A Novel Class of Functionally Tuneable Star-Shaped Molecules for Interaction with Multiple Proteins

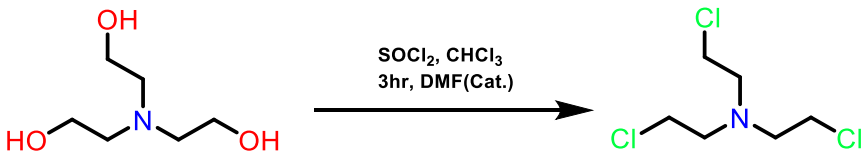
Contents

1. Materials and methods

All the chemicals were purchased from sigma Aldrich, Spectrochem, SRL and used for the synthesis without any further purification. ^{1}H NMR were recorded on n Bruker AV III 500 MHz. The data were analyzed by MestReNova (version 8.1.1) (https://mestrelab.com/software/mnova/nmr/). ^{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. 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 and 254 nm with positive mode for mass detection. Solvents for LC-MS were water with 0.1% acetic acid (solvent A) and acetonitrile with 0.1% acetic acid (solvent B). Compounds were eluted at a flow rate of 0.7 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 acetonitrile and water. Compounds were eluted at a flow rate of 19 mL/min with a gradient of 20%, 60%, 75%, 90% and 20% of acetonitrile over 26 minutes. Before injecting the sample to HPLC the column was washed with 5 min for each gradient (20%, 60%, 75%, 90% and 20%) of acetonitrile. Fluorescence was recorded on Perkin Elmer FL 6500. All fluorescence spectra are recorded at 25^o C. Fourier transform IR spectroscopy

(FT-IR) was recorded in a Shimadzu IR Tracer 100 in KBr pellet method. All the spectra were plotted Origin Pro 8.5.1.

Synthesis of tris(2-chloroethyl) amine



Scheme S1: Synthesis of tri-chloro precursor

The synthesis of tris(2-chloroethyl) amine starts with triethanol amine. First triethanol amine (1 mmol) was dissolved in 20 mL of $CHCl_3$, then it was treated slowly with thionyl chloride ($SOCl_2$) (10 mmol) over the 5 minutes. Followed by the addition of $SOCl_2$, dimethyl formamide (2 mmol) was added as catalyst to the reaction mixture. The reaction was stirred for 3 hrs at 60°C. The completion of reaction was monitored by TLC in ethyl acetate and hexane (1:9). After the completion of reaction, the reaction mixture was washed with saturated sodium bicarbonate ($NaHCO_3$) solution and chloroform (1:1). The organic layer chloroform ($CHCl_3$) was dried over anhydrous sodium sulphate and concentrated under the reduced pressure. The product was purified by column chromatography with 2% ethyl acetate and hexane. A greenish oil will form with 84% yield.

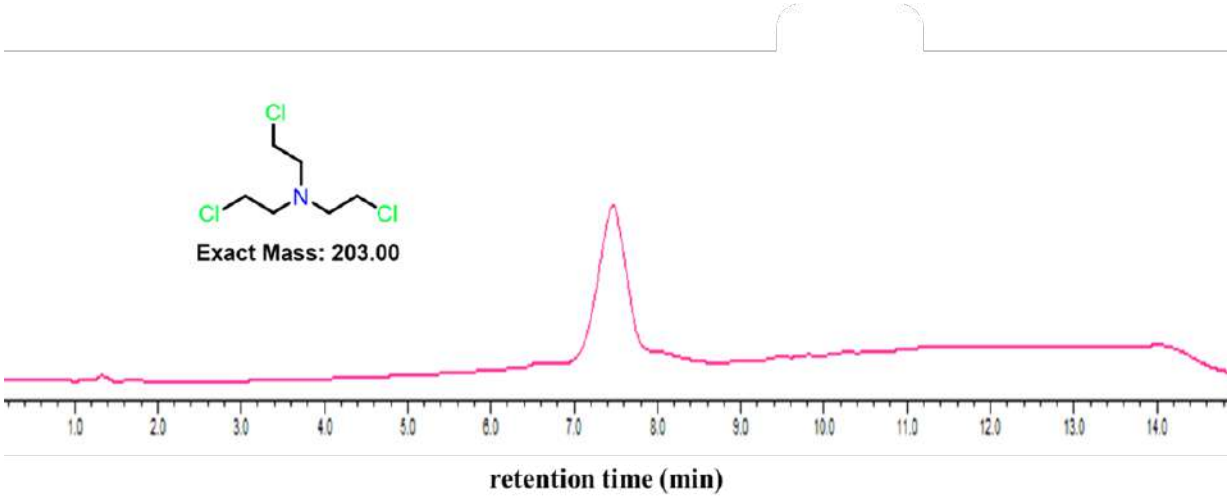


Figure S1: The HPLC trace of tris-chloro precursor.

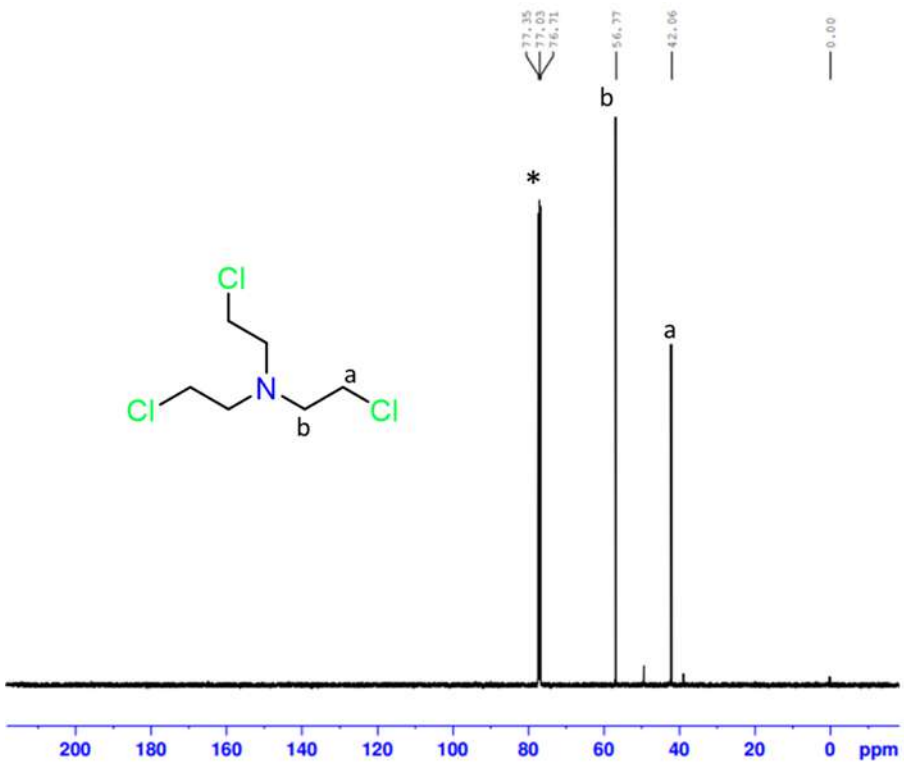
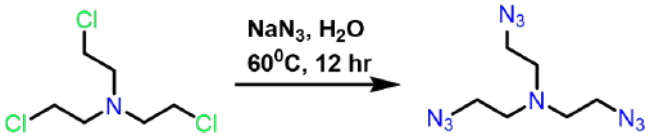


Figure S2: ^{13}C NMR of tris-chloro precursor, 400MHz in $CDCl_3$: δ (ppm): 42.06, 56.77, 76.71-77.35. * is for $CDCl_3$

Synthesis of tris(2-azidoethyl) amine



Scheme S2: Synthesis of tri-azide precursor

Tris (2-chloroethyl) amine (1mmol) was treated with sodium azide (5 mmol) followed by the addition of 20 mL water. The reaction mixture was stirred for 12 hrs at 60^0C. Completion of reaction was monitored by TLC with ethyl acetate and hexane (2:8) and a single UV active spot was observed. Then the reaction mixture was washed with ethyl acetate and water (1:1). The organic layer (ethyl acetate) was dried over sodium sulphate and concentrated under reduced pressure. A yellowish oil was formed with 98% yield.

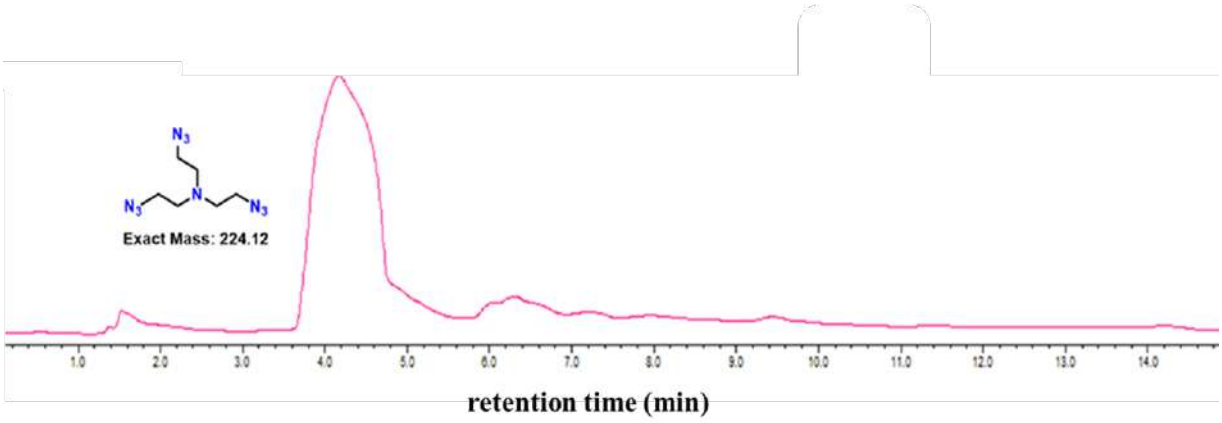


Figure S3: The HPLC trace of tris-azide precursor.

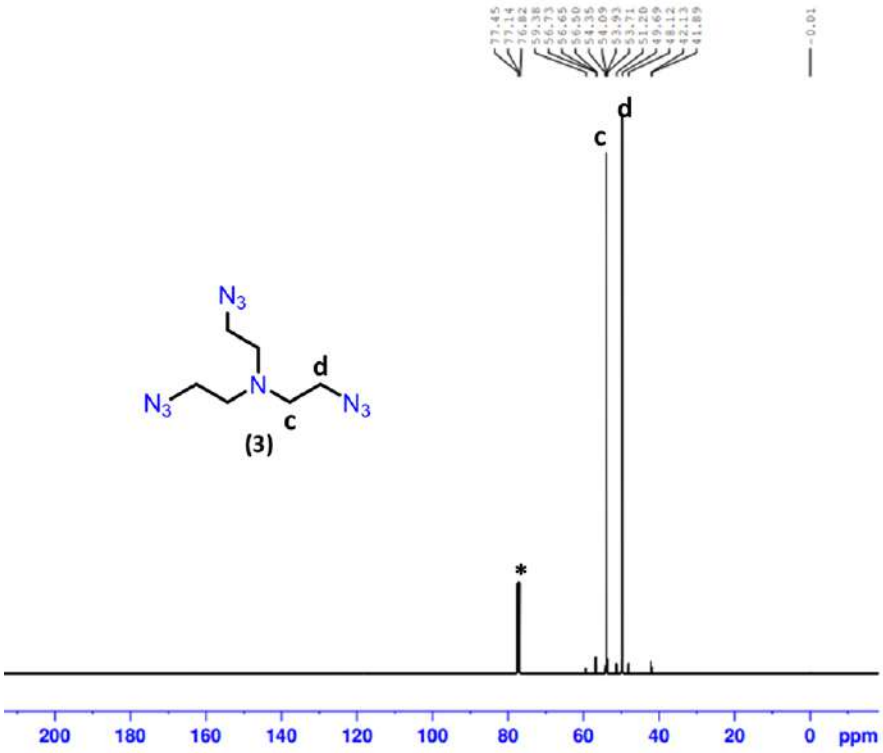
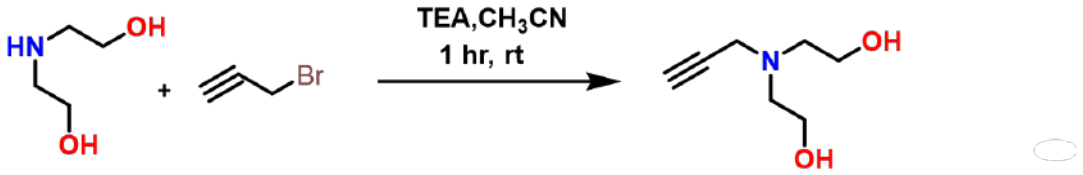


Figure S4: ^{13}C NMR of tris-azide precursor, 400MHz in $CDCl_3$: δ (ppm): 49.61, 53.93-54.35, 76.82-77.45.

Synthesis of alkyne precursor



Scheme S3: Synthesis of alkyne precursor

Ethanolamine (1mmol) was treated with propargyl bromide (1.2 mmol) followed by the addition of base triethyl amine (1.5 mmol) in 20 mL acetonitrile (CH_3CN) solvent. Then the reaction mixture was stirred at room temperature for 1 hr. Completion of reaction was

monitored by TLC with ethyl acetate and methanol (9:1). After the completion of reaction, the solvent acetonitrile was evaporated under reduced pressure. The product was obtained in round bottom flask which was washed with ethyl acetate and water (1:1). The organic layer (ethyl acetate) was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The reaction mixture was purified by column chromatography with ethyl acetate and hexane (10% hexane) mixture.

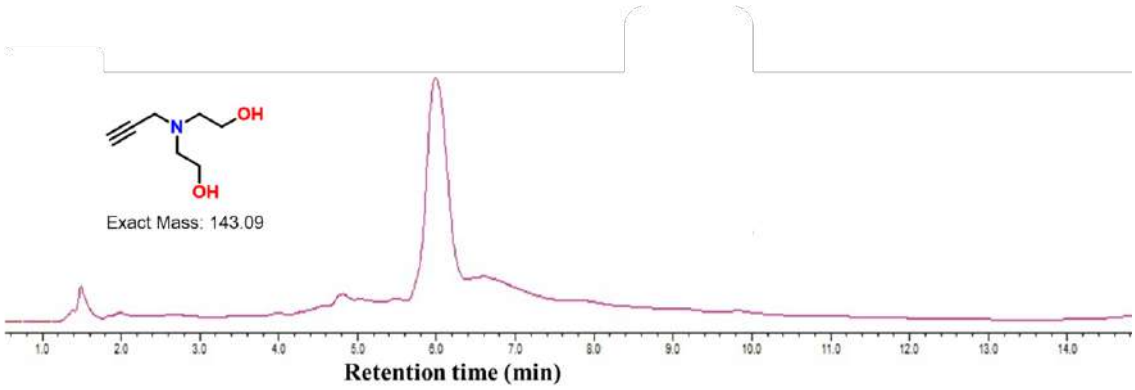
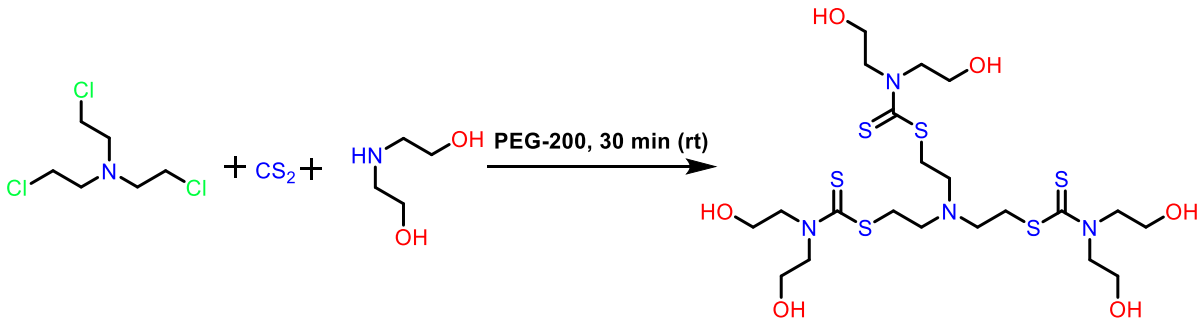


Figure S5: The HPLC trace of alkyne precursor and MS data showing experimental $[M+H]^+$: 144.05 Da and calculated $[M+H]^+$: 144.05 Da.

Synthesis of DTC-SSM



Scheme S4: Synthesis of DTC-SSM.

DTC based star shaped macromolecule (DTC-SSM) was synthesized by a three-component organic reaction. Diethanol amine (3 mmol) was treated with carbon sulfide (CS_2) (6 mmol) followed by the addition of solvent polyethylene glycol-200 (2 mL). Then the reaction mixture was stirred for 5 minutes for the synthesis of in-situ sulfide ion. Thereafter, tris(2-chloroethyl) amine (1 mmol) was slowly added and stirred for 30 minutes. The completion of reaction was monitored by TLC with ethyl acetate and hexane (1:9). The final mechanism was followed by the replacement of chlorine atom from tris(2-chloroethyl) amine by the in-situ sulfide ion. After completion, the reaction mixture was washed with water and ethyl acetate (1:1). The organic layer (ethyl acetate) was dried over sodium sulphate and concentrated under reduced pressure. Thereafter, the reaction mixture was purified by HPLC (High performance liquid chromatography) with 60% acetonitrile and water.

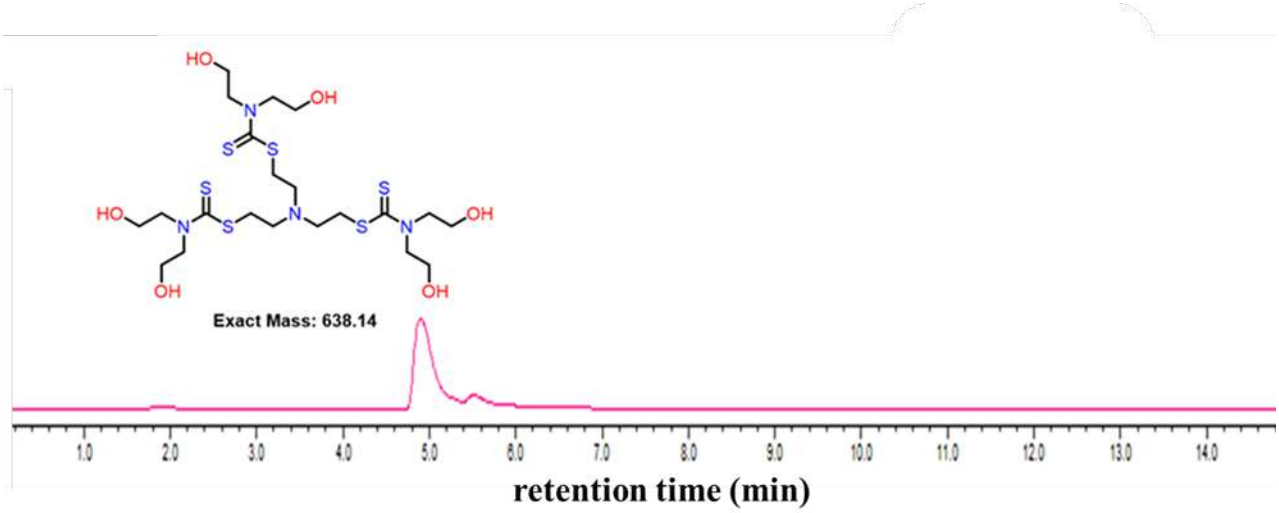


Figure S6: The HPLC trace of DTC-SSM and MS data showing experimental $[M+H]^+$: 639.25 Da and calculated $[M+H]^+$: 639.14 Da.

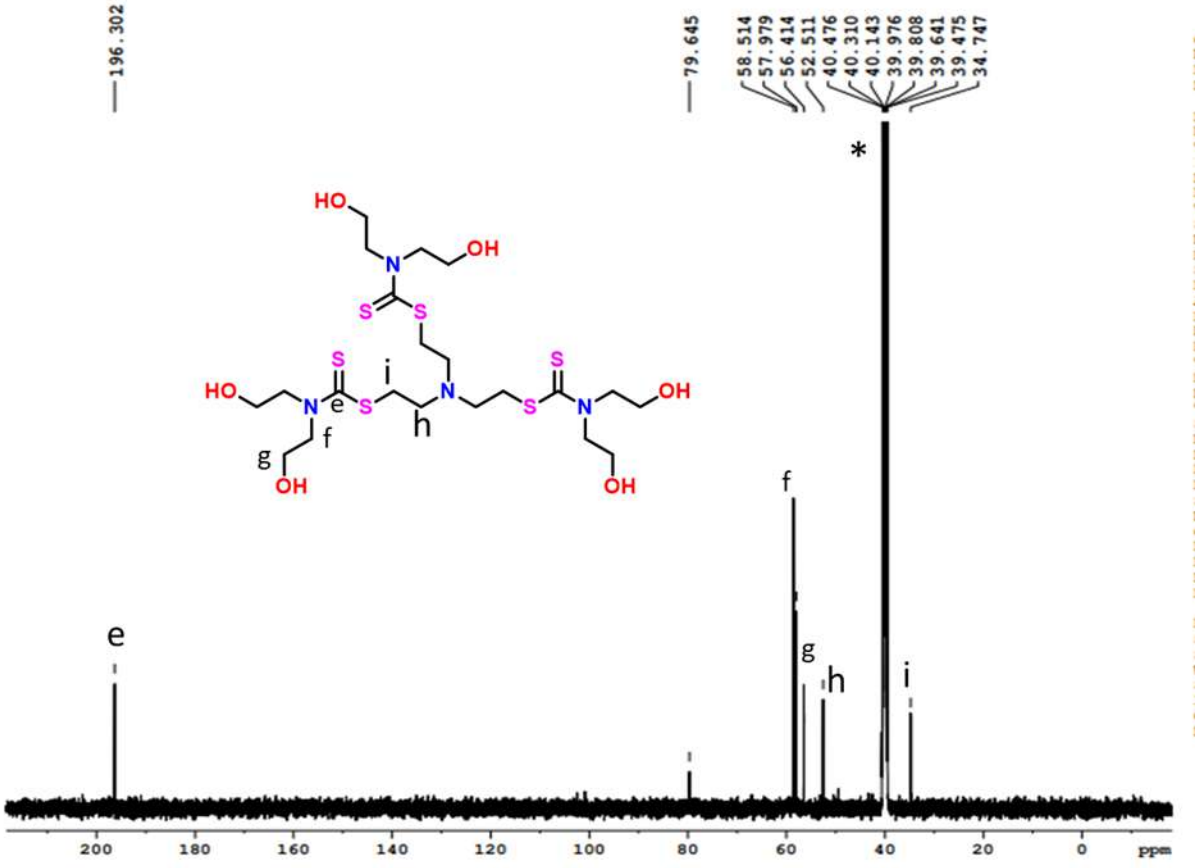


Figure S7: 13C-NMR (500MHz) of DTC-SSM in DMSO: δ (ppm): 34.747, 39.475-40.476, 52.511, 56.414, 57.979, 58.514, 79.645, 196.302.

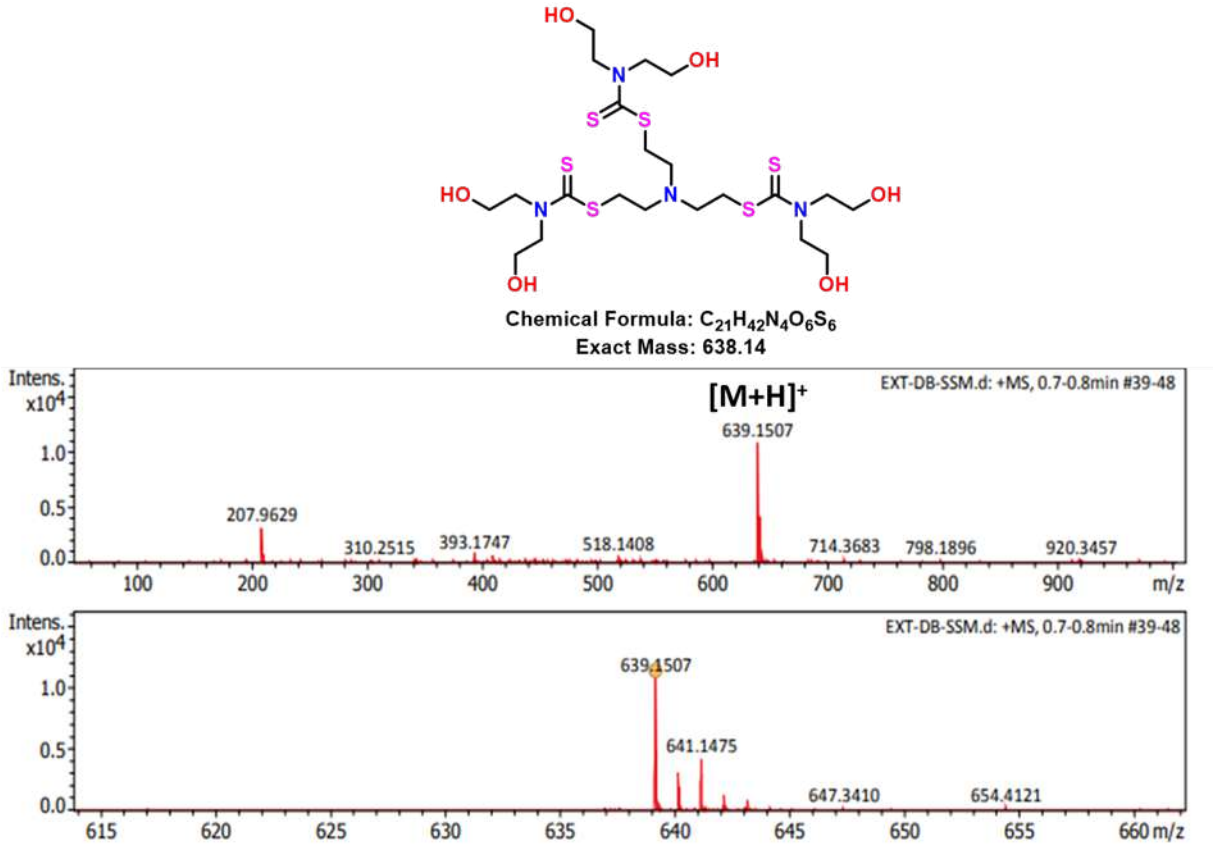


Figure S8: HRMS spectra of DTC-SSM.

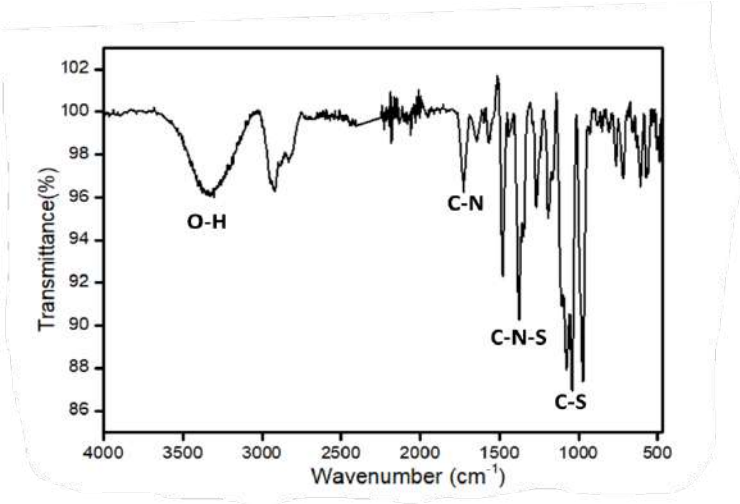
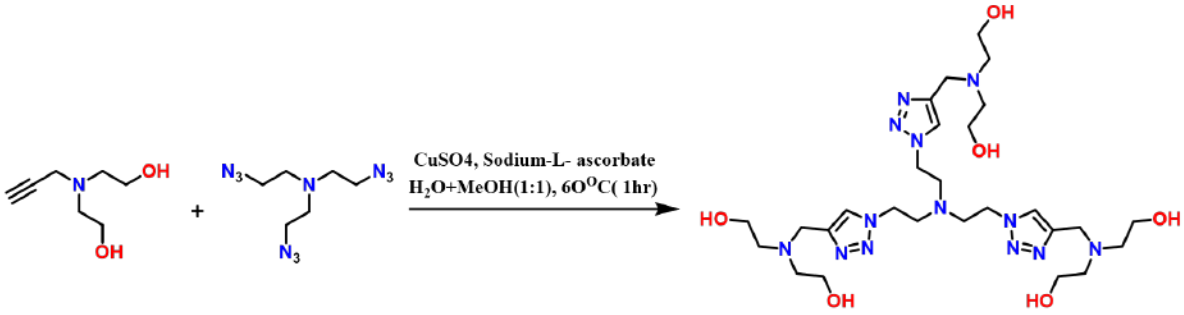


Figure S9: FT-IR spectrum of DTC-SSM.

Synthesis of T-SSM:



Scheme S4: Synthesis of T-SSM.

Alkyne precursor (Scheme S3) (3 mmol) was added to tri-azide (S2) (1mmol) followed by the addition 5 mL methanol. Thereafter, aqueous solution of sodium-L- ascorbate (1.2 mmol in 2.5 ml water) was added to the reaction mixture. Then aqueous solution of copper sulphate (0.2 mmol in 2.5 mL water) was added. The reaction mixture has stirred for 1 hr at room temperature. Completion of the reaction was monitored by TLC with ethyl acetate and methanol (9:1). The reaction mixture was filtered to remove the sodium ascorbate. Followed by the filtration, the reaction mixture was concentrated under reduced pressure. Then the reaction mixture was washed with water and ethyl acetate (1:1). The product was obtained in the water layer and concentrated under reduce pressure.

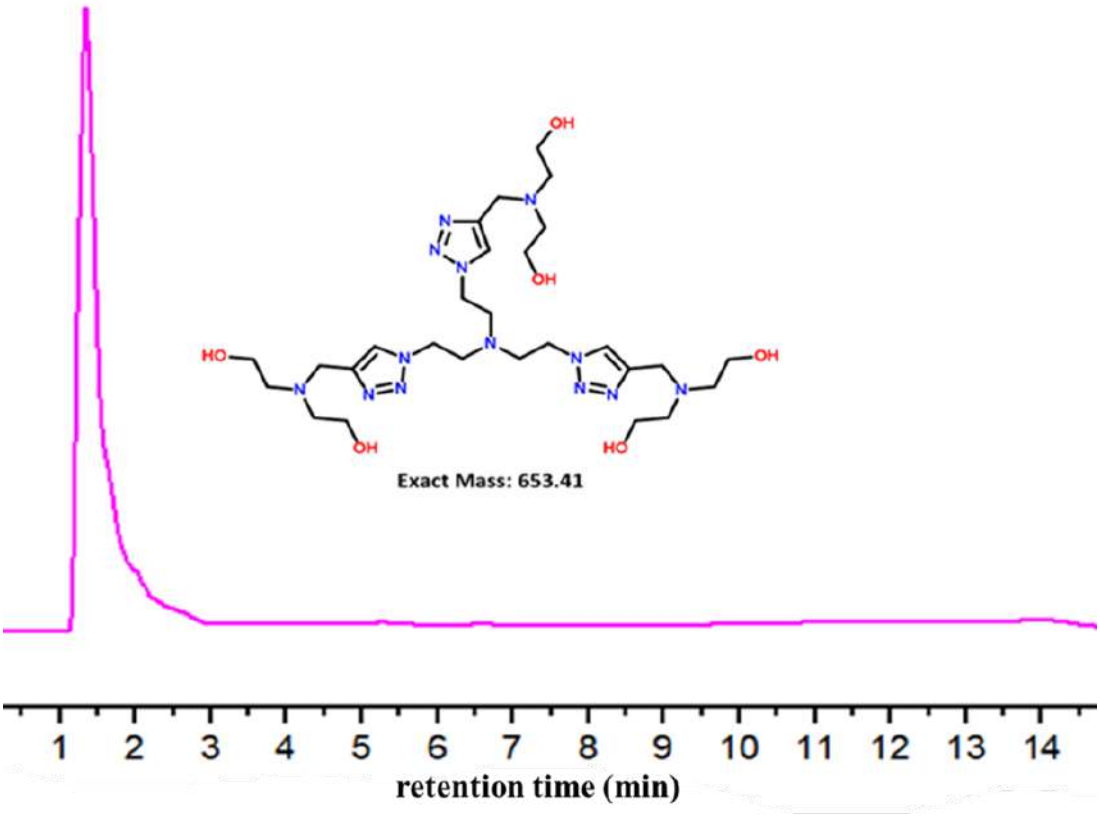


Figure S10: The HPLC trace of T-SSM and MS data showing experimental $[M+H]^+$: 654.55 Da and calculated $[M+H]^+$: 654.41 Da.

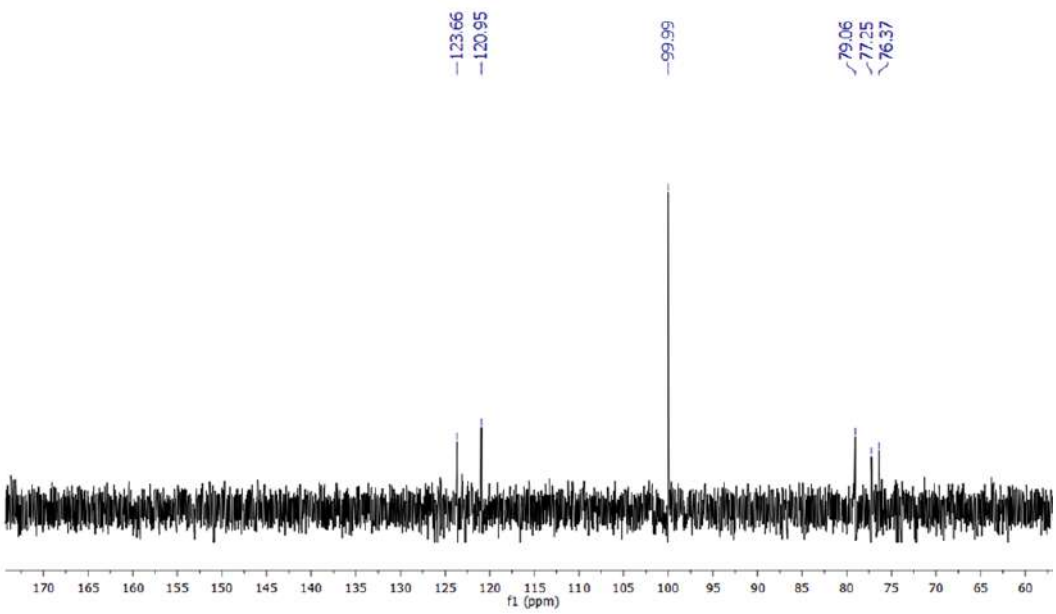


Figure S11: ^{13}C-NMR (400MHz) of T-SSM in D_2O: δ (ppm): 76.37, 77.25, 79.06, 99.99, 120.95, 123.66.

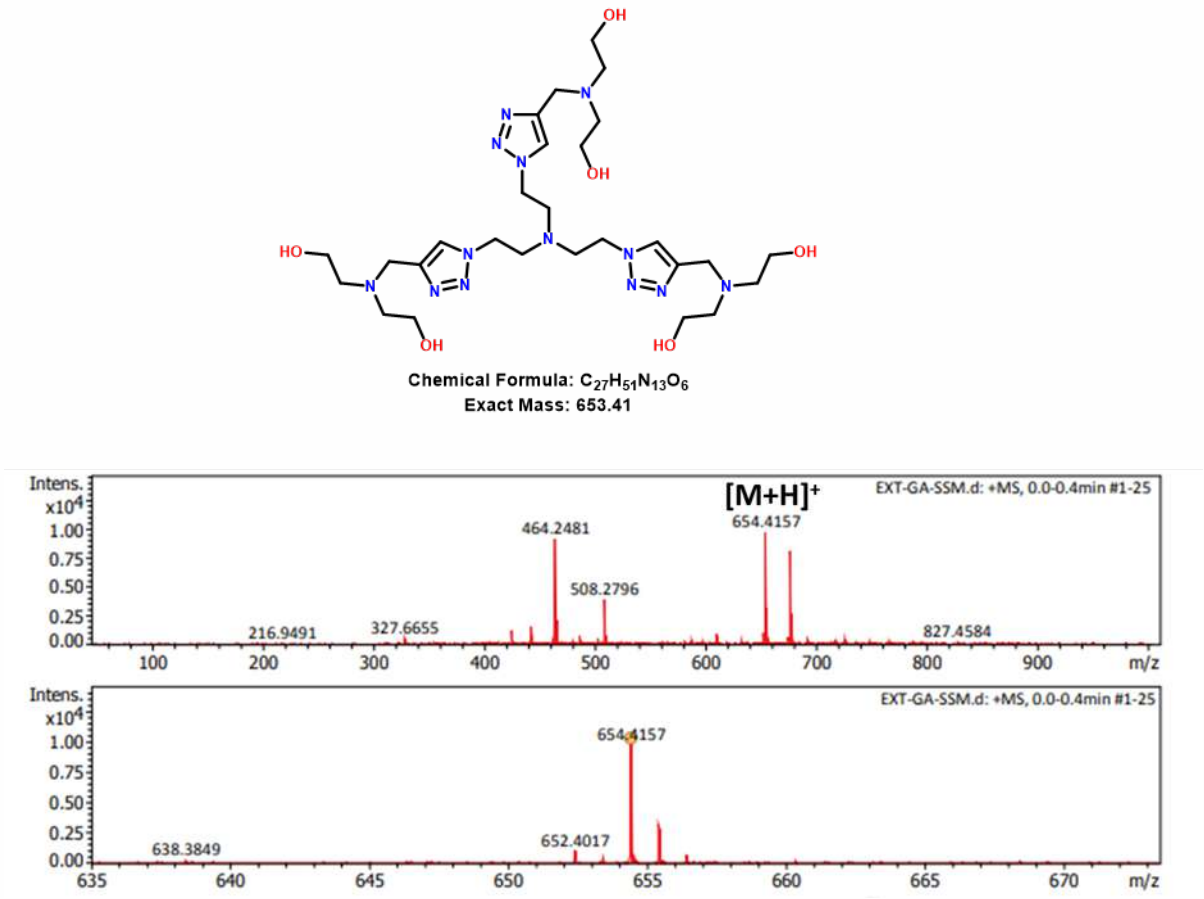


Figure S12: HRMS spectra of T-SSM.

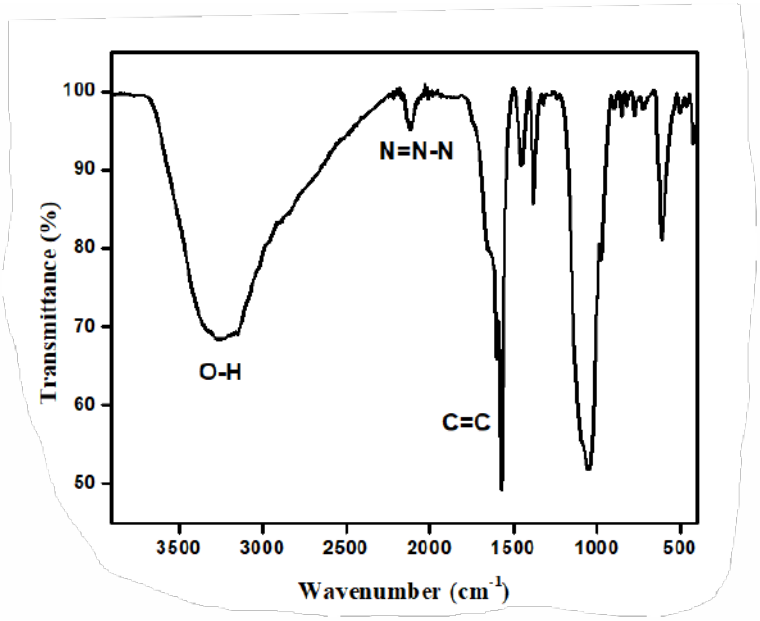


Figure S13: FT-IR spectrum of DTC-SSM.

Protein Binding study of DTC-SSM

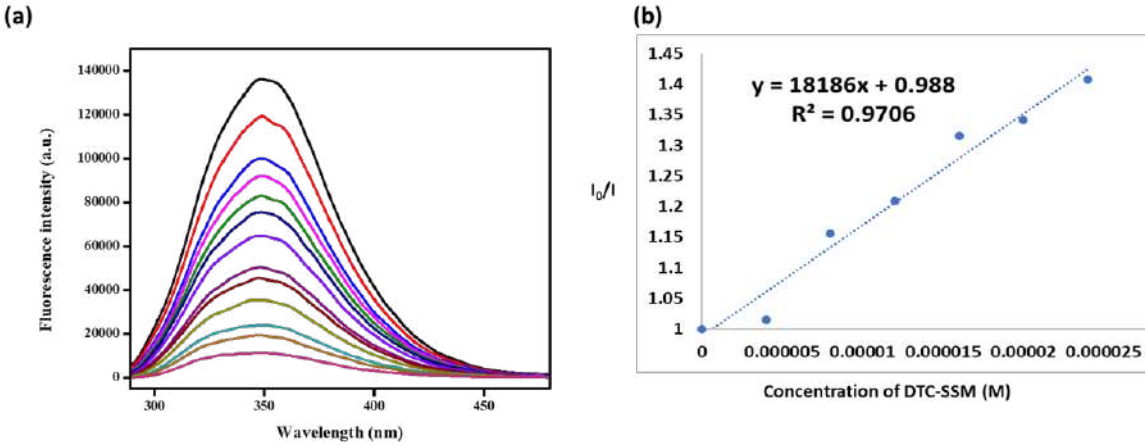


Figure S14: a. Fluorescence titration spectra of BSA with DTC-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of DTC-SSM was added at 25 °C. b. The plot of I_o/I vs concentration of DTC-SSM. The slope of the graph is attributed as binding constant, which is 1.81×10^4 M^{-1}.

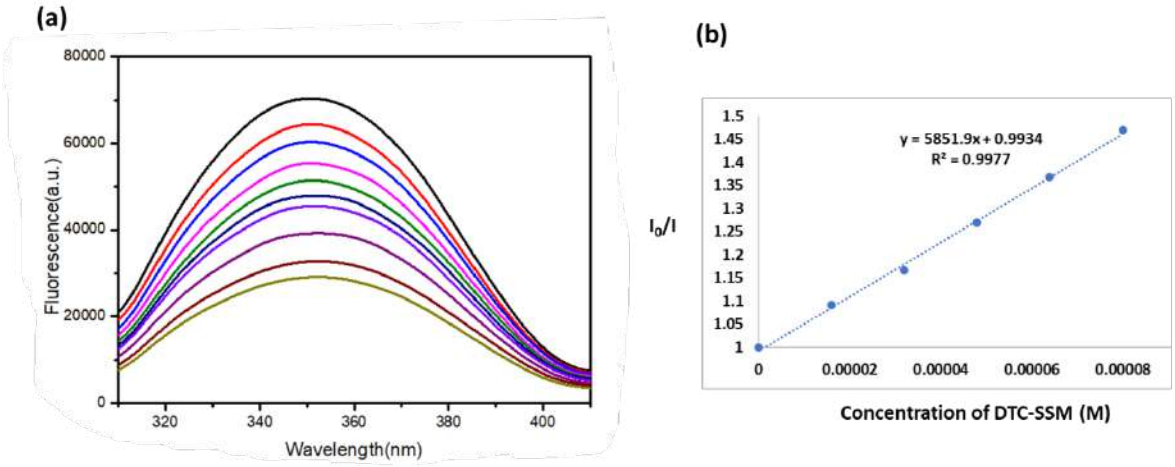


Figure S15: a. Fluorescence spectrum of DTC-SSM on interaction with Haemoglobin excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C. b. The plot of I_o/I vs concentration of DTC-SSM The slope of the graph is attributed as binding constant, which is $5.8 \times 10^3\ M^{-1}$.

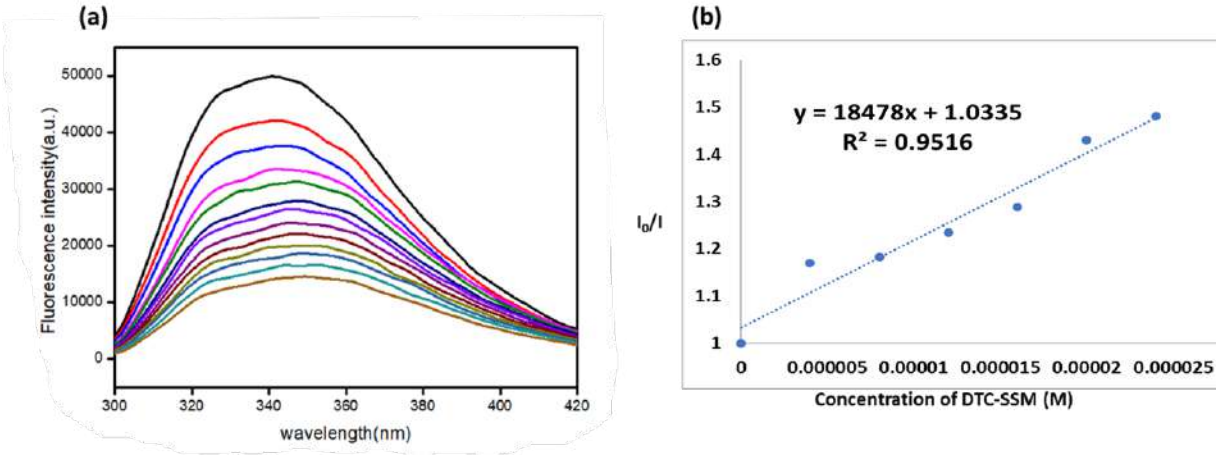


Figure S16: a. Fluorescence spectrum of DTC-SSM on interaction with Trypsin, excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C. b. The plot of I_o/I vs concentration of DTC-SSM The slope of the graph is attributed as binding constant, which is $1.84 \times 10^4\ M^{-1}$.

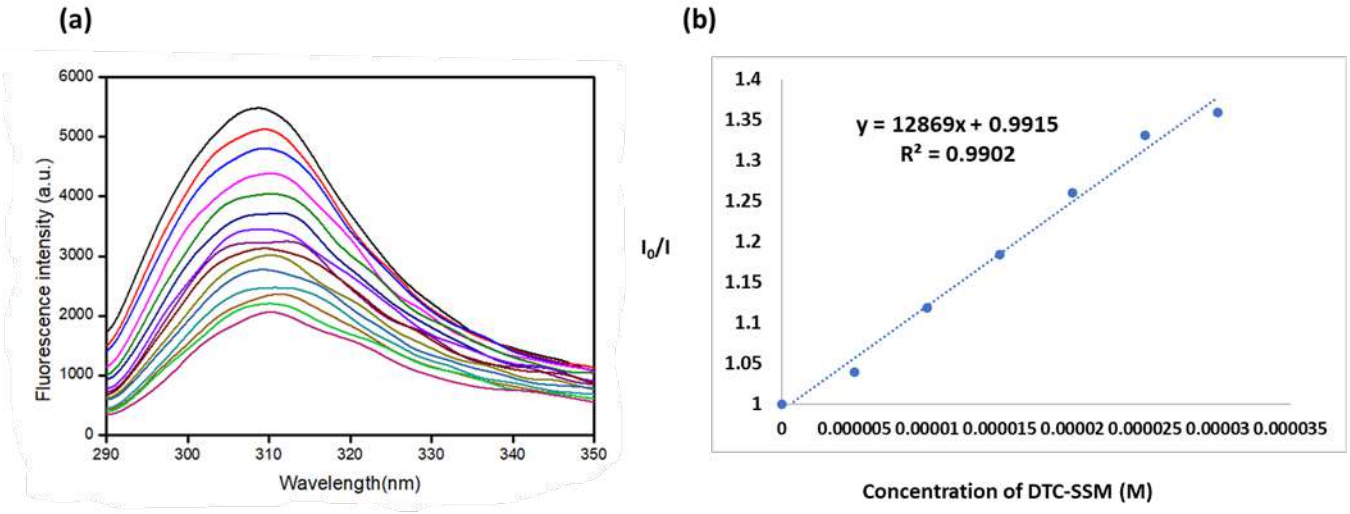


Figure S17: a. Fluorescence spectrum of DTC-SSM on interaction with Ribonuclease, excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C. b. The plot of I_o/I vs concentration of DTC-SSM The slope of the graph is attributed as binding constant, which is $1.28 \times 10^4\ M^{-1}$.

Protein Binding study of T-SSM

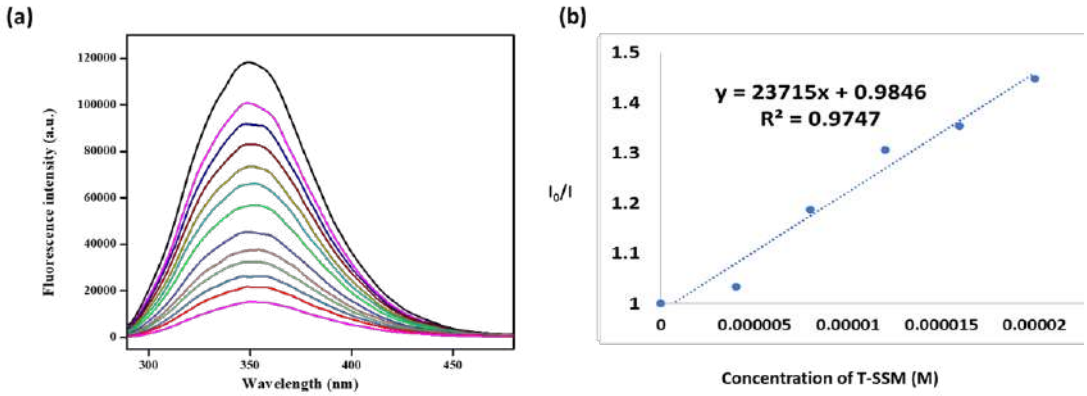


Figure S18: a. Fluorescence spectrum of T-SSM on interaction with BSA, excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C. b. The plot of I_o/I vs concentration of T-SSM The slope of the graph is attributed as binding constant, which is $2.37 \times 10^4\ M^{-1}$.

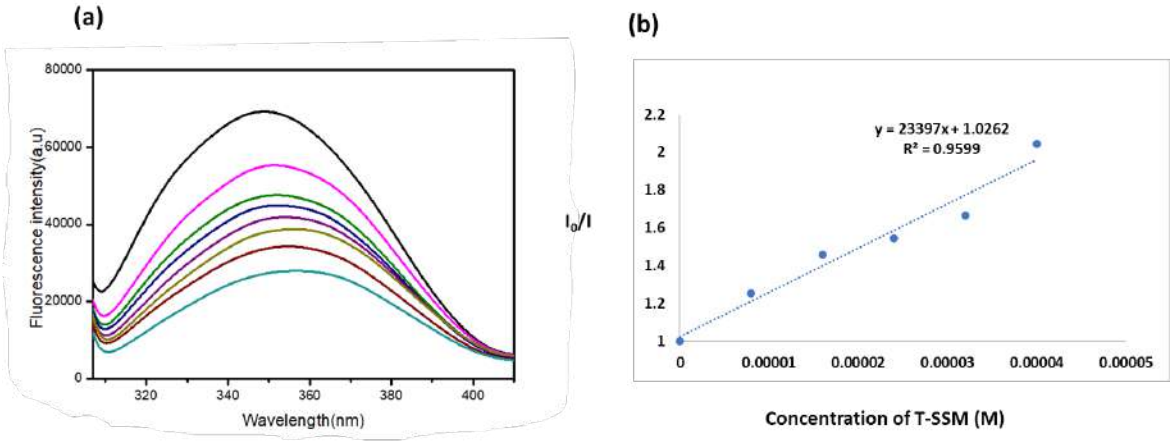


Figure S19: a. Fluorescence spectrum of T-SSM on interaction with haemoglobin, excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C. b. The plot of I_o/I vs concentration of T-SSM The slope of the graph is attributed as binding constant, which is $2.3 \times 10^4\ M^{-1}$.

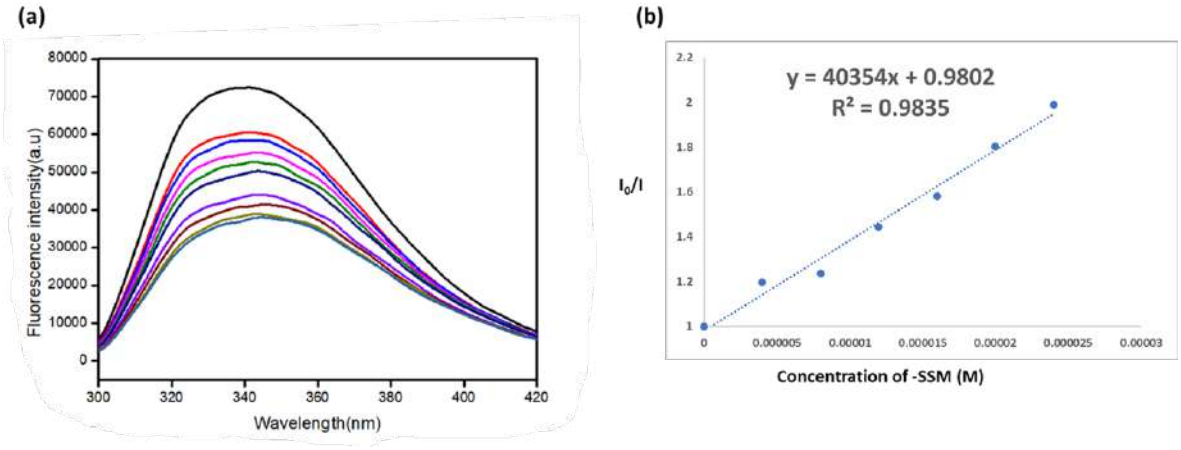


Figure S20: a. Fluorescence spectrum of T-SSM on interaction with Trypsin, excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C. b. The plot of I_o/I vs concentration of T-SSM The slope of the graph is attributed as binding constant, which is $4.03 \times 10^4\ M^{-1}$.

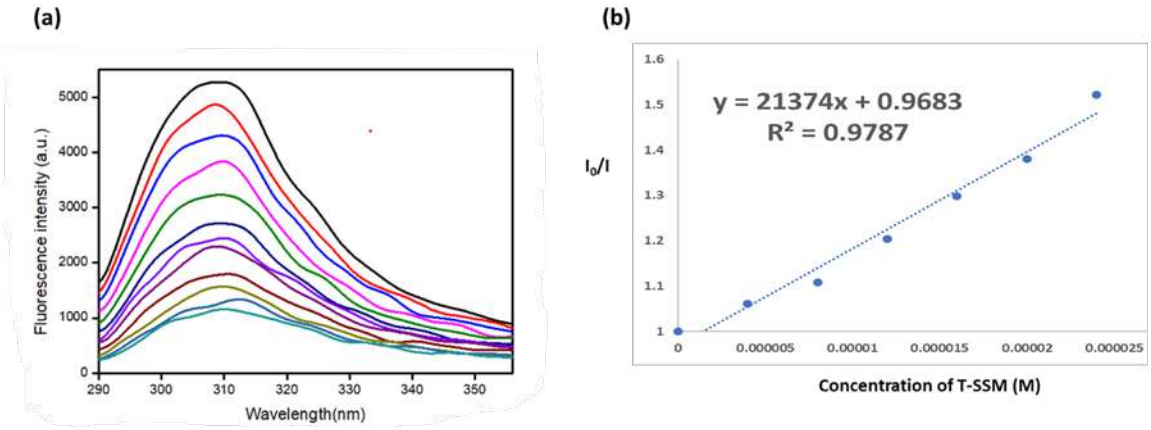


Figure S21: a. Fluorescence spectrum of T-SSM on interaction with Ribonuclease, excited at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of macromolecules solution was added at 25 °C). b. The plot of I_o/I vs concentration of T-SSM The slope of the graph is attributed as binding constant, which is $2.13 \times 10^4\ M^{-1}$.

Synchronous study

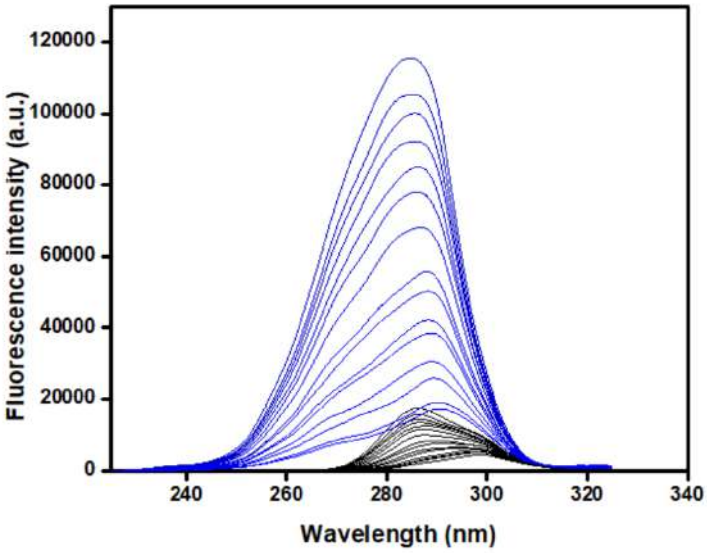


Figure S22: Fluorescence Synchronous titration spectra of BSA with DTC-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of DTC-SSM was added at 25 °C. (blue line spectra $\Delta\lambda$ = 60 nm:, black line spectra: $\Delta\lambda$ = 15 nm).

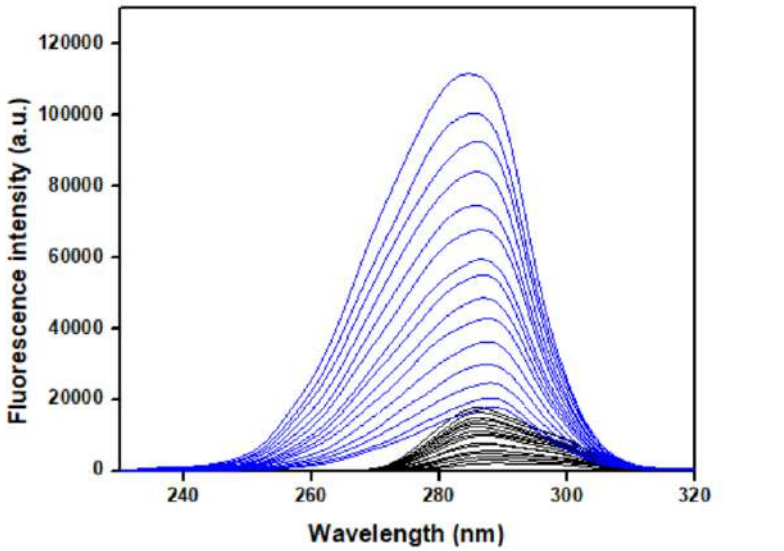


Figure S23: Fluorescence Synchronous titration spectra of BSA with T-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of T-SSM was added at 25 °C (blue line spectra Δλ = 60 nm:, black line spectra: Δλ = 15 nm).

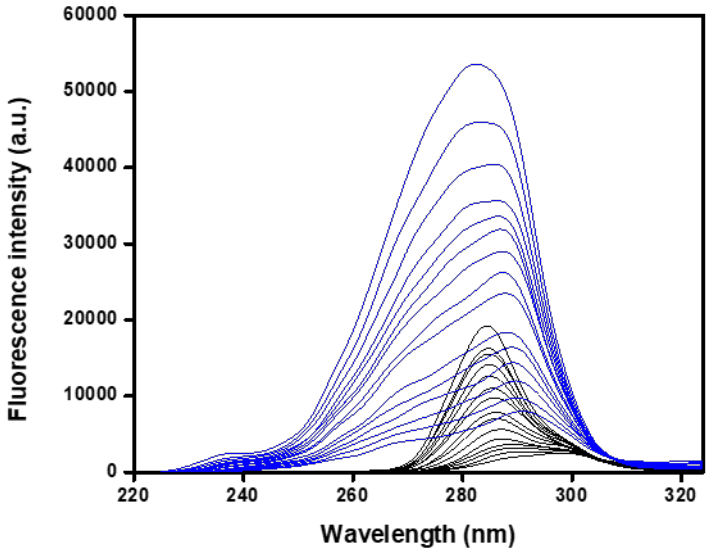


Figure S24: Fluorescence Synchronous titration spectra of HSA with DTC-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of DTC-SSM was added at 25 °C (blue line spectra Δλ = 60 nm:, black line spectra: Δλ = 15 nm).

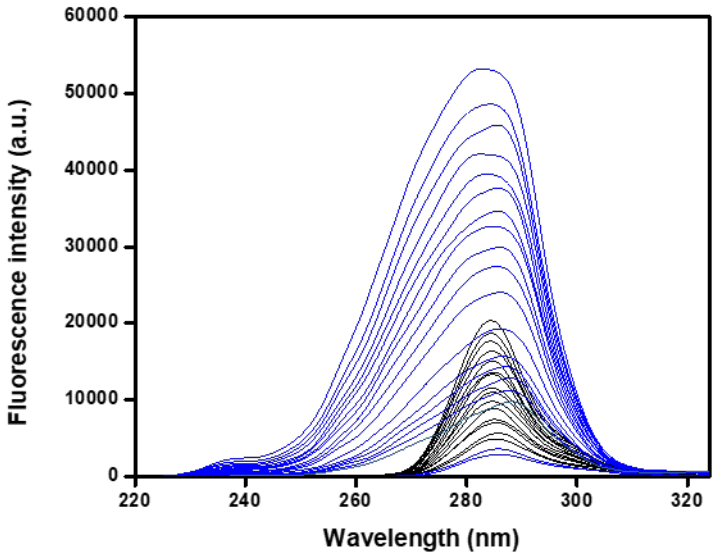


Figure S25: Fluorescence Synchronous titration spectra of HSA with T-SSM, excitation at 280 nm (10 μM protein solution in phosphate buffer (pH 7.4), 2–100 μM of T-SSM was added at 25 °C (blue line spectra $\Delta\lambda = 60$ nm:, black line spectra: $\Delta\lambda = 15$ nm).

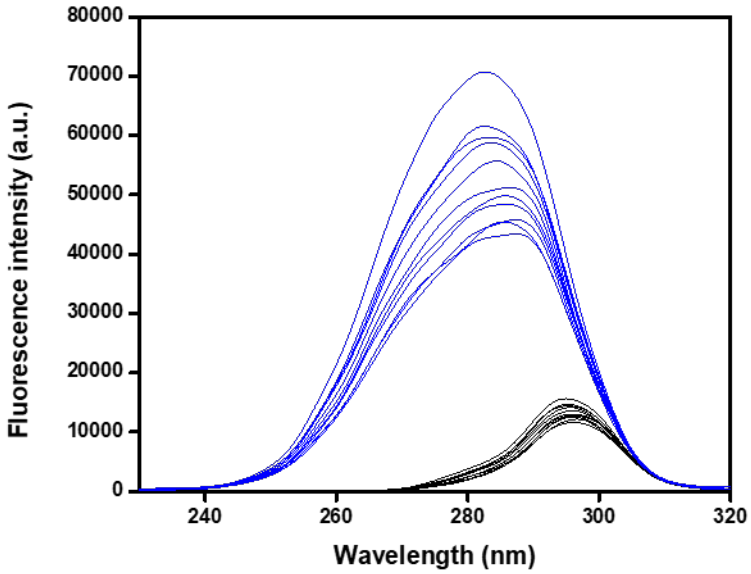


Figure S26: Fluorescence Synchronous titration spectra of trypsin with DTC-SSM, excitation at 280 nm (10 μM protein solution in phosphate buffer (pH 7.4), 2–100 μM of DTC-SSM was added at 25 °C (blue line spectra $\Delta\lambda = 60$ nm:, black line spectra: $\Delta\lambda = 15$ nm).

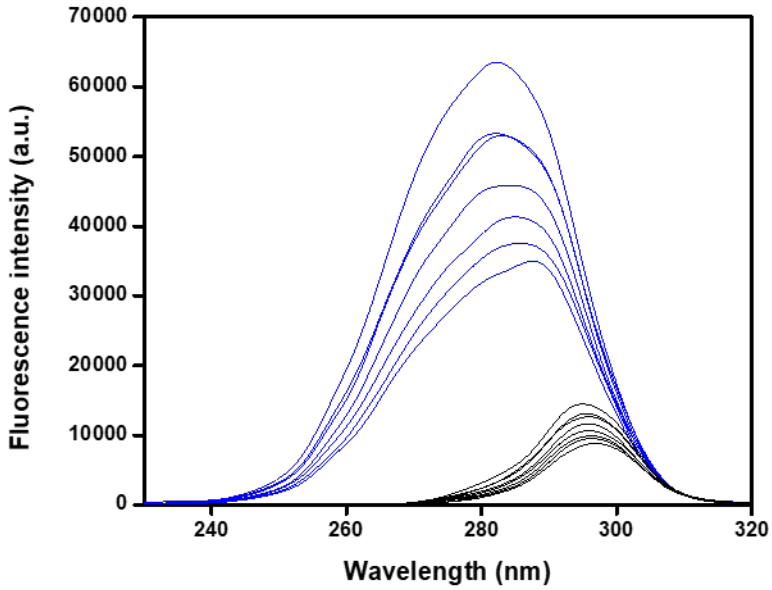


Figure S27: Fluorescence Synchronous titration spectra of trypsin with T-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of T-SSM was added at 25 °C (blue line spectra Δλ = 60 nm:, black line spectra: Δλ = 15 nm).

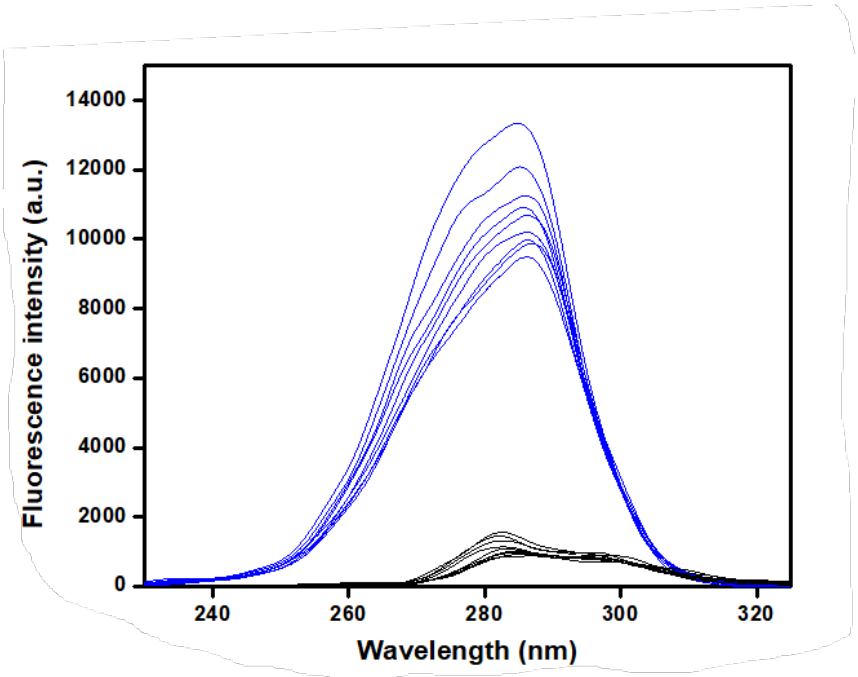


Figure S28: Fluorescence Synchronous titration spectra of haemoglobin with DTC-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of DTC-SSM was added at 25 °C (blue line spectra Δλ = 60 nm:, black line spectra: Δλ = 15 nm).

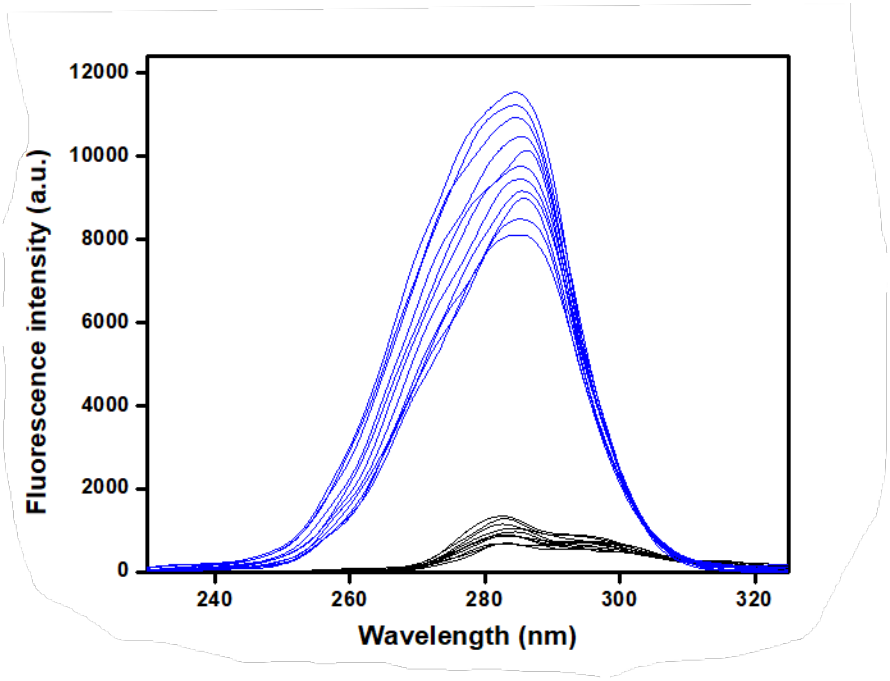


Figure S29: Fluorescence Synchronous titration spectra of haemoglobin with T-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of T-SSM was added at 25 °C (blue line spectra Δλ = 60 nm:, black line spectra: Δλ = 15 nm).

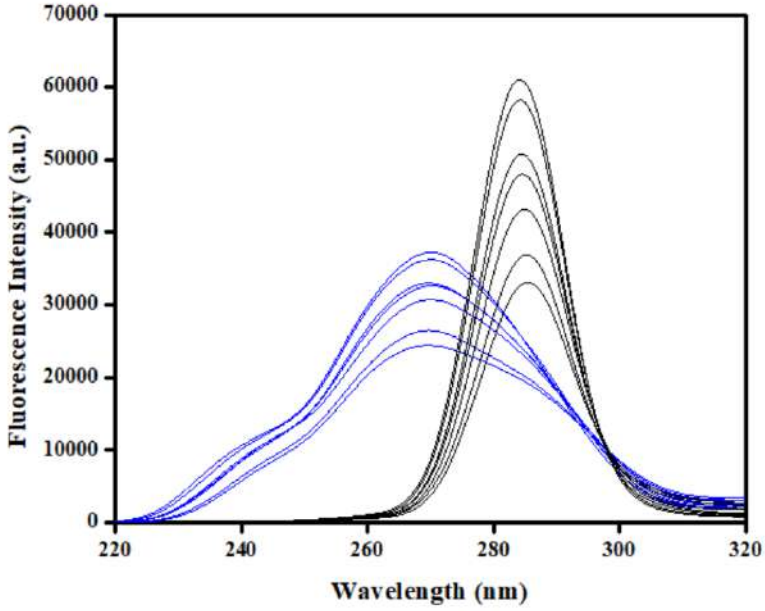


Figure S30: Fluorescence Synchronous titration spectra of ribonuclease with DTC-SSM, excitation at 280 nm (10 µM protein solution in phosphate buffer (pH 7.4), 2–100 µM of DTC-SSM was added at 25 °C (blue line spectra Δλ = 60 nm:, black line spectra: Δλ = 15 nm).

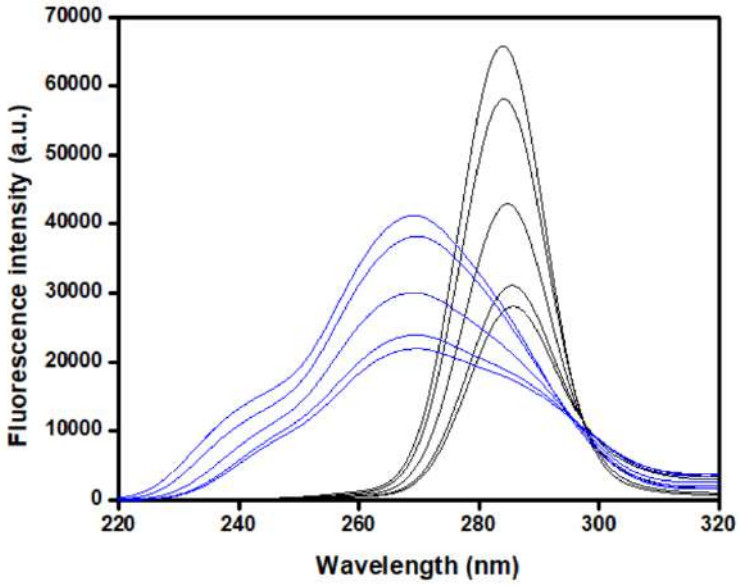


Figure S31: Fluorescence Synchronous titration spectra of ribonuclease with T-SSM, excitation at 280 nm (10 μM protein solution in phosphate buffer (pH 7.4), 2–100 μM of T-SSM was added at 25 °C (blue line spectra $\Delta\lambda$ = 60 nm:, black line spectra: $\Delta\lambda$ = 15 nm).

Molecular docking studies

Molecular docking studies was carried out by auto dock vina and Perl script for integration executables. The protein structure was collected from protein bank for BSA (4F5V, chain A), HSA (1BM0, chain A), trypsin (1TRN, chain A), haemoglobin (4 HHB, chain A) and ribonuclease (2G8Q, chain A). The grid box were used of size for BSA (X: 34.167, Y: 24.806, Z: 41.472) centred at (62,80,74) , HSA (X: 29.607, 31.782, 23.488) centred at (60, 60, 60) , trypsin (X: 2.494, Y: 7.537, Z: 21.194) centred at (42, 36, 52), Haemoglobin (X: 14.422, Y: 67.713, Z: 4.181) centred at (100, 90, 110) and ribonuclease (X: 5.325, -26.182, -15.471) centred at (84,72,116). The ligand structure was minimized by Argus lab (4.0.1), The docked structure was visualised by Pymol and interaction of ammino acid residues with the ligand determined by Discovery studio.

Interaction of macromolecules with proteins

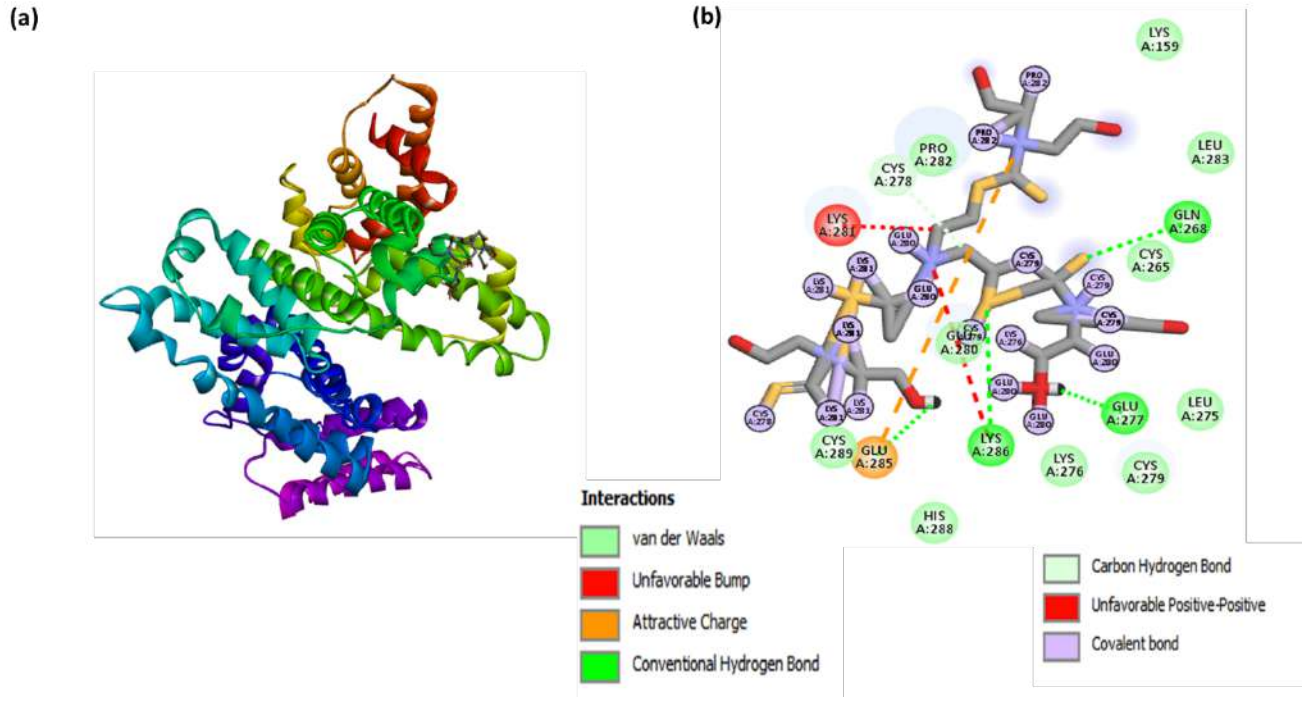


Figure S32: (a) Docking of DTC-SSM with HSA. (b) 2-D interaction of DTC-SSM and amino acid residues of HSA.

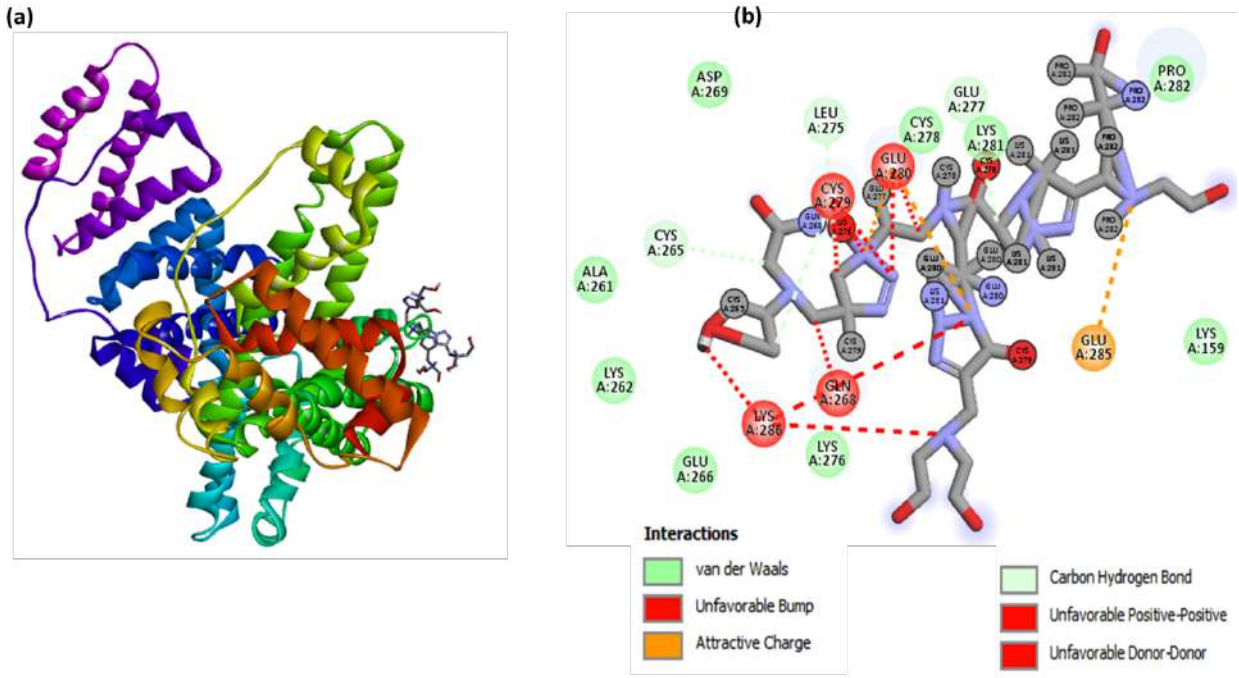


Figure S33: (a) Docking of T-SSM with HSA. (b) 2-D interaction of T-SSM and amino acid residues of HSA.

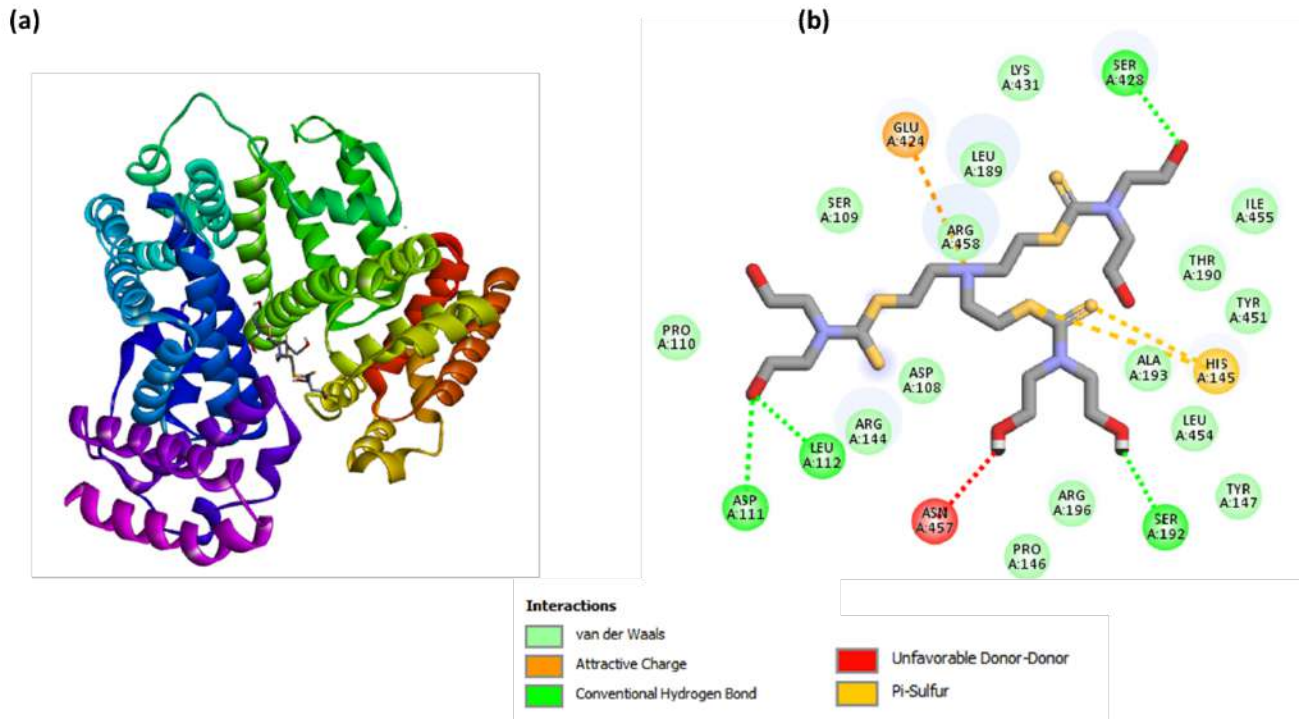


Figure S34: (a) Docking of DTC-SSM with BSA. (b) 2-D interaction of DTC-SSM and amino acid residues of BSA.

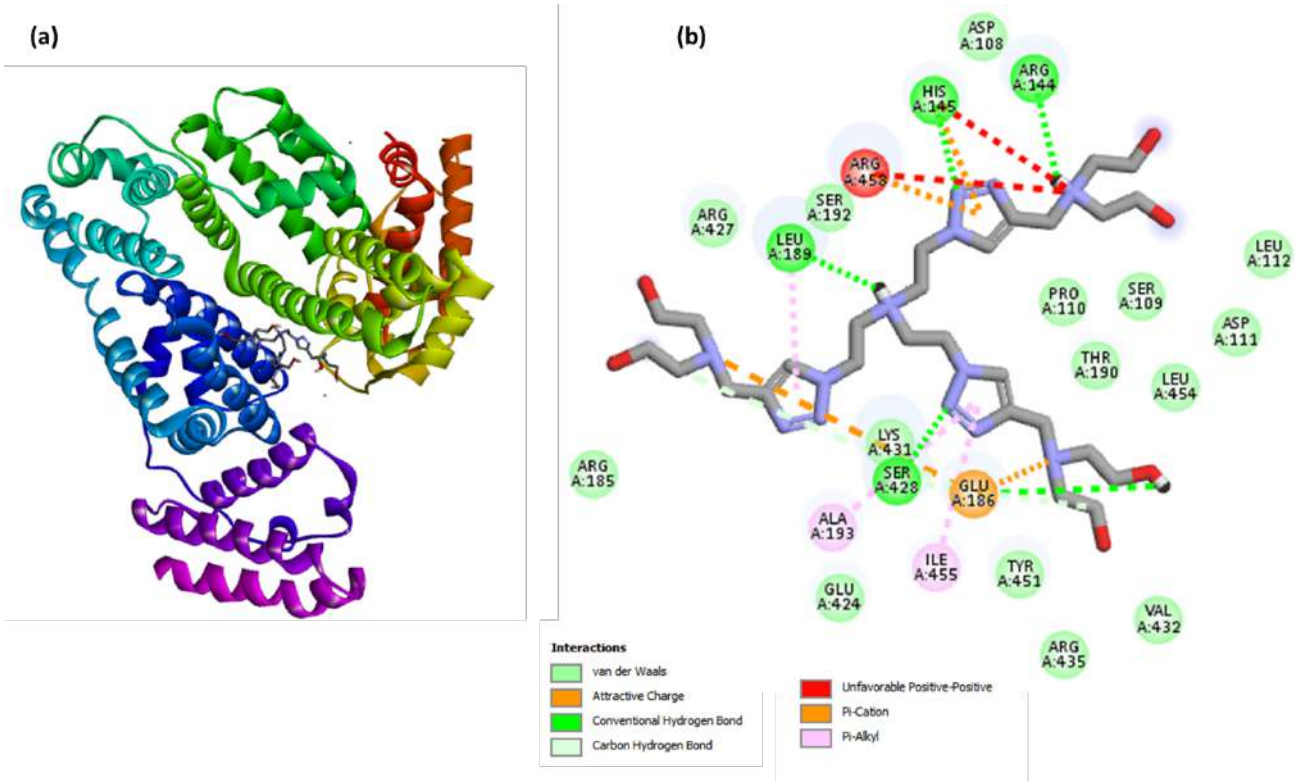


Figure S35: (a) Docking of T-SSM with BSA. (b) 2-D interaction of T-SSM and amino acid residues of BSA.

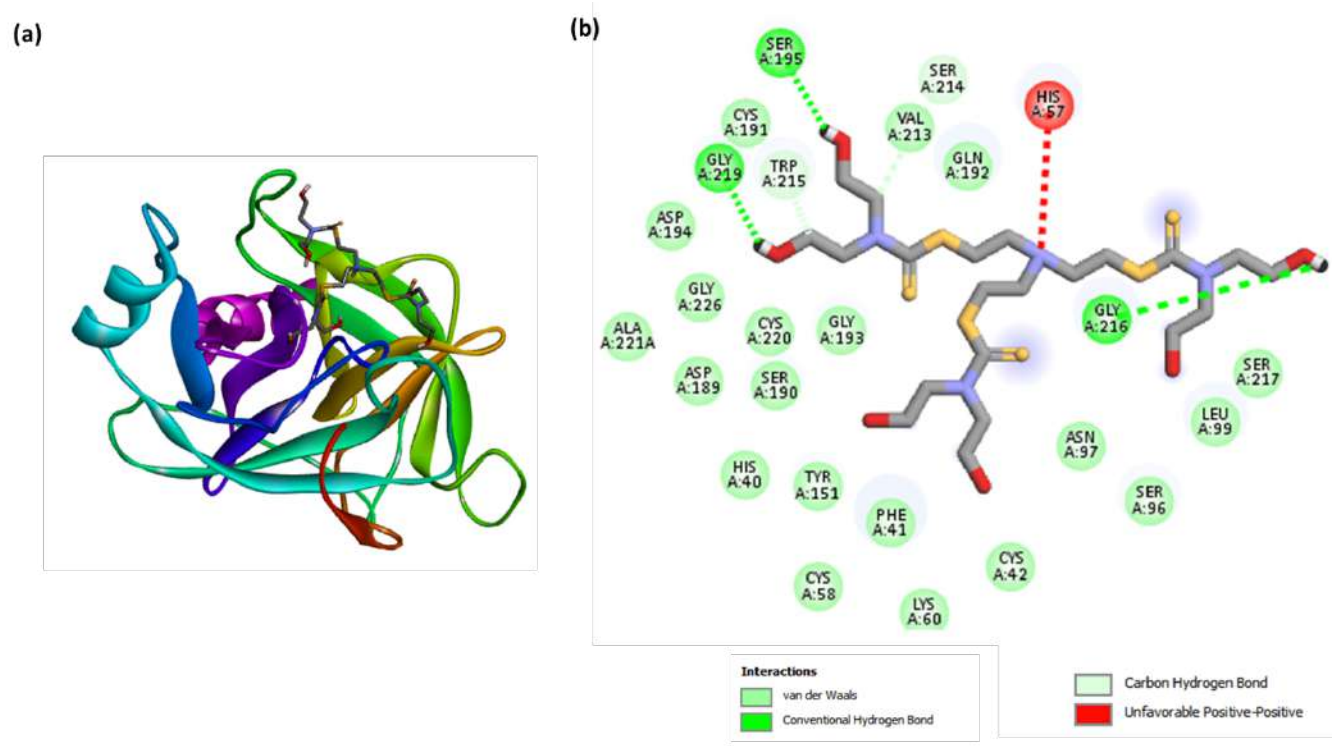


Figure S36: (a) Docking of DTC-SSM with trypsin. (b) 2-D interaction of DTC-SSM and amino acid residues of trypsin.

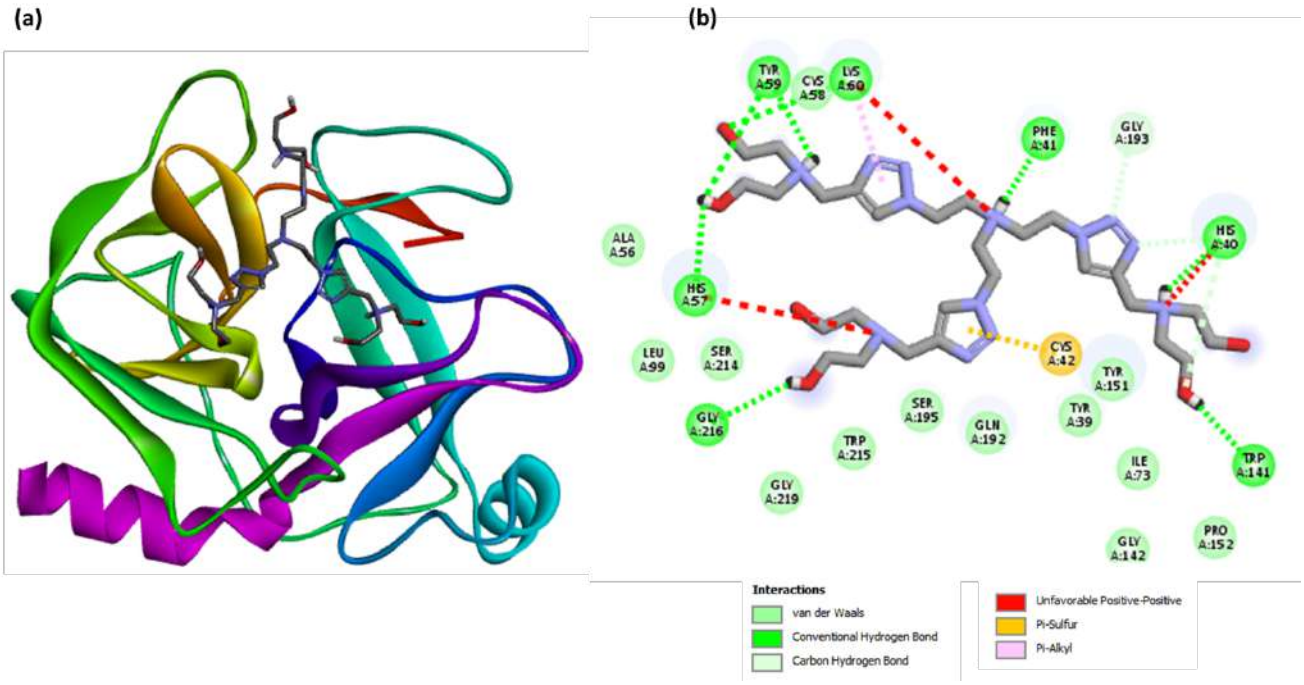


Figure S37: (a) Docking of T-SSM with trypsin. (b) 2-D interaction of T-SSM and amino acid residues of trypsin.

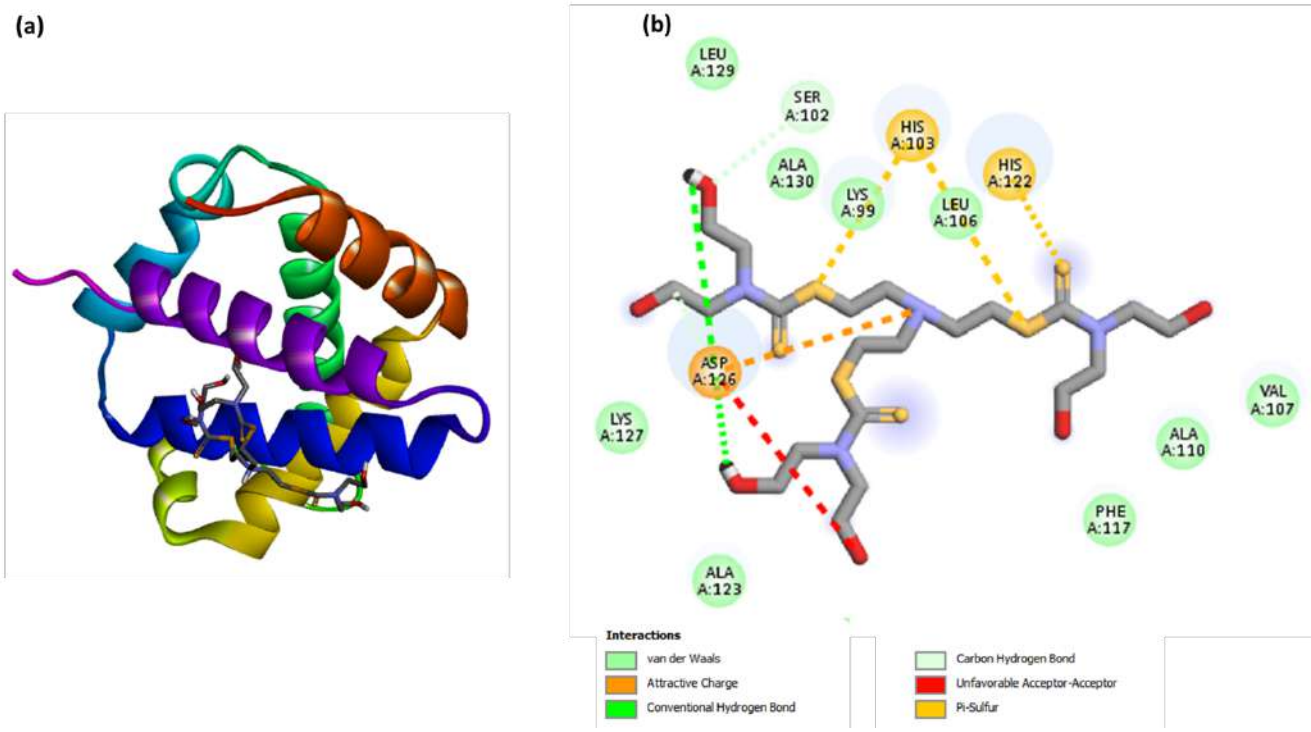


Figure S38: (a) Docking of DTC-SSM with haemoglobin. (b) 2-D interaction of DTC-SSM and amino acid residues of haemoglobin.

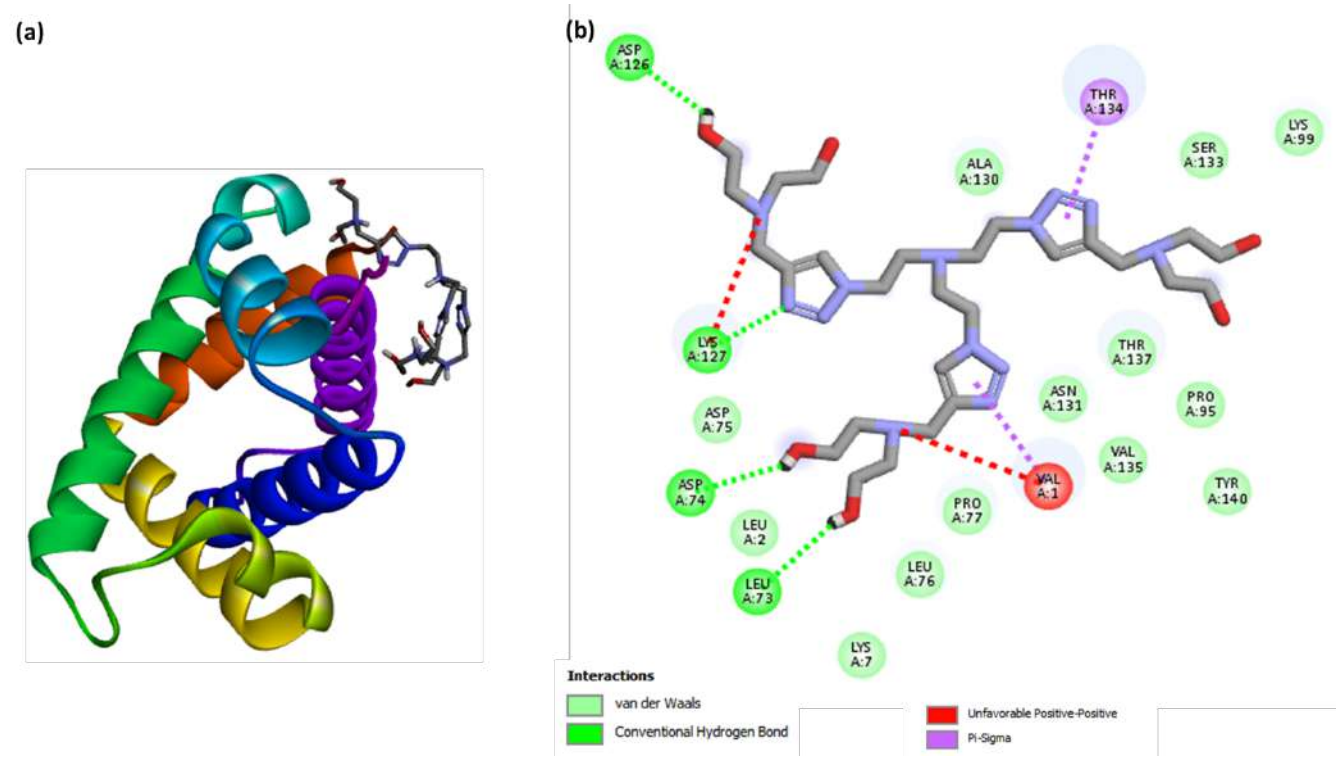


Figure S39: (a) Docking of T-SSM with haemoglobin. (b) 2-D interaction of T-SSM and amino acid residues of haemoglobin.

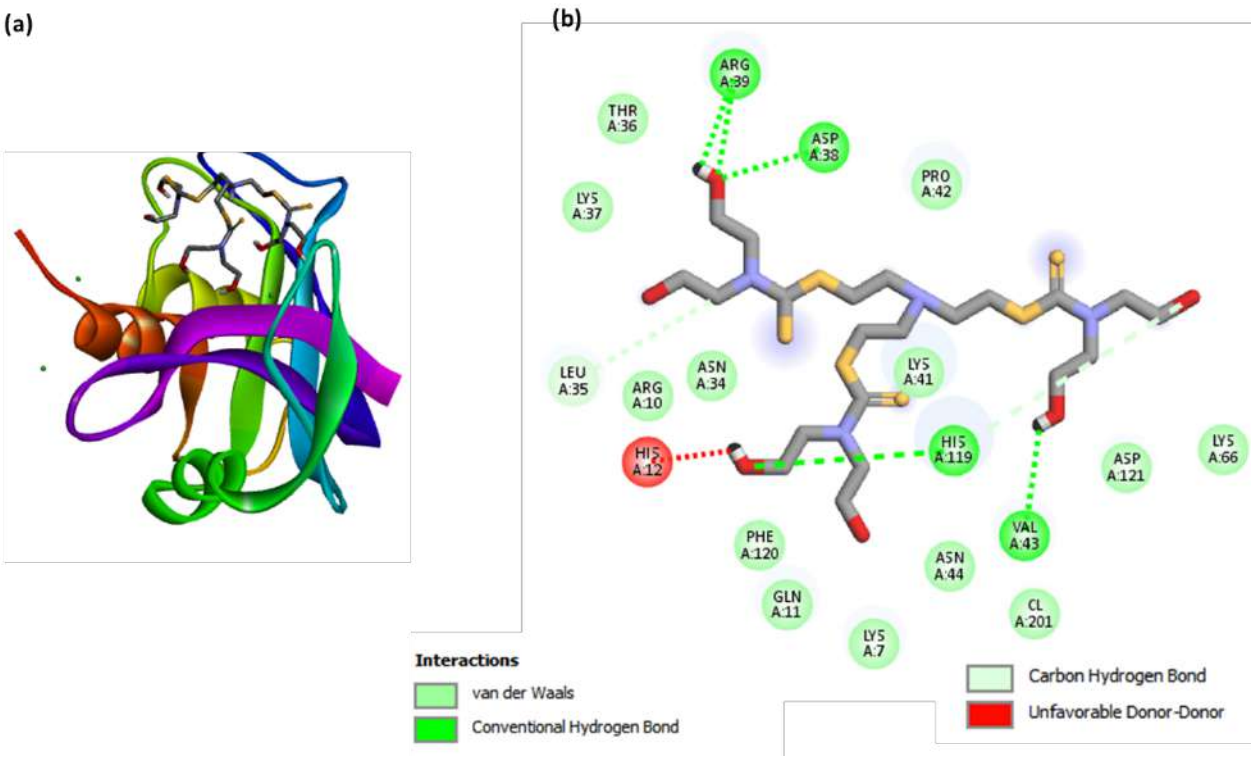


Figure S40: (a) Docking of DTC-SSM with Ribonuclease. (b) 2-D interaction of DTC-SSM and amino acid residues of Ribonuclease.

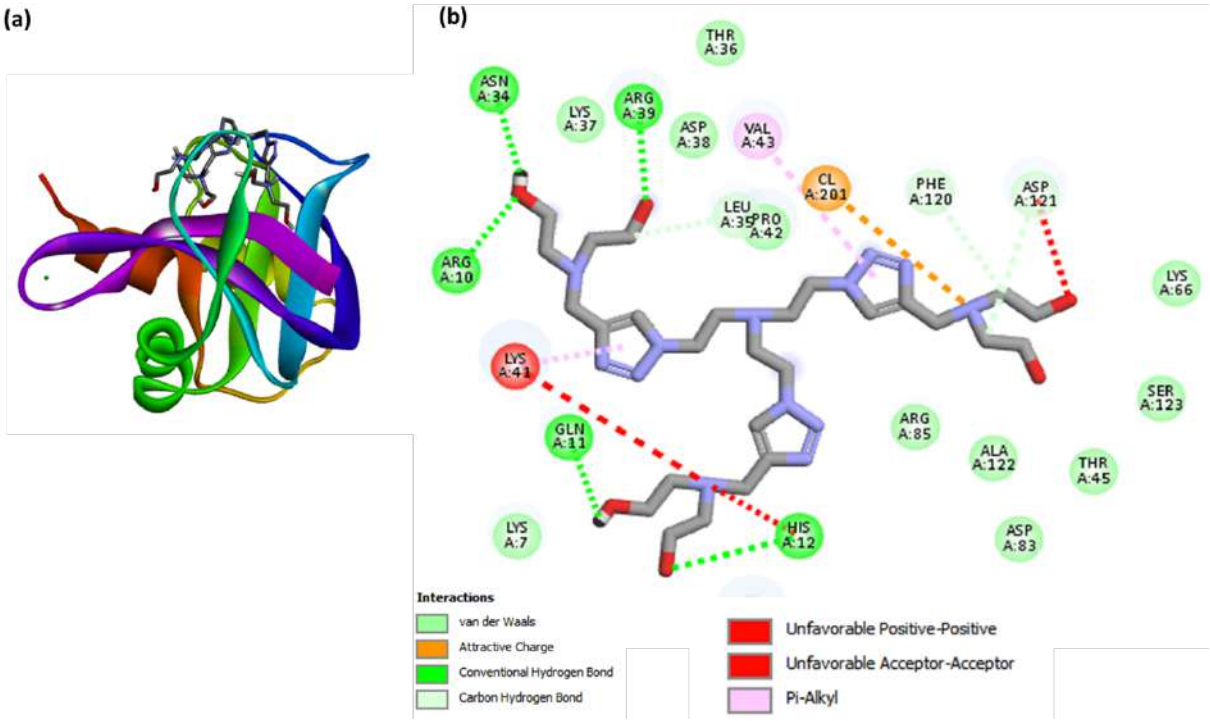


Figure S41: (a) Docking of T-SSM with Ribonuclease. (b) 2-D interaction of T-SSM and amino acid residues of Ribonuclease.