Supporting information

Selective and sensitive staining of serum albumin in protein gel electrophoresis via fluorescent tagged sequence-defined oligo-dithiocarbamate

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Materials and methods

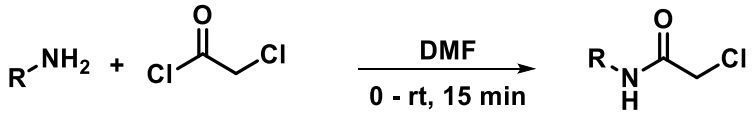
All the chemicals were purchased from Sigma Aldrich, Alfa Aeser, Spectrochem, Merck and TCI and used without further purification. LC-MS experiments were carried out on a Shimadzu LC-MS-8045 with a Sprite TARGA C18 column (40 × 2.1 mm, 5 µm) monitoring at 210 nm and 254 nm with positive mode for mass detection. Solvents for LC-MS were water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). Compounds were eluted at a flow rate of 0.5 mL/min with a gradient of 5% solvent B for 2 min, followed by a linear gradient from 5% to 40% solvent B over 4 min, followed by changing the solvent B from 40% to 60% for 10 min and finally, it was brought down to 5% solvent B in 2min and then continued till for 2 min before the method stopped. The column was washed with 50% Solvent B followed by 95% Solvent B always before sample injection. The purification by HPLC is performed on Shimadzu HPLC-20AP instrument by using the same solvent system as that of LC-MS. The method for preparative HPLC is as follows: The HPLC column was washed with 5% solvent B for 20 min, followed by 50% (15 min) and then by 95% (15 min) before sample injection. Afterwards, the compound was loaded and was eluted at a flow rate of 0.7 mL/min. For the first 5 min, the solvent B gradient was 5% solvent B for 2 min, followed by a linear gradient from 5% to 40% solvent B over 10 min. This was followed by an increase in the solvent B from 40% to 60% for next 10 min and finally, it was brought down to 5% solvent B in 2min and then continued till for 2 min before the method stopped. ^{1}H NMR spectra were recorded on Bruker AV III 500 MHz. The data were analyzed by MestReNova (version 8.1.1). ^{1}H NMR shifts are reported in units of ppm relative to tetramethyl silane. The data are presented in the order: chemical shift, peak multiplicity (s=singlet, d=doublet, t=triplet, m=multiplet) and proton number. Fluorescence was recorded on Perkin Elmer FL 6500. All fluorescence spectra are recorded at 25 ^{o}C with an excitation wavelength of 320 nm and slit width of 5 nm for excitation and emission. The fluorescence spectra were plotted in OriginPro 8.5.1

General procedure for docking analysis: Molecular docking investigations were carried out using Autodock Vina17 with the integration of executables facilitated by a Perl Script. On 23 December 2022, the protein data bank was accessed to retrieve the PDB structures of HSA (1BM0, Chain A), BSA (4F5V, Chain A), proteinase (4NKK), trypsin (2STB), lysozyme (5LVK), hemoglobin (1GZX), and amylase (3VX0). To prepare the structures, hydrogen atoms were added and Gasteiger charges were assigned using Autodock tools 4. For the docking simulations, grid sizes were selected as follows: HSA with dimensions (60 × 60 × 70) and center at (x29.61, y31.78, z23.48), BSA with dimensions (62 × 80 × 74) and center at (x34.16, y24.80, z41.47), proteinase with dimensions (64 × 104 × 126) and center at (x2.45, y8.34, z25.63), trypsin with dimensions (42 × 36 × 52) and center at (x2.49, y7.53, z21.19), lysozyme with dimensions (100 × 90 × 110) and center at (x24.21, y31.71, z10.18), hemoglobin with dimensions (100 × 90 × 90) and center at (x14.42, y67.71, z4.18), and amylase with dimensions (54 × 54 × 53) and center at (x21.79, y15.32, z40.94). Prior to docking, the ligands underwent energy minimization utilizing Argus lab 4.0.1. Subsequently, the docked structures were visualized using Pymol, and interactions with amino acid residues were explored through Discovery studio20.Autodock Vina was used to perform the molecular docking studies with a Perl Script for integration executables. The pdb structure of the proteins were retrieved from the protein data bank on 12 May 2022 (http://www.rcsb.org). Autodock tools 4 was used to

add hydrogen atoms and to compute Gasteiger charges. The ligands used were energy-minimized using Argus lab 4.0.1. The docked structures were visualized in Pymol, and the amino acid residue interactions were studied using Discovery studio.

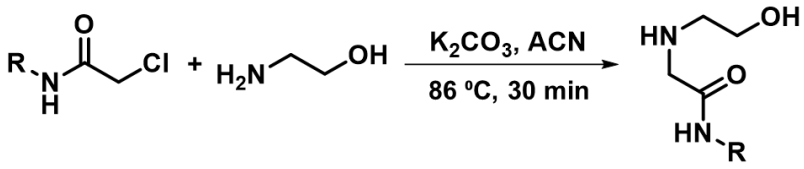
Synthesis of monomers

The monomers were synthesized through two consecutive reactions. Details of the reactions are given below.



Scheme S1. Synthesis of chloro-terminal amides

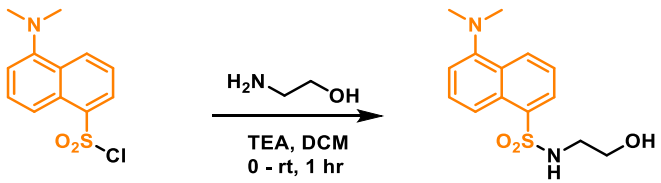
Chloro-terminal amides were synthesized by the reaction of substituted amines (1 mmol) and chloroacetyl chloride (1.5 mmol) in DMF (5 mL). The reaction was carried out at 0 ºC during the addition of chloroacetyl chloride and then brought to room temperature. After completion of the reaction (monitored by TLC), the excess chloroacetyl chloride was quenched by the addition of sodium bicarbonate ($NaHCO_3$). The reaction mixture was extracted in 1:1 ethyl acetate/water. Ethyl acetate layer was dried over anhydrous Na_2SO_4 and the product was isolated from ethyl acetate layer. The solvent was removed under reduced pressure and the products were obtained with high purity (>95%).



Scheme S2. Synthesis of amine hydroxy monomer

The monomers were prepared by the reaction of chloro-terminal amides (1 mmol) and ethanolamine (5 mmol) in acetonitrile (5 mL) in presence of potassium carbonate (K_2CO_3, 10 mmol) as base. The reaction mixture was refluxed for 30 min. Thereafter, the reaction mixture was extracted in1:1 ethyl acetate/water mixture. Ethyl acetate layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and product was obtained at good yield (90-95%) with >95% purity. Monomers were directly used for polymerization reaction without further purification.

Synthesis of dansyl ethanol amine



Scheme S3: Synthesis of dansyl ethanol amine

Procedure: Dansyl chloride (1 mmol) was dissolved in 10 mL of DCM and was kept in an ice bath for 5 minutes. To this triethylamine (1.5 mmol) was added followed by dropwise addition of ethanolamine (1 mmol). The reaction mixture was allowed to stir for 1 hour. The completion of the reaction was monitored by TLC. Once the reaction was complete, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the pure product with 96% yield.

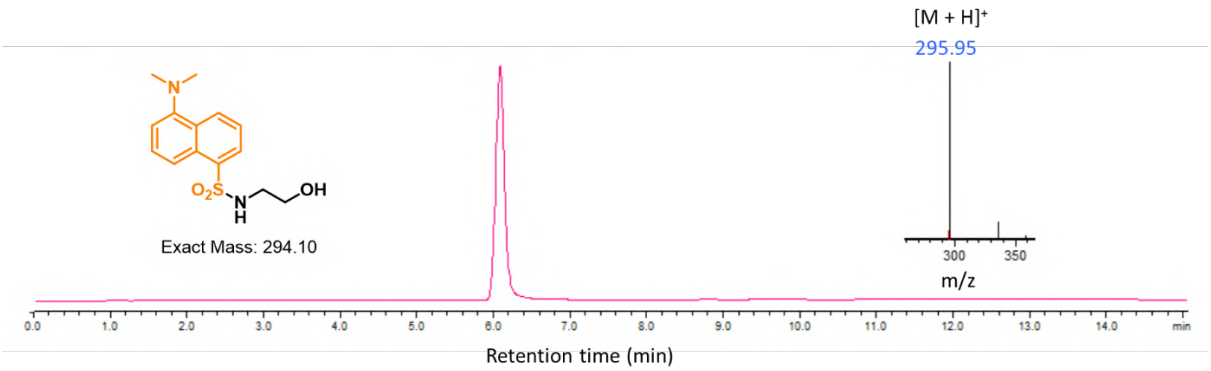
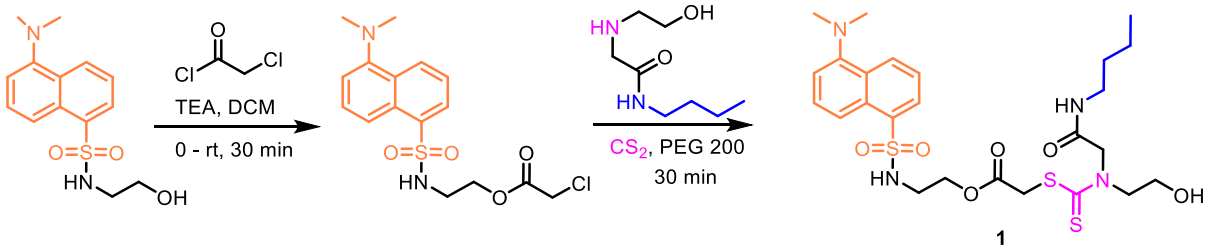


Figure S1: The HPLC trace and MS data showing experimental $[M + H]^+$: 295.95 Da and calculated $[M+H]^+$: 295.10 Da

Synthesis of 1



Scheme S4: Synthesis of **1**

Procedure: The dansyl ethanol amine from previous reaction (1 mmol) was dissolved in 10 mL of DCM and was kept in an ice bath for 5 minutes. To this triethylamine (1.5 mmol) was added followed by dropwise addition of chloroacetylchloride (1.5 mmol). The reaction mixture was allowed to stir for 30 minutes. The completion of the reaction was monitored by TLC. Once the reaction was complete, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the product with 95% yield. The chloroacetyl derivative (1 mmol) was dissolved in 2 mL PEG, carbon disulphide (2 mmol) and monomer (1.5 mmol) were added and was allowed to stir for 30 minutes. The reaction was monitored by TLC (Rf = 0.5 in 7: 3 ethyl acetate: hexane) and after the completion of the reaction, it was extracted with 1: 1 ethyl acetate: water mixture. The product was obtained in the ethyl acetate layer and was evaporated to obtain the product with 70 % yield. The product was column purified using silica gel of 60-120 mesh size. The product eluted in 85: 15 ethyl acetate: hexane mixture.

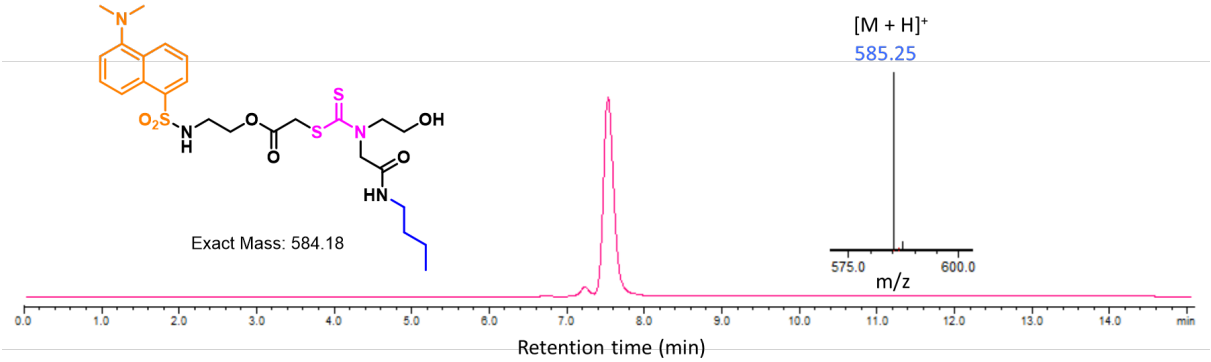
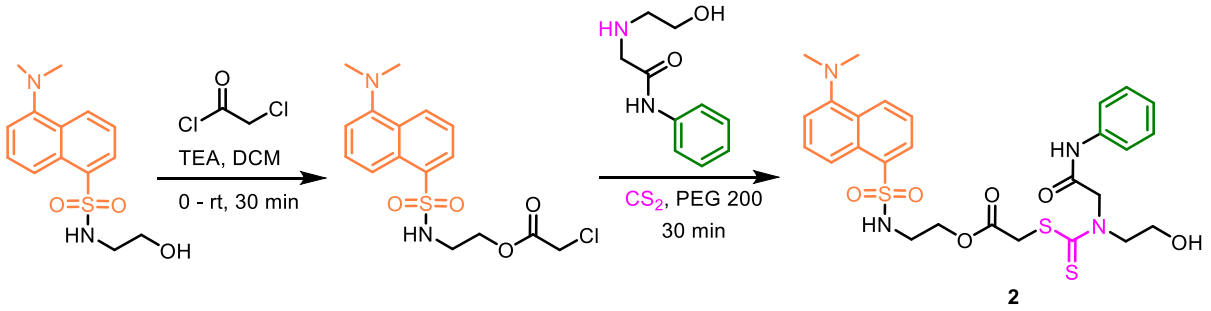


Figure S2: The HPLC trace of **1** and MS data showing experimental $[M + H]^+$: 585.25 Da and calculated $[M+H]^+$: 585.18 Da

Synthesis of 2



Scheme S5: Synthesis of **2**

Procedure: Similar procedure for the synthesis of **1** was followed to prepare **2**. The acid chloride derivative (1 mmol) was dissolved in 2 mL PEG, carbon disulphide (2 mmol) and monomer (1.5 mmol) were added and was allowed to stir for 30 minutes. The reaction was monitored by TLC (Rf = 0.6 in 7: 3 ethyl acetate: hexane) and after the completion of the reaction, it was extracted with 1: 1 ethyl acetate: water mixture. The product was obtained in the ethyl acetate layer and was evaporated to obtain the product with 70 % yield. The product was column purified using silica gel of 60-120 mesh size. The product eluted in 80: 20 ethyl acetate: hexane mixture.

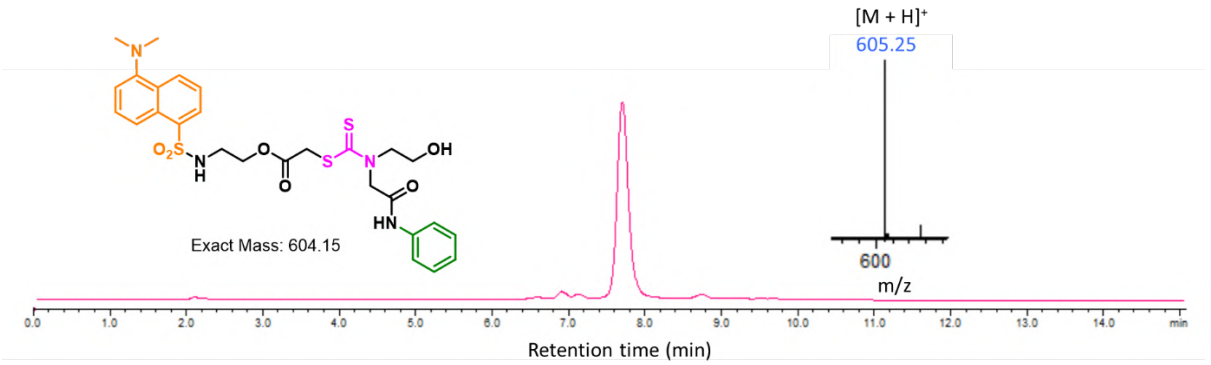
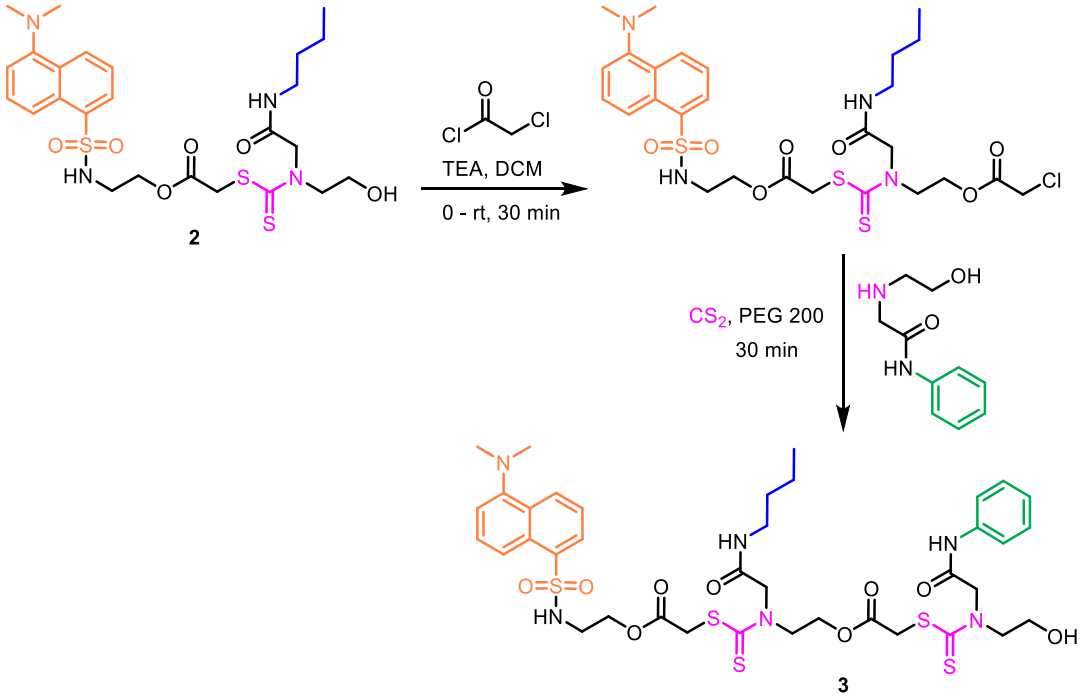


Figure S3: The HPLC trace of **2** and MS data showing experimental $[M + H]^+$: 605.25 Da and calculated $[M+H]^+$: 605.15 Da

Synthesis of 3



Scheme S6: Synthesis of **3**

Procedure: The product **1** (1 mmol) was dissolved in 10 mL of DCM and was kept in an ice bath for 5 minutes. To this triethylamine (1.5 mmol) was added followed by dropwise addition of chloroacetylchloride (1.5 mmol). The reaction mixture was allowed to stir for 30 minutes. The completion of the reaction was monitored by TLC (Rf = 0.5 in 7: 3 ethyl acetate: hexane). Once the reaction was complete, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the product with 90% yield. The acid chloride derivative **(**1 mmol) was dissolved in 2 mL PEG. To this carbon disulphide (2 mmol) and monomer (1.5 mmol) were added and was allowed to stir for 30 minutes. The reaction was monitored by TLC and after the completion of the reaction, it was extracted with 1: 1 ethyl acetate: water mixture. The product was obtained in the ethyl acetate layer and was evaporated to obtain the product with 70 % yield. The product was column purified using silica gel of 60-120 mesh size. The product eluted in 90:10 ethyl acetate: hexane mixture.

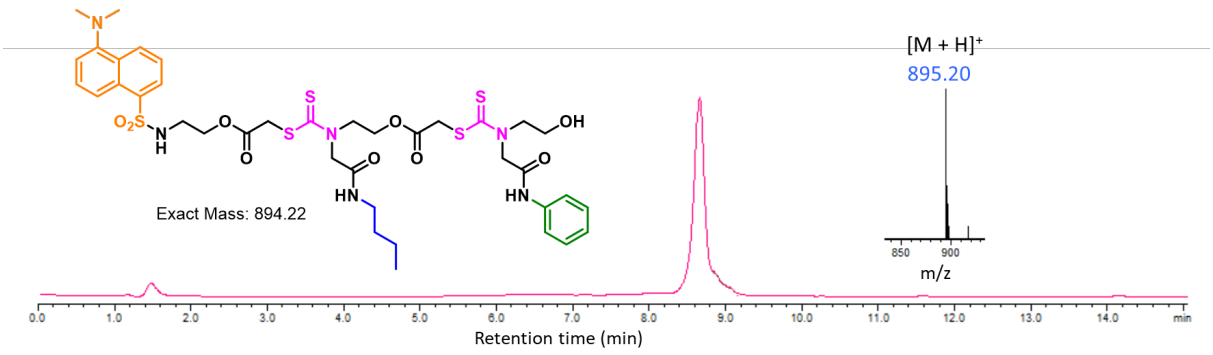
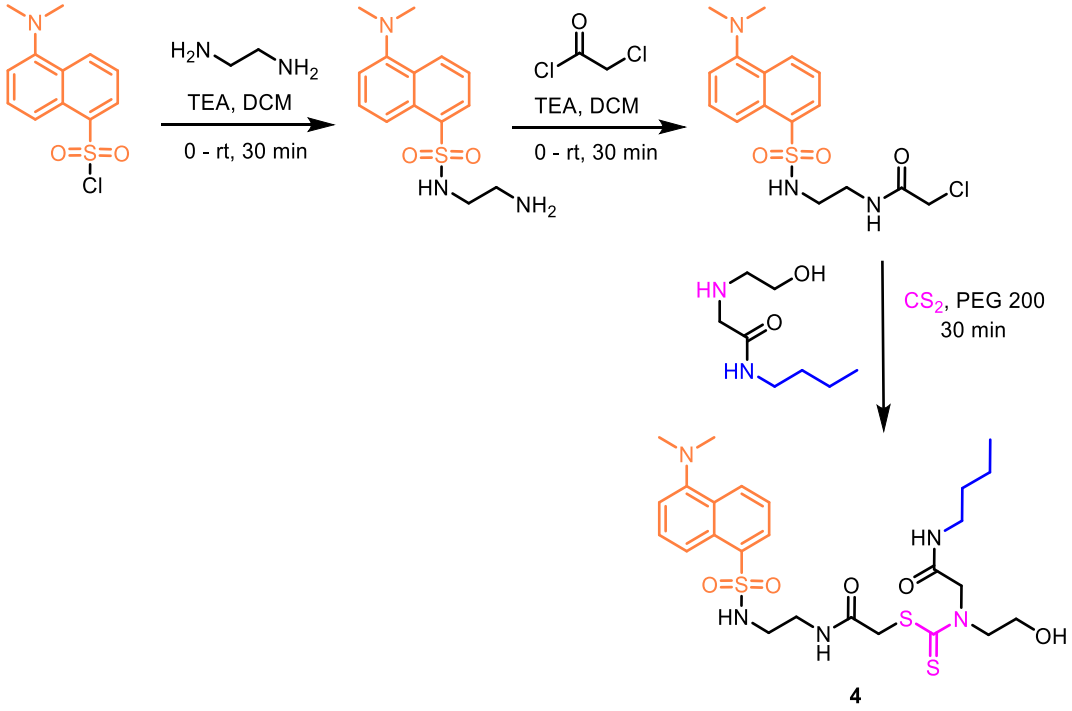


Figure S4: The HPLC trace of **3** and MS data showing experimental $[M + H]^+$: 895.20 Da and calculated $[M+H]^+$: 895.22 Da

Synthesis of 4



Scheme S7: Synthesis of **4**

Procedure: Dansyl chloride (1 mmole) was dissolved in 10 mL of DCM and was kept in an ice bath for 5 minutes. To this, triethylamine (1.5 mmole) was added followed by dropwise addition of ethane diammine (1 mmole). The reaction mixture was allowed to stir for 1 hour. The completion of the reaction was monitored by TLC. Once the reaction was complete, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the pure product, with 96% yield. The product (1 mmole) was dissolved in 10 mL of DCM and was kept in an ice bath for 5 minutes. To this triethylamine (1.5 mmole) was added followed by dropwise addition of chloroacetylchloride (1.5 mmole). The reaction mixture was allowed to stir for 30 minutes. The completion of the reaction was monitored by TLC. Once the reaction was complete, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the product with 95% yield. This chloroacetyl derivative (1 mmole) was dissolved in 2 mL PEG and then carbon disulphide (2 mmole) and monomer (1.5 mmole) were added. It was allowed to stir for 30 minutes. The reaction was monitored by TLC (Rf = 0.4 in 7: 3 ethyl acetate: hexane) and after the completion of the reaction, it was extracted with 1: 1 ethyl acetate: water mixture. The product was obtained in the ethyl acetate layer and was evaporated to obtain the product with 70 % yield. The product was column purified using silica gel of 60-120 mesh size. The product eluted in 85: 15 ethyl acetate: water mixture.

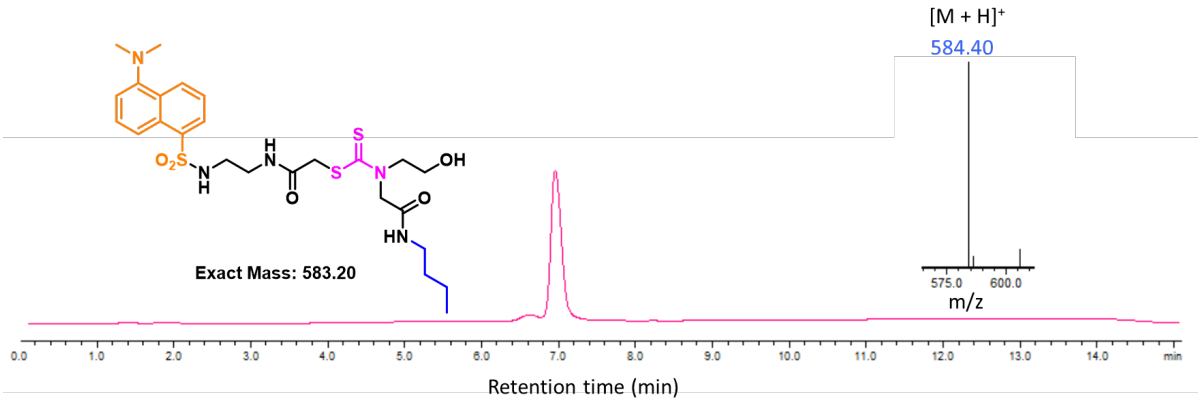
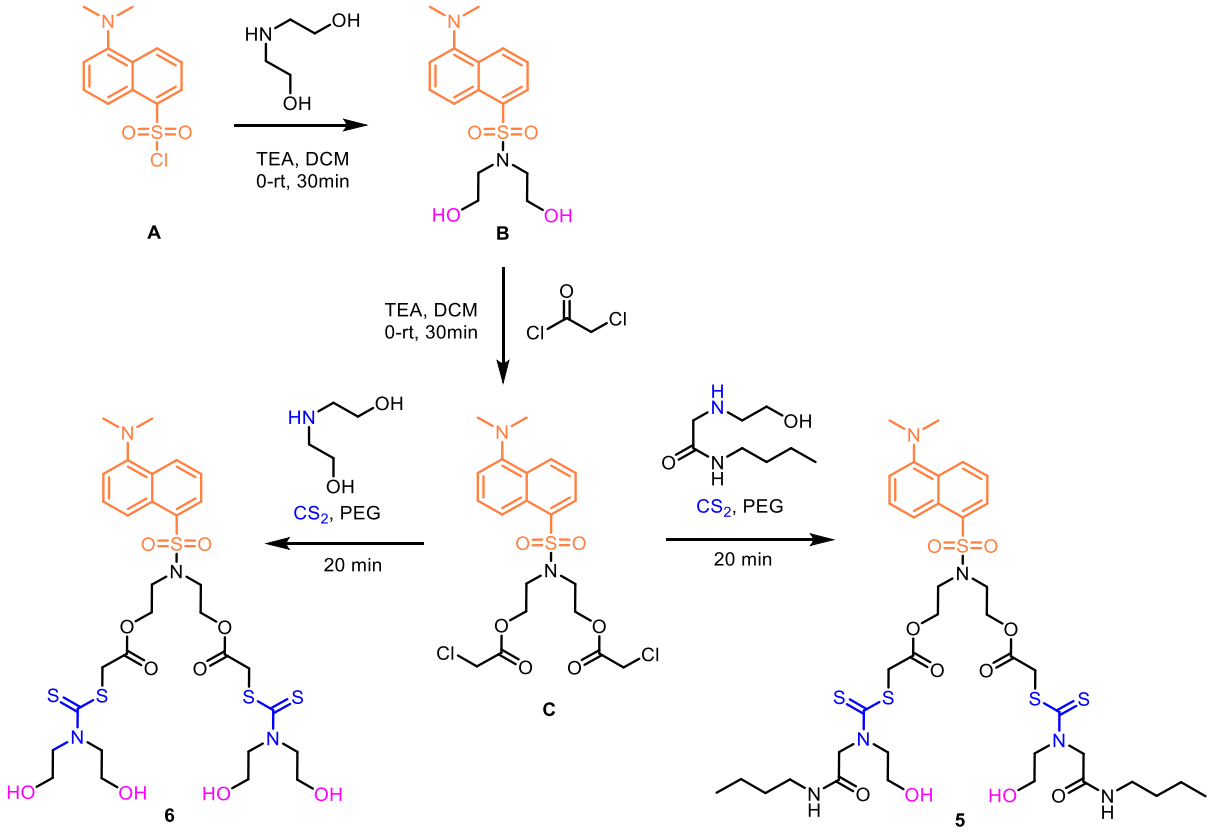


Figure S5: The HPLC trace of **4** and MS data showing experimental $[M + H]^+$: 584.40 Da and calculated $[M+H]^+$: 584.20 Da

Synthesis of 5 and 6



Scheme S8: Synthesis of **5** and **6**

Procedure: Dansyl chloride (1 mmole) was dissolved in 10 mL of DCM and was kept in an ice bath for 5 minutes. To this, triethylamine (1.5 mmole) was added followed by dropwise addition of diethanolamine (1 mmole). The reaction mixture was allowed to stir for 1 hour. After the completion of the reaction, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the pure product, **B**, with 98% yield. The product **B** (1 mmole) was dissolved in 10 mL of DCM and was kept in an ice

bath for 5 minutes. To this triethylamine (1.5 mmole) was added followed by dropwise addition of chloroacetylchloride (1.5 mmole). The reaction mixture was allowed to stir for 30 minutes. The completion of the reaction was monitored by TLC. Once the reaction was complete, it was extracted with 1: 1 DCM: water mixture. The product was obtained in the DCM layer and was evaporated to obtain the product with 96% yield. This chloroacetyl derivative (1 mmole) was dissolved in 2 mL PEG and to this either (a) carbon disulphide (2 mmole) and butyl substituted monomer (1.5 mmole) or (b) carbon disulphide (2 mmole) and diethanol amine were added to obtain **5** and **6** respectively. The reaction mixture was stirred for 30 minutes. After the completion of the reaction, it was extracted with 1: 1 ethyl acetate: water mixture. The product was obtained in the ethyl acetate layer and was evaporated to obtain the product **5** (Rf = 0.3 in 8: 2 ethyl acetate: hexane) and **6** (Rf = 0.2 in 9: 1 ethyl acetate: hexane) with 70% and 68% yield respectively. The products were column purified using silica gel of 60-120 mesh size.

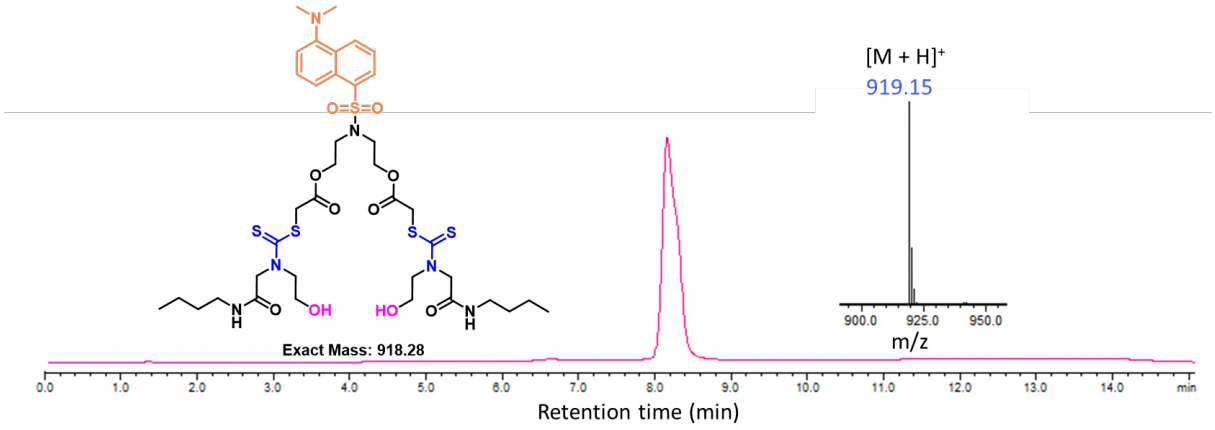


Figure S6: The HPLC trace of **5** and MS data showing experimental $[M + H]^+$: 919.15 Da and calculated $[M+H]^+$: 919.28 Da

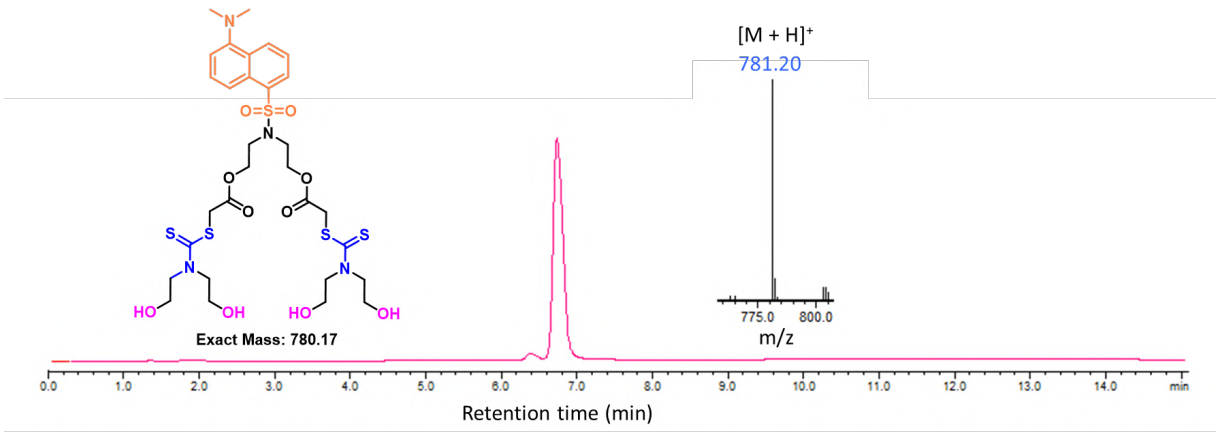


Figure S7: The HPLC trace of **6** and MS data showing experimental $[M + H]^+$: 781.20Da and calculated $[M+H]^+$: 781.17 Da

Characterisation: ^{1}H NMR

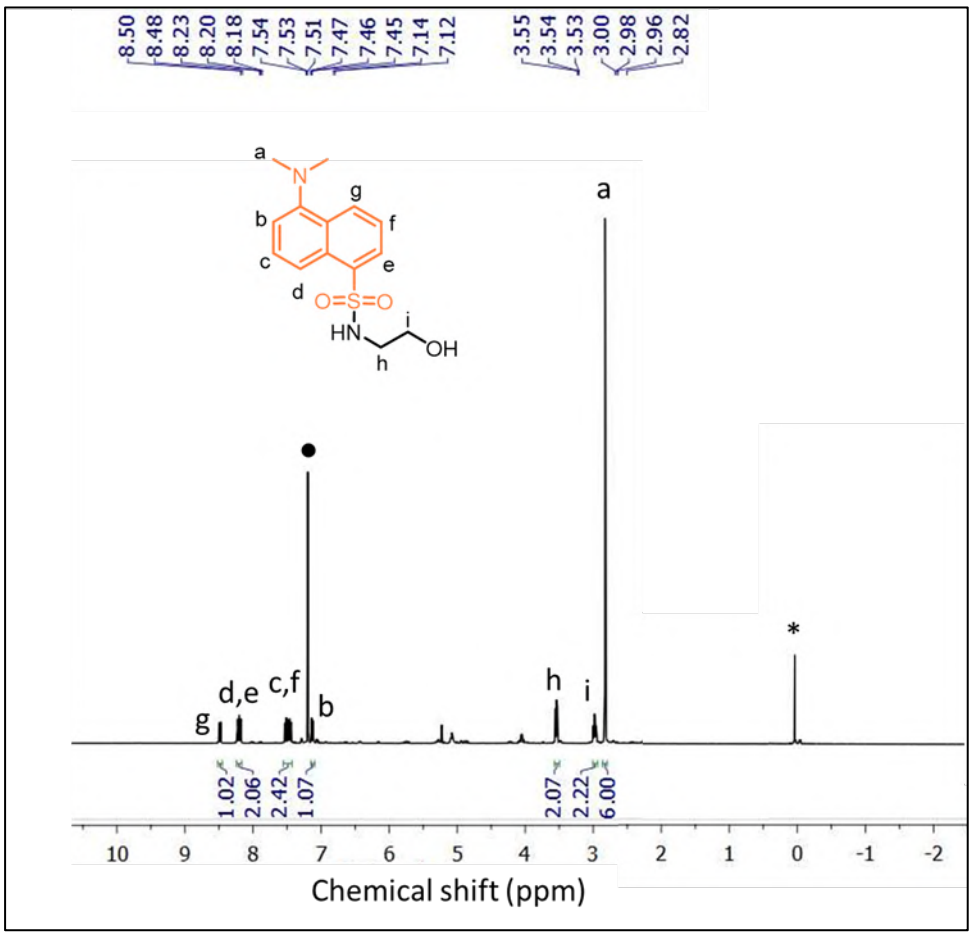


Figure S8: ^{1}H-NMR (500MHz) in $CDCl_3$: δ (ppm) 2.82 (s, 6H), 2.96- 3.00 (t, 2H), 3.53-3.55 (t, 3H), 7.12- 7.14 (d, 1H), 7.45- 7.54 (m, 2H), 8.18- 8.23 (m, 2H), 8.48 -8.50 (d, 1H). * and • represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

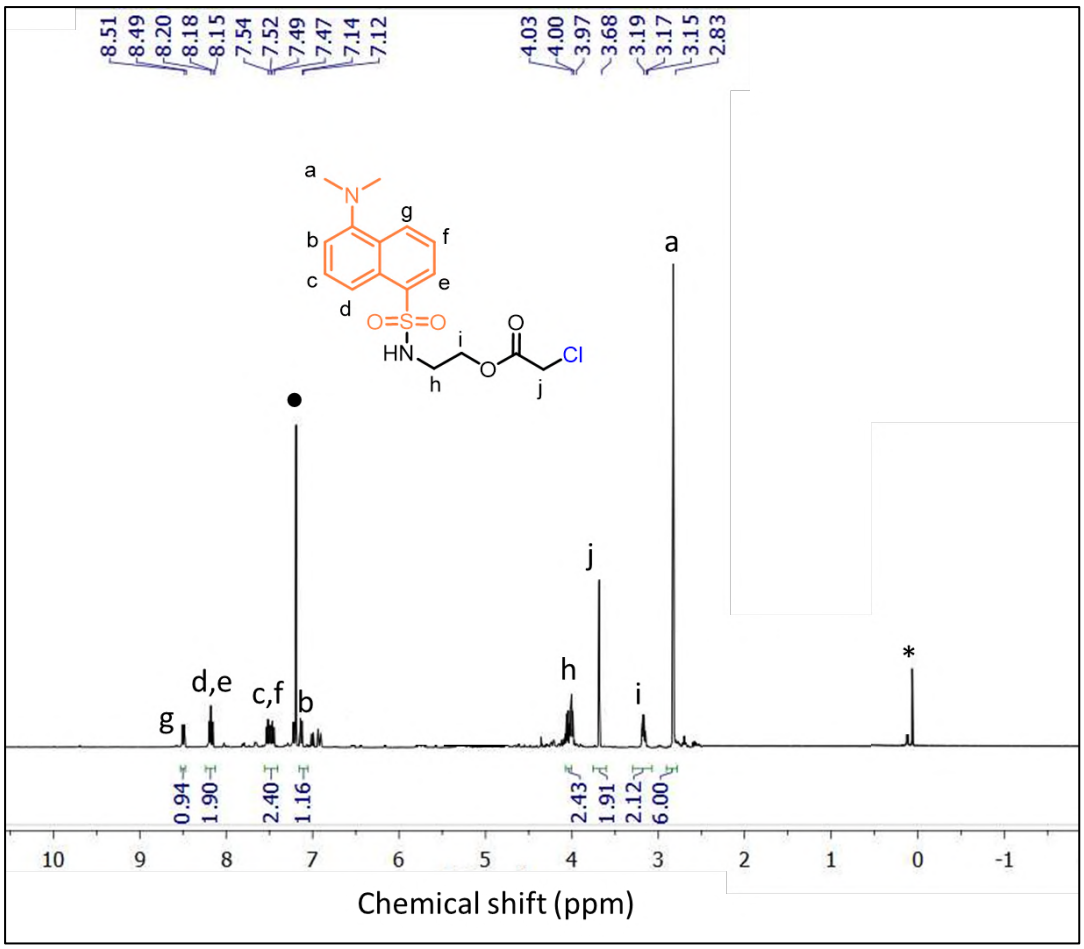


Figure S9: ^{1}H-NMR (500MHz) in $CDCl_3$: δ (ppm) 2.83 (s, 6H), 3.15-3.19 (t, 2H), 3.68 (s, 2H), 3.97-4.03 (t, 3H), 7.12- 7.14 (d, 1H), 7.47- 7.54 (m, 2H), 8.15- 8.20 (m, 2H), 8.49 -8.51 (d, 1H). * and • represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

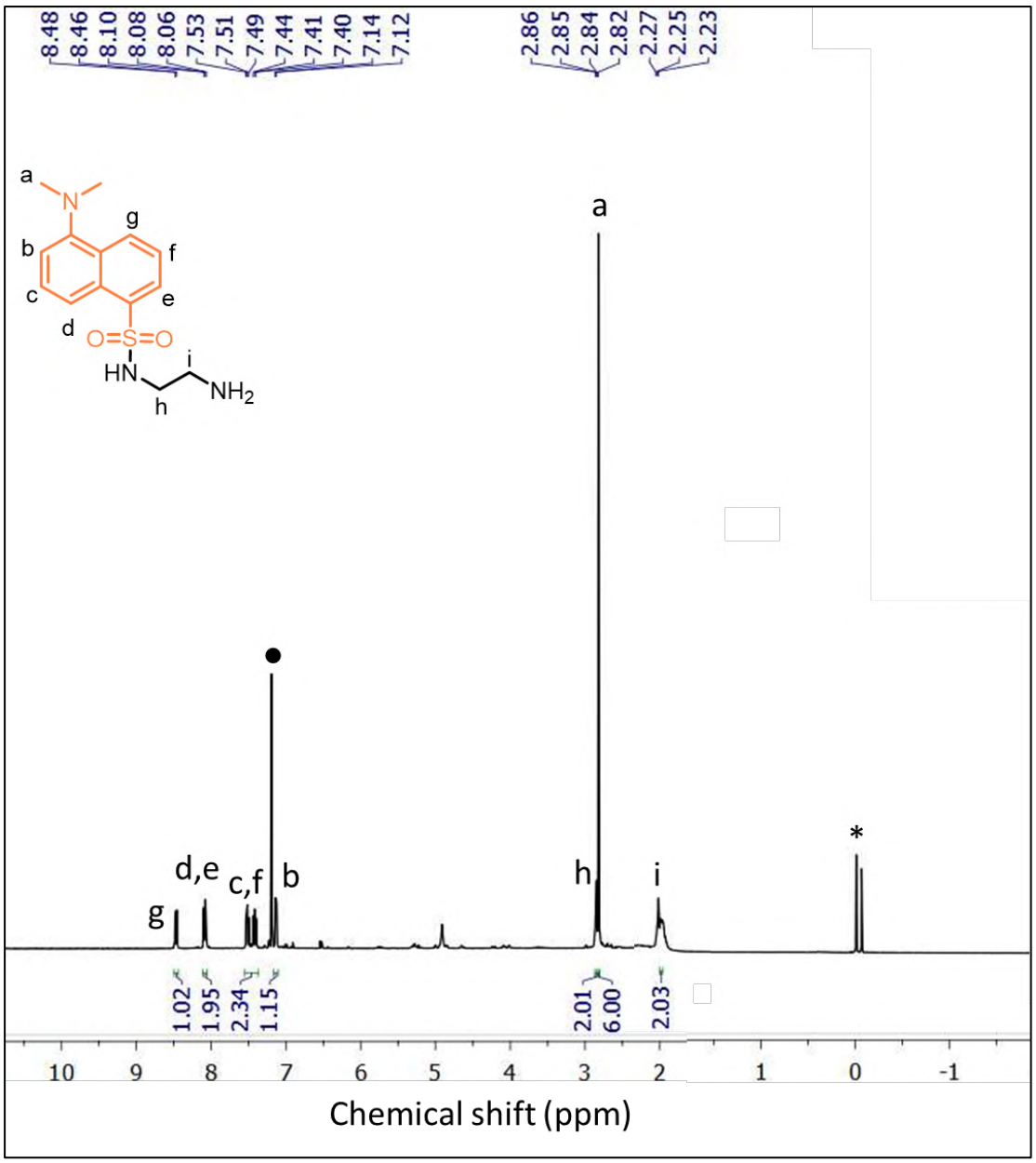


Figure S10: ^{1}H-NMR (500MHz) in $CDCl_3$: δ (ppm)2.23- 2.27 (t, 2H), 2.82 (s, 6H), 2.84-2.86 (t, 2H), 7.12- 7.14 (d, 1H), 7.40- 7.53 (m, 2H), 8.06- 8.10 (m, 2H), 8.46 -8.48 (d, 1H). * and • represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

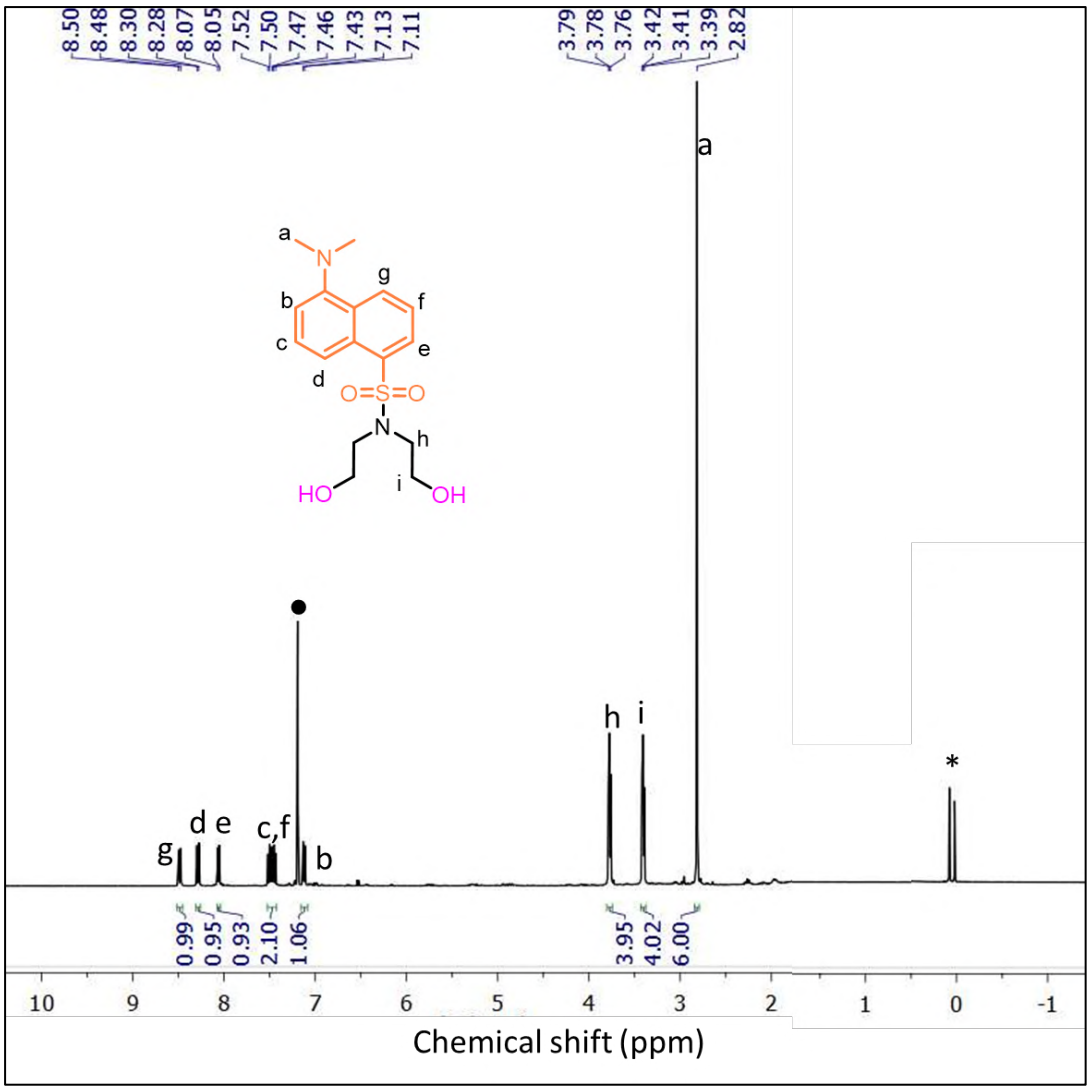


Figure S11: ^{1}H-NMR (500MHz) in $CDCl_3$: δ (ppm) 2.82 (s, 6H), 3.39- 3.42 (t, 4H), 3.76- 3.79 (t, 4H), 7.11- 7.13 (d, 1H), 7.43- 7.52 (m, 2H), 8.05- 8.07 (d, 1H), 8.28 -8.30 (d, 1H), 8.48 - 8.50 (d, 1H). * and • represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

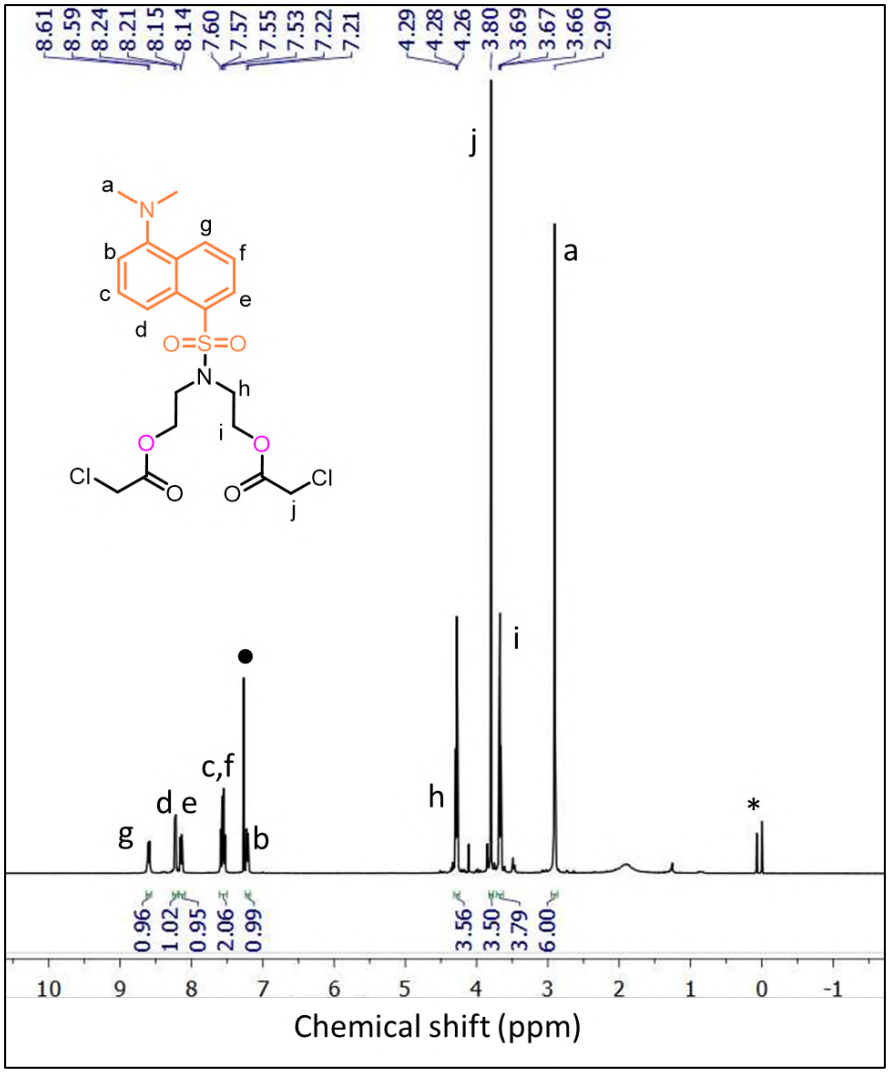


Figure S12: ^{1}H-NMR (500MHz) in $CDCl_3$: δ (ppm) 2.90 (s, 6H), 3.66- 3.69 (t, 4H), 3.80 (s, 4H), 4.26- 4.29 (t, 4H), 7.21- 7.22 (d, 1H), 7.53- 7.60 (m, 2H), 8.14- 8.15 (d, 1H), 8.21 -8.24 (d, 1H), 8.59 -8.61 (d, 1H). * and • represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

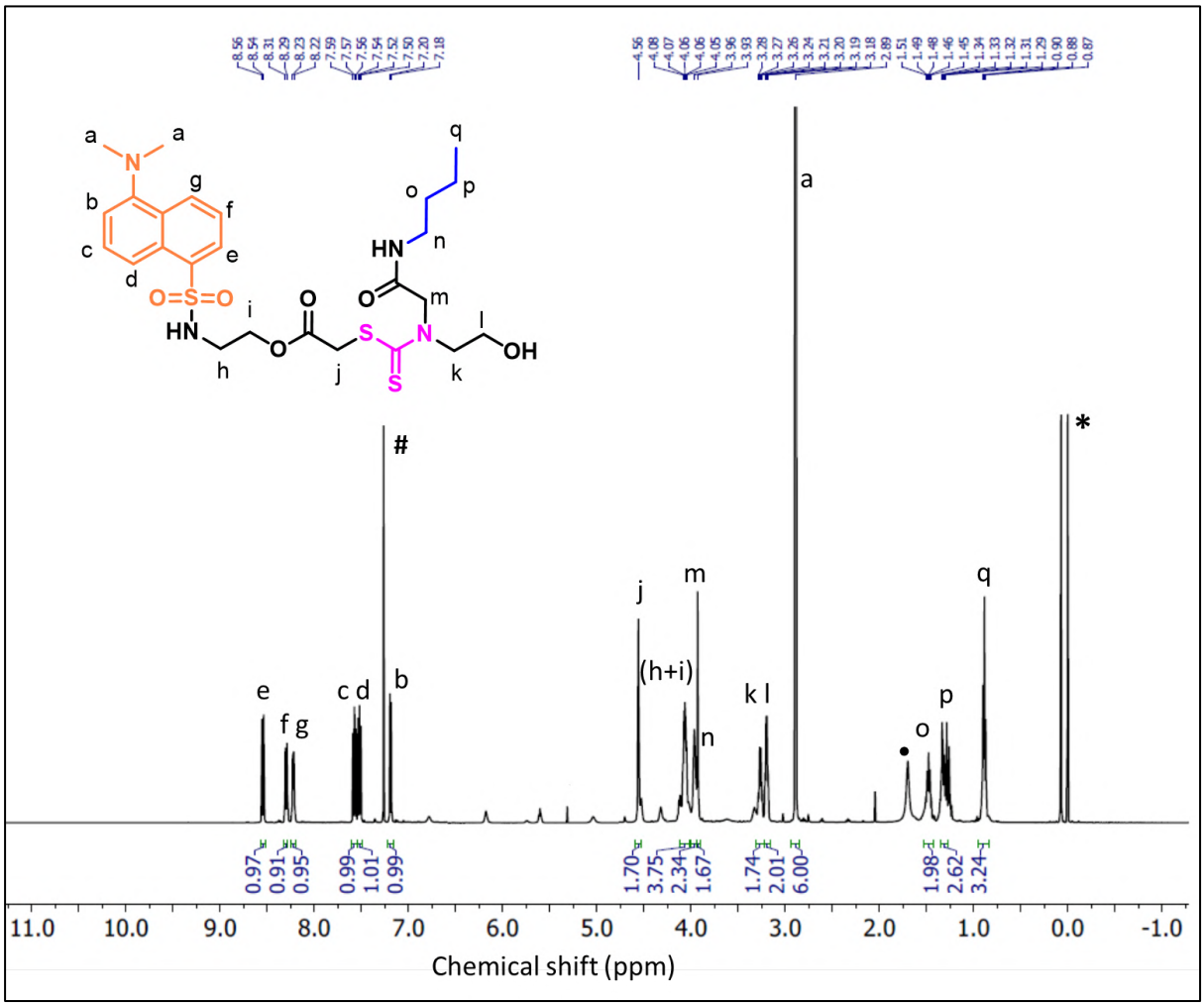


Figure S13: ^{1}H-NMR (500MHz) of **1** in $CDCl_3$: δ (ppm) 0.87-0.90 (t, 3H), 1.29 -1.33 (m, 2H), 1.34-1.51 (m, 2H), 2.89 (s, 6H), 3.18 -3.20 (t, 2H), 3.21 -3.26 (t, 2H), 3.28 (s, 2H), 3.93 -4.08 (m, 6H), 4.56 (s, 2H), 7.18 -7.20 (d, 1H), 7.50 -7.54 (d, 1H), 7.56 -7.99 (d, 1H), 8.22 -8.23 (d, 1H), 8.29 -8.31 (d, 1H), 8.54 -8.56 (d, 1H), *, • and # represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

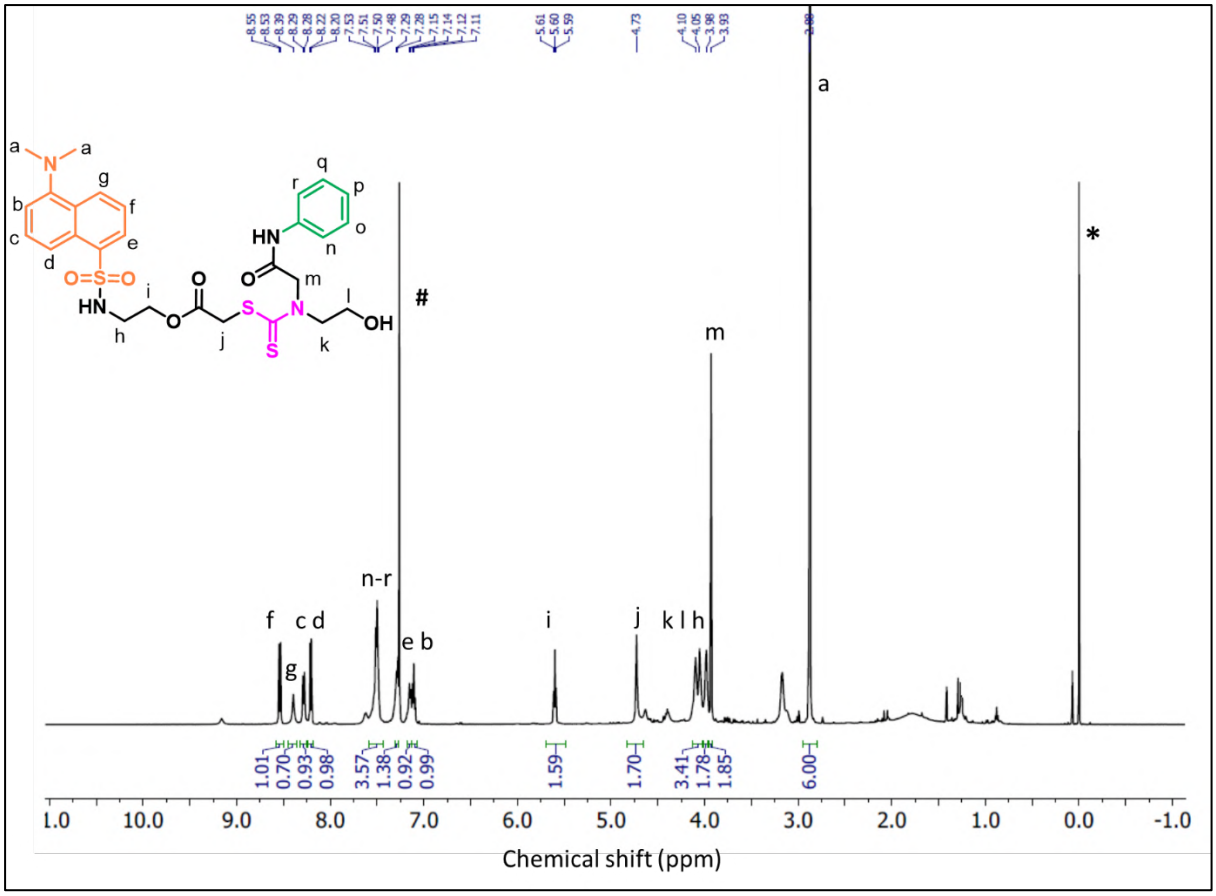


Figure S14: ^{1}H-NMR (500MHz) of **2** in $CDCl_3$: δ (ppm) 2.88 (s, 6H), 3.93 (s, 2H), 3.98 -4.10 (m, 6H), 4.73 (s, 2H), 5.99 -5.61 (t, 2H), 7.11 -7.12 (d, 1H), 7.14 -7.15 (d, 1H), 7.28 -7.29 (d, 1H), 7.48 -7.50 (d, 1H), 7.51 -7.53 (d, 1H), 8.20 -8.22 (d, 1H), 8.28 -8.29 (d, 1H), 8.39 (s, 1H), 8.53 -8.55 (d, 1H), * and # represents the residual protons of internal standard tetramethyl silane and chloroform respectively.

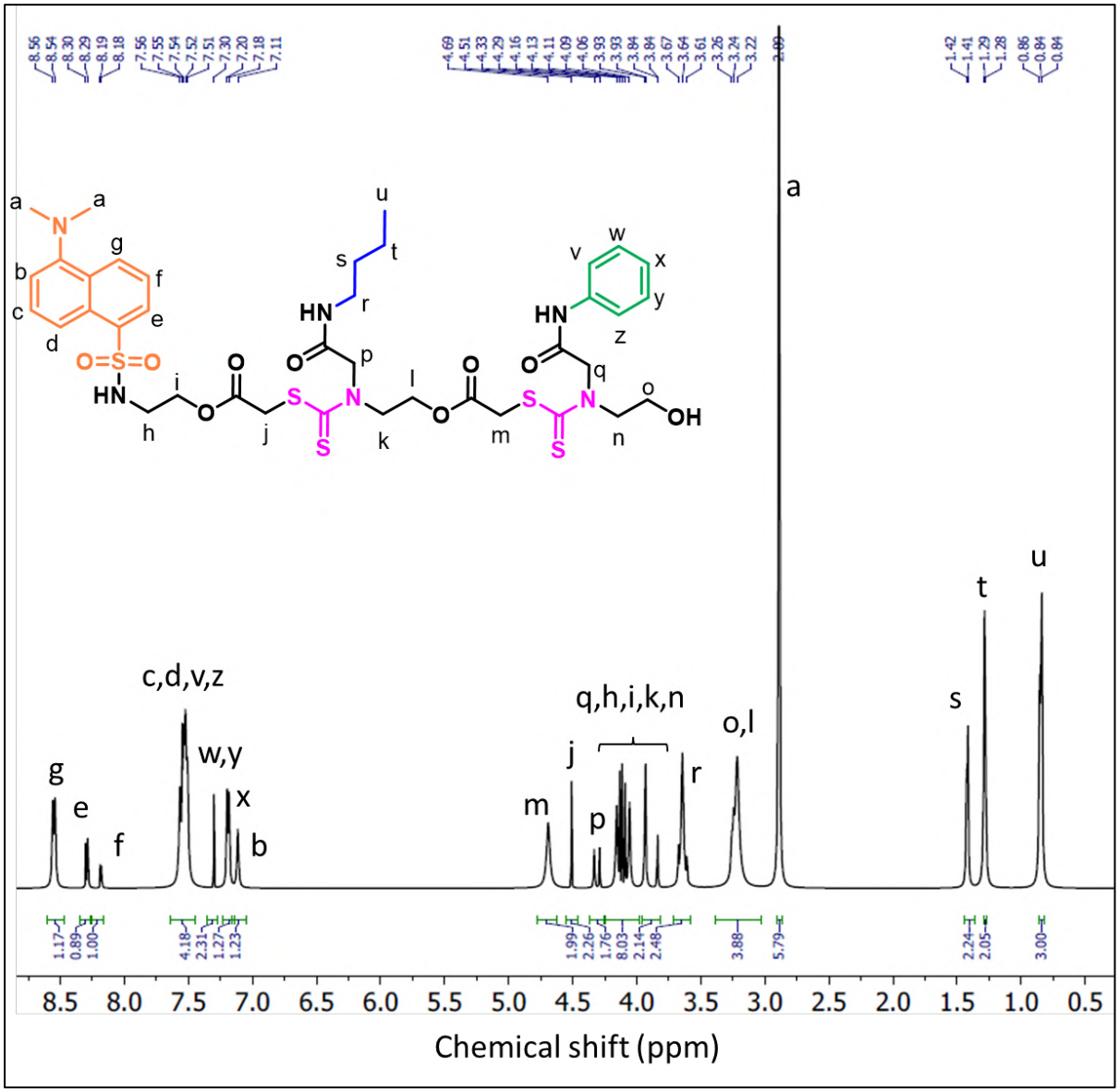


Figure S15: ^{1}H-NMR (500MHz) of **3** in $CDCl_3$: δ (ppm) 0.84-0.86 (t,3H), 1.28-1.29 (m, 2H), 1.41-1.42 (m, 2H), 2.89 (s, 6H), 3.22-3.26 (m, 4H), 3.61-3,67 (t, 3H), 3.84-4.09 (m, 10H), 4.11-4.16 (s,2H), 4.29-4.33 (s,2H), 4.51-4.60 (s, 2H), 7.11 (s, 1H), 7.18 -7.20 (s, 1H), 7.20 (s, 1H), 7.30 (s, 1H), 7.51-7.56 (m, 4H), 8.18 -8.19 (d, 1H), 8.29 – 8.30 (d, 1H), 8.54 -8.56 (d, 1H).

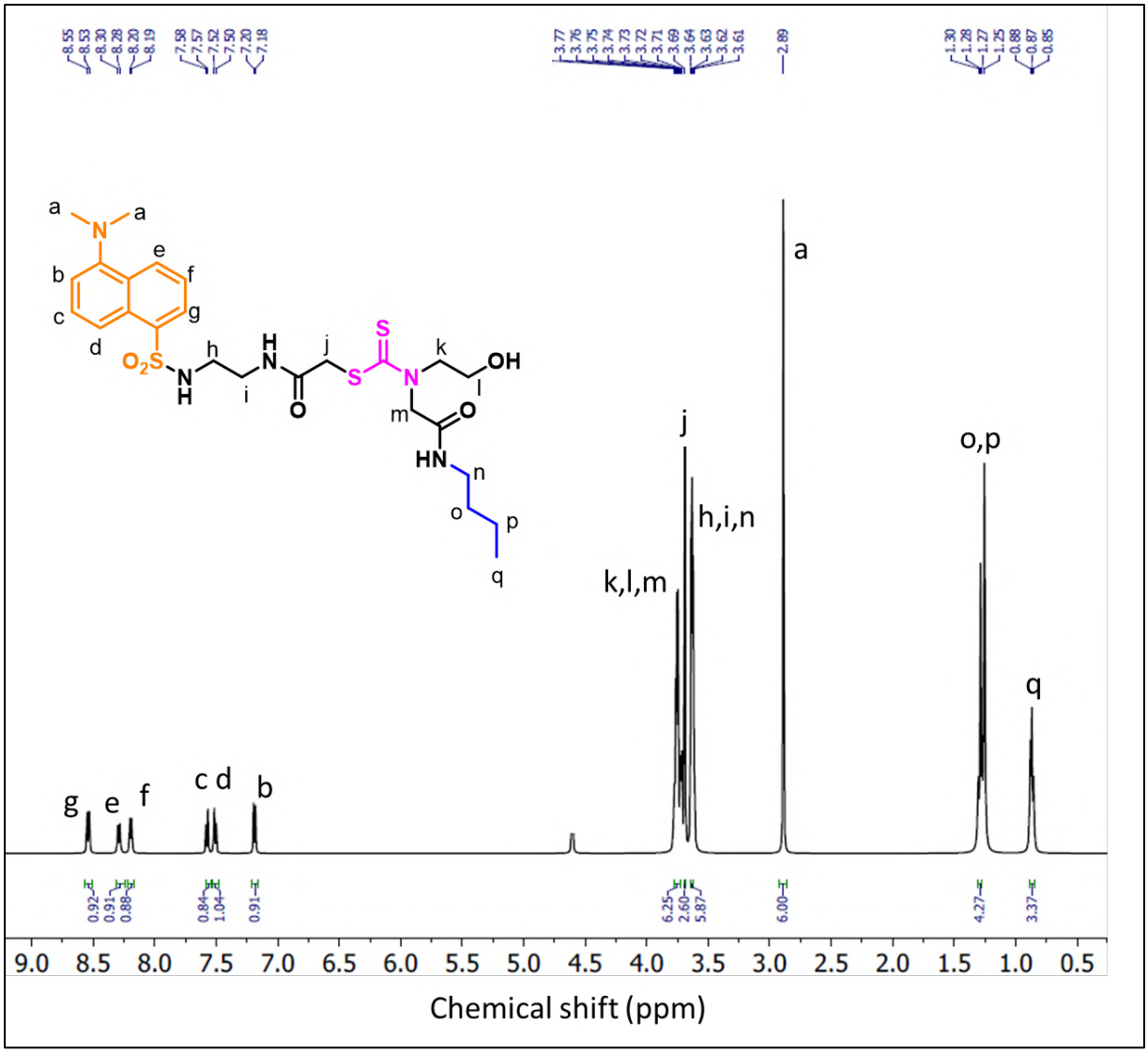


Figure S16: ^{1}H-NMR (500MHz) of **4** in $CDCl_3$: δ (ppm) 0.85-0.88 (t,3H), 1.25-1.30 (m, 4H), 2.89 (s, 6H), 3.61-3,64 (m, 6H), 3.69-3.71 (s, 2H), 3.72-3.77 (m, 6H), 7.18 -7.20 (d, 1H), 7.50 -7.52 (d, 1H), 7.57 -7.58 (d, 1H), 8.19-8.20 (d, 1H), 8.28-8.30 (d, 1H), 8.53-8.55 (d, 1H)

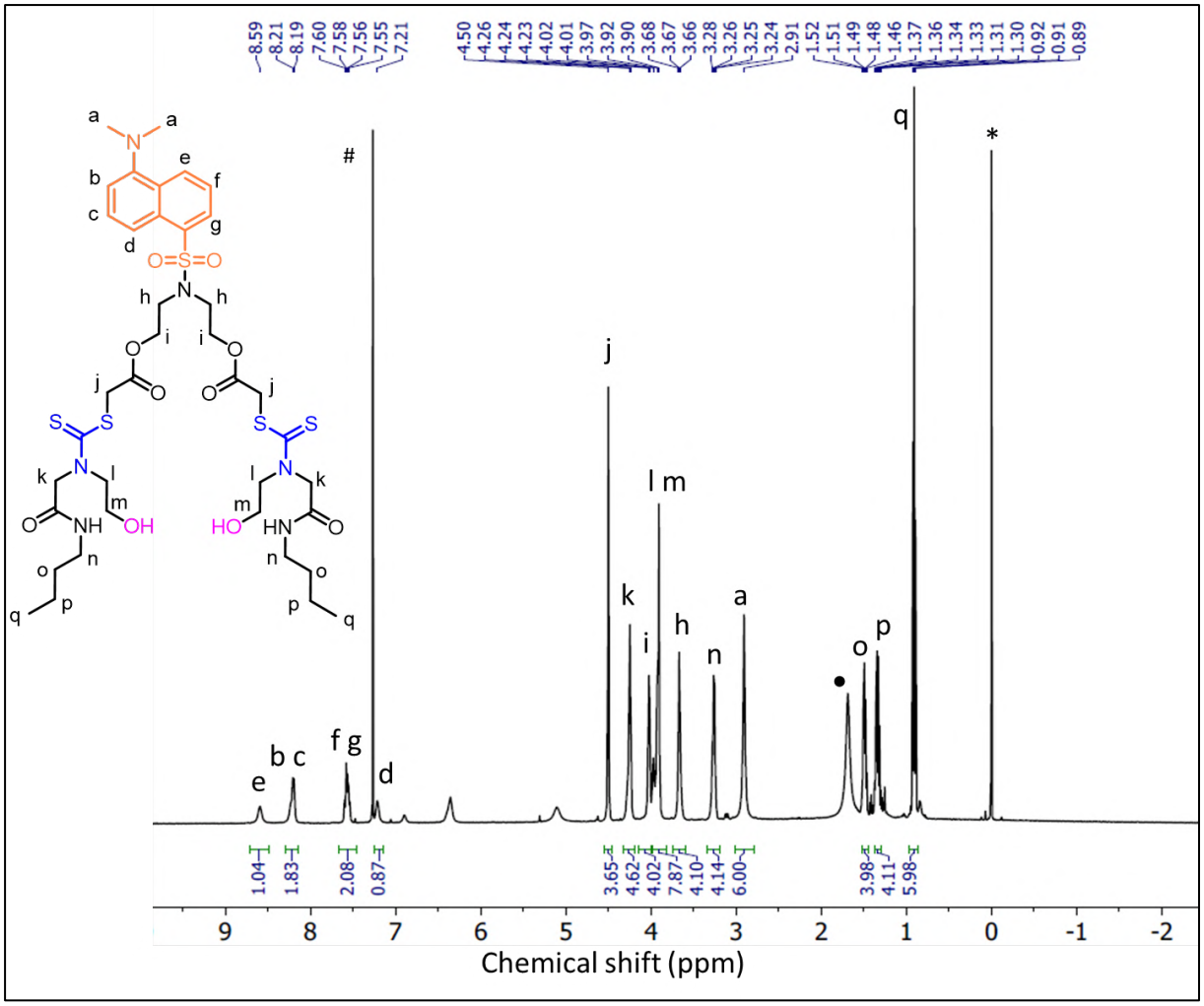


Figure S17: ^{1}H-NMR (500MHz) of **5** in $CDCl_3$: δ (ppm) 0.89-0.92 (t, 6H), 1.30-1.37 (m, 4H), 1.46-1.52 (m, 4H), 2.91 (s, 6H), 3.24-3.28 (t, 4H) 3.66-3.68 (t, 4H), 3.90-4.02 (m, 8H), 4.23-4.24 (t, 4H), 4.26 (s, 4H), 4.50 (s,4H), 7.21 (s, 1H), 7.55-7.60 (m,2H), 8.19-8.21 (d,2H), 8.59 (s, 1H). *, • and # represents the residual protons of internal standard tetramethyl silane tetrahydrofuran and chloroform respectively.

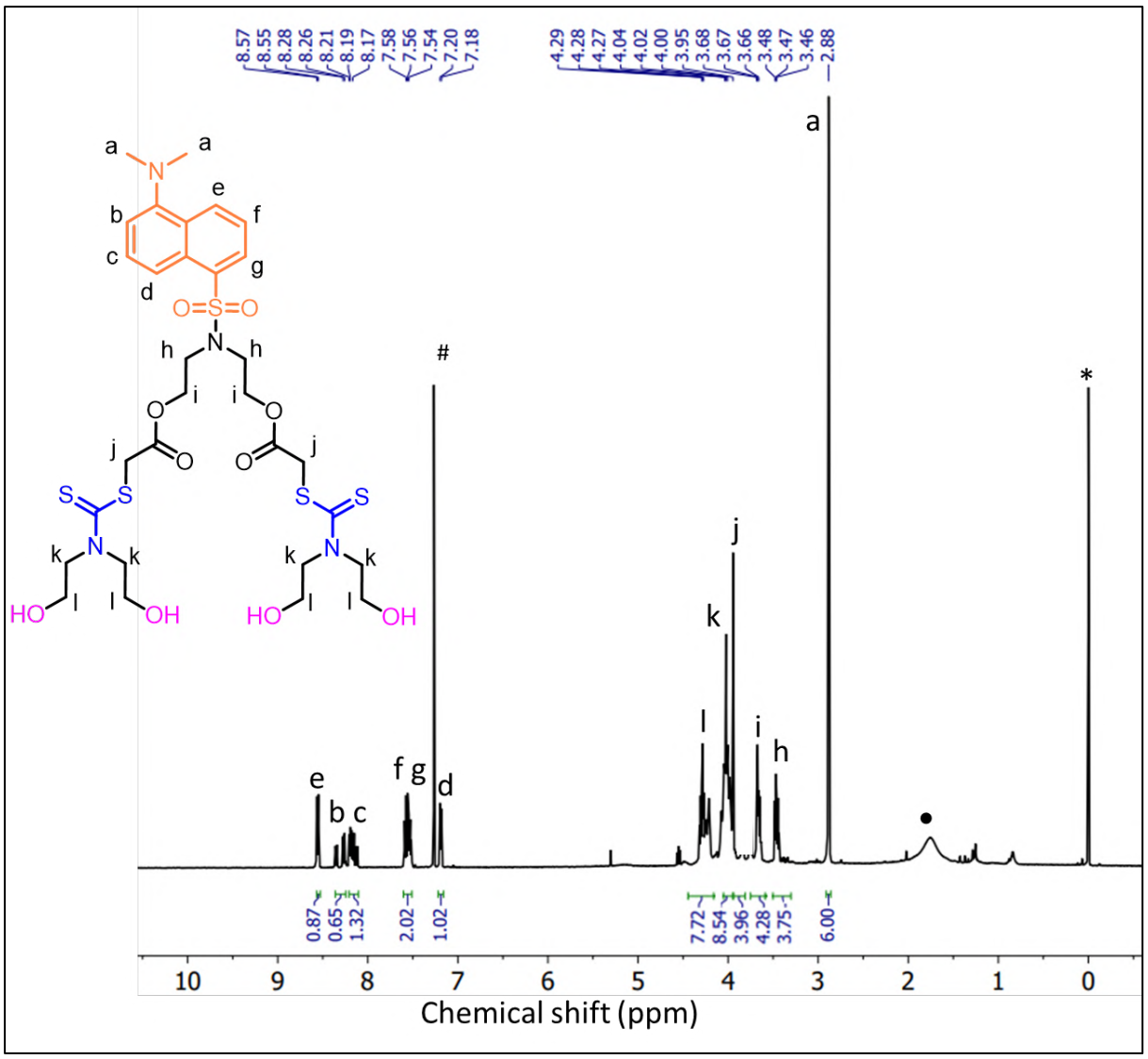


Figure S18: ^{1}H-NMR (500MHz) of **6** in $CDCl_3$: δ (ppm) 2.88 (s, 6H), 3.46- 3.48 (t, 4H), 3.66-3.68 (t, 4H), 3.95 (s, 4H), 4.00-4.04 (t, 8H), 4.27-4.29 (t, 8H), 7.18-7.20 (d, 1H), 7.54-7.58 (m, 2H), 8.17-8.21 (d, 1H), 8.26-8.28 (d, 1H), 8.55- 8.57 (d, 1H), 8.55-8.57 (d, 1H), *, • and # represents the residual protons of internal standard tetramethyl silane tetrahydrofuran and chloroform respectively.

FRET between HSA and SDO 1

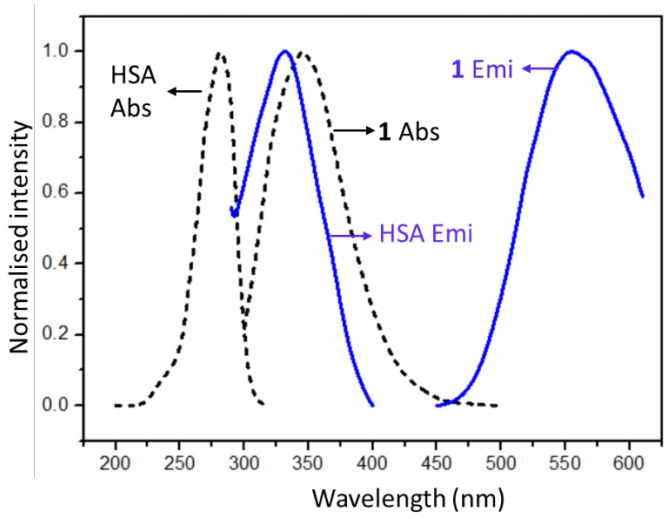


Figure S19: The normalised emission and absorption spectra of HSA (10 μM) and dansyl-SDO, **1** (10 μM) in phosphate buffer at 25 ºC. The excitation wavelength for HSA and **C** was 280 nm and 350 nm respectively.

Fluorescence titration for the interaction of 2 with proteins

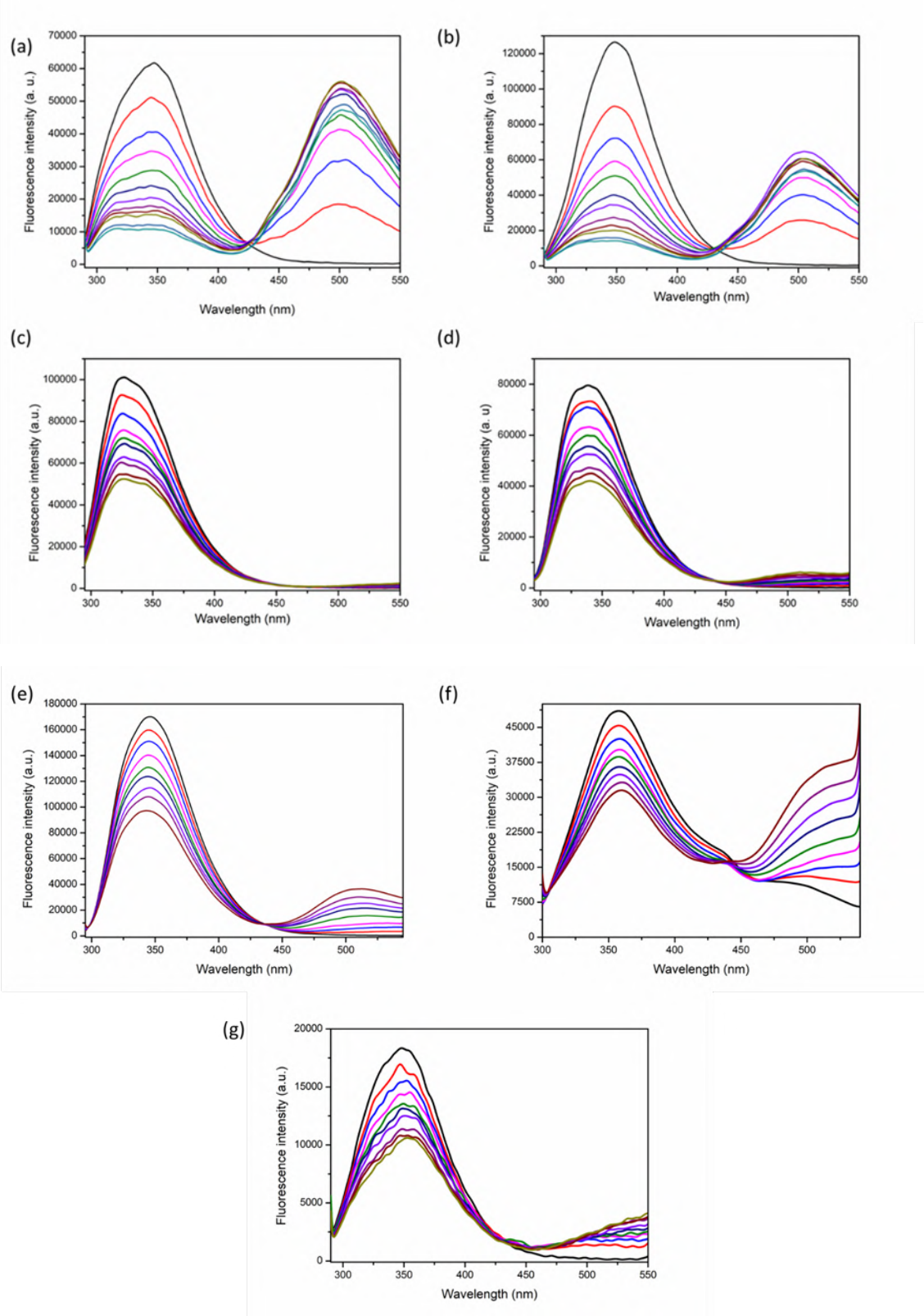


Figure S20: The fluorescence emission spectra for the titration of **2** (4.25 μM to 51 μM) with 10 μM each of (a) HSA, (b) BSA, (c) proteinase, (d) trypsin, (e) lysozyme, (f) haemoglobin, and (g) amylase in phosphate buffer at 25 °C with an excitation wavelength of 280 nm.

Fluorescence titration for the interaction of 3 with proteins

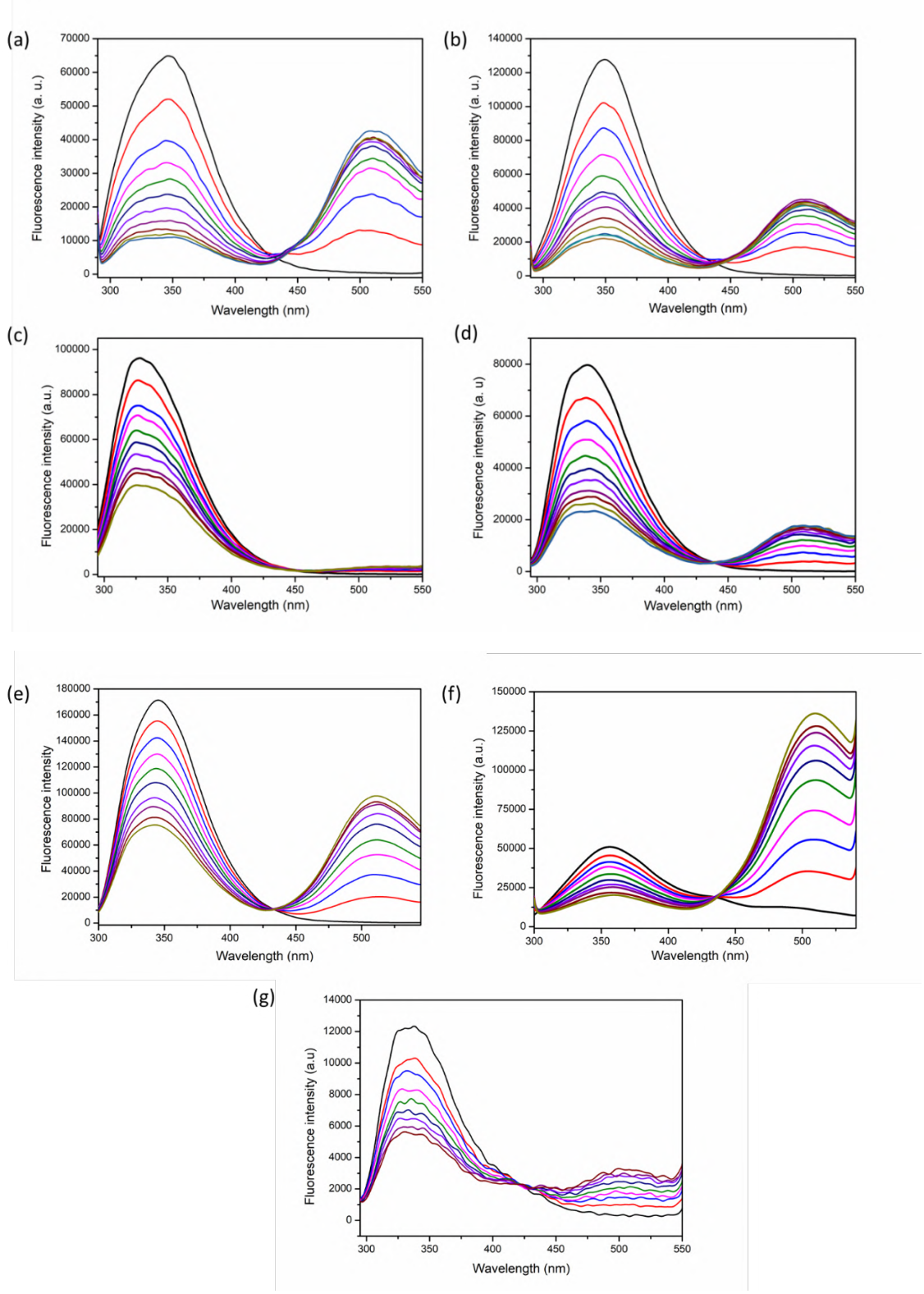


Figure S21: The fluorescence emission spectra for the titration of **3** (4.25 µM to 51 µM) with 10 µM each of (a) HSA, (b) BSA, (c) proteinase, (d) trypsin, (e) lysozyme, (f) haemoglobin, and (g) amylase in phosphate buffer at 25 ^{o}C with an excitation wavelength of 280 nm.

Fluorescence titration for the interaction of 4 with proteins

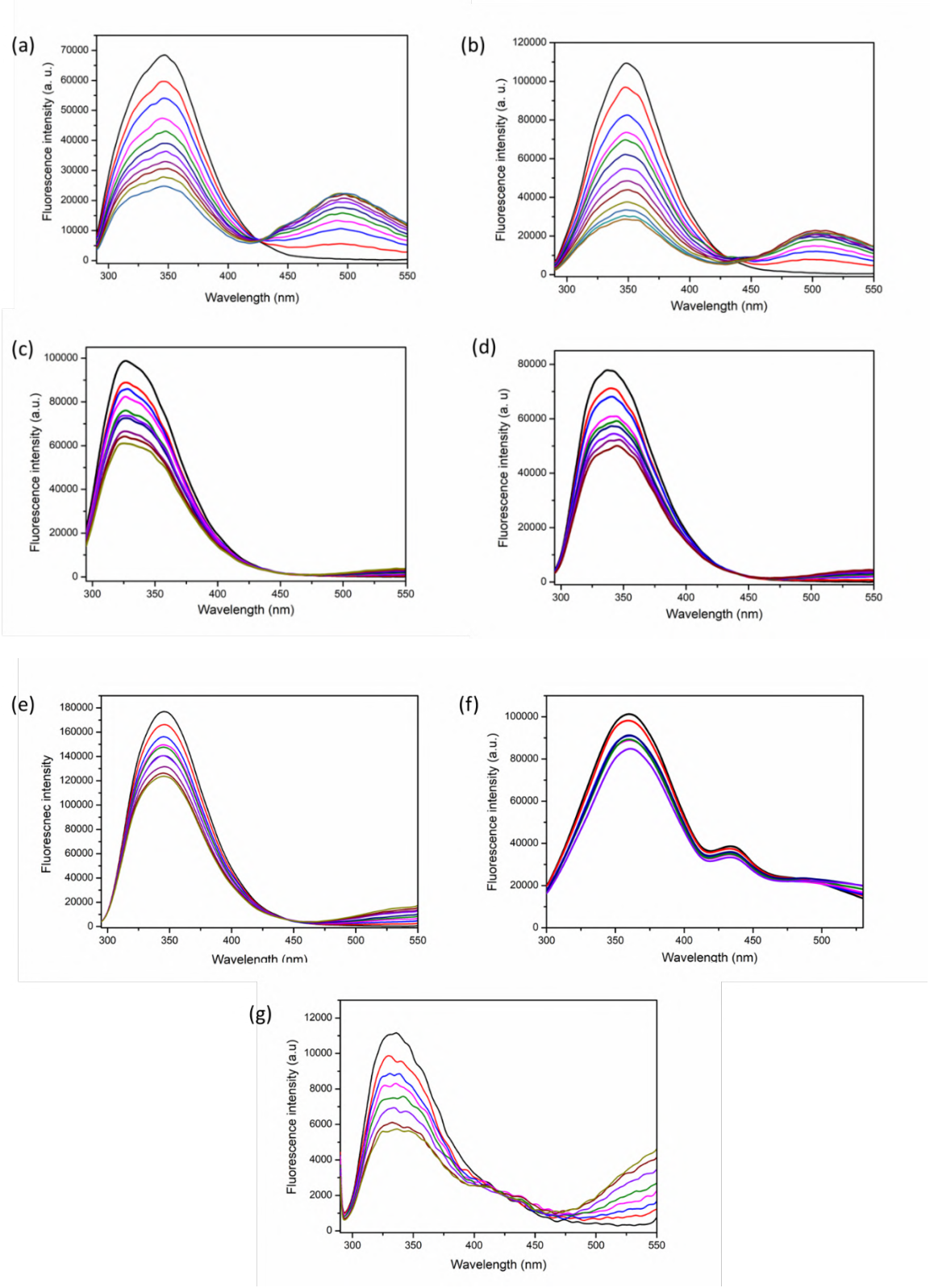


Figure S22: The fluorescence emission spectra for the titration of **4** (4.25 μM to 51 μM) with 10 μM each of (a) HSA, (b) BSA, (c) proteinase (d) trypsin, (e) lysozyme (f) haemoglobin, and (g) amylase in phosphate buffer at 25 ^{o}C with an excitation wavelength of 280 nm.

Fluorescence titration for the interaction of 5 with proteins

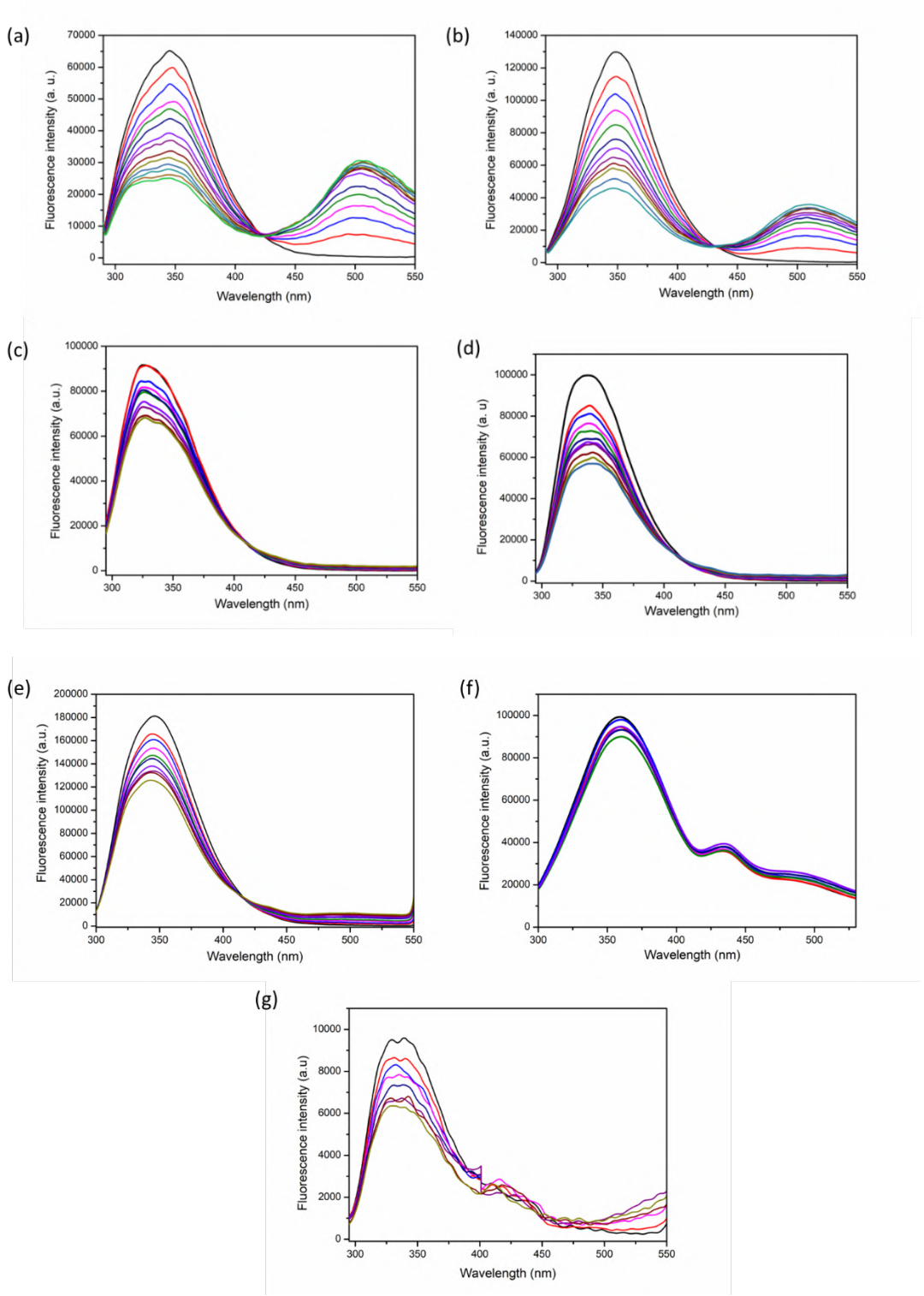


Figure S23 The fluorescence emission spectra for the titration of **5** (4.25 μM to 51 μM) with 10 μM each of (a) HSA, (b) BSA, (c) proteinase (d) trypsin, (e) lysozyme (f) haemoglobin, and (g) amylase in phosphate buffer at 25 ºC with an excitation wavelength of 280 nm.

Fluorescence titration for the interaction of 6 with proteins

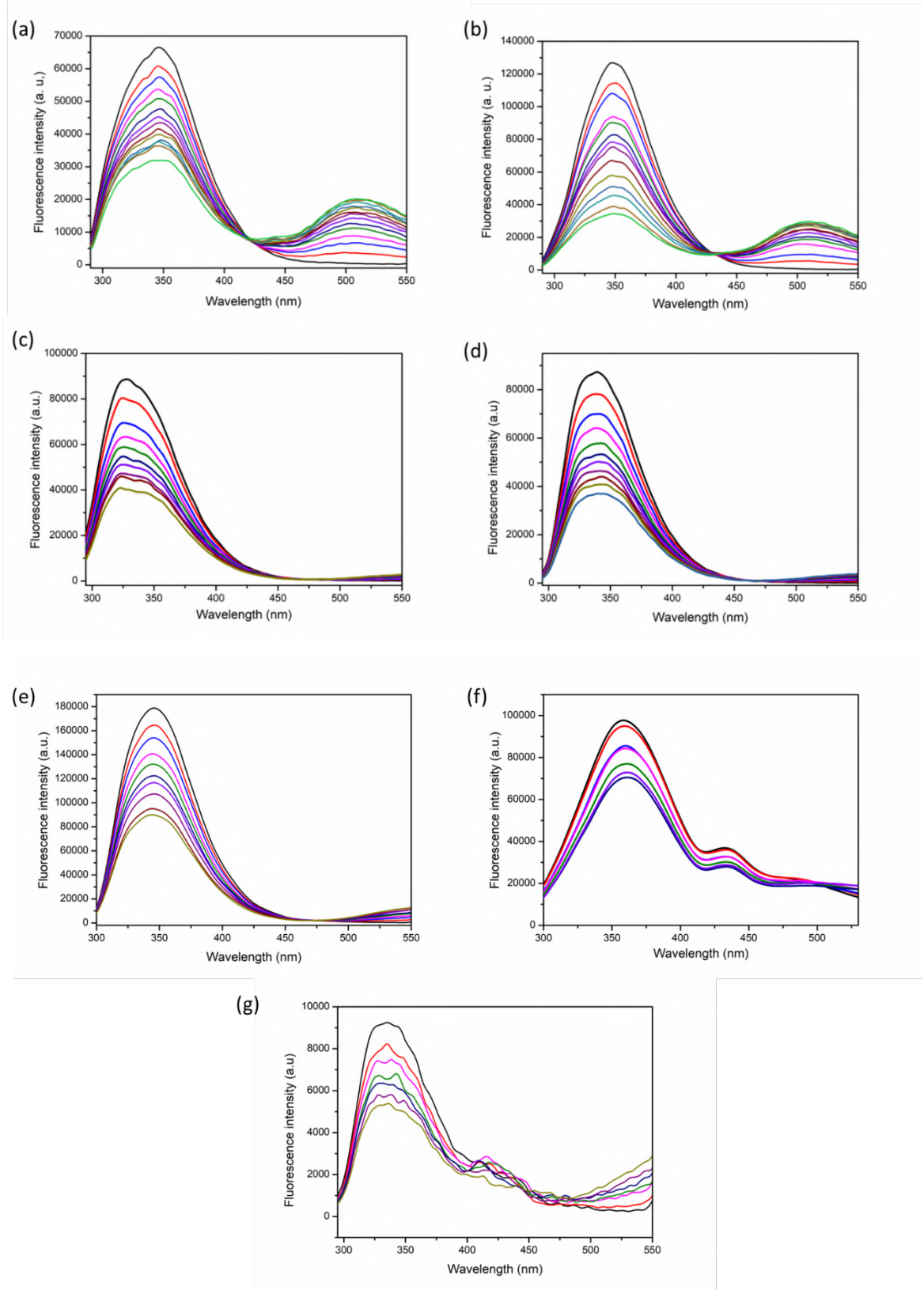


Figure S24: The fluorescence emission spectra for the titration of **6** (4.25 μM to 51 μM) with 10 μM each of (a) HSA, (b) BSA, (c) proteinase (d) trypsin, (e) lysozyme (f) haemoglobin, and (g) amylase in phosphate buffer at 25 °C with an excitation wavelength of 280 nm.

Fluorescence titration of all SDOs with each protein

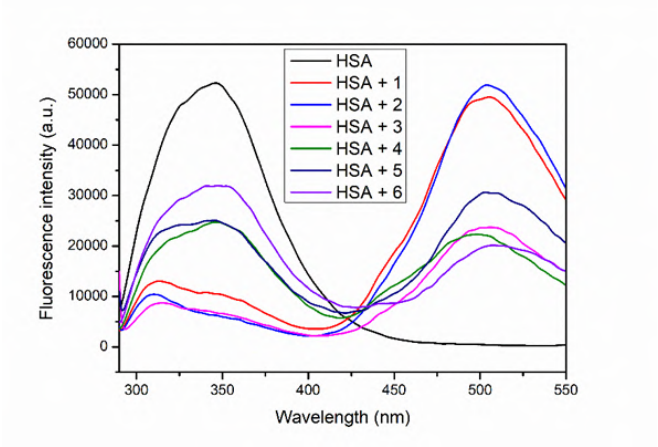
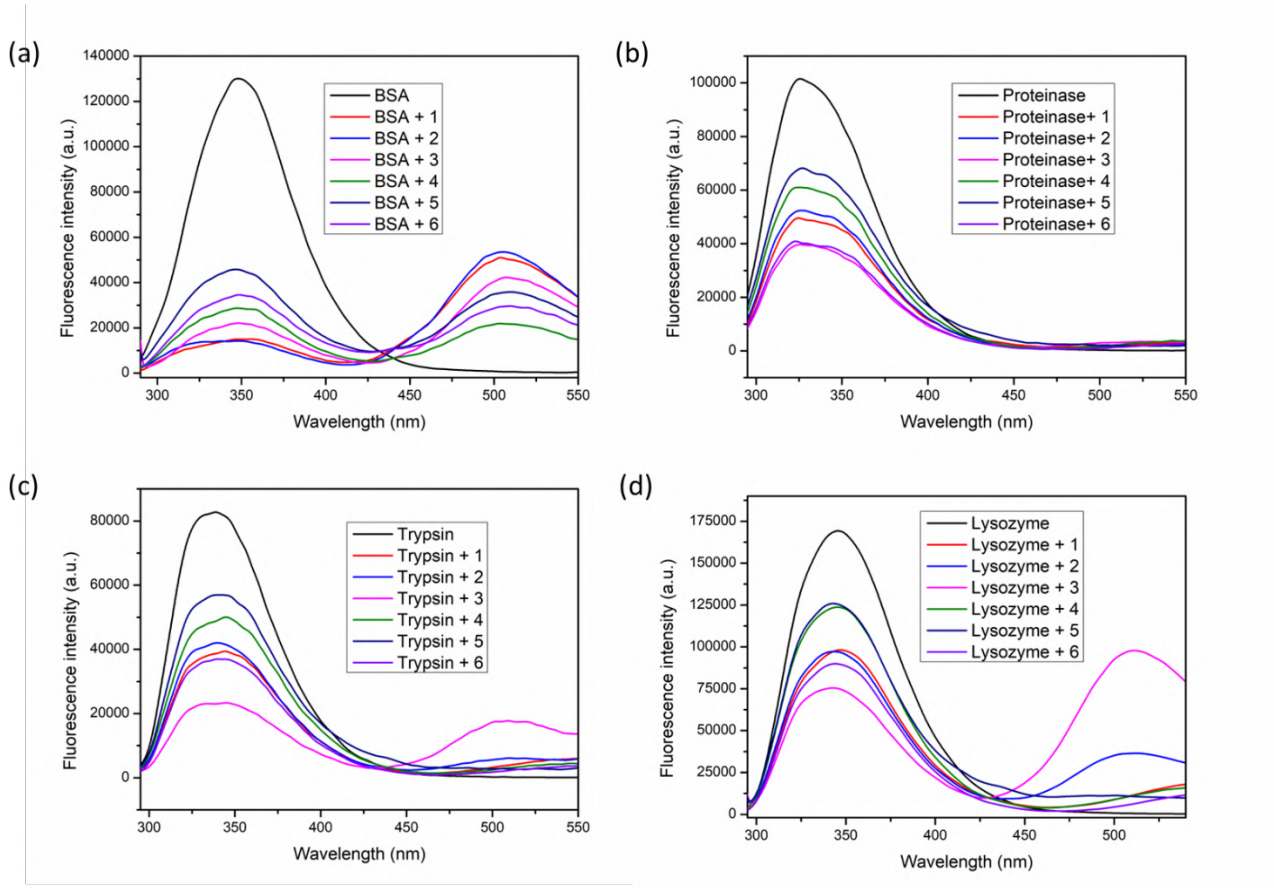


Figure S25: The fluorescence intensity of 10 µM each of HSA upon addition of 50 µM each of **1**, **2**, **3**, **4**, **5**, and **6** in phosphate buffer at 25 °C with an excitation wavelength of 280 nm.



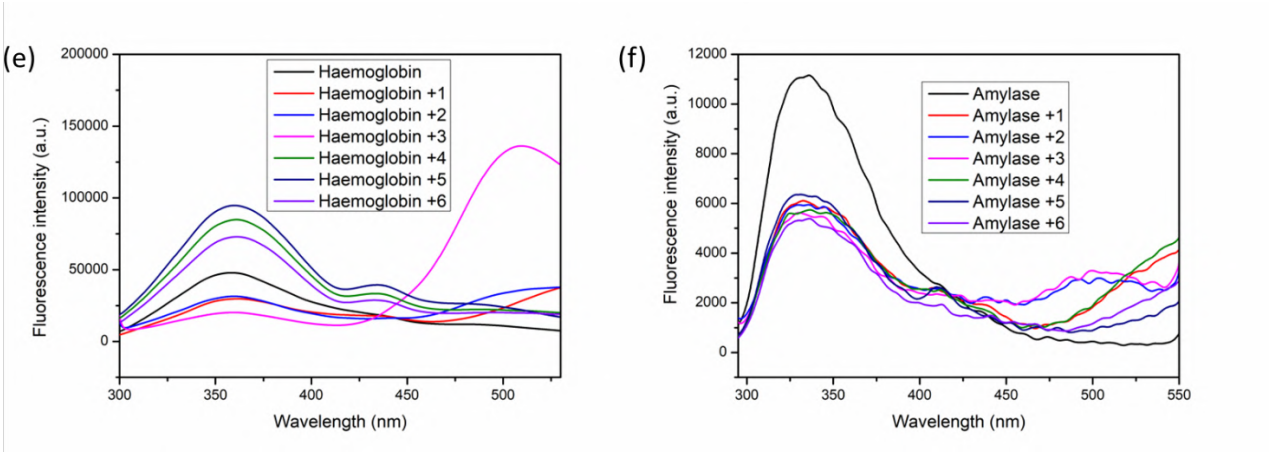


Figure S26: The fluorescence intensity of 10 μM each of (a) BSA, (b) proteinase (c) trypsin, (d) lysozyme (e) haemoglobin, and (f) amylase upon addition of 50 μM each of **1**, **2**, **3**, **4**, **5**, and **6** in phosphate buffer at 25 °C with an excitation wavelength of 280 nm.

Docking studies with BSA

1) Docking of **1** with BSA

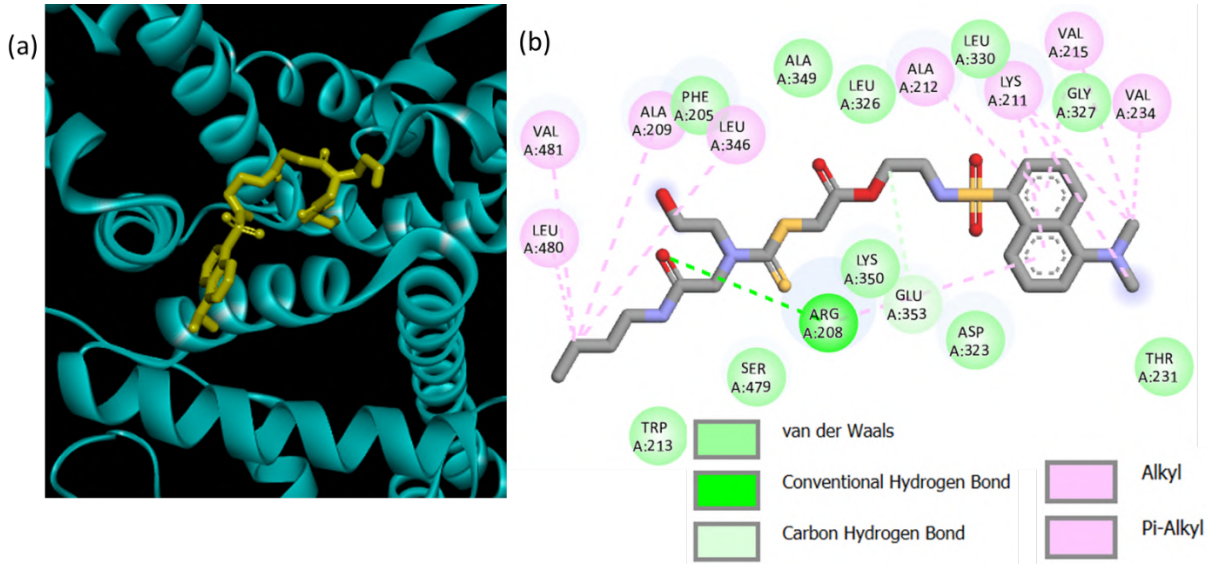


Figure S27: (a) Docking of **1** with BSA and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in BSA.

2) Docking of **2** with BSA

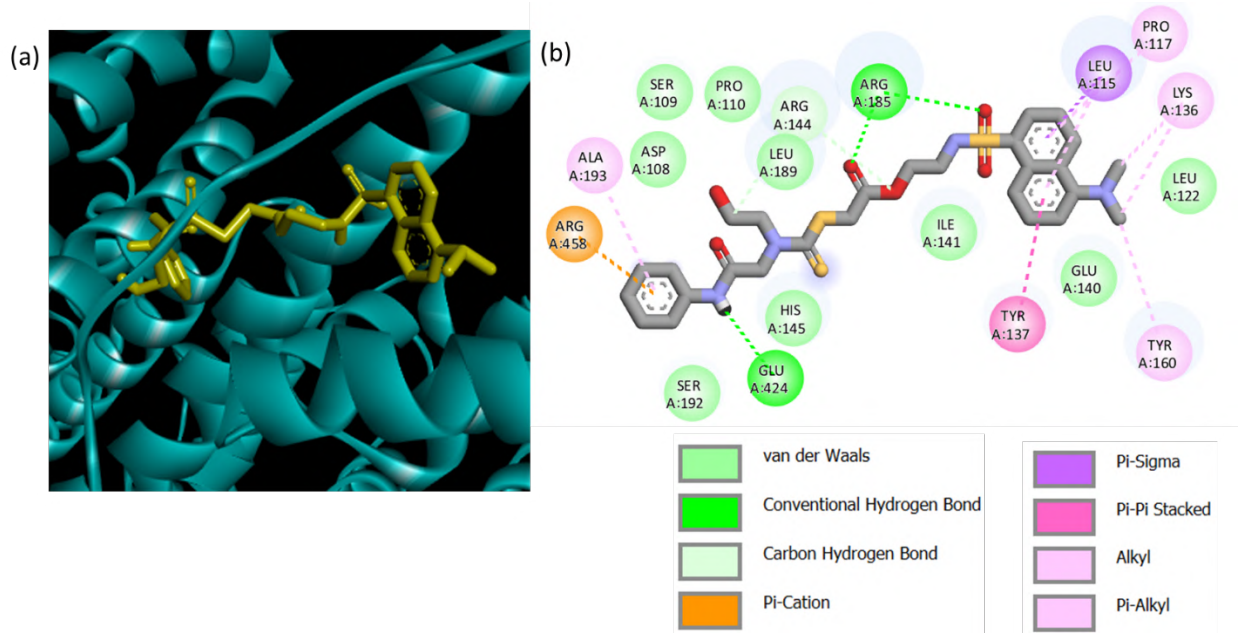


Figure S28: (a) Docking of **2** with BSA and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in BSA.

3) Docking of **3** with BSA

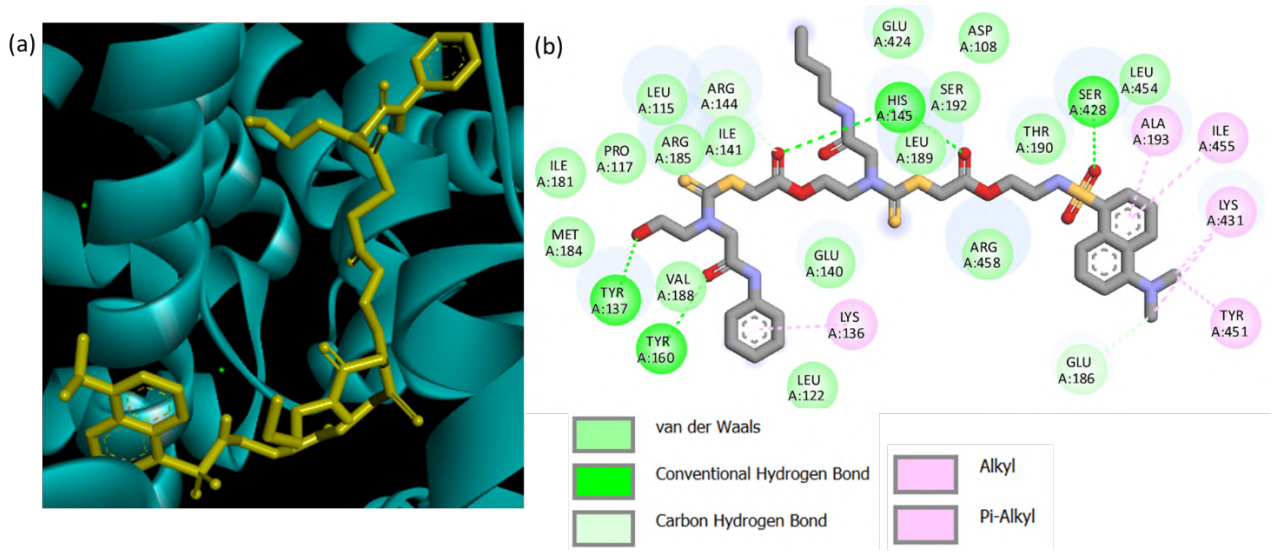


Figure S29: (a) Docking of **3** with BSA and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in BSA.

4) Docking of **4** with BSA

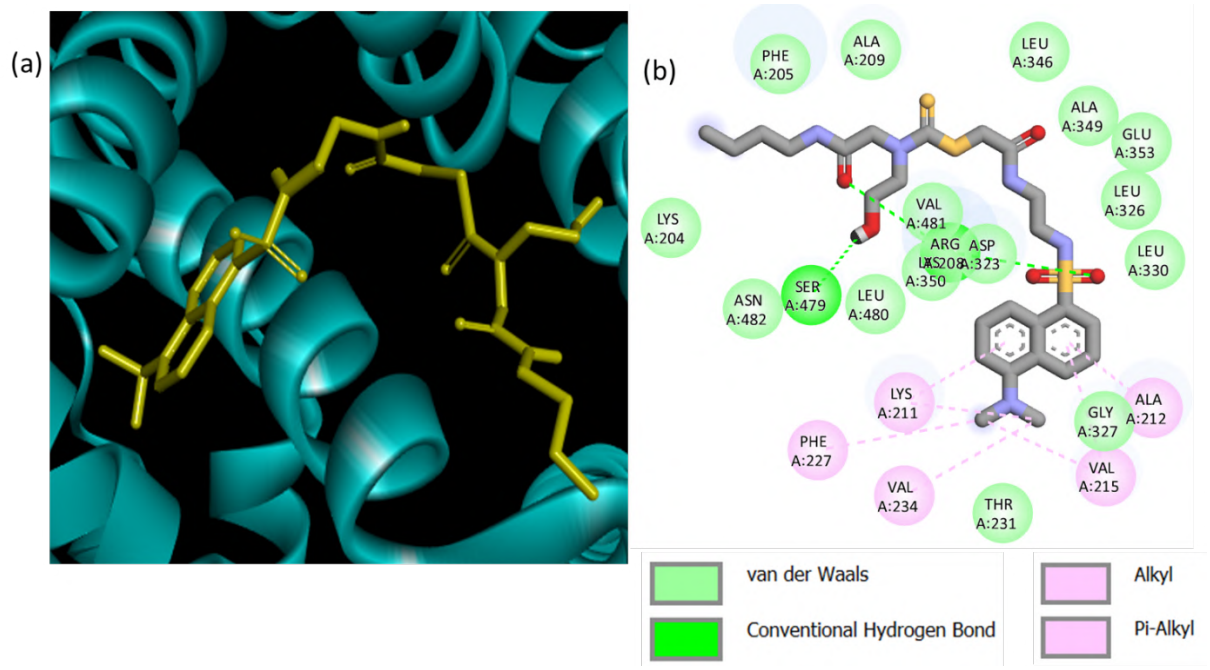


Figure S30: (a) Docking of **4** with BSA and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in BSA.

5) Docking of **5** with BSA

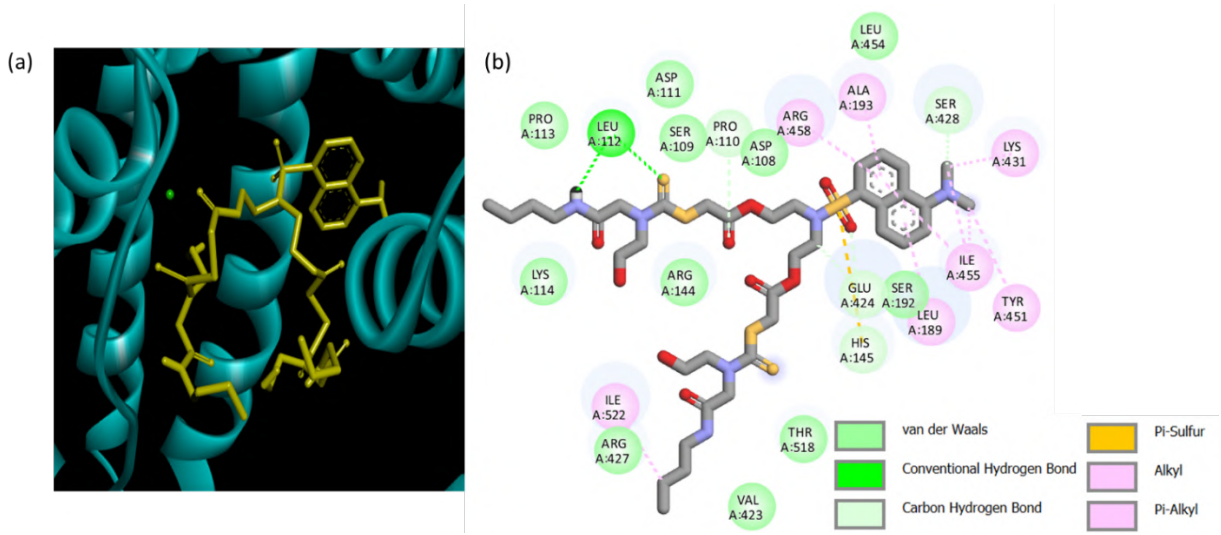


Figure S31: (a) Docking of **5** with BSA and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in BSA.

6) Docking of **6** with BSA

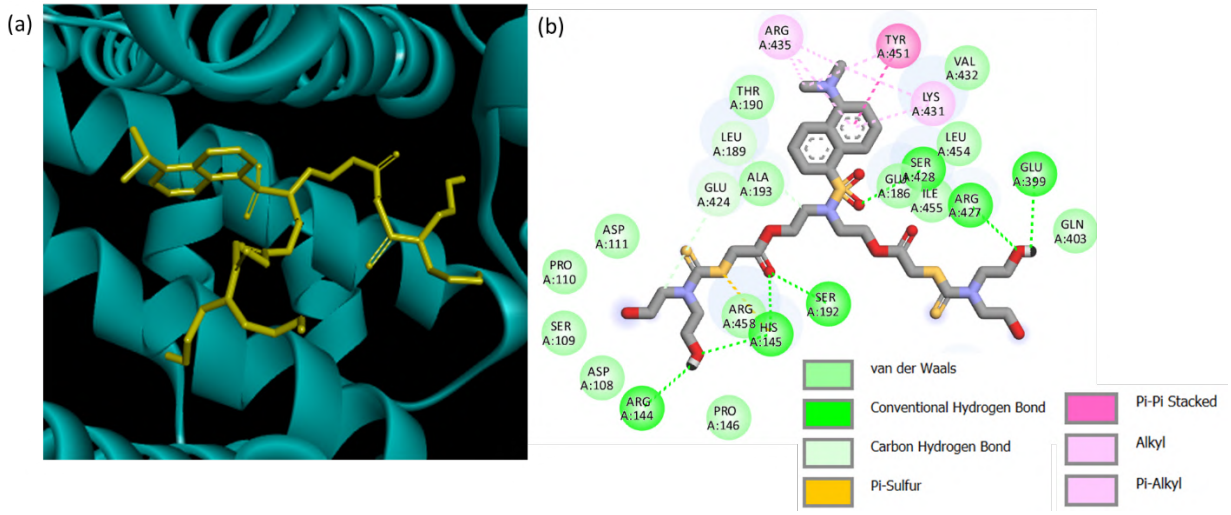


Figure S32: (a) Docking of **6** with BSA and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in BSA.

Docking studies with HSA

1) Docking of **1** with HSA

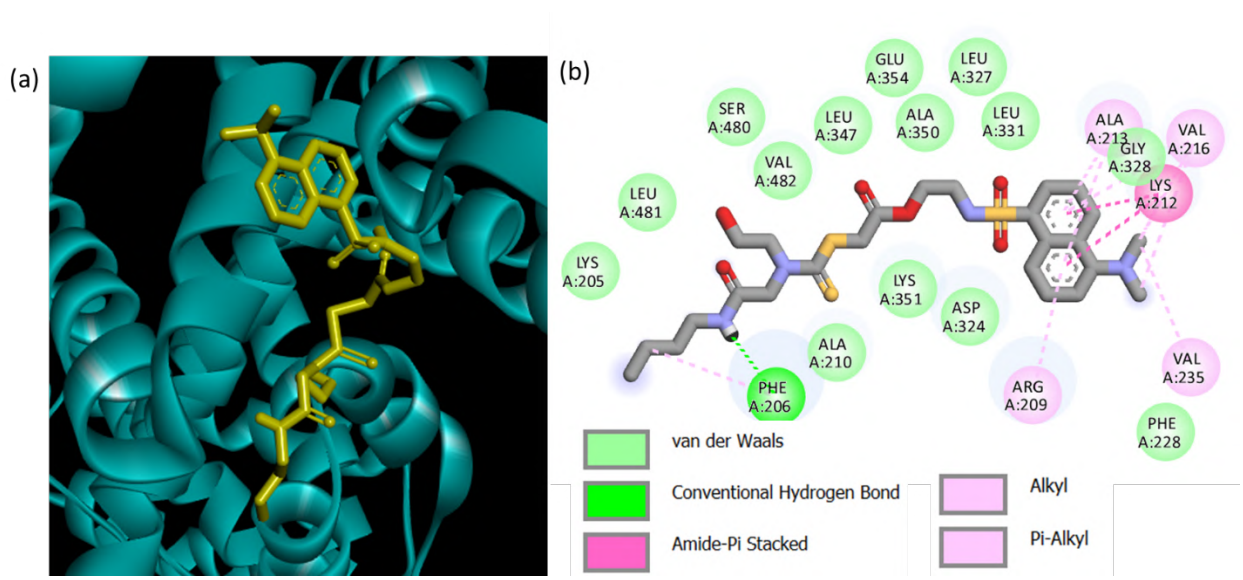


Figure S33: (a) Docking of **1** with HSA and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in HSA.

2) Docking of **2** with HSA

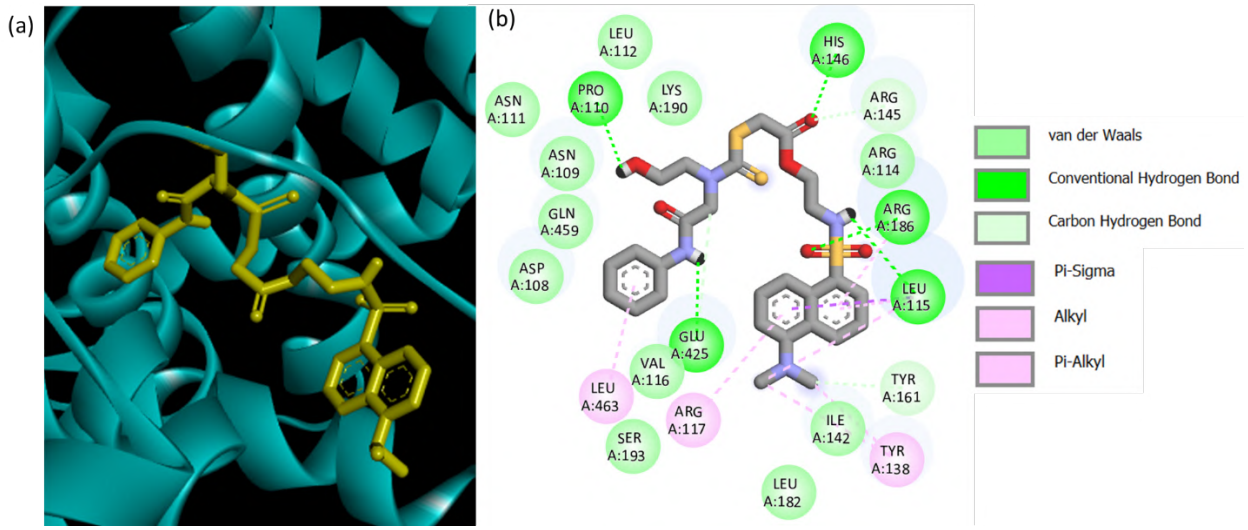


Figure S34: (a) Docking of **2** with HSA and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in HSA.

3) Docking of **3** with HSA

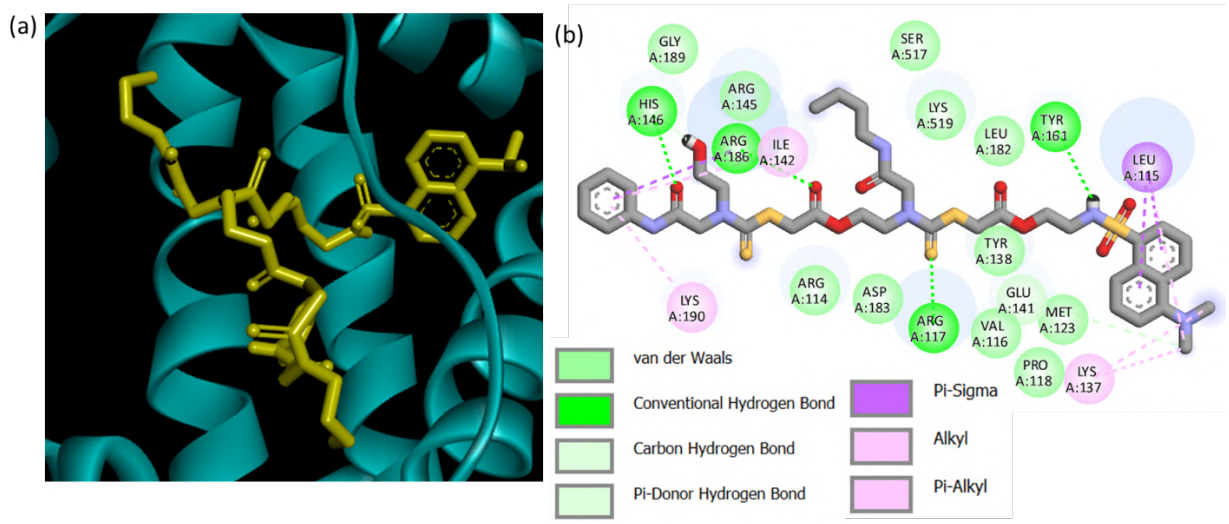


Figure S35: (a) Docking of **3** with HSA and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in HSA.

4) Docking of **4** with HSA

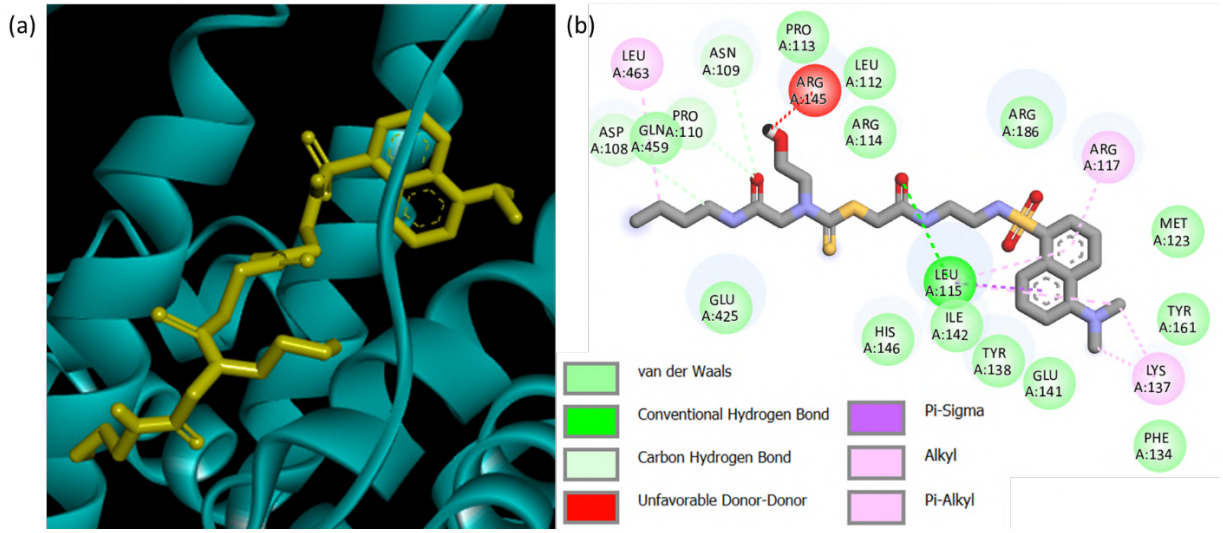


Figure S36: (a) Docking of **4** with HSA and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in HSA.

5) Docking of **5** with HSA

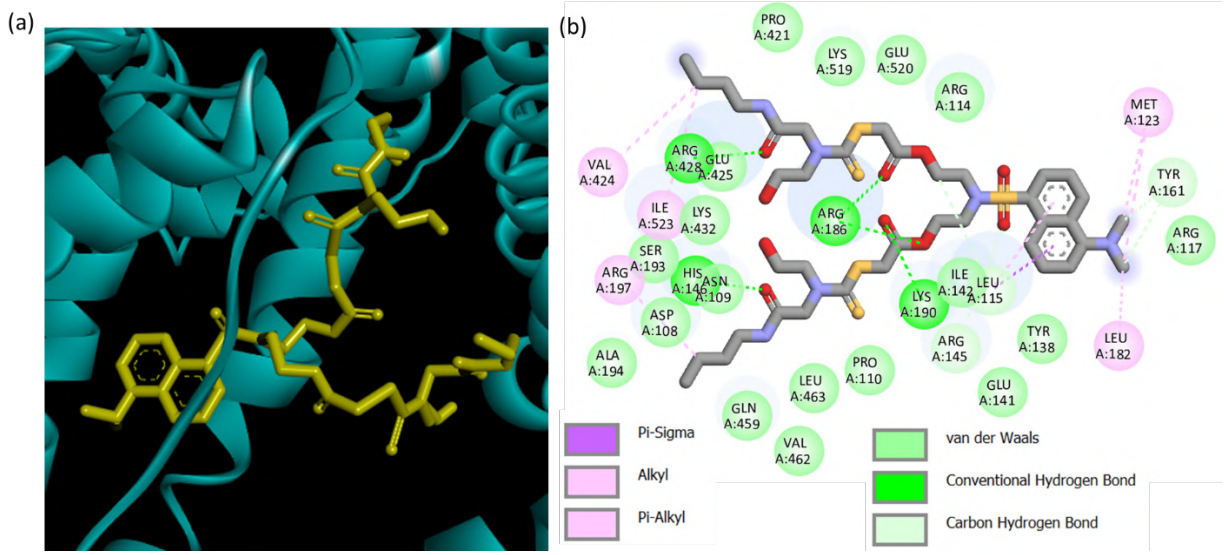


Figure S37: (a) Docking of **5** with HSA and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in HSA.

6) Docking of **6** with HSA

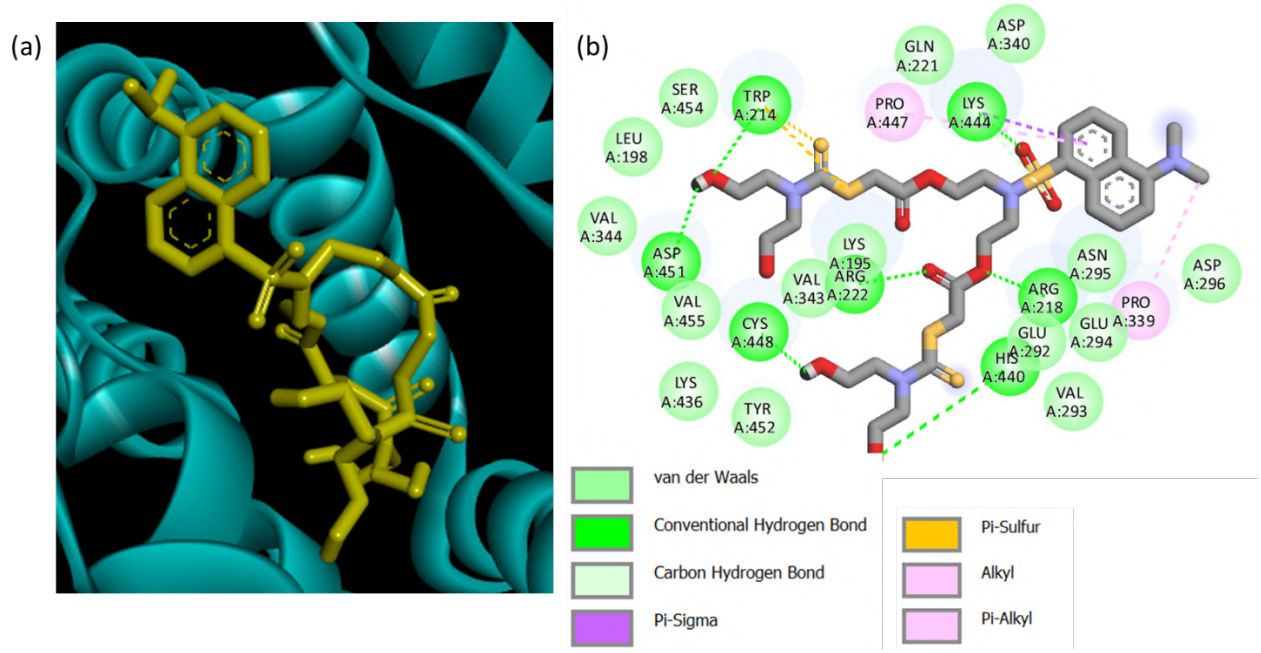


Figure S38: (a) Docking of **6** with HSA and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in HSA.

Docking studies with trypsin

1) Docking of **1** with trypsin

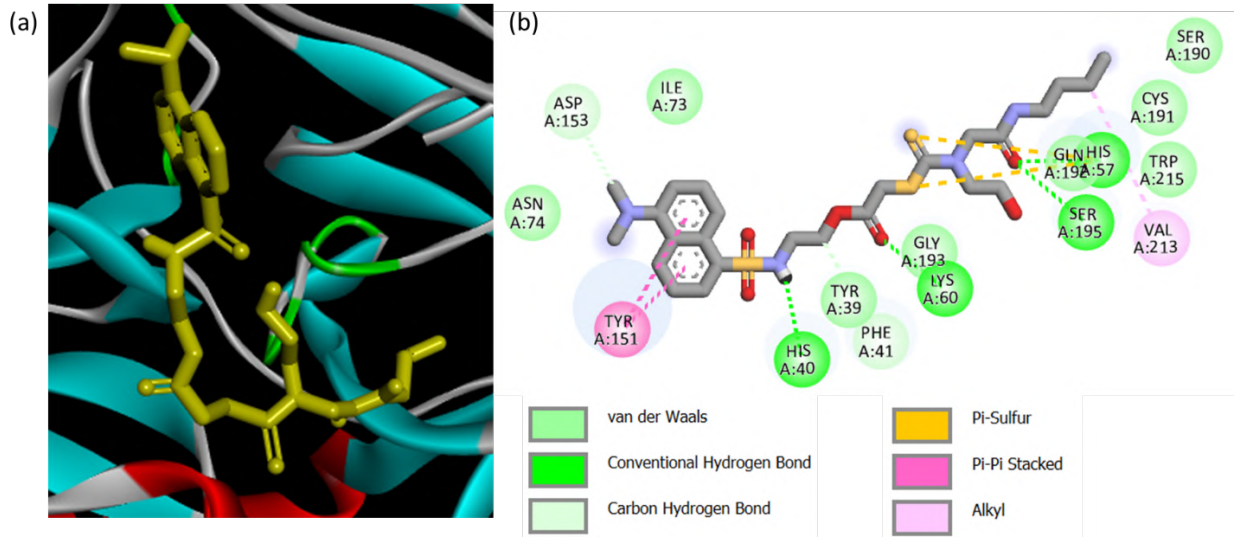


Figure S39: (a) Docking of **1** with trypsin and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in trypsin.

2) Docking of **2** with trypsin

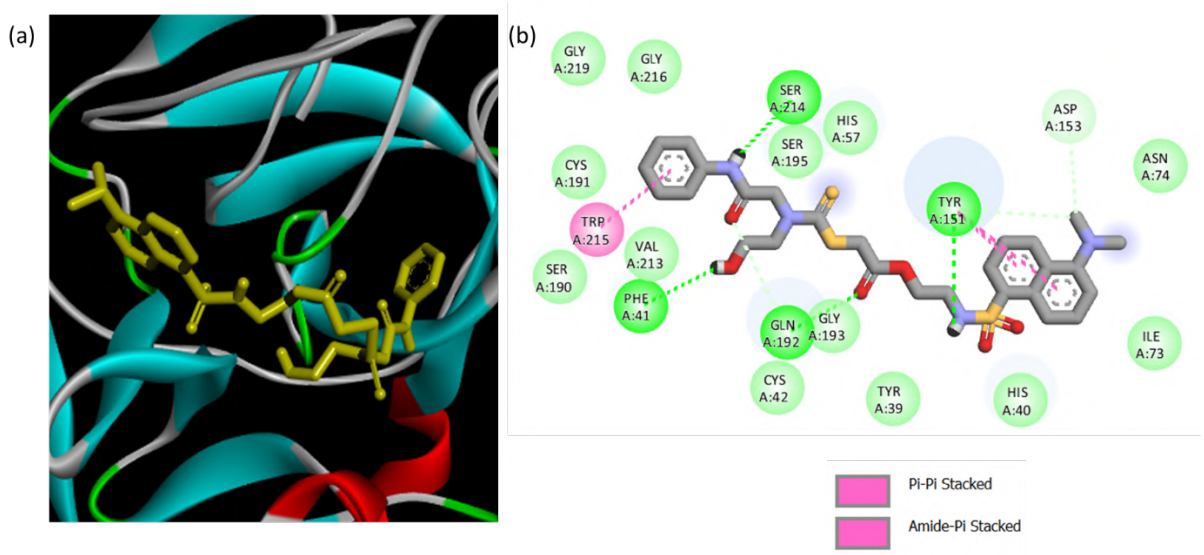


Figure S40: (a) Docking of **2** with trypsin and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in trypsin.

3) Docking of **3** with trypsin

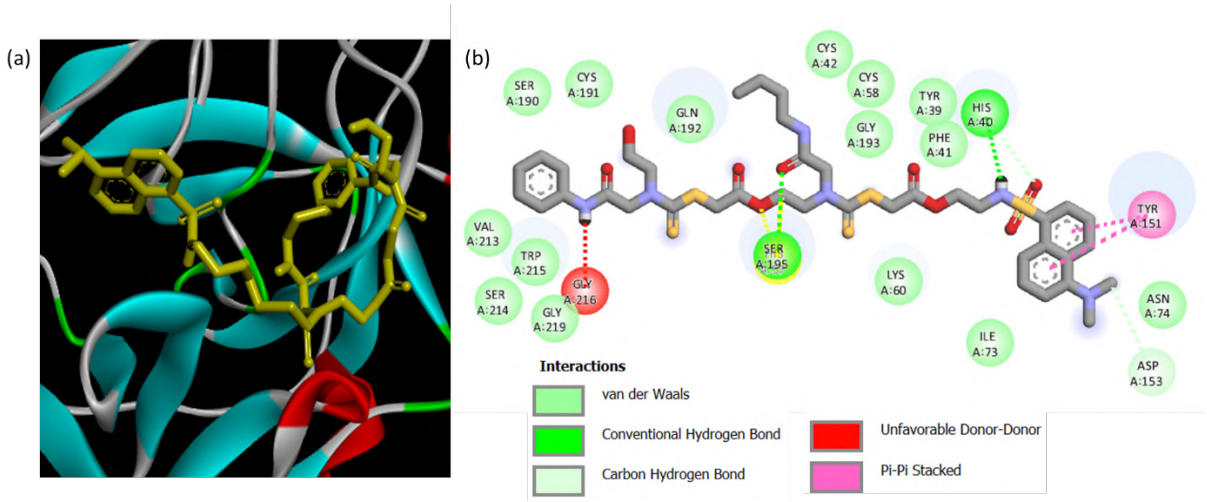


Figure S41: (a) Docking of **3** with trypsin and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in trypsin.

4) Docking of **4** with trypsin

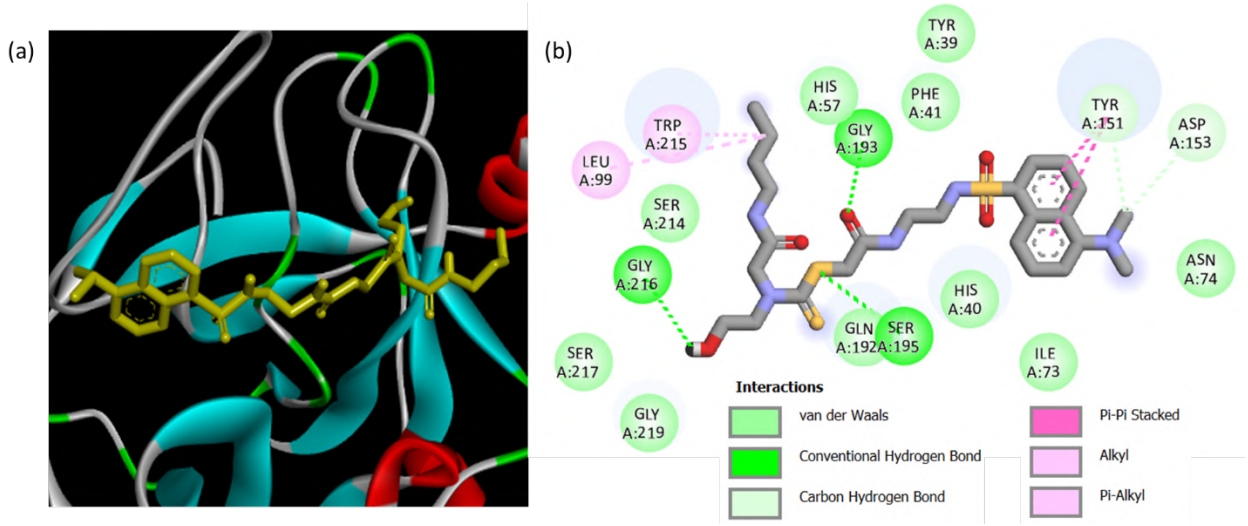


Figure S42: (a) Docking of **4** with trypsin and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in trypsin.

5) Docking of **5** with trypsin

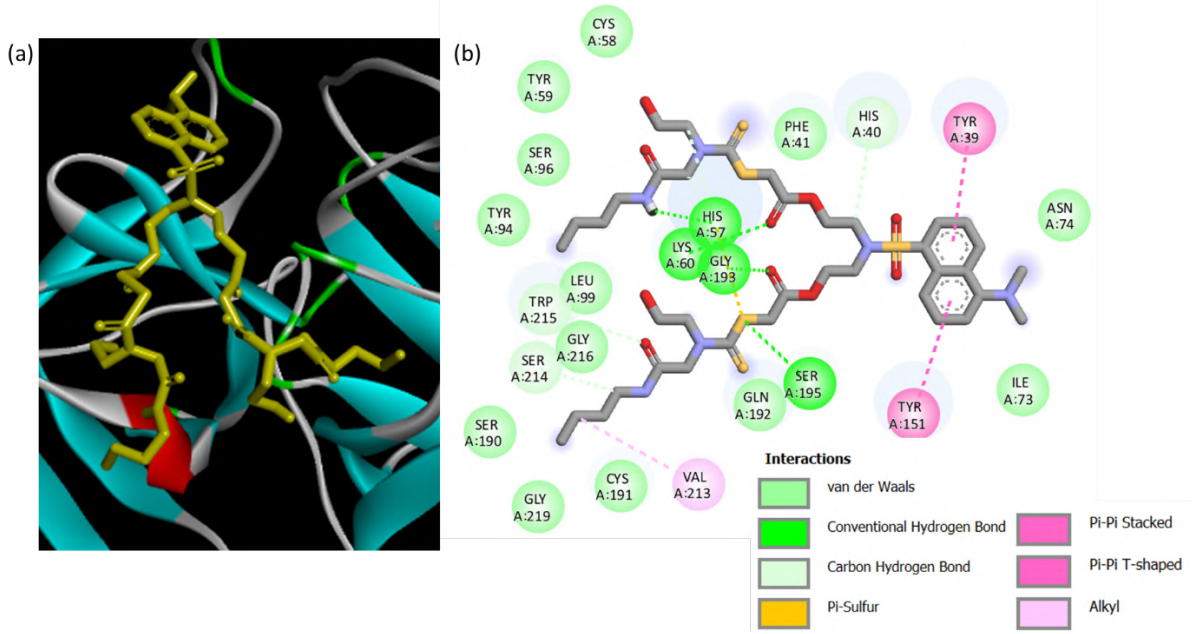


Figure S43: (a) Docking of **5** with trypsin and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in trypsin.

6) Docking of **6** with trypsin

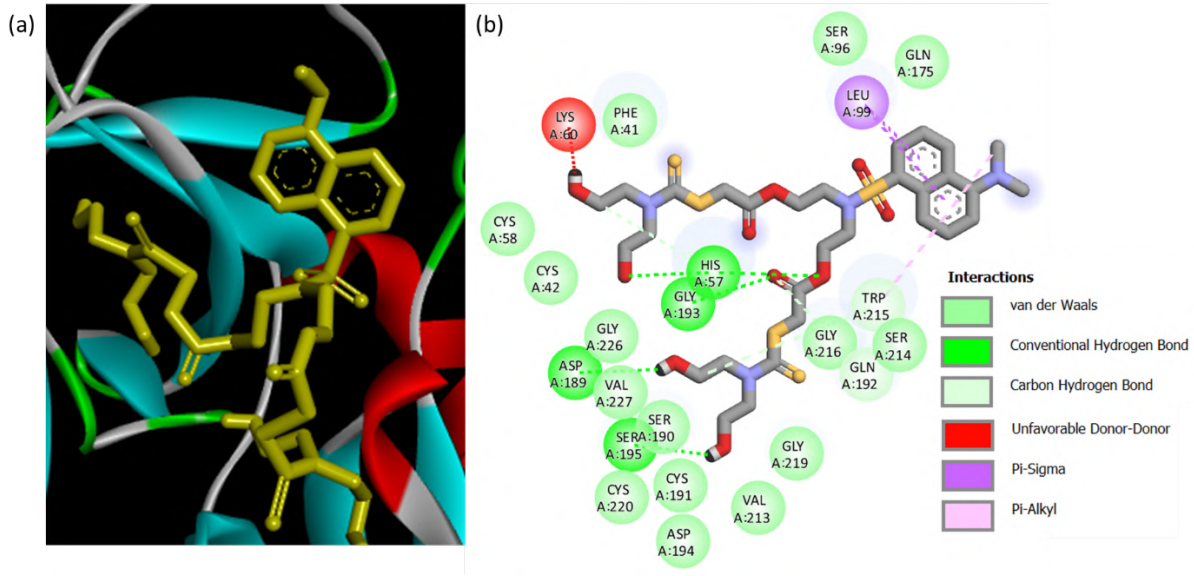


Figure S44: (a) Docking of **6** with trypsin and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in trypsin.

Docking studies with amylase

1) Docking of **1** with amylase

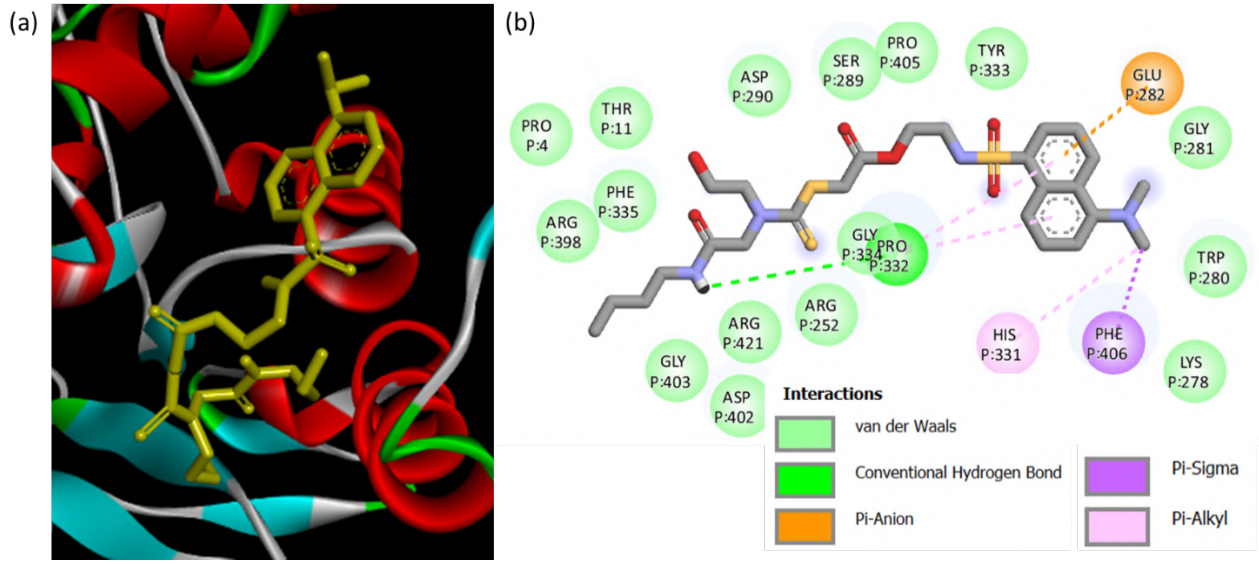


Figure S45: (a) Docking of **1** with amylase and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in amylase.

2) Docking of **2** with amylase

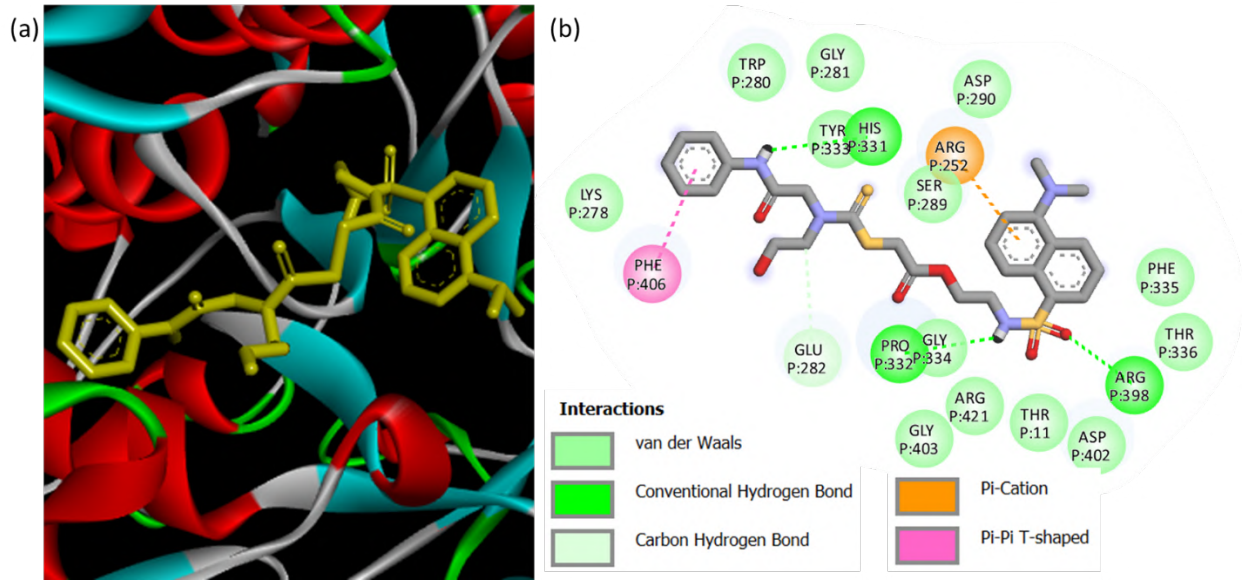


Figure S46: (a) Docking of **2** with amylase and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in amylase.

3) Docking of **3** with amylase

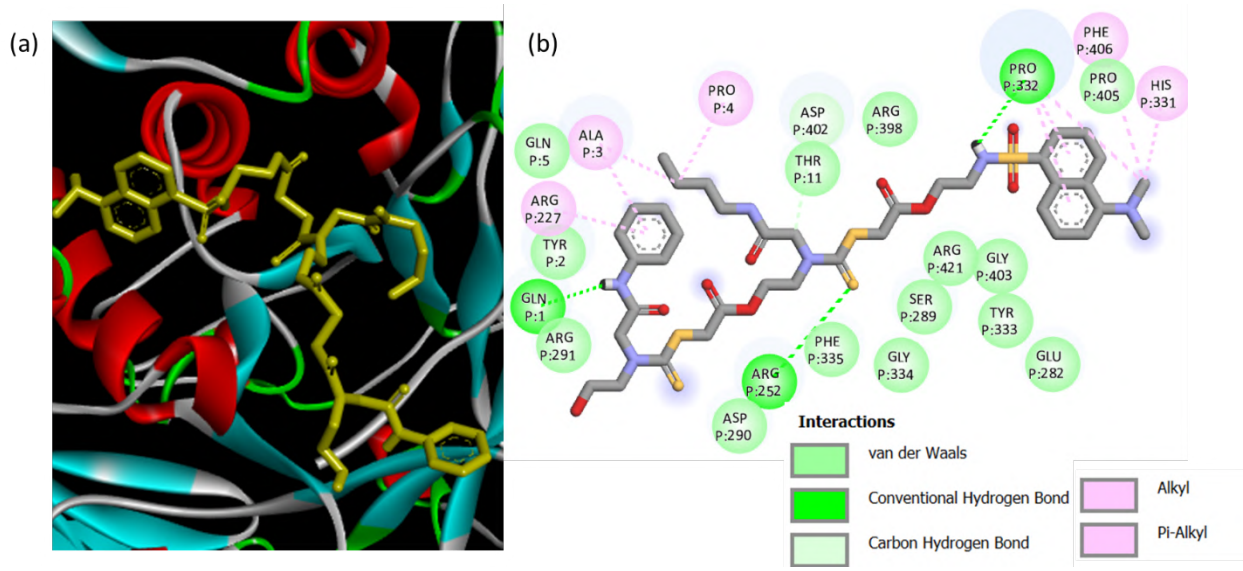


Figure S47: (a) Docking of **3** with amylase and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in amylase.

4) Docking of **4** with amylase

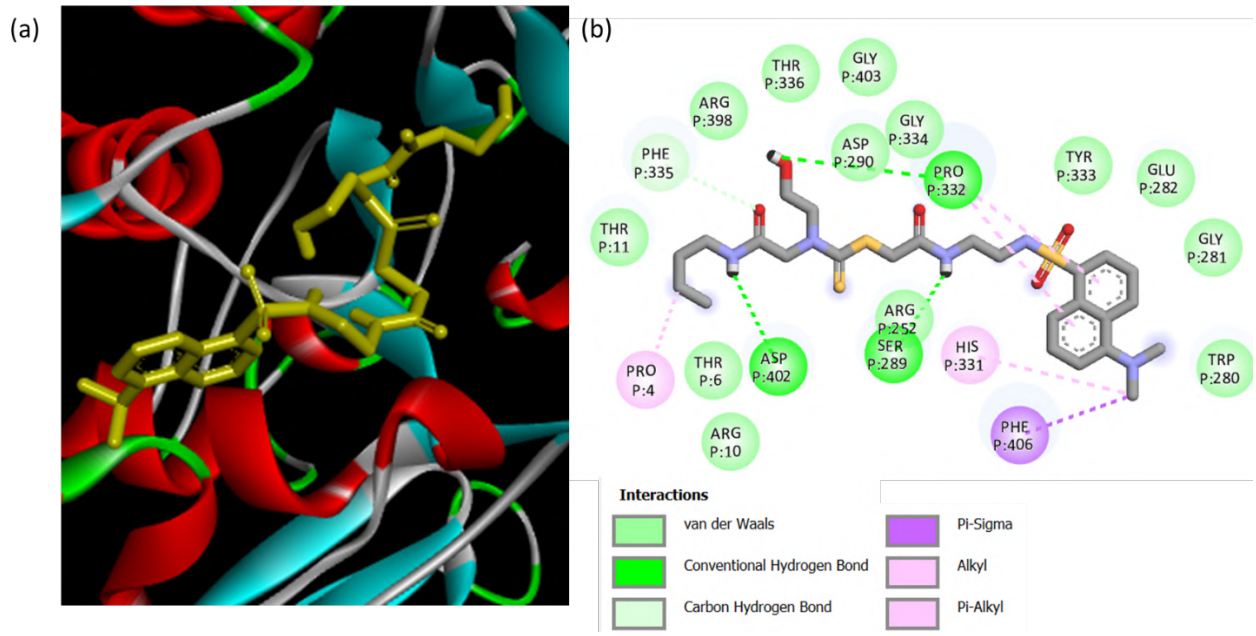


Figure S48: (a) Docking of **4** with amylase and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in amylase.

5) Docking of **5** with amylase

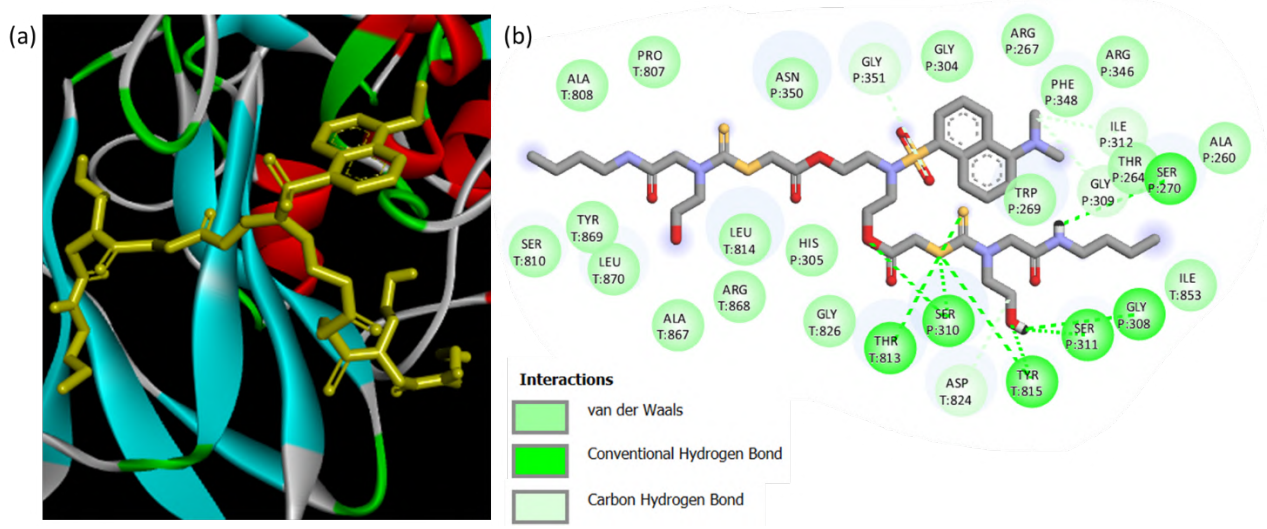


Figure S49: (a) Docking of **5** with ribonuclease and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in amylase.

6) Docking of **6** with amylase

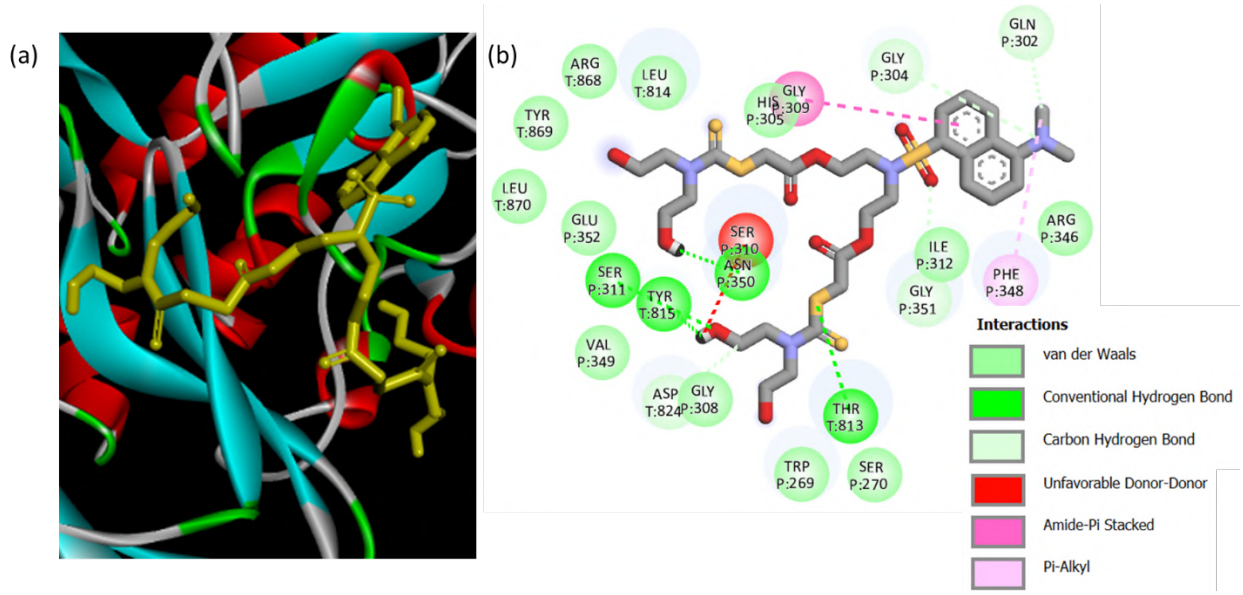


Figure S50: (a) Docking of **6** with amylase and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in amylase.

Docking studies with proteinase

1) Docking of **1** with proteinase

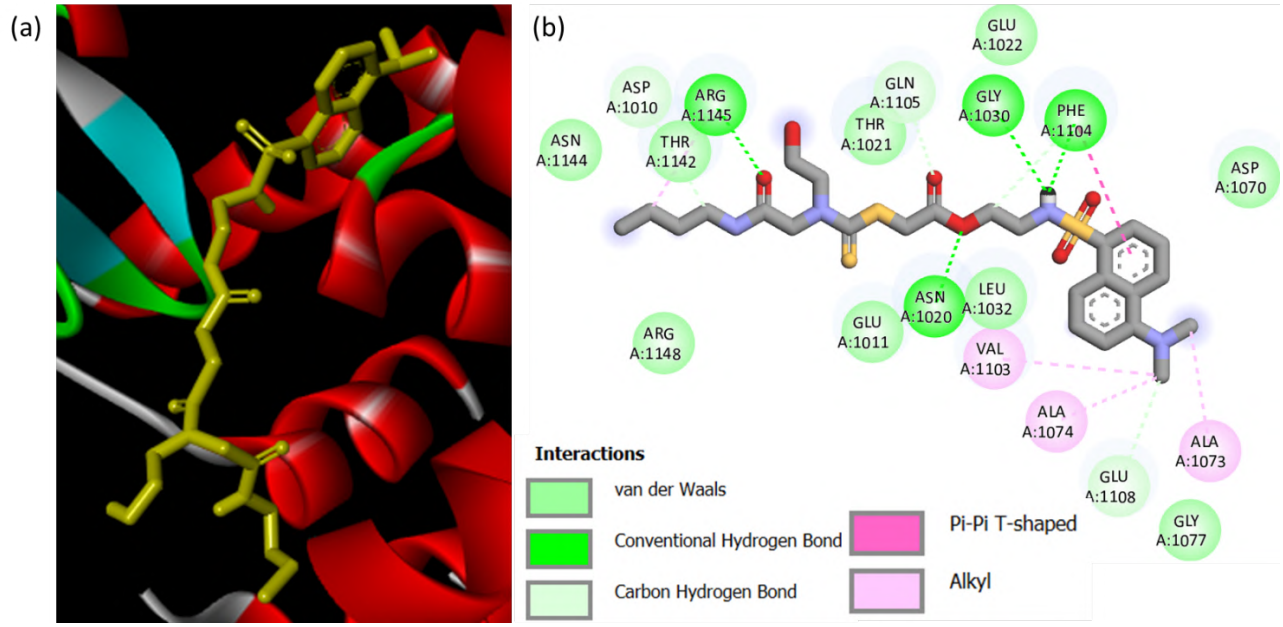


Figure S51: (a) Docking of **1** with proteinase and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in proteinase.

2) Docking of **2** with proteinase

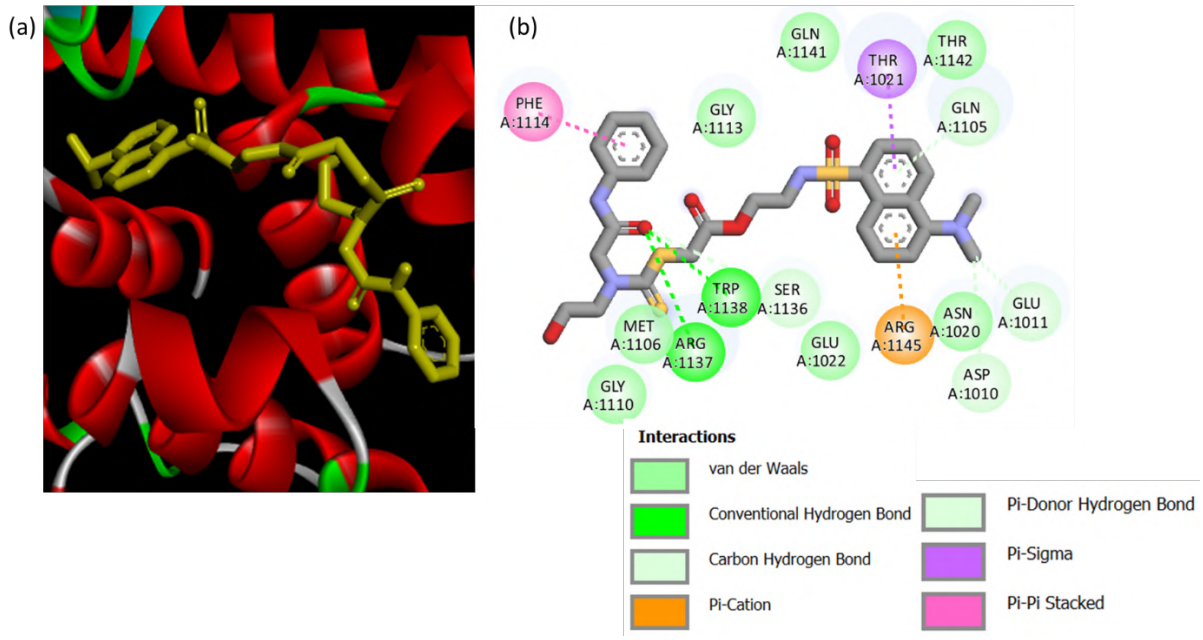


Figure S52: (a) Docking of **2** with proteinase and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in proteinase.

3) Docking of **3** with proteinase

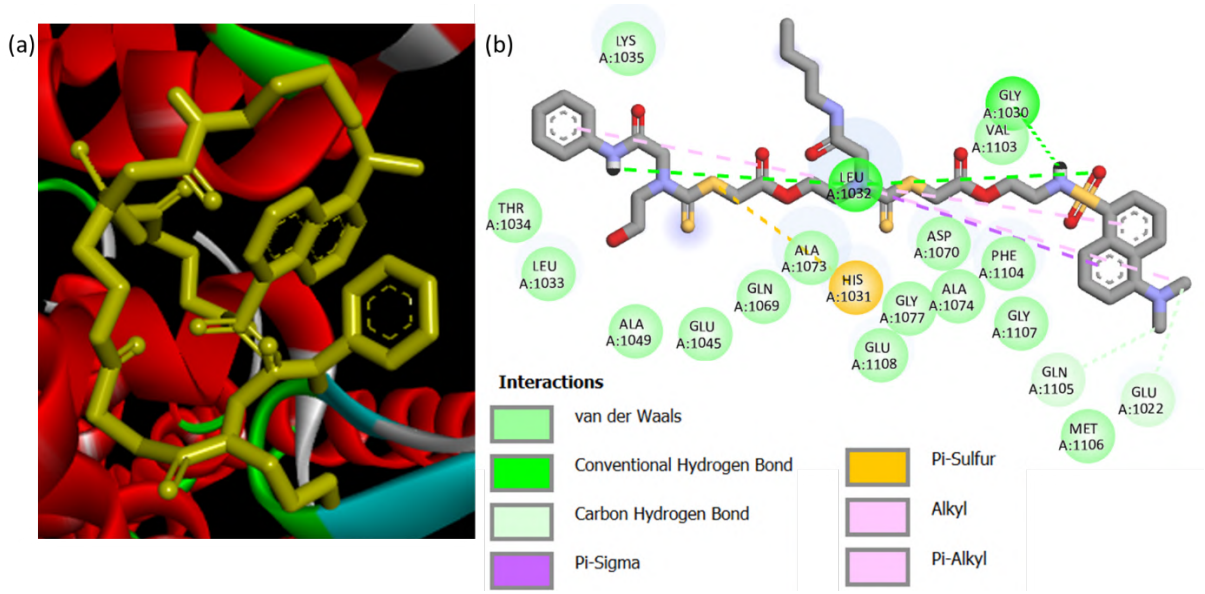


Figure S53: (a) Docking of **3** with proteinase and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in proteinase.

4) Docking of **4** with proteinase

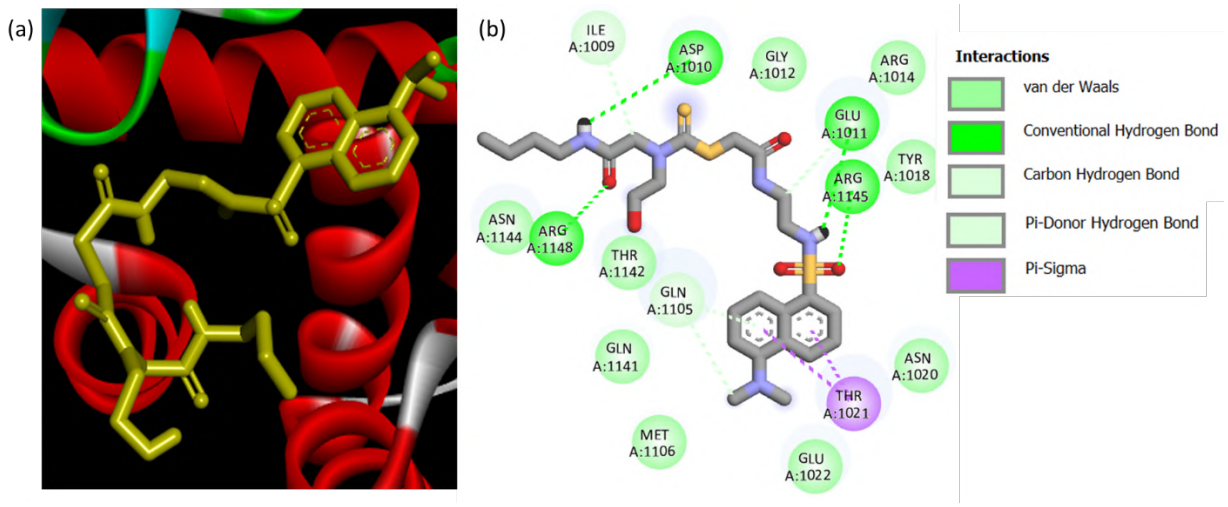


Figure S54: (a) Docking of **4** with proteinase and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in proteinase.

5) Docking of **5** with proteinase

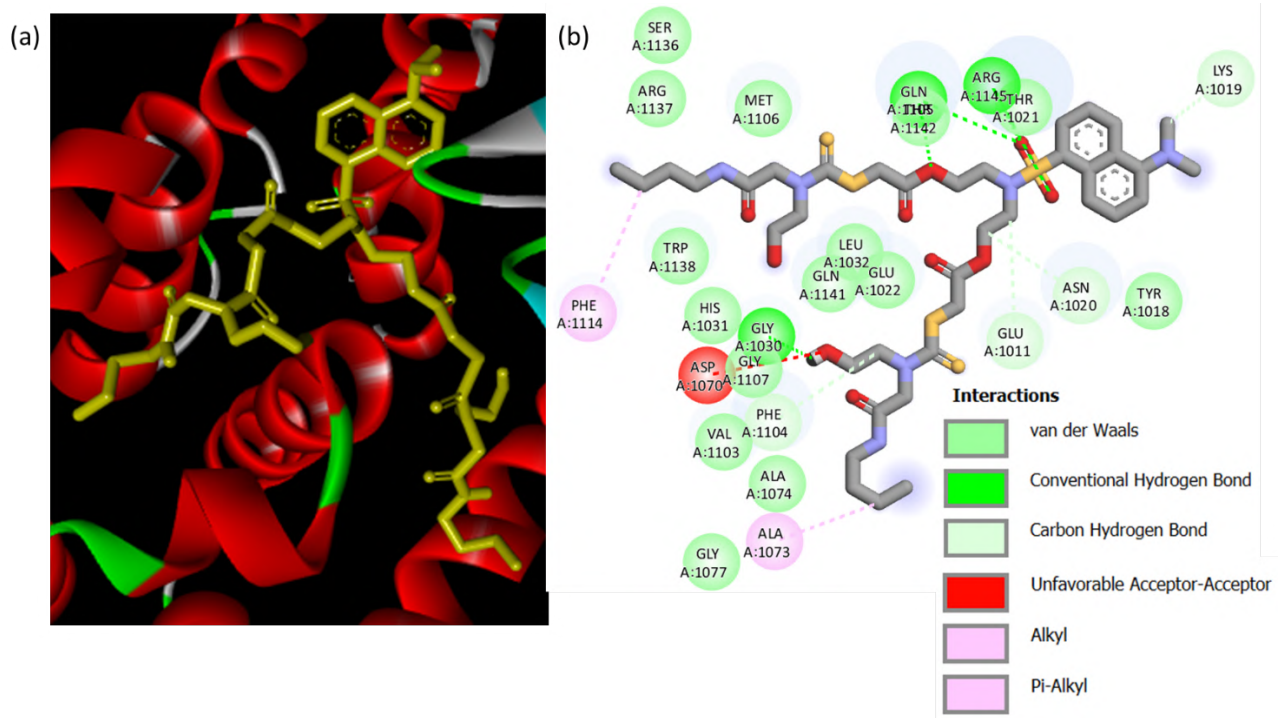


Figure S55: (a) Docking of **5** with proteinase and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in proteinase.

6) Docking of **6** with proteinase

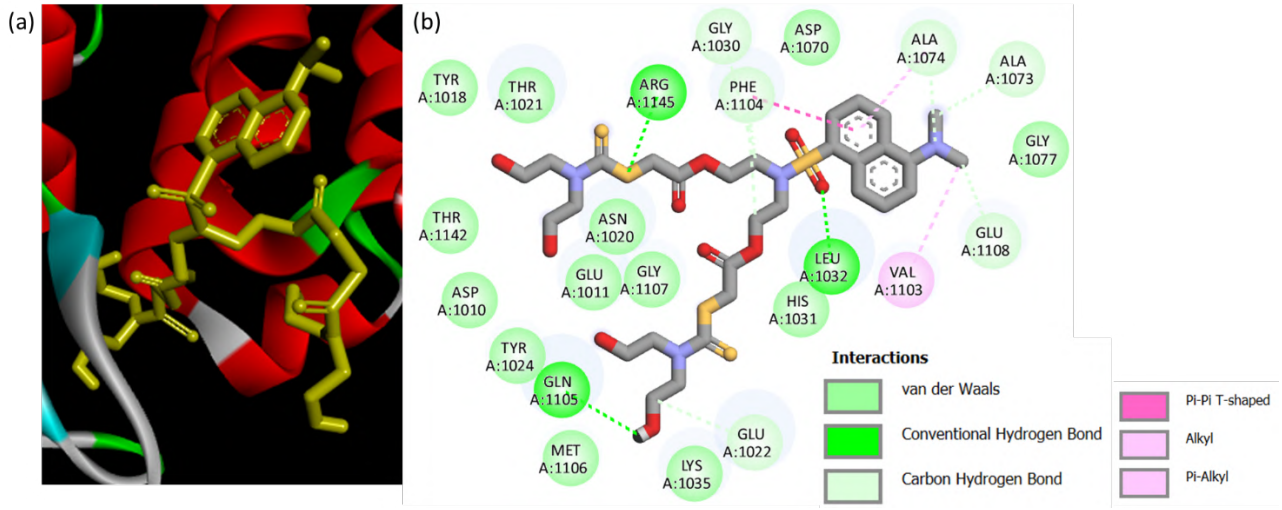


Figure S56: (a) Docking of **6** with proteinase and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in proteinase.

Docking studies with lysozyme

1) Docking of **1** with lysozyme

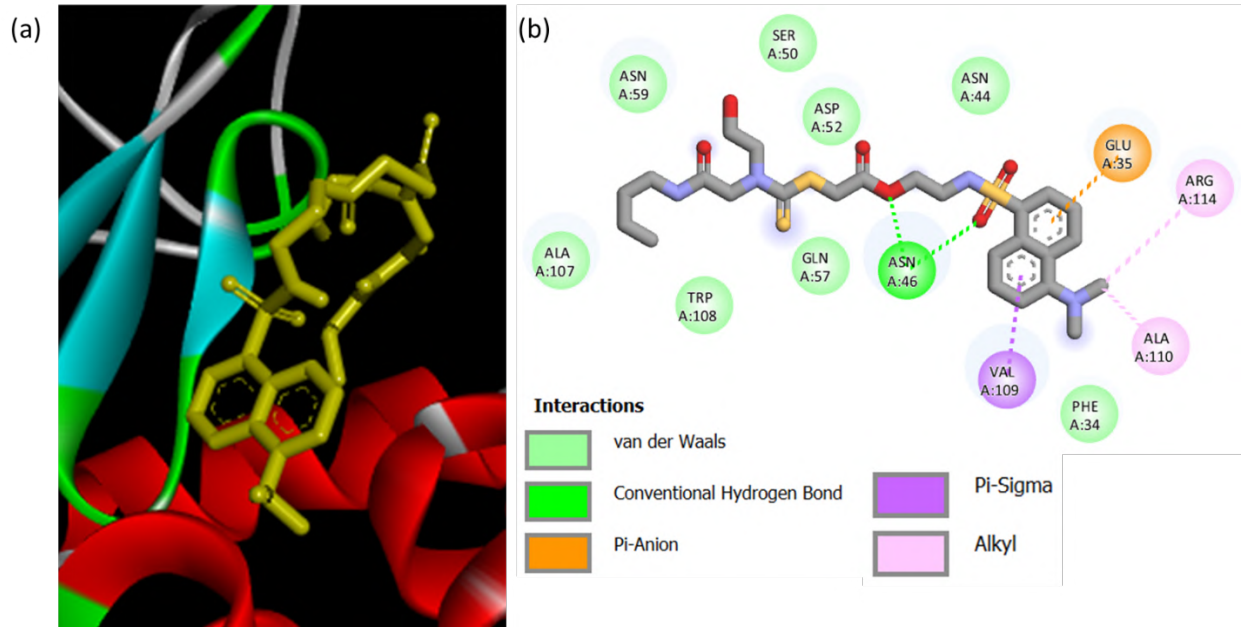


Figure S57: (a) Docking of **1** with lysozyme and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in lysozyme.

2) Docking of **2** with lysozyme

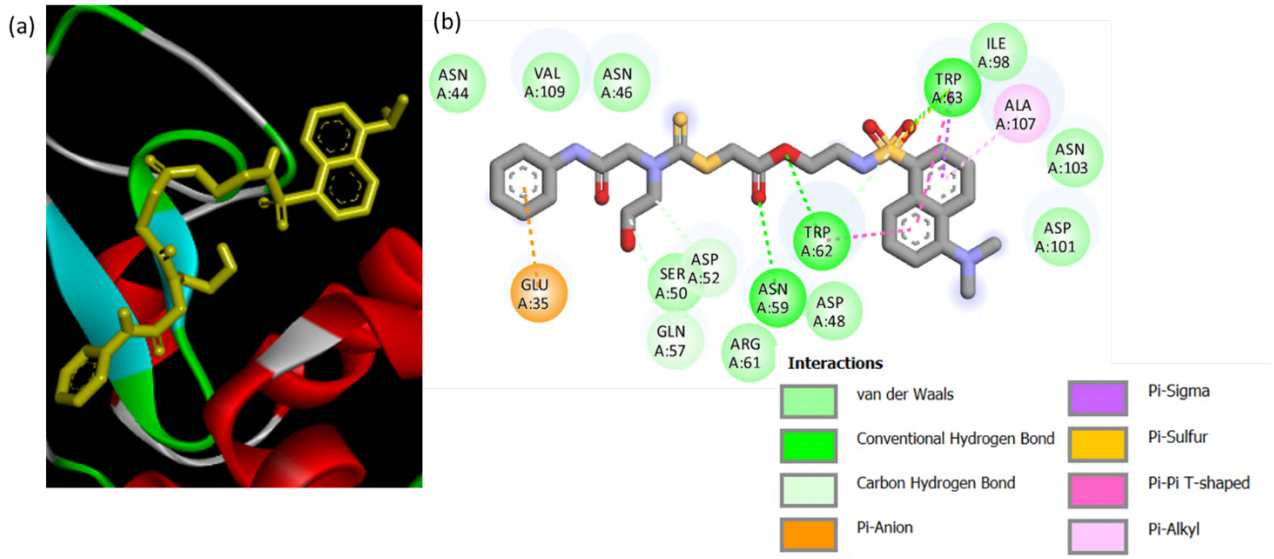


Figure S58: (a) Docking of **2** with lysozyme and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in lysozyme.

3) Docking of **3** with lysozyme

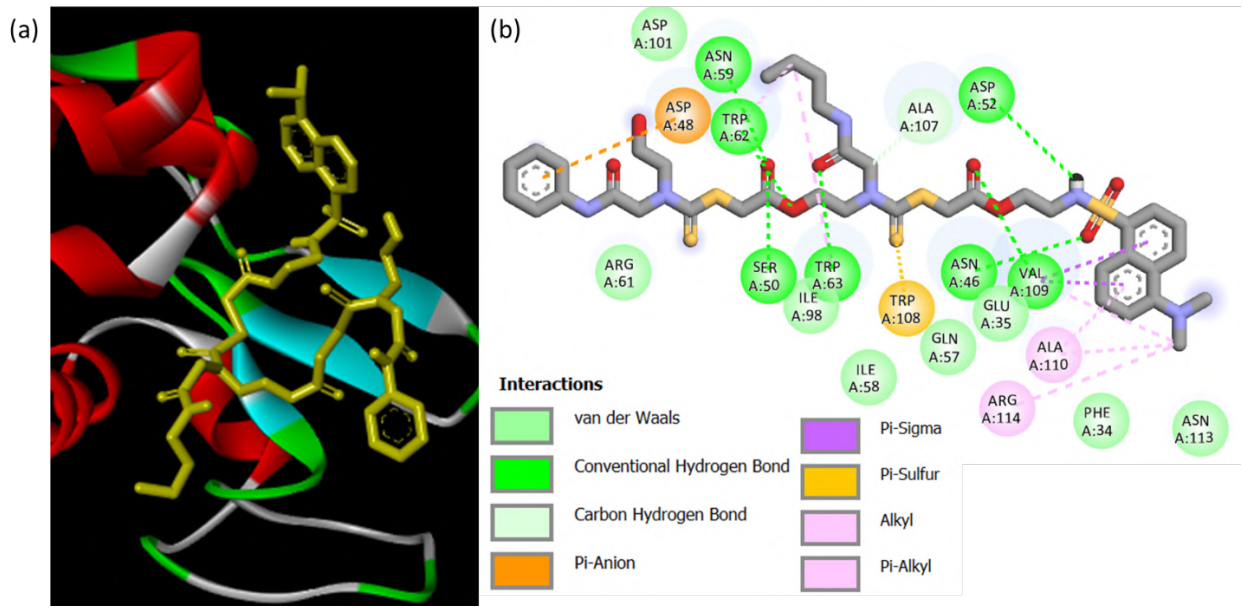


Figure S59: (a) Docking of **3** with lysozyme and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in lysozyme.

4) Docking of **4** with lysozyme

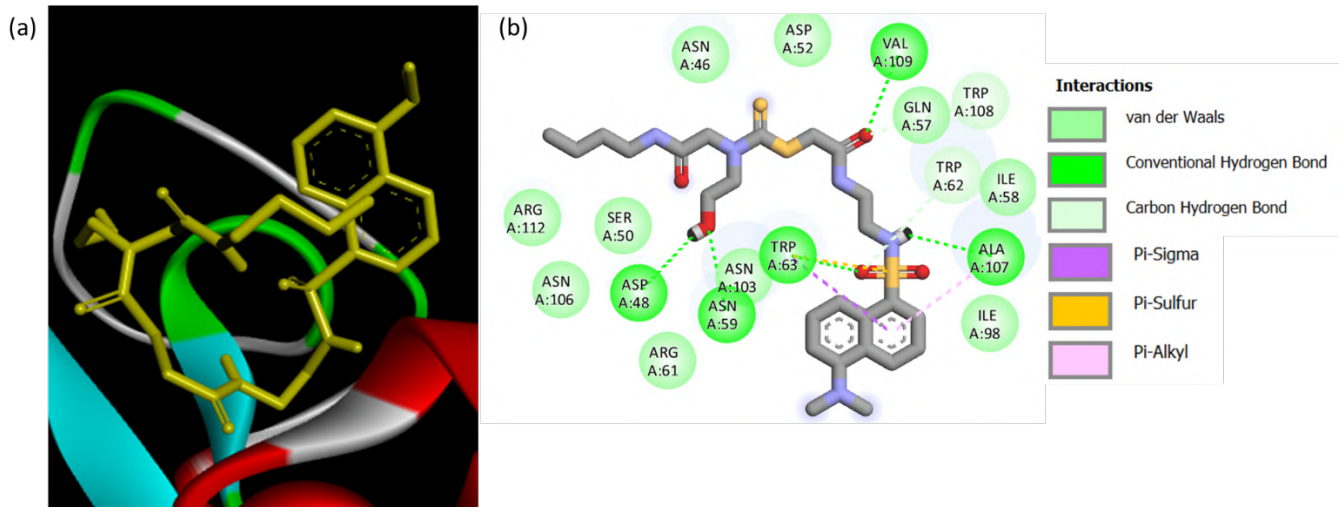


Figure S60: (a) Docking of **4** with lysozyme and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in lysozyme.

5) Docking of **5** with lysozyme

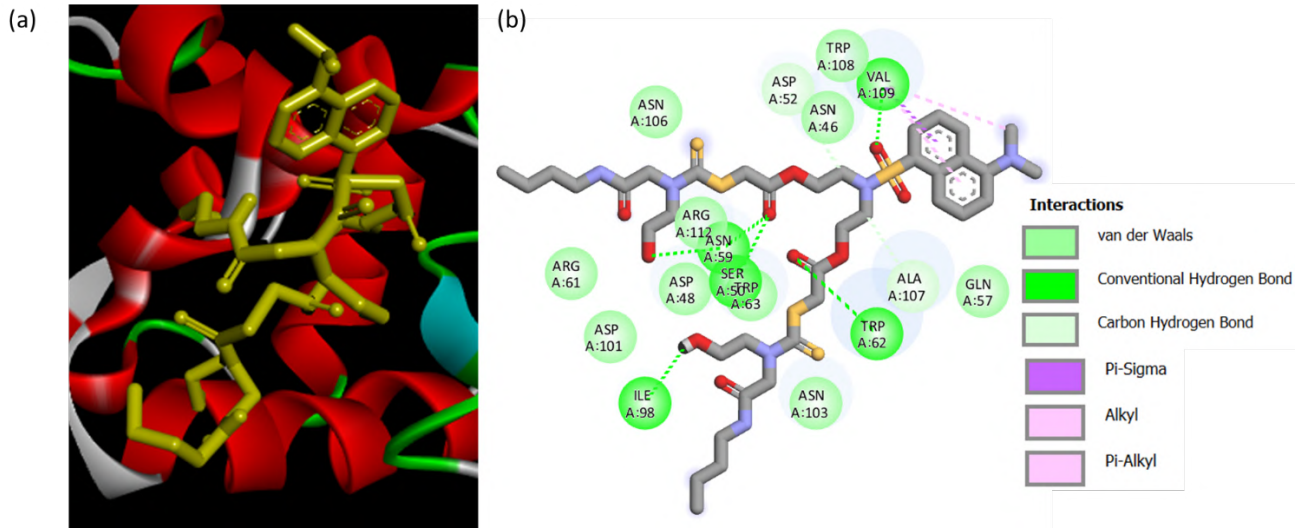


Figure S61: (a) Docking of **5** with lysozyme and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in lysozyme.

6) Docking of **6** with lysozyme

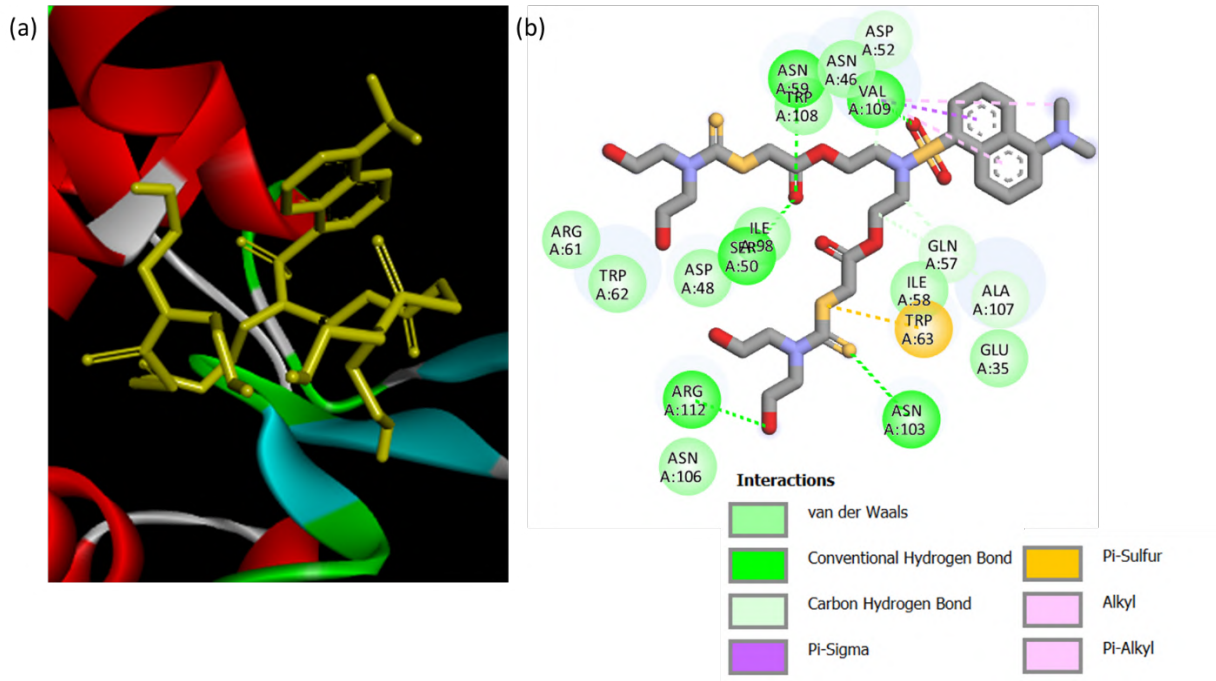


Figure S62: (a) Docking of **6** with lysozyme and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in lysozyme.

Docking studies with Haemoglobin

1) Docking of **1** with Haemoglobin

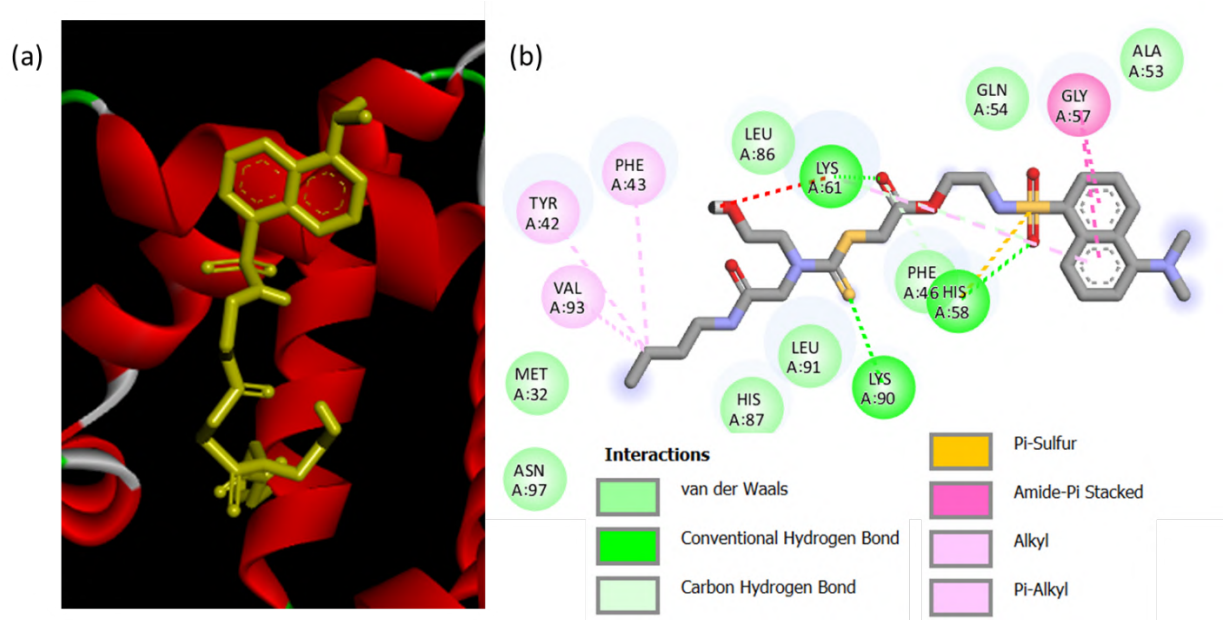


Figure S63: (a) Docking of **1** with haemoglobin and (b) the 2-D representation for the interaction of **1** with the amino acid residues present in haemoglobin.

2) Docking of **2** with haemoglobin

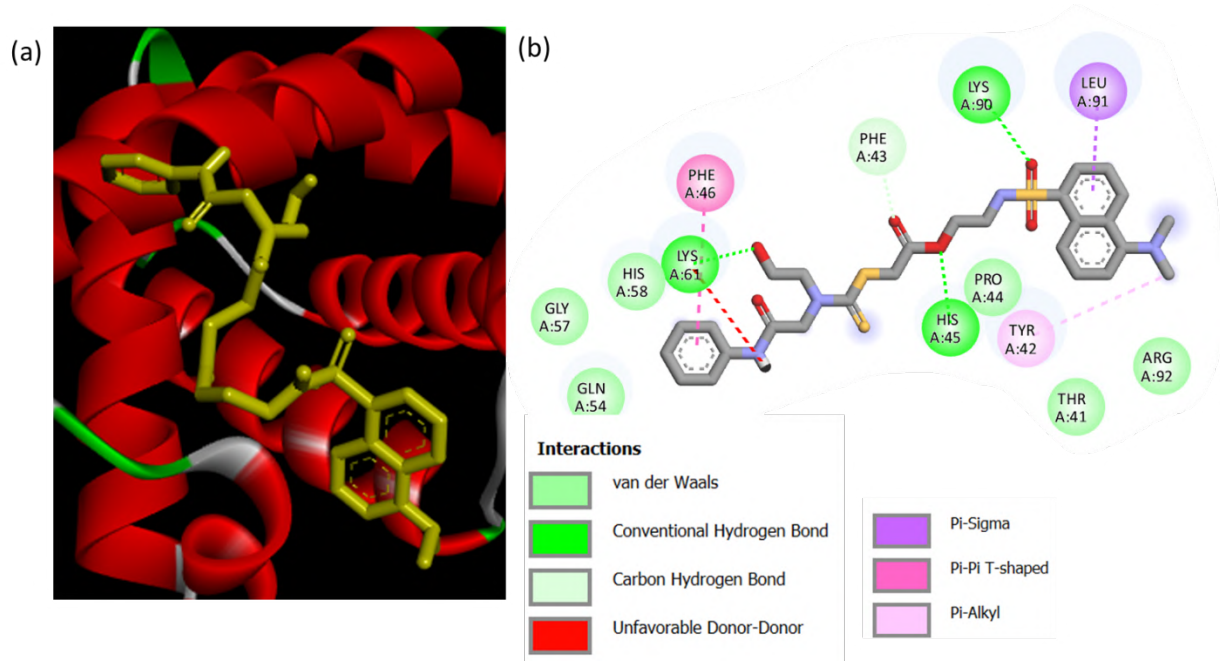


Figure S64: (a) Docking of **2** with haemoglobin and (b) the 2-D representation for the interaction of **2** with the amino acid residues present in haemoglobin.

3) Docking of **3** with haemoglobin

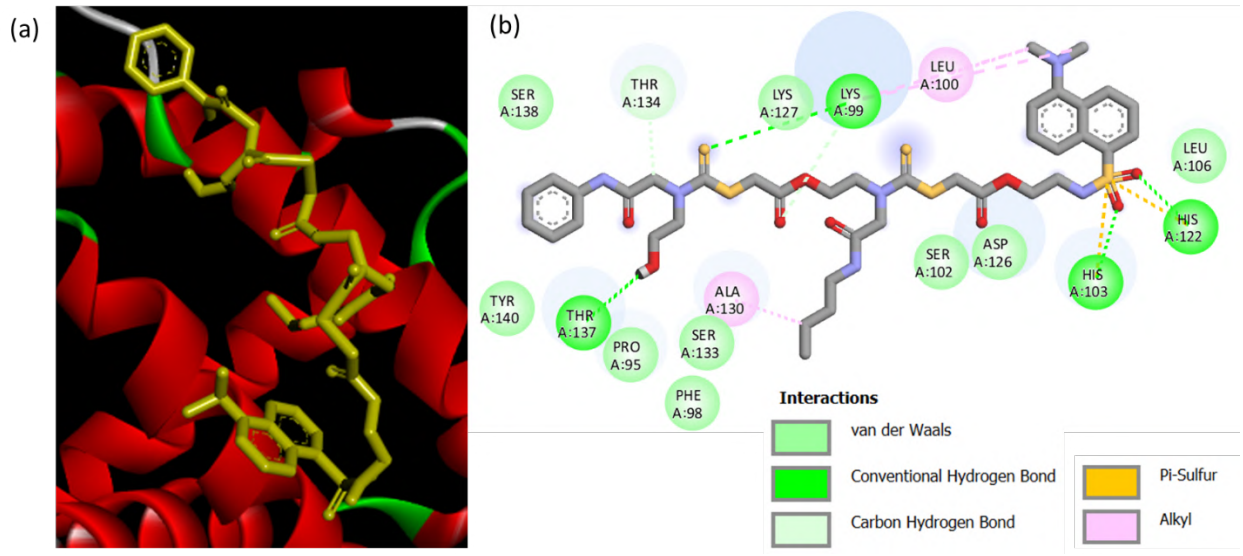


Figure S65: (a) Docking of **3** with haemoglobin and (b) the 2-D representation for the interaction of **3** with the amino acid residues present in haemoglobin.

4) Docking of **4** with haemoglobin

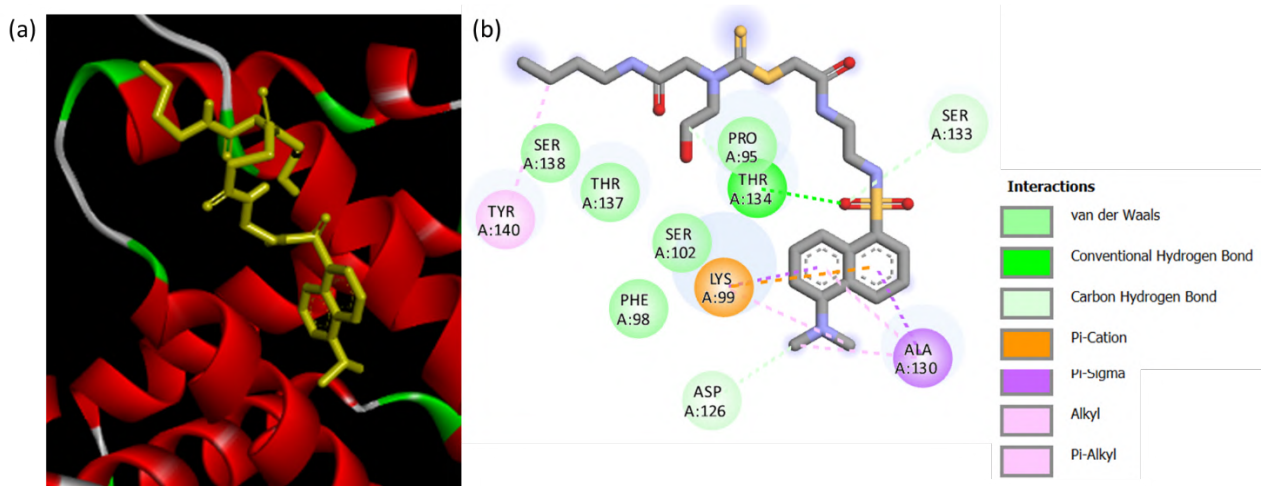


Figure S66: (a) Docking of **4** with haemoglobin and (b) the 2-D representation for the interaction of **4** with the amino acid residues present in haemoglobin.

5) Docking of **5** with haemoglobin

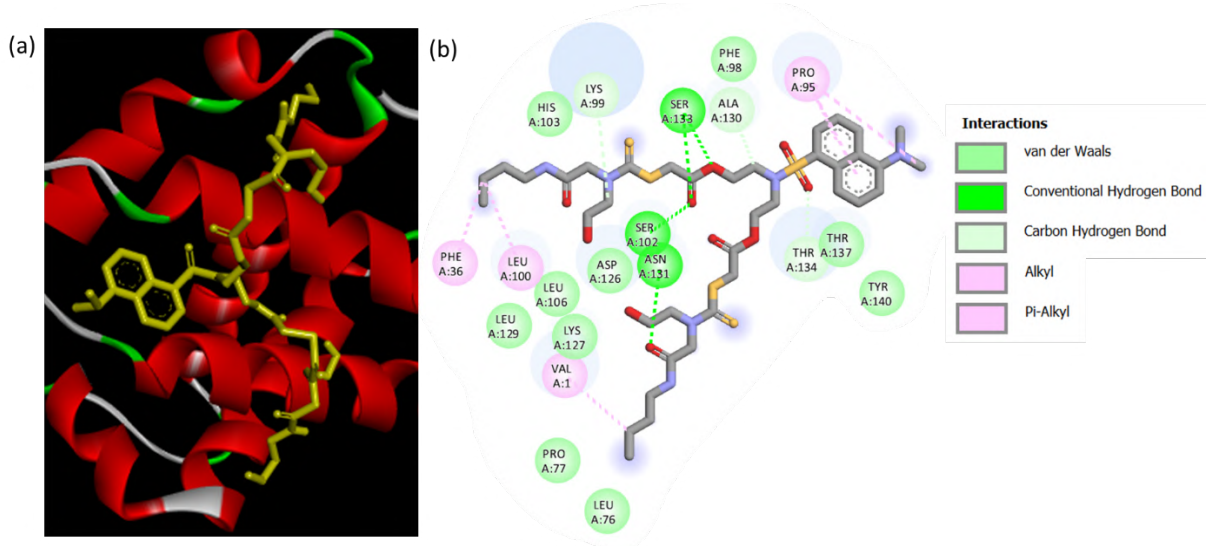


Figure S67: (a) Docking of **5** with haemoglobin and (b) the 2-D representation for the interaction of **5** with the amino acid residues present in haemoglobin.

6) Docking of **6** with haemoglobin

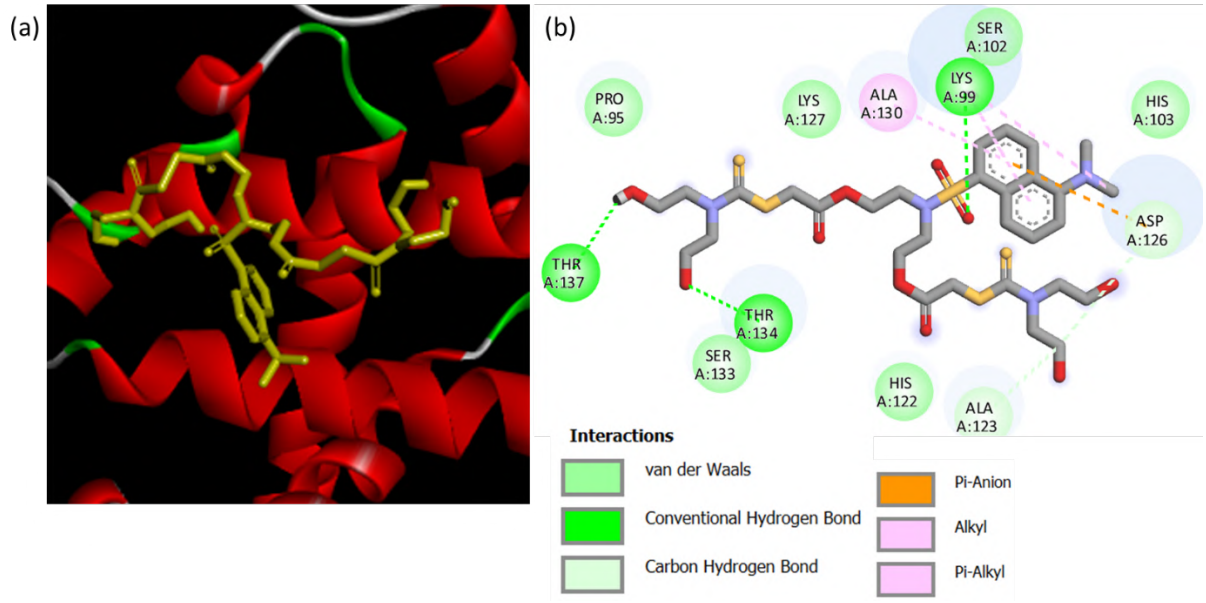


Figure S68: (a) Docking of **6** with haemoglobin and (b) the 2-D representation for the interaction of **6** with the amino acid residues present in haemoglobin.

Table S1: The dissociation constant calculated for all the SDOs

Protein	Dissociation constant (M) obtained for the SDOs					
	1	**2**	**3**	**4**	**5**	**6**
HSA	9.86×10^{-6}	2.18×10^{-6}	1.85×10^{-6}	1.81×10^{-5}	2.13×10^{-5}	6.69×10^{-5}
BSA	9.86×10^{-6}	8.04×10^{-6}	5.80×10^{-6}	1.54×10^{-5}	2.13×10^{-5}	2.13×10^{-5}
Proteinase	2.61×10^{-5}	4.82×10^{-5}	1.09×10^{-4}	7.87×10^{-5}	5.68×10^{-5}	9.26×10^{-5}
Trypsin	2.22×10^{-5}	1.81×10^{-5}	2.51×10^{-5}	1.81×10^{-5}	6.69×10^{-5}	1.09×10^{-4}
Amylase	5.88×10^{-5}	1.31×10^{-5}	1.77×10^{-4}	2.96×10^{-5}	1.28×10^{-4}	1.28×10^{-4}
Lysozyme	2.53×10^{-4}	1.54×10^{-5}	2.96×10^{-5}	6.69×10^{-5}	1.51×10^{-4}	1.77×10^{-4}
Haemoglobin	3.07×10^{-5}	2.96×10^{-5}	7.87×10^{-5}	9.26×10^{-5}	9.26×10^{-5}	3.48×10^{-5}

Procedure for preparation of native gel

Composition for preparation of 3 gels of 10%

Components	Separating gel	Stacking gel
Distilled water	5.9 mL	2.1 mL
30% acrylamide	5.0 mL	0.5 mL
1.5 M Tris HCl	3.8 mL (8.8 pH)	0.38 mL (6.8 pH)
Distilled water	0.15 mL	0.03 mL
10% APS	0.15 mL	0.03 mL
TEMED	0.006 mL	0.003 mL

Procedure for preparing gel

1. In two separate falcon tubes prepare the separating gel and stacking gel solutions. Add water, acrylamide, and tris buffer to each tube. APS and TEMED should be added at the time when they are required, because the addition of these will initiate polymerisation
2. Now fix the glass plates in the Bio rad apparatus, add water, and see if its leaking. If not leaking, then remove water and add the separating gel into it.
3. After addition of separating gel add a little of IPA to avoid polymerisation at the top layer.
4. Once the separating gel is set, remove the IPA, wash two or three times with water, then add stacking gel and fix the comb.
5. Wait for 15 min for the gel to set.
6. Different concentrations of the sample were prepared in the meantime using PBS buffer and 4X loading dye.
7. Once the gel was set, the comb was removed and the wells are inserted in the electrophoresis tank filled with running buffer.
8. Samples are loaded in the wells and were run against a ladder. After loading, the lid was closed and a constant power supply was given.
9. The gel was run until the samples reaches the bottom of the gel.
10. After this the gels were removed from between the plates and was immersed in the staining solutions to visualise it.
11. For staining with Coomassie blue destaining was done following staining. For the staining with **1** no destaining is required.
12. After staining, the gel treated with Coomassie blue was viewed under normal light and the one stained with **1** was viewed under transilluminator.

Preparation of running buffer

1. For the preparation of 1L of running buffer, take 3g of Tris HCl and 14.4 g glycine. Mix it well and add around 800 mL of distilled water.
2. Check the pH of this solution, and adjust the pH to 8.3 by adding concentrated HCl.
3. Then make up to 1L

Preparation of 4X loading dye

1. To prepare 1 mL of 4X loading dye, add 200 µL of 1M Tris HCl (6.8 pH), 4mg of bromophenol blue and 400 µl glycerol
2. Dissolve all these and then make up to 1 mL

*Preparation of staining solution using **1***

1. For 20 mL of staining solution, 2 mg of SDO **1** was dissolved in 10 mL methanol.
2. To this 2 mL of glacial acetic acid was added and made up to 8 mL by adding water.

Preparation of Coomassie blue staining solution

1. Coomassie blue, 50 mg (0.25%) was dissolved in 10 mL (50%) methanol solution
2. To this 2mL (10%) glacial acetic acid and 8 mL (40%) water is added.

Images of Native PAGE

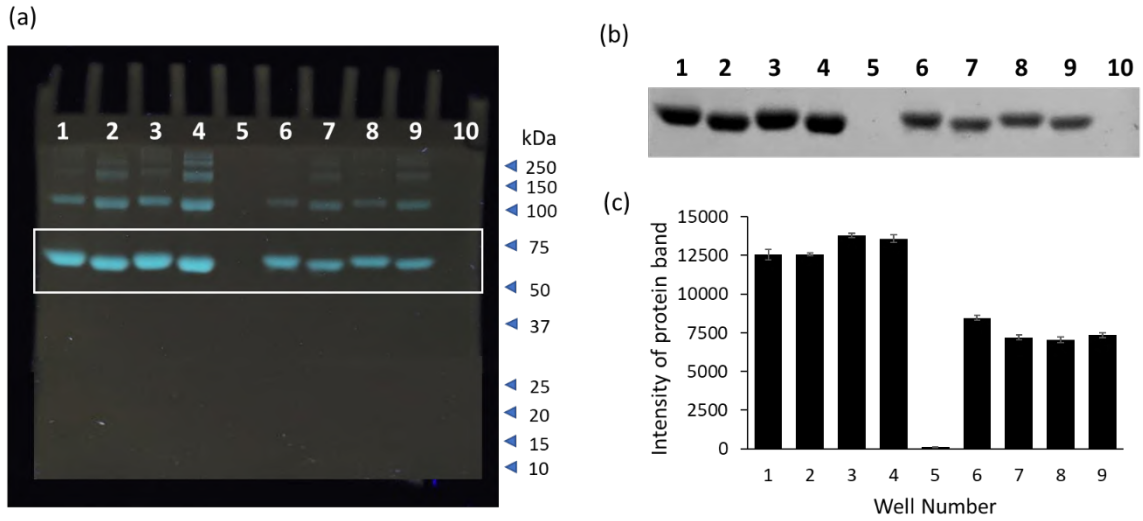


Figure S69: (a) Native-PAGE of a mixture of BSA, HSA, amylase, lysozyme, and haemoglobin. The proteins loaded in each well are; 10- ladder, 9-BSA (20 µg), 8-HSA (20 µg), 7- BSA (20 µg) + mixture (2.5 µg each), 6- HSA (20 µg) + mixture (2.5 µg each), 5- mixture (2.5 µg each) of proteins without BSA/HSA, 4- BSA (40 µg), 3-HSA (40 µg), 2- BSA (40 µg) + mixture (5 µg each), 1- HSA (40 µg) + mixture (5 µg each) stained with SDO **1**. (b) The protein band and (c) their intensity obtained from ImageJ software.

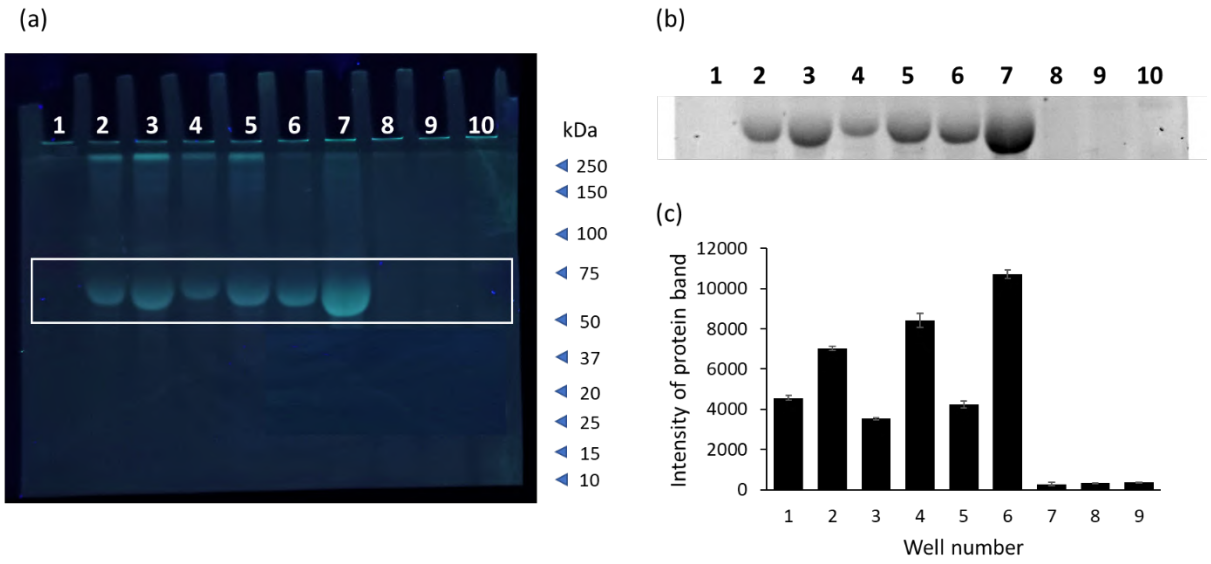


Figure S70: (a) Native-PAGE of a BSA, HSA, fetal serum, amylase, lysozyme, and haemoglobin. The proteins loaded in each well are; 1- ladder, 2- BSA (20 µg), 3- BSA (40 µg), 4-HSA (20 µg), 5- HSA (40 µg), 6-fetal serum (20 µg), 7- fetal serum (40 µg), 8- lysozyme (20 µg), 9- amylase (20 µg), 10- haemoglobin (20 µg) stained with SDO **1**. (b) The protein band and (c) their intensity obtained from ImageJ software.

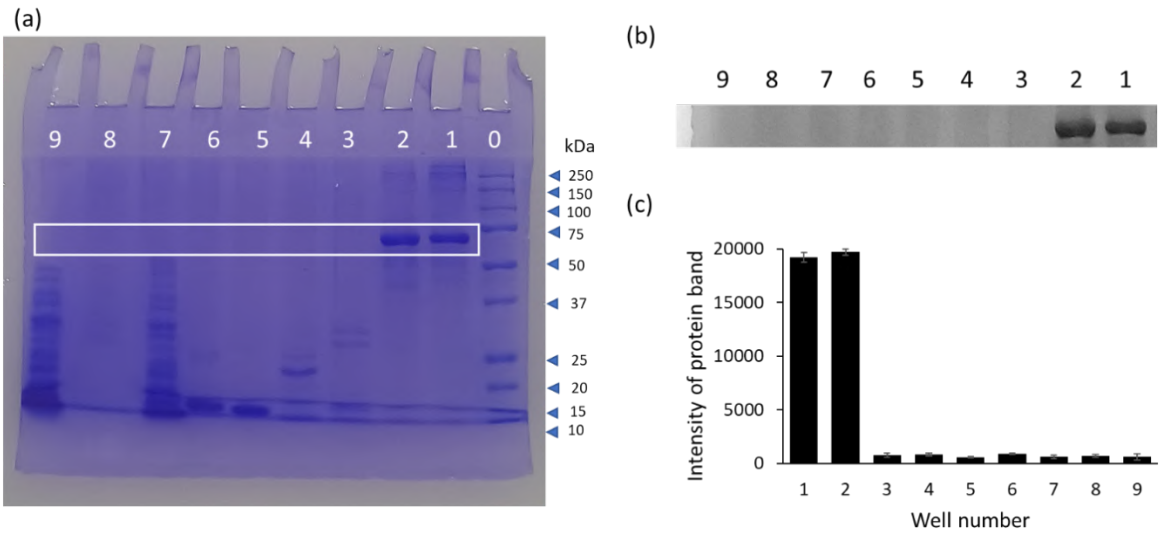


Figure S71: (a) Native PAGE for (0) ladder (1) BSA (40 µg), (2)-HSA (40 µg), (3) proteinase (4) trypsin (5) lysozyme (6) cytochrome (7) mixture of proteinase, lysozyme, cytochrome (2.5 µg each) (8) blank and (9) mixture of proteinase, trypsin, lysozyme, cytochrome (5 µg each) stained with Coomassie blue, (b) the protein band and (c) their intensity obtained from ImageJ software.

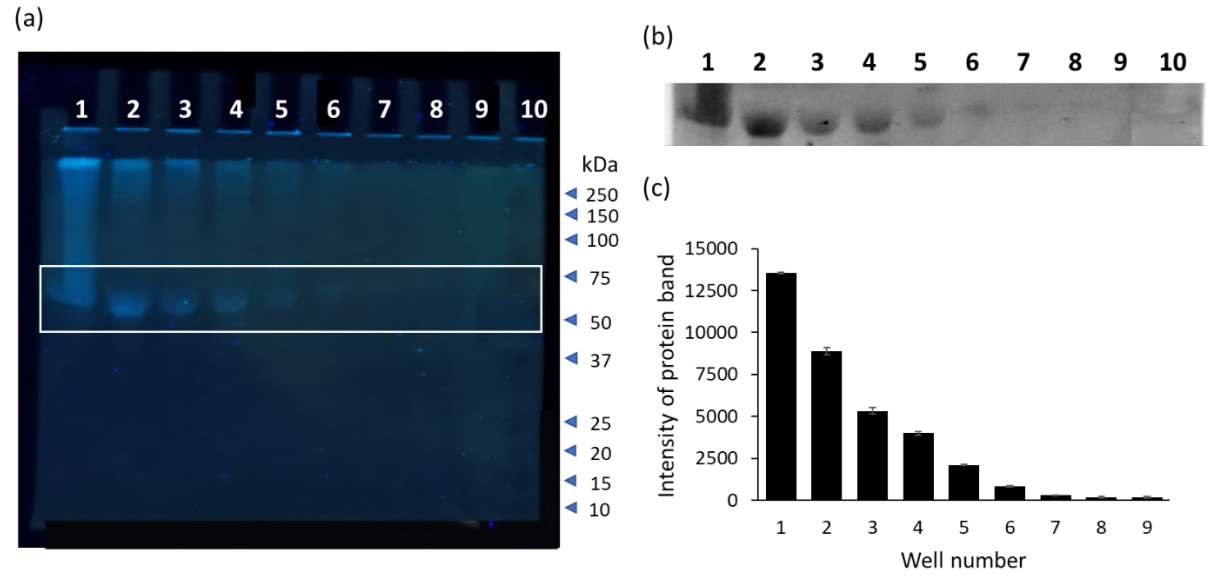


Figure S72: (a) Native-PAGE of BSA with varying concentrations (1) 75 µg, (2) 50 µg, (3) 30 µg, (4) 20 µg, (5) 10 µg, (6) 5 µg, (7) 3 µg, (8) 2 µg, (9) 1 µg, (10) ladder stained with SDO **1**, (b) the protein band and (c) their intensity obtained from ImageJ software.