Electronic Supplementary Material(ESI)

Lysine-based non-cytotoxic ultrashort self-assembling peptides with antimicrobial activity

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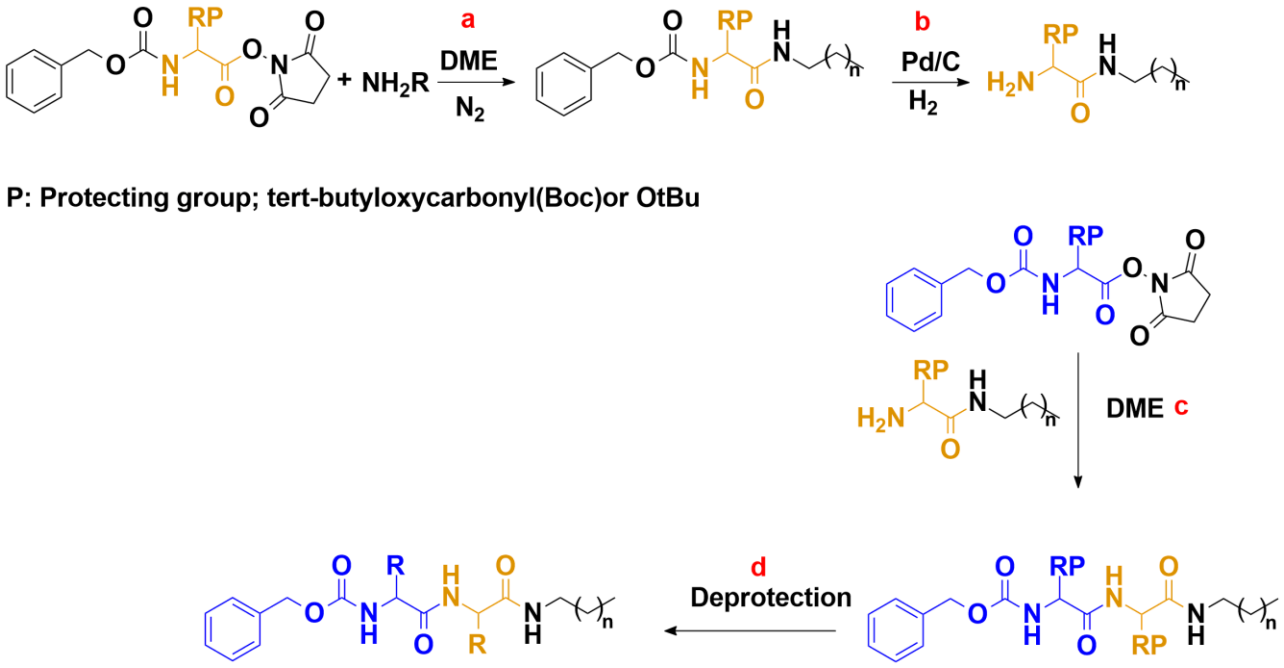
Synthesis and characterization of 1-4

SCHEME S1



a) Coupling of Z-Lys(Boc)-Osu

The N-hydroxysuccinimide ester(Z-Lys(Boc)-OSu, 10.47 mmol, 1eq.) was dissolved in DME(20 mL), then corresponding amine with the C-terminal group as propylamine(12.56 mmol, 1.2 eq) was dissolved in DME(10 mL) and added drop wise. The resulting solution was stirred for 48 h in total. The solvent was evaporated by rotary, the resulting solid(white matter) was dissolved in CH_2Cl_2(20 mL) and washed with 0.1 M Na_2CO_3, 0.1 M HCl and water respectively. The final product was dried under vacuum.

b) N-benzyloxycarbonyl deprotection of the Z-Lys(Boc)-$NHCH_2CH_2CH_3$ (general procedure for N-benzyloxycarbonyl deprotection)

The corresponding N-benzyloxycarbonyl protected peptide derivative (Z-Lys(Boc)-$NHCH_2CH_2CH_3$, 9.29 mmol) and catalytic amount of Pd over activated carbon(5-10 % w/w) were placed in a two necked round bottom flask and suspended in MeOH(30 mL). The system

was purged to remove the air with N_2 and connected to H_2 atmosphere.The black-grey suspension was continued 24h in total. The black suspension was filtered over Celite and the solvent was evaporated by rotary, the resulting oil was dried further in vacuum pump overnight.

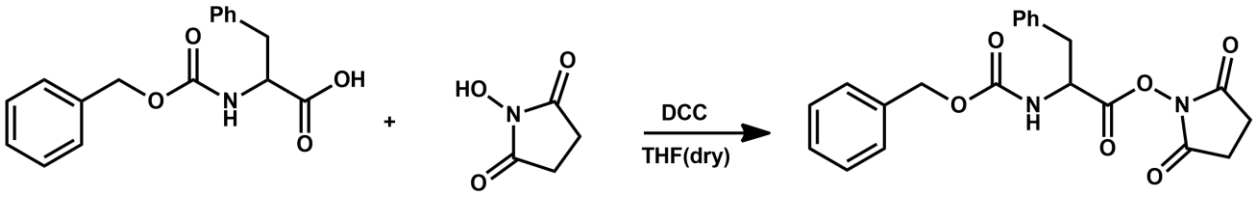
c) Coupling of Z-Phe-Osu

The N-hydroxysuccinimide ester(Z-Phe-OSu, 10 mmol, 1.1eq.) was dissolved in DME(30 mL), then corresponding amine with the C-terminal group as an amine-H2N-Lys(Boc)$NHCH_2CH_2CH_3$(9.1 mmol, 1eq.) was dissolved in DME(10 mL) and around 10 mL of THF was added to solve the reaction mixture well. The resulting solution was stirred at room temperature overnight. The matter(white solid) was a concentrated precipitate, then it was filtered on the crucible(N:3) and washed with 0.1 M Na_2CO_3, 0.1 M HCl and water respectively. The final product was dried under vacuum overnight at 40 °C.

d) Boc group deprotection of Z-Phe-Lys(Boc)NHC_3. HCl

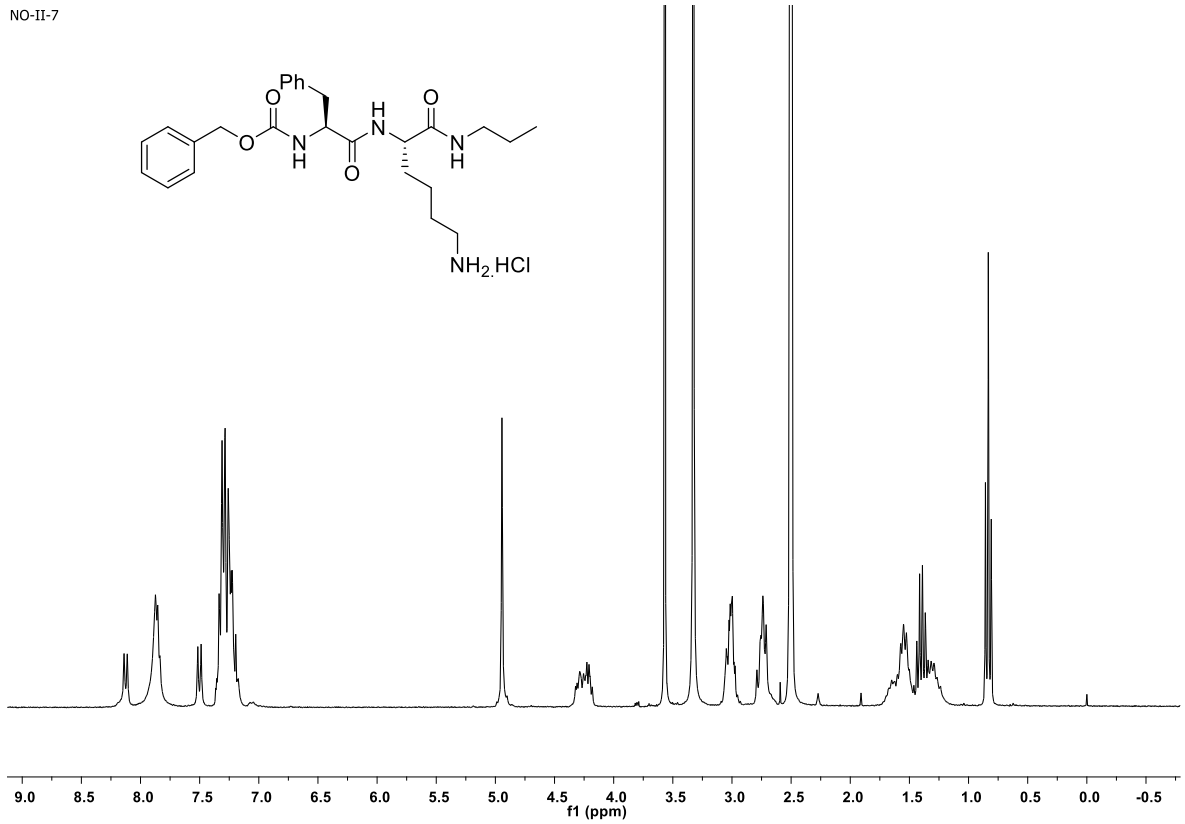
Z-Phe-Lys(Boc)NHC_3 (6.82 mmol) was dissolved in 1,4-dioxane(30 mL) under the N_2 atmosphere, then 4M HCl in 1,4-dioxane(15.7 mL) added dropwise. The reaction mixture was stirred at room temperature overnight. Resulting matter filtered on crucible(No:3) and dried under the vacuum at 40 °C overnight.

Activation of Z-Phe-OH

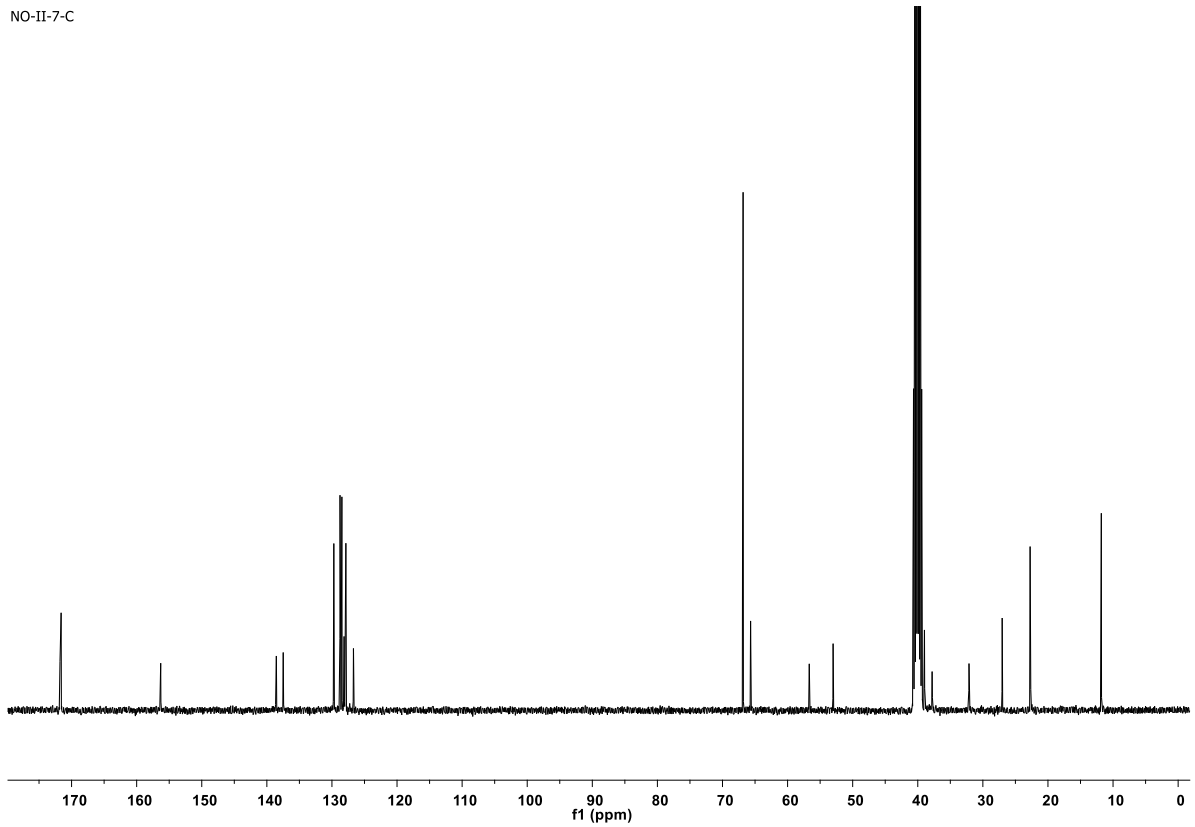


The corresponding Z-protected amino acid (Z-Phe-OH;33.4 mmol, 1eq.) and N-hydroxysuccinimide (33.4 mmol, 1eq.) were dissolved in dry THF at 0 °C (~ 30 min). Once a clear solution is obtained, dicyclohexylcarbodiimide(DCC) (33.4 mmol, 1eq.) in anhyrous THF was added drop by drop and the resulting solution was stirred at 0-5 °C in ice-bath for 1 h, and allowed to warm up room temperature with vigorous stirring 48h. The dicyclohexylurea(DCU) formed was filtered off and the filtrate was concentrated to dryness through the rotary. The crude product recrystallized from 2-propanol to furnish the pure product. Then it was washed some 2-propanol via crucible then dried into the oven at 40 °C overnight.

1

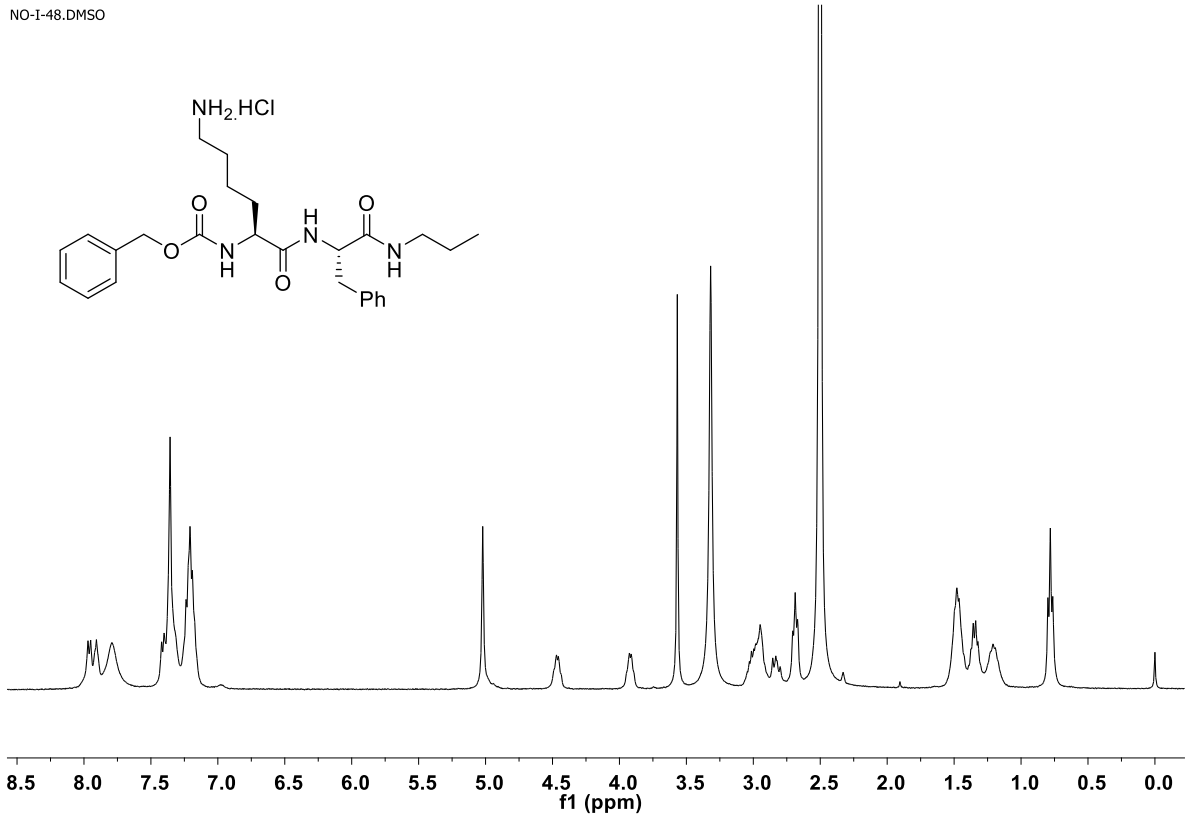


^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.13 (d, *J* = 8.2 Hz, 1H), 8.01 – 7.72 (m, 1H), 7.5 (d, *J* = 8.4 Hz, 1H), 7.4 – 7.10 (m, 10H), 4.94 (s, 2H), 4.36 – 4.15 (m, 2H), 2.99 (m, 3H), 2.75 (m, 3H), 1.73 – 1.45 (m, 4H), 1.47 – 1.20 (m, 4H), 0.83 (t, *J* = 7.4 Hz, 3H).

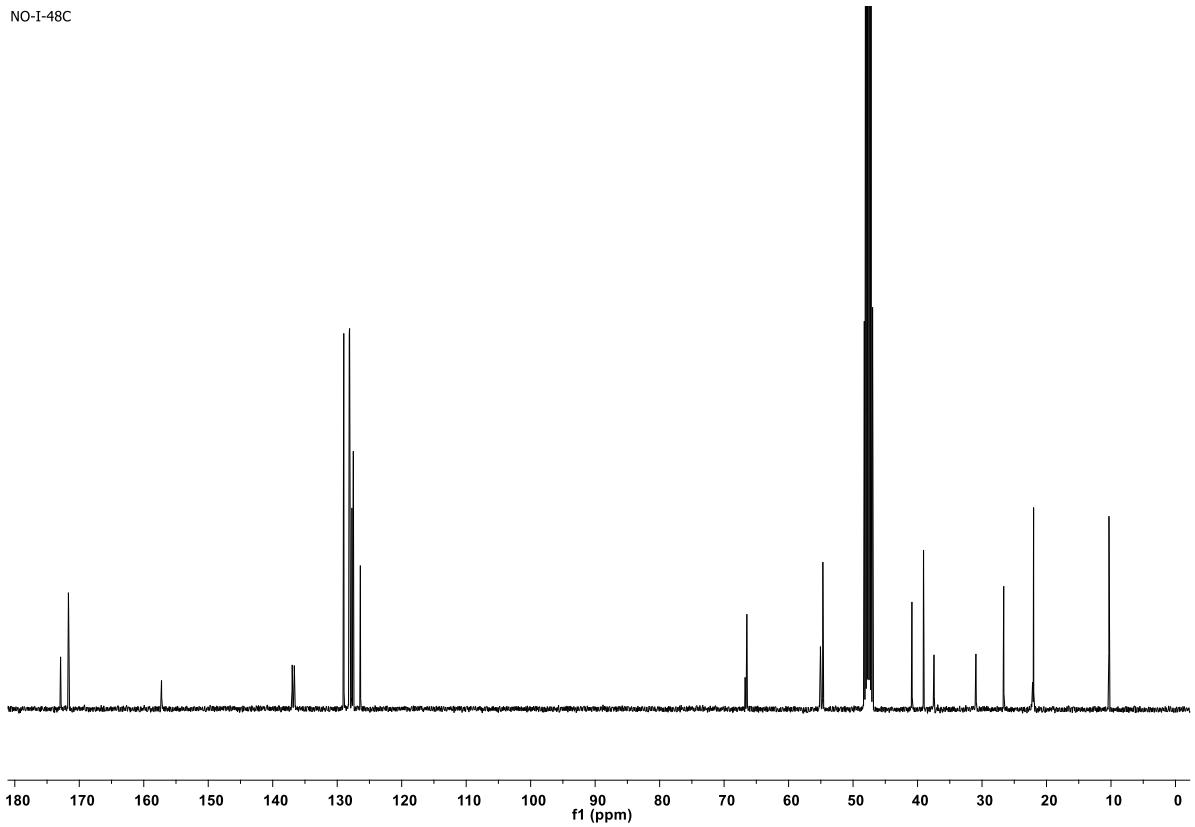


^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.7 (2C), 156.3, 138.5, 137.5, 128.3 (10C), 66.8, 65.7, 56.7, 53.0, 37.8, 32.1, 27.0, 22.7 (2C), 11.8.

2

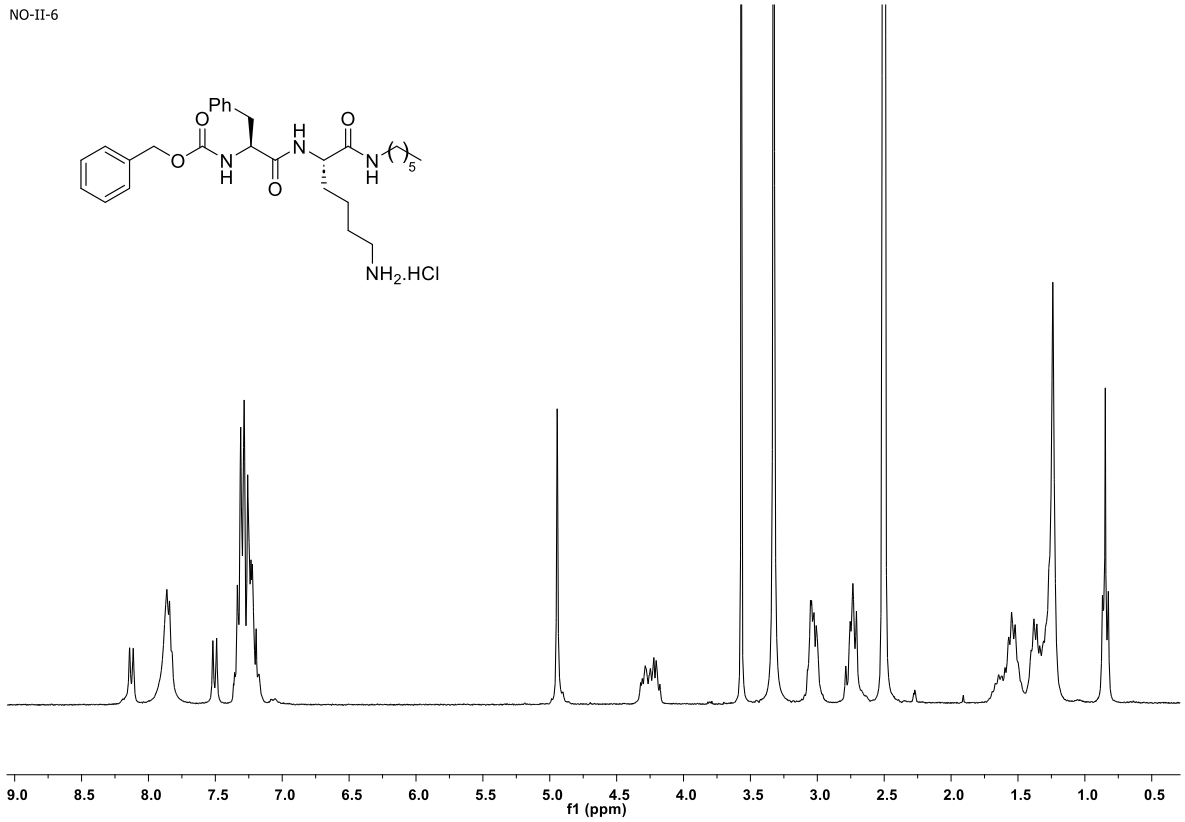


^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.96 (d, *J* = 7.9 Hz, 1H), 7.91 (s, 1H), 7.8 (s, 1H), 7.47 – 7.11 (m, 10H), 5.02 (s, 2H), 4.46 (m, 1H), 3.92 (m, 1H), 2.98 (m, 3H), 2.89 – 2.76 (m, 1H), 2.75 – 2.62 (m, 2H), 1.59 – 1.39 (m, 4H), 1.40 – 1.28 (m, 4H), 1.21 (s, 1H), 0.78 (t, *J* = 7.2 Hz, 3H).

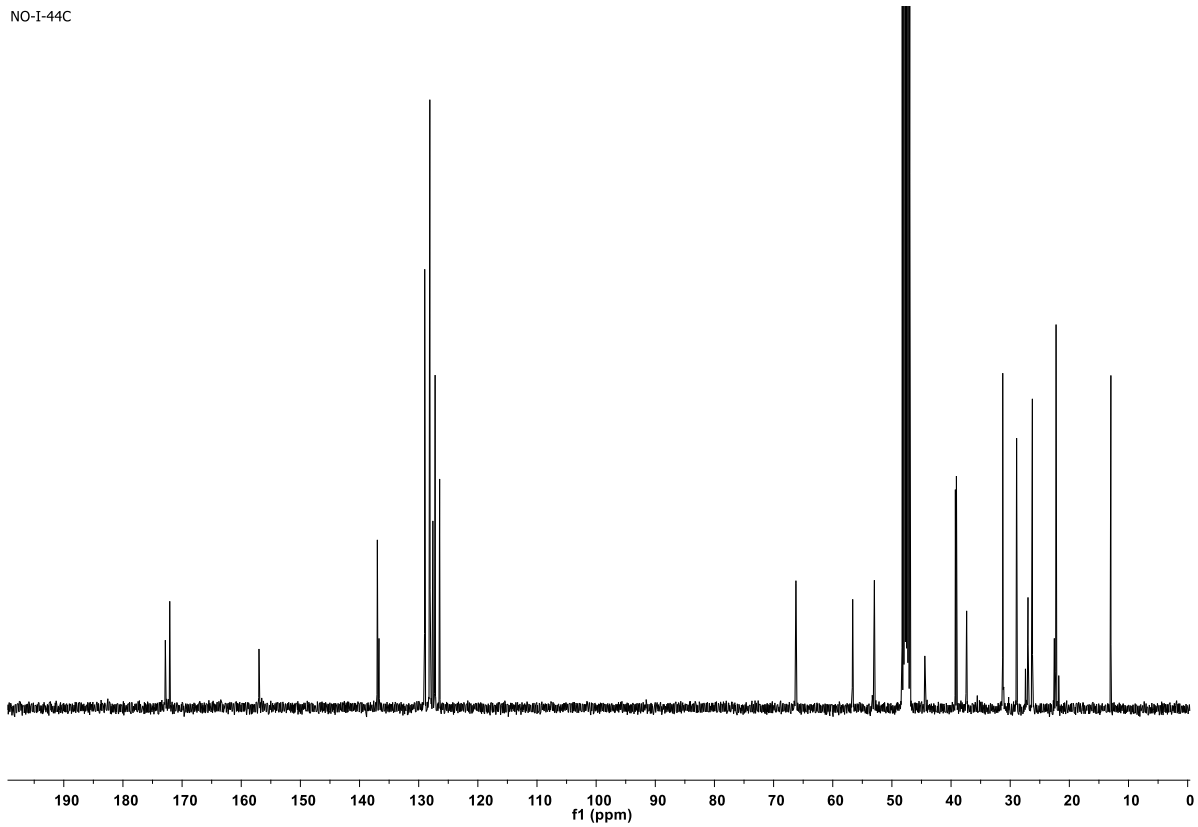


^{13}C NMR (101 MHz, MeOD-d_4) δ = 172.3 (2C), 157.2, 136.8 (2C), 130 – 124.6 (10C), 66.5, 54.9 (2C), 40.9, 39.1, 37.4, 30.9, 26.6, 22.1 (2C), 10.3.

3

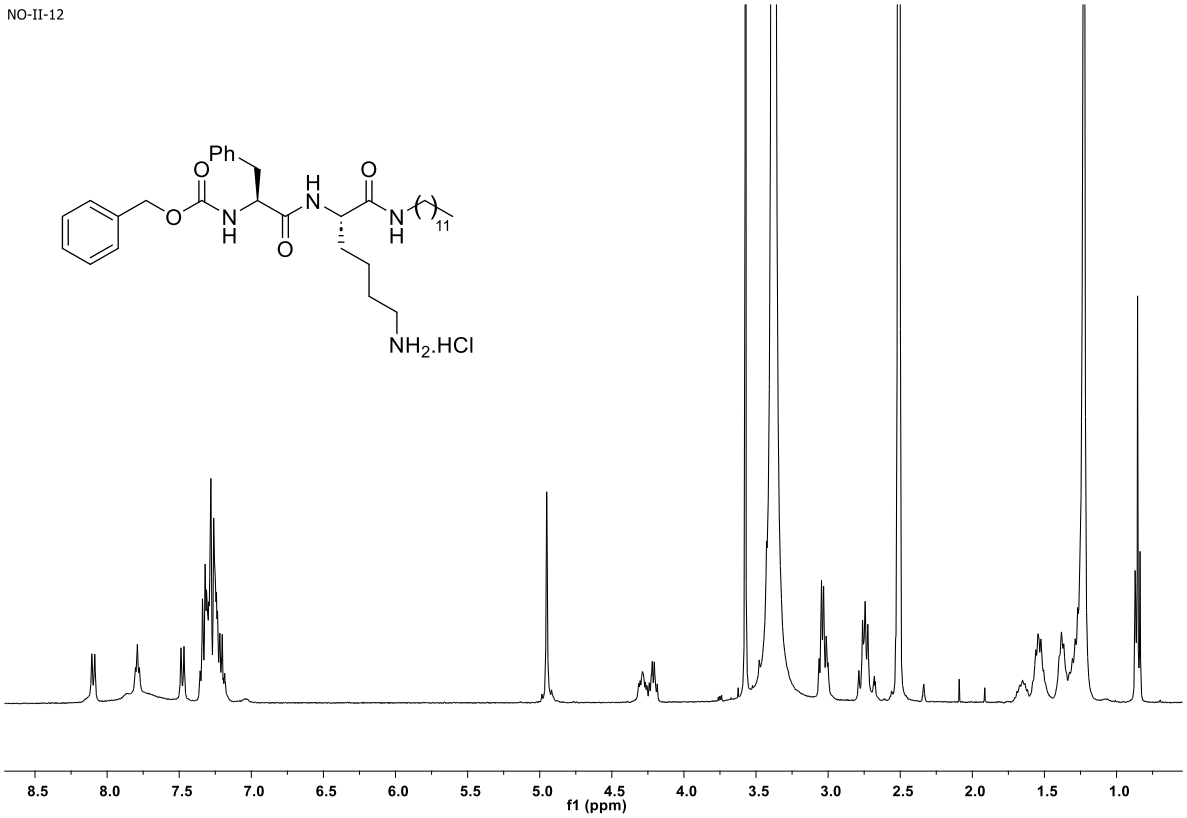


^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.11 (d, *J* = 8.1 Hz, 1H), 7.83 (m, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.39 – 7.11 (m, 10H), 4.93 (s, 2H), 4.34 – 4.25 (m, 1H), 4.23 – 4.13 (m, 1H), 3.1 – 2.99 (m, 3H), 2.80 – 2.66 (m, 3H), 1.63 (m, 1H), 1.52 (m, 3H), 1.37 (m, 3H), 1.25 (m, 7H), 0.85 (t, *J* = 6.7 Hz, 3H).

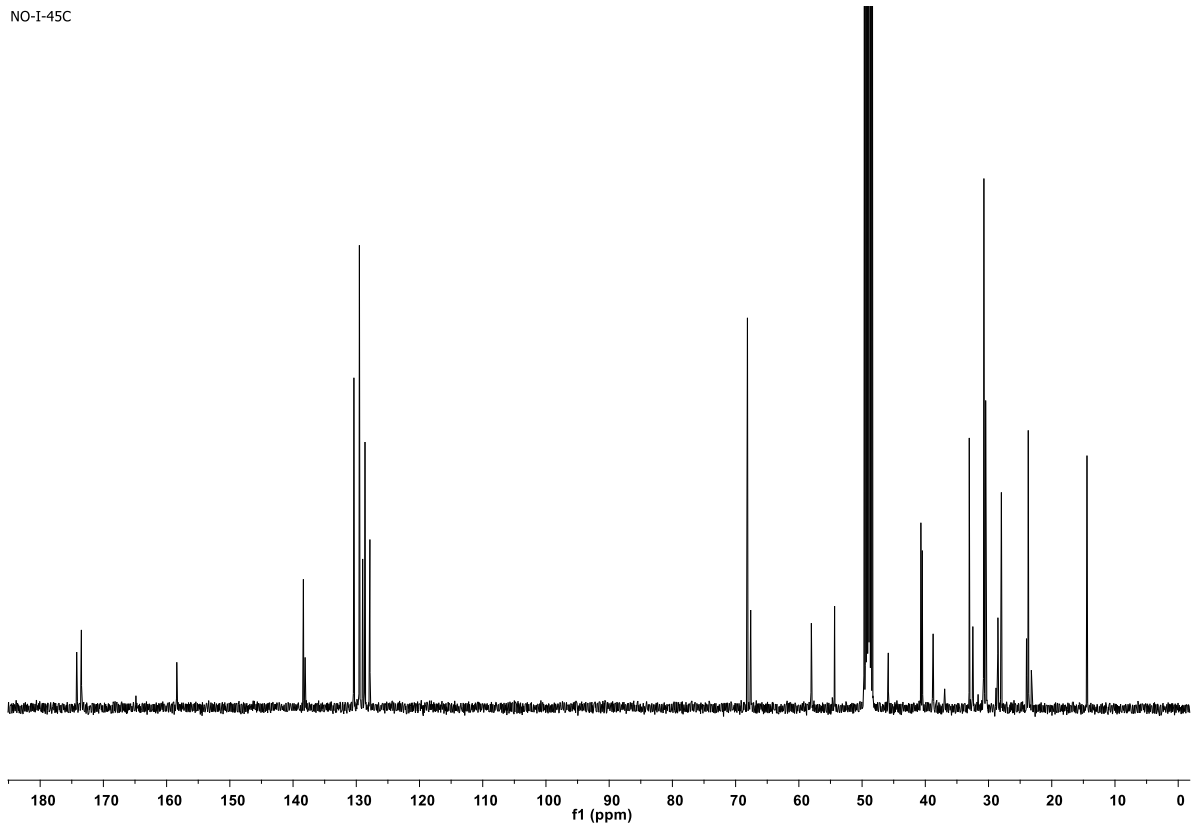


^{13}C NMR (101 MHz, MeOD-d_4) δ = 172.8, 172.1, 157.0, 136.8 (2C), 129.6 – 125.6 (10C), 66.2, 56.6, 53.0, 39.3, 39.1, 37.4, 31.2 (2C), 28.9, 27.0, 26.2, 22.3 (2C), 13.0.

4



^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.12 (d, *J* = 8.1 Hz, 1H), 7.82 (m, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.39 – 7.14 (m, 10H), 4.95 (s, 2H), 4.28 (m, 1H), 4.22 (m, 1H), 3.04 (m, 3H), 2.71 (m, 3H), 1.63 (s, 1H), 1.58 – 1.47 (m, 3H), 1.38 (m, 3H), 1.23 (m, 19H), 0.86 (t, *J* = 6.8 Hz, 3H).



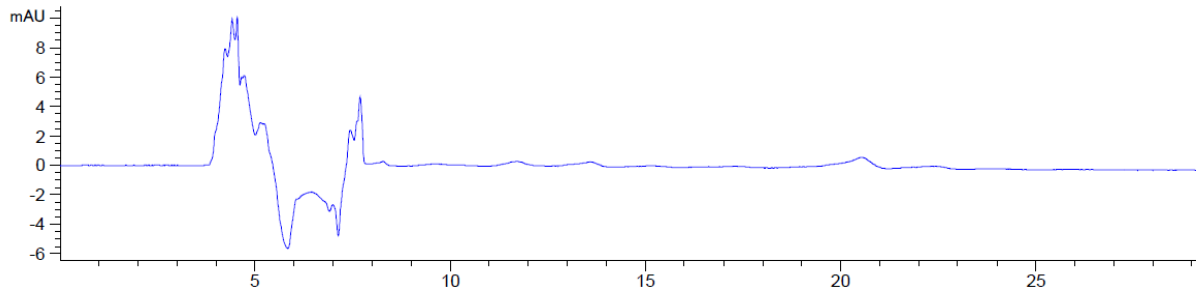
^{13}C NMR (101 MHz, MeOD-d_4) δ = 172.8, 172.1, 157.0, 137.0, 136.7, 129.7 – 126.4 (10C), 66.7, 56.6, 53.0, 39.3, 39.1, 37.4, 31.7, 31.1, 29.2(7C), 27.1, 26.6, 22.3 (2C), 13.0.

High Performance Liquid Chromatography(HPLC)

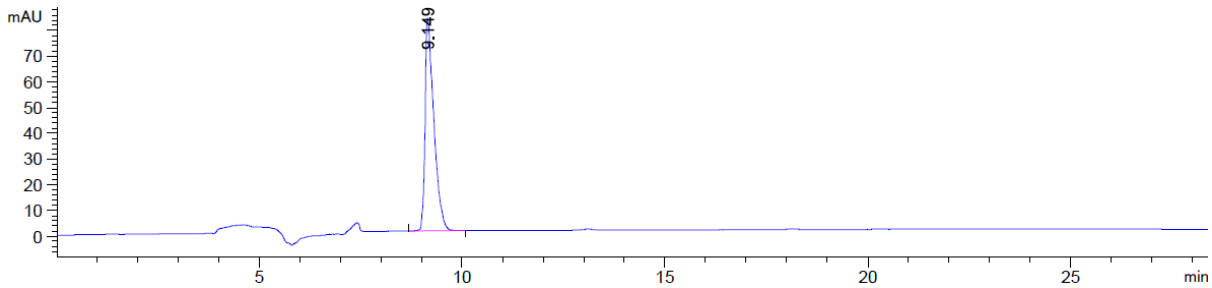
The purity of the compounds was confirmed by reverse-phase analytical HPLC on a Prontosil NC (250 × 4.6 mm) C18 column (3 µm pore size). A mixture of water (50%) and acetonitrile (50%) with 0.5 % TFA at 0.5 mL/min was used to elute the compounds **1**-**3**. Compound **4** was eluted in a mixture of water (20%) and acetonitrile (80%) with 0.5 % TFA at 0.5 mL/min. The eluent was monitored by measuring the UV absorbance at 254 nm. The column temperature was maintained at 30 °C.

HPLC chromatograms

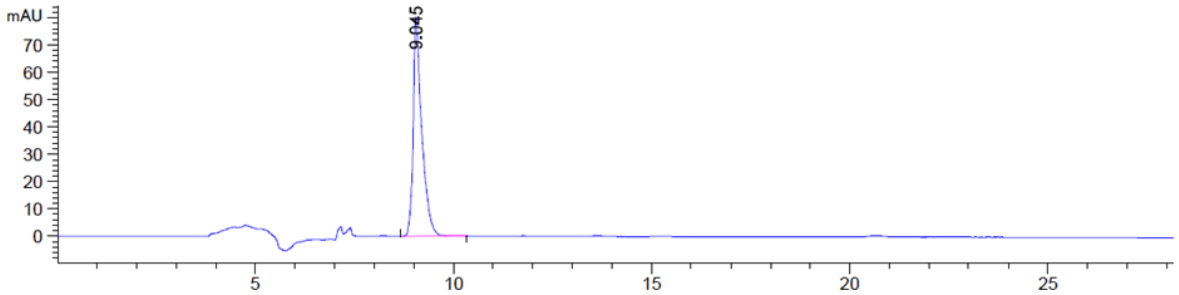
Methanol



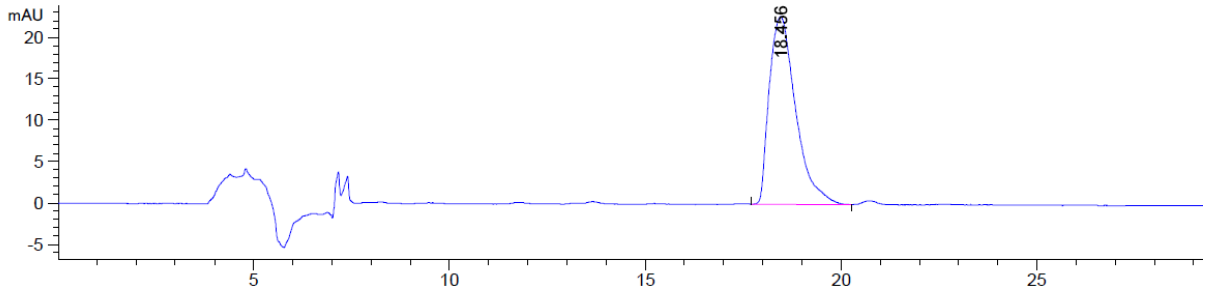
Compound 1



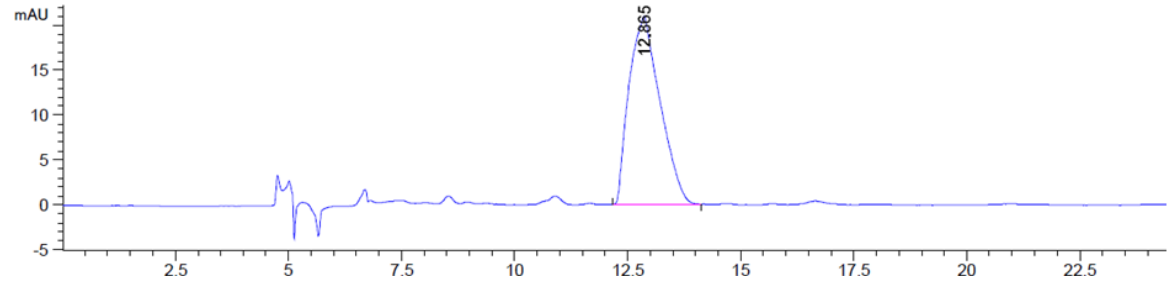
Compound 2



Compound 3

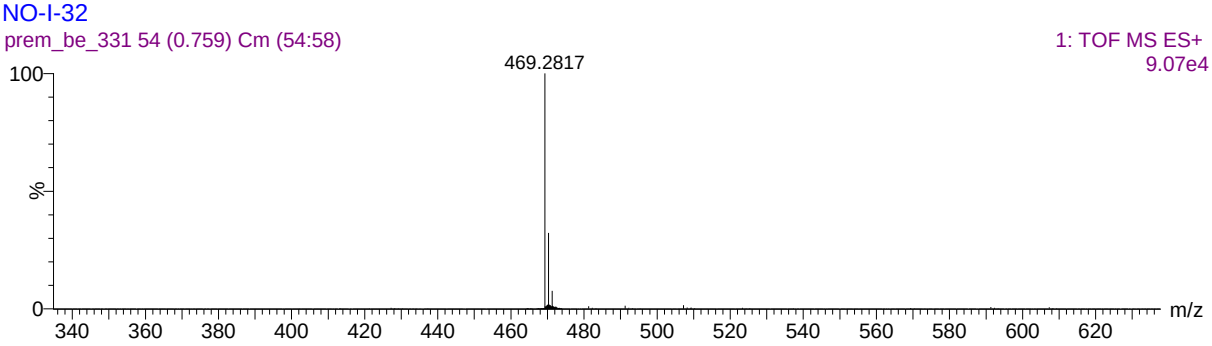


Compound 4



Mass Spectroscopy Results

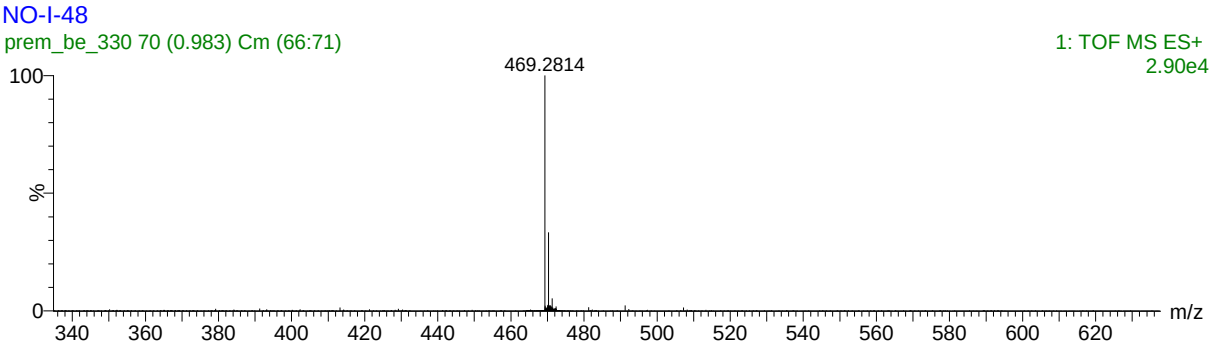
Compound 1



$[M+H]^+$=469.2814

$[M+H]^+$=469.2817 (0.4 ppm)

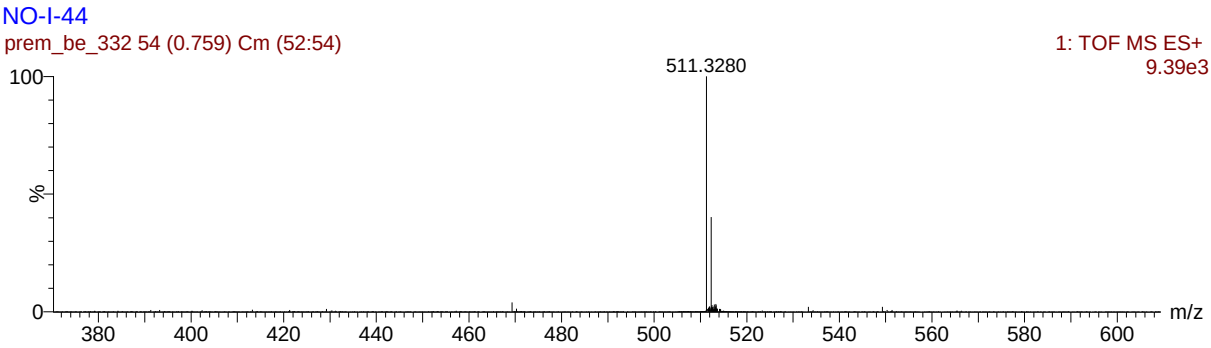
Compound 2



$[M+H]^+$=469.2814

$[M+H]^+$=469.2815 (0.2 ppm)

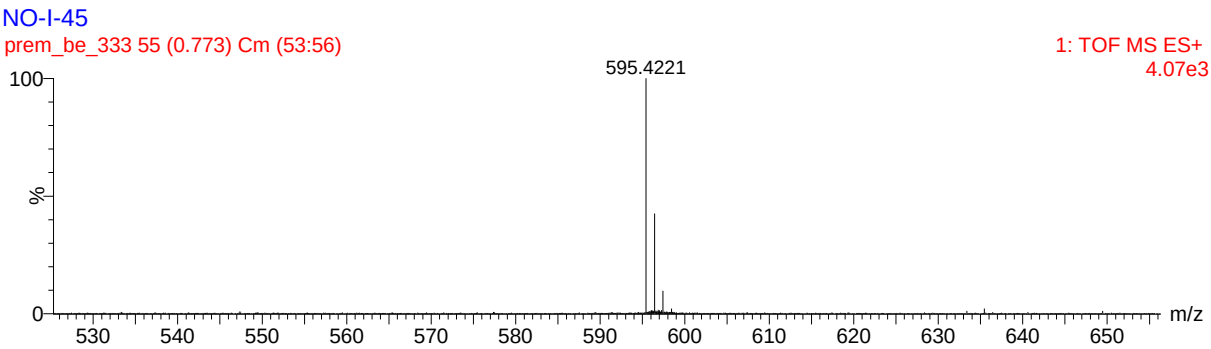
Compound 3



$[M+H]^+$=511.3284

$[M+H]^+$=511.3280 (0.8 ppm)

Compound 4



$[M+H]^+$=595.4223

$[M+H]^+$=595.4221 (0.3 ppm)

Table S1: Determination of the minimum gel concentrations (mgc) and critical aggregation concentration (cac, in 0.1 M PBS pH = 7.4) for compounds **1-4**

Compound	mgc	Cac
1	10 mM	1 mM
2	2.85 mM	1.5 mM
3	0.5 mM	0.2 mM
4	suspension[a]	0.47 mM

a: samples studied between 1 to 15 mM

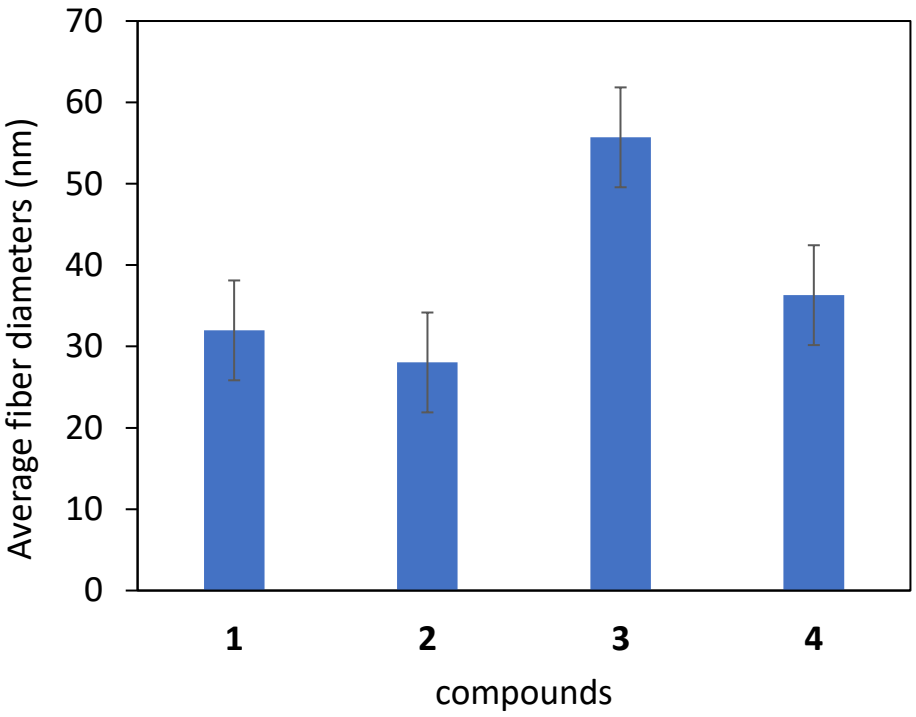
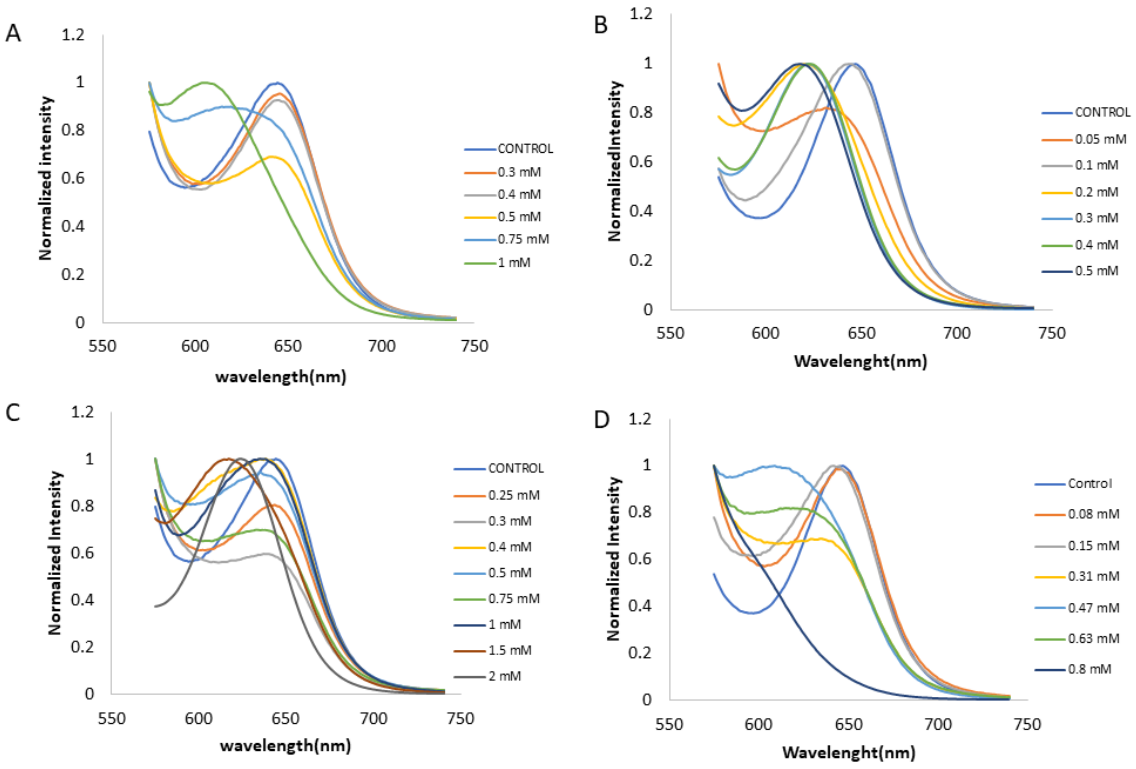


Figure S1: Average fiber diameters of **4**(1.6 mM) and **1**-**3** at their mgc in PBS.

Determination of critical aggregation concentration (cac): samples at different concentrations for each sample (below than mgc) were prepared in PBS and transferred to the 1 cm path length PMMA fluorescence cells (final volume 2 mL). After, the hydrophobic probe Nile red 4 µM was added to each sample and fluorescence spectra were recorded from 575 to 740 nm with the excitation at 550 nm. The shift at λ_{max} is a useful way to monitor the participation of the aromatic fragment in π-stacking interactions and indicating the cac for each sample.



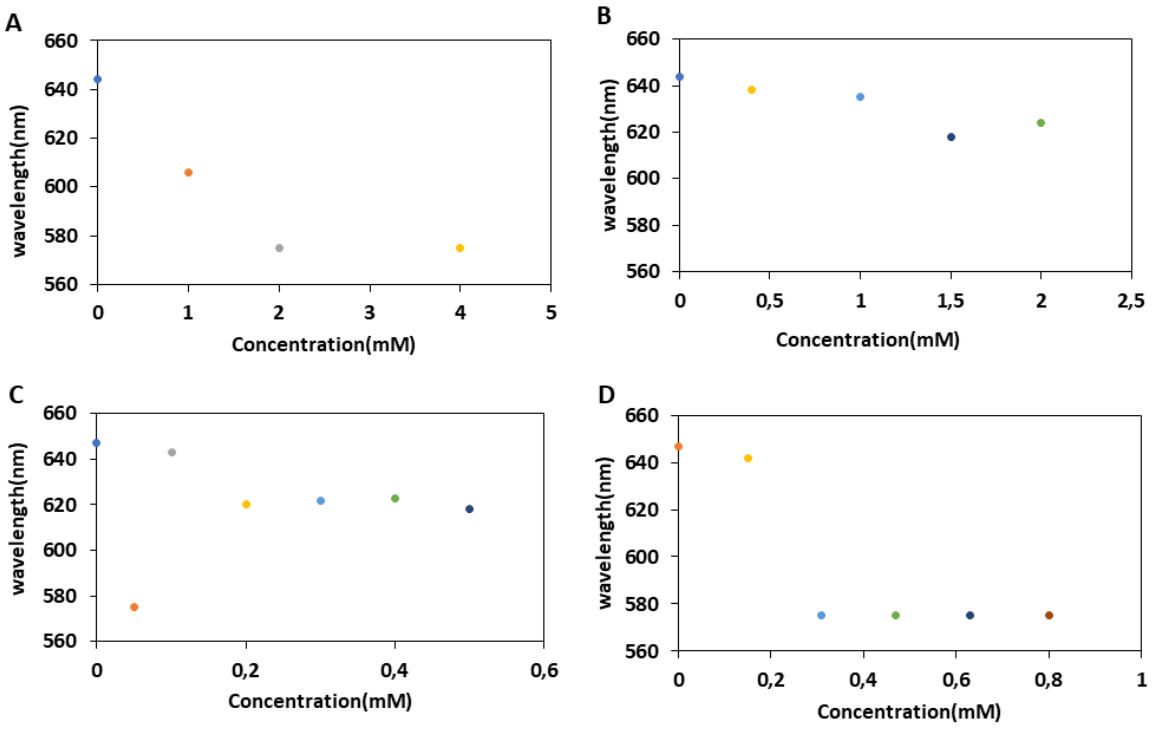


Figure S2: Determination of critical aggregation concentration (cac) against to the control (0.1 M PBS, pH 7.4; λ_{max} = 644 nm) with Nile Red **A**) **1;** 1 mM; λ_{max} = 606 nm **B**) **2**; 1.5 mM; λ_{max} = 615 nm, **C**) **3**; 0.2 mM; λ_{max} = 620 nm and **D**) **4;** 0.47 mM; λ_{max} = 609 nm**.**

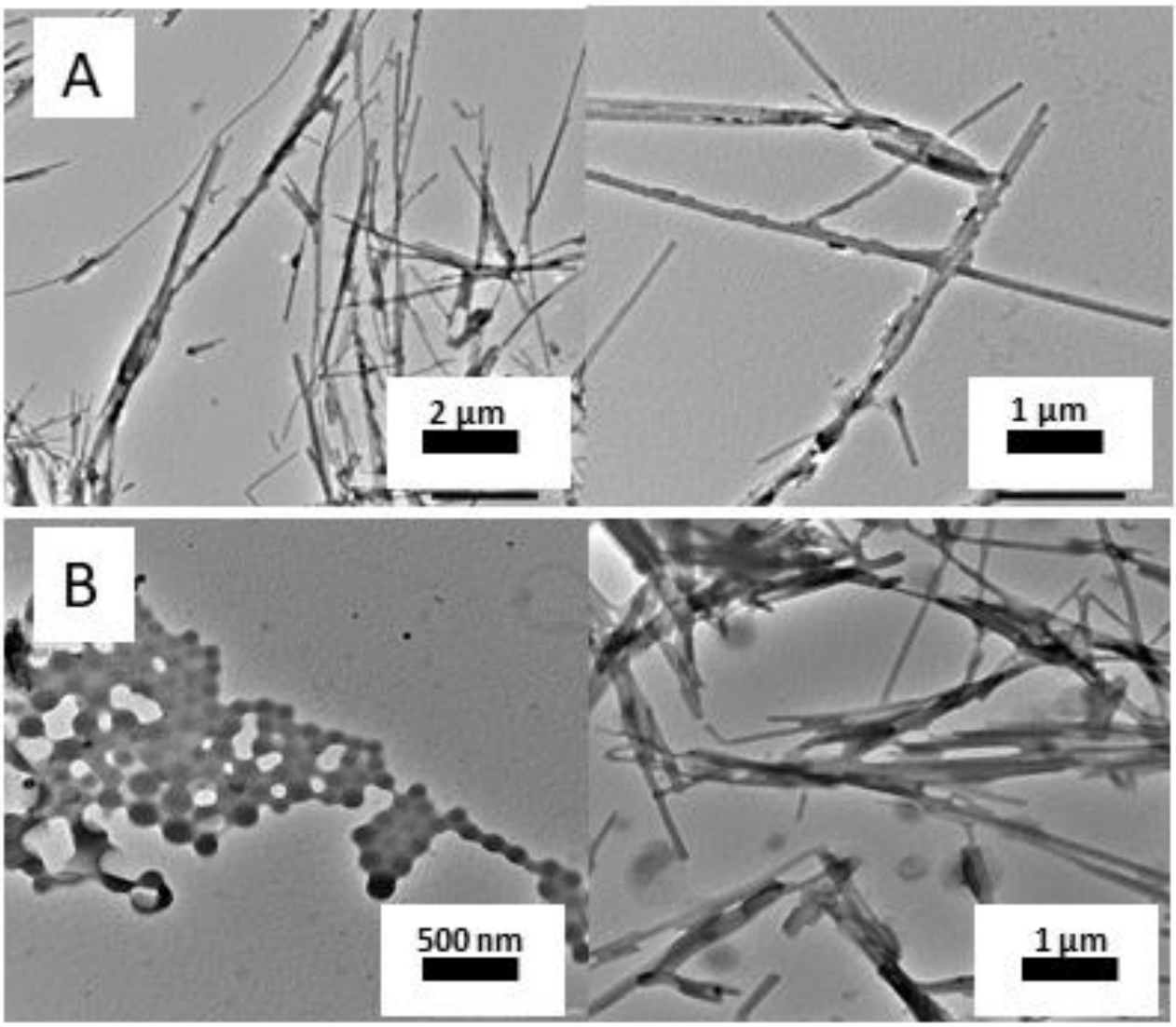


Figure S3: TEM micrographs of **4** at 10 mM in PBS (0.1 M, pH = 7.4) (A) the sample was dissolved by heating and (B) the sample was only vortexed.

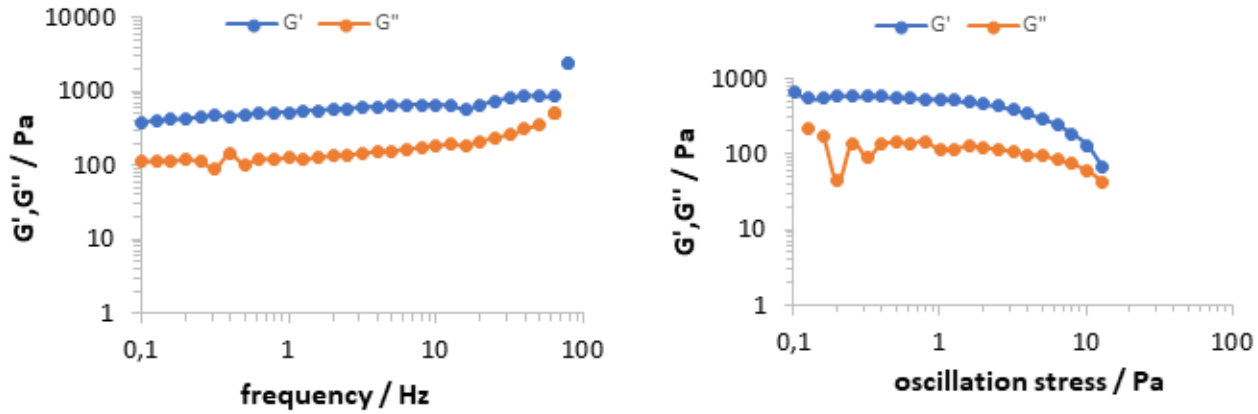


Figure S4: Oscillatory rheology data for hydrogels of **1**; 10 mM in PBS.

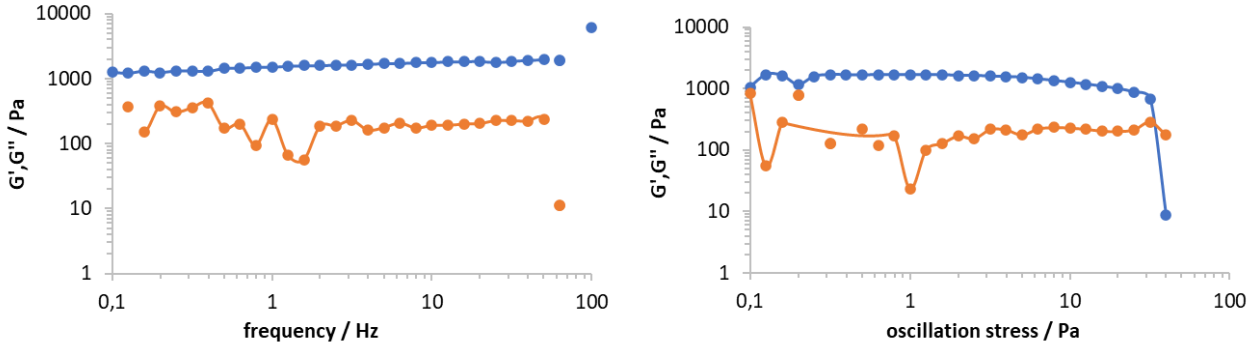


Figure S5: Oscillatory rheology data for hydrogels of **2**; 2.85 mM in PBS.

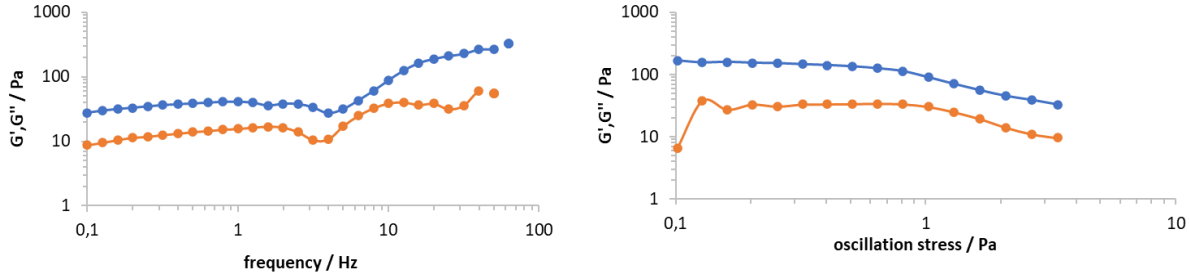


Figure S6: Oscillatory rheology data for hydrogels of **3**; 0.5 mM in PBS.

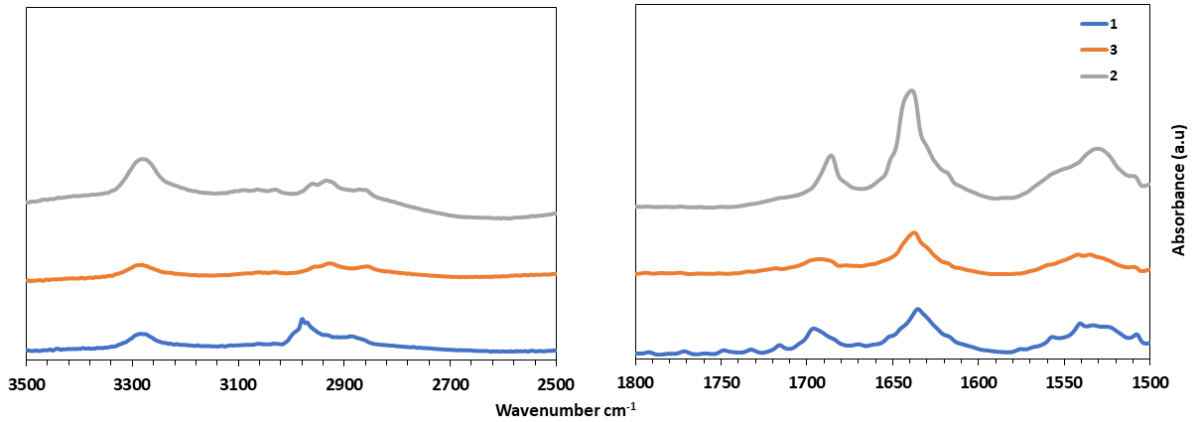


Figure S7: FT-IR spectra of lyophilized hydrogels in PBS; **1**(10 mM - blue line), **3**(0.5 mM- orange line) and **2**(2.85 mM – grey line)

Table S2: Selected FT-IR vibration bands.

xerogel	wavenumber (cm^{-1})
1	1539 1633 1695 (m) 2977 (s) 3328-3240 (b)
2	1527 1637 1685 (m) 2928 (w) 3314-3237 (b)
3	1531 1634 1704-1680 (b,w) 2920 (w) 3320-3219 (b, w)

Band intensity: (s) strong, (m) medium, (w) weak and (b) broad.

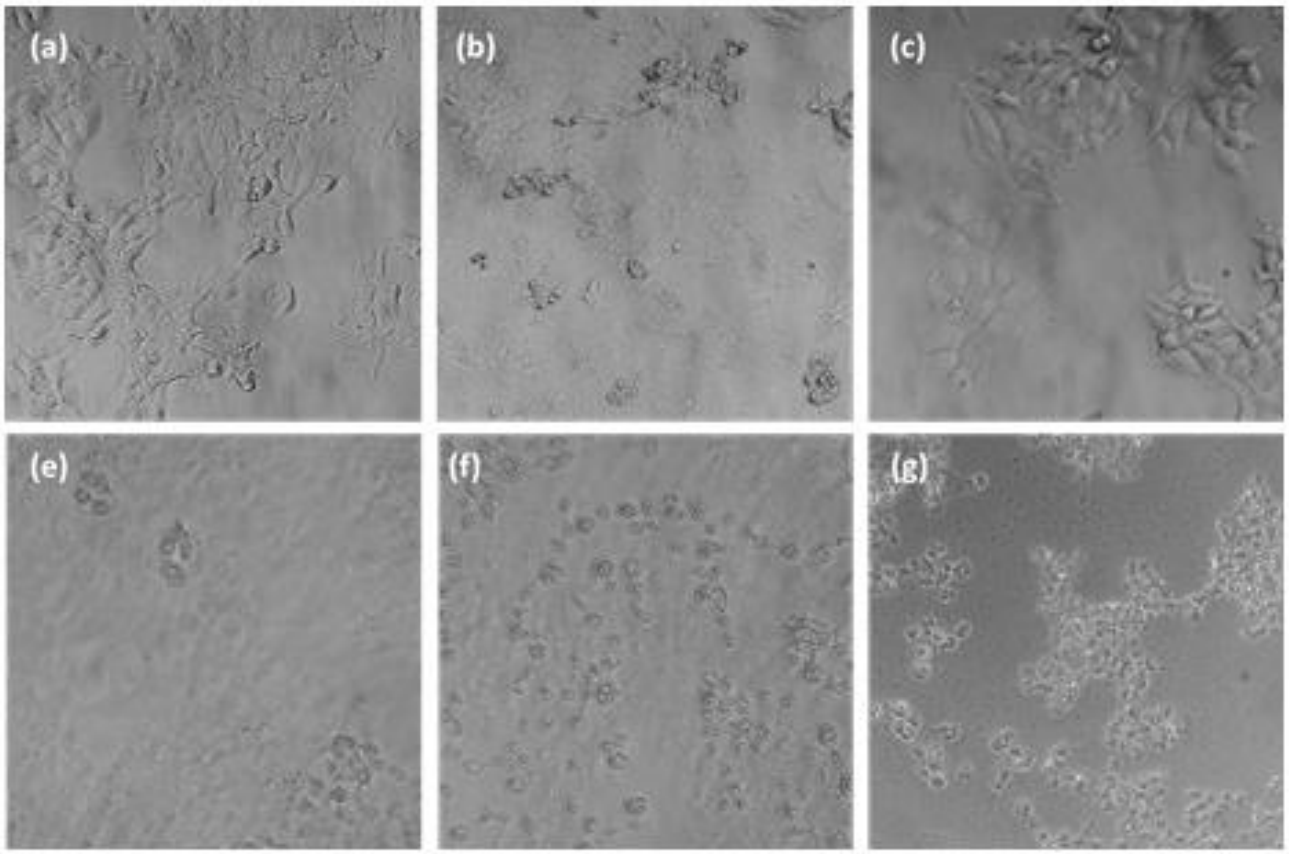


Figure S8: Morphological alterations in HEK-293 cells after 48 hours of treatment. (a) Control; (b) **4** at 400 µM; (c) **4** at 200 µM; (e) **3** at 400 µM (f) **3** at 200 µM; (g) **3** at 100 µM. (magnifications. ×20)

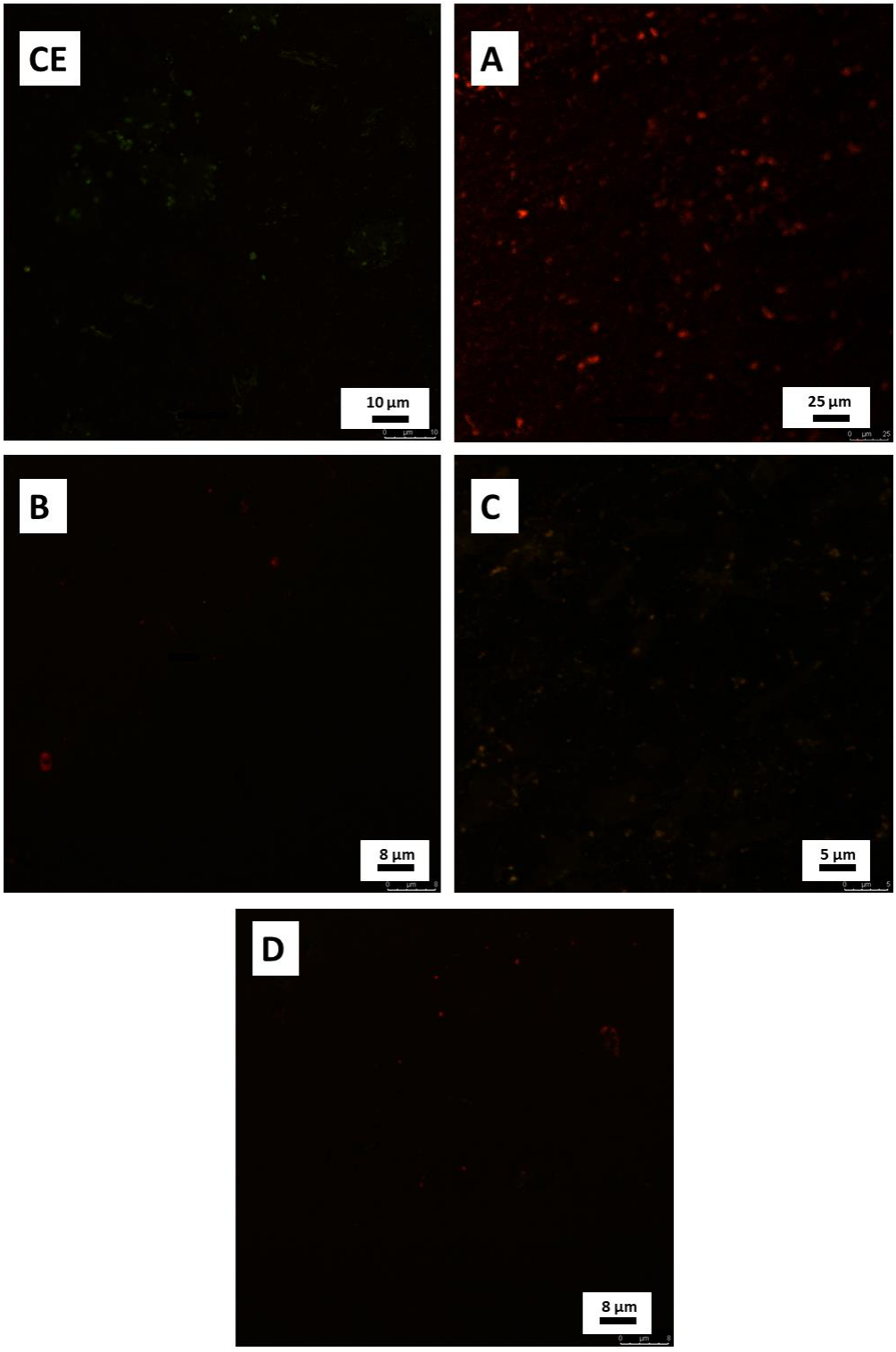


Figure S9: Confocal images of **1-4**; **1**(**A**), **2**(**B**), **3**(**C**) and **4**(**D**) against *E. coli*. The bacteria remained alive in the controls (green spots) and were damaged in the treatment (red staining). Scale bars = 5 to 25 µm.

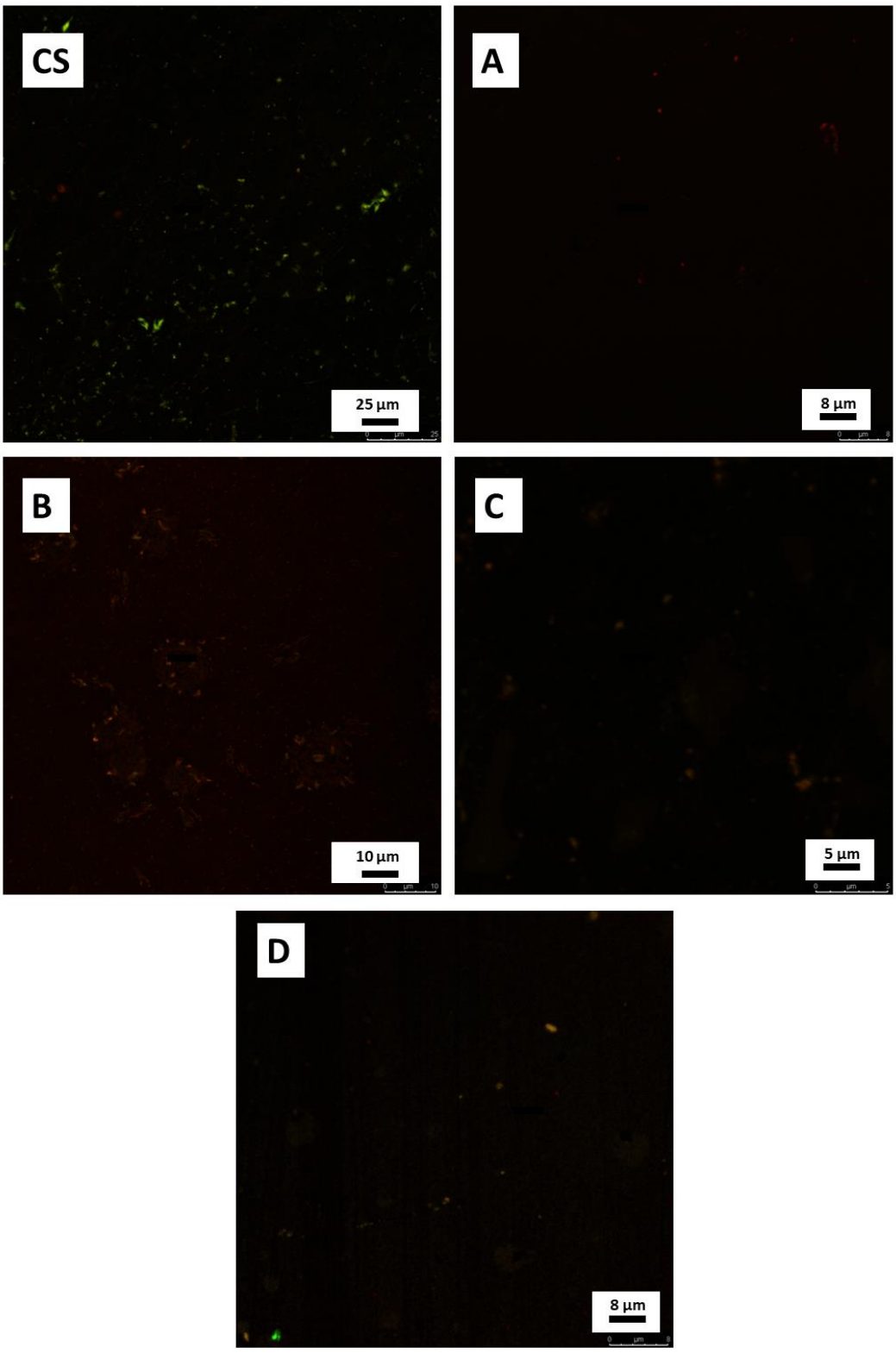


Figure S10: Confocal images of **1**-**4**; **1**(**A**), **2**(**B**), **3**(**C**) and **4**(**D**) against *S. aureus*. The bacteria remained alive in the controls (green spots) and were damaged in the treatment (red staining). Scale bars = 5 to 25 µm.

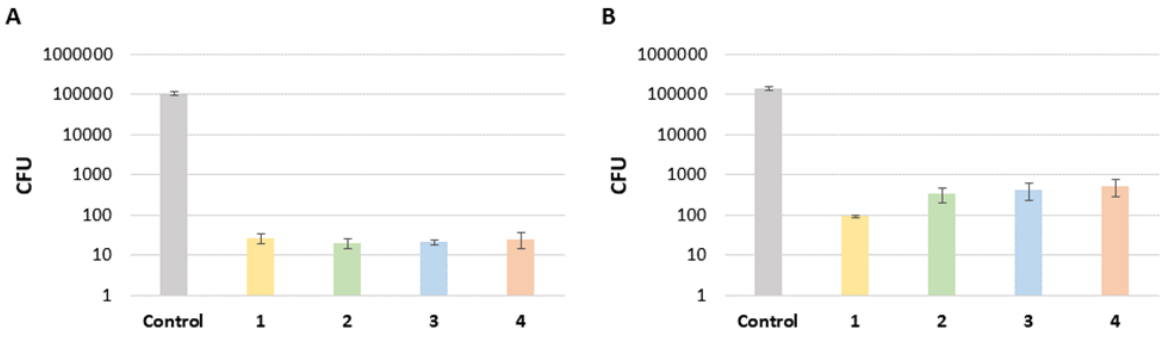


Figure S11. Antibacterial effect of **1**-**4** on the survival of *E. coli* (A) and *S. aureus* (B). CFU reduction achieved with each compound compared to control. The assays were performed in LB medium diluted 1:50.