Electronic Supplementary Information (ESI)

Rhodamine derived colorimetric and fluorescence mercury(II) sensor for human breast cancer cell (MCF7) imaging

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UV-Vis and fluorescence titration

The path length of cells used for absorption and emission studies is 1 cm. For UV-Vis and fluorescence titrations, stock solution of **RDHDNAP** (20 μM) has been prepared in HEPES buffered (0.1 M) solution (ethanol/water = 3/7, v/v, pH 7.4). Working solutions of **RDHDNAP** and $Hg(NO_3)_2$. H_2O are prepared from their respective stock solutions. Fluorescence measurements are performed using 5 nm × 5 nm slit width.

Quantum yield measurements

The fluorescence quantum yield has been determined using Rhodamine B as a reference with a known φ_{ref} value of 0.65 in basic CH_3OH. The area of the emission spectrum is integrated using the software available in the instrument and the quantum yield is calculated according to the following equation:

$$\varphi_{sample} = \varphi_{ref} \times [A_{sample}/A_{ref}] \times [OD_{ref}/OD_{sample}]$$

Where φ_{sample} and φ_{ref} are the fluorescence quantum yield of the sample and reference, respectively; A_{sample} and A_{ref} are the area under the fluorescence spectra of the sample and the reference, respectively; OD_{sample} and OD_{ref} are the corresponding optical densities of the sample and the reference solution at the wavelength of excitation.

Job's plot from fluorescence measurements

A series of solutions containing **RDHDNAP** and $Hg(NO_3)_2$,H_2O are prepared such that the total concentration of Hg^{2+} and **RDHDNAP** remain constant (20 μM) in all the sets. The mole fraction (X) of **RDHDNAP** is varied from 0.1 to 0.8. The emission intensity at 591 nm is plotted against the mole fraction of **RDHDNAP**.

Determination of detection limit

To determine the detection limit, fluorescence titration of RDHDNAP with Hg^{2+} is carried out by adding aliquots of micro-molar concentration of Hg^{2+}. From the concentration at which there is a sharp change in the fluorescence intensity multiplied with the concentration of RDHDNAP gives the detection limit.[3] Equations used for the calculation of detection limit (DL): DL = CL × CT, Where CL is the concentration of ligand, CT is the concentration of Hg^{2+} at which fluorescence enhanced. Thus: DL for RDHDNAP = 10^{-6}M × (3×10^{-7}) M = 3 × 10^{-7}M

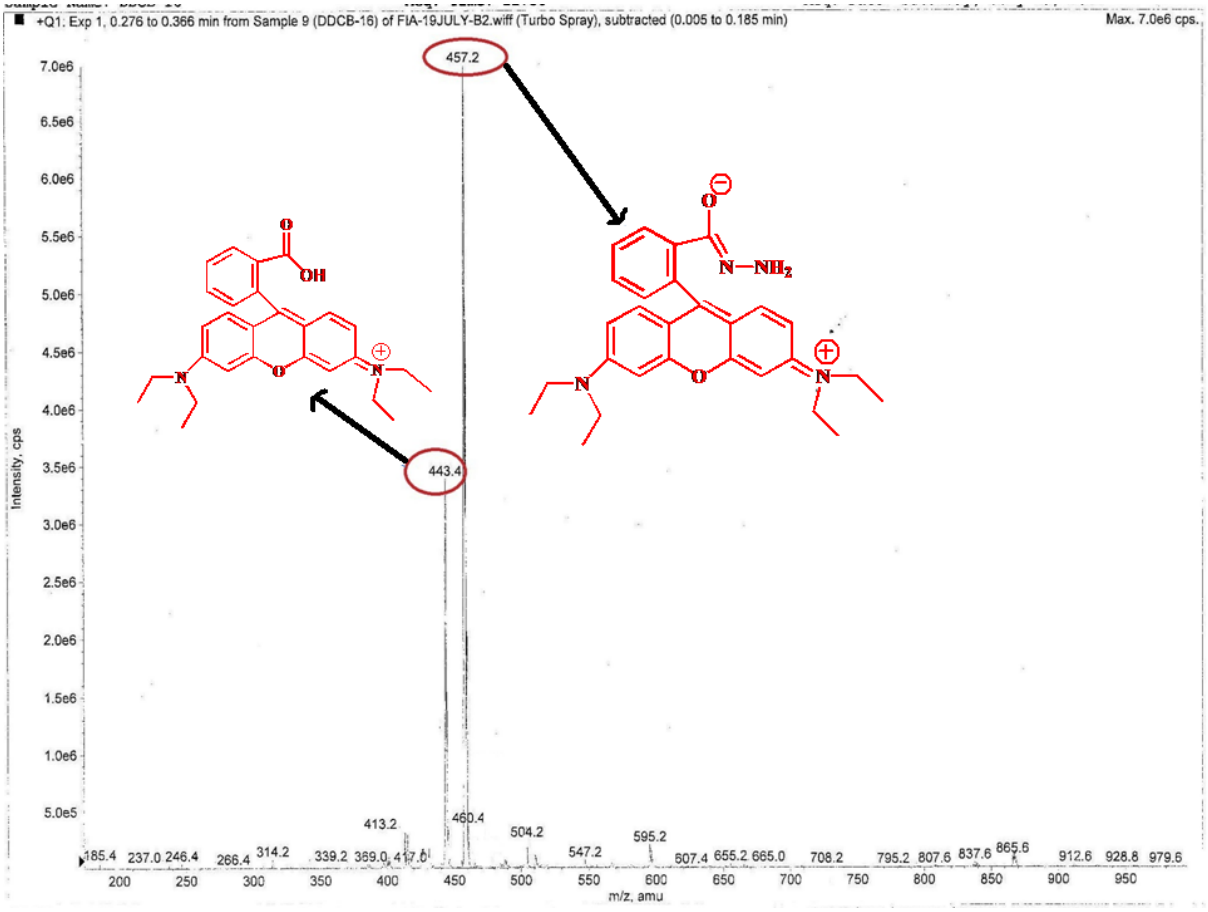


Fig.S1 QTOF MS spectrum of the [**RDHDNAP**–Hg^{2+}] system.

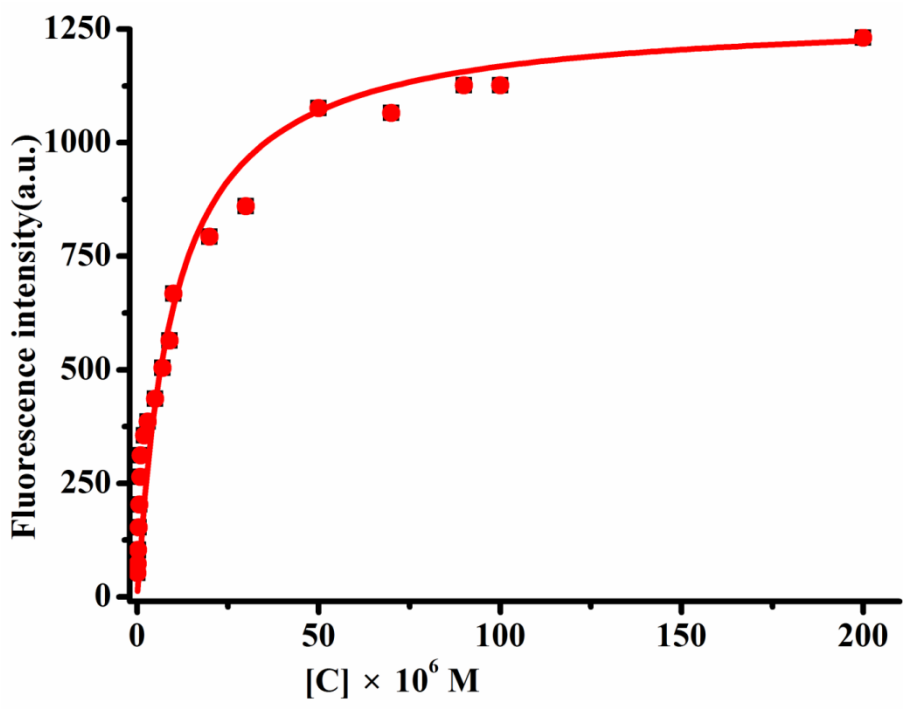


Fig.S2 Emission intensity of **RDHDNAP** *vs.* externally added Hg^{2+} (0.01–800.0 μM, λ_{ex}, 550 nm; λ_{em}, 591 nm).

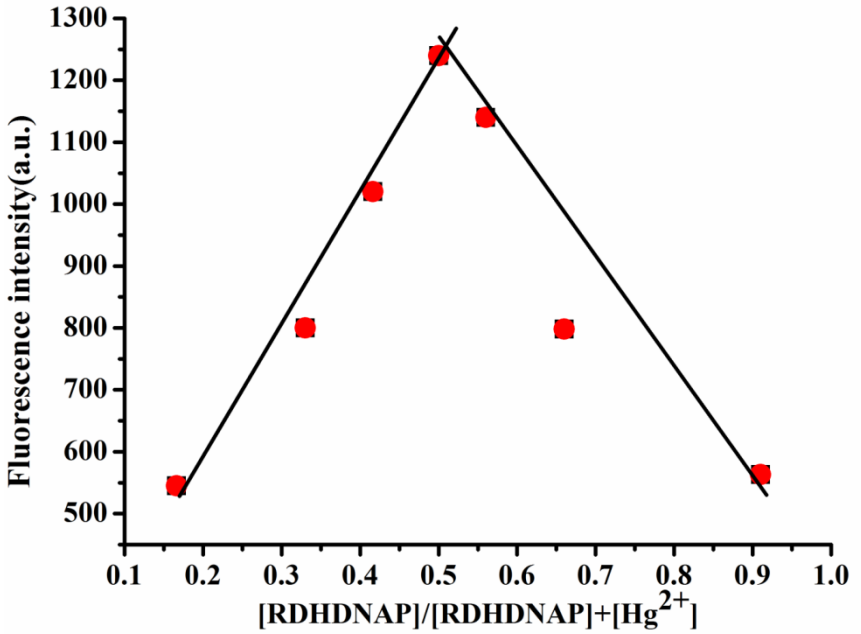


Fig.S3 Job's plot for stoichiometry determination between **RDHDNAP** and Hg^{2+} in HEPES buffered (0.1 M) solution (ethanol/water = 3/7, v/v, pH 7.4)

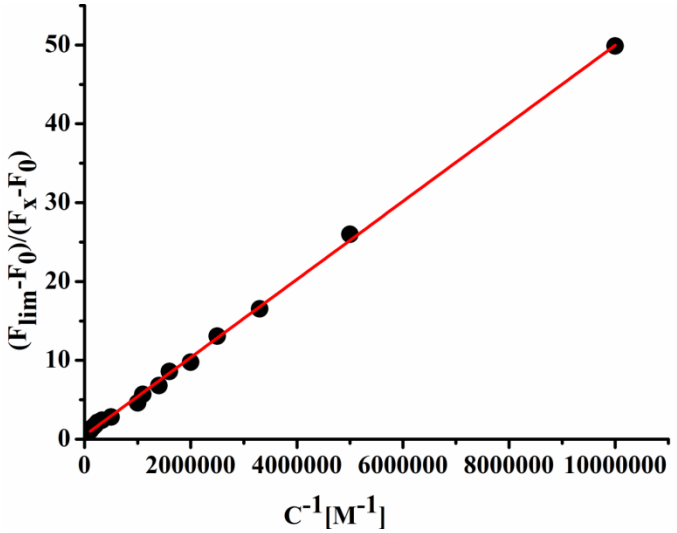


Fig.S4 Determination of the association constant of **RDHDNAP** for Hg^{2+} using $(F_{max} - F_0)/(F_x - F_0) = 1 + (1/K) \times (1/[M]^n)$, where F_0, F_x, and F_{lim} are the emission intensities of **RDHDNAP** in absence of Hg^{2+}, at an intermediate Hg^{2+} concentration, and at a concentration of complete interaction with Hg^{2+} (λ_{ex}, 550 nm; λ_{em}, 591 nm).

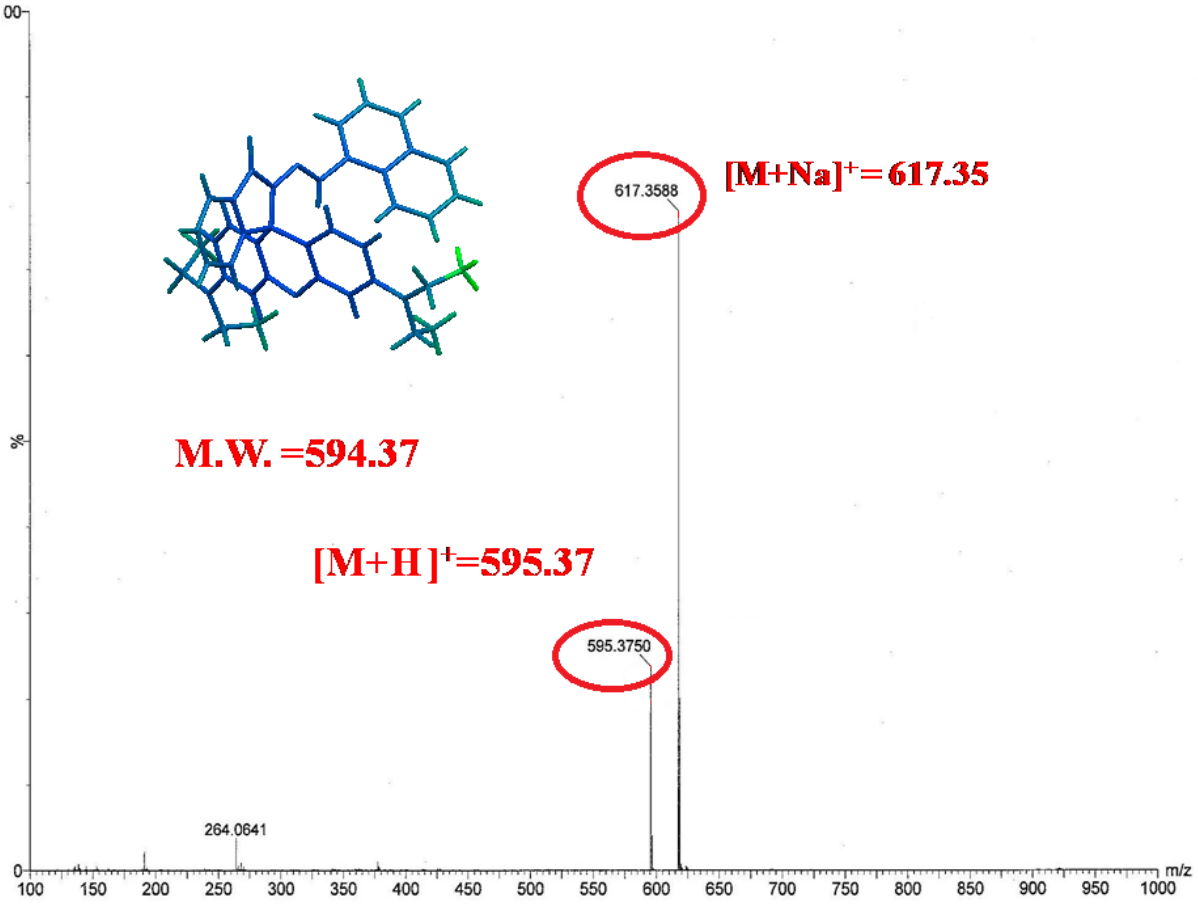


Fig.S5 QTOF MS spectrum of **RDHDNAP**

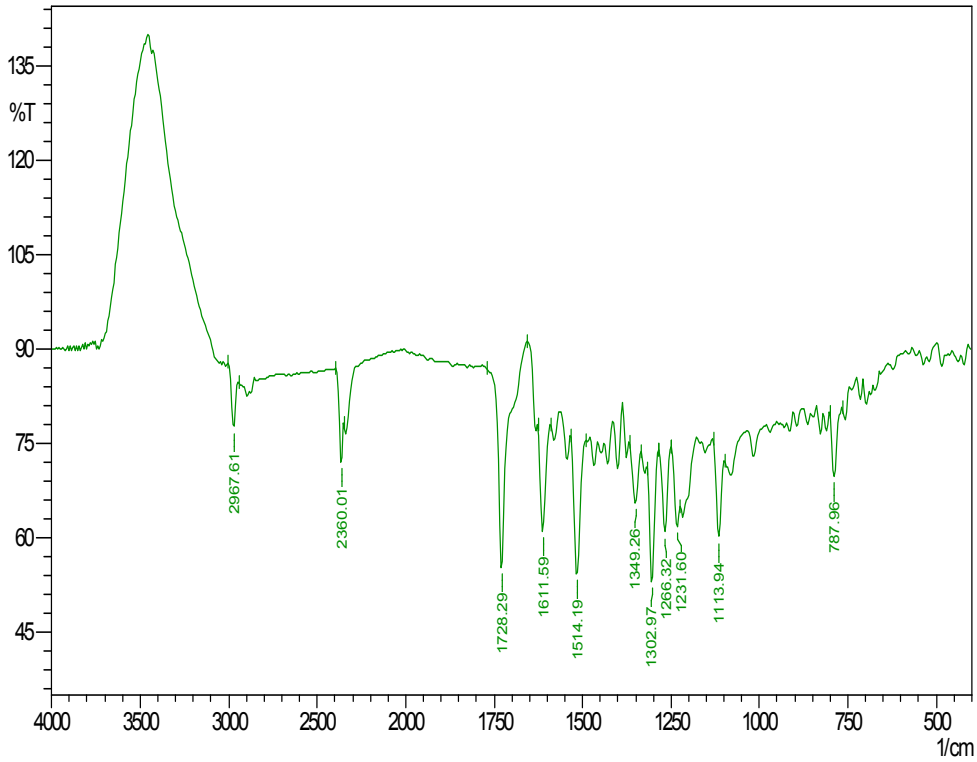


Fig. S6 FTIR spectrum of **RDHDNAP**

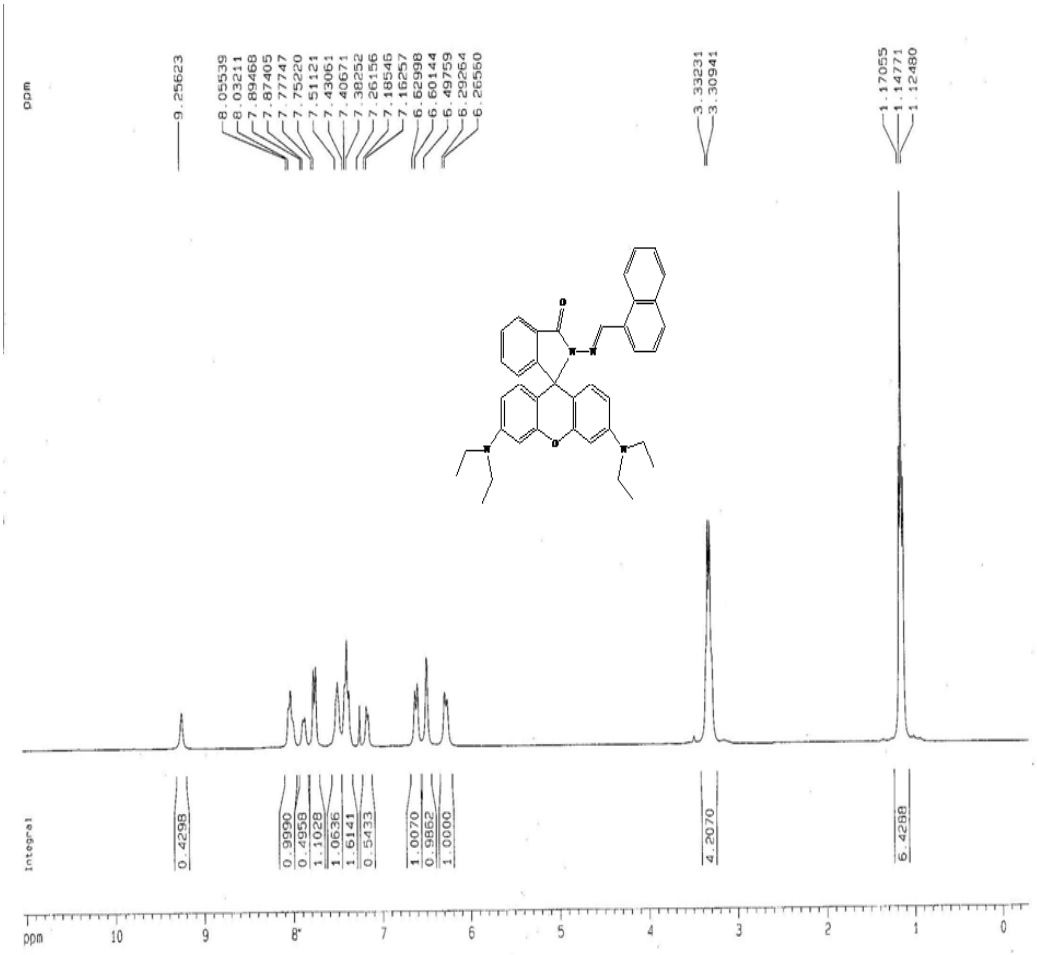


Fig S7 ^{1}H NMR spectrum of **RDHDNAP** in $CDCl_3$

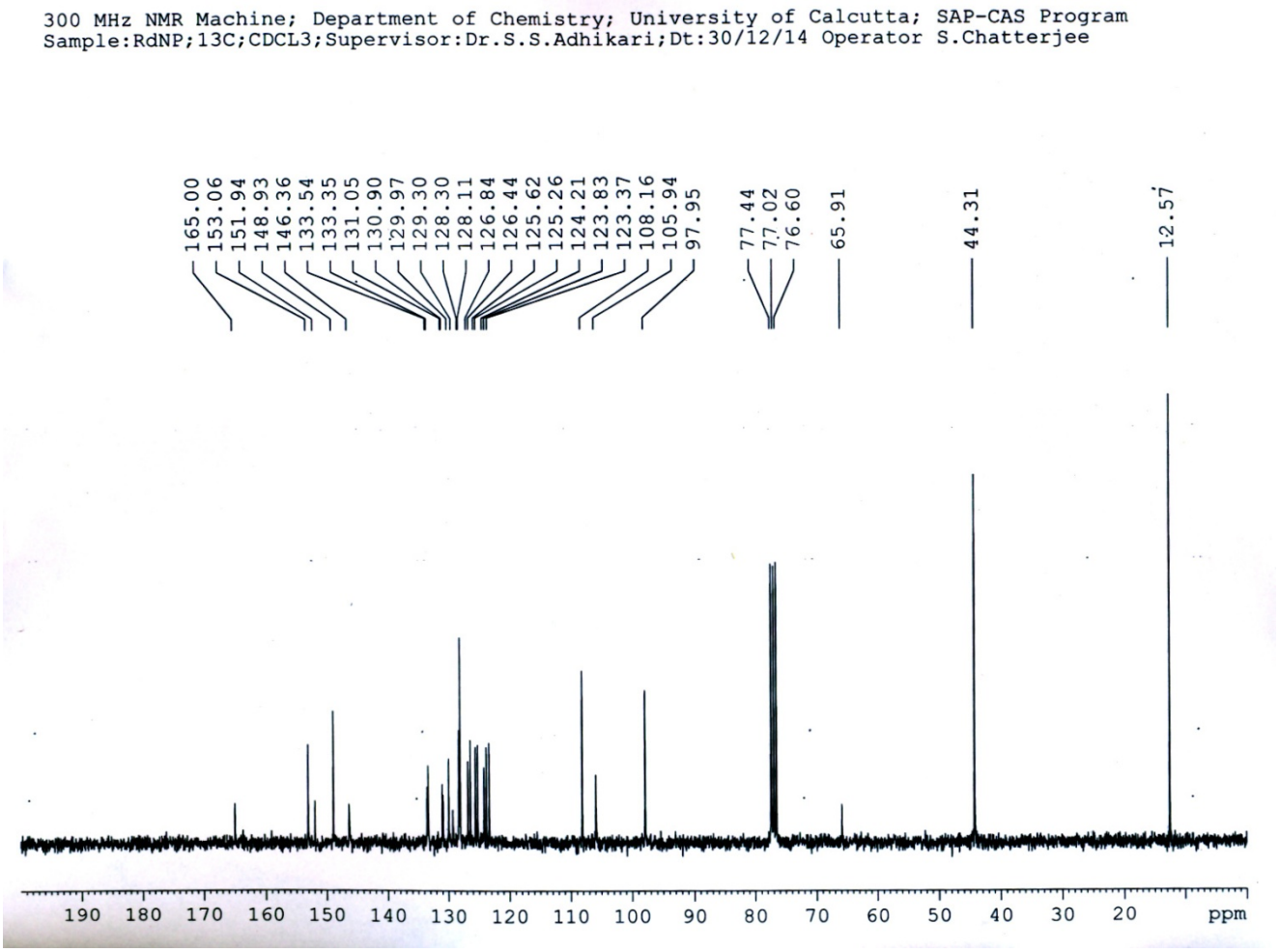


Fig S8 ^{13}C NMR spectrum of **RDHDNAP** in $CDCl_3$

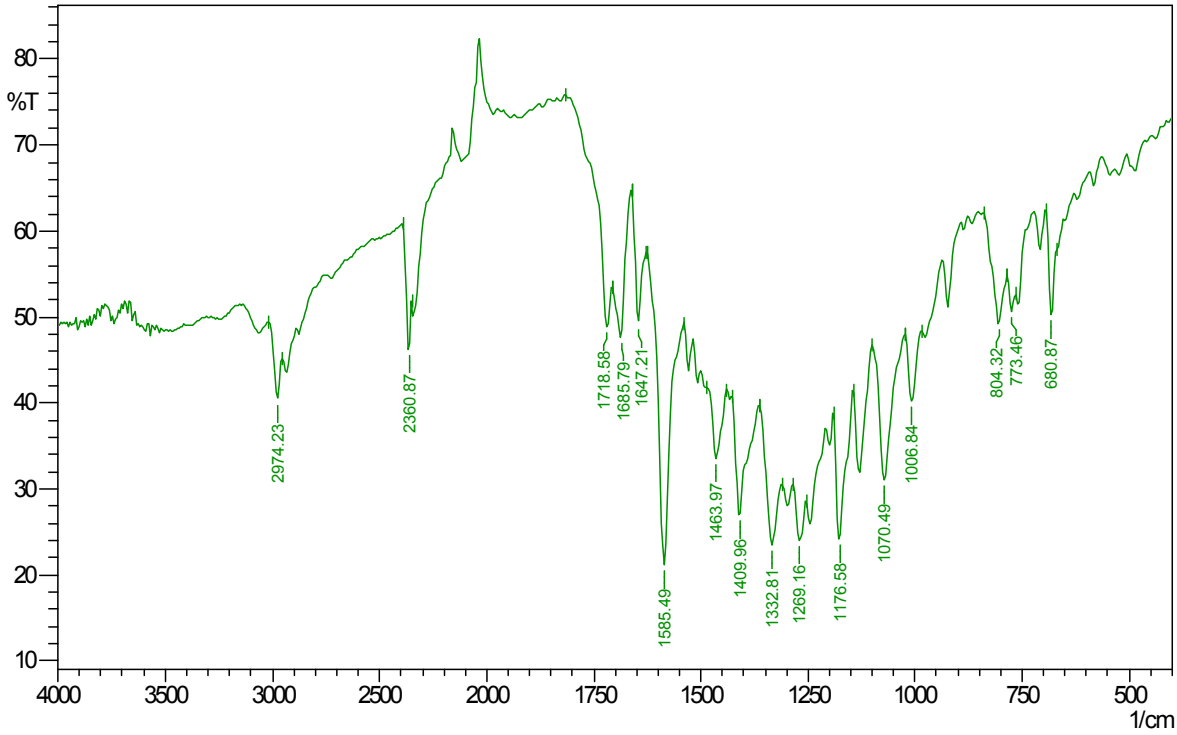


Fig.S9 FTIR spectrum of [**RDHDNAP**–Hg^{2+}] adduct

Table S1 Selected cell parameters of **RDHDNAP** and **RDHDPY**

Empirical formula	$C_{45}H_40N_4O_2$	$C_{39}H_{38}N_4O_2$
Formula weight	668.81	594.73
Temperature	296(2)	150(2)
Wavelength	0.71073	0.71073
Unit cell dimensions	9.273(4)	11.5411(14)
	16.808(7)	11.5412(10)
	23.315(9	23.608(2)
	90.00	90.00
	100.688(15)	92.592(4)
	90.00	90.00
Volume	3571(2)	3141.4(6)
Z	4	4
Density (calculated)	1.244	1.258
Absorption coefficient	0.077	0.078
F(000)	1416	1264
Crystal size		
Theta range for data collection	1.50, 26.37	1.93, 26.42
Index ranges	-11,h 9-20, k 20-28,l 29	
Reflections collected	3738	4434

Table S2 Selected bond lengths [Å] for **RDHDNAP**

O1 C2	1.386(3)	C25 C30	1.382(3)
O1 C14	1.386(3)	C26 C27	1.396(4)
C2 C7	1.377(3)	C27 C28	1.385(4)
C2 C3	1.390(3)	C28 C29	1.372(4)
C3 C4	1.403(3)	C29 C30	1.400(4)
C4 N15	1.374(3)	C30 C31	1.469(4)
C4 C5	1.400(4)	C31 O33	1.214(3)
C5 C6	1.370(4)	C31 N32	1.391(3)
C6 C7	1.398(3)	N32 N34	1.389(3)
C7 C8	1.509(3)	N34 C35	1.272(4)
C8 N32	1.506(3)	C35 C36	1.499(4)
C8 C25	1.522(4)	C36 C37	1.385(4)
C8 C9	1.524(3)	C36 C51	1.409(4)
C9 C14	1.383(3)	C37 C38	1.379(4)
C9 C10	1.385(3)	C38 C39	1.395(5)
C10 C11	1.374(4)	C39 C48	1.419(4)
C11 C12	1.417(4)	C39 C40	1.438(5)
C12 N20	1.387(3)	C40 C41	1.352(6)
C12 C13	1.396(4)	C41 C42	1.433(6)
C13 C14	1.391(3)	C42 C43	1.380(6)
N15 C16	1.466(4)	C42 C47	1.442(5)
N15 C16	1.466(4)	C43 C44	1.388(6)
N15 C18	1.469(4)	C44 C45	1.402(6)
C16 C17	1.521(5)	C45 C46	1.406(5)
C18 C19	1.504(5)	C46 C47	1.401(5)
N20 C21	1.463(4)	C46 C49	1.433(5)
N20 C23	1.481(4)	C47 C48	1.438(5)
C21 C22	1.513(5)	C48 C51	1.411(4)
C23 C24	1.444(5)	C49 C50	1.348(5)
C25 C26	1.377(4)	C50 C51	1.452(4)

Table S3 Selected bond angles [°] for **RDHDNAP**

C2 O1 C14	118.60(17)	C25 C26 C27	118.4(3)
C7 C2 O1	123.0(2)	C28 C27 C26	120.2(3)
C7 C2 C3	122.5(2)	C29 C28 C27	121.5(3)
O1 C2 C3	114.5(2)	C28 C29 C30	118.3(3)
C2 C3 C4	120.9(2)	C25 C30 C29	120.3(3)
N15 C4 C5	121.9(2)	C25 C30 C31	110.1(2)
N15 C4 C3	121.3(2)	C29 C30 C31	129.6(2)
C5 C4 C3	116.8(2)	O33 C31 N32	125.3(3)
C6 C5 C4	120.8(2)	O33 C31 C30	129.3(3)
C5 C6 C7	123.2(2)	N32 C31 C30	105.3(2)
C2 C7 C6	115.8(2)	N34 N32 C31	117.9(2)
C2 C7 C8	122.1(2)	N34 N32 C8	127.9(2)
C6 C7 C8	121.9(2)	C31 N32 C8	114.0(2)
N32 C8 C7	111.37(19)	C35 N34 N32	119.7(2)
N32 C8 C25	99.61(19)	N34 C35 C36	120.6(3)
C7 C8 C25	111.6(2)	C37 C36 C51	119.1(3)
N32 C8 C9	111.38(19)	C37 C36 C35	120.4(3)
C7 C8 C9	110.91(19)	C51 C36 C35	120.4(3)
C25 C8 C9	111.5(2)	C38 C37 C36	121.9(3)
C14 C9 C10	116.3(2)	C37 C38 C39	120.4(3)
C14 C9 C8	121.4(2)	C38 C39 C48	118.8(3)

C10 C9 C8	122.3(2)	C38 C39 C40	121.9(4)
C11 C10 C9	122.8(2)	C48 C39 C40	119.4(4)
C10 C11 C12	120.9(2)	C41 C40 C39	121.1(4)
N20 C12 C13	121.8(2)	C40 C41 C42	121.9(4)
N20 C12 C13	121.8(2)	C43 C42 C41.	123.2(5)
N20 C12 C11	121.7(2)	C43 C42 C47	118.4(4)
C13 C12 C11	116.5(2)	C41 C42 C47	118.4(4)
C14 C13 C12	120.8(2)	C42 C43 C44	122.0(5)
C9 C14 O1	123.02(19)	C43 C44 C45	119.9(5)
C9 C14 C13	122.7(2)	C44 C45 C46	119.9(5)
O1 C14 C13	114.3(2)	C47 C46 C49	118.2(3)
C4 N15 C16	121.1(2)	C45 C46 C49	122.0(4)
C4 N15 C18	120.6(2)	C46 C47 C48	120.4(3)
C16 N15 C18	118.3(2)	C46 C47 C42	119.9(4)
N15 C16 C17	115.0(3)	C48 C47 C42	119.7(4)
N15 C18 C19	112.9(3)	C51 C48 C39	120.3(3)
C12 N20 C21	120.5(3)	C51 C48 C47	120.1(3)
C12 N20 C23	121.5(2)	C39 C48 C47	119.6(3)
C21 N20 C23	117.4(2)	C50 C49 C46	122.4(3)
N20 C21 C22	113.4(3)	C49 C50 C51	120.5(3)
C24 C23 N20	111.7(3)	C36 C51 C48	119.4(3)
C26 C25 C30	121.3(3)	C36 C51 C50	122.2(3)
C26 C25 C8	127.8(2)	C48 C51 C50	118.3(3)
C30 C25 C8	110.9(2)		

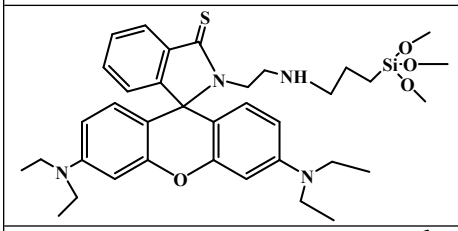
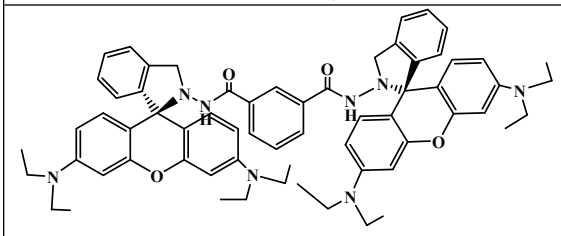
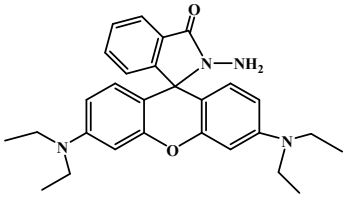
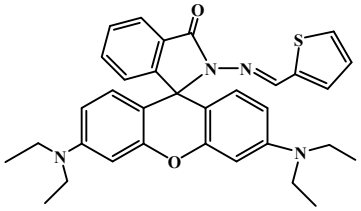
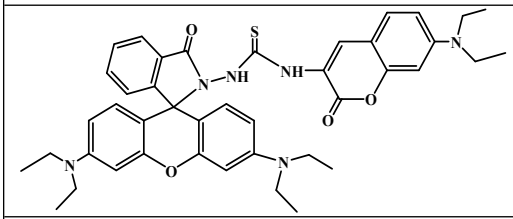
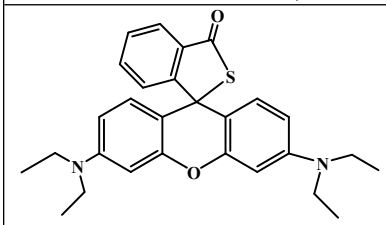
Table S4 Selected bond lengths [Å] for **RDHDPY**

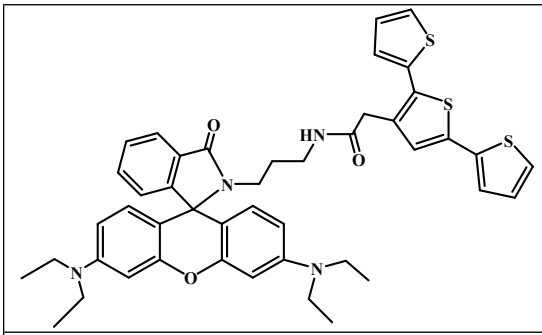
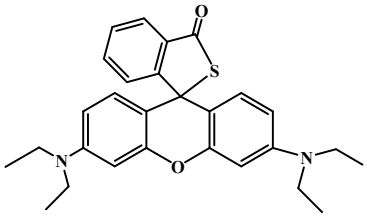
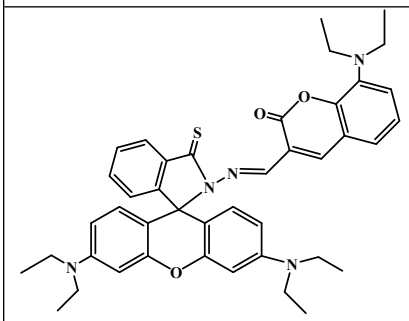
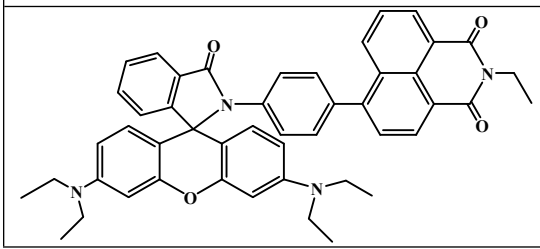
O1 C2	1.3829(19)	C21 C22	1.517(3)
O1 C14	1.3871(18)	C23 C24	1.512(3)
C2 C3	1.386(2) .	C25 C30	1.380(2)
C2 C7	1.388(2)	C25 C26	1.386(2)
C3 C4	1.402(2)	C26 C27	1.379(2)
C4 N15	1.379(2)	C27 C28	1.388(3)
C4 C5	1.417(2)	C28 C29	1.380(3)
C5 C6	1.374(2)	C29 C30	1.390(2)
C6 C7	1.395(2)	C30 C31	1.469(2)
C7 C8	1.519(2)	C31 O33	1.220(2)
C8 N32	1.494(2)	C31 N32	1.386(2)
C8 C9	1.517(2)	N32 N34	1.3760(19)
C8 C25	1.527(2)	N34 C35	1.281(2)
C9 C14	1.388(2)	C35 C36	1.469(2)
C9 C10	1.391(2)	C36 C45	1.373(2)
C10 C11	1.377(2)	C36 C37	1.431(2)
C11 C12	1.410(2)	C37 C38	1.416(2)
C12 N20	1.379(2)	C37 C42	1.426(2)
C12 C13	1.399(2)	C38 C39	1.368(3)
C13 C14	1.386(2)	C39 C40	1.408(3)
N15 C18	1.451(2)	C40 C41	1.355(3)
N15 C16	1.458(2)	C41 C42	1.419(3)
C16 C17	1.471(3)	C42 C43	1.414(3)
C18 C19	1.518(3)	C43 C44	1.364(3)
N20 C23	1.464(2)	C44 C45	1.400(3)
N20 C21	1.464(2)		

Table S5 Selected bond angles [°] for **RDHDPY**

C2 O1 C14	117.68(12)	C23 N20 C21	117.25(14)
O1 C2 C3	114.79(14)	N20 C21 C22	114.34(16)
O1 C2 C7	122.52(14)	N20 C23 C24	114.16(16)
C3 C2 C7	122.64(15)	C30 C25 C26	120.30(15)
C2 C3 C4	120.67(15)	C30 C25 C8	111.16(15)
N15 C4 C3	121.14(15)	C26 C25 C8	128.53(15)
N15 C4 C5	121.83(15)	C27 C26 C25	118.08(17)
C3 C4 C5	117.03(14)	C26 C27 C28	121.52(18).
C6 C5 C4	120.46(16)	C29 C28 C27	120.65(16)
C5 C6 C7	122.95(15)	C28 C29 C30	117.62(17)
C2 C7 C6	116.14(14)	C25 C30 C29	121.82(17)
C2 C7 C8	120.83(14)	C25 C30 C31	109.30(14)
C6 C7 C8	123.03(14)	C29 C30 C31	128.82(16)
N32 C8 C7	112.44(13)	O33 C31 N32	125.89(16)
C9 C8 C7	109.82(12)	O33 C31 C30	128.19(15)
N32 C8 C25	99.53(12)	N32 C31 C30	105.92(14)
C9 C8 C25	111.43(14)	N34 N32 C31	117.24(14)
C7 C8 C25	110.64(12)	N34 N32 C8	127.55(12)
C14 C9 C10	116.15(15)	C31 N32 C8	114.07(13)
C14 C9 C8	120.60(14)	C35 N34 N32	118.14(14)
C10 C9 C8	123.03(14)	N34 C35 C36	119.11(16)
C11 C10 C9	122.50(15)	C45 C36 C37	120.05(17)
C10 C11 C12	121.11(15)	C45 C36 C35	120.89(16)
N20 C12 C13	121.64(15)	C37 C36 C35	119.07(15)
N20 C12 C11	121.75(15)	C38 C37 C42	118.46(16)
C13 C12 C11	116.61(15)	C38 C37 C36	122.97(16)
C14 C13 C12	120.94(15)	C42 C37 C36	118.51(16)
C13 C14 O1	114.99(14)	C39 C38 C37	120.93(18)
C13 C14 C9	122.58(14)	C38 C39 C40	120.34(19)
O1 C14 C9	122.43(14)	C41 C40 C39	120.44(19)
C4 N15 C18	121.40(15)	C40 C41 C42	121.00(18)
C4 N15 C16	122.39(14)	C43 C42 C41	122.05(17)
C18 N15 C16	115.98(14)	C43 C42 C37	119.12(17)
N15 C16 C17	113.44(17)	C41 C42 C37	118.82(17)
N15 C18 C19	114.25(16)	C44 C43 C42	120.92(18)
C12 N20 C23	120.32(14)	C43 C44 C45	120.44(18)
C12 N20 C21	120.79(15)	C36 C45 C44	120.94(18)

Table S6. Detection limits along with related important parameters of reported rhodamine based Hg^{2+} sensors useful for cell imaging studies

Probe	**Medium**	**LOD**	**Cell imaging**	**Reference**
	HEPES buffered water	$1X10^{-6}M$	NA	1
	CH_3CN:H2O (3:1, *v*/*v*)	$4X10^{-7}M$	NA	2
	H_2O:CH_3OH (90:10, v/v)	$2X10^{-7}M$	NA	3
	Ethanol:H_2O (50 : 50, v/v)	$1.72X10^{-9}M$	MCF-7	4
	HEPES:CH_3CN, 20:80 (v/v)	0.42 ppb	A375 cells	5
	Na_2HPO_4–NaH_2PO_4 buffer (pH 7) containing 0.5% (v/v) of 1,4-dioxane.	$2.0X10^{-8}M$	NA	6

	DMSO:H_2O 50:50(v/v)	1.34X10^{-6}M	NA	7
	H_2O:MeCN 99 : 1 (v/v)	2.1 X10^{-9}M	NA	8
	Water:ethanol 50:50 (v/v)	4.0×10^{-8}M	NA	9
	THF:H_2O (9.5:0.5, v/v)	2X10^{-6}	PC3	10

NA = Not available/ not performed

References

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