Electronic Supplementary Information

Tuning fork-shaped bisbenzothiazole derivative as a pH-responsive digital fluorescent probe and its ex-vivo evaluation

Priya Ranjan Sahoo, [a] Nikhil Kumar,[b] Keloth Sairam,[a] L. K. Gulati,[a] G. K. Gulati,[a] Anupama Datta,[b] Satish Kumar*

*[a]*Department of Chemistry, St. Stephen's College, University Enclave, Delhi-110007, India.*

E-mail: satish@ststephens.edu

[b]Institute of Nuclear Medicine and Allied Sciences, DRDO, Brig. SK Majumdar Marg, Delhi 110054, India.
E-mail udatta@gmail.com

General Information and Remarks:

The Nuclear Magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a 400 MHz *Jeol NMR* ECX 400 NMR spectrometer. UV–visible spectra were recorded on an Ocean Optics USB 4000 UV–visible spectrometer. Fluorescence measurements were performed using a Cary Eclipse Fluorescence spectrophotometer. Time-resolved fluorescence measurements were recorded on Horiba Vyon with an automated polarizer. The powder X-ray Diffraction pattern was recorded on a pan-analytical X-ray diffractometer. The Melting Point was determined using the ALTIS melting point apparatus and was uncorrected. Di-formyl tertbutyl phenol was prepared according to the reported literature. 2-Amino thiophenol was directly purchased from Spectrochem India Pvt. Ltd.

Procedures:

X-ray structure determination:

The detailed crystal data, collection, and structure refinement parameters for **1** are listed in Table S1.[1] Olex2[2] GUI was employed to get a solution of the structure with the help of ShelXT/direct method,[3] and refinement was accomplished using ShelXL/least square [4] program. All the non-hydrogen atoms in all the structures were refined anisotropically. The aromatic H atoms in all structures were placed at the calculated positions (C-H = 0.93 Å) and refined with the help of the riding model with $U_{iso}(H) = 1.2U_{eq}(C)$. Mercury software [5] The CH_3 group protons (C-H = 0.96Å) were placed at the position and refined using a riding model such that U_{iso} (H) = $1.5U_{eq}(C)$, The methyl group was refined as an idealized rotor. was used to prepare the graphics material for publication.

Procedure for UV-visible studies at different pH

The pH (2.0×10^{-5} M) range between 1 to 12 was set by using an ELICO pH meter; 1 to 12. The UV-visible data of probe **1** at different pHs were recorded on an Ocean Optics UV-visible spectrometer.

Procedure for fluorescence studies at different pH

The different pH of probe **1** (2.0×10^{-5} M) was set in an ELICO pH meter from pH = 1 to 12. The solutions were inserted in a quartz cuvette and the fluorescence spectrum was recorded on a VARIAN CARY Eclipse Fluorescence spectrometer. The slit width was set at 5.0 nm.

Synthesis of the probe 1

In a 100 mL round bottom flask, diformyl *tert*-butyl phenol (1 g, 4.84 mmol) was mixed with 2-amino thiophenol (1.0 mL, 9.6 mmol) and was stirred in 30 mL of ethanol for 24 h. The resulting reaction mixture was filtered and the solid product was purified by flash column chromatography (10% EA/hexane) to give a yellow solid. Yield: 82% m.pt.230-234ºC ^{1}H-NMR (400MHz, $CDCl_3$): 13.96 (s, 1H), 8.26 (s, 2H), 8.07(d, 3H, *J* = 8.4Hz), 7.94(d, 3H, *J* = 8.4Hz), 7.51(t, 3H, *J* = 7.6Hz), 7.40(t, 3H, *J* = 7.6Hz), 1.45(s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): 154.08, 151.78, 128.6, 126.4, 125.22, 122.5, 121.45, 34.47, 31.45. FT-IR (v/cm-1): 3743.98, 3678.36, 2959.27, 1611.11, 1492.88, 1366.24, 1308.16, 1240.42, 1104.05, 1062.99, 1017.20, 927.31, 884.64, 757.97, 653.79. HRMS *m/z* Calculated for $C_{24}H_{20}N_2OS_2$: 416.1017, found: $[C_{24}H_{20}N_2OS_2+H^+]$: 417.1061.

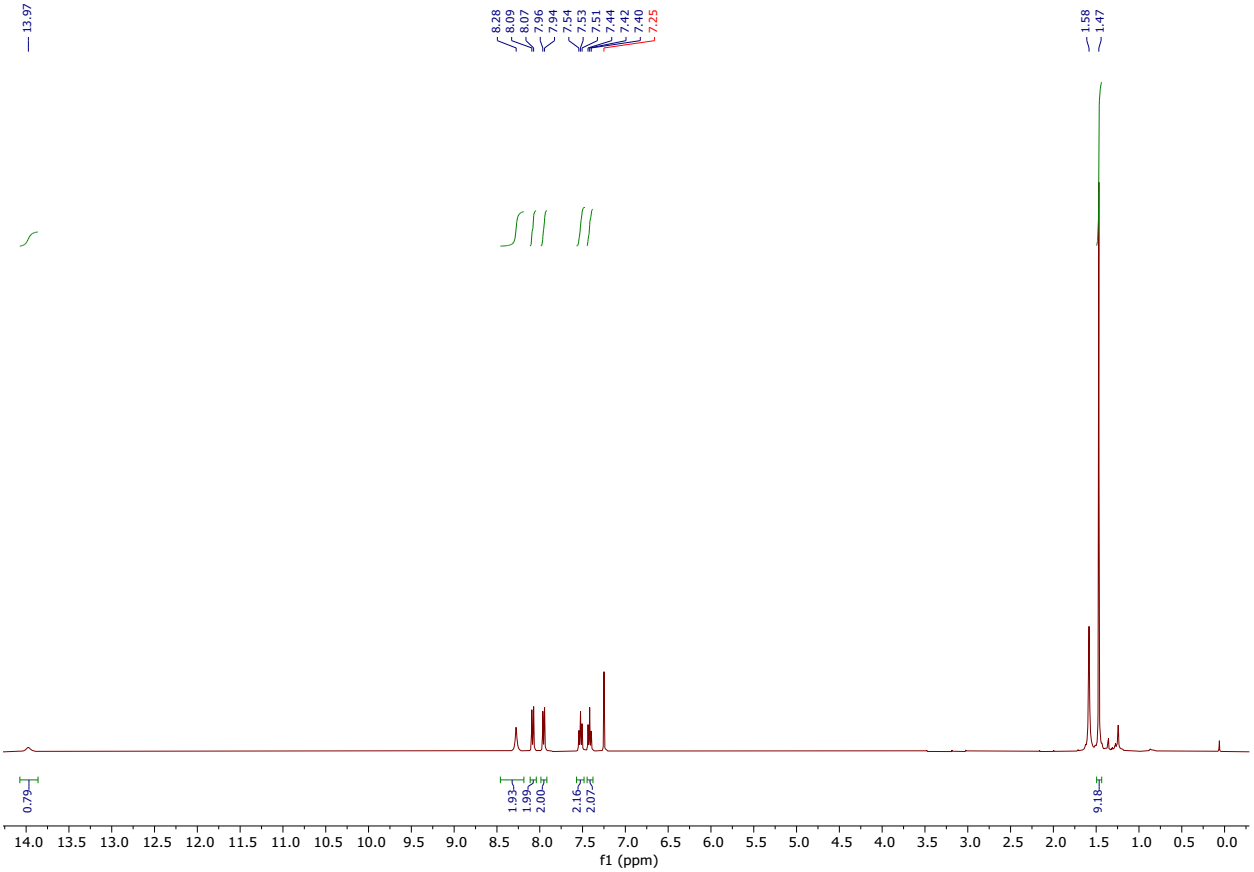


Fig. S1. ^{1}H-NMR spectrum of the probe **1**.

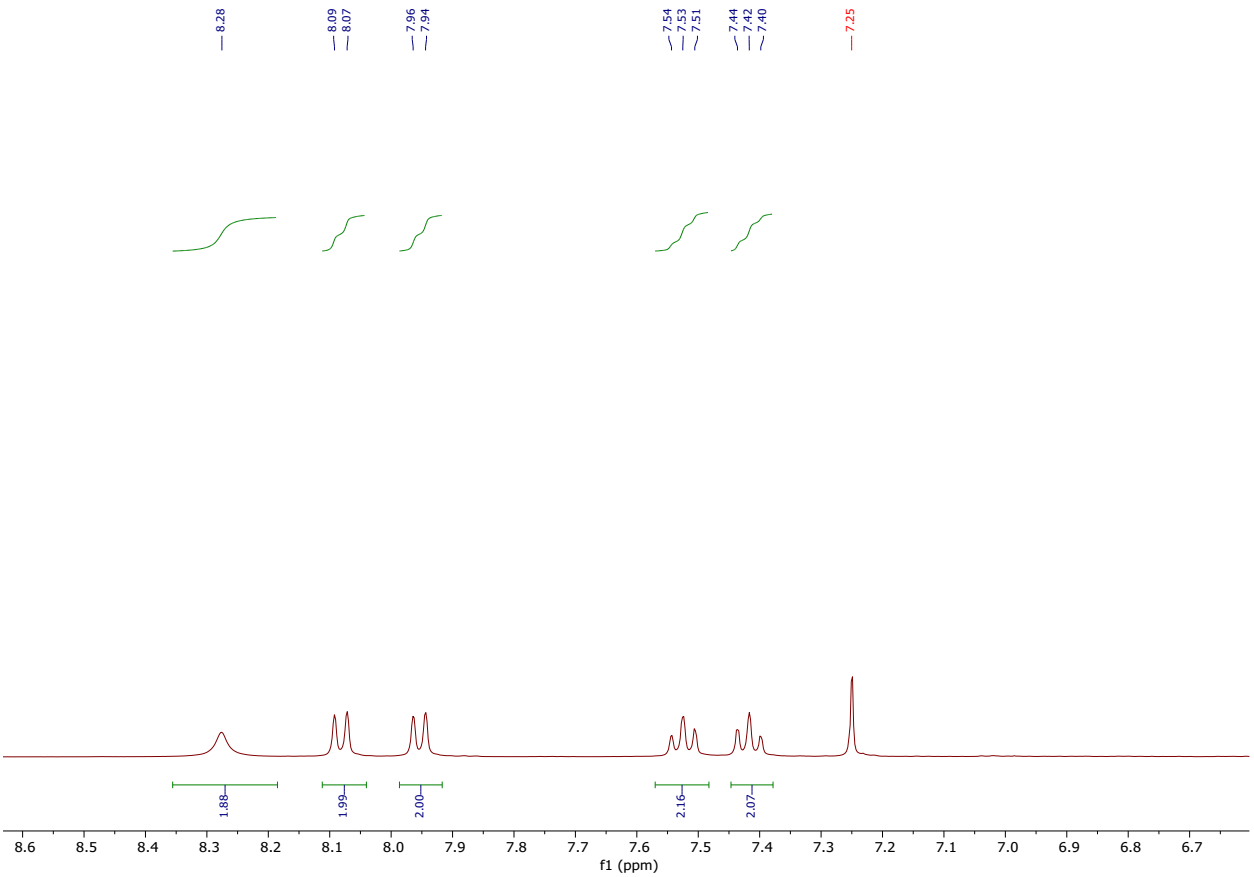


Fig. S1a. Partial ^{1}H-NMR spectrum of the probe **1**.

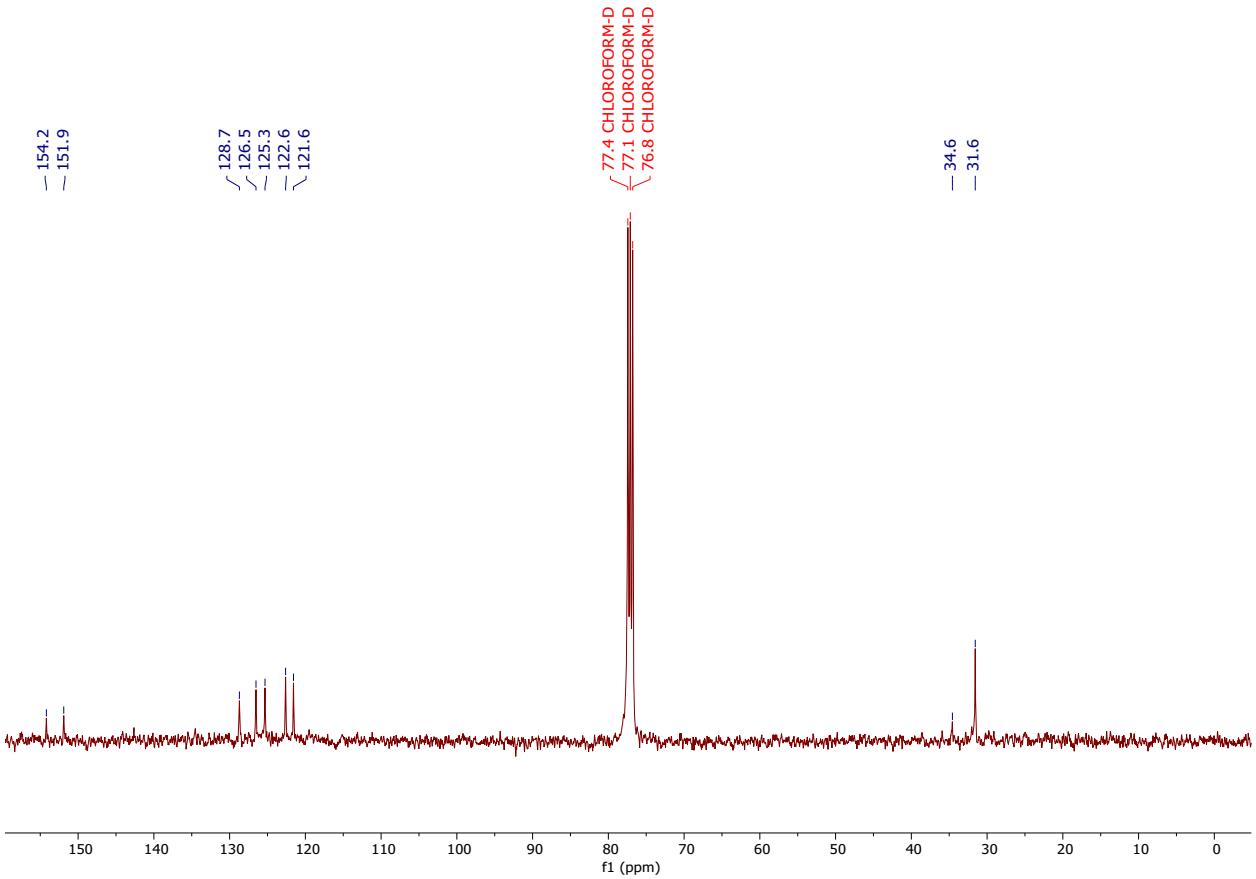


Fig. S2. ^{13}C-NMR spectrum of the probe **1**.

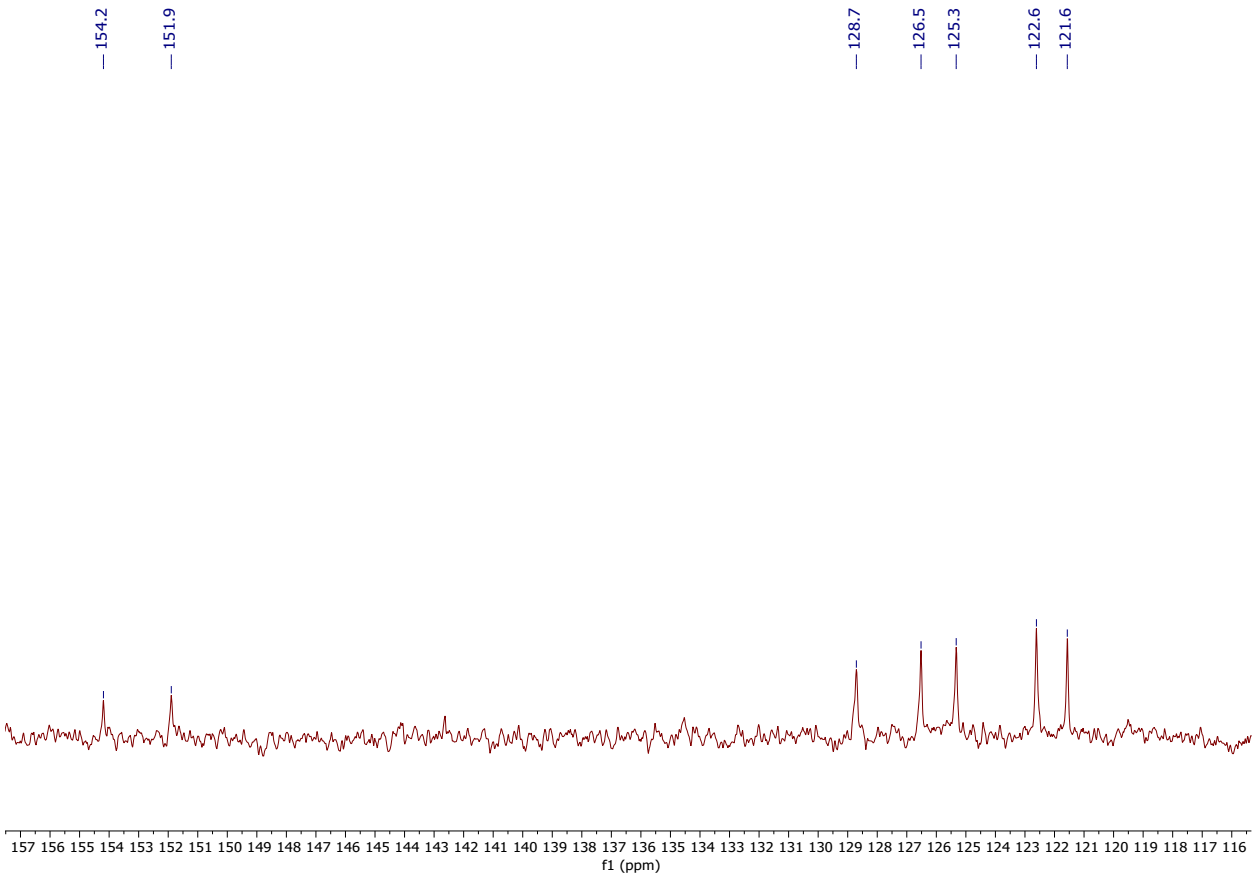


Fig. S2a. Partial ^{13}C-NMR spectrum of the probe **1**.

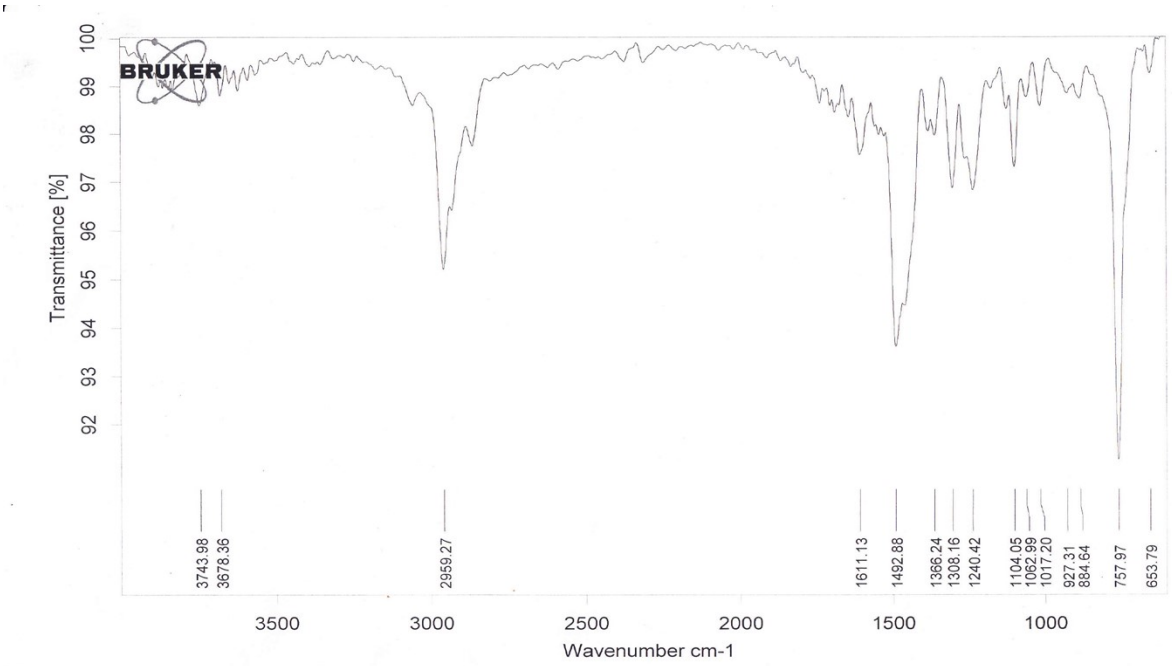


Fig. S3. Fourier Transform Infrared (FT-IR) spectrum of the probe **1**.

Qualitative Compound Report

Data File	5PS-131.d	**Sample Name**	5PS-131
Sample Type	Sample	**Position**	P1-A7
Instrument Name	Instrument 1	**User Name**	
Acq Method	29.10.2014.m	**Acquired Time**	10-10-2016 13:41:16
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

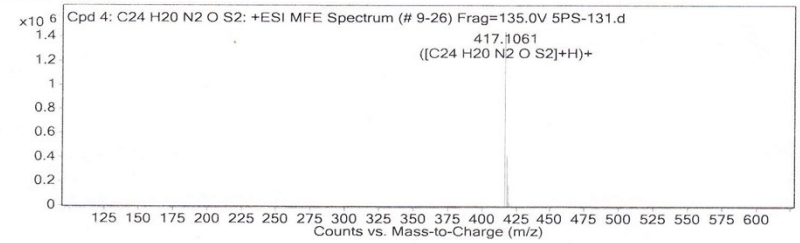
Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125)

Compound Table

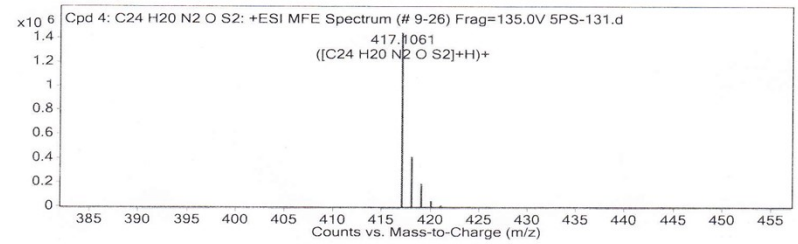
Compound Label	RT	Mass	Formula	MFG Formula	MFG Diff (ppm)	DB Formula
Cpd 4: C24 H20 N2 O S2	13	416.0988	C24 H20 N2 O S2	C24 H20 N2 O S2	7.03	C24 H20 N2 O S2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 4: C24 H20 N2 O S2	417.1061	13	Find by Molecular Feature	416.0988

MFE MS Spectrum



MFE MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
417.1061	1	1442653.88	C24 H20 N2 O S2	(M+H)+
418.109	1	413013.28	C24 H20 N2 O S2	(M+H)+
419.105	1	177140.24	C24 H20 N2 O S2	(M+H)+
420.1058	1	38913.94	C24 H20 N2 O S2	(M+H)+
421.1052	1	7809.22	C24 H20 N2 O S2	(M+H)+
422.1047	1	1455.23	C24 H20 N2 O S2	(M+H)+

--- End Of Report ---

Fig. S4. High-Resolution Mass spectrum of the probe **1**.

Haemolysis Assay

A hemolysis assay was performed as per the procedure reported earlier in the literature using heparinized blood from healthy volunteers. Haemolysis experiment and serum stability assays were conducted using human serum by the procedure approved by Institutional Human Ethical Committee at INMAS, DRDO. Blood serum was obtained by centrifugation followed by repeated washing of the RBC pellet with PBS (pH = 7.4). The pellet was suspended again in PBS and added to different vials in equal amounts. Similarly, variable concentrations of the probe were taken in equal volumes and incubated with the RBC pellet in an 8:2 ratio at 37 °C. The Triton X-100 (2% v/v) and phosphate buffer saline were used as positive and negative controls, respectively, which were also incubated with RBCs at similar experimental conditions. The solvent (100 μL) was centrifuged and withdrawn at the following intervals: 1 h, 2 h, 4h, and 24 h. The supernatant solution was used to measure absorbance at 540 nm in a 96-well plate by a Synergy 2 Multi-Mode Reader (BioTek Instruments, United States). The experiments were performed three times and the degree of hemolysis was calculated using the following formula (reference: https://doi.org/10.1021/acsami.6b15203):

$$\text{Hemolysis ratio} = ((\lambda(\text{test}) - \lambda(\text{negative control}))/(\lambda(\text{positive control}) - \lambda(\text{negative control}))) \times 100\%$$

where $\lambda(\text{test})$ represents the absorbance values of the test sample; $\lambda(\text{negative control})$ is a measure of absorbance values of phosphate buffer saline and $\lambda(\text{positive control})$ represents the absorbance values for Triton X-100.

Table S1: Crystallographic Parameters of the probe **1** (CCDC 1509581).

Crystal data	
Chemical formula	$C_{23.96}H_{19.89}N_2OS_2$
M_r	416.05
Crystal system, space group	Monoclinic, $P2_1/n$
Temperature (K)	297
a, b, c (Å)	13.6239 (16), 7.8788 (11), 19.179 (3)
β (°)	92.723 (11)
V (Å^3)	2056.4 (5)
Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.28
Crystal size (mm)	0.02 × 0.01 × 0.01
Data collection	
Diffractometer	Xcalibur, Sapphire3
Absorption correction	Multi-scan *CrysAlis PRO*, Agilent Technologies, Version 1.171.36.32 (release 02-08-2013 CrysAlis171 .NET) (compiled Aug 2 2013,16:46:58) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.872, 1.000
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	28368, 3784, 1057
R_{int}	0.307
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.606
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.087, 0.215, 0.87
No. of reflections	3784
No. of parameters	266
No. of restraints	12
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement

$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.31, –0.35

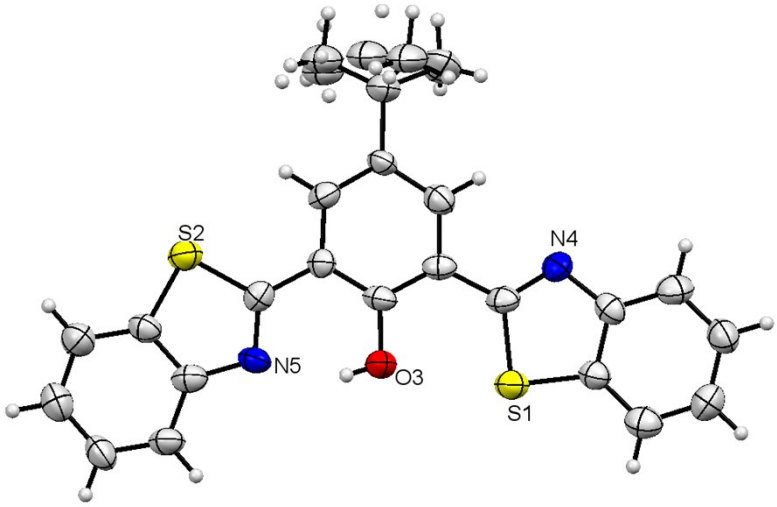


Fig. S5. ORTEP representation of the probe **1**. Ellipsoids are shown at 50% Probability level.

Table S2. Hydrogen-Bond geometry (Å, °) for **1**.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O_3	H_3	N_5	0.76(5)	1.90(5)	2.598(5)	153.6
C_{10}	H_{10}	S_2	0.93	2.69	3.118(5)	108.8
C_{13}	H_{13}	N_4	0.93	2.44	2.789(6)	102.5
C_{23}	H_{23}	O_3^1	0.93	2.95	3.662(6)	134.3

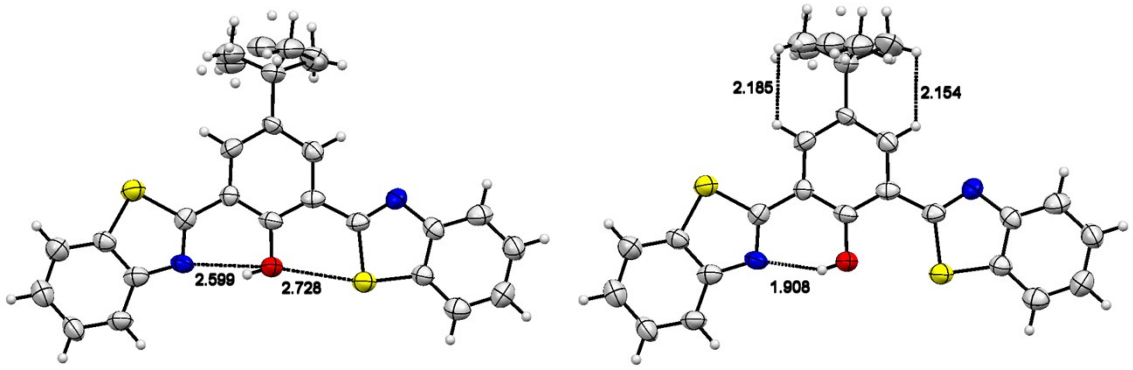


Fig. S6. Hydrogen Bonding and Short Intramolecular Contacts of the probe **1**. Ellipsoids are shown at 50% Probability level.

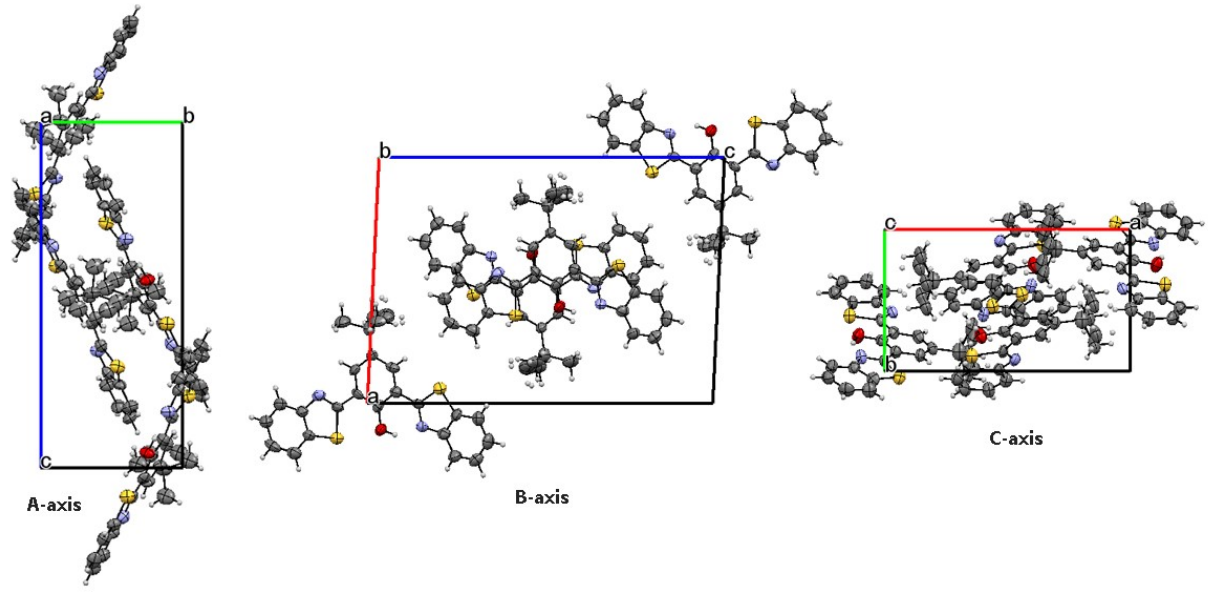


Fig. S7. Crystal packing diagram of probe **1** along the a-axis, b-axis, and c-axis respectively. Ellipsoids are shown at 50% Probability level.

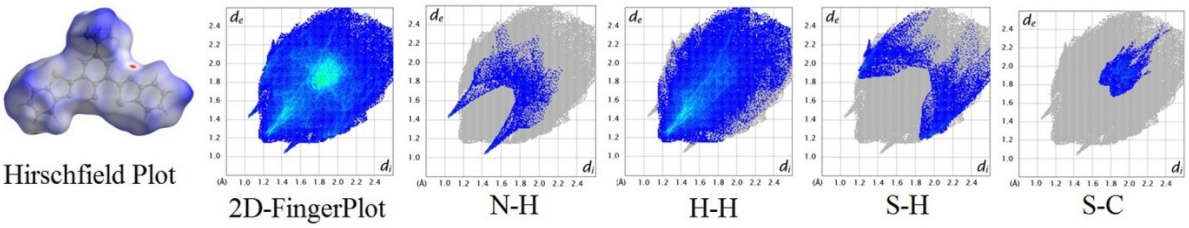


Fig. S8. The Hirschfield surface, a 2D-fingerprint plot showing complete, N-H, H-H, S-H, and S-C interactions respectively using Crystal Explorer 3.0 software.

Table S3. The different lattice energy components were calculated using the CLP-PIXEL method.

	E_{coul} (kJ/mol)	E_{pol} (kJ/mol)	E_{disp} (kJ/mol)	E_{rep} (kJ/mol)	E_{tot} kJ/mol)
1	*3.1*	*-43.0*	*-173.6*	*28.1*	*-185.4*

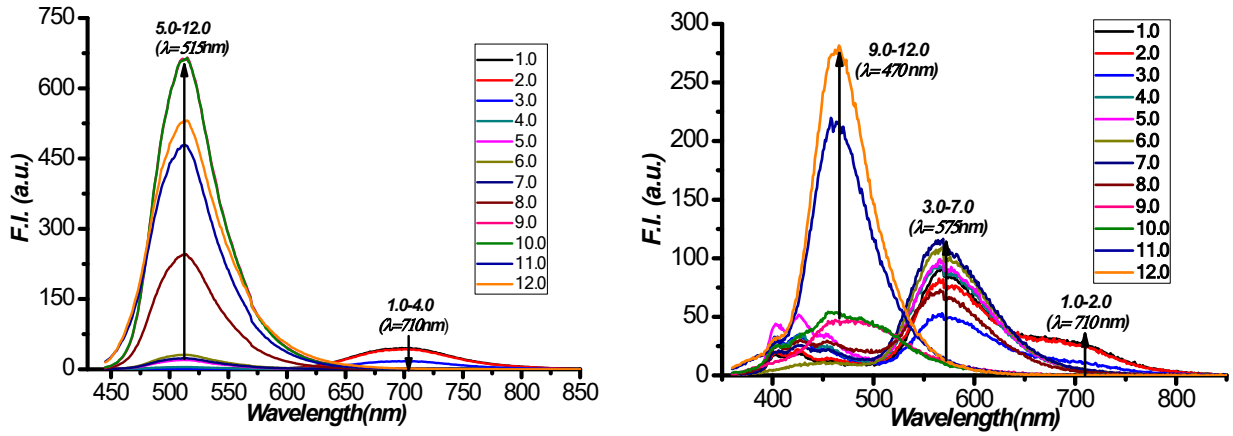


Fig. S9. Fluorescence Intensity response of probe 1 towards different pH at two different excitation wavelengths (a) λ_{ex} = 435nm and (b) λ_{ex} = 350nm in Methanol-TRIS.

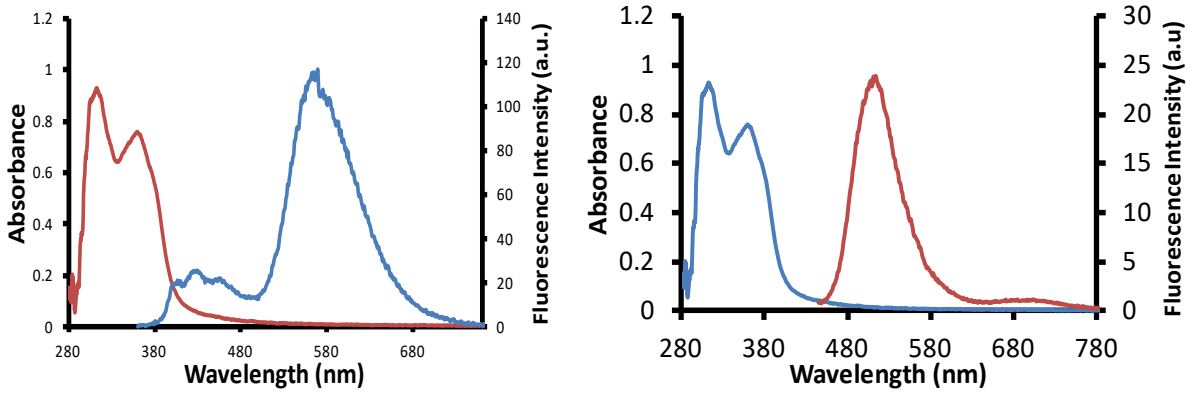


Fig. S10. Absorption and Fluorescence spectra of probe **1** ([**1**] = 2.0 × 10^{-5} M) in MeOH: water, (a) λ_{ex} = 350 nm, (b) λ_{ex} = 435 nm system.

Table. S4 Absorbance and Emission of the probe **1** at different pH.

pH	Abs, ʎ(nm)	FI, ʎ(nm)	Lifetime(ns)
1	0.69,349	44.24,699	
2	0.71,352	42.61,697	2.92
3	0.73,357	17.18,695	
4	0.79,360	4.89,510	3.19
5	0.77,360	23.16,510	
6	0.74,362	23, 511	3.29
7	0.07,425	31, 511	

8	0.15,428	244,512	
9	0.53,432	475,512	
10	0.56,435	531,513	3.29
11	0.75,437	660,513	
12	0.85,439	662,513	

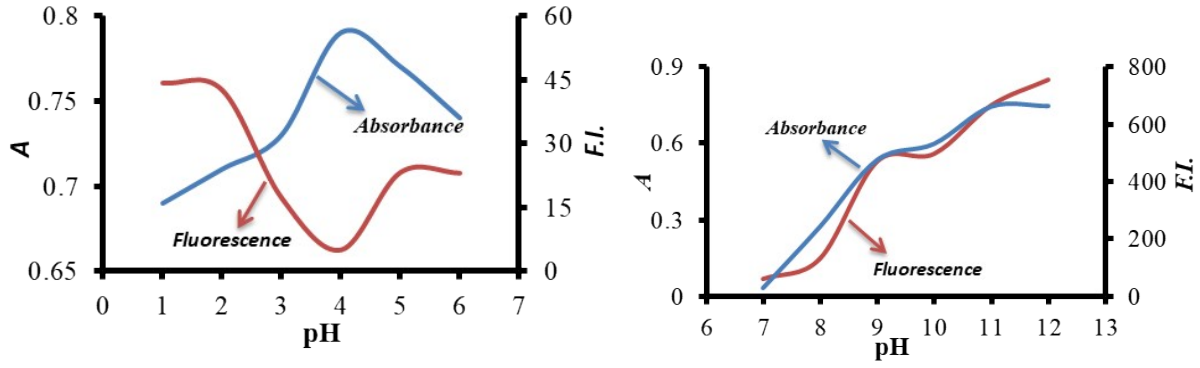


Fig. S11. Comparison of Absorbance and Fluorescence Emission of probe **1** at different pH.

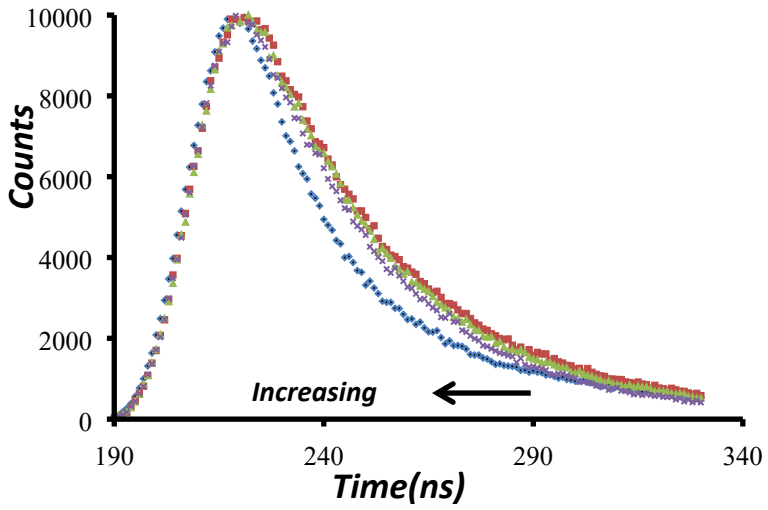


Fig. S12.Lifetimes decay profile of probe **1** at different pH at an excitation wavelength at λ_{ex} = 350nm in Methanol-Tris.

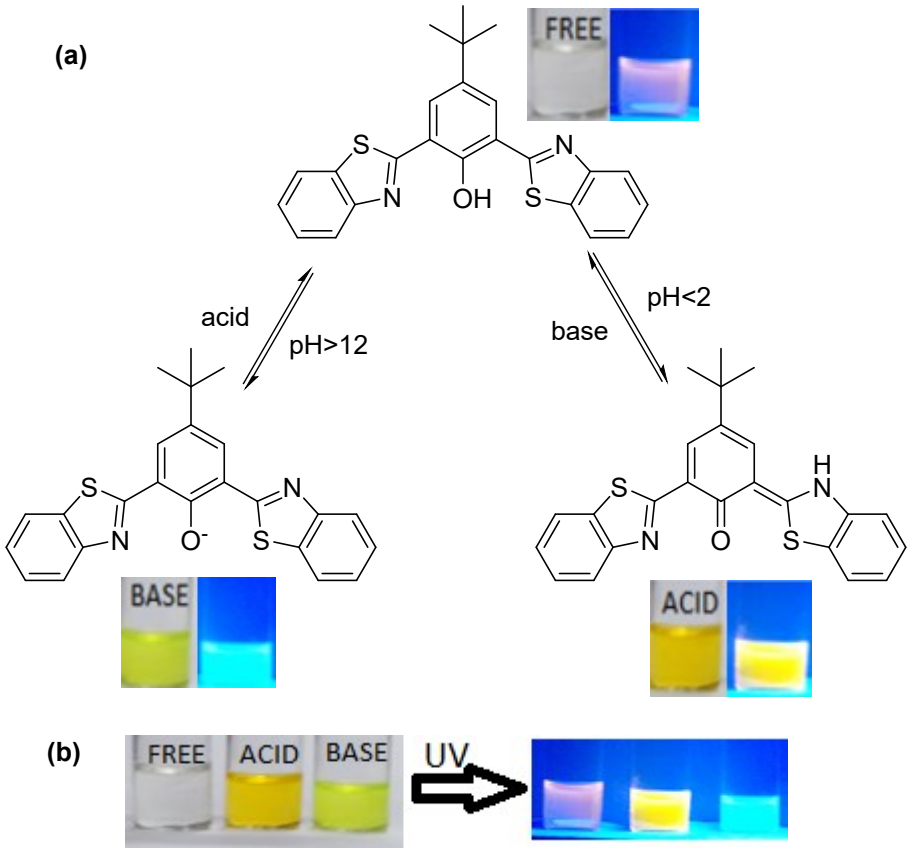


Fig. S13. (a) Plausible Mechanism of the probe **1** under acidic and basic environment. **(b)** Colorimetric and Fluorometric view of probe **1** under day and UV light (365nm) under acidic and basic conditions.

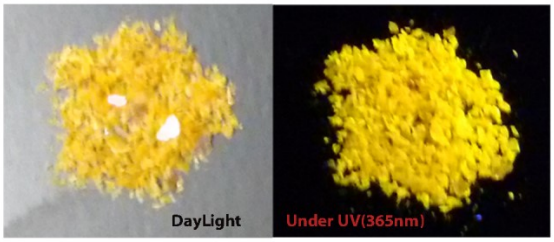


Fig.S14. Snapshots of the crude product under (DayLight) and under (UV-365nm) of the probe **1**.

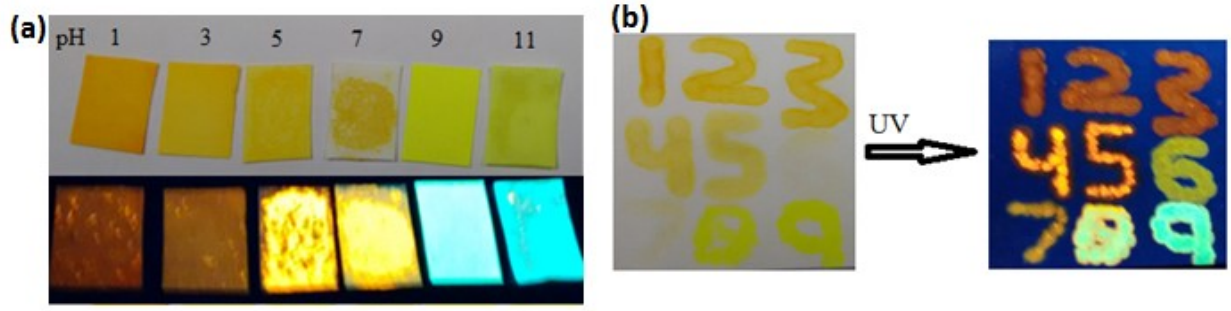


Fig. S15. (left) Fabrication of pH strips following impregnation of probe **4.2** (right) digital response of probe **4.2** with encoded numerical 1 to 9 in MeOH: water (1:1, pH = 1-12, 1.0 mM HEPES).

References:

1. S. Parsons, H. D. Flack and T. Wagner, *Acta Cryst. B*, 2013, **69**, 249-259.
2. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. Howard and H. Puschmann, *J. Appl. Crystallogr.*, 2009, **42**, 339-341.
3. G. M. Sheldrick, *Acta Cryst. A*, 2015, **71**, 3-8.
4. G. M. Sheldrick, *Acta Cryst. C*, 2015, **71**, 3-8.
5. C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. v. Streek and P. A. Wood, *J. Appl. Crystallogr.*, 2008, **41**, 466-470.