Electronic Supplementary Information for

Fluorometric sensing of hydroxylamine in an aqueous medium utilizing a diphenyl imidazole-based probe

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1. Materials and preparation of the test samples

All the solvents and reagents (analytical grade and spectroscopic grade) were obtained from Merck (India), Spectrochem Pvt. Limited (India) and Sigma-Aldrich and were used without further purification. A 1.0 mM **DIB** stock solution was prepared in UV-grade dimethyl sulphoxide (DMSO) and subsequently diluted to 50 μM in 5% DMSO phosphate buffer solution at pH 7.4 for various photophysical studies. Stock solutions of metal ions such as Ag^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Cr^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Pd^{2+}, Zn^{2+} were prepared at 1.0 mM concentration in Millipore water from their respective perchlorate, nitrate or chlorides salts for cation interaction studies. Similarly for anionic interaction studies, 1.0 mM stock solutions of anions such as F^{-}, Cl^{-}, Br^{-}, I^{-}, H_2PO4^{-}, $HSO4^{-}$,OH^{-}, AcO^{-} (as tetrabutyl ammonium salts), CN^{-} (as KCN salt) and S^{2-} (as Na_2S salt) were prepared in Millipore water. The interaction of neutral amines such as urea, thiourea, aniline, triethylamine, ammonium hydroxide, hydroxyl amine, ethylene diamine, piperidine, hydrazine hydrate and methyl amine were studied by preparing stock solutions in the range of 10-100 mM concentration in Millipore water. The aggregation induced emission (AIE) characteristics of **DIB** (50 μM) was studied in UV-grade DMSO with varying water fractions. The solvatofluorism behaviour of **DIB** (50 μM) was examined with various solvents such as benzene, toluene, tetrahydrofuran, dioxane, acetone, methanol, acetonitrile and DMSO.

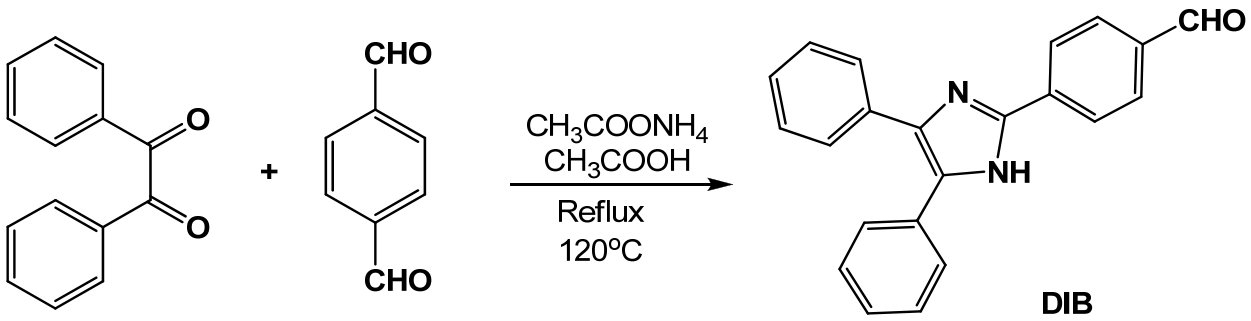
2. General methods

^{1}H NMR was recorded on an Avance III-400 MHz Bruker spectrometer. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent (DMSO-d_6: 2.5 ppm) resonance as the internal standard. Data are reported as follows: chemical shifts, multiplicity (s singlet, (d=doublet, t=triplet, m=multiplet), coupling constant (Hz). ^{13}C NMR (100 MHz) spectra were recorded on an Avance III–400 MHz Bruker spectrometer in proton decoupling mode. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (DMSO-d_6: 39.51 ppm). Fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. Hydrodynamic diameters were measured using Malvern Zetasizer instrument. Mass spectrum was measured on Xevo G2S QTof Mass analyser (Waters). FTIR spectra were recorded on Eco-ATR, Alpha, Bruker Optik GmbH, Ettlingen, Germany. pH readings were measured on UTECH CON-700 digital pH meter. Elemental analysis was carried out by using Elementar Vario EL III CHNS. Chromatographic purification

was done using 60–120 mesh silica gels (Merck). For reaction monitoring, manually coated silica gel-60 thin layer chromatography (TLC) glass plates were used.

3. Synthesis of the probe DIB

The probe 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzaldehyde (**DIB**) was synthesized through a straightforward protocol as depicted in Scheme S1. 0.2 gm (0.95 mmol) of benzil and 0.1276 gm (0.95 mmol) of terephthaldehyde were taken in a 100 ml round bottom flask and dissolved in 30 ml of glacial acetic acid. Then the mixture was kept at room temperature (27 ^{o}C) for 2 hours under continuous stirring. After two hours, ammonium acetate (0.5 gm) was added and the mixture was further refluxed at 120 ^{o}C on an oil bath till completion of the reaction as monitored by thin layer chromatography. After end of the starting materials, the reaction mixture was poured into ice cold water and the precipitates obtained were washed with water and dried. Then the residues were purified by silica gel column chromatography with hexane/ethylacetate (95:5, v/v) as eluent to afford the probe **DIB** as yellow colour solid (154 mg, 47%) MP: 237-239°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 13.03 (1H, s, NH), 10.03 (1H, s, CHO), 8.30 (2H, d, J = 8 Hz, Ar-H), 8.01 (2H, d, J = 8 Hz, Ar-H), 7.58-7.23 (m, 10H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 193.0, 144.7, 138.6, 135.9, 135.8, 135.2, 131.1, 130.6, 130.0, 129.2, 129.0, 128.8, 128.6, 127.6, 127.3, 125.9. FTIR (KBr, υ cm^{-1}) 3355 (-NH str.), 1695 (-C=O str), 1442 (C=C str), 1203, 1164, 1073, 969, 833, 775, 697. ESI-MS *m/z*: calcd for $C_{22}H_{16}N_2O$ $[M+H]^+$: 325.38; found 325.33. Elemental analysis: $C_{22}H_{16}N_2O$: calcd. C 81.46, H 4.97, N 8.64; found: C 81.35, H 5.07, N 8.53%.



Scheme S1. Synthesis protocol for probe **DIB**

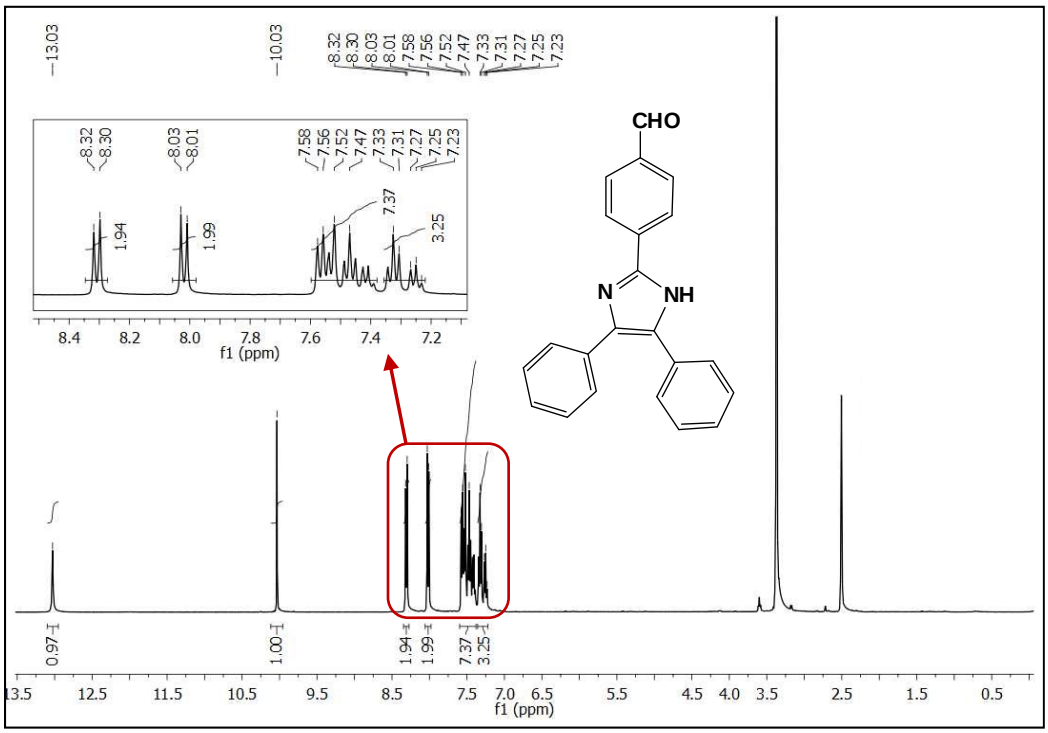


Fig. S1. ^{1}H NMR spectrum of **DIB in** DMSO-d_6

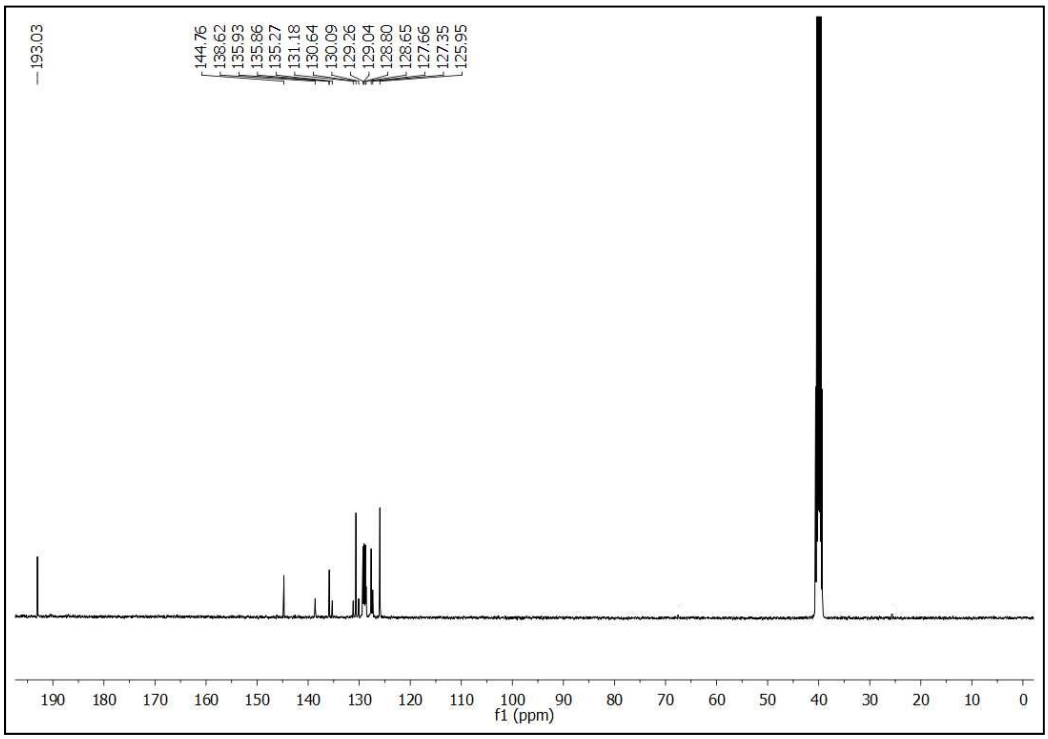


Fig. S2. ^{13}C NMR spectrum of **DIB in** DMSO-d_6

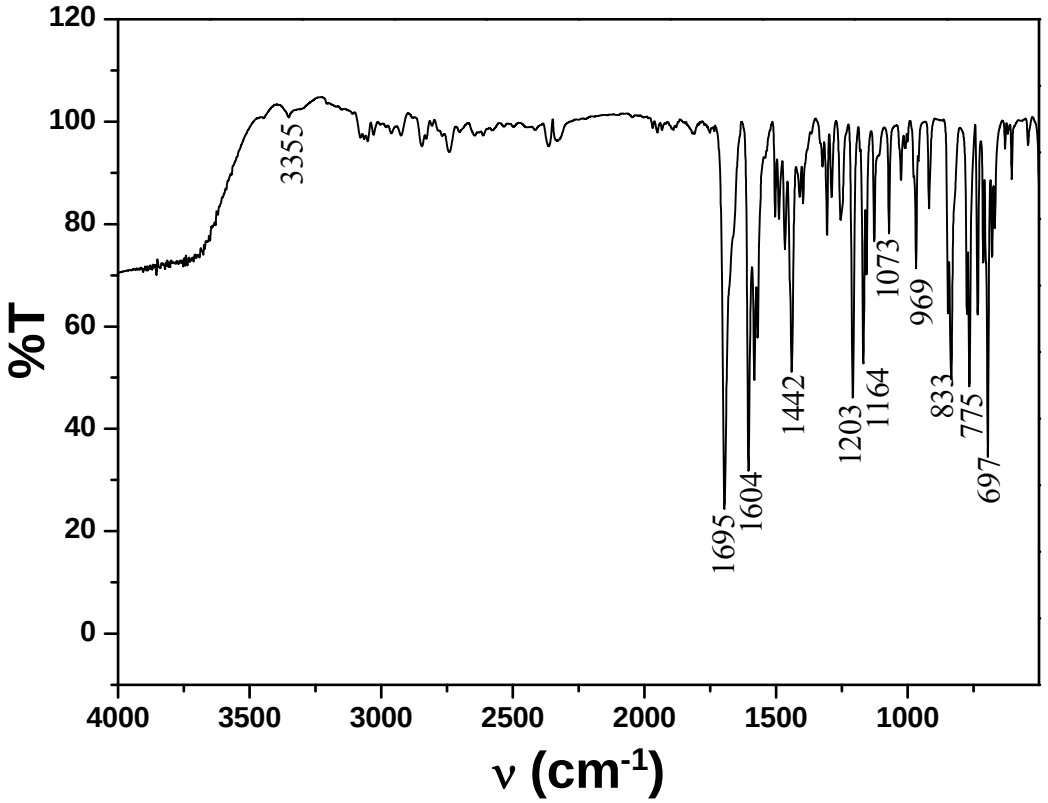


Fig. S3. FTIR spectra of **DIB**

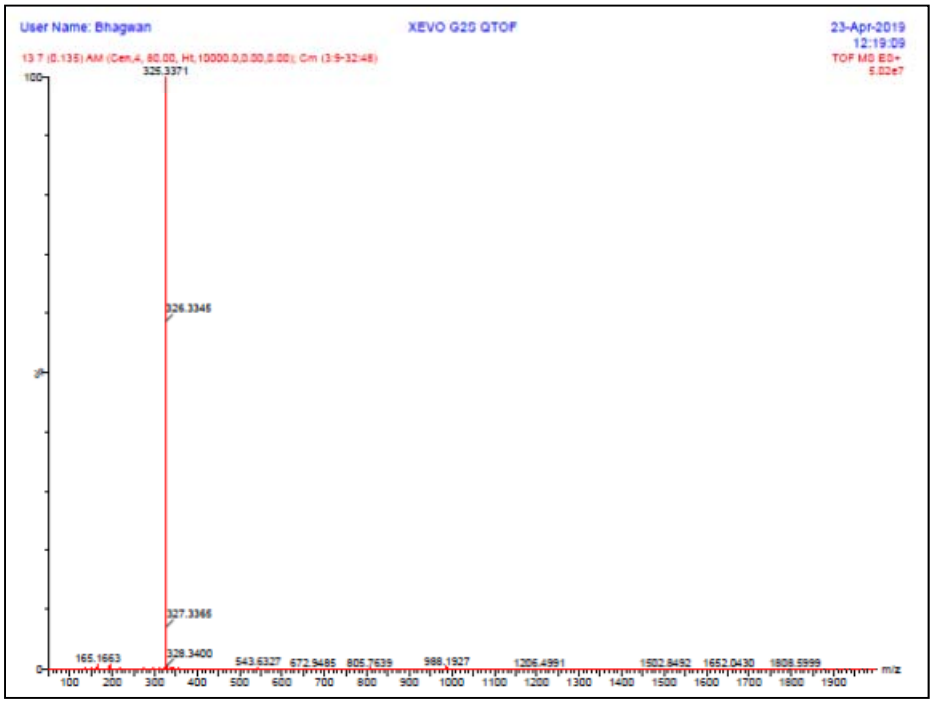


Fig. S4. ESI mass spectrum of **DIB**

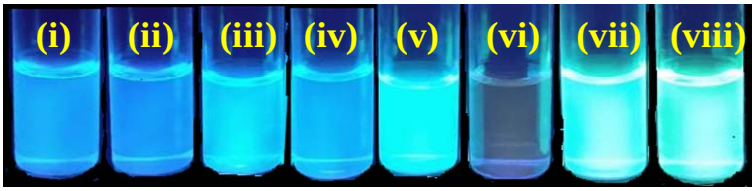


Fig. S5. Visual photograph of **DIB (**50 μM) under UV light at 365 nm in (i) benzene (ii) toluene (iii) tetrahydrofuran (THF) (iv) dioxane (v) acetone (vi) methanol (vii) acetonitrile (viii) dimethyl sulphoxide (DMSO) solvent medium.

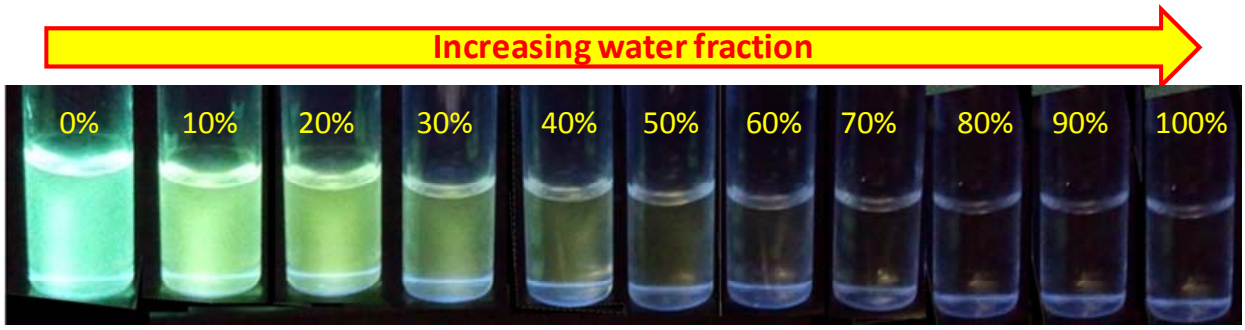


Fig. S6. Visual observation of probe **DIB** (50 μM) at various DMSO-Water fractions under UV-lamp at 365 nm.

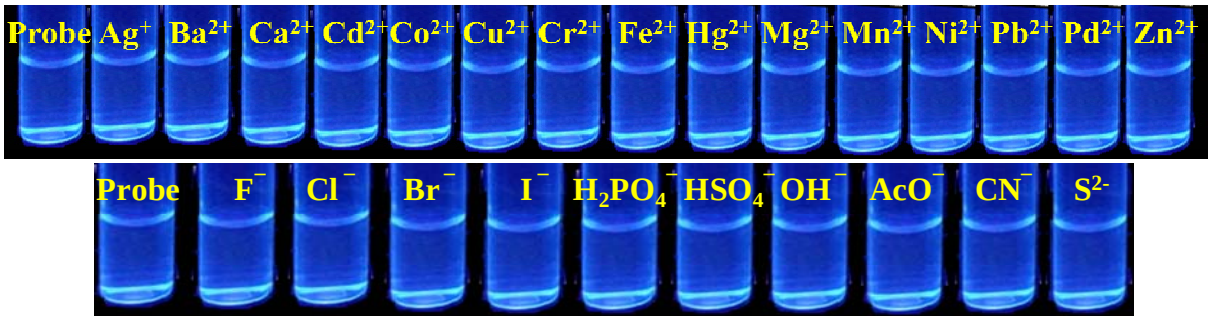


Fig. S7. Visual observation of interaction of **DIB (**50 μM**)** in 5% DMSO phosphate buffer solution at pH 7.4 with various metal ions and anions (20 equiv.) under UV light of 365 nm.

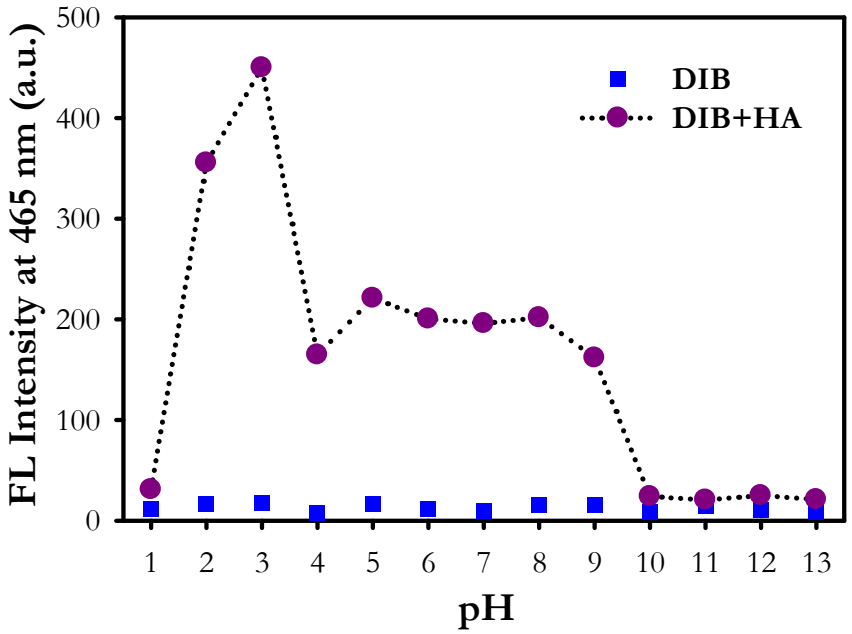


Fig. S8. Fluorescence intensity of **DIB** (50 μM) in absence (blue square) and in presence (purple circle) of HA (20 equiv.) at 465 nm within pH 1-13.

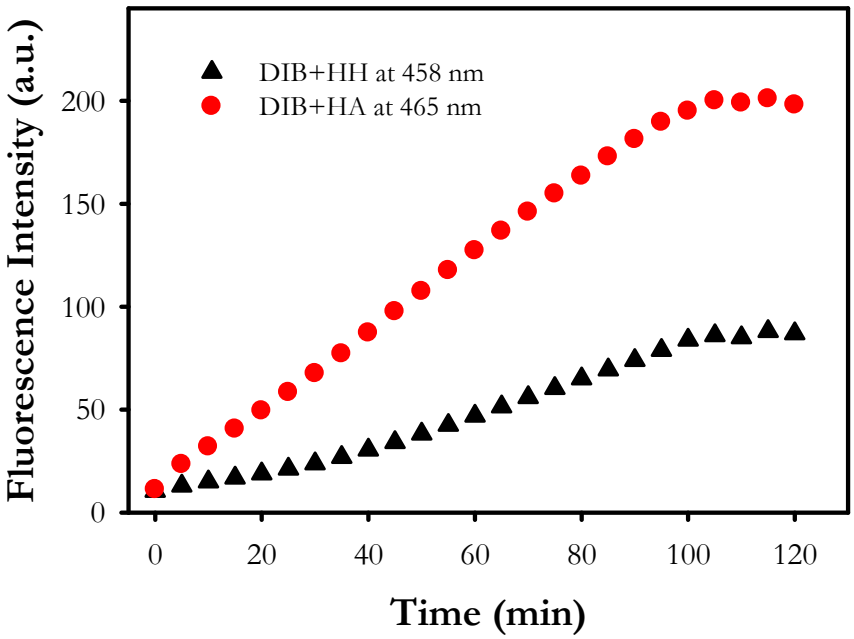


Fig. S9. Fluorescence response time of **DIB** (50 μM) in 5% DMSO phosphate buffer solution at pH 7.4 in presence of 20 equiv. of hydroxyl amine (HA) and hydrazine hydrate (HH) studied upto 120 minutes.

Fig. S10. Fluorescence colour changes for **DIB** in presence of 0.0 to 20.0 equiv. of HA after 2 hrs under UV-light at 365 nm in 5% DMSO phosphate buffer solution at pH 7.4.

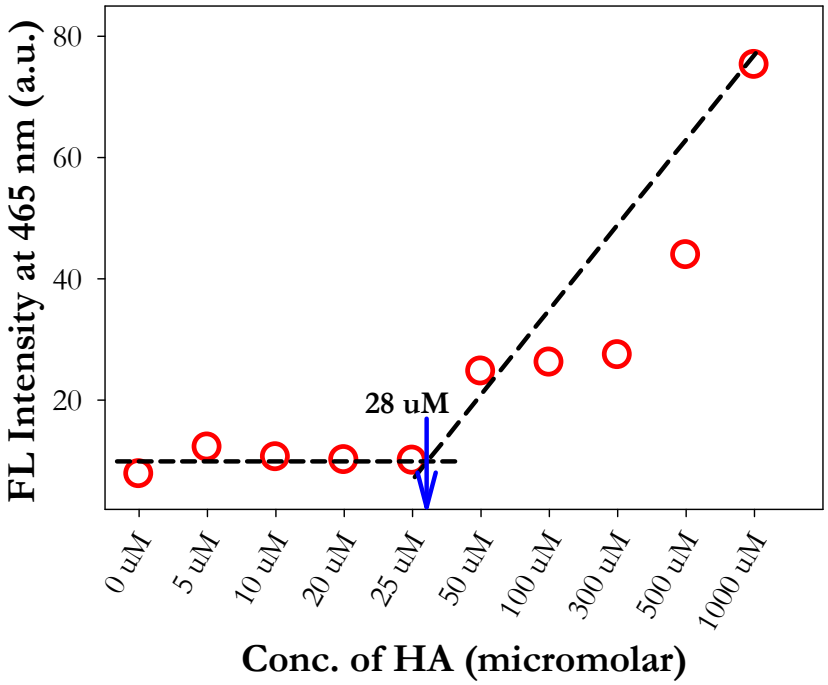


Fig. S11. Fluorescence intensity of DIB (50 μM) in 5% DMSO phosphate buffer solution at pH 7.4 in presence various concentration of HA.

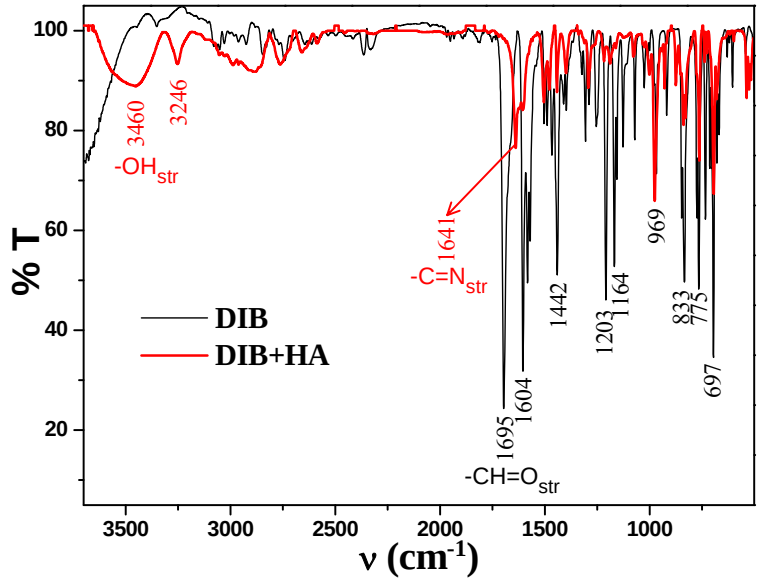


Fig. S12. Overlay FTIR spectra of **DIB** and Product of **DIB**+HA.

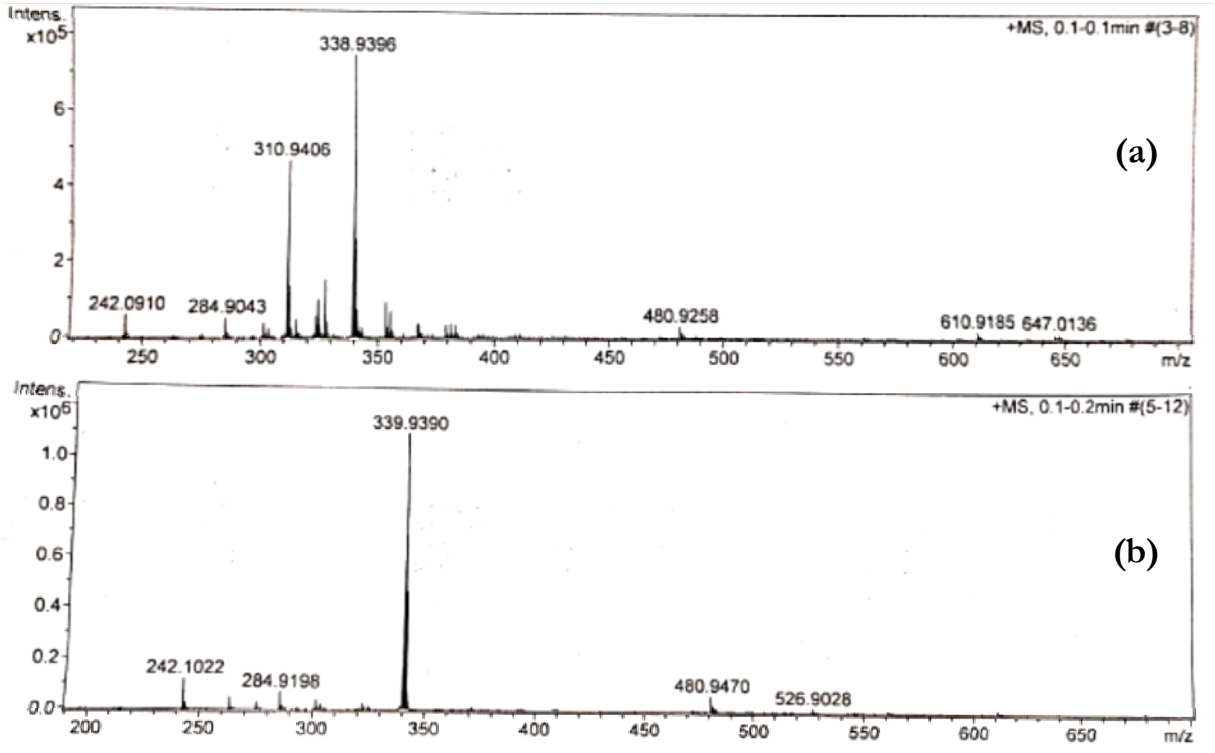


Fig. S13. ESI-MS of (a) hydrazone product (**C**) and (b) oxime product (**D**).

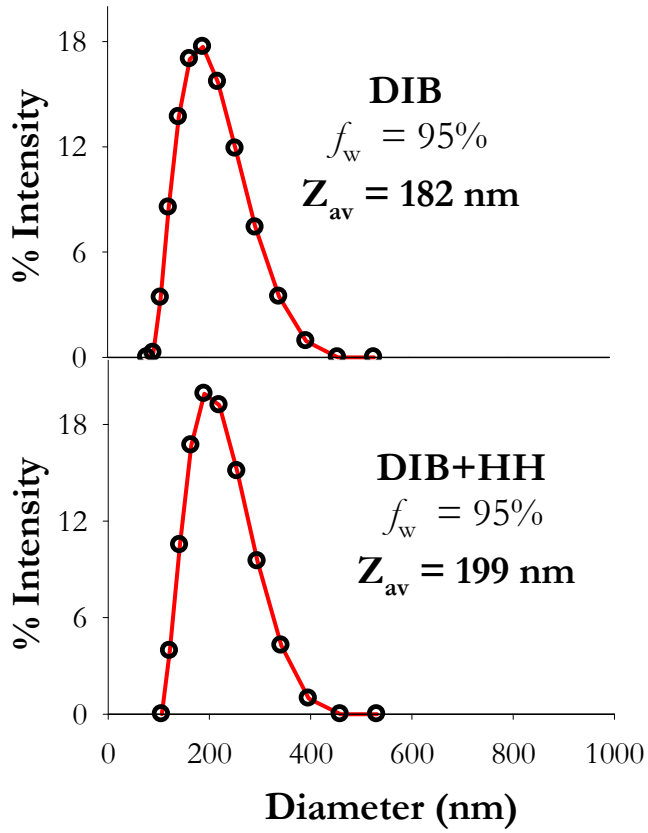


Fig. S14. Size distribution by DLS of **DIB** (50 μM, Top Fig.) and **DIB**+HH (50 μM each, Bottom Fig.) after an incubation period of 5 hr in 5% DMSO in Water.

Atomic coordinates of optimized structures

Coordinates of DIB probe

No	Atom	x-	y-	z-
1	N	-1.896	2.333	0
2	C	-2.753	3.447	0.064
3	N	-2.027	4.565	0.139
4	C	-0.675	4.185	0.112
5	C	-0.576	2.763	0.067
6	H	-2.189	1.372	0.076
7	C	0.331	5.211	0.023
8	C	-0.033	6.546	0.379
9	C	1.655	4.976	-0.457
10	C	0.896	7.576	0.309
11	H	-1.048	6.722	0.712
12	C	2.572	6.017	-0.533
13	H	1.935	3.988	-0.799
14	C	2.205	7.32	-0.143
15	H	0.611	8.581	0.598
16	H	3.571	5.828	-0.914
17	H	2.927	8.128	-0.205
18	C	0.522	1.818	0.131
19	C	0.449	0.587	-0.573
20	C	1.658	2.059	0.948
21	C	1.479	-0.35	-0.479
22	H	-0.397	0.391	-1.226
23	C	2.682	1.116	1.036
24	H	1.707	2.967	1.538
25	C	2.601	-0.091	0.323
26	H	1.413	-1.278	-1.038
27	H	3.537	1.312	1.676
28	H	3.399	-0.823	0.398
29	C	-4.204	3.347	0.06
30	C	-4.865	2.093	-0.045
31	C	-4.992	4.521	0.173
32	C	-6.252	2.021	-0.033
33	H	-4.3	1.168	-0.147
34	C	-6.377	4.451	0.186
35	H	-4.489	5.48	0.249
36	C	-7.046	3.194	0.084
37	H	-6.746	1.056	-0.117
38	H	-6.983	5.346	0.273
39	C	-8.496	3.11	0.096
40	H	-8.922	2.095	0.011
41	O	-9.276	4.111	0.198

Coordinates of Intermediate A

No	Atom	x-	y-	z-
1	N	-0.146	0.812	0
2	C	-1.016	1.921	0.158
3	N	-0.29	3.051	0.283
4	C	1.038	2.709	0.206
5	C	1.171	1.263	0.089
6	H	-0.41	-0.149	0.163
7	C	2.051	3.737	0.12
8	C	1.757	5.038	0.614
9	C	3.311	3.52	-0.507
10	C	2.694	6.061	0.521
11	H	0.788	5.207	1.069
12	C	4.239	4.558	-0.607
13	H	3.534	2.556	-0.948
14	C	3.944	5.827	-0.087
15	H	2.461	7.044	0.917
16	H	5.19	4.381	-1.1
17	H	4.671	6.629	-0.162
18	C	2.275	0.359	0.152
19	C	2.161	-0.96	-0.408
20	C	3.487	0.681	0.851
21	C	3.208	-1.869	-0.307
22	H	1.274	-1.227	-0.975
23	C	4.521	-0.244	0.943
24	H	3.572	1.634	1.36
25	C	4.401	-1.523	0.363
26	H	3.109	-2.852	-0.758
27	H	5.423	0.018	1.488
28	H	5.211	-2.241	0.444
29	C	-2.441	1.819	0.153
30	C	-3.104	0.576	-0.085
31	C	-3.241	2.97	0.403
32	C	-4.494	0.504	-0.064
33	H	-2.534	-0.322	-0.305
34	C	-4.625	2.883	0.42
35	H	-2.743	3.917	0.58
36	C	-5.281	1.644	0.186
37	H	-4.982	-0.45	-0.242
38	H	-5.218	3.772	0.611
39	C	-6.783	1.533	0.243
40	H	-7.11	0.553	-0.135
41	O	-7.3	1.492	1.625
42	H	-7.051	2.322	2.086
43	N	-7.411	2.641	-0.461
44	N	-8.803	2.565	-0.725
45	H	-9.11	1.672	-1.113
46	H	-9.35	2.844	0.08
47	H	-6.894	3.014	-1.247

Coordinates of Intermediate B

No	Atom	x-	y-	z-
1	N	-1.208	1.375	0
2	C	-2.074	2.489	0.149
3	N	-1.342	3.617	0.268
4	C	-0.014	3.264	0.193
5	C	0.111	1.821	0.086
6	H	-1.478	0.416	0.171
7	C	1.001	4.289	0.105
8	C	0.707	5.593	0.594
9	C	2.261	4.069	-0.521
10	C	1.646	6.613	0.5
11	H	-0.263	5.763	1.046
12	C	3.191	5.105	-0.621
13	H	2.483	3.104	-0.959
14	C	2.897	6.376	-0.105
15	H	1.414	7.598	0.893
16	H	4.142	4.926	-1.113
17	H	3.625	7.177	-0.181
18	C	1.211	0.909	0.146
19	C	1.088	-0.406	-0.417
20	C	2.424	1.223	0.843
21	C	2.13	-1.322	-0.321
22	H	0.197	-0.666	-0.982
23	C	3.454	0.292	0.93
24	H	2.517	2.175	1.353
25	C	3.325	-0.985	0.347
26	H	2.024	-2.303	-0.774
27	H	4.359	0.548	1.474
28	H	4.131	-1.707	0.425
29	C	-3.499	2.391	0.149
30	C	-4.166	1.146	-0.075
31	C	-4.296	3.547	0.391
32	C	-5.555	1.076	-0.049
33	H	-3.599	0.245	-0.291
34	C	-5.68	3.463	0.416
35	H	-3.796	4.495	0.555
36	C	-6.34	2.221	0.196
37	H	-6.046	0.122	-0.22
38	H	-6.27	4.357	0.594
39	C	-7.84	2.111	0.254
40	H	-8.187	1.144	-0.12
41	O	-8.378	2.143	1.619
42	H	-8.026	2.927	2.093
43	N	-8.45	3.223	-0.49
44	H	-8.027	3.349	-1.41
45	O	-9.861	2.886	-0.806
46	H	-10.374	3.305	-0.085

Coordinates of Product C

No	Atom	x-	y-	z-
1	N	-3.438	2.646	0
2	C	-4.197	1.462	-0.075
3	N	-3.352	0.397	-0.135
4	C	-2.069	0.881	-0.102
5	C	-2.083	2.321	-0.059
6	H	-3.8	3.576	-0.144
7	C	-0.947	-0.047	-0.02
8	C	-1.092	-1.345	-0.567
9	C	0.259	0.274	0.648
10	C	-0.056	-2.274	-0.479
11	H	-2.027	-1.598	-1.054
12	C	1.29	-0.664	0.743
13	H	0.373	1.244	1.119
14	C	1.143	-1.938	0.174
15	H	-0.178	-3.261	-0.913
16	H	2.204	-0.406	1.269
17	H	1.947	-2.663	0.245
18	C	-1.069	3.339	-0.133
19	C	-1.314	4.637	0.414
20	C	0.167	3.129	-0.813
21	C	-0.363	5.649	0.307
22	H	-2.229	4.822	0.971
23	C	1.108	4.15	-0.915
24	H	0.358	2.174	-1.29
25	C	0.856	5.418	-0.355
26	H	-0.564	6.621	0.748
27	H	2.036	3.969	-1.448
28	H	1.593	6.211	-0.44
29	C	-5.617	1.414	-0.073
30	C	-6.421	2.591	0.061
31	C	-6.291	0.157	-0.221
32	C	-7.8	2.524	0.041
33	H	-5.95	3.562	0.198
34	C	-7.67	0.096	-0.241
35	H	-5.691	-0.741	-0.316
36	C	-8.473	1.272	-0.114
37	H	-8.399	3.421	0.148
38	H	-8.163	-0.867	-0.355
39	C	-9.905	1.178	-0.139
40	H	-10.346	0.18	-0.258
41	N	-10.684	2.238	-0.026
42	N	-12.035	2.041	-0.063
43	H	-12.453	1.121	-0.154
44	H	-12.61	2.858	0.038

Coordinates of Product D

No	Atom	x-	y-	z-
1	N	-0.167	0.333	0
2	C	0.594	-0.849	0.066
3	N	-0.237	-1.909	0.132
4	C	-1.537	-1.419	0.106
5	C	-1.519	0.014	0.066
6	H	0.206	1.265	0.102
7	C	-2.641	-2.351	0.022
8	C	-2.427	-3.692	0.446
9	C	-3.909	-2.009	-0.525
10	C	-3.451	-4.631	0.372
11	H	-1.45	-3.955	0.832
12	C	-4.924	-2.959	-0.607
13	H	-4.076	-1.014	-0.917
14	C	-4.706	-4.271	-0.15
15	H	-3.277	-5.646	0.714
16	H	-5.881	-2.688	-1.039
17	H	-5.501	-5.008	-0.214
18	C	-2.532	1.041	0.134
19	C	-2.325	2.295	-0.506
20	C	-3.721	0.867	0.896
21	C	-3.275	3.311	-0.41
22	H	-1.44	2.446	-1.118
23	C	-4.664	1.89	0.987
24	H	-3.874	-0.057	1.442
25	C	-4.451	3.116	0.334
26	H	-3.106	4.254	-0.921
27	H	-5.558	1.742	1.583
28	H	-5.186	3.911	0.41
29	C	2.031	-0.881	0.068
30	C	2.815	0.302	-0.051
31	C	2.712	-2.128	0.203
32	C	4.198	0.254	-0.03
33	H	2.336	1.272	-0.175
34	C	4.094	-2.177	0.226
35	H	2.125	-3.036	0.287
36	C	4.886	-0.993	0.113
37	H	4.783	1.162	-0.125
38	H	4.597	-3.135	0.331
39	C	6.321	-1.074	0.142
40	H	6.793	-2.05	0.249
41	N	7.079	0.002	0.042
42	O	8.496	-0.422	0.106
43	H	8.984	0.422	0.027