Supporting Information for

Can Conformational Flexibility Influence the Self-Assembly Behavior and Sensing Efficacy of Fluorogenic Amphiphiles? A Case Study with Bisbenzimidazole based Probes

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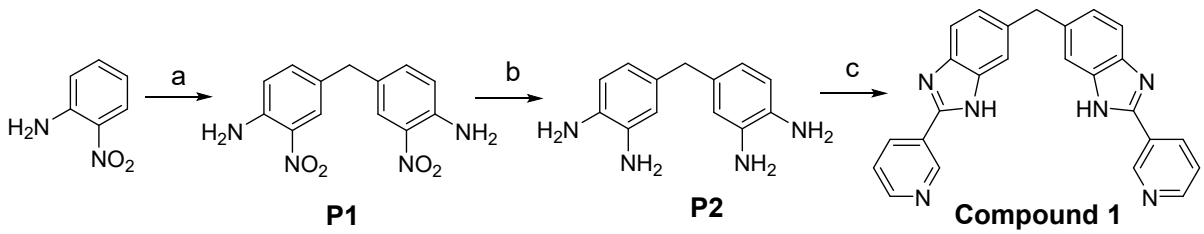
Synthesis and Characterization of Compound 1



Reaction conditions. (a) HCHO, HCl, reflux, 5 h (b) Zn powder, NaOH, reflux. (c) 3-pyridine aldehyde, dry nitrobenzene, 140 °C.

Compounds **P1 and P2** have been synthesized following the procedure reported in the literature.

Compound 1. 300 mg (2.8 mmol, 2 eq.) of 3-pyridinecarbaxaldehyde was dissolved in 20 ml 0f ethanol and to it 264 mg $Na_2S_2O_5$, dissolved in water, was added, and stirred for 15 minute and to this 320 mg (1.4mmol, 1 eq.) of compound **P2** was added and refluxed for 24 h. The pure product was isolated by neutral alumina column chromatography with 2.5-3.5 % MeOH/$CHCl_3$ as eluent.

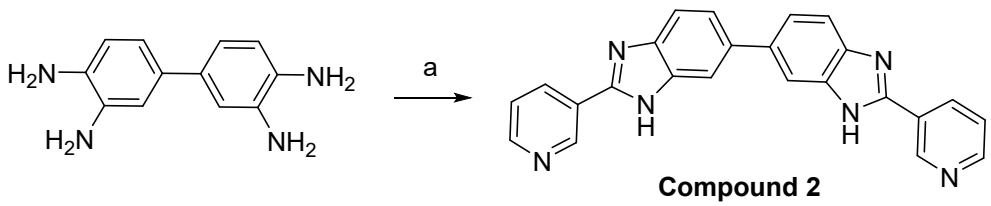
^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm: 12.99 (s, 1H, NH); 12.93 (s, 1H, NH); 9.30 (d, *J* = 4.4 Hz, 2H); 8.65 (d, *J* = 4.4 Hz, 2H); 8.44 (m, 2H); 7.57 (m, 4H); 7.47 (dd, *J* = 4, 8.2, 1H); 7.4 (s, 2H); 7.15 (t, 2H); 4.19 (t, 2H).

^{13}C-NMR (DMSO-d_6, 400 MHz): δ ppm: 150.8, 149.2, 147.8, 144.4, 142.5, 137.4, 135.4, 134.0, 126.6, 124.4, 119.2, 111.6, 41.8.

HRMS: m/z= 425.1492 $[M+Na]^+$ (Calcd. : 425.1491)

Ref: S. Mondal and N. Dey*, Eur J Inorg Chem., **2023,** 26, e202300147

Synthesis and Characterization of Compound 2



Reaction conditions. (a) 3-pyridine aldehyde, dry nitrobenzene, 120 °C, 8-10 h.

2,2'-di(pyridin-3-yl)-3H,3'H-5,5'-bibenzo[d]imidazole (Compound 2). 3-pyridine aldehyde (2.5 eq.) was dissolved in nitrobenzene (5 mL). 3,3'-Diaminobenzidine (1 eq.) was then added. The solution was heated at 120 ^{o}C for 8–10 h. Once all the 3,3′-diaminobenzidine had reacted, the reaction mixture was cooled to room temperature. Petroleum ether was added to precipitate the crude solid. The solvent was decanted off. This process was repeated several times until almost all the nitrobenzene was removed. The compounds were purified by column chromatography over silica gel (mesh 60–120). The pure product was obtained as a yellow powdery solid (R_f = 0.4 in 6% MeOH in $CHCl_3$ on TLC plate coated with silica GF254). The compound was further purified by dissolving it in $CHCl_3$/MeOH mixture and precipitating it with petroleum ether. The pure compounds were obtained in 65–70% yield.

1H NMR (300 MHz, DMSO-d_6, 25 ^{o}C, TMS): d=9.3 (s, 2H), 8.67 (d, J=5.1 Hz, 2H), 8.55 (d, J=8.3 Hz, 2H), 7.83 (s, 2H), 7.67–7.53 ppm (m, 6H); HRMS: m/z calcd: 389.1514 $[M+H]^+$; found:389.1523.

Ref: Bhattacharya S.; Chaudhuri P. Metal-Ion-Mediated Tuning of Duplex DNA Binding by Bis (2-(2-pyridyl)-1H-benzimidazole). Chem. Asian J. **2007**, 2(5), 648-55.

Experimental Details

Materials and Methods. All necessary chemicals (precursors, starting materials, reagents, and solvents) were purchased from best-known suppliers and used as received. FT-IR spectra were recorded on a PerkinElmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). ^1H NMR spectra were recorded on a Bruker-400 Advance NMR spectrometer. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane. Mass spectra were recorded on a Micromass Q-TOF Micro TM spectrometer.

Sampling Procedure of Sensing. The sensing studies with metal ions (added in the forms of nitrate and perchlorate salts in water and acetonitrile medium respectively) was carried out by adding 10 μL DMSO solution of **1** and **2** from stock (1×10^{-3} M) in acetonitrile or water medium to make the final volume of 1 mL (conc. = 1×10^{-5} M) followed by addition of DMSO or water solutions of metal ions respectively. In this case, the final concentration of DMSO in the solution did not exceed 1%.

UV−Vis and Fluorescence Spectroscopy. The UV−vis and fluorescence spectroscopy were recorded on a Shimadzu model 2100 spectrometer and Cary Eclipse spectrofluorimeter respectively. The slit-width for the fluorescence experiment was kept at 5 nm (excitation) and 5 nm (emission) and the excitation wavelength was set at 320 nm.

Dynamic Light Scattering Studies (DLS). DLS measurements were done using a Malvern Zetasizer NanoZS particle sizer (Malvern Instruments Inc., MA) instrument. Samples (**1**) were prepared and examined under dust free conditions. Reported mean hydrodynamic diameters were obtained from Gaussian analysis of the intensity weighted particle size distributions.

Detection limit determination. The method used for the calculation of the detection limit is known as the blank variation method. In this method, the calibration curve was prepared by fluorescence titration of **1** (10 μM) with Hg^{2+} ion in Water medium. The fluorescence signals of the compound without the added Hg^{2+} were considered as blank reading. The standard deviation value was calculated from the blank readings and fluorescence titration data. Using this standard deviation value, we calculated limit of decision by this following equation.

$L_C = t_C \times s \times (1 + 1/N)^{1/2}$…….…...…….. (1)

where, N = the number of blank replicates taken; the value of tc for 10 blank readings is 1.833; and s = the standard deviation value. The detection limit (L_D) was calculated as the double of the decision limit obtained,

$L_D = 2\ L_c$ ……...…. (2)

^{1}H NMR Titration Studies. ^1H NMR titration studies with probe **1** was performed upon dissolving **1** (5 mM) in $DMSOd_6$ medium. To that Hg^{2+} were added independently gradually added (- 1.2 equiv.)

and the spectra were recorded using identical parameters. The chemical shifts have been represented as 'δ ppm'.

Scanning Electron Microscopy: Solution of **1** (concentration 10 μM) in water medium with and without Hg^{2+} were drop cast over double-sided tapes attached onto the brass stubs and air-dried for 48 h. The samples were then coated with gold vapor and analysed on a Quanta 200 SEM operated at 15 kV.

Stoichiometry determination by Job plot: The Job plot is a method of continuous variation for determining the stoichiometry of interaction between the two species. The total molar concentration of the two binding species (here, **1** and Hg^{2+} and ions) was kept constant (1×10^{-4} M) and the mole fraction was varied. Further the change in absorbance was plotted against the mole-fraction. The maxima or minima thus obtained gave the stoichiometry of interaction. In all cases, we have plotted ΔA*[analyte] vs [**1**] / {[analyte] + [**1**]}. Where, $\Delta A = A - A_0$, A = absorbance of probe molecule after addition of analyte at specific wavelengths and A_0 = absorbance of probe molecule without the analyte. [**1**] / {[analyte] + [**1**]} is the mole fraction of probe molecule in the mixture, analyte = Hg^{2+} etc.

Cell Culture. HeLa cells were cultured in Dulbecco'sModified Eagle's Medium (DMEM, CELL clone, India)containing 10% FBS (Invitrogen, USA) and antibiotics (100units/mL penicillin and 100 μg/mL streptomycin). Cells wereincubated in a humidified 5% CO_2 incubator (Sanyo, UK) at 37°C.

Cell Viability Assay. Cytotoxicity of the compound **1** was evaluated in HeLa cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In a typicalexperiment, cells were seeded at a density of ~10000 cells/well in the 96 well plates and incubated for 48 h. Then, the cellswere treated with different concentrations of the probe. Finally, cells were washed, and 20 μL of MTT (5 mg/mL in DMEM) was added in each well and incubated for 3 h in the dark.Finally, whole medium was removed from the wells, andDMSO (100 μL) was added. The cells were incubated for 5min in the dark, and absorbance was measured at 560 nm.

Cell Imaging. For the blank reading, the cells wereincubated with 10 μM of compound 1 for 20 min at 37 °C.Then, the medium was removed, and the cells were washedmultiple times with pH 7.4 DMEM buffer. To check Hg^{2+}-induced emission quenching, the cells were incubated with 20 μM of Hg^{2+} for 2 h, followed by treatment with compound **1** for 20 min. Finally, theflorescence images were obtained with and without thepresence of UA using fluorescence microscopy.

Additional Spectroscopic Data

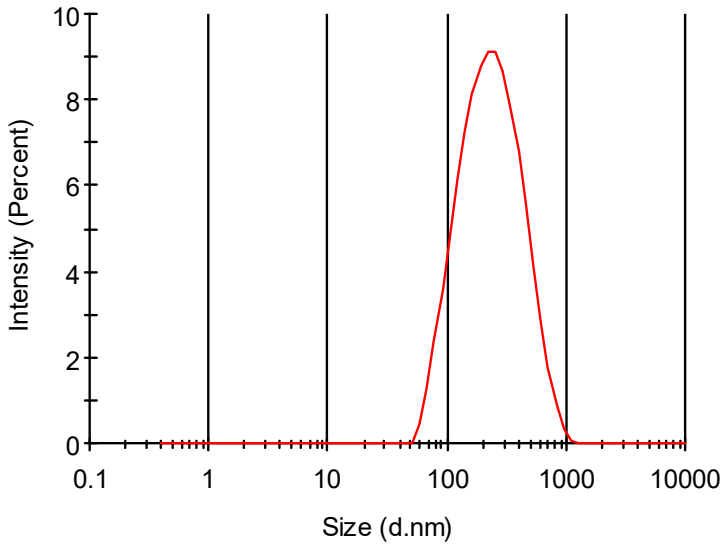


Figure S1. Determination of hydrodynamic diameter of **1** (10 μM) by dynamic light scattering measurement in aqueous medium.

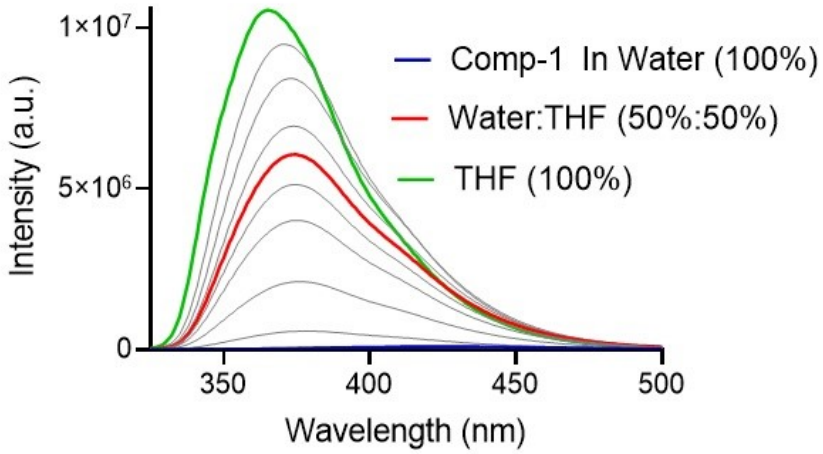


Figure S2. Fluorescence spectra of compound **1** (10 μM, λex = 310 nm) in different Water-THF mixture.

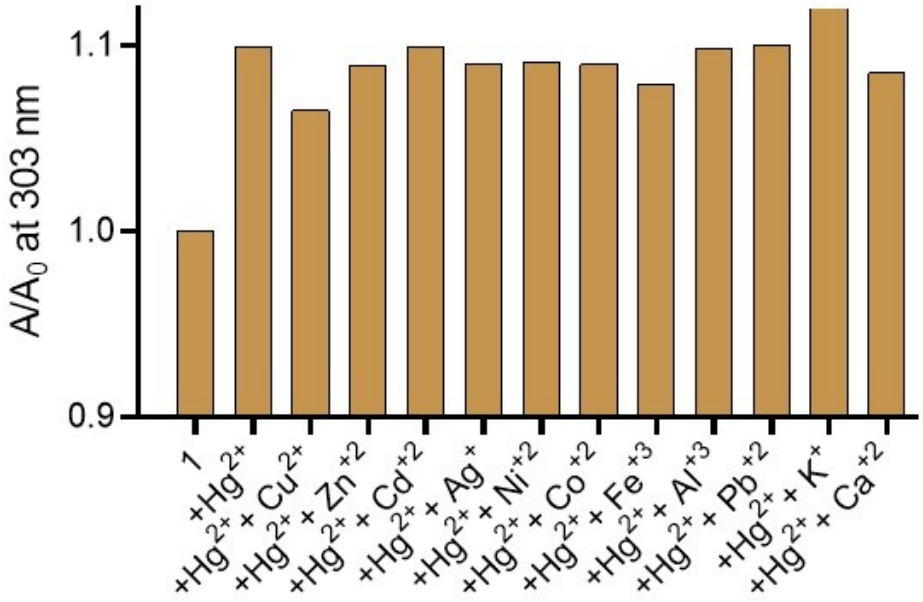


Figure S3. Changes in absorbance of **1** (10 μM) at 330 nm upon addition of Hg^{2+} ion (20 μM) in the presence of other metal ions.

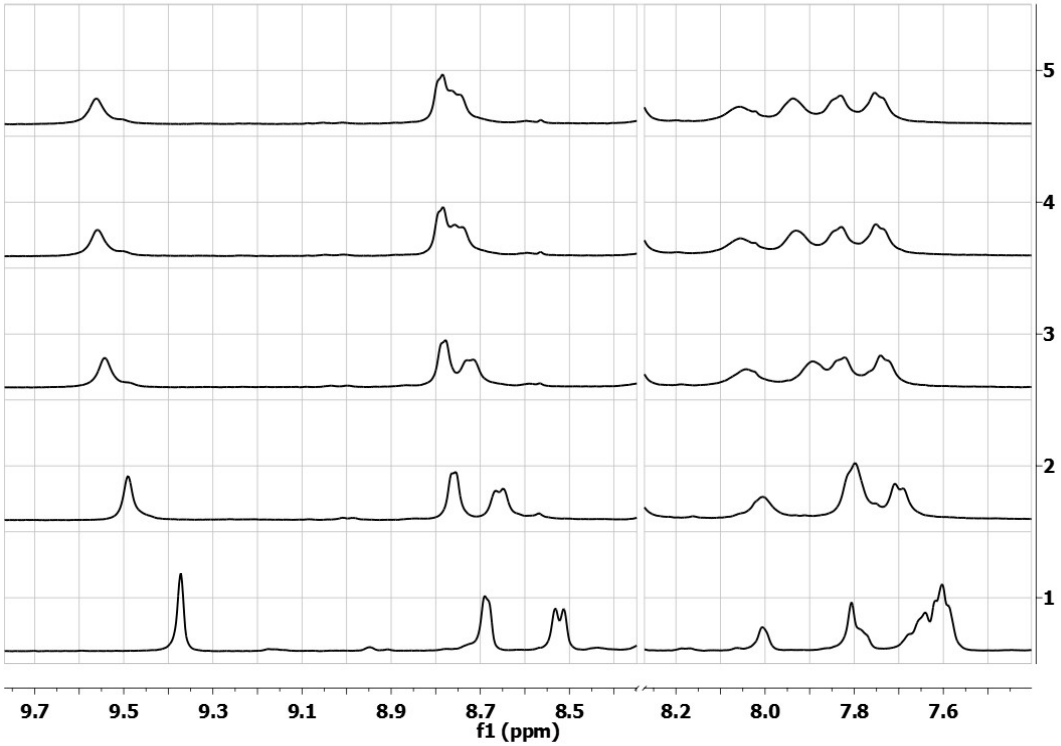


Figure S4. Partial ^{1}H-NMR spectra of **2** (8 mM) with Hg^{2+} (0, 0.25, 0.5, 0.75 and 1.0 equiv.) in DMSO-d6 medium.

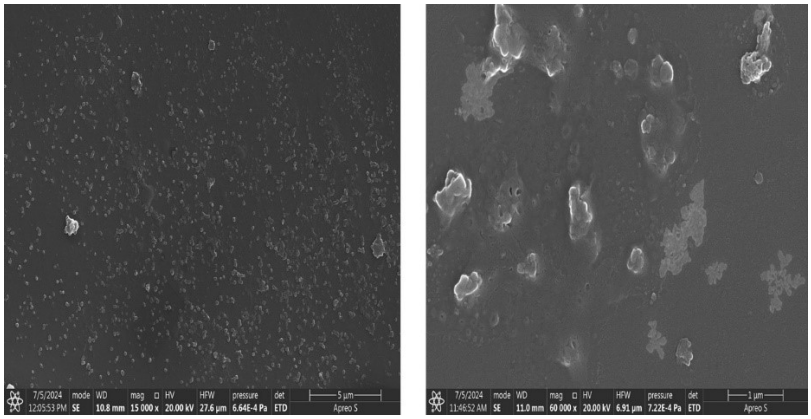


Figure S5. FESEM images of compound **2** and compound **2** + Hg^{2+}

System	Method	Time of incubation	Solvent	LOD	Application	Reference
Benzimidazole Substituted BODIPY	Florescence	20 Min	ACN/PBS (7:3 V/V 7.4 pH)	0.77 μM	Detection Hg(II) in human breast adenocarcinoma cells.	Inorg. Chem. 2013, 52, 11136−11145
functionalized gold nanoparticles	Florescence	30 Min	Water	2 μM	heavy metal ions detection in water environment	Chemosphere 303 (2022) 135174
Quantum Dot-Based	Florescence	Not reported	PBS pH 7.3	Not reported	Water samples	ACS Omega 2023, 8, 29468−29474
crown-ether modified azo dye	Florescence	Not reported	1:1 MeOH/ H_2O	1 mM	industrial water sample	Water SA Vol. 40 No. 1 January 2014
BODIPY- imine based fluorescence	Florescence	Not reported	DMSO-H_2O (9/1 v/v)	0.90 μM	Alzheimer's disease	Journal of Photochemistry & Photobiology, A: Chemistry 451 (2024) 115541
Bisbenzimidazole based Probes	Florescence	Not required	water	9 ppb	Tap, Pond, Sea water detection of Hg(II) in HeLa cells	Present work

Table S1. Table representing Hg (II) detection using various probes based on Florescence method.