Supporting Information

Dansyl-triazole-based fluorescent macrocycle for selective nitro-antibiotics sensing in water/paper-strips and its interaction with multiple proteins

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1. Materials and Methods

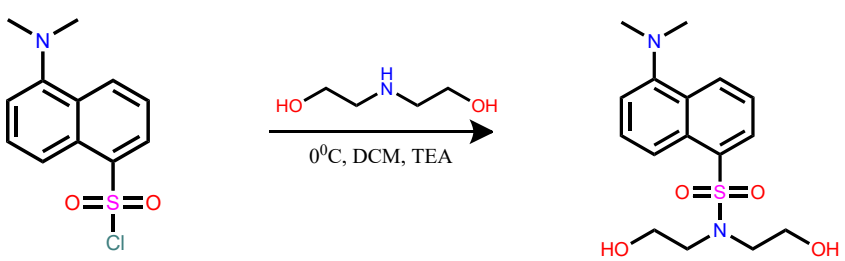
All the chemicals were purchased from Sigma Aldrich, Alfa Aeser, Spectrochem, Merck and TCI and used without further purification. LC-MS experiments were carried out on a Shimadzu LC-MS-8045 with a Sprite TARGA C18 column (40 × 2.1 mm, 5 µm) monitoring at 210 nm

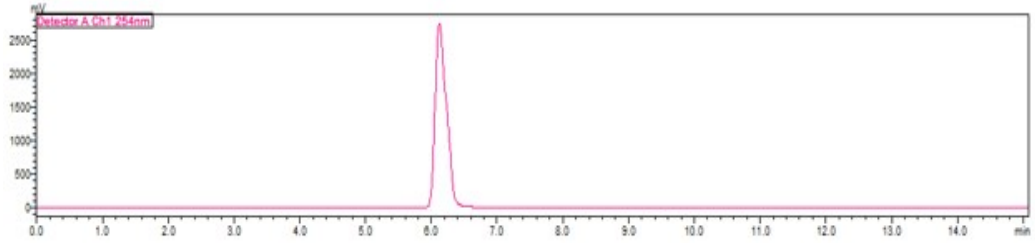
and 254 nm with positive mode for mass detection. Solvents for LC-MS were water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). Compounds were eluted at a flow rate of 0.5 mL/min with a gradient of 5% solvent B for 2 min, then linearly from 5% to 40% solvent B over 4 min, then from 40% to 60% for 10 min, and lastly, it was brought down to 5% solvent B in 2 min, and then the procedure proceeded till for 2 min before stopping. Before injecting the sample, the column was washed twice, once with 50% Solvent B and once with 95% Solvent B.The purification by HPLC is performed on Shimadzu HPLC-20AP instrument by using the same solvent system as that of LC-MS. Over the course of 26 minutes, compounds were eluted at a rate of 19 mL/min using a gradient of 20%, 60%, 75%, 90%, and 20% acetonitrile. ^{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. The fluorescence spectra were plotted in OriginPro 8.5.1. 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 compound was 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 protein. 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).

2. Synthesis and characterization of the dansyl-triazole-based macrocycle

2.1. Dansyl diethanolamine (**3**)

To the commercially available diethanolamine (46.6 mg, 42.7 ul, 0.444 mmol, 2.4 equiv.) 15 mL of dichloromethane (DCM) was added. Then the solution was placed in an ice bath (0^0C) for 5 minutes. The base triethylamine (22.4 mg, 32.02 µl, 0.222 mmol, 1.2 equiv.) was added to the solution and stirred well. After 2 minutes, dansyl chloride (50 mg, 0.185 mmol, 1 equiv.) was added, and the reaction was completed in 3 hours. Completion of the reaction was monitored by TLC. The reaction mixture was quenched by adding water to it and extracted with DCM and water. Dansyl diethanolamine was obtained without any further purification. LC-MS calculated $[M+H]^+$: 339.13 Da, observed $[M+H]^+$: 339.15 Da. ^{1}H-NMR (500 MHz, CDCl3 δ (ppm) 2.88 (s, 6H), 3.46 (t, J=5Hz, 4H), 3.71(s, 2H), 3.84 (t, J=5Hz, 4H), 7.19(m, J=5Hz, 1H), 7.54 (m, J= 5Hz, 2H), 8.10 (m, J=5Hz, 1H), 8.36 (m, J= 5Hz, 1H), 8.55 (m, J=5Hz, 1H). "#" and "*" represent the residual proton of internal standard tetramethyl silane and $CDCl_3$ respectively.





Scheme S1. Synthesis of Dansyl diethanolamine

Figure S1. LC of dansyl diethanolamine

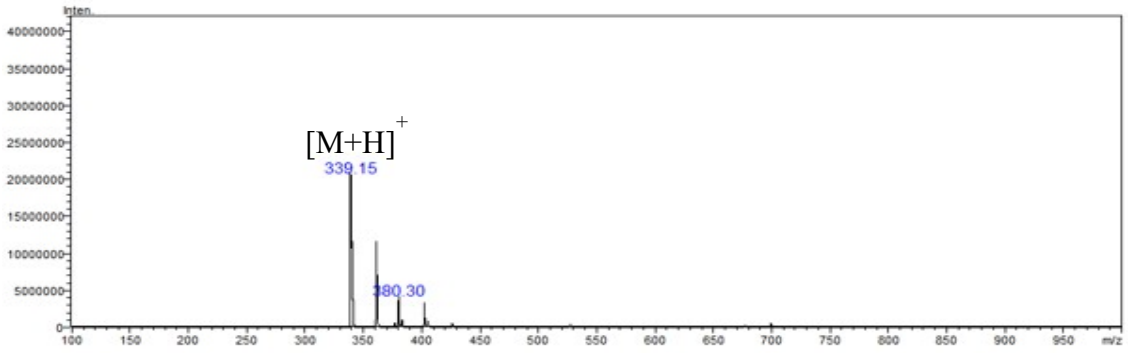


Figure S2. MS Calculated $[M+H]^+$: 339.13 Da Observed $[M+H]^+$: 339.15

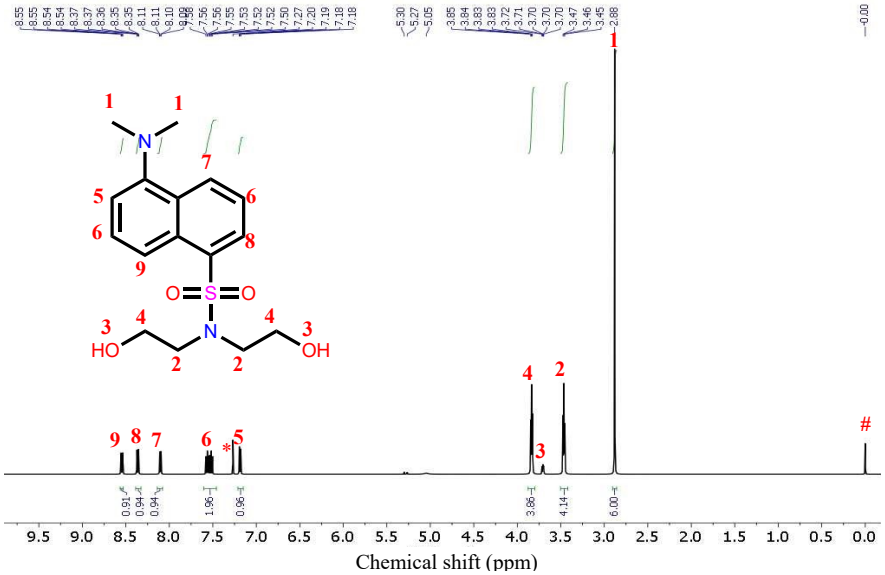
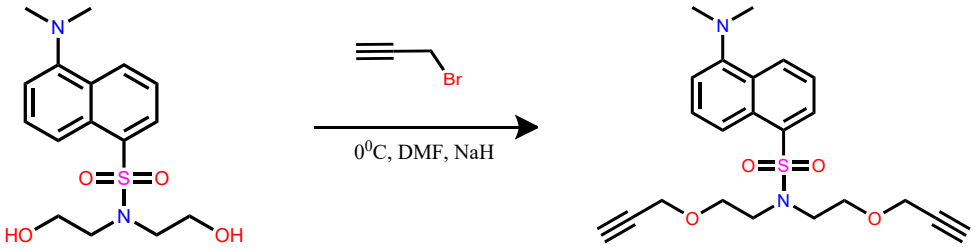


Figure S3. ^{1}H-NMR (500 MHz) of dansyl diethanolamine in $CDCl_3$

2.2. Dansyl diethanolamine dialkyne (**5**)

To dansyl diethanolamine (38 mg, 0.112 mmol, 1 equiv.) 1 mL of N, N'-dimethylformamide (DMF) was added, and kept the solution in an ice bath for 5-10 minutes. Sodium hydride (10.75

mg, 0.448 mmol, 4 equiv.) was added in different parts over the interval of 5 minutes. Then the solution is stirred for 5 minutes. Propargyl bromide (33.3 mg, 21.22 µl, 0.28 mmol, 2.5 equiv.) was added dropwise to this and kept the reaction complete for 3 hours. Completion of the reaction was monitored by TLC. The reaction was quenched with water and extracted with ethyl acetate and water. The pure product was obtained without any further purification. LC-MS calculated $[M+H]^+$: 415.16 Da, observed $[M+H]^+$: 415.25 Da. ^{1}H-NMR (500 MHz, CDCl3 δ (ppm) 2.39 (s, 2H), 2.88 (s, 6H), 3.60(t, J=5Hz, 4H), 3.63 (t, J=5Hz, 4H), 4.04(s, 4H), 7.19 (m, J= 10Hz, 1H), 7.51 (m, J=5Hz, 2H), 8.18(m, J= 5Hz, 1H), 8.31 (m, J=5Hz, 1H), 8.52 (m, J=5Hz,1H). "#" and "*" represent the residual proton of internal standard tetramethyl silane and $CDCl_3$ respectively.



Scheme S2. Synthesis of dansyl diethanol dialkyne

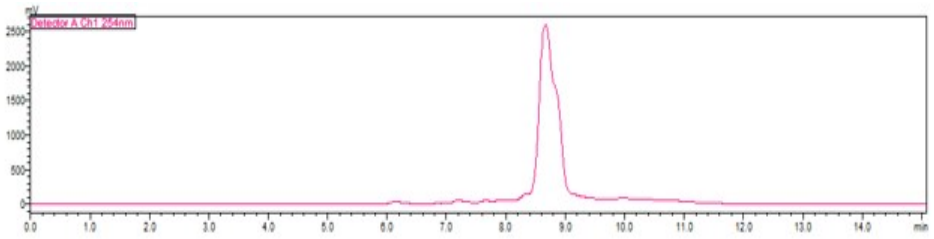


Figure S4. LC Synthesis of dansyl diethanolamine dialkyne

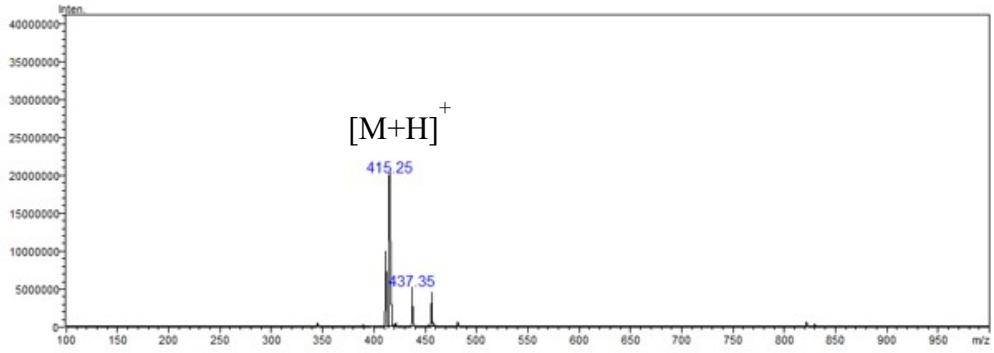


Figure S5. MS Calculated $[M+H]^+$: 415.16 Da Observed $[M+H]^+$: 415.25 Da

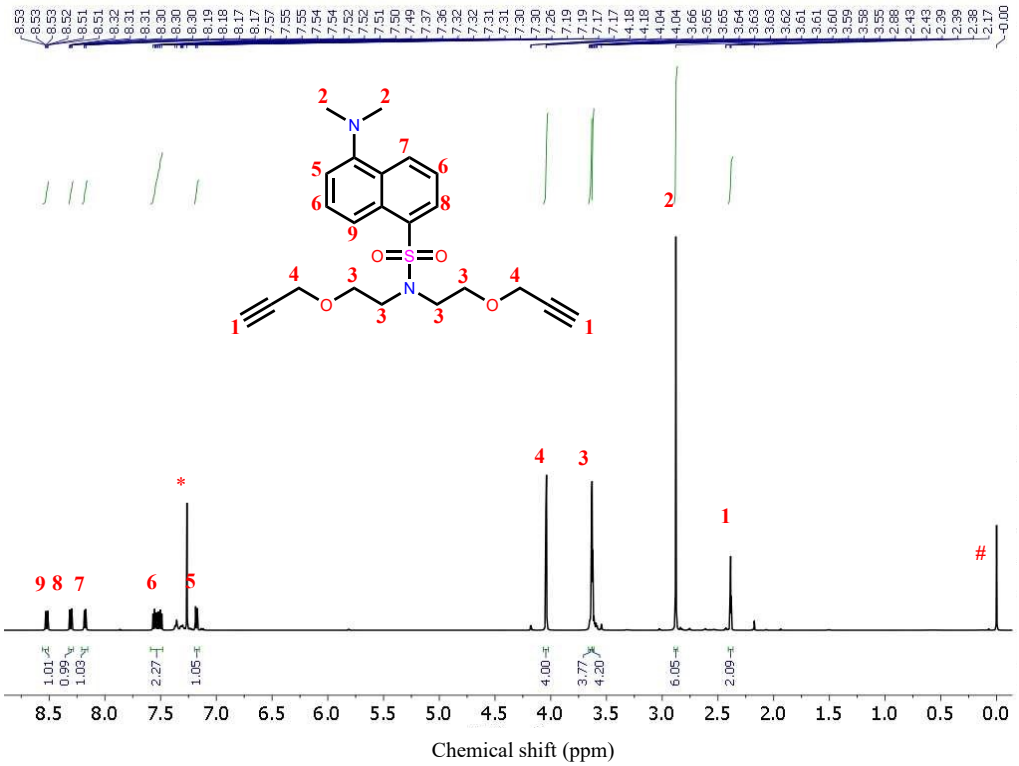
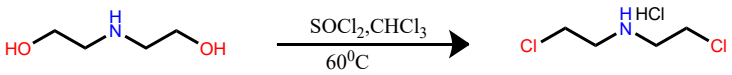


Figure S6. ^{1}H-NMR (500 MHz) of diethanolamine dialkyne in $CDCl_3$

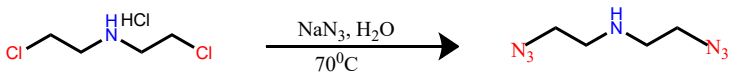
2.3. Diethanolamine diazide (**6**)

Diethanolamine (500 mg, 458 µl, 4.761 mmol, 1 equiv.) was chlorinated by using thionyl chloride (2.2g, 1.37 mL, 19.044 mmol, 4 equiv.). To the diethanolamine 10 mL of chloroform was added and kept in an oil bath (60 ^{0}C). After that 1.37 mL of thionyl chloride was added. At that time a white-colored solution was formed. Then 100 µl N, N' dimethylformamide was added and the reaction mixture was refluxed for 30 minutes. Chloroform was evaporated and got a solid mass as the product.

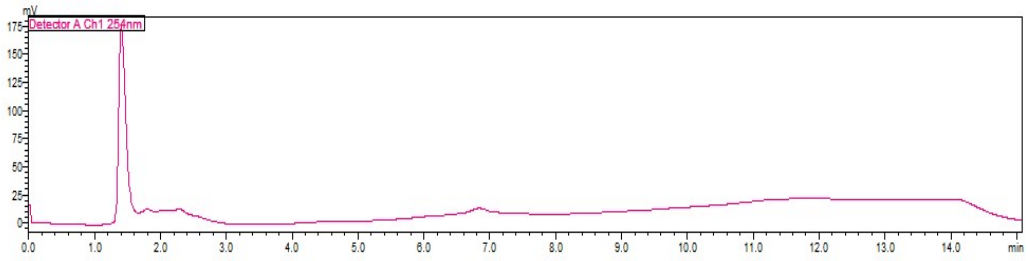


Scheme S3. Synthesis of dichlorodiethylamine

The dichlorodiethylamine (0.89g, 4.9893 mmol, 1 equiv.) was dissolved in 18 mL of water. To this sodium azide was added and refluxed at 70^{0}C for 12 hours. The completion of the reaction was checked with TLC. The product formation was confirmed by a spot visible in iodine.



Scheme S4. Synthesis of diethanolamine diazide



Retention time (min)

Figure S7. LC of diethanolamine diazide

$[M+H]^+$

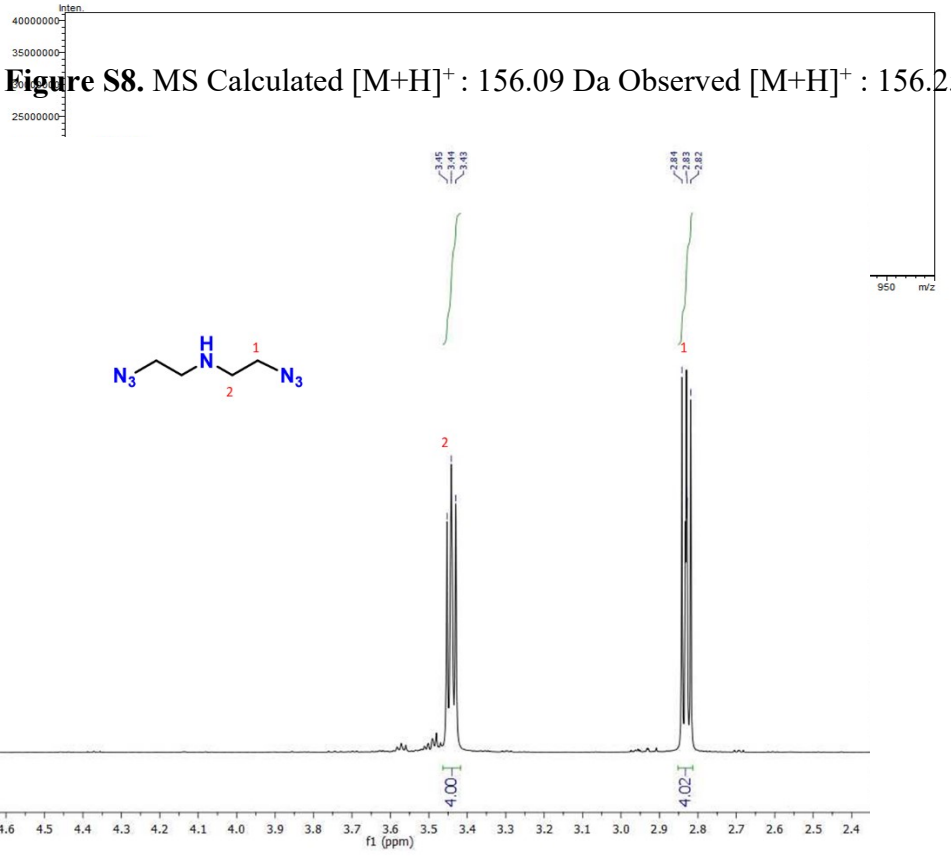
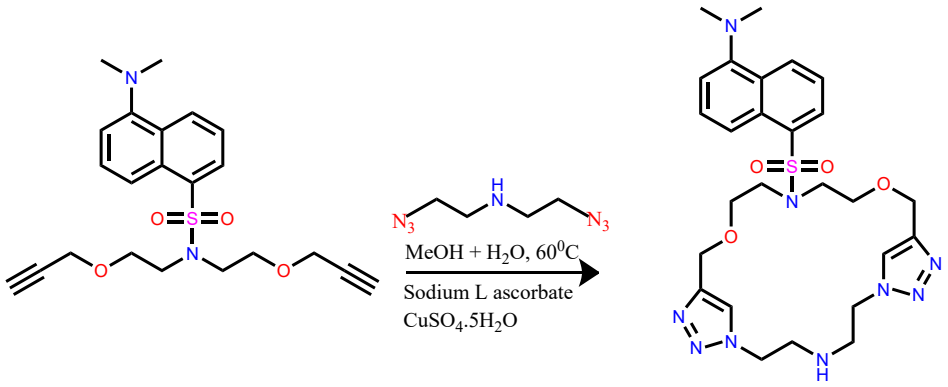


Figure S8. MS Calculated $[M+H]^+$: 156.09 Da Observed $[M+H]^+$: 156.25 Da

Figure S9. ^{1}H-NMR (500 MHz) of diethanolamine diazide in $CDCl_3$: δ (ppm) 2.83 (t, 3H), 3.44 (t, 3H)

2.4. Dansyl – triazole macrocycle (DTMC)

To the diethanolamine alkyne (30 mg, 0.072 mmol, 1 equiv.) diethanolamine diazide (11.16 mg, 0.172 mmol, 1 equiv.) was added. Sodium L ascorbate (57.45 mg, 0.36 mmol, 5 equiv.) was used as the catalyst and $CuSO_4.5H_2O$ was used as the reducing agent for this reaction and was added to the mixture. Methanol and water were added in a 1:1 ratio and kept the reaction in an oil bath (60^0C) for 30 minutes. Completion of the reaction was monitored by TLC and the pure macrocycle was obtained after HPLC purification. ^{1}H-NMR (500 MHz, CDCl3 δ (ppm) 2.64 (s, 1H), 2.88 (s, 6H), 3.10 (t, 4H), 3.54 (t, J=10Hz, 4H), 3.62 (t, J=5Hz, 4H), 4.38 (t, J= 5Hz, 4H), 4.60 (s, 4H), 7.18 (m, J= 10Hz, 1H), 7.48 (s, 2H), 7.54 (m, J= 5Hz, 2H), 8.12 (m, J= 5Hz, 1H), 8.31 (m, J= 5Hz, 1H), 8.53 (m, J= 5Hz, 1H) "#" and "*" represent the residual proton of internal standard tetramethyl silane and $CDCl_3$ respectively. ^{13}C NMR (126 MHz, CDCl3) δ 151.81, 134.69, 130.39, 130.09, 129.26, 128.18, 123.56, 123.17, 119.41, 115.27, 69.94, 64.46, 50.57, 48.91, 45.44. LC-MS calculated $[M+H]^+$: 570.25 Da, observed $[M+H]^+$: 570.40 Da. IR (ATR) υ~ = 3442 cm^{-1}(stretching, N-H), 2929 cm^{-1} (stretching, alkane C-H), 2102 cm^{-1} (stretching, N=N-N), 1705 cm^{-1}(stretching, C=C).



Scheme S5. Synthesis of DTMC

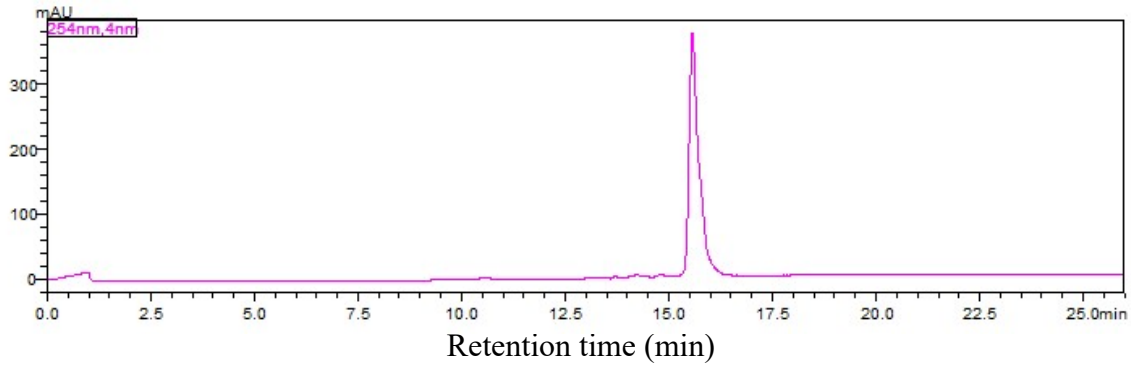


Figure S10. LC of DTMC

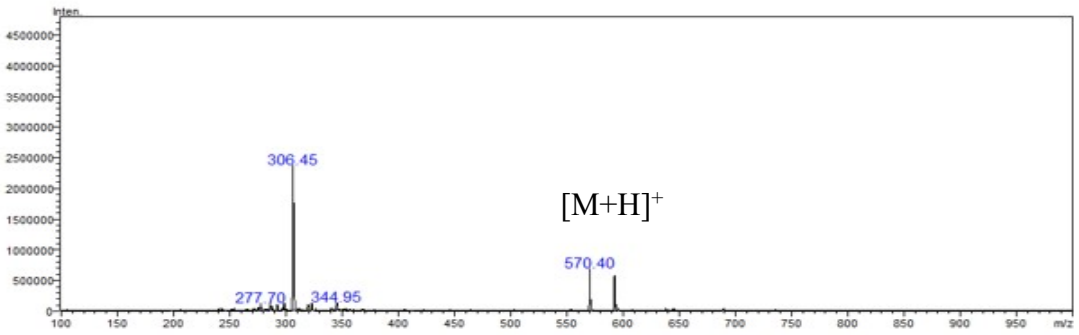


Figure S11. MS Calculated $[M+H]^+$: 570.25 Da Observed $[M+H]^+$: 570.40 Da

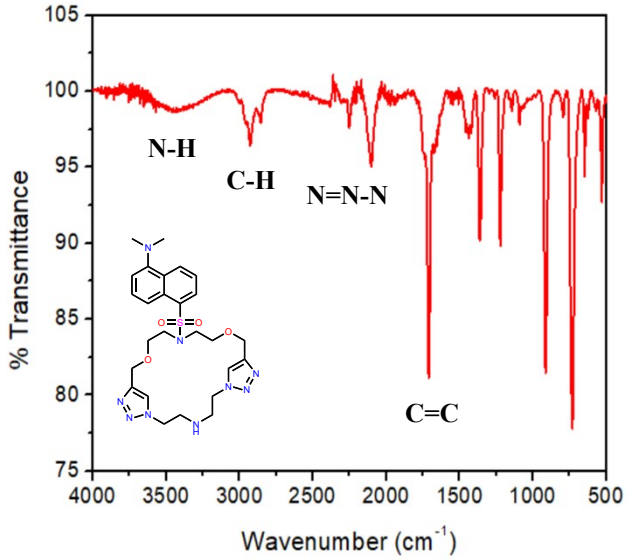


Figure S12. FT-IR of DTMC

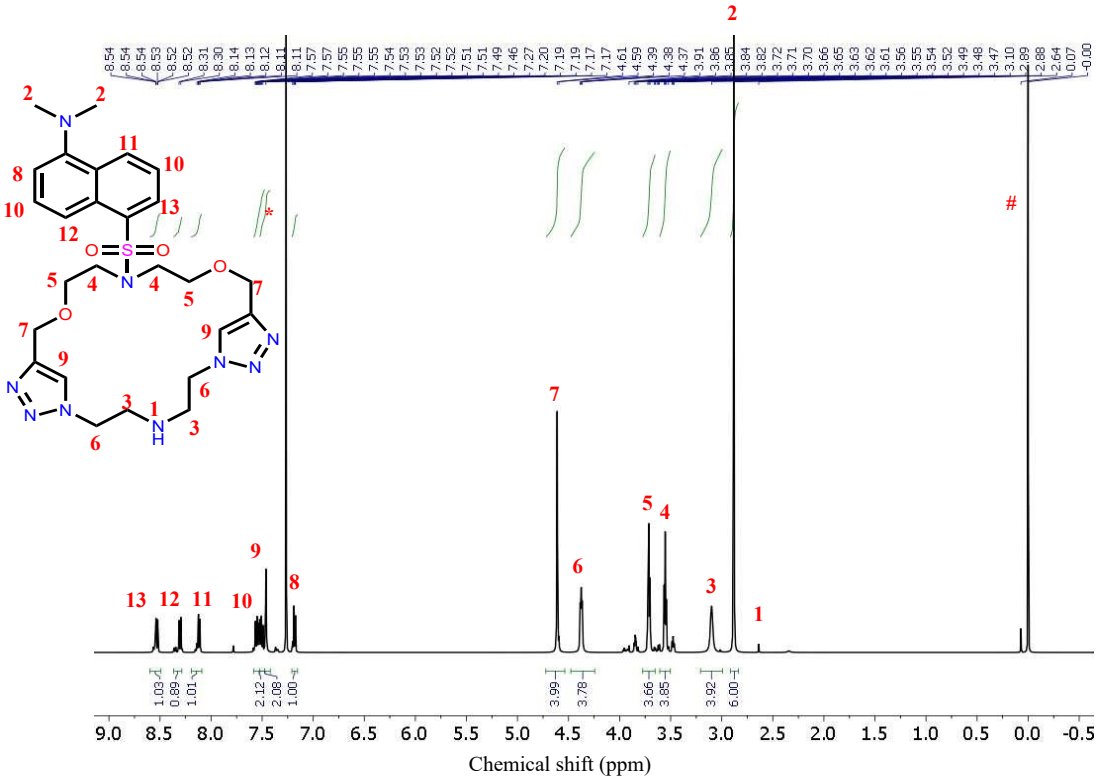


Figure S13. ^{1}H-NMR (500 MHz) of DTMC in $CDCl_3$

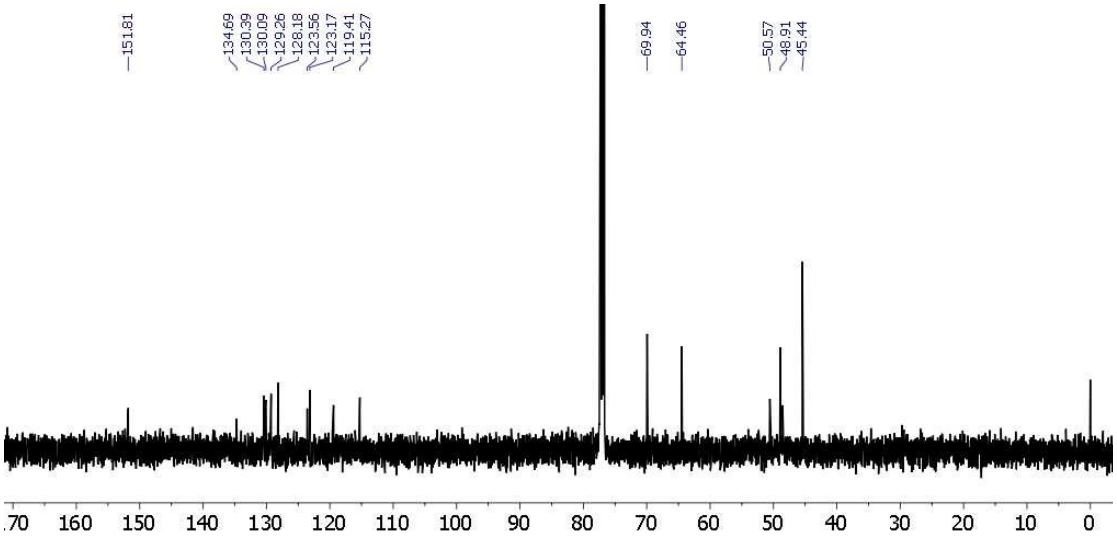


Figure S14. ^{13}C-NMR of DTMC in $CDCl_3$

3. Photophysical studies

3.1. Excitation-emission spectrum

A stock solution of 2 mM of DTMC was prepared in DMSO and diluted with water to get the required concentration to check the emission for all the cases at 25 °C. First, the absorption spectrum was taken with 10 µM of DTMC solution in a UV-visible spectrophotometer. The emission spectrum was taken from the maximum wavelength obtained at an excitation

wavelength of 330 nm. And, the excitation spectrum was taken at the emission wavelength at 560 nm.

3.2. Solvatochromism

For the photograph, 1 mg/mL of DTMC solution was prepared in different solvents like hexane, toluene, chloroform, acetone, and DMSO. And the vial with these solutions are visualized under UV lamp. The prepared solutions were diluted in same solvent to make a 10 µM solution for the fluorescence spectrum recorded at the excitation wavelength 330 nm at 25°C.

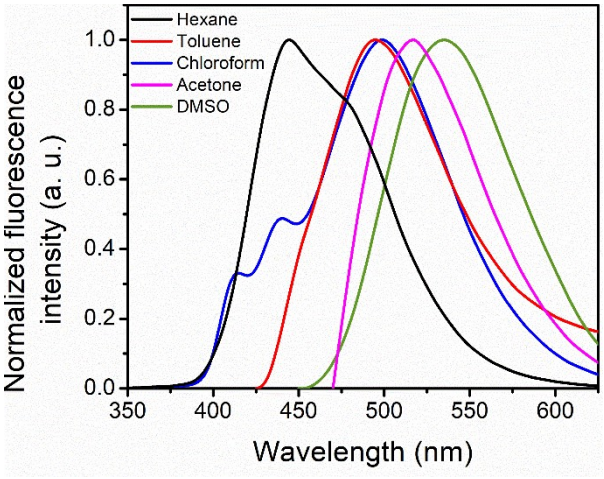


Figure S15. Fluorescence emission of DTMC at 25°C in various solvents like hexane, toluene, chloroform, acetone, and DMSO recorded at an excitation wavelength of 330 nm.

3.3. Quantum yield calculation

The quantum yield was calculated using the following equation:

$$Q_S = Q_R \frac{I_S A_R \eta_S^2}{I_R A_S \eta_R^2}$$

Where Q_S and Q_R represent the quantum yield of the sample and the reference respectively. I_S and I_R represents the integrated area under the emission spectra of sample and reference, A_R and A_S represents the absorption of reference and sample and η represents the refractive index of the medium. Using quinine sulphide as the reference, the quantum yield of the macrocycle was estimated using the reference technique. The reference quinine sulphide has a quantum of 0.54. The integrated area under the curve for the quinine sulphide and macrocycle by using emission spectra are 2.103×10^6 and 9.110×10^7 respectively. The absorbance of quinine sulphide and DTMC was calculated are 0.14 and 0.1064 respectively. By using the above equation, the quantum yield of the macrocycle is 0.308.

(a) (b)

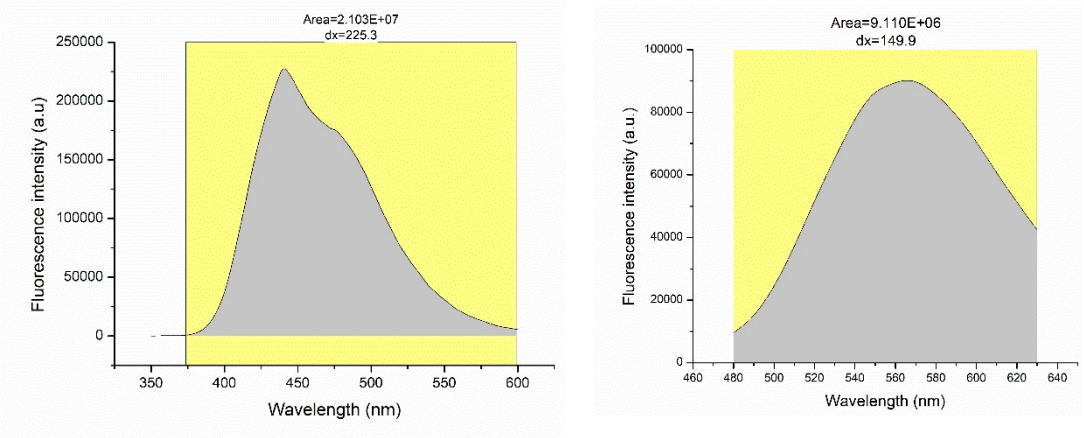
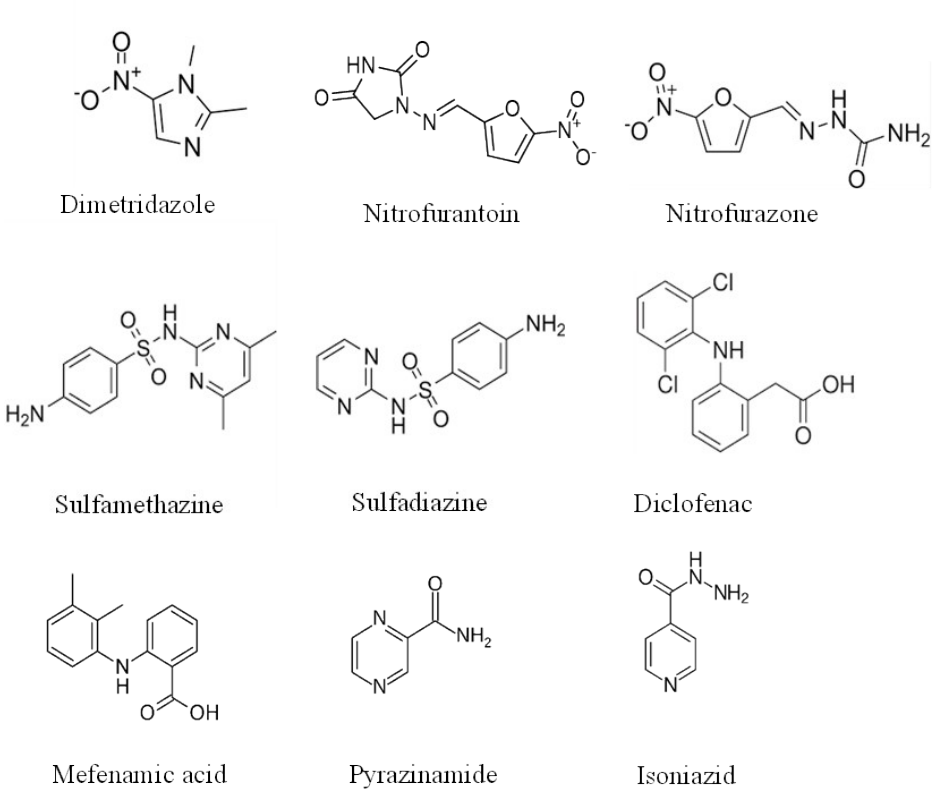


Figure S16. The integrated area under the curve for the (a) quinine sulphide (b) DTMC

4. Sensing of pharmaceuticals

4.1. Selectivity studies

The fluorescence spectrum of 10 µM of DTMC prepared in water from a 5 mM stock solution in DMSO was taken for this study. 5 mM of drugs solutions of dimetridazole (DMI), nitrofurantoin (NFT), nitrofurazone (NFZ), sulfamethazine (SMZ), sulfadiazine (SDZ), diclofenac (DCL), mefenamic acid (MFA), pyrazinamide (PZA), and isoniazid (INZ), and 5 mM of NaCl, KCl, $MgSO_4$, $NaHCO_3$, glucose, urea, and creatinine were prepared in water and added the required volume of these solutions to the cuvette. The bar diagram was plotted with 10 µM of DTMC and 200 µM of the drug solution. For the photograph, a 100 µM solution of



the DTMC and 1 mM of the analytes were used. The structures of the drugs used for the study are given below.

Figure S17. The chemical structure of pharmaceutical drugs used for the selectivity study

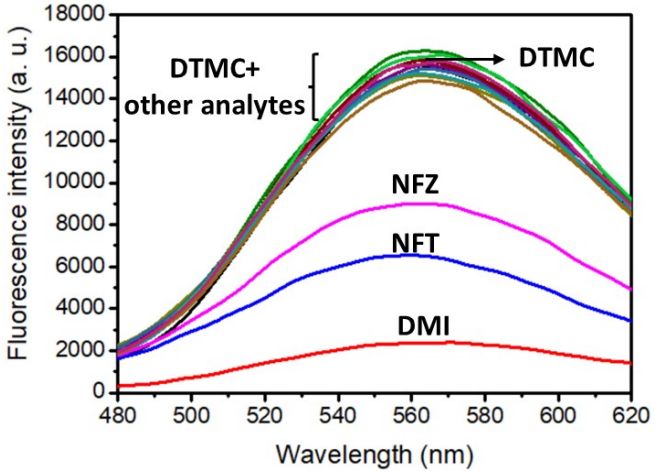


Figure S18. The selectivity in fluorescence quenching of DTMC (control: 10 µM in 0.2 % DMSO in water) and in presence of various analytes in water (200 µM) (a) fluorescence spectrum

4.2. Competitivity studies

The fluorescence spectrum of 10 µM of DTMC prepared in water from a 5 mM stock solution in DMSO was taken for this study. To this, 200 µM of competing analytes were added separately and measured the emission. Fluorescence was measured by adding 200 µM of DMI, NFT, and SMZ on separate experiments.

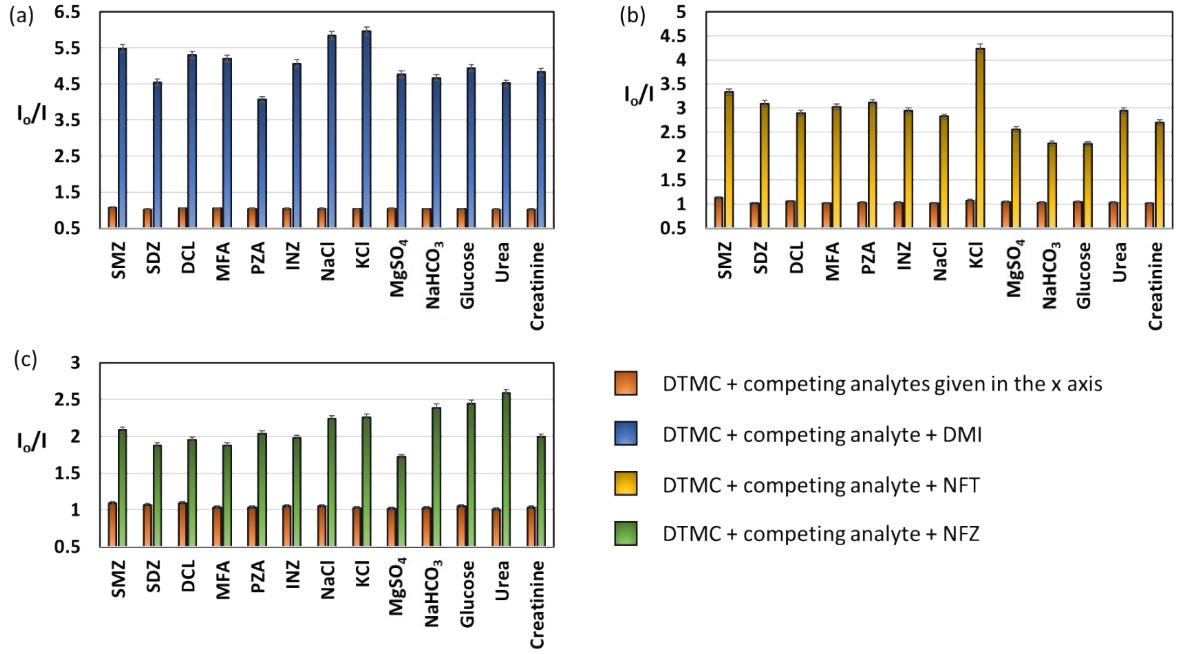


Figure S19. The competitivity studies in fluorescence quenching of DTMC (10 µM in 0.2 % DMSO and water) in presence of 200 µM of the analyte given in x axis and 200 µM of (i) DMI (ii) NFT and (iii) NFZ

4.3. Stern-Volmer plot

To a 10 µM of DTMC, the drugs solutions of DMI, NFT, and NFZ was added separately in a wide range of concentrations. The plot of concentration of drug versus I_o/I resulted in a non-linear curve which fitted well with exponential equation of Stern-Volmer equation given below:

$$I_o/I = A\, e^{k[Q]} + B,$$

where A, B, and k are the constants and [Q] is the concentration of the quencher/drug, and the Stern-Volmer quenching constant was calculated from the multiplication of A and k (Wei, W.; Lu, R.; Tang, S.; Liu, X., *J. Mater. Chem. A,* **2015**, 3, 4604−4611; R. Das.; S. Bej.; H. Hirani.; P. Banerjee., *ACS Omega*, **2021**, 6, 14104−14121). The Stern-Volmer quenching constant calculated for the addition of DMI, NFT and NFZ to DTMC are 13.4 (±1.18) x10^3 M^{-1}, 5.74 (±0.26) x10^3 M^{-1} and 4.57 (±0.25) x10^3 M^{-1} respectively. The errors are calculated by the standard deviations from the replica experiments.

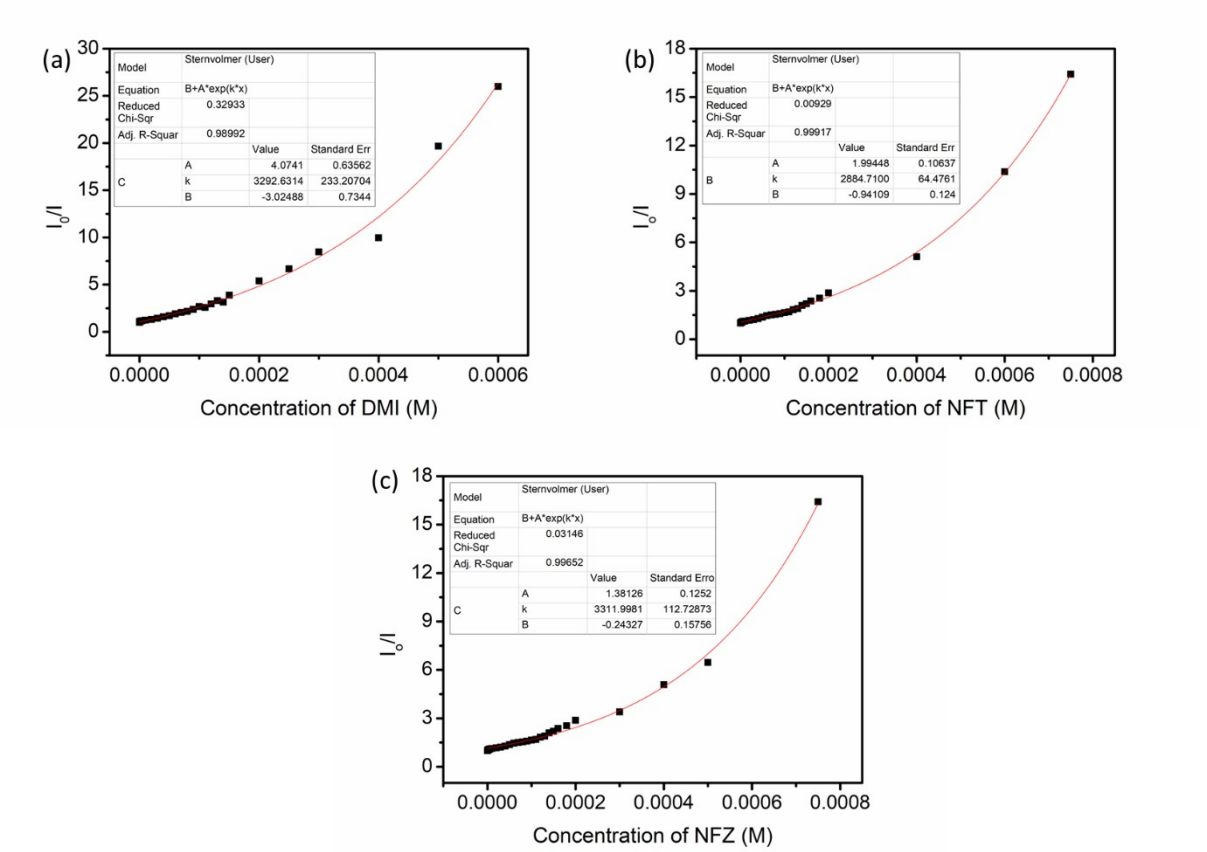


Figure S20. Stern-Volmer plots of DTMC upon the addition of (a) DMI, (b) NFT, and (c) NFZ

4.4. Sensing on paper strip

Whatman 1 filter paper was cut into three thin strips around 5 cm in length. The solution of dansyl-triazole macrocycle in dimethyl sulfoxide (5 mM, 5 µL) was coated at the two ends of the paper strip. After one hour of drying at room temperature, nitroaromatic drug solutions like dimetridazole, nitrofurantoin, and nitrofurazone in water (5 mM, 20 µL) were added on one end of the three paper strips into the macrocycle coated area. After another hour of drying, the strip was examined using a UV lamp. The strip coated with the sensor showed bright green colour and upon the addition of nitro-containing aromatic drugs quenching of the colour was observed.

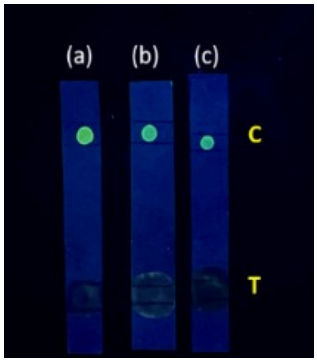


Figure S21. Photograph of paper strips with DTMC (5 µL of 5 mM solution in DMSO) dropped as the control (C) and the drugs (20 µL of 5 mM solution in water) (a) DMI, (b) NFT, and (c) NFZ added on the top of DMCT marked as test (T)

The stability of the paper strip sensor was confirmed by monitoring the change in the paper strip after 10 days.

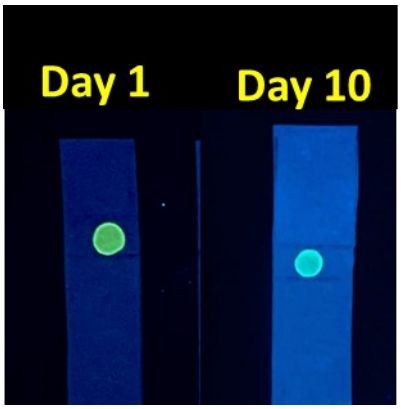


Figure S22. The digital photograph of the paper strip sensor on Day 1 and Day 10

4.5. Sensing of real samples

5 mM drug solutions of DMI, NFT, and NFZ were prepared in different samples like lake water, tap water, HSA (10 µM in phosphate buffer of pH=7.4) and milk (diluted 10 times). The fluorescence was recorded with 10 µM of DTMC and 200 µM of the drug solution at the excitation wavelength 330 nm at 25 °C.

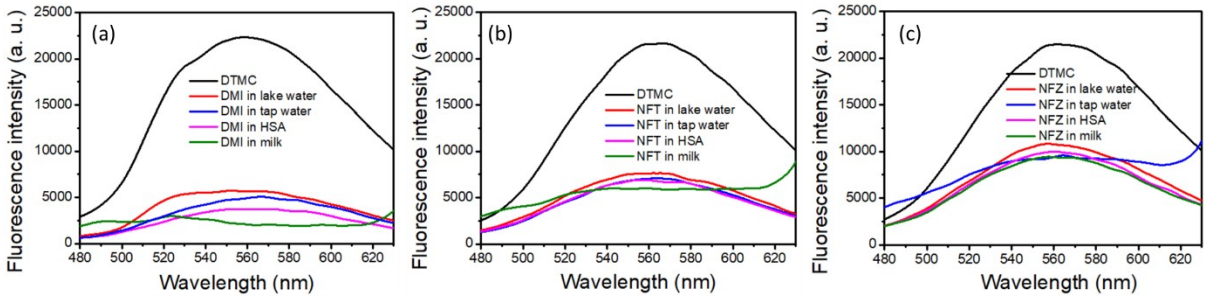


Figure S23. Fluorescence spectrum of 10 µM of DTMC upon the addition of 200 µM of (a) DMI, (b) NFT, and (c) NFZ spiked in various samples like lake water, tap water, HSA and milk recorded at an excitation wavelength 330 nm at 25 °C

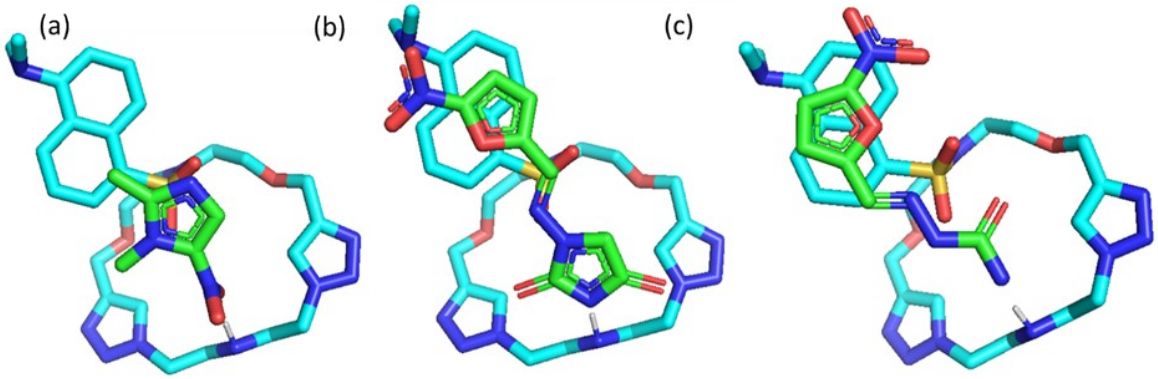


Figure S24. The optimized structure of DTMC (cyan backbone structure) bound with the drugs (green backbone structure) (a) DMI, (b) NFT, and (c) NFZ. The structure was created using molecular docking by Autodock Vina and visualized in PyMol.

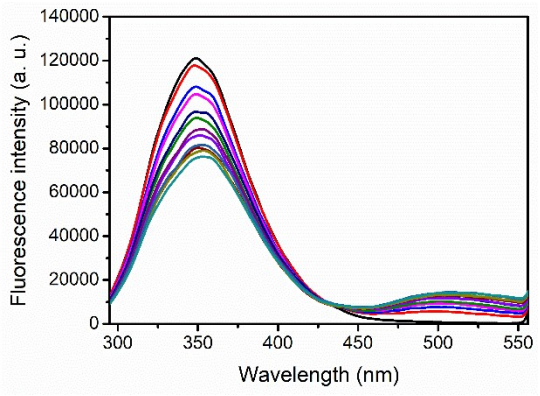
4.6. Sensing of other nitro-containing compounds

5 mM solutions of various nitro-containing compounds 2-chloro-6-nitrobenzothiazole (CNB), 5-nitroisoquinoline (NIQ), 2-amino-6-nitro-benzothiazole (ANB), 2-mercapto-5-nitrobenzimidazole (MNB), and 2-nitrothiophene (NTP) were prepared in DMSO. To a 10 µM solution of DTMC, a required volume of these solutions were added individually to obtain the total concentration of 200 µM. The fluorescence spectrum were recorded in excitation wavelength 330 nm at 25 ^{o}C.

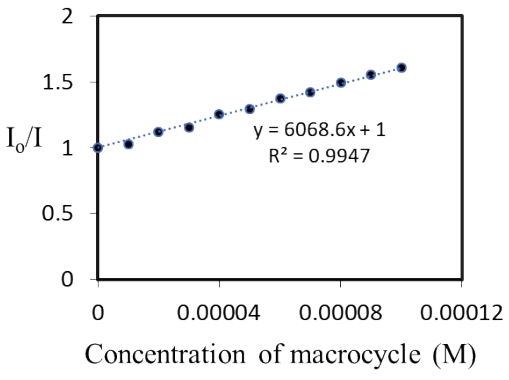
5. Interaction of DTMC with multiple proteins

5.1. Fluorescence spectroscopy

10 µM stock solutions of bovine serum albumin (BSA), human serum albumin (HSA), lysozyme, proteinase and trypsin were prepared in phosphate buffer of pH = 7.4. From the stock solution of DTMC (2 mM), required volume was added to the protein to make the conctrantions ranging from 2 µM to 100 µM. The total volume of DMSO in the mixture was kept below 2 %. The fluorescence were recorded in 280 nm excitation wavelength at 25 ^{o}C. Stern-Volmer plot corresponding the experiment was also plotted



(a)



(b)

Figure S25. (a) Fluorescence spectrum of macrocycle on interaction with BSA recorded at excitation wavelength 280 nm. (b)The plot of I_o/I vs concentration of macrocycle. The slope of the graph is attributed as quenching constant, which is 6.068×10^3 M^{-1}.

(a) (b)

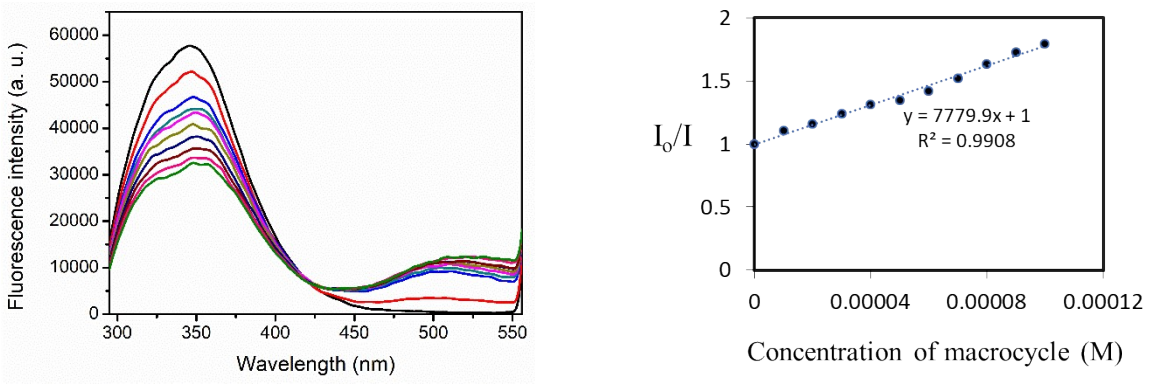
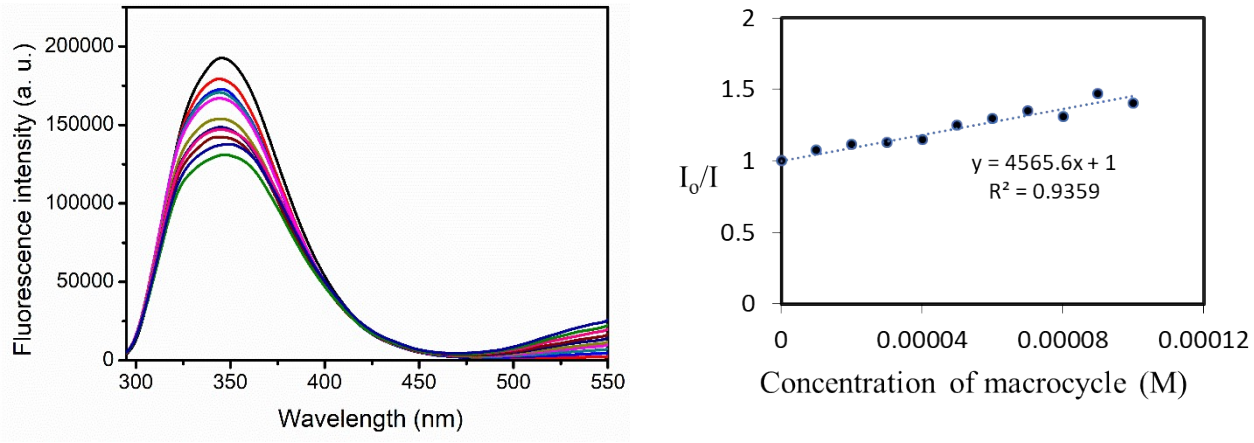
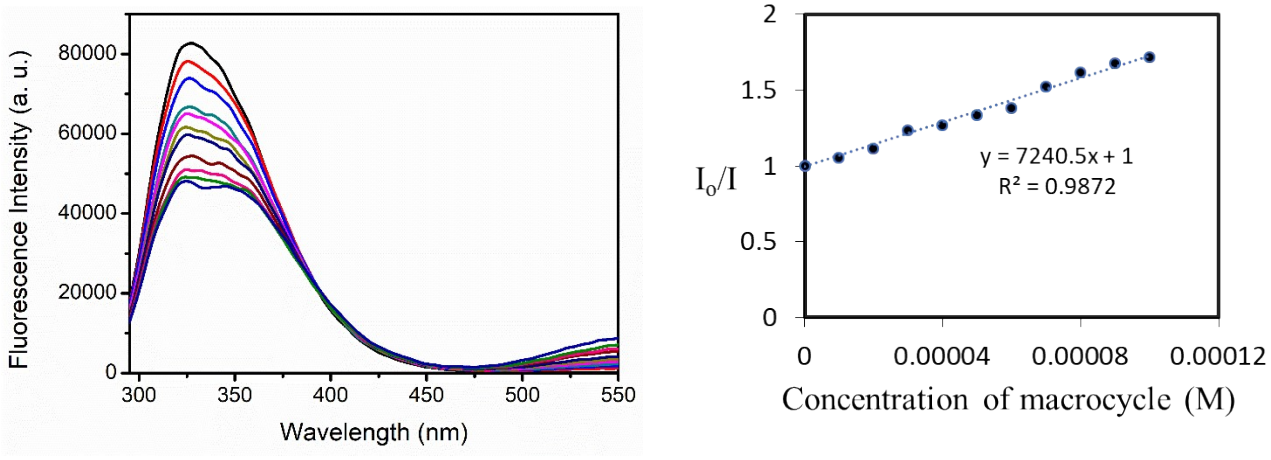


Figure S26. (a) Fluorescence spectrum of macrocycle on interaction with HSA recorded at excitation wavelength 280 nm. (b) The plot of I_o/I vs concentration of macrocycle. The slope of the graph is attributed as quenching constant, which is $7.779 \times 10^3\ M^{-1}$.



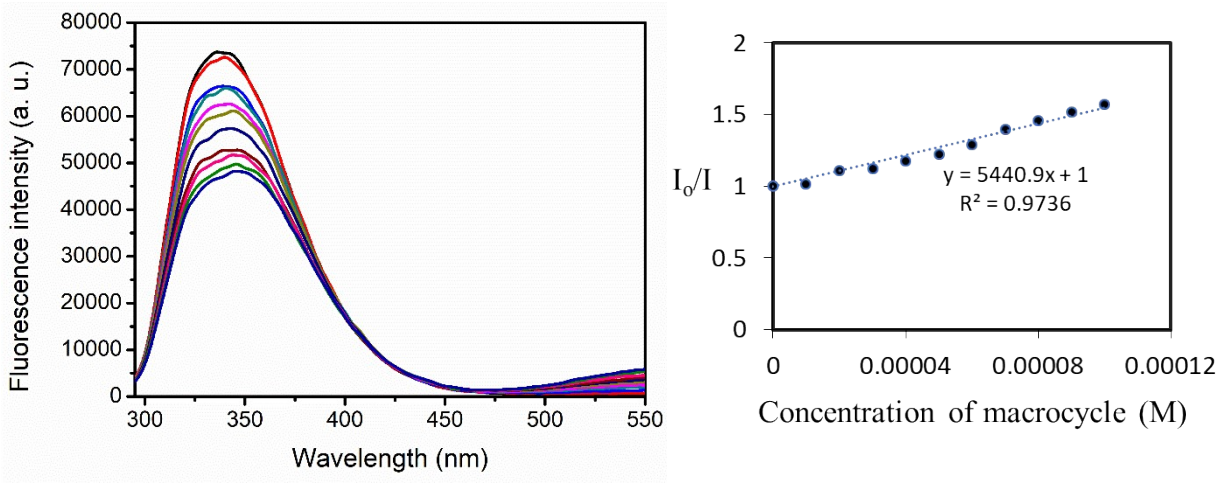
(a) (b)

Figure S27. (a) Fluorescence spectrum of macrocycle on interaction with lysozyme recorded at excitation wavelength 280 nm. (b) The plot of I_o/I vs concentration of macrocycle. The slope of the graph is attributed as quenching constant, which is $4.565 \times 10^3\ M^{-1}$.



(a) (b)

Figure S28. (a) Fluorescence spectrum of macrocycle on interaction with proteinase recorded at excitation wavelength 280 nm. (b) The plot of I_o/I vs concentration of macrocycle. The slope of the graph is attributed as quenching constant, which is $7.240 \times 10^3\ M^{-1}$.



(a) (b)

Figure S29. (a) Fluorescence spectrum of macrocycle on interaction with trypsin recorded at excitation wavelength 280 nm. (b) The plot of I_o/I vs concentration of macrocycle. The slope of the graph is attributed as quenching constant, which is $5.440 \times 10^3\ M^{-1}$.

5.2. Molecular docking studies

Table S1. Binding energies of protein-DTMC complexes. The PDB ID of the proteins used were added in the parentheses.

Protein (PDB ID)	Binding energy (kcal/mol)
BSA (4f5v)	-9.9
HSA (1bm0)	-10.8
Lysozyme (2lyz)	-7.9
Proteinase (3vw7)	-8.3
Trypsin (2ptn)	-8.1

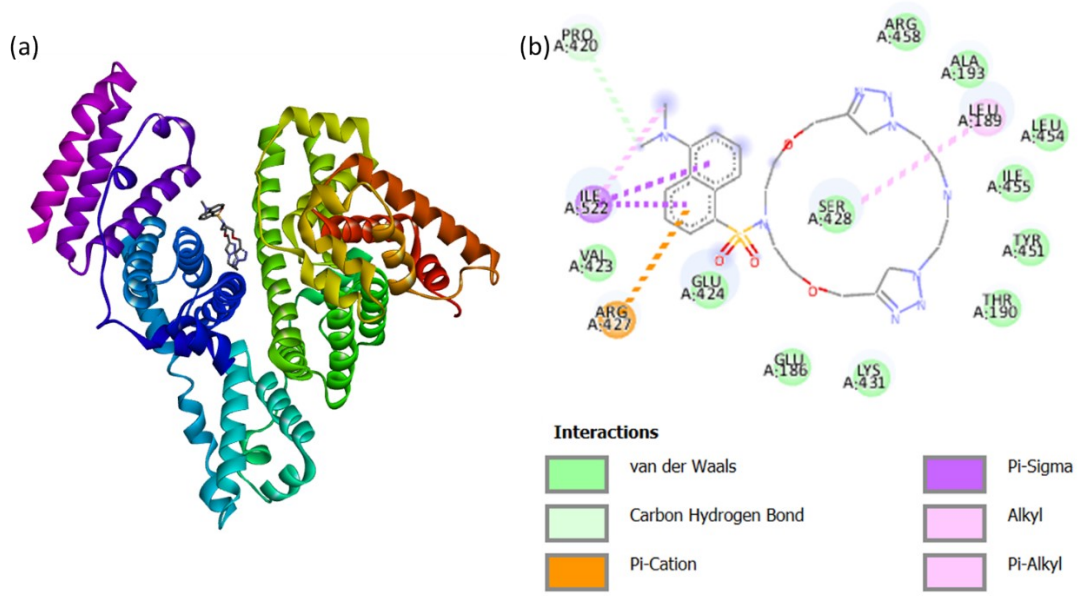


Figure S30. (a) Docked structure of DTMC with BSA (b) 2D plot showing the interaction of DTMC with BSA

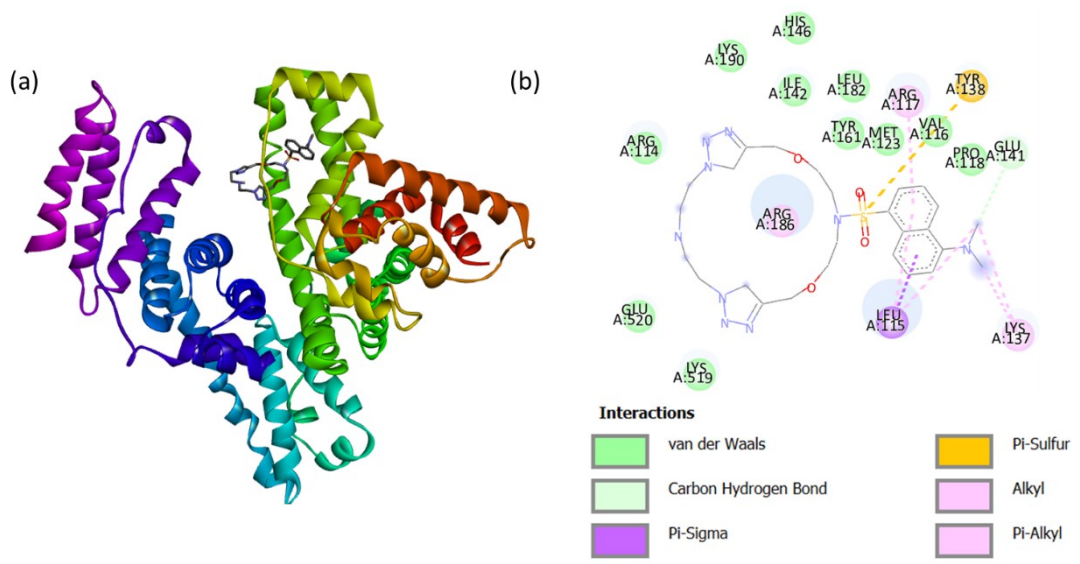


Figure S31. (a) Docked structure of DTMC with HSA (b) 2D plot showing the interaction of DTMC with HSA

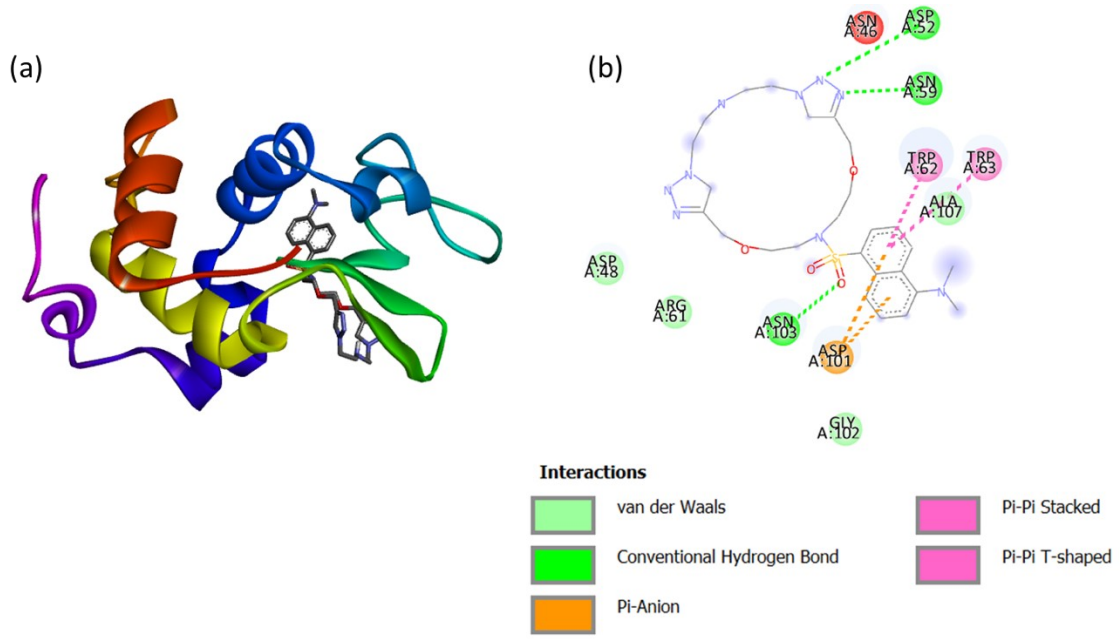


Figure S32. (a) Docked structure of DTMC with lysozyme (b) 2D plot showing the interaction of DTMC with lysozyme

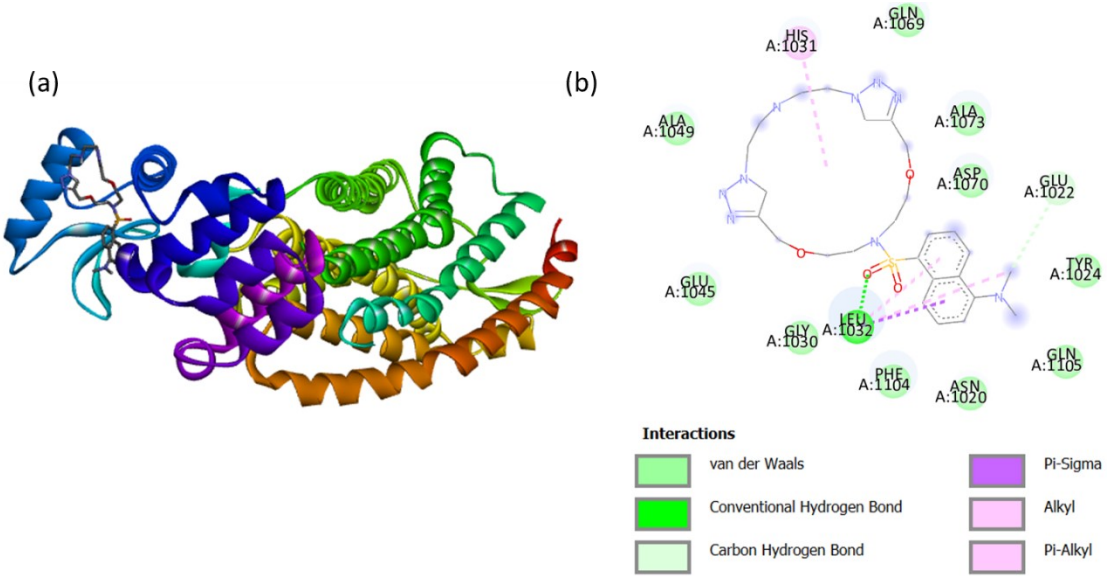


Figure S33. (a) Docked structure of DTMC with proteinase (b) 2D plot showing the interaction of DTMC with proteinase

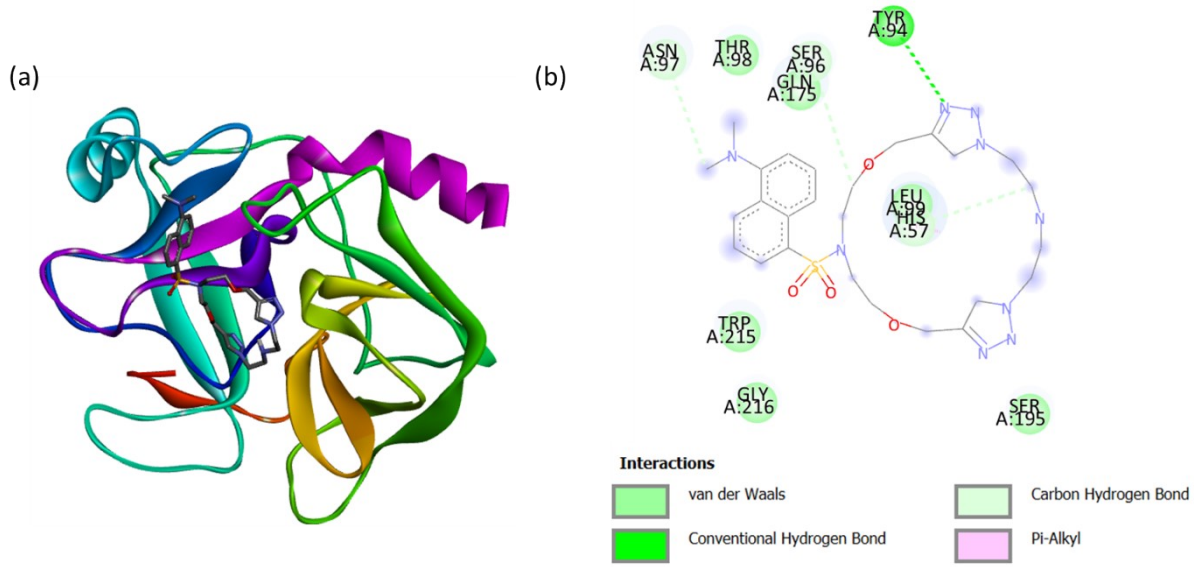


Figure S34. (a) Docked structure of DTMC with trypsin (b) 2D plot showing the interaction of DTMC with trypsin