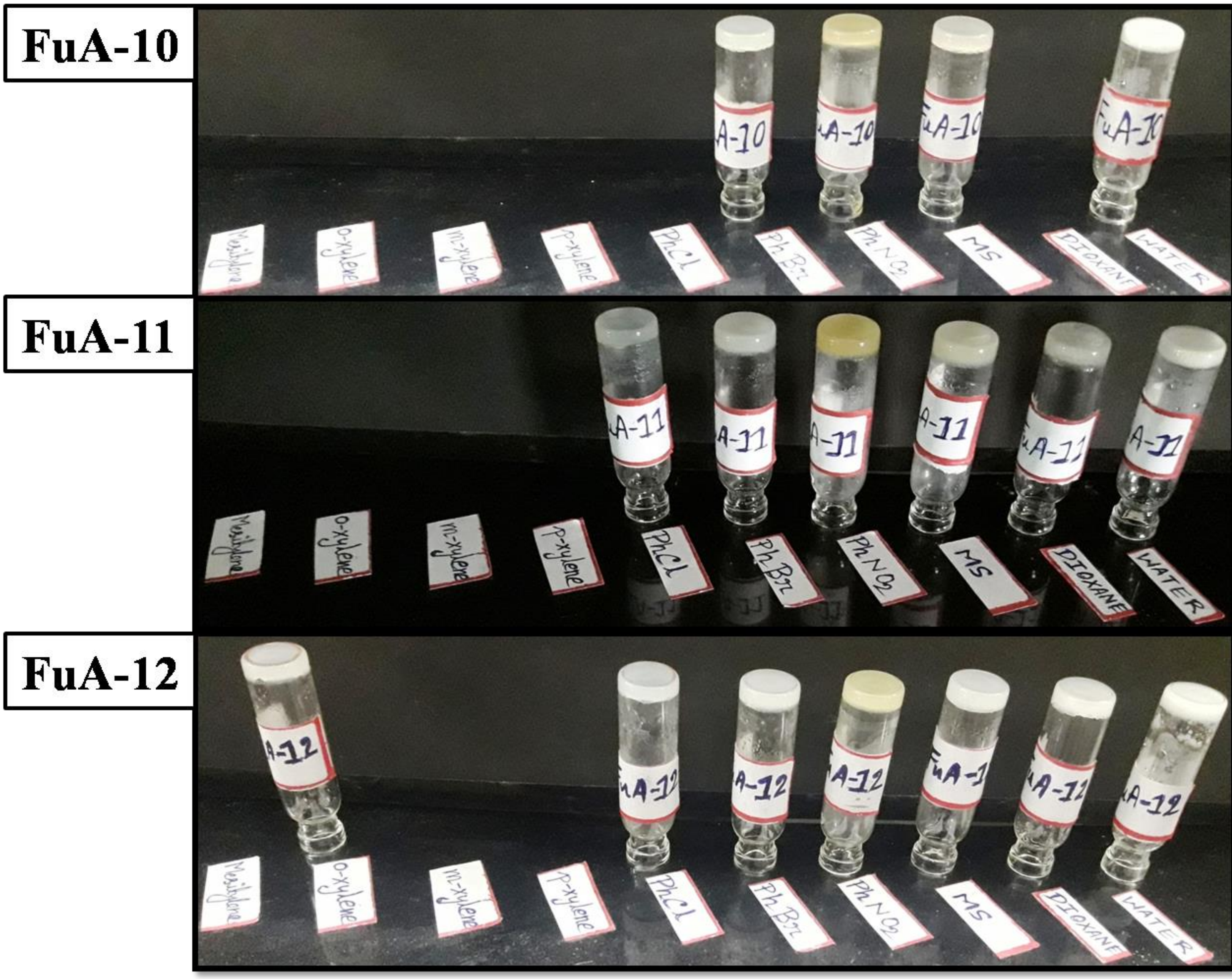


Figure S20(a): Characterization of gels (**FuA-10**, **FuA-11**, **FuA-12**): tube-inversion method.

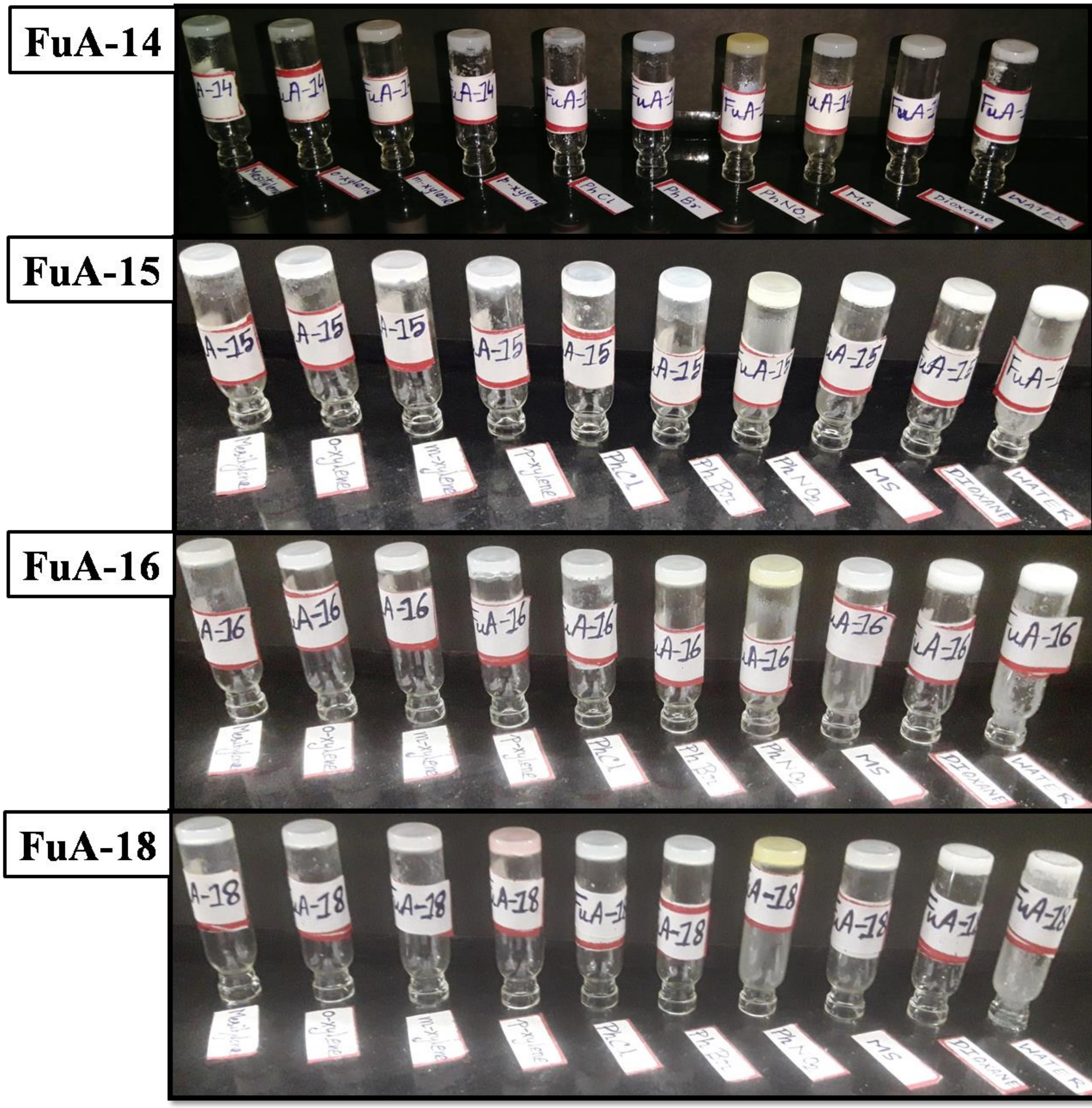


Figure S20(b): Characterization of gels (**FuA-14**, **FuA-15**, **FuA-16**, **FuA-18**): tube-inversion method.

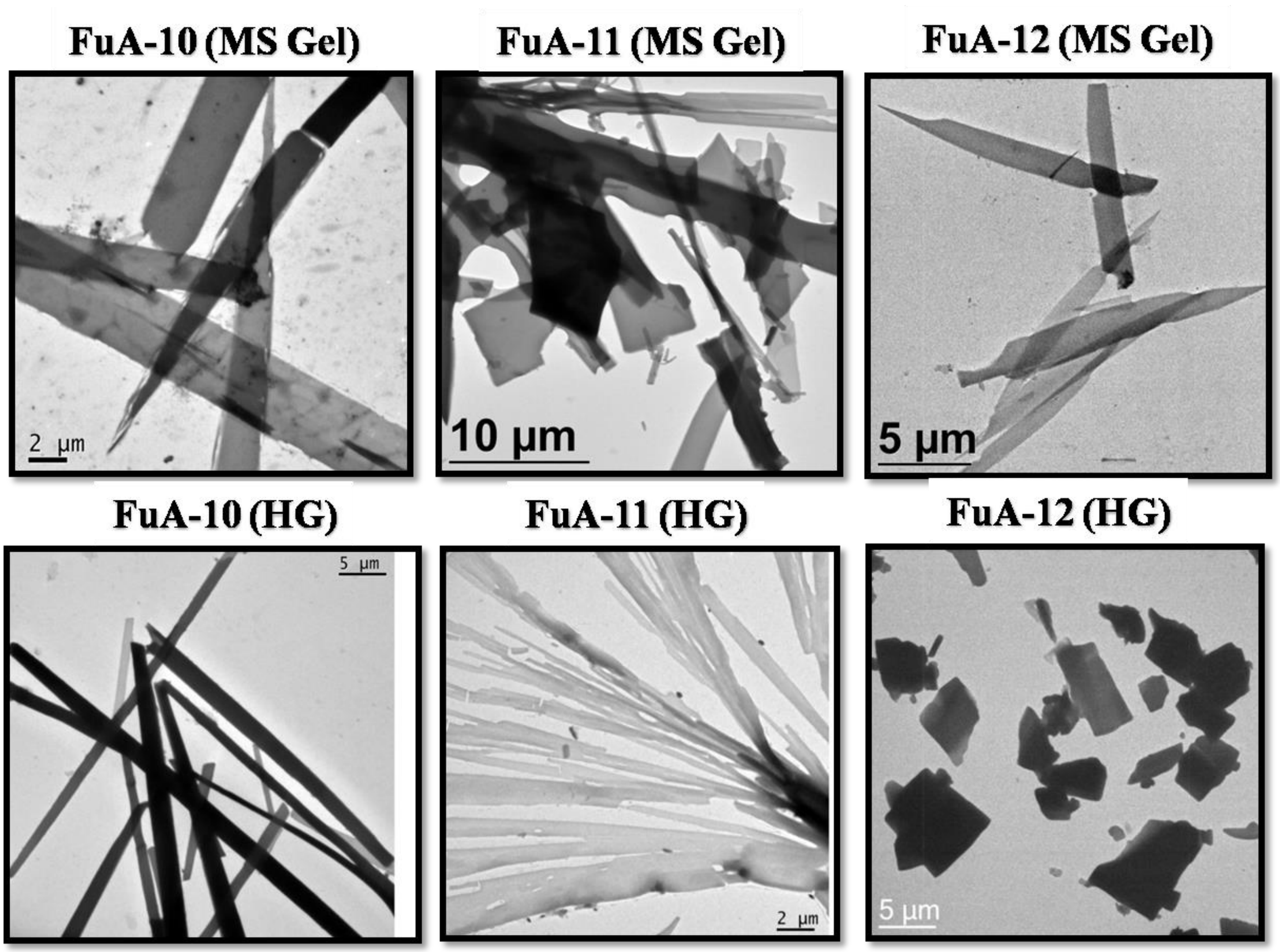


Figure S21(a): Morphology of the Gels (Hydro and MS gels of **FuA-10**, **FuA-11**, **FuA-12**) as observed in Transmission Electron Microscopy (TEM).

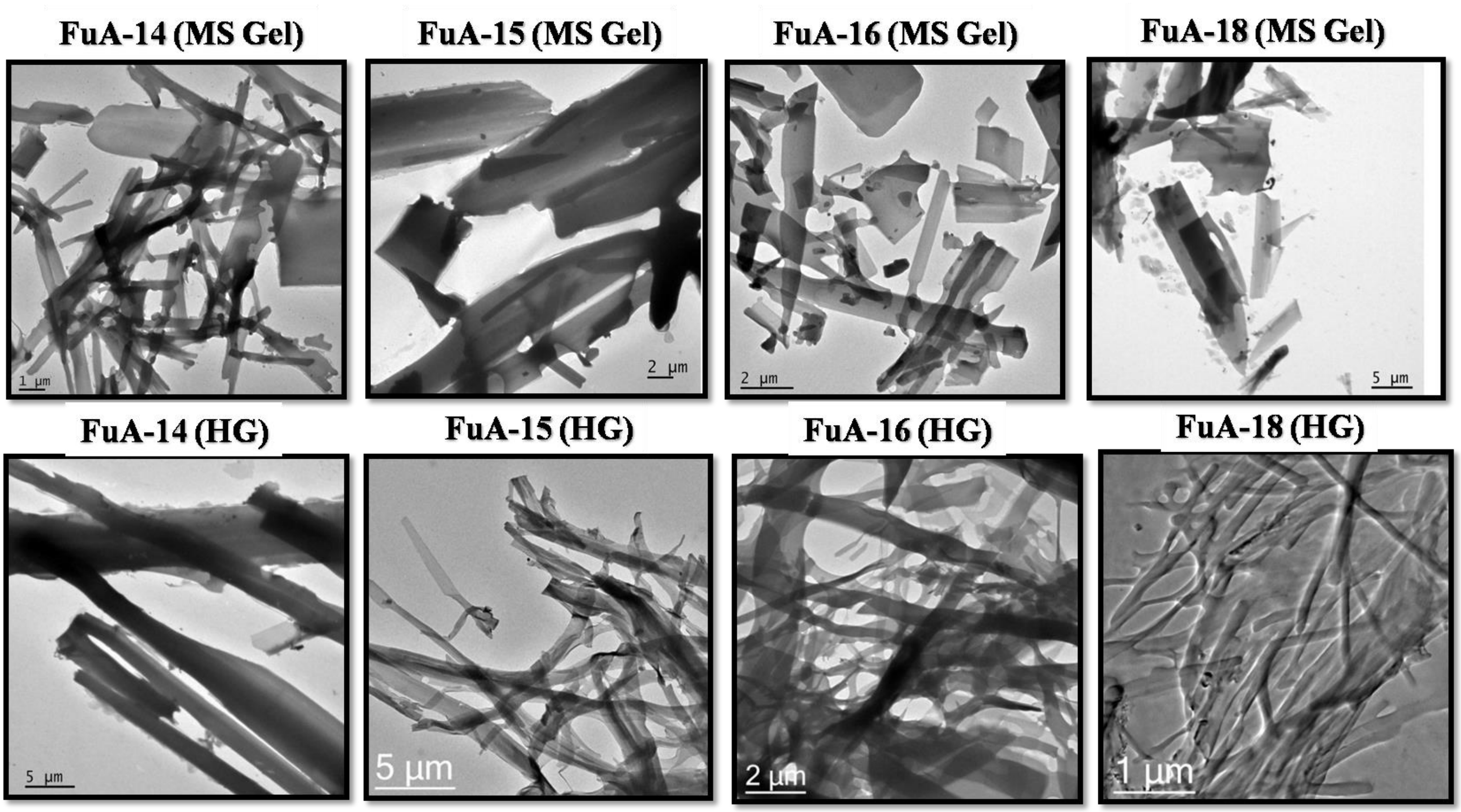


Figure S21(b): Morphology of the Gels (Hydro and MS gels of **FuA-14**, **FuA-15**, **FuA-16**, **FuA-18**) as observed in Transmission Electron Microscopy (TEM).

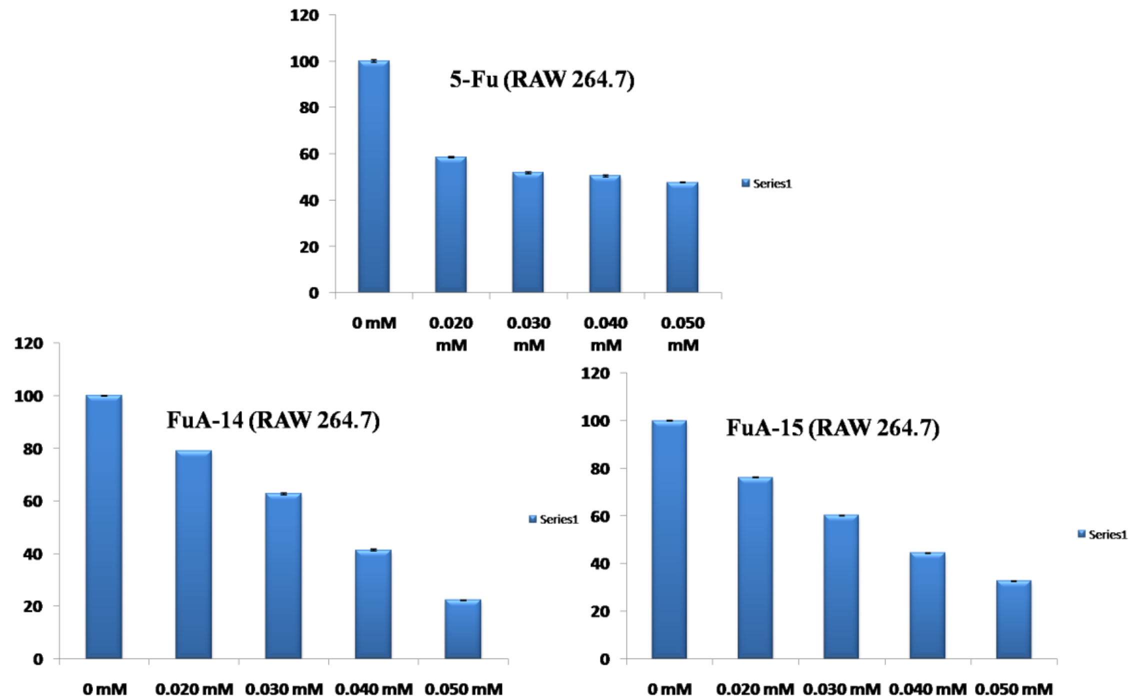


Figure S22: Cell viability assay (MTT Assay) done in Raw 264.7 cell line.

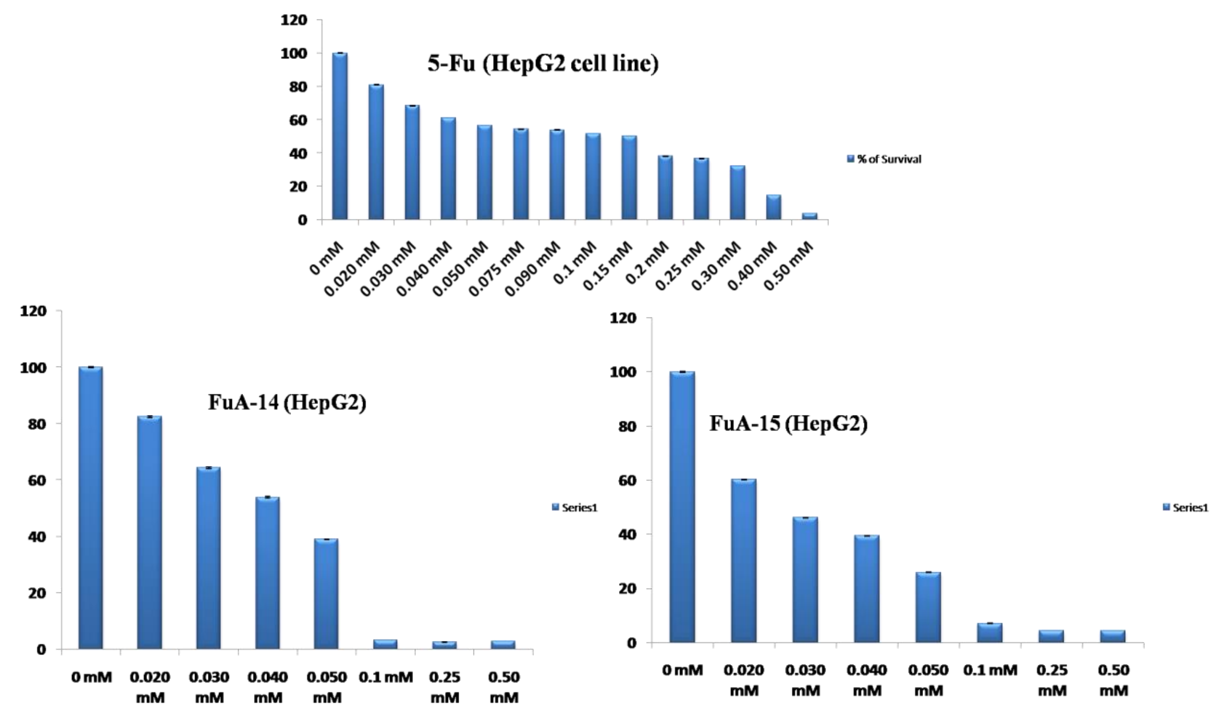


Figure S23(a): Cell viability assay (MTT Assay) done in HepG2 cell line.

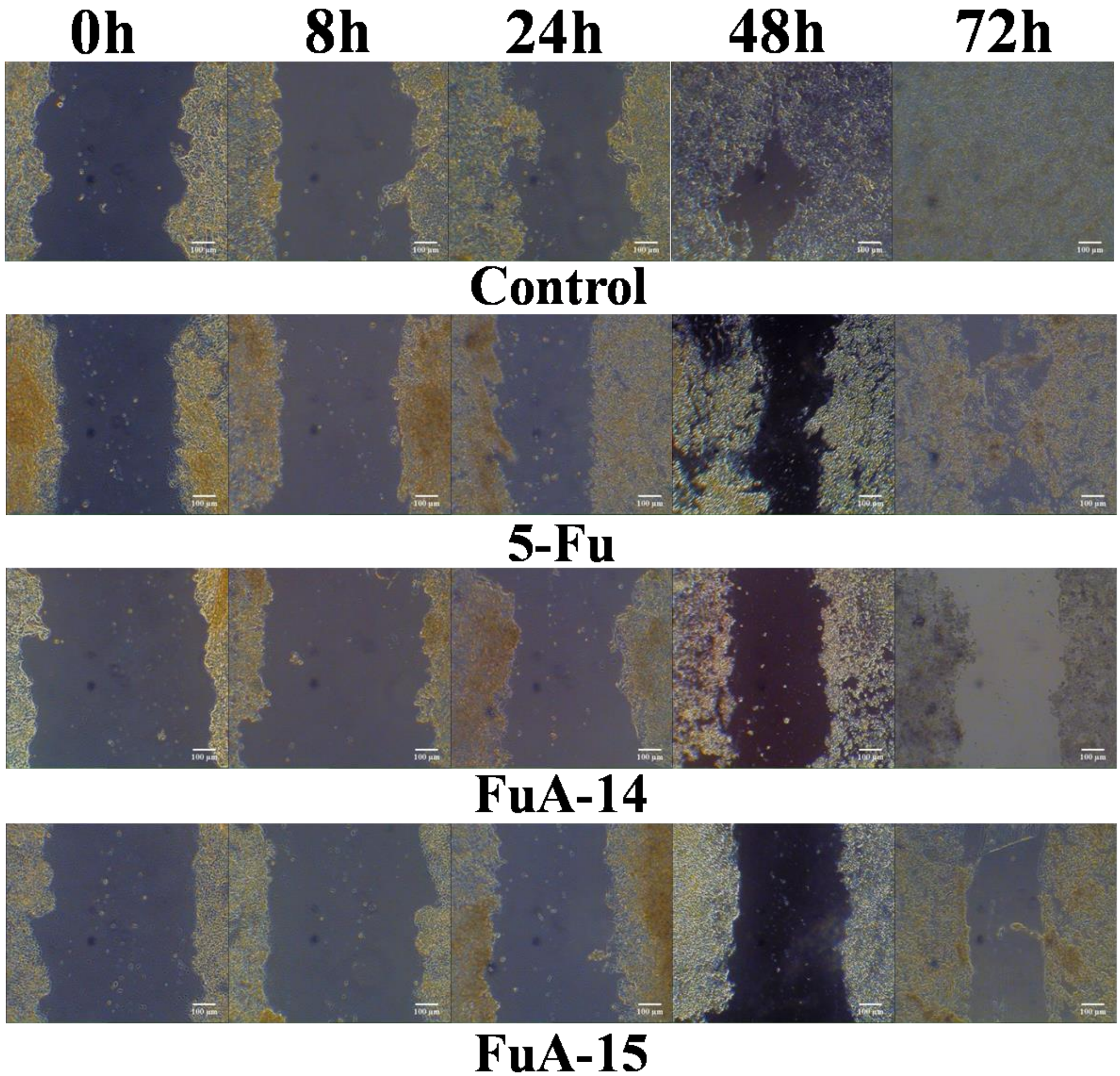


Figure S23(b): Cell Migration assay (Scratch Assay) done in HepG2 cell line.

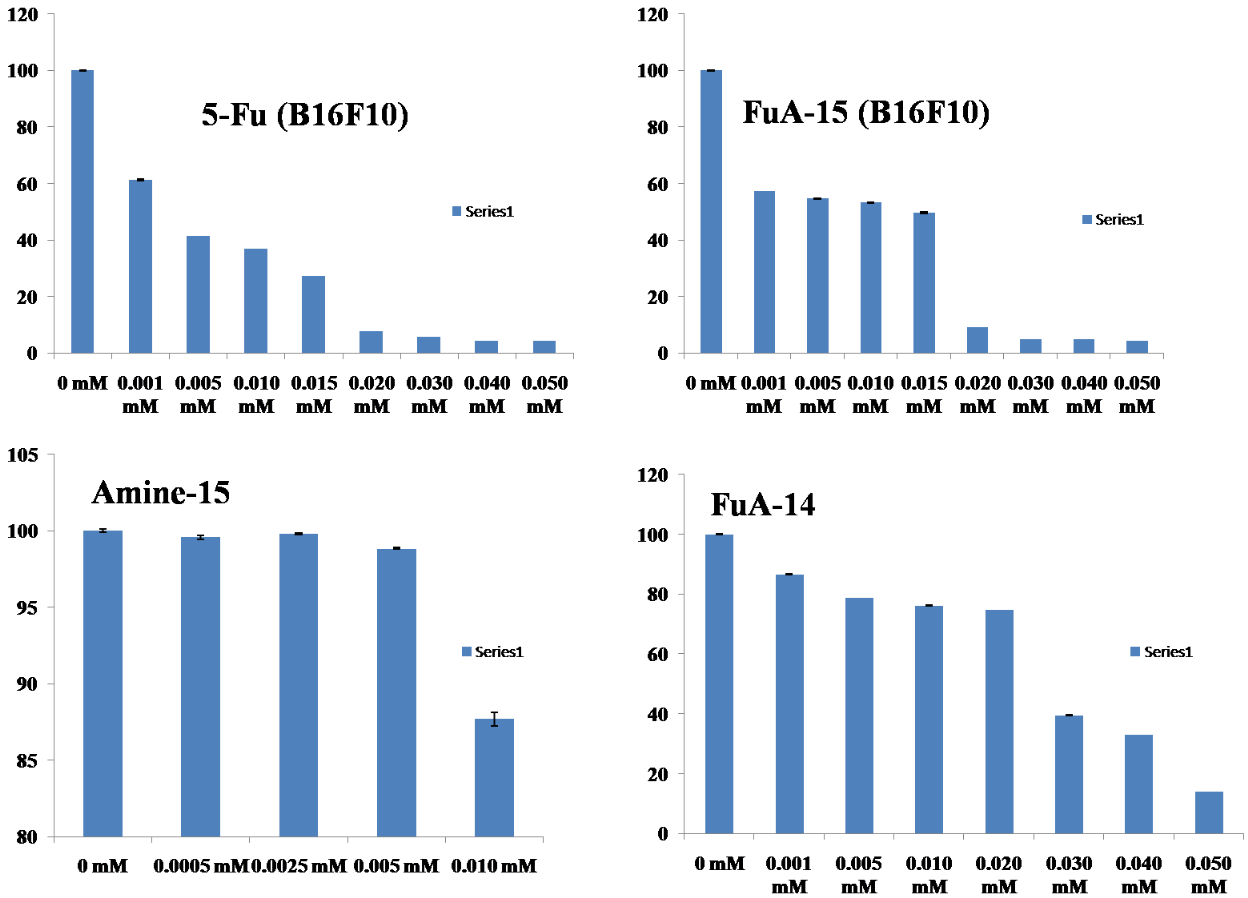


Figure S24: Cell viability assay (MTT Assay) done in B16F10 cell line.

Table S4(a): Table of comparison of the anticancer behaviour between the mother drug (**5-Fu**) and the salts (**FuA-14** and **FuA-15**) in HepG2 Cell Line

Compounds	Concentration (mM)	% of HepG2 cell death	IC50 in RAW 264.7 (mM)
5-Fu	0.1	48.0	0.03
5-Fu	0.5	96.0	0.03
FuA-14 (Salt)	0.1	97	0.03
FuA-15 (Salt)	0.1	93	0.03

Table S4(b): Table of comparison of the anticancer behaviour between the mother drug (**5-Fu**) and the salts (**FuA-14** and **FuA-15**) in B16F10 Cell Line

Compounds	Concentration (mM)	% of B16F10 cell death
5-Fu	0.02	92
FuA-14 (Salt)	0.02	26
FuA-15 (Salt)	0.02	91

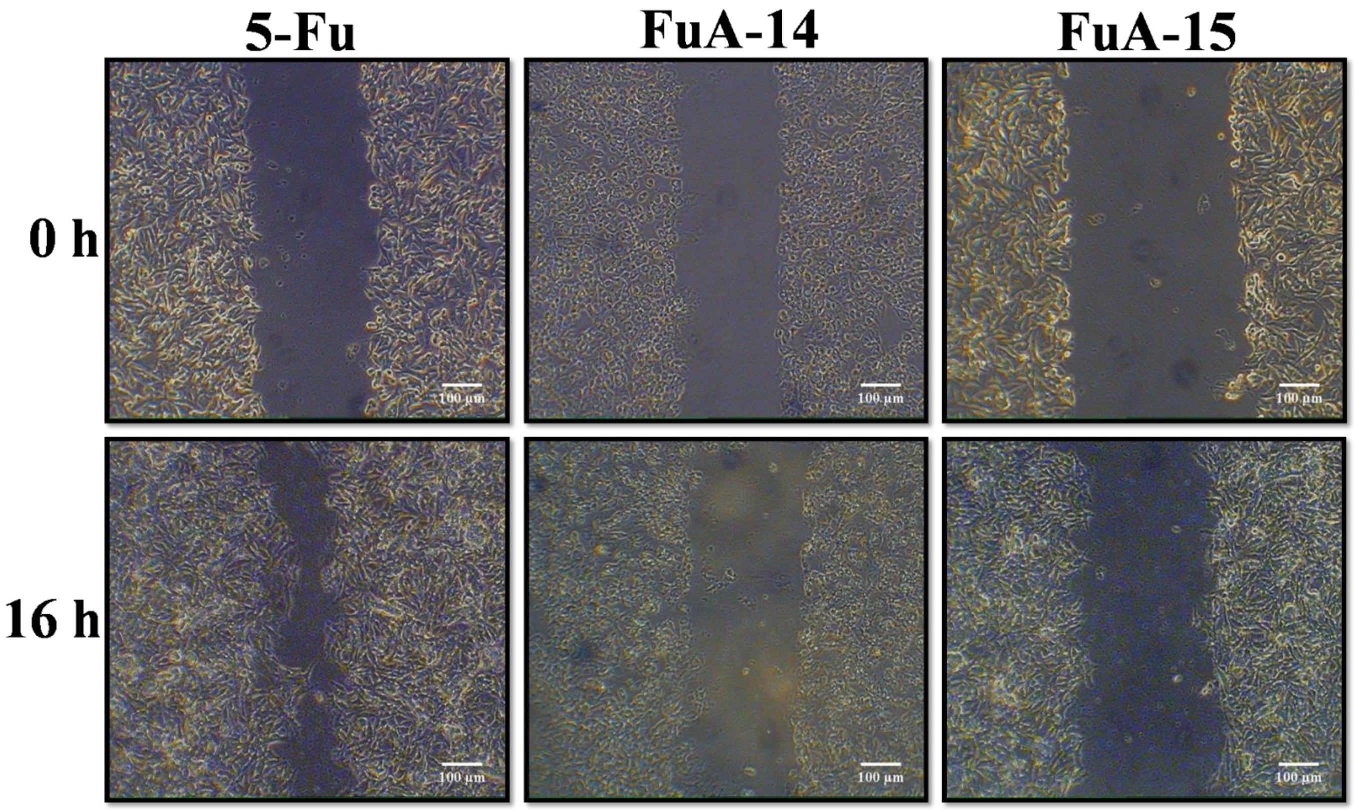


Figure S25: The morphology images of cell upon treatment of **5-Fu**, **FuA-14** and **FuA-15** in B16F10 cell line.

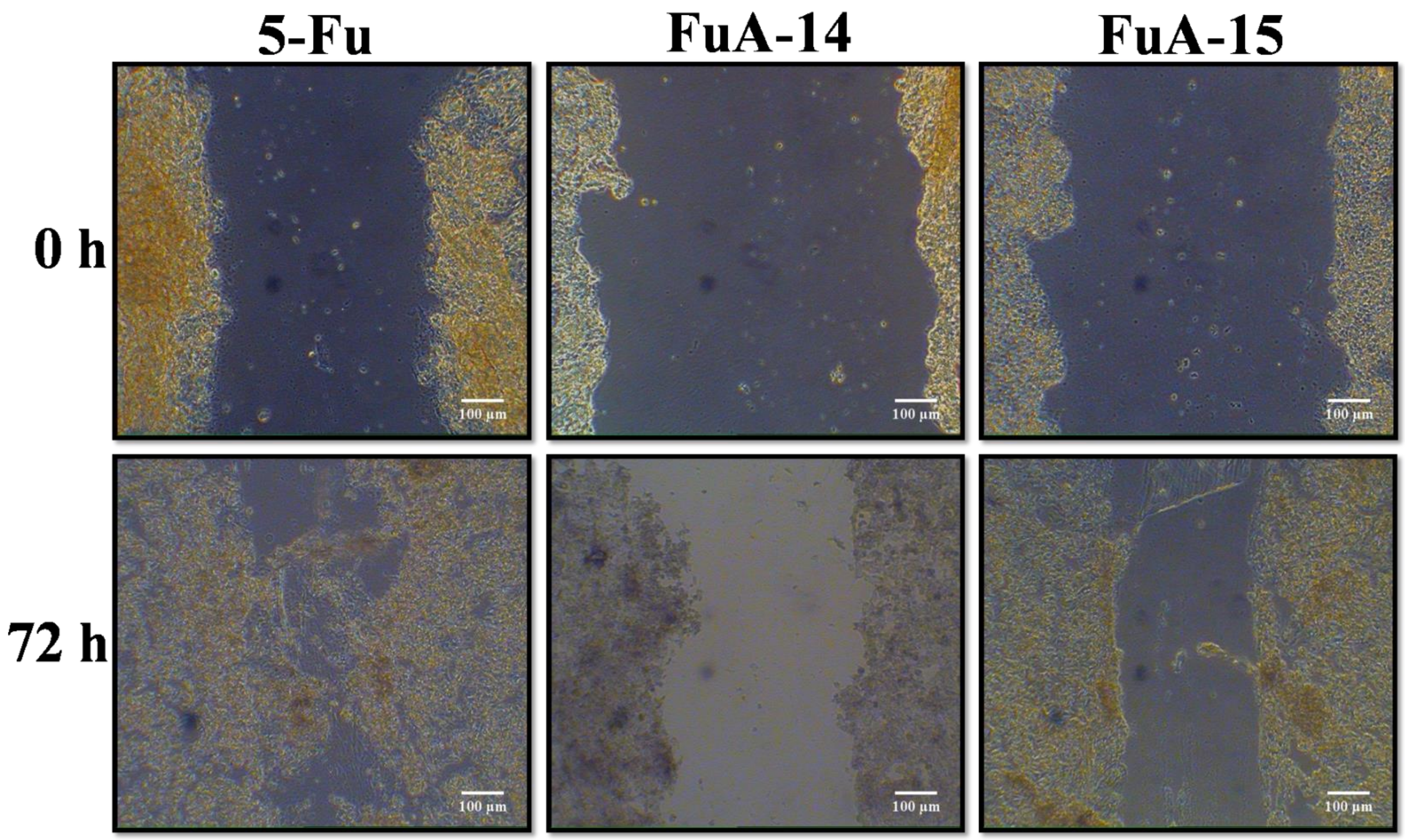


Figure S26: The morphology images of cell upon treatment of **5-Fu**, **FuA-14** and **FuA-15** in HepG2 cell line.

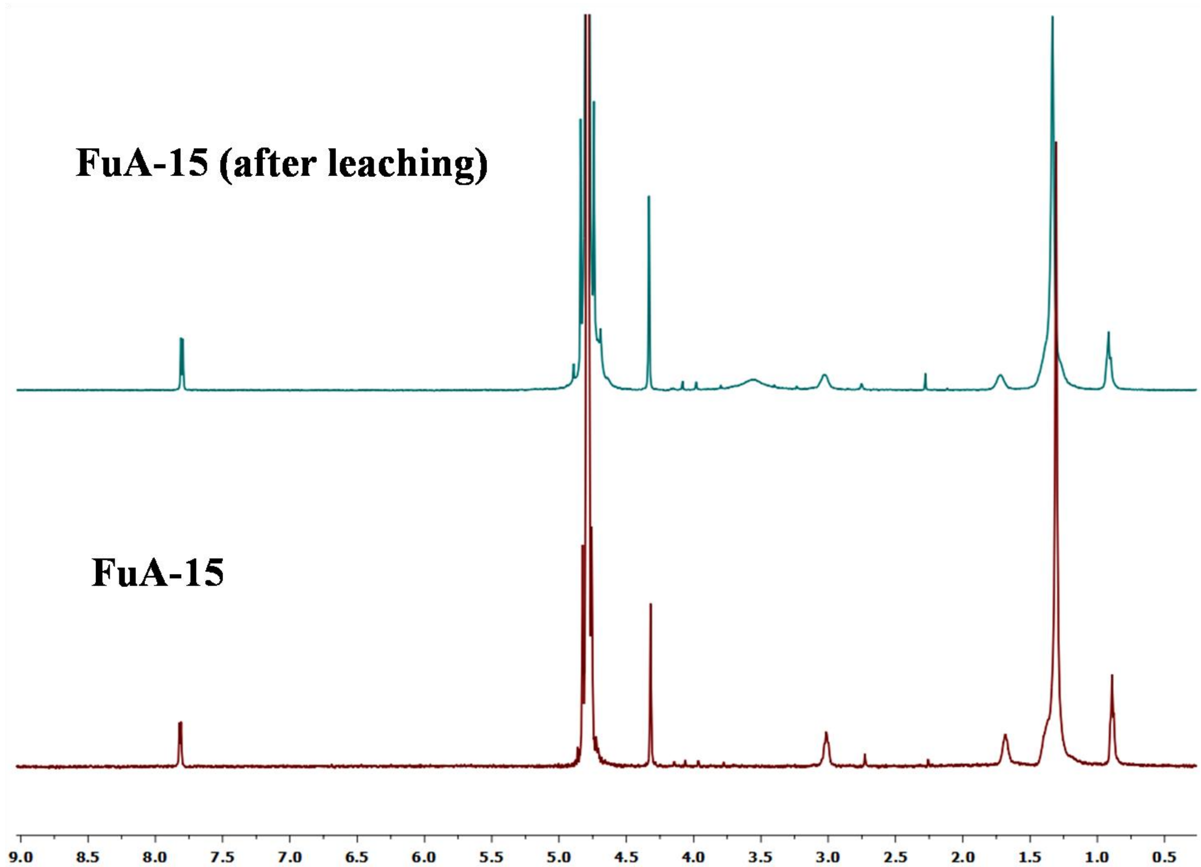


Figure S27: NMR Data to prove the existence of **FuA-15** in leached out solution (D_2O).

Leaching of FuA-15 from the corresponding hydrogel

To probe the leaching of **FuA-15** from its corresponding hydrogel, ^{1}HNMR experiments were performed. For this purpose, we prepared a D_2O gel of FuA-15 (2.5 wt %, 2 mL) and on top of it, 2 mL D_2O was layered and kept at room temperature for 30 h. The top D_2O layer was then subjected to ^{1}H NMR that clearly showed the existence of FuA-15 (Fig. S27).

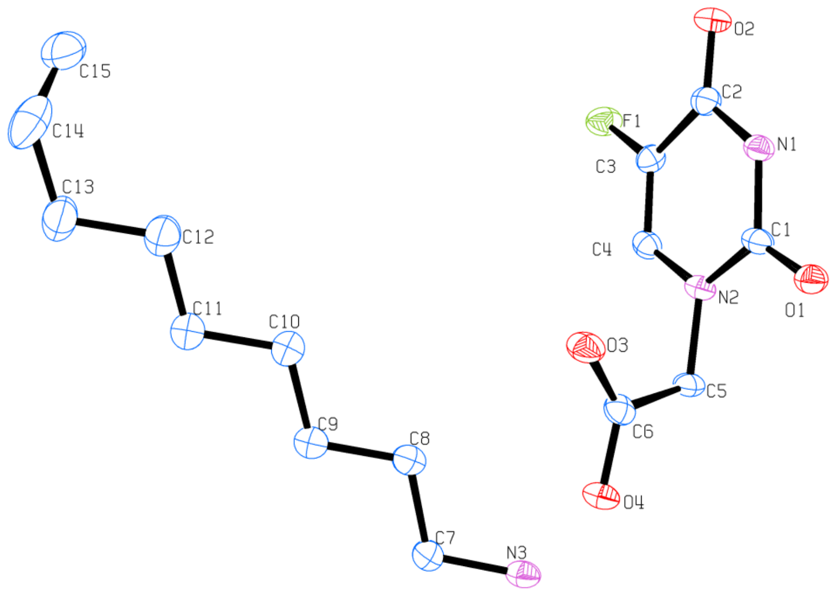


Figure S28: ORTEP Plot of **FuA-9** (50% probability).

Table S5: Hydrogen Bonding Parameter table of **FuA-9**

Table S5 : Hydrogen Bonds for **FuA-9**						
D	**H**	**A**	**d(D-H)/Å**	**d(H..A)/Å**	**d(D..A)/Å**	**<D-H-A/°**
N3	H3A	O4[1]	0.890	1.865	2.743	168.87
N3	H3B	O1[2]	0.890	2.515	3.292	146.18
N3	H3B	O2[3]	0.890	2.442	2.940	115.76
N3	H3C	O4	0.890	1.924	2.812	175.59
C5	H5A	O4[4]	0.970	2.524	3.355	143.71
C5	H5B	O3[5]	0.970	2.429	3.295	148.49
C4	H4	O3[6]	0.930	2.258	3.101	150.33
C7	H7A	F1[7]	0.970	2.587	3.278	128.37
C7	H7A	O2[8]	0.970	2.646	3.306	125.61
N1	H1	O1[9]	0.968	1.822	2.789	177.38

[1][x-1, y, z], [2][-x+1, -y, -z], [3][x-1, y-1, z], [4][-x+1, -y, -z], [5][x-1, y, z], [6][x-1, y, z], [7][x, y-1, z], [8][x, y-1, z], [9][-x+2, -y+1, -z].

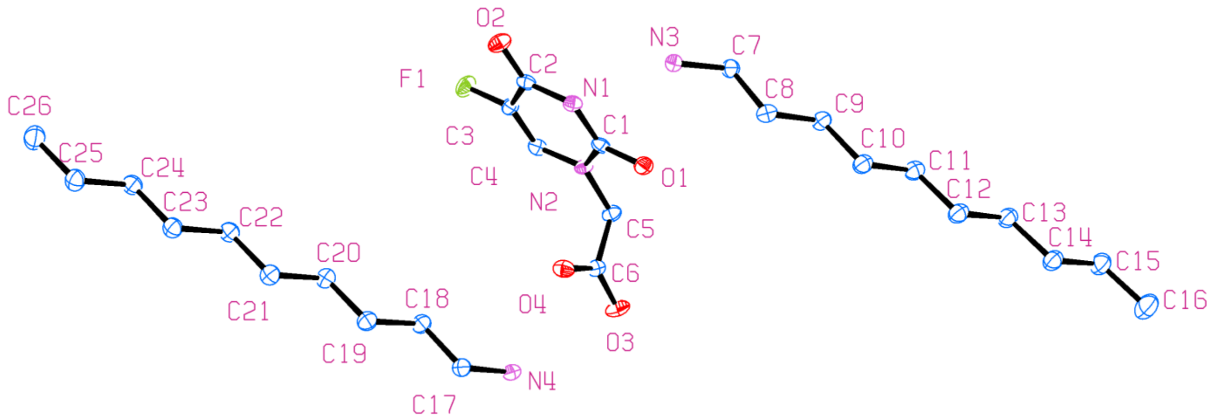


Figure S29: ORTEP Plot of **FuA-10** (50% probability).

Table S6: Hydrogen Bonding Parameter table of **FuA-10**

Table S6 : Hydrogen Bonds for **FuA-10**						
D	**H**	**A**	**d(D-H)/Å**	**d(H..A)/Å**	**d(D..A)/Å**	**<D-H-A/°**
N3	H3A	O1	0.890	2.213	2.985	144.84
N3	H3A	N1	0.890	2.376	3.013	128.57
N3	H3B	O3[1]	0.890	1.890	2.740	159.21
N3	H3C	O4[2]	0.890	1.996	2.811	151.66
N4	H4A	O2[3]	0.890	1.822	2.711	176.27
N4	H4A	N1[4]	0.890	2.648	3.252	126.01
N4	H4B	O1[5]	0.890	1.938	2.805	164.00
N4	H4C	O4	0.890	1.940	2.811	165.81
C4	H4	O3[6]	0.930	2.339	3.264	172.92
C7	H7B	F1[7]	0.970	2.608	3.520	156.86

[1][x-1, y, z], [2][-x+1, -y+1, -z+1], [3][x+1, y, z], [4][x+1, y, z], [5][-x+2, -y+1, -z+1], [6][-x+2, -y, -z+1], [7][-x+1, -y, -z+1].

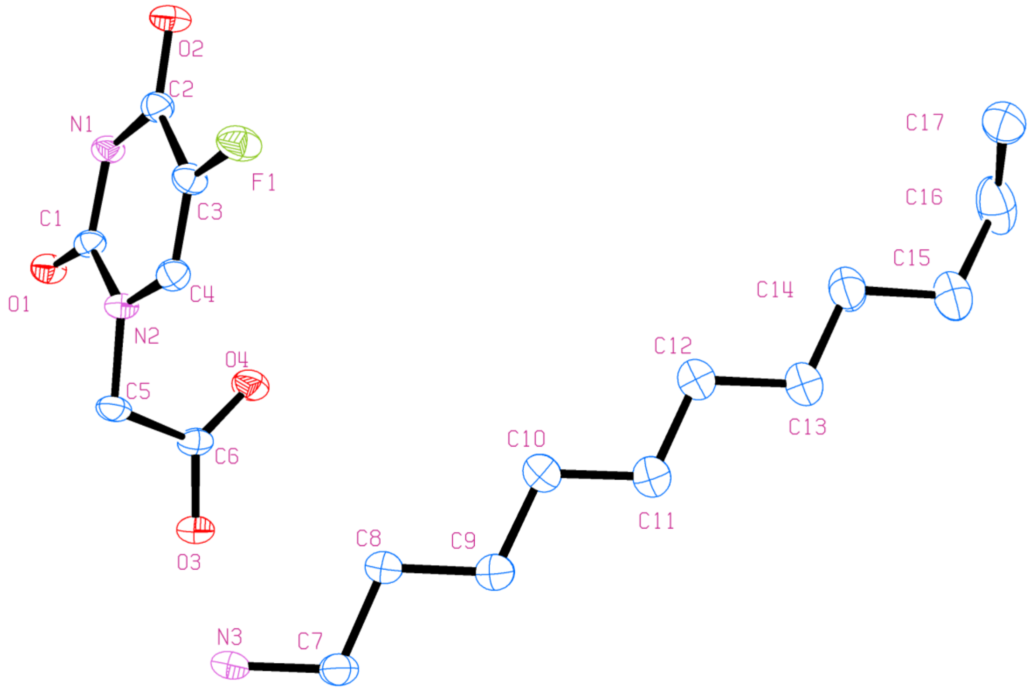


Figure S30: ORTEP Plot of **FuA-11** (50% probability).

Table S7: Hydrogen Bonding Parameter table of **FuA-11**

Table S7 : Hydrogen Bonds for **FuA-11**						
D	**H**	**A**	**d(D-H)/Å**	**d(H..A)/Å**	**d(D..A)/Å**	**<D-H-A/°**
N1	H1	O1[1]	0.880	1.920	2.800	177.35
N3	H3A	O1[2]	0.910	2.528	3.316	145.09
N3	H3A	O2[3]	0.910	2.427	2.930	114.96
N3	H3B	O3	0.910	1.905	2.814	176.45
N3	H3C	O3[4]	0.910	1.846	2.744	168.80
C4	H4	O4[5]	0.950	2.245	3.108	150.51
C5	H5A	O3[6]	0.990	2.502	3.353	143.89
C5	H5B	O4[7]	0.990	2.428	3.307	147.64
C7	H7A	F1[8]	0.990	2.568	3.271	127.98
C7	H7A	O2[9]	0.990	2.647	3.306	124.20

[1][-x+1, -y+1, -z], [2][-x, -y, -z], [3][x-1, y-1, z], [4][x-1, y, z], [5][x-1, y, z], [6][-x, -y, -z], [7][x-1, y, z], [8][x, y-1, z], [9][x, y-1, z].

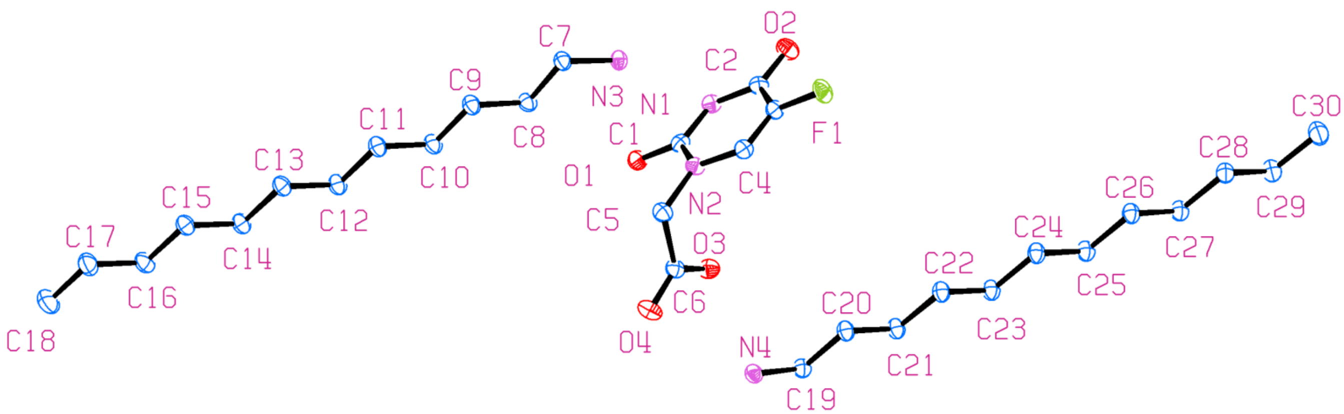


Figure S31: ORTEP Plot of **FuA-12** (50% probability).

Table S8: Hydrogen Bonding Parameter table of **FuA-12**

Table S8 : Hydrogen Bonds for **FuA-12**						
D	**H**	**A**	**d(D-H)/Å**	**d(H..A)/Å**	**d(D..A)/Å**	**<D-H-A/°**
N3	H3A	O3[1]	0.910	1.962	2.804	152.99
N3	H3B	O1	0.910	2.208	2.990	143.65
N3	H3B	N1	0.910	2.372	3.013	127.40
N3	H3C	O4[2]	0.910	1.869	2.736	158.34
N4	H4A	O3	0.910	1.924	2.813	165.16
N4	H4B	O2[3]	0.910	1.804	2.713	176.65
N4	H4B	N1[4]	0.910	2.642	3.254	125.31
N4	H4C	O1[5]	0.910	1.923	2.809	164.16
C4	H4	O4[6]	0.950	2.317	3.262	172.84
C7	H7B	F1[7]	0.990	2.602	3.532	156.35

[1][-x+1, -y+1, -z+1], [2][x-1, y, z], [3][x+1, y, z], [4][x+1, y, z], [5][-x+2, -y+1, -z+1], [6][-x+2, -y, -z+1], [7][-x+1, -y, -z+1].

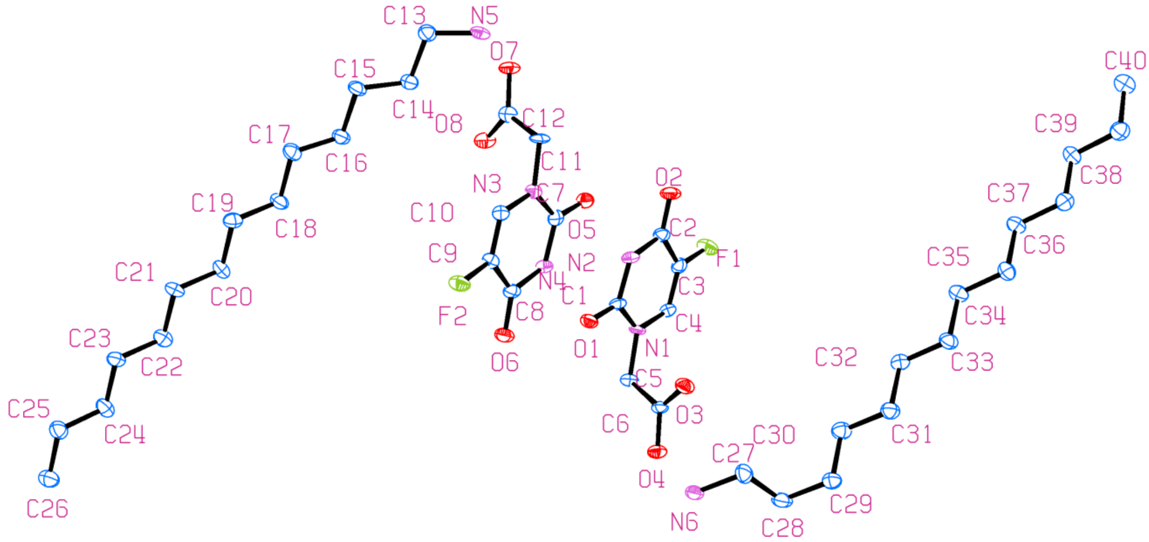


Figure S32: ORTEP Plot of **FuA-14** (50% probability).

Table S9: Hydrogen Bonding Parameter table of **FuA-14**

Table S9 : Hydrogen Bonds for **FuA-14**						
D	**H**	**A**	**d(D-H)/Å**	**d(H..A)/Å**	**d(D..A)/Å**	**<D-H-A/°**
N2	H2	O5	0.880	1.974	2.854	177.42
N4	H4	O1	0.880	1.900	2.779	178.15
N5	H5A	O7	0.910	1.882	2.776	167.07
N5	H5B	O1[1]	0.910	2.556	3.322	142.06
N5	H5B	O6[2]	0.910	2.479	2.986	115.51
N5	H5C	O7[3]	0.910	1.938	2.845	174.68
N6	H6A	O4[4]	0.910	2.003	2.819	148.41
N6	H6B	O2[5]	0.910	2.644	3.123	113.72
N6	H6C	O4	0.910	1.865	2.767	170.87
C10	H10	O8[6]	0.950	2.256	3.120	150.79
C11	H11A	O4[7]	0.990	2.414	3.276	145.21
C11	H11B	O8[8]	0.990	2.383	3.290	152.03
C5	H5D	O3[9]	0.990	2.374	3.280	151.95
C5	H5E	O7[10]	0.990	2.513	3.349	142.02
C4	H4A	O3[11]	0.950	2.239	3.105	151.24
C14	H14B	O8	0.990	2.459	3.403	159.31
C28	H28B	F1[12]	0.990	2.528	3.467	158.35
C13	H13A	F2[13]	0.990	2.579	3.244	124.44
C27	H27B	O3	0.990	2.615	3.352	131.23

[1][x, y+1, z], [2][x, y+1, z], [3][x+1, y, z], [4][x-1, y, z], [5][x-1, y-1, z], [6][x-1, y, z], [7][x-1, y+1, z], [8][x-1, y, z], [9][x+1, y, z],[10][x+1, y-1, z], [11][x+1, y, z], [12][x-1, y-1, z], [13][x+1, y+1, z].

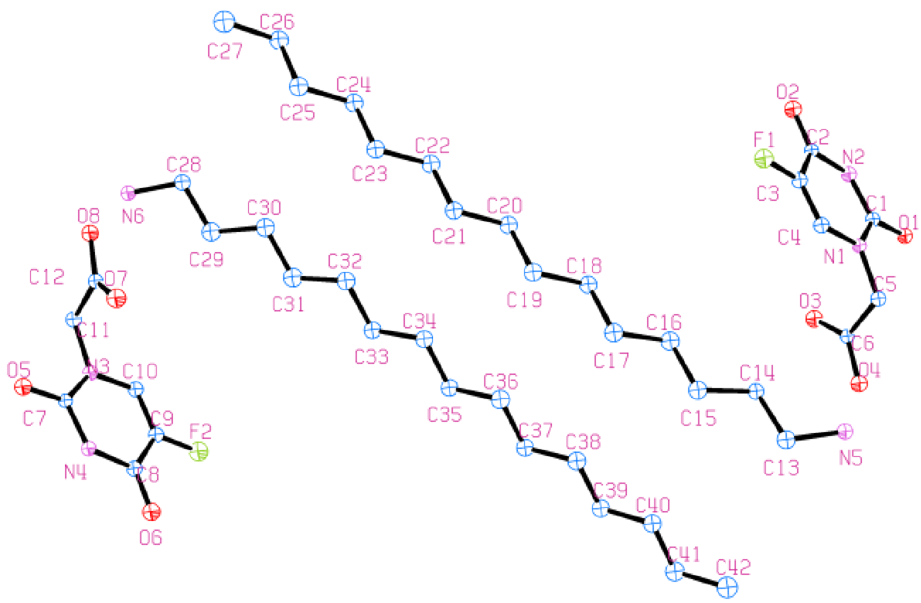


Figure S33: ORTEP Plot of **FuA-15** (50% probability).

Table S10: Hydrogen Bonding Parameter table of **FuA-15**

Table S10 : Hydrogen Bonds for FuA-15						
D	**H**	**A**	**d(D-H)/Å**	**d(H..A)/Å**	**d(D..A)/Å**	**<D-H-A/°**
N4	H4A	O5[1]	0.880	1.931	2.810	177.94
N5	H5A	O1[2]	0.910	2.519	3.259	138.78
N5	H5A	O2[3]	0.910	2.543	2.992	110.98
N5	H5B	O4[4]	0.910	1.867	2.773	173.94
N5	H5C	O4	0.910	1.947	2.854	174.60
N6	H6A	O8[5]	0.910	1.924	2.828	172.36
N6	H6B	O6[6]	0.910	2.450	2.948	114.63
N6	H6B	O5[7]	0.910	2.512	3.259	139.55
N6	H6C	O8	0.910	1.890	2.785	167.07
N2	H2	O1[8]	0.880	1.930	2.809	176.94
C14	H14B	O3[9]	0.990	2.398	3.340	158.72
C4	H4	O3[10]	0.950	2.244	3.106	150.46
C11	H11A	O8[11]	0.990	2.455	3.321	145.76
C11	H11B	O7[12]	0.990	2.346	3.248	151.00
C10	H10	O7[13]	0.950	2.231	3.080	148.34
C5	H5D	O3[14]	0.990	2.414	3.313	150.63
C5	H5E	O4[15]	0.990	2.476	3.328	143.97
C29	H29A	O7[16]	0.990	2.611	3.179	116.55

[1][-x+2, -y+1, -z+1], [2][-x+1, -y+1, -z], [3][x+1, y-1, z], [4][x+1, y, z], [5][x-1, y, z], [6][x-1, y+1, z], [7][-x+1, -y+2, -z+1], [8][-x, -y+2, -z], [9][x+1, y, z], [10][x+1, y, z], [11][-x+1, -y+2, -z+1], [12][x-1, y, z], [13][x-1, y, z], [14][x+1, y, z], [15][-x+1, -y+1, -z], [16][x-1, y, z].