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Supporting Information

Dithiocarbamate based linear versus macrocyclic architecture: Comparative studies and applications in protein interaction and heavy metals removal

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Contents

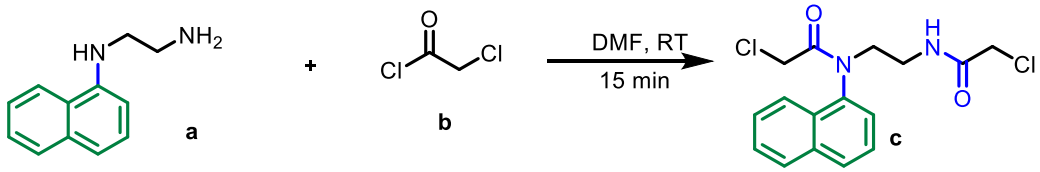
1. Materials and Methods

All the chemicals were purchased from Alfa Aeser, Spectrochem, Nice 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 254 nm (unless not specified) 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%, 60%, 90% and again 5% of acetonitrile over the time of 15 minutes. The purification by HPLC is performed on Shimadzu HPLC-20AP instrument by using the same solvent system of that of LC-MS. 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. ^{1}H NMR spectra were recorded on INOVA-400 spectrometer and and 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. Fluorescence was recorded on Perkin Elmer FL 6500. All fluorescence spectra are recorded at 25^{o}C with an excitation wavelength of 280 nm and slit width of 5 nm for excitation and emission. The fluorescence spectra were plotted in OriginPro 8.5.1 (https://www.originlab.com/). HRMS was measured in Waters ACQUITY H-CLASS + UPLC/XevoG2 XS QTOF instrument. Fourier

Transformed IR Spectroscopy (FT-IR) was recorded in Shimadzu IR Tracer 100 in KBr pellet method and spectra were plotted in OriginPro 8.5.1. The software comprising of Autodock Tools, Autodock Vina was used to perform molecular docking of proteins with the compounds. The crystal structure of proteins were downloaded from Protein Data Bank and the linear and macrocyclic compounds were energy minimized by ArgusLab 4.0.1. by using Chimera. Chain A of the proteins were used for docking by removing chain B and water molecules. Polar hydrogens and partial Kollmann charges were added by merging the nonpolar hydrogens to BSA. The output of the results was estimated using Lamarckian genetic algorithm. PyMol was used to get the pdb format of the docked structure and BIOVIA Discovery Studio Visualizer 2021 was used for the visualization of the docked structure (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/visualization). SEM images were taken using Carl Zeiss - Gemini SEM 300 with 2000000 X magnification. The quantitative analysis of heavy metals was carried out using inductively coupled plasma-optical emission spectroscopy (ICP-OES) instrument (Perkin Elmer Optima 5300 DV). Thermal gravimetry analysis was carried out in Perkin Elmer Inc Thermal Analyzer STA 8000,

2. Synthetic procedure and characterization

2.1.*Chloroacetylated N-Naphthyl ethylene diamine*



Scheme 1. Synthesis of **c**.N-naphthyl ethylene diamine, **a** was taken in 15 ml DMF and chloroacetyl chloride was added very slowly to afford **b**; white solid (Yield = 800 mg, 96 %). ^{1}H NMR (400MHz, $CDCl_3$) spectrum of **c**: δ (ppm) 3.71 (t, 2H), 3.88 (t, 3H), 4.10 (s, 4H),

7.35-7.98 (m, 7H) and '*' represents the residual solvent peak of internal standard tetramethyl silane.

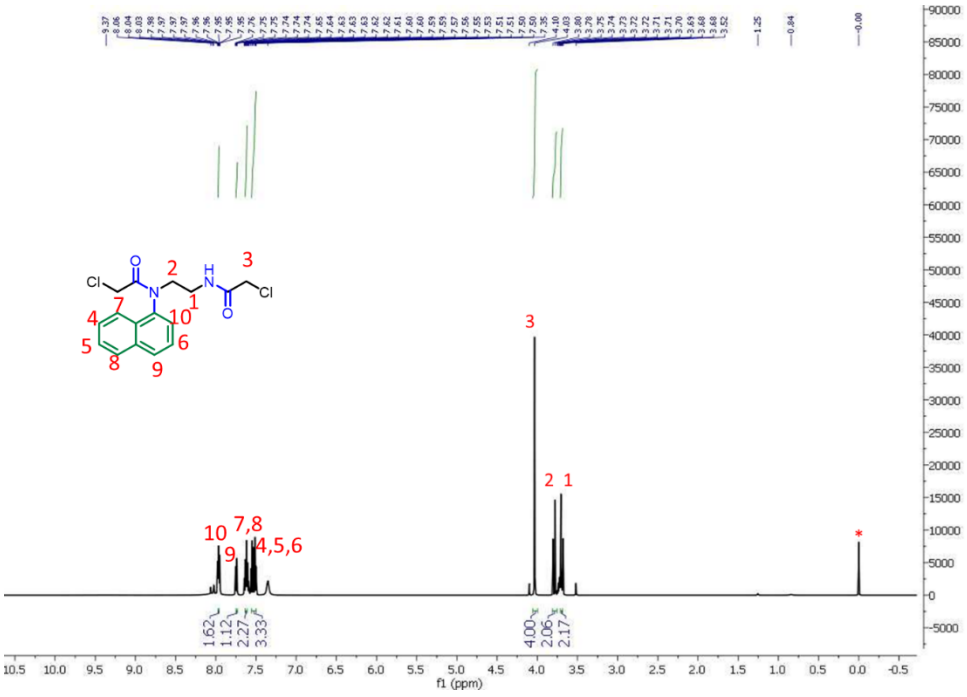


Figure S1. ^{1}H NMR (500MHz, $CDCl_3$) spectrum of **a**

*2.2.Synthesis of linear compound **1.***

Chloroacetylated diamide (**c**) (2.94 mmol, 1 eq) was taken in 10 ml of Polyethylene glycol (PEG)-200; CS_2 (29.4 mmol, 10 eq., 1.8 ml) and propylamine (8.82 mmol, 3 eq., 0.72 ml) was added to this and stirred for 15 minutes under room temperature. The progress of the reaction was monitored by TLC in 1:1 ethyl acetate and hexane solution and visualized under UV light and iodine. After completing the reaction, the reaction mixture was extracted by using water and ethyl acetate. The organic layer was passed through anhydrous Na_2SO_4. The solvent was removed under low pressure and the product was taken for further purification. The purification was carried out by column chromatography with silica gel 60-120 mesh. The pure product was eluted in the solvent ratio 50 % of ethyl acetate and 50 % of hexane. LC-MS calculated $[M+H]^+$: 537.14 Da, observed $[M+H]^+$: 537.30 Da, $[M+Na]^+$: 559.25 D. IR (KBr) $\upsilon^{\sim}$ = 1638 cm^{-1} (C=O), 1150 cm^{-1} (symmetric stretch, C=S) and 1064 cm^{-1} (asymmetric stretch, C=S). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.03 (t, 6H), 1.77 (m, 4H), 3.42 (t, 4H), 3.43 (s, 4H), 3.69 (t, 2H), 3.72 (t, 2H), 7.48-8.03 (m, 7H). "*", "#" and "**" represent the residual proton signal of internal standard tetramethyl silane, silica grease impurity and $CDCl_3$ respectively. ^{13}C NMR (11.62, 21.54, 37.19, 38.49, 38.87, 47.32, 49.31, 121.59, 125.83, 127.08, 127.22, 127.32, 129.05, 129.38, 130.14, 134.96, 135.74, 169.82, 171.94, 194.92

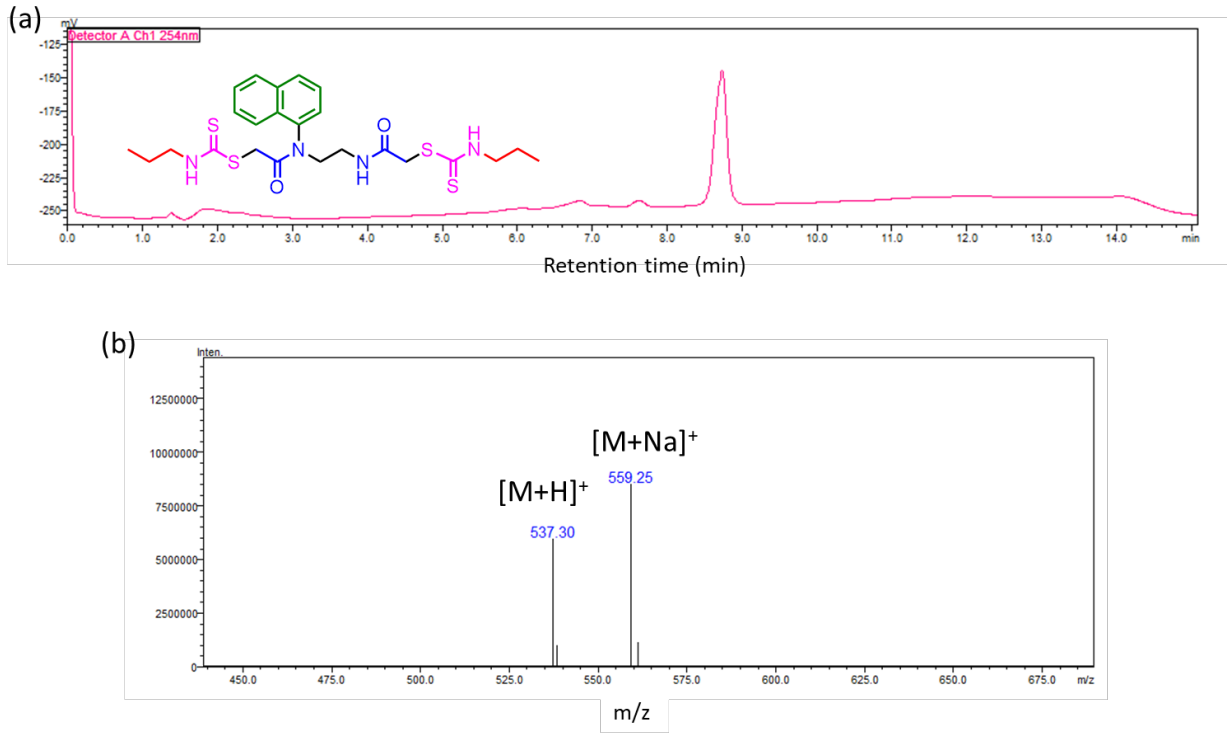


Figure S2. a: HPLC of linear **1** eluted in acetonitrile and water gradient recorded at 254 nm. **b**: Full range MS spectrum in positive ion mode.

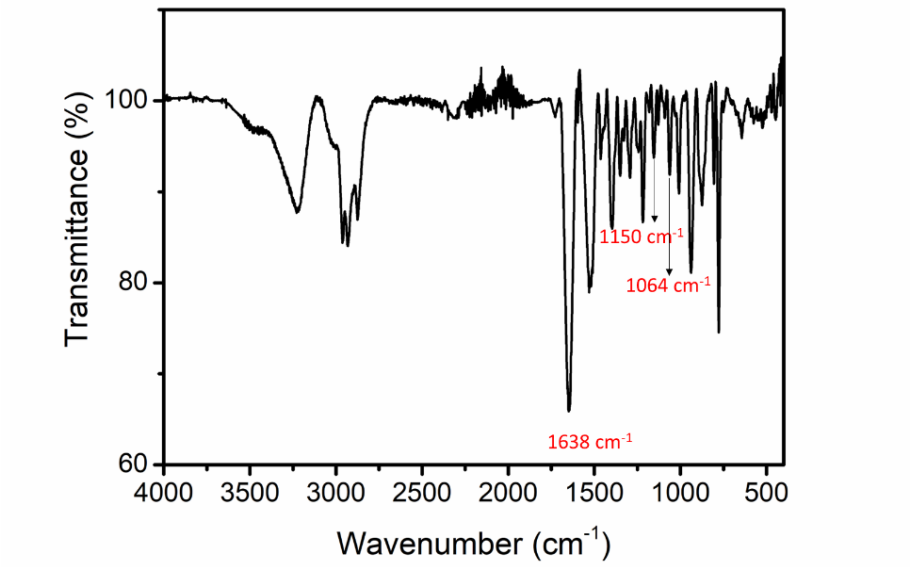


Figure S3: FT-IR spectrum of linear **1.**

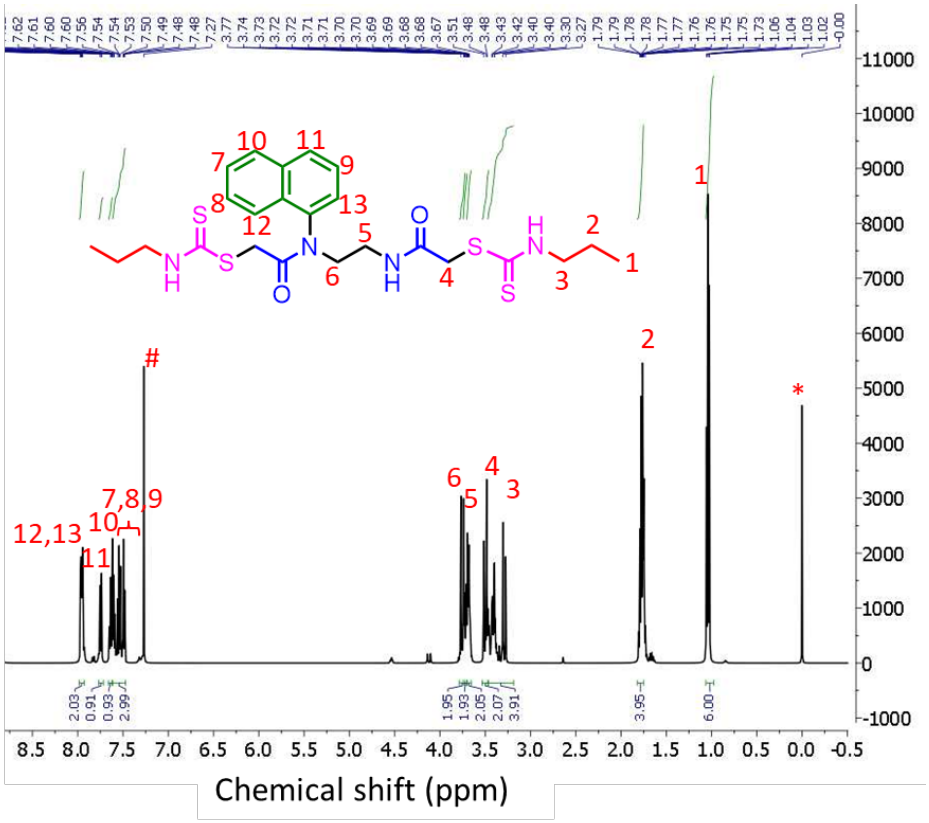


Figure S4. ^{1}H-NMR (500 MHz) of linear (**1**) in $CDCl_3$

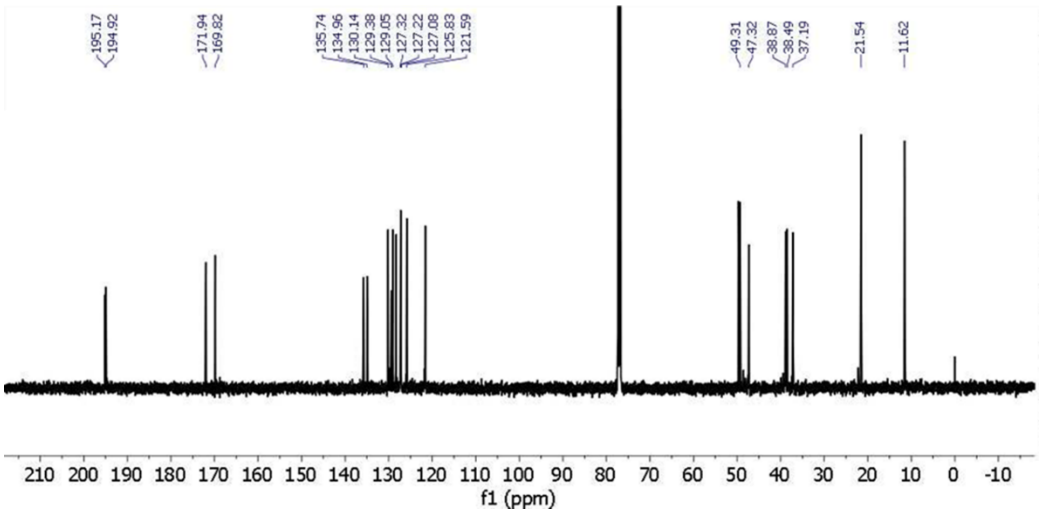


Figure S5. ^{13}C-NMR of linear (**1**) in $CDCl_3$

*2.3.Synthesis of macrocycle **2***

Chloroacetylated diamide (**c**) (1.5 mmol, 1 eq) was taken in 10 ml of Polyethylene glycol (PEG)-200; CS_2 (14.8 mmol, 10 eq., 0.8 ml) and 1,3-diaminopropane (2.21 mmol, 1.5 eq., 0.2 ml) was added to this and stirred for 15 minutes under room temperature. The progress of the reaction was monitored by TLC in 7:3 ethyl acetate and hexane solution and visualized under UV light and iodine. After completing the reaction, the reaction mixture was extracted by using

water and ethyl acetate. The organic layer was passed through anhydrous Na_2SO_4. The solvent was removed under low pressure and the product was taken for further purification. The purification was carried out by column chromatography with silica gel 200-400 mesh. The pure product was eluted in the solvent ratio 60 % of ethyl acetate and 40 % of hexane. LC-MS calculated $[M+H]^+$: 493.07 Da, observed $[M+H]^+$: 493.20 Da, $[M+Na]^+$: 515.15 D. IR (KBr) $\upsilon^{\sim} = 1716\ cm^{-1}$ (C=O), 1160 cm^{-1} (symmetric stretch, C=S) and 1071 cm^{-1} (asymmetric stretch, C=S). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.25 (m, 2H), 2.10 (t, 4H), 3.63 (t, 2H), 3.68 (s, 4H) 3.79 (t, 2H), 7.29-8.03 (m, 7H). "*", "#" and "**" represent the residual proton signal of internal standard tetramethyl silane, silica grease impurity and $CDCl_3$ respectively. ^{13}C NMR (125 MHz, $CDCl_3$) δ24.62, 36.12, 37.68, 38.31, 47.03, 67.71, 121.30, 126.03, 127.34, 128.36, 129.10, 130.28, 134.72, 170.20, 173.34, 195.22, 196.88

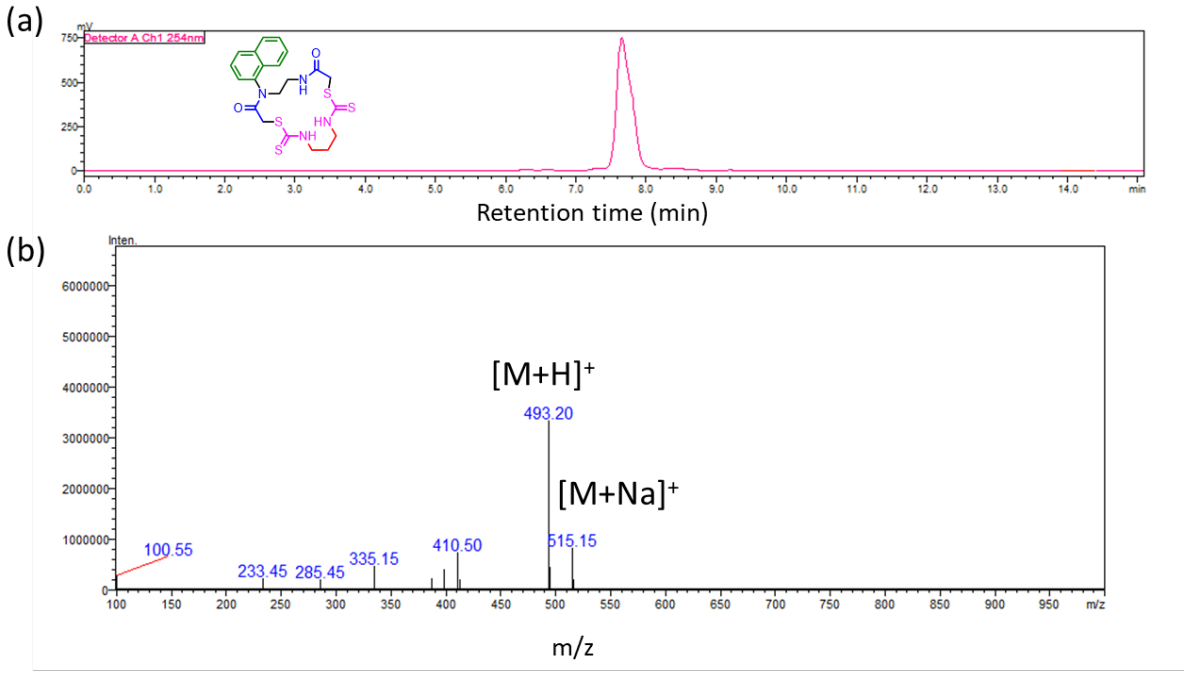


Figure S6. a: HPLC of macrocycle **2** eluted in acetonitrile and water gradient recorded at 254 nm. **b**: Full range MS spectrum in positive ion mode

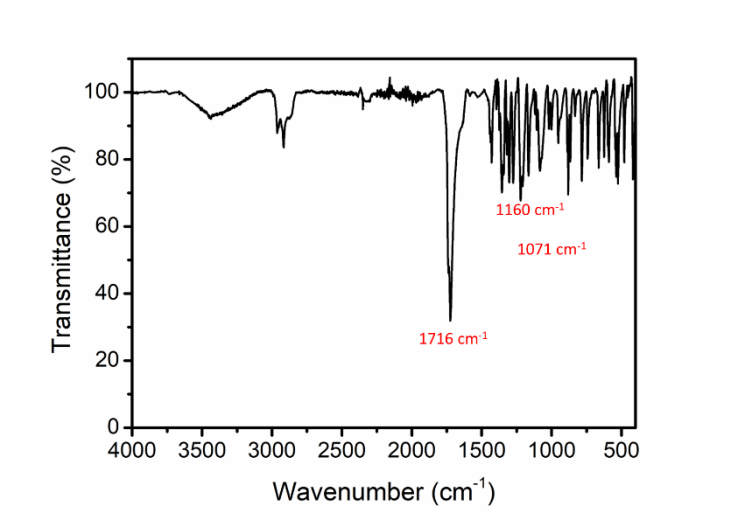


Figure S7. FT-IR spectrum of macrocycle **2.**

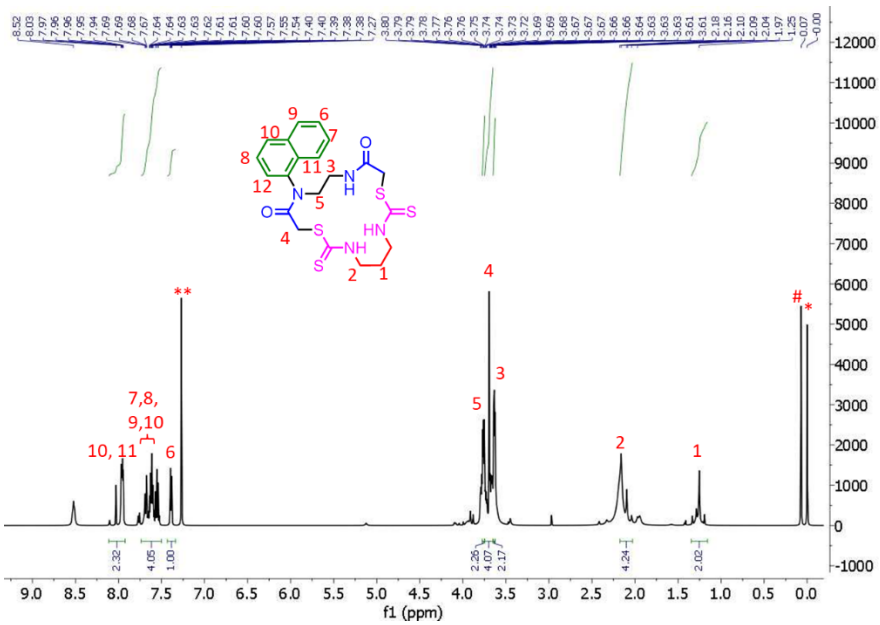


Figure S8. ^{1}H-NMR (500 MHz) of macrocycle (**2**) in $CDCl_3$

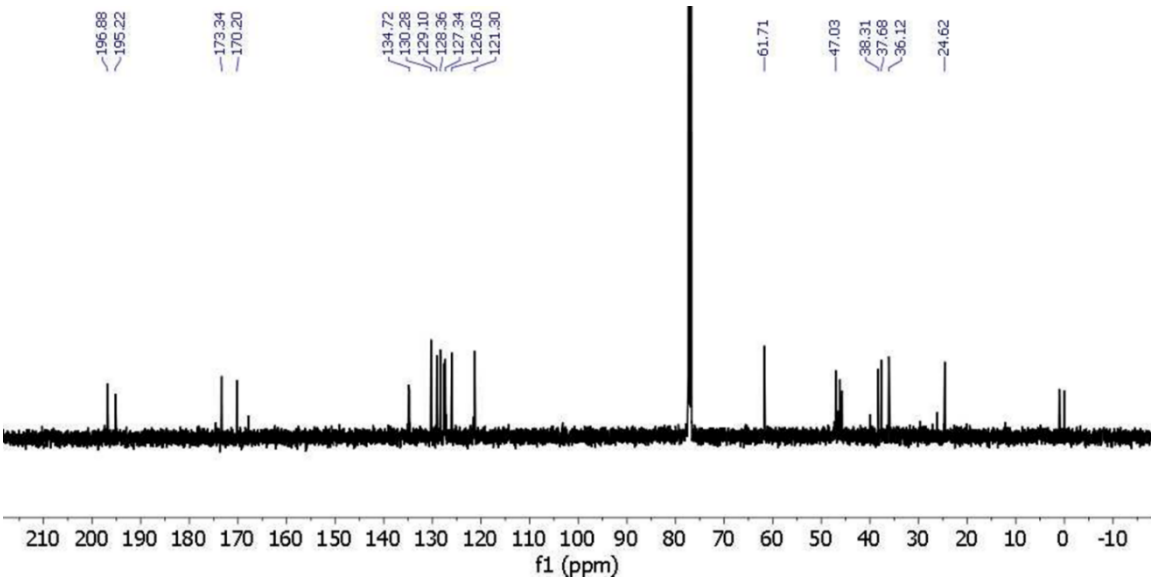


Figure S9. ^{13}C-NMR of macrocycle (**2)** in $CDCl_3$

3. Physicochemical studies of 1 and 2

Table S1. Physicochemical properties of the linear and macrocyclic structures; MW: Molecular Weight, Log P: lipophilicity (log of partition coefficient in octanol and water), PSA: polar surface area, HBD: number of hydrogen bond donors, HBA: number of hydrogen bond acceptors, RB: number of rotatable bonds, and K_{SP}: solubility product.

	Linear	**Macrocycle**
MW	536.8	492.7
Log P	4.41	2.64
HBD	3	3
HBA	2	2
RB	18	1
Solubility (S) (mol/L)	2.48×10^{-6}	3.66×10^{-6}
K_{SP}	6.20×10^{-6}	13.7×10^{-6}
log S	-5.61 (moderately soluble)	-5.44 (moderately soluble)
Violation from Lipinsky rule	2(MW, RB)	0

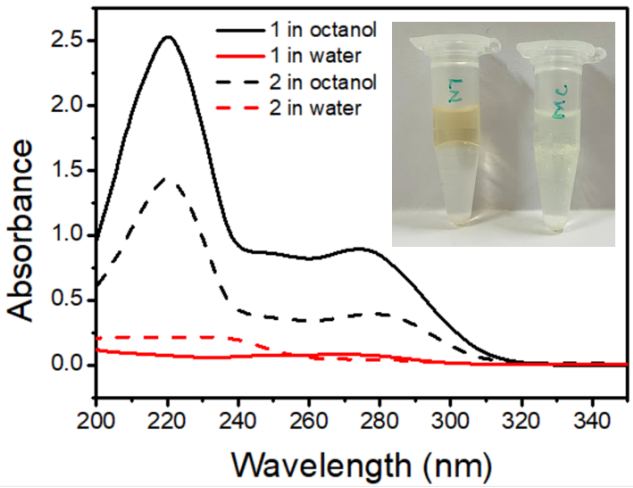


Figure S10. The UV-Visible spectrum of linear and macrocycle in octanol and water. Inset: Photograph of linear and macrocycle partitioned in octanol and water

4. Thermal studies of 1 and 2

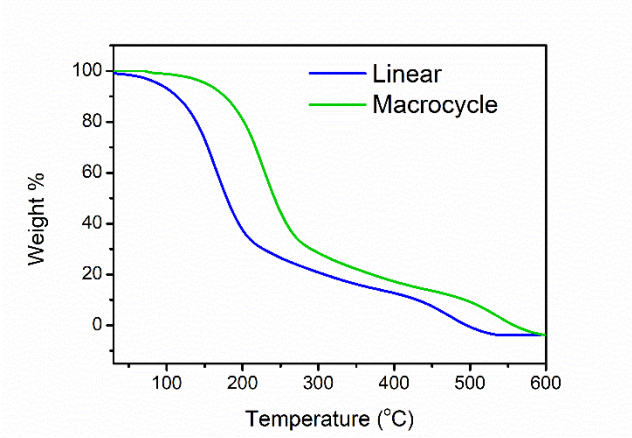


Figure S11. Thermogravimetric analysis spectrum of linear and macrocycle

5. Stability studies

A stock solution of both linear and macrocycle in DMSO + water mixture (5 μM) was made and used in the studies. The absorbance of these solutions was taken in various intervals of time such as 0 hr, 24 hrs, 48 hrs, and 72 hrs by UV-Visible spectroscopy. It was observed that there is no change in the absorbance of these compounds in even in solution state.

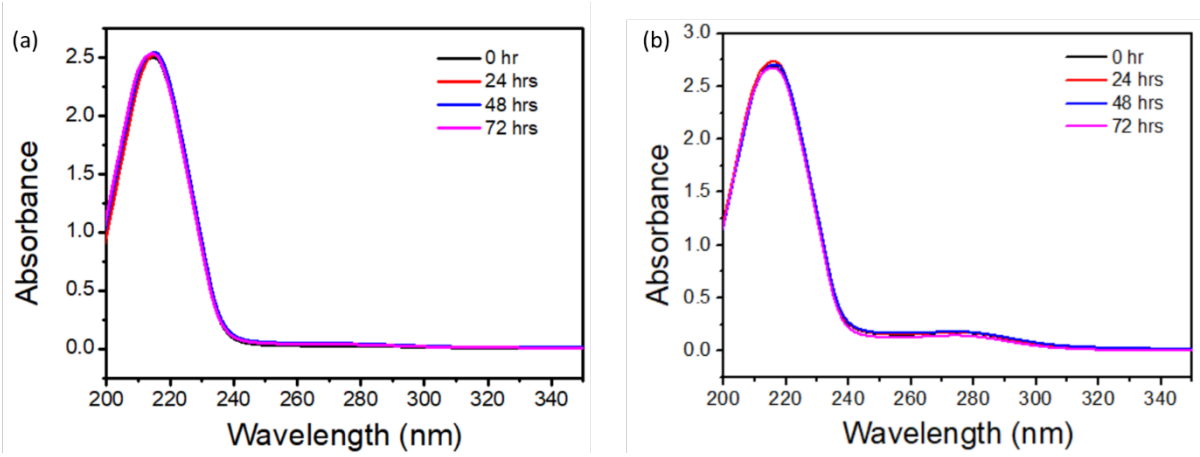


Figure S12. UV-visible spectrum of (a) linear and (b) macrocycle solutions over different intervals of time

6. Removal of toxic heavy metals

To the 25 mM **1** and **2,** 12.5 mM of metal salt solutions of Hg^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+} and Ni^{2+} were added separately. The precipitate formed was centrifuged and filtered to the detoxified water. The concentration of this solution was measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and the % removal was calculated by the equation:

$$\% \text{ removal} = \frac{(C_i - C_f) * 100}{C_f}$$

where C_i is the initial concentration of the metal solution and C_f is the concentration after the removal. The dilution factor was considered during the concentration calculation.

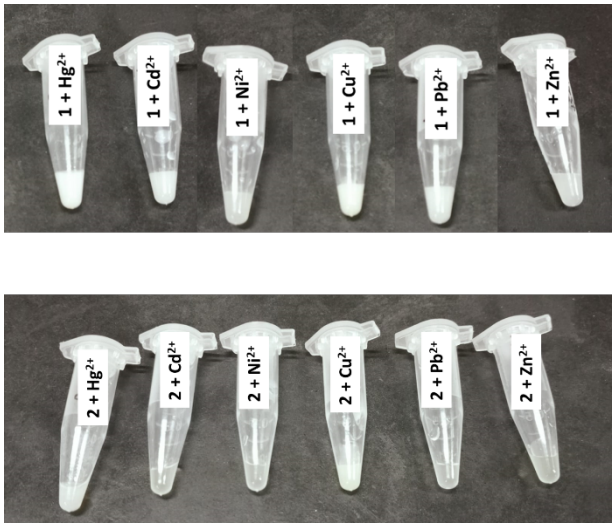


Figure S13. Photograph of metals precipitated immediately taken upon the addition of **1** (linear) and **2** (macrocycle)**.**

Table S2. % Removal by linear and macrocycle from the mixture of heavy metals

Metal	% Removal by linear	% Removal by macrocycle
Hg^{2+}	99.88	99.82
Cu^{2+}	94.0	90.05
Cd^{2+}	89.69	88.13
Pb^{2+}	89.59	88.87

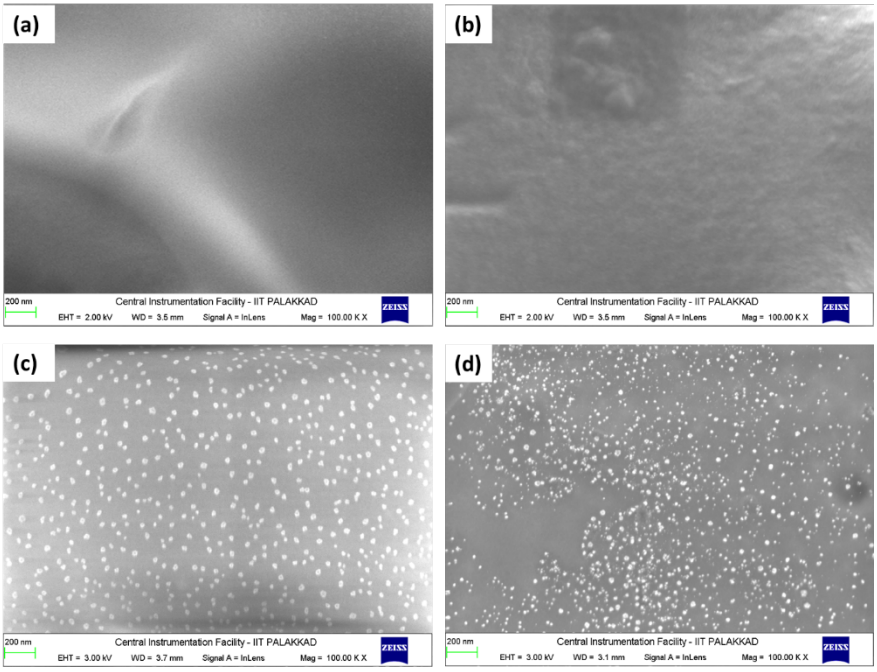


Figure S14. (a,b) SEM image of **1** and **2 (c,d)** SEM image of the precipitate formed upon the interaction of **1** and **2** with the mixture of metal solution.

7. Fluorescence spectroscopy for protein interaction studies

10 µM protein was prepared by dissolving in 10 ml of phosphate buffer of pH 7.4. The compounds were dissolved in DMSO to make the stock solution of 2 mM concentration. 2 ml of the protein control solution was taken in the fluorescence cuvette to take the emission at zero concentration of the compound exciting at 280 nm. The titration was carried out by varying the concentration of **1** and **2** from 2 µM to 100 µM. An equilibration time of 3 minutes is given for each measurement after the addition of the solution. Binding constant were estimated from the Stern-Volmer plot by plotting I_o/I vs concentration of the macrocycle. The slope of the graph was the attributed as the binding constant.

Table S3. Binding constant of **1** and **2** with the selected proteins calculated by Stern-Volmer equation

Protein	**Binding constant ($x10^3$ M^{-1})**	
	Linear	*Macrocycle*

BSA	13.695	14.111
HSA	16.786	16.892
Haemoglobin	6.477	8.828
Lysozyme	8.601	11.258
Proteinase	10.230	12.027
Ribonuclease	10.447	10.731
Trypsin	12.364	13.837

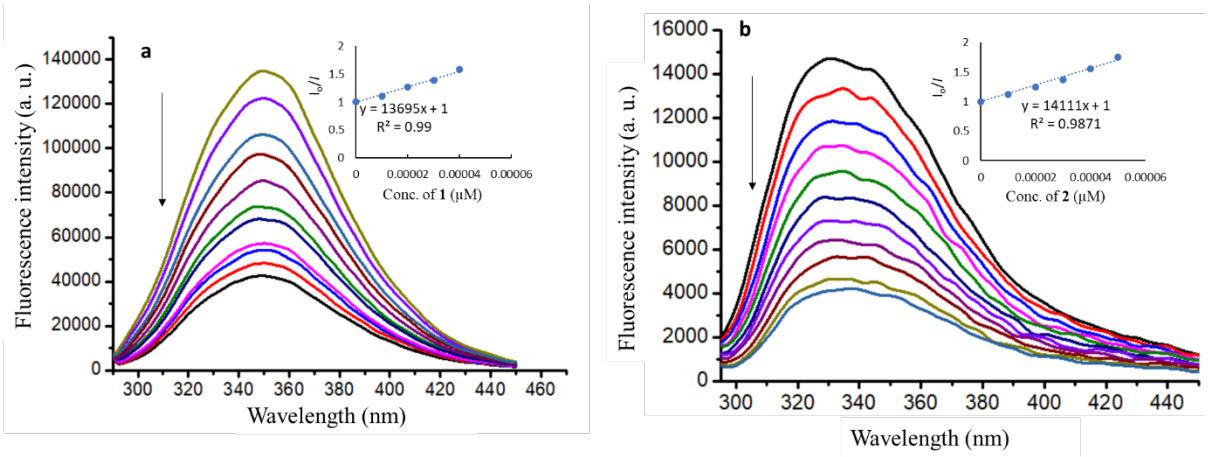


Figure S15. Fluorescence spectrum of **(a)** linear and **(b)** macrocycle on interaction with BSA. Inset: Stern Volmer plot for the calculation of binding constant.

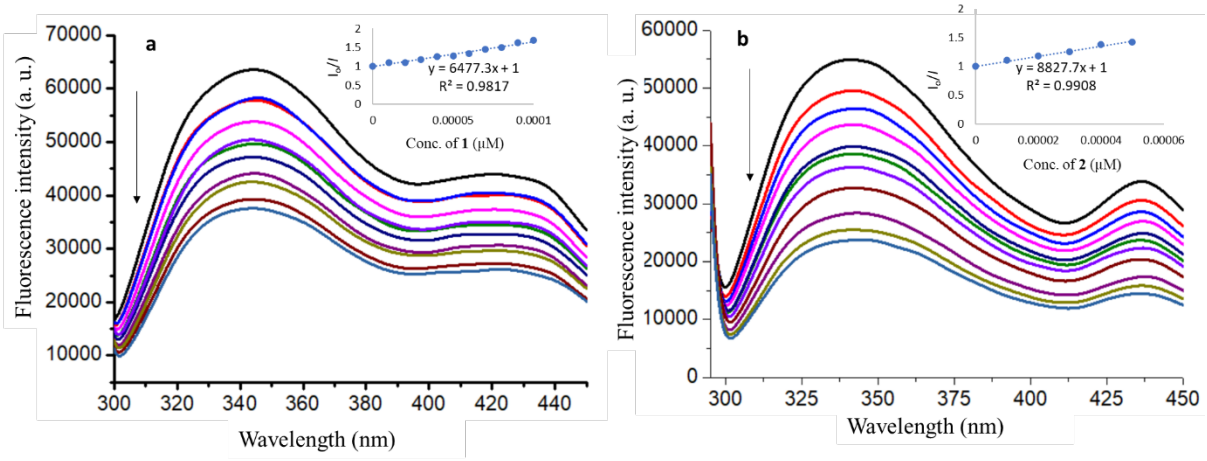


Figure S16. Fluorescence spectrum of **(a)** linear and **(b)** macrocycle on interaction with hemoglobin. Inset: Stern Volmer plot for the calculation of binding constant.

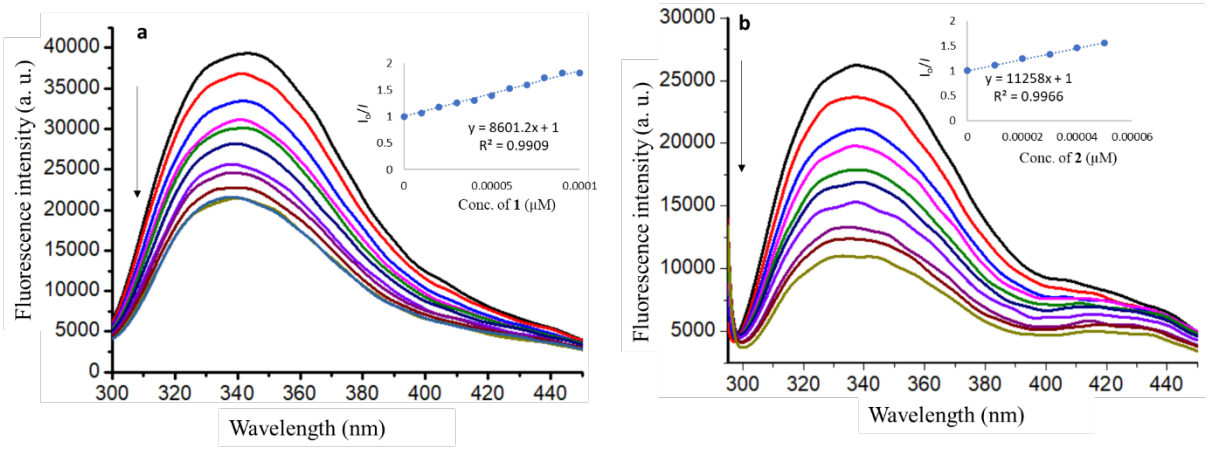


Figure S17. Fluorescence spectrum of **(a)** linear and **(b)** macrocycle on interaction with lysozyme. Inset: Stern Volmer plot for the calculation of binding constant.

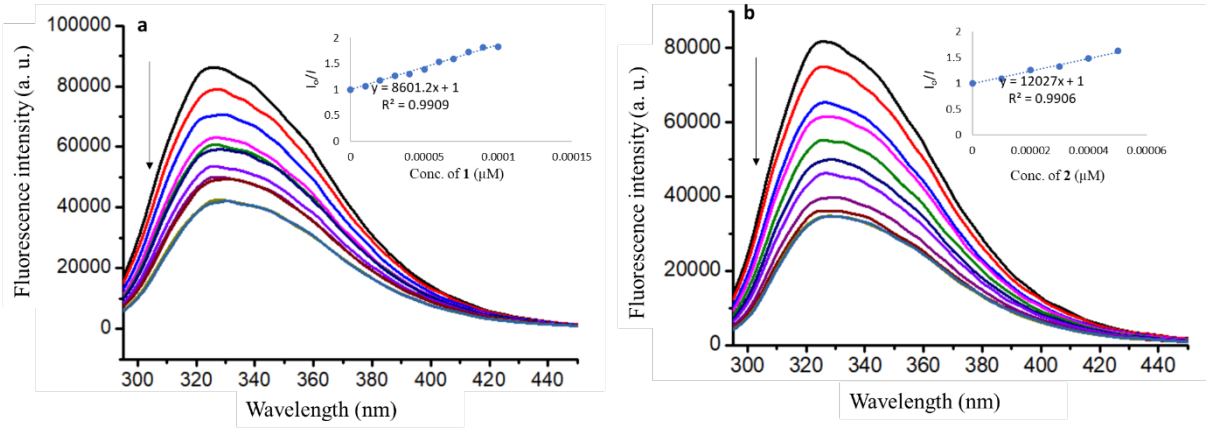


Figure S18. Fluorescence spectrum of **(a)** linear and **(b)** macrocycle on interaction with proteinase. Inset: Stern Volmer plot for the calculation of binding constant.

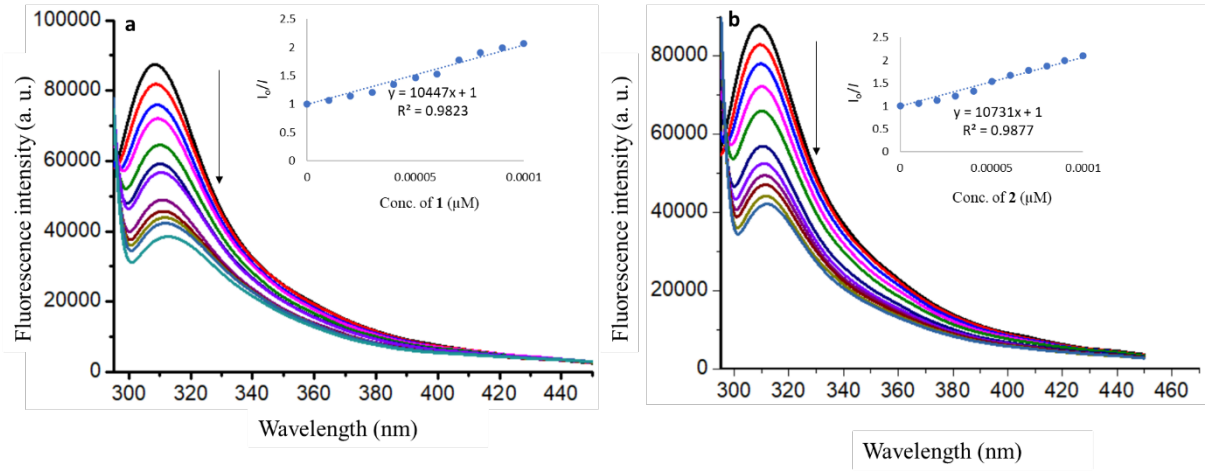


Figure S19. Fluorescence spectrum of **(a)** linear and **(b)** macrocycle on interaction with ribonuclease. Inset: Stern Volmer plot for the calculation of binding constant.

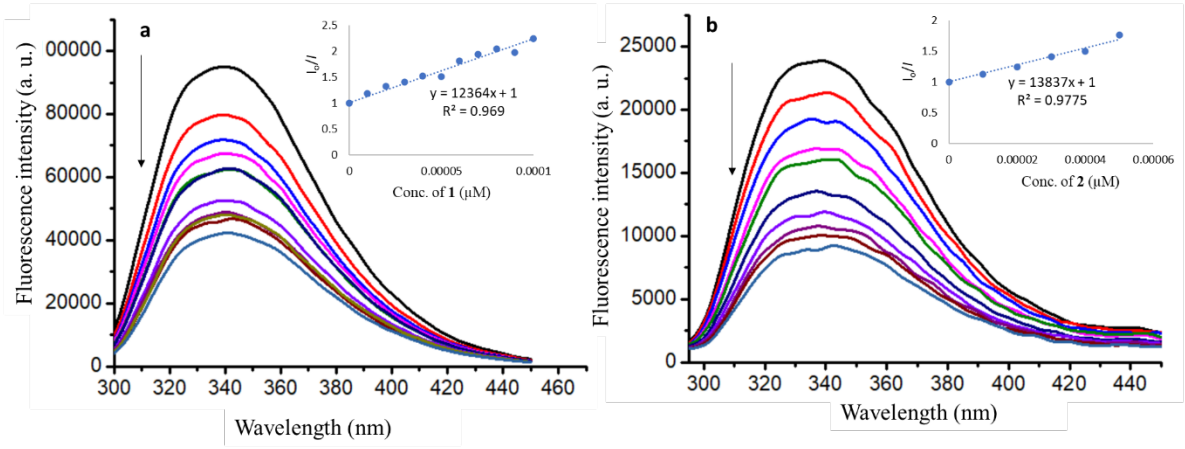


Figure S20. Fluorescence spectrum of **(a)** linear and **(b)** macrocycle on interaction with trypsin. Inset: Stern Volmer plot for the calculation of binding constant.