Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

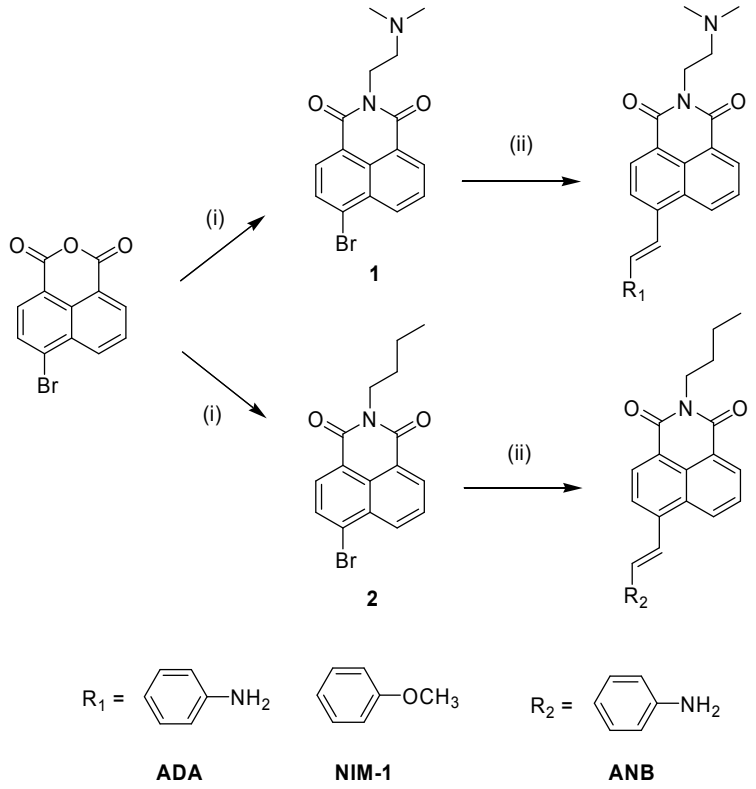
Supporting Information

for

A fluorescent pH probe for acidic organelle in living cells

General procedure for the synthesis of naphthalimide derivatives (Scheme 1).

Synthesis of the naphthalimide derivative is shown in the steps (i), (ii) of **Scheme 1**. The first stage of the reaction, in which commercial starting material 4-bromo-1,8-naphthalic anhydride was reacted with N,N-dimethylethane-1,2-diamine or N-butylamine, was performed conveniently in ethanol under room temperature. Next, the samples were subjected to the Heck coupling reaction with 4-vinylaniline or 4-methoxystyrene under catalyst $Pd(OAc)_2$. Details of the synthesis of NIM-1 [1] and ABN [2] are described in the previous results.



Scheme 1 Reagents and conditions: (i) N,N-dimethylethane-1,2-diamine (ADA, NIM-1) or n-butylamine (ANB), EtOH, r.t. 24 h; (ii) $Pd(OAc)_2$/(*o*-tol)$_3$P, 4-vinylaniline (ADA, ANB), 4-methoxystyrene (NIM-1), Et_3N/MeCN, N_2, reflux, 48 h. (ref: (1) H. H. Lin, Y. C. Chan, J. W. Chen and C. C. Chang, J. Mater. Chem., 2011, 21, 3170-3177. (2) Y. T. Tung, C. C. Chang, Y. L. Lin, S. L. Hsieh, G. J. Wang, Biosensors and Bioelectronics, 2016, 77, 90–98.)

2-(6-bromo-1, 3-dioxo-1H-benzo[de] isoquinolin-2(3H)-yl)-N, N-dimethyl ethanamine ***(compound 1)****:*

4-Bromo-1, 8-naphthalic anhydride (10 mmole) and N, N-dimethylethane-1, 2-diamine (12 mmole) was stirred in ethanol solution (20 mL) under room temperature for 12h. The system was filtered to remove the excess N, N-dimethylethane-1, 2-diamine and the collected precipitate was crystallized from acetone/ethanol to get white solid (yield: 70%). Data for **compound 1**: ^{1}HNMR (400Hz, $CDCl_3$, δ in ppm)= 8.65 (d, J = 8

Hz, 1H), 8.56 (d, *J* = 8 Hz, 1H), 8.41 (d, *J* = 8 Hz, 1H), 8.03 (d, *J* = 8 Hz, 1H), 7.84 (t, *J* = 8 Hz, 1H), 4.34 (t, *J* = 8 Hz, 2H), 2.72 (t, *J* = 8 Hz, 2H), 2.40 (s, 6H).

N-butyl-4-bromo-1,8-naphthalimide ***(compound 2)****:*

4-Bromo-1, 8-naphthalic anhydride (10 mmole) and N-butylamine (12 mmole) was stirred in ethanol solution (20 mL) under room temperature for 12h. The system was filtered to remove the excess amine and the collected precipitate was crystallized from acetone/ethanol to get white solid (yield: 78%). Data for **compound 2**: ^{1}HNMR (400Hz, $CDCl_3$, δ in ppm)= 8.65 (d, *J* = 8 Hz, 1H), 8.56 (d, *J* = 8 Hz, 1H), 8.41 (d, *J* = 8 Hz, 1H), 8.03 (d, *J* = 8 Hz, 1H), 7.85 (t, J = 8 Hz, 1H), 4.17 (t, J = 8 Hz, 2H), 1.69 (m, 2H), 1.44 (m, 2H), 0.97 (t, J = 8 Hz, 3H).

*2-(6-(4-aminostyryl)-1,3-dioxo-1H-benzo[de] isoquinolin-2(3H)-yl)-N, N-dimethylethanamine (****ADA****):*

Compound **1** (5 mmole) was added into to a high pressure bottle containing the a mixture of palladium (II) acetate (8 mg, Strem) and tri-*o*-tolyl phosphine (80 mg, Aldrich), then to which was added the solvent pair (triethylamine 5 ml / acetonitrile 15 ml) and 4-vinylaniline (7 mmole, Acros). The bottle was then sealed after bubbling with nitrogen for 10 min. After keeping the system under ~105°C for 48 h, the system was cooled to room temperature and then extracted with CH_2Cl_2 / H_2O twice. The organic layer was then dried by with $MgSO_4$ and evaporated in vacuum. The residue was subjected to chromatography on a silica gel by using acetone / hexane (1/2, Rf = 0.4). The deep-red solid was then obtained by recrystallizing with acetone / hexane (yield: 50%). Data for **ADA**: ^{1}H NMR（400 Hz, DMSO-d_6）：δ = 8.96 (d, *J* = 8 Hz, 1H), 8.51 (d, *J* = 8 Hz, 1H), 8.41 (d, *J* = 8 Hz, 1H), 8.15 (d, *J* = 8 Hz, 1H), 7.86 (d, *J* = 8 Hz, 1H), 7.86 (t, *J* = 16 Hz, 1H), 7.55 (d, *J* = 6.8 Hz, 2H), 7.47 (d, *J* = 16 Hz, 1H), 6.61 (d, *J* = 6.8 Hz, 2H), 5.59 (s, 2H), 4.15 (t, *J* = 8 Hz, 2H), 2.50 (t, *J* = 8 Hz, 2H), 2.20 (s, 6H) ppm. ^{13}C NMR (400.00 MHz, DMSO-d6): δ= 164.10, 163.77, 152.06, 150.76, 142.76, 140.77, 137.72, 136.89, 136.64, 131.48, 129.98, 129.67, 129.30, 128.80, 127.28, 127.01, 124.96, 122.80, 122.54, 119.72, 117.44, 117.18, 114.99, 114.57, 114.35, 110.37, 106.46, 101.15, 57.38, 57.16, 46.38, 46.16, 45.95 ppm. HRMS (ESI, m/z): $[M+H]^+$ 386.18; found, 386.7. EA: Anal. Calcd. For $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90 (%). Found: C, 73.75; H, 6.09; N, 10.75 (%).

Figure S1

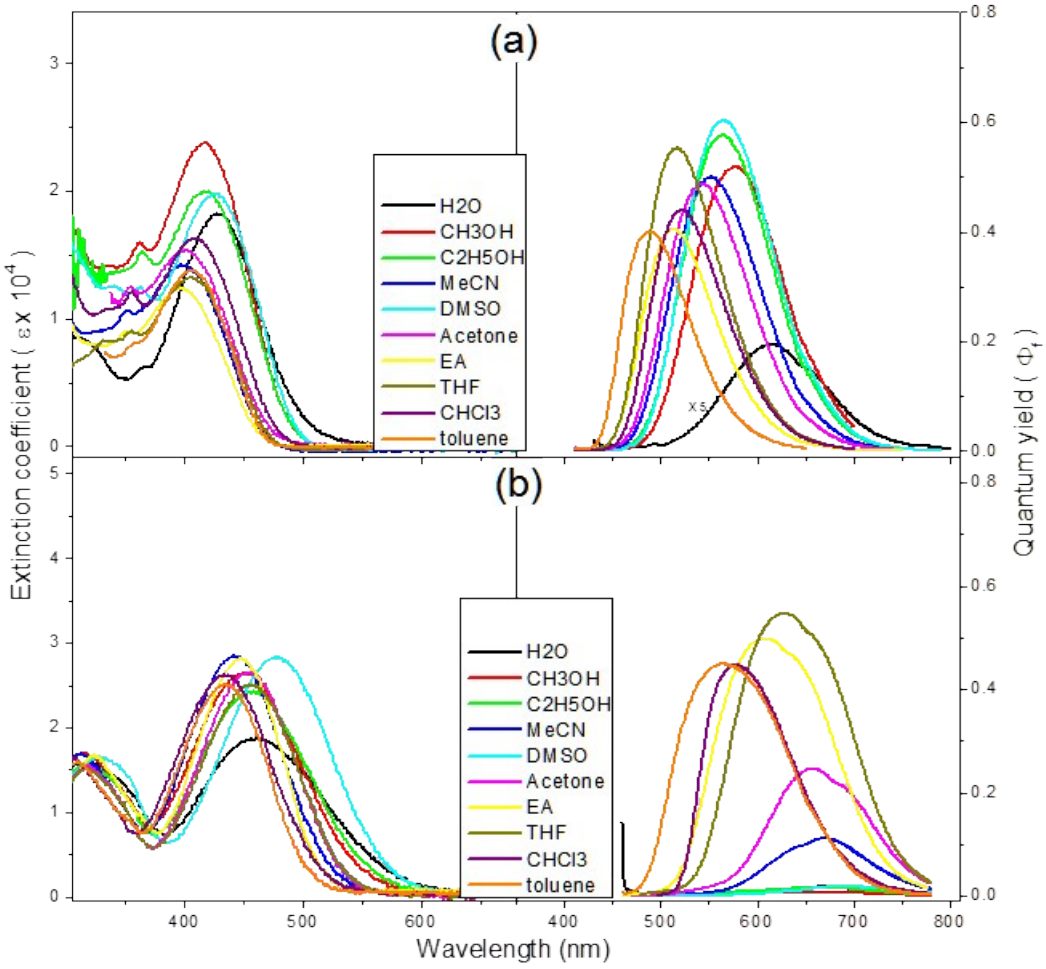


Figure S1: Solvent effects of compounds (a) NIM-1 and (b) ANB in neutral conditions, represented by extinction coefficient for absorbance and quantum yield for emission spectra.

Figure S2

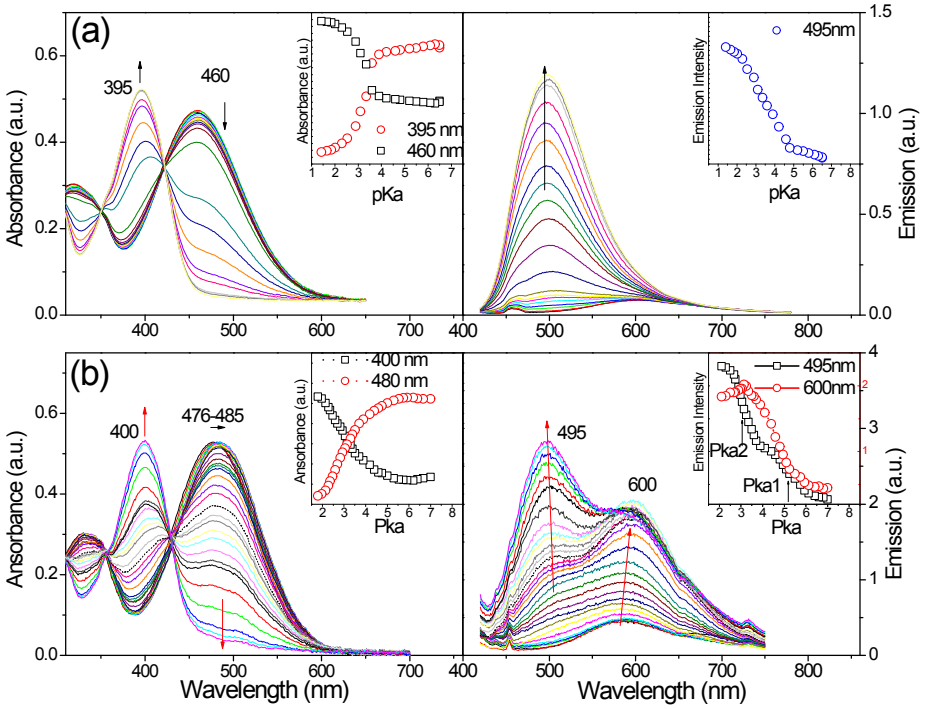


Figure S2: ADA (30 μM) absorption (left) and emission (right) spectral variations as a function of addition of [H^+] in (a) DMSO/H_2O=1/1 and (b) DMSO/H_2O=1000/1 in v/v. Inserts show peak intensities plots against pH. (Excited wavelength= 400 nm).

Figure S3

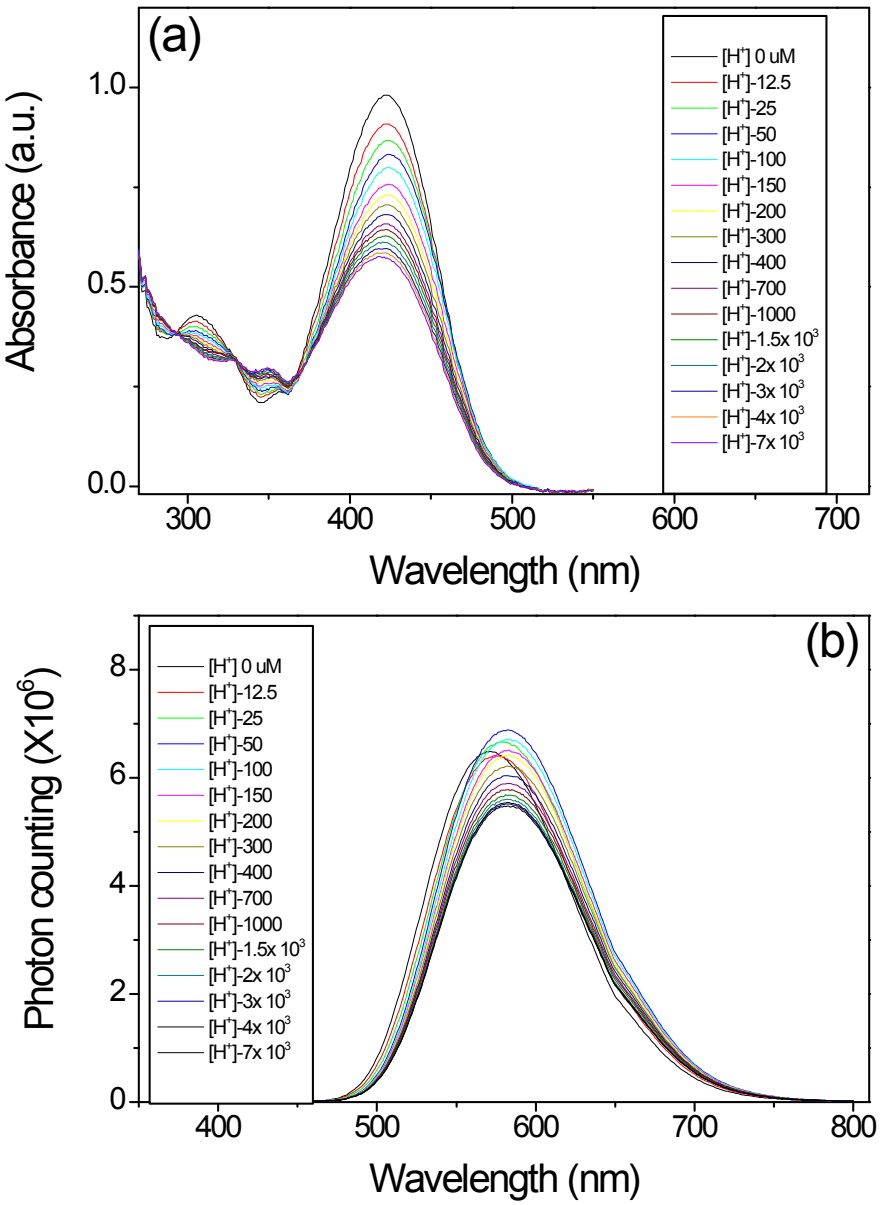


Figure S3: Compound NIM-1 (30 μM) absorption (a) and emission (b) spectral variations as a function of adding [H^+] in DMSO. (excited wavelength= 420 nm in (b))

Figure S4

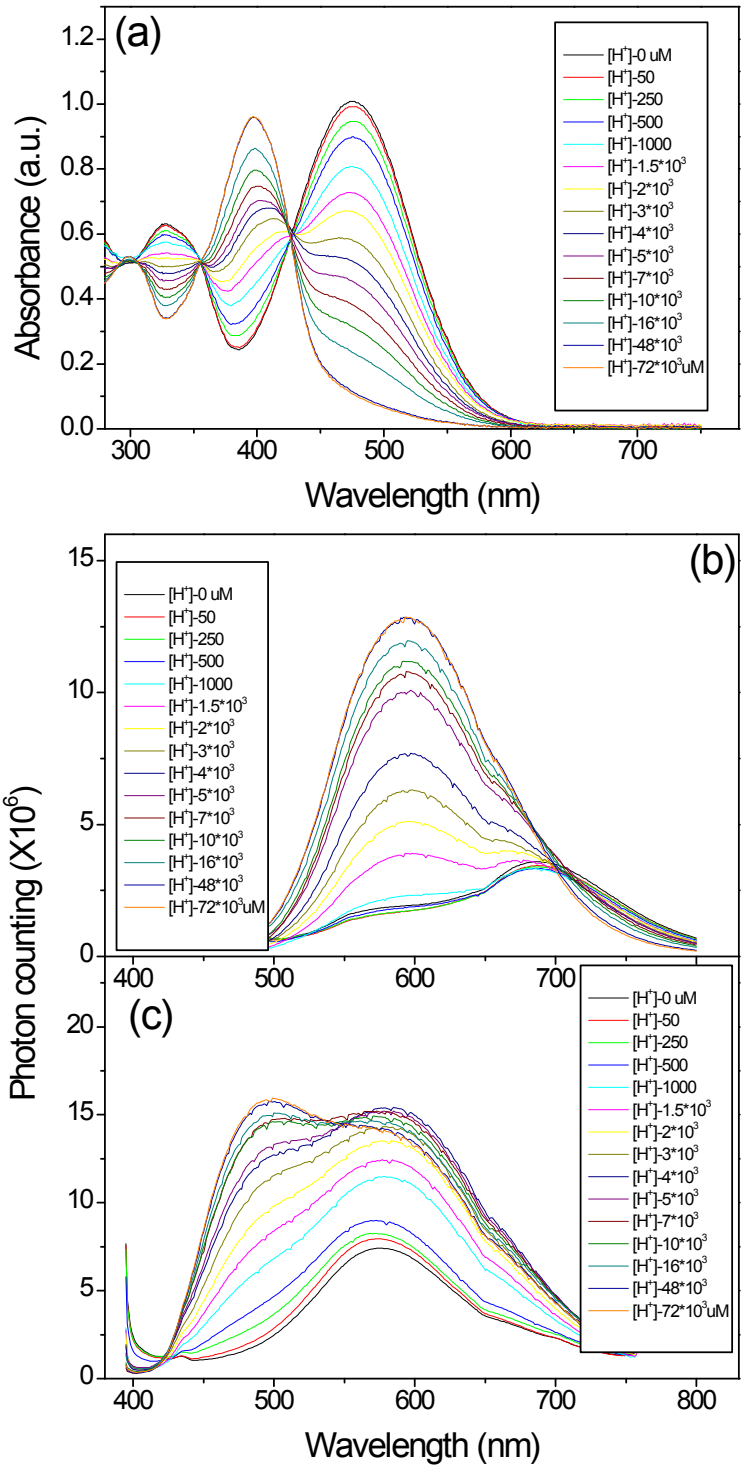


Figure S4: Compound ANB (30 μM) absorption (a) and emission (b) (c) spectral variations as a function of adding [H^+] in DMSO. Inserts show the peak intensity plots against pH. (excited wavelength= 480 nm in (b); 400 nm in (c))

Figure S5

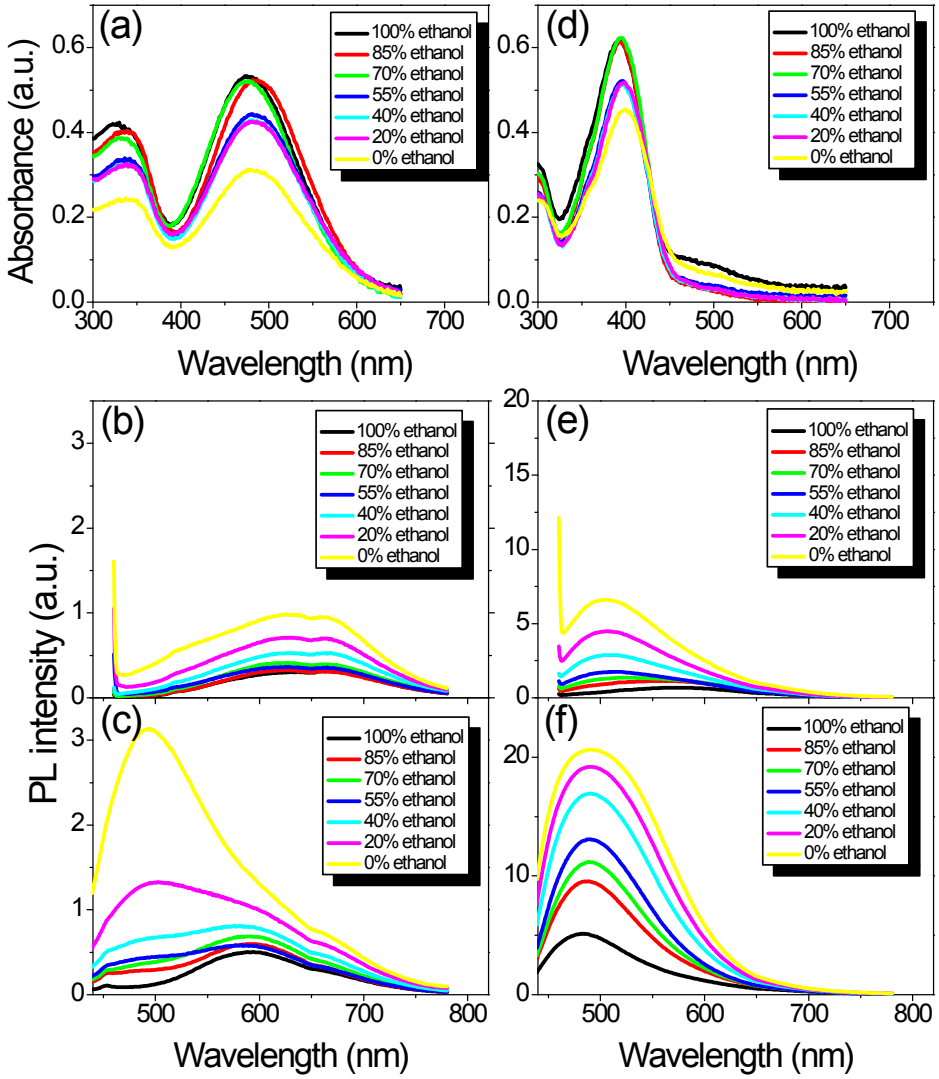


Figure S5: Compound ADA (30 μM) absorption (a) and emission (b) (c) spectral in variable ratios of neutral ethanol/glycerol solutions. (d)(e)(f) Represent the ADA in variable ratios of 0.1 M [H^+] ethanol/glycerol solutions. (excited wavelength= 460 nm in (b); 400 nm in (c))

Figure S6

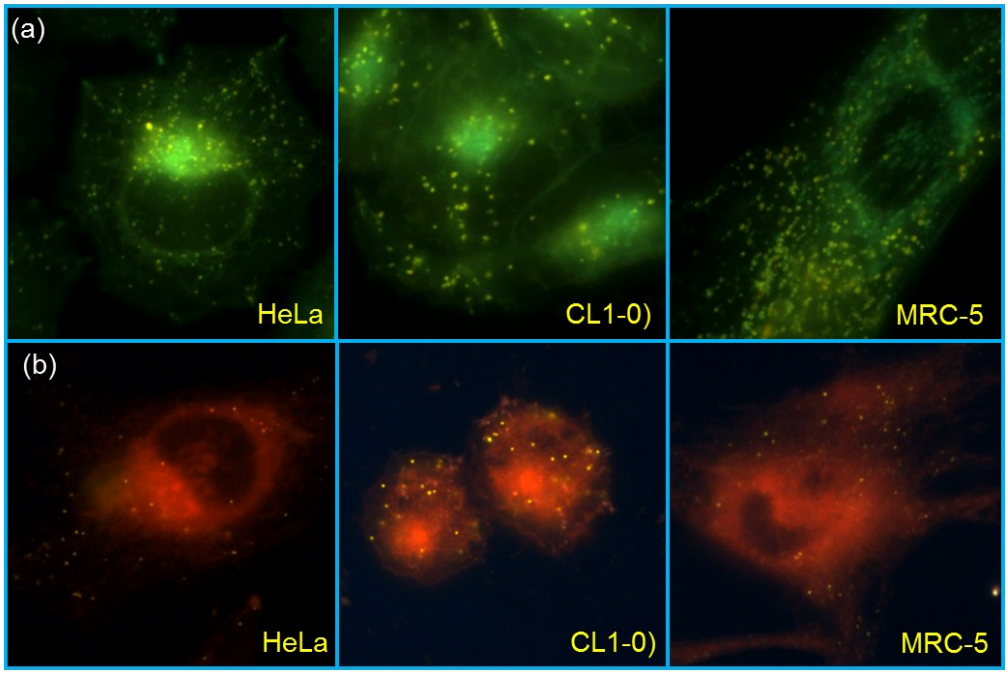


Figure S6: Fluorescent images of HeLa, CL1-0 cancer cells and MRC-5 normal cell stained with (a) NIM-1 and (b) ANB (5 μM) for 4 hour. (cube for (a): ex, 390/10 nm; em, 410 nm lp filter); (cube for (b): ex, 470/20 nm; em, 515 nm lp filter)

Figure S7

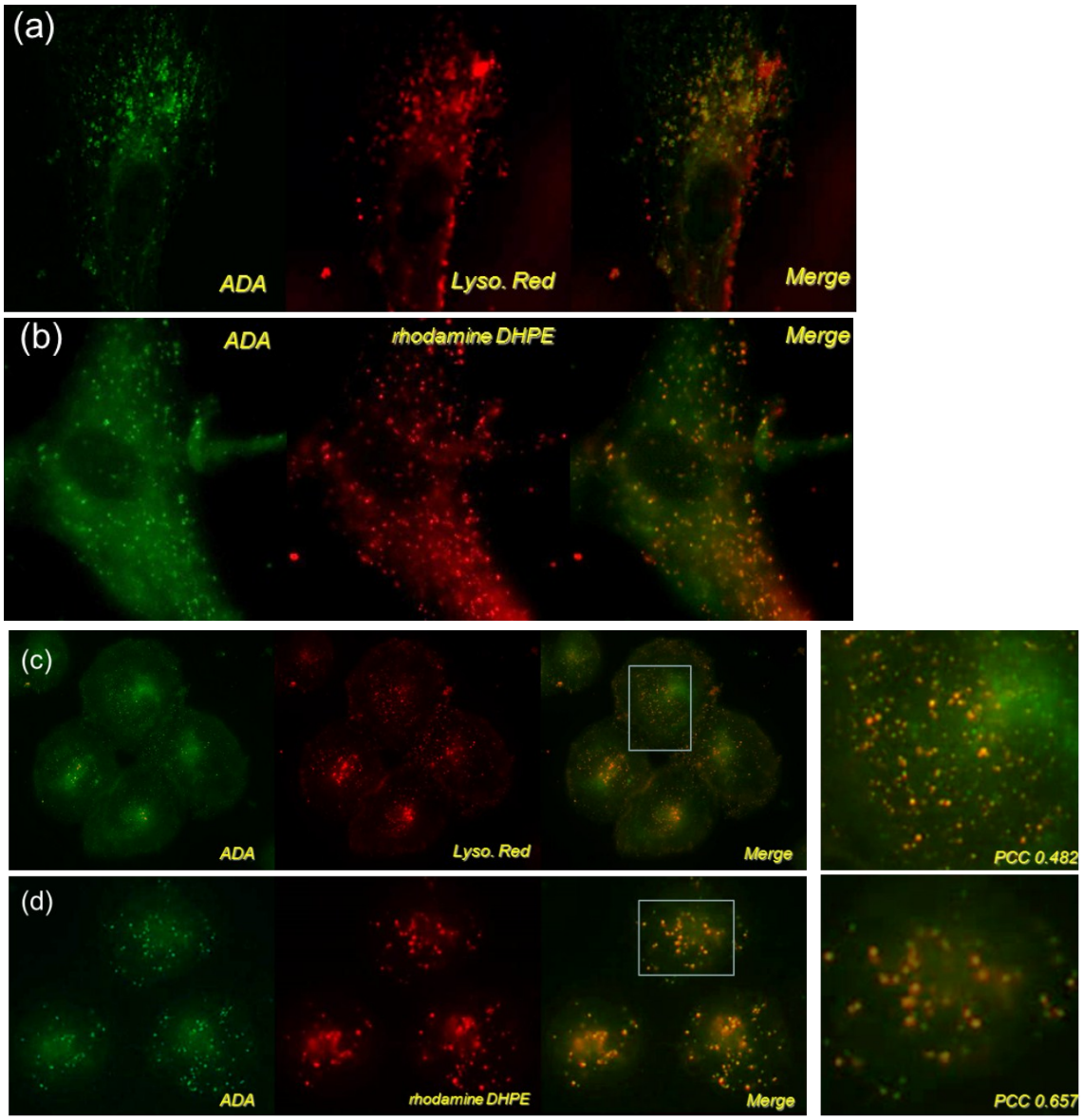


Figure S7. Sub-cellular localization of 5 μM ADA in MRC-5 normal cells (a) (b) and CL1-0 cancer cells (c) (d). The images of tracker red were excited by a green light cube that passed light through a 530±20 nm bp filter and emission was collected through a 590 nm lp filter. The green images of the compound ADA were excited by a UV light cube that passed light through a 390±10 nm bp filter and emission was collected through a 410 nm lp filter. Co−localization was analyzed by the Pearson correlation coefficient (PCC)

Figure S8

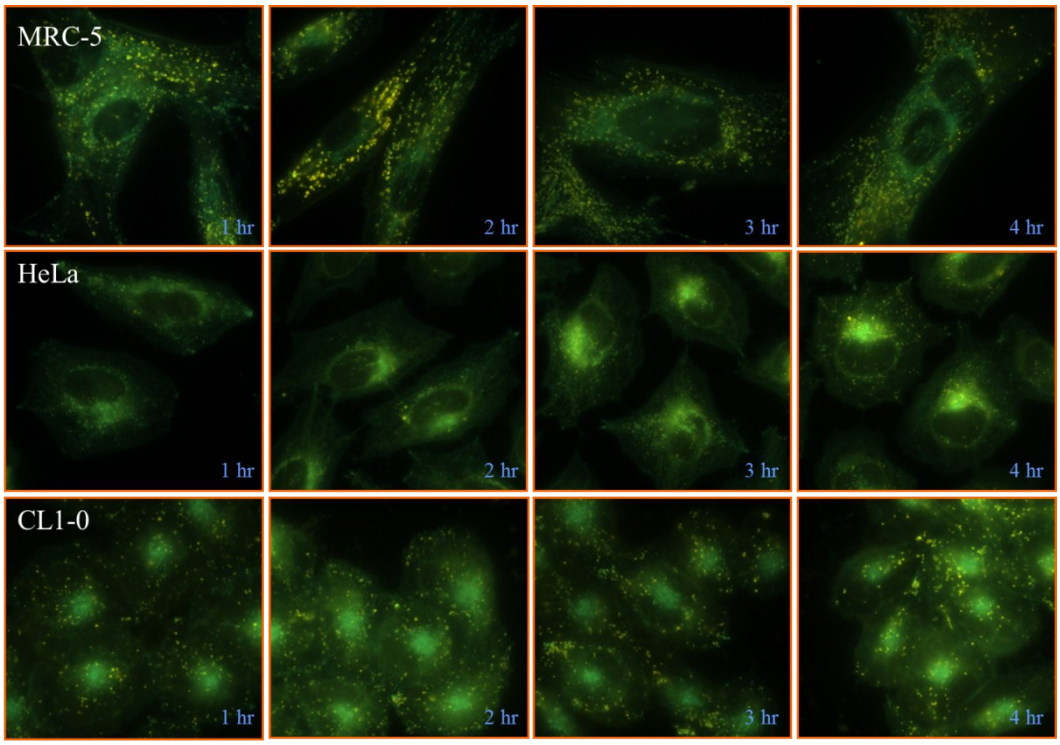


Figure S8: A time-dependent intracellular accumulation illustration of 5 μM NIM-1 in MRC-5 normal cells, HeLa cancer cells and CL1-0 cancer cell, respectively. The UV cube (ex 390/10 nm, 410 long pass filter) was used to collect images.

Figure S9

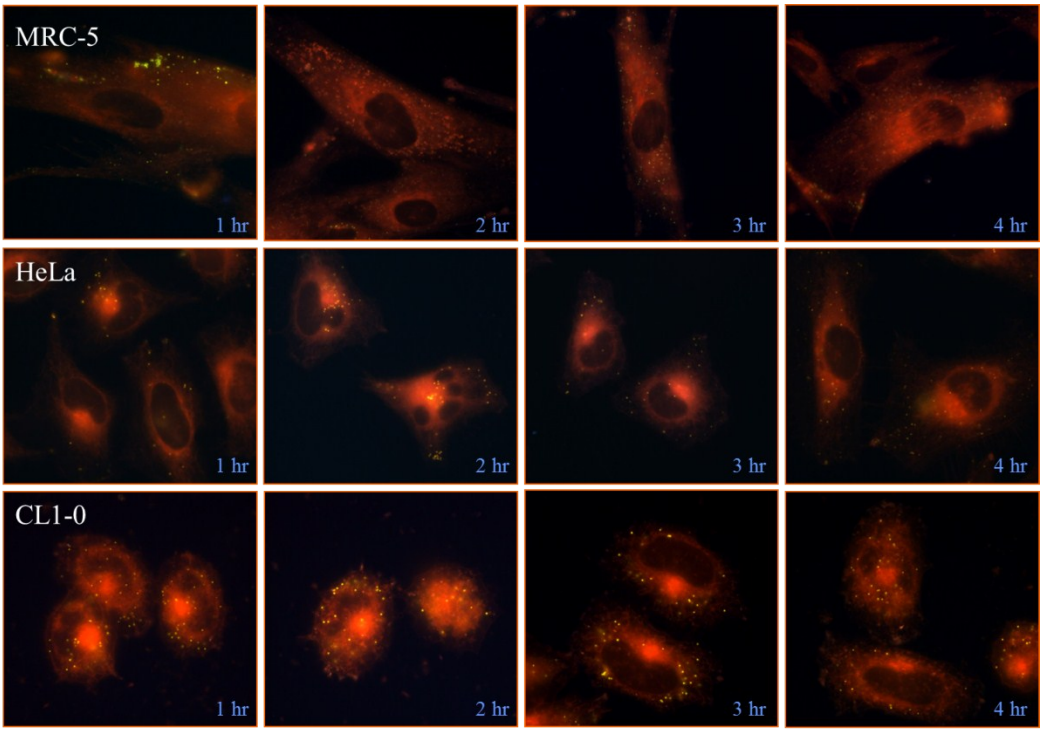


Figure S9: A time-dependent intracellular accumulation illustration of 5 μM ANB in MRC-5 normal cells, HeLa cancer cells and CL1-0 cancer cell, respectively. The blue cube (ex 470/20 nm, 515 long pass filter) was used to collect images.

Figure S10

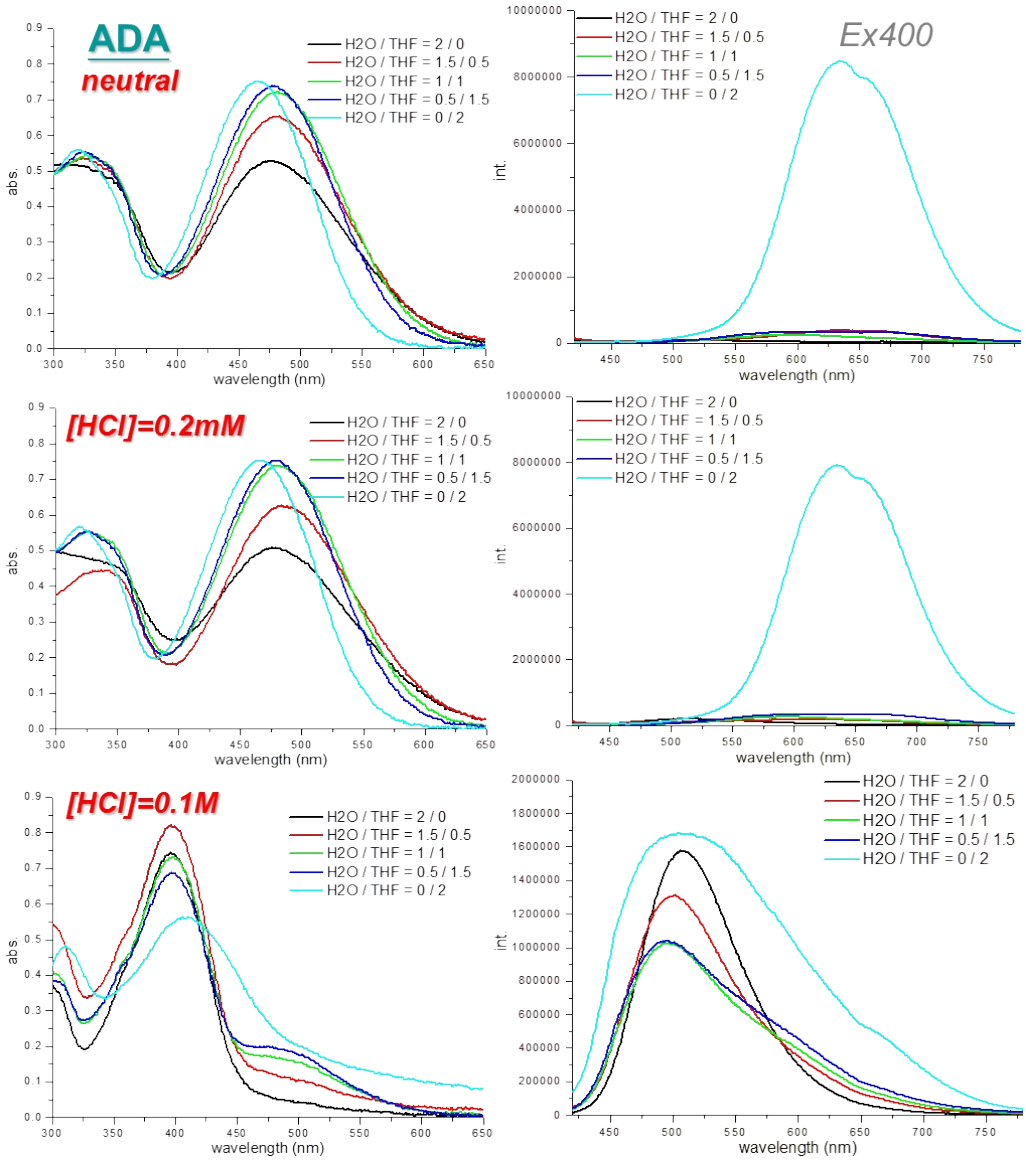
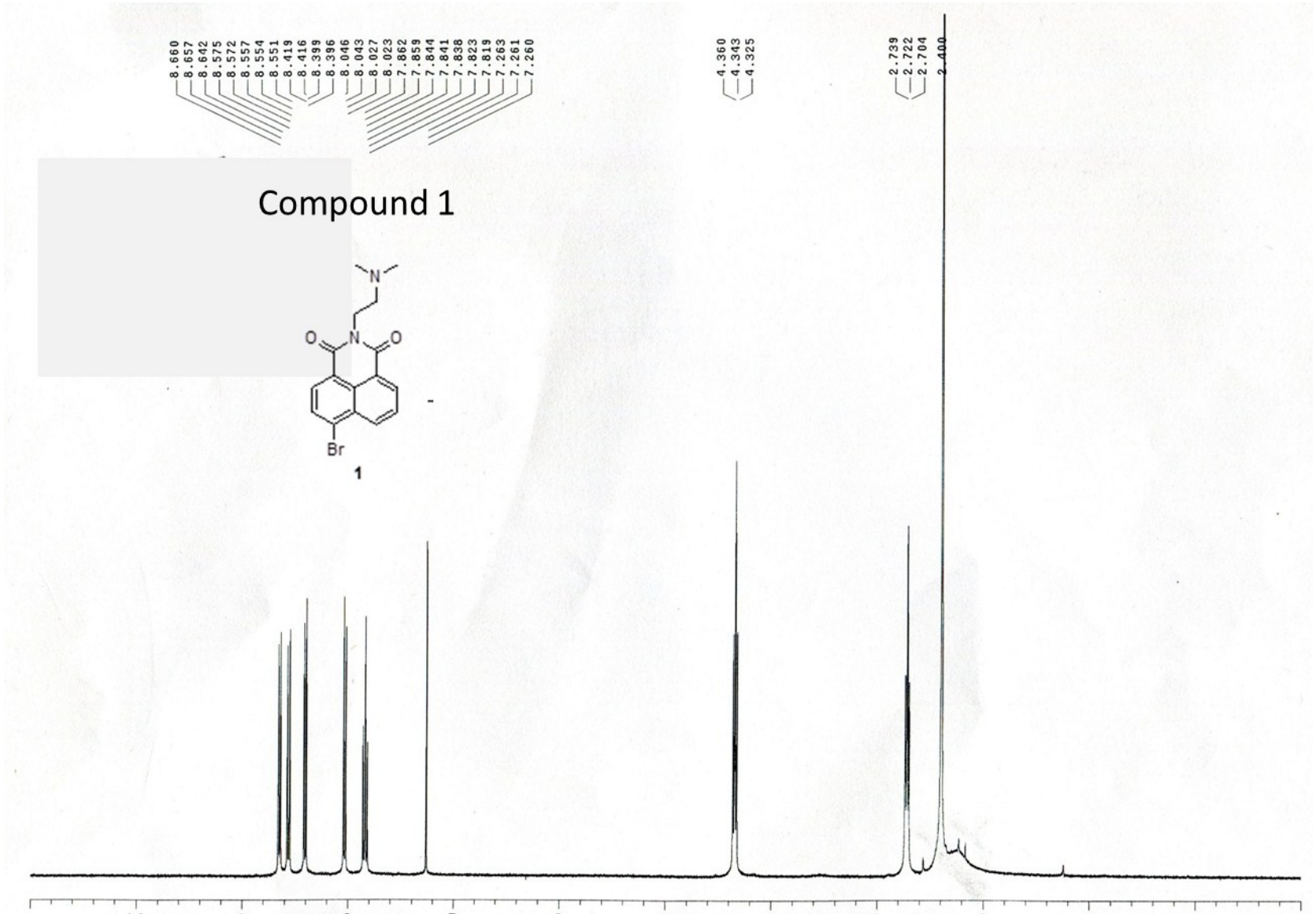
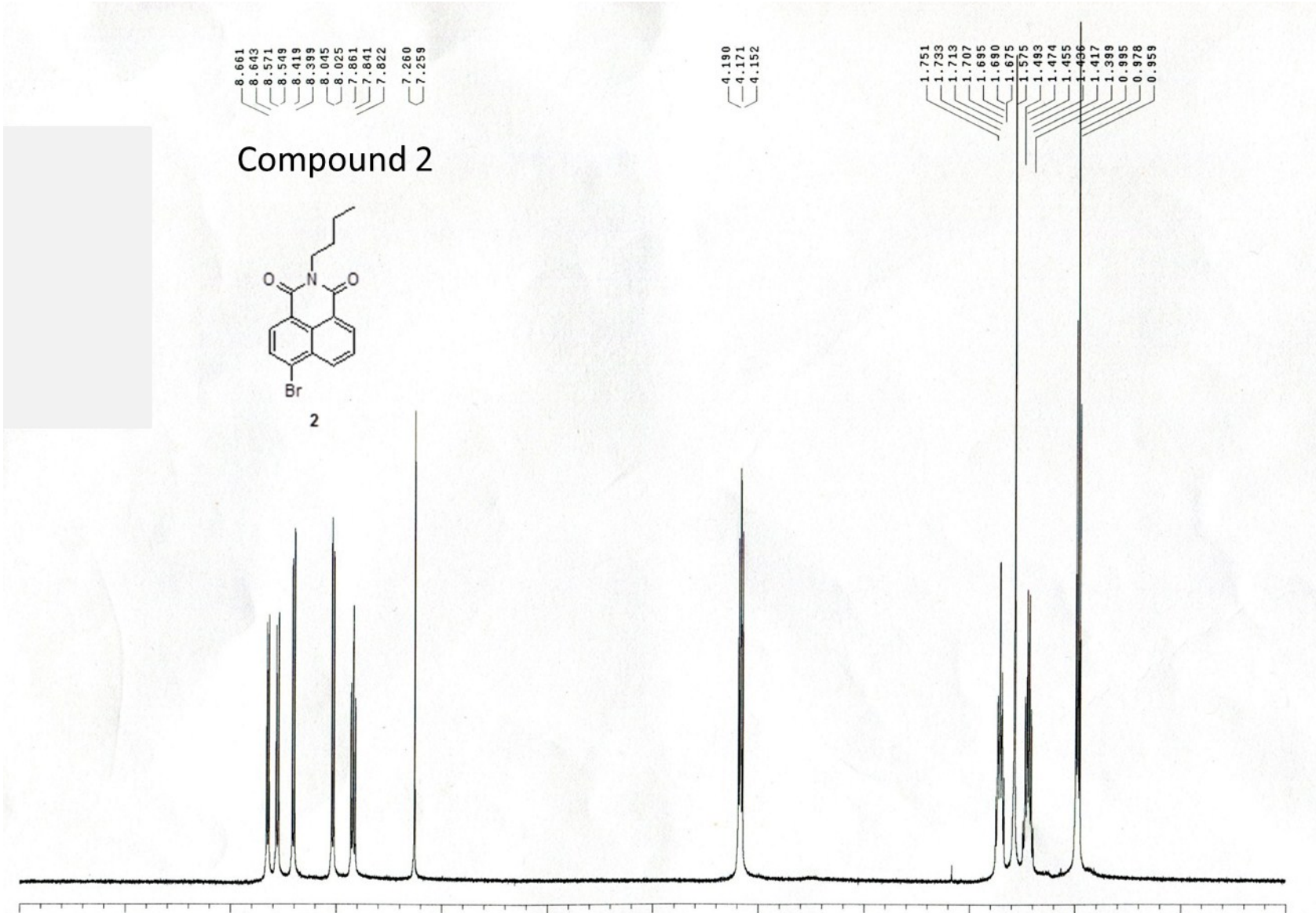
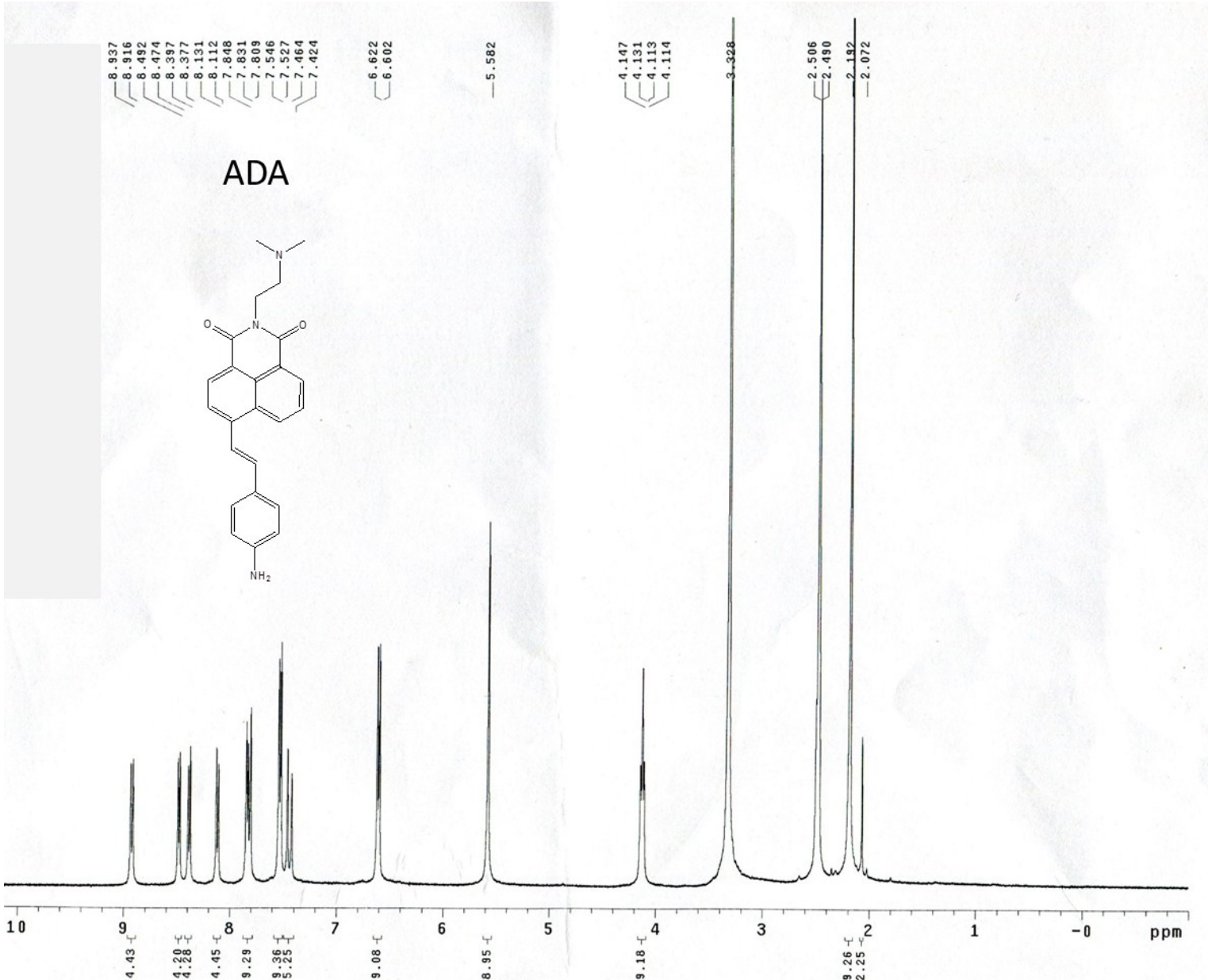

ADA
neutral
[HCl]=0.2mM
[HCl]=0.1M
Ex400
H2O / THF = 2 / 0
H2O / THF = 1.5 / 0.5
H2O / THF = 1 / 1
H2O / THF = 0.5 / 1.5
H2O / THF = 0 / 2
abs.
int.
wavelength (nm)

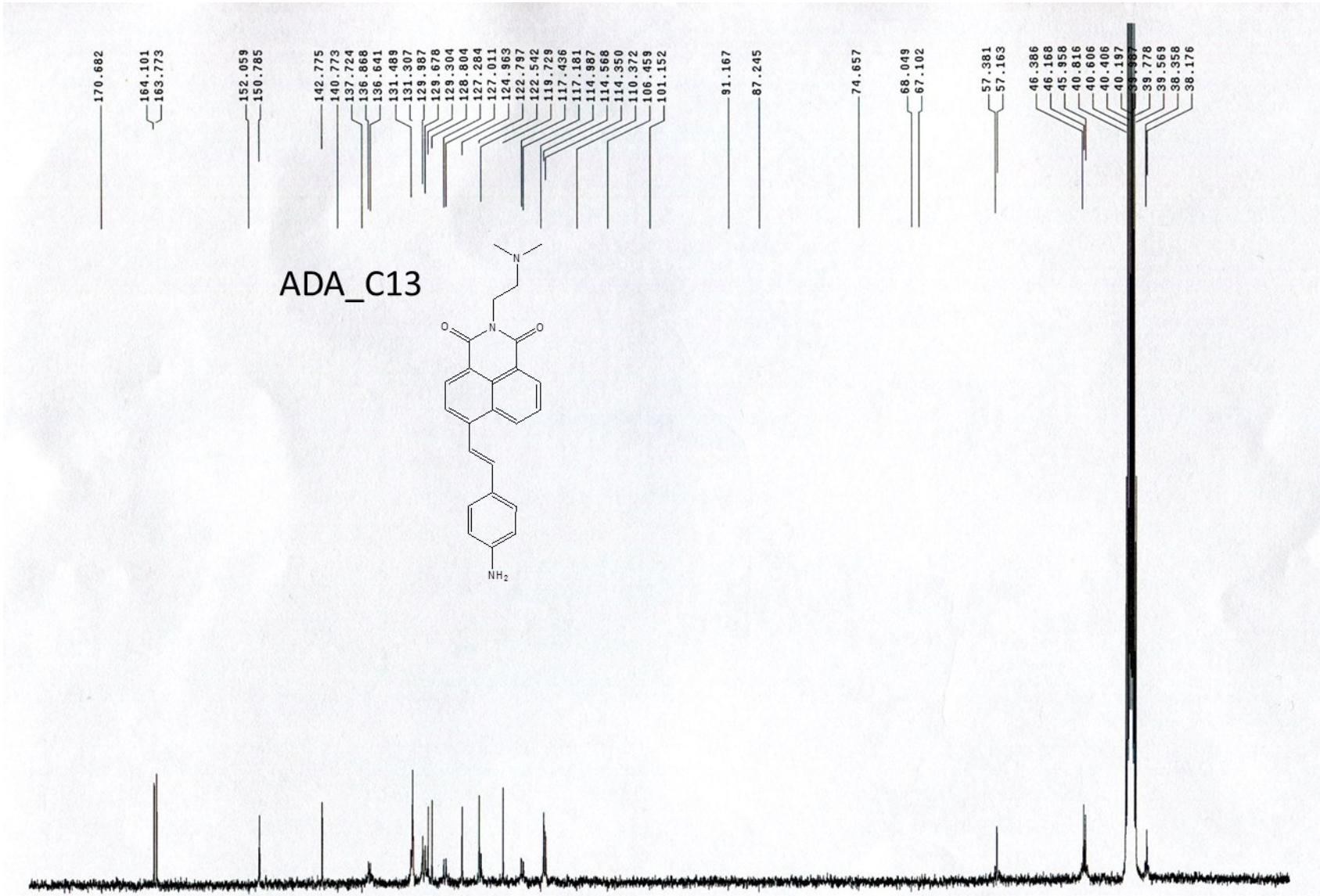
Table S1: Solvent effect of compound ADA in neutral and 150, 400 and 3000 μM of $[H^+]$ acidic conditions, represented by emission wavelengths and intensities.

	$[H^+]$ (μM)			
solvent Fluo(nm)/int.	0	150	400	3000
H_2O (Ex:440)	nd	520/11903 (Em/Intensity)	515/17428	515/50218
MeOH (Ex:445)	670/14486	nd	nd	nd
EtOH (Ex:450)	675/42853	675/21342	675/20864	nd
MeCN (Ex:440)	670/205700	675/41982	675/37068	480/104324
DMSO (Ex:480)	695/59646	700/27380	700/27379	600/92586
Acetone (Ex:450)	665/570345	600,665/ 270415,203594	595,665/ 174390,127773	490/215950
EA (Ex:440)	630/1859730	635/113240	555/101656	nd
THF (Ex:450)	630/2816900	640/1756388	640/1319990	540/250632
Chloroform (Ex:435)	605/3167790	610/1636480	610/1294440	515/38445
Toluene (Ex:430)	575/2104045	570/716793	570/655714	nd


8.660
8.657
8.642
8.575
8.572
8.557
8.554
8.551
8.419
8.416
8.399
8.396
8.046
8.043
8.027
8.023
7.862
7.859
7.844
7.841
7.838
7.823
7.819
7.263
7.261
7.260
4.360
4.343
4.325
2.739
2.722
2.704
2.400
Compound 1
N
O
N
O
Br
1


8.661
8.643
8.571
8.549
8.419
8.399
8.045
8.025
7.861
7.841
7.822
7.260
7.259
4.190
4.171
4.152
1.751
1.733
1.713
1.707
1.695
1.690
1.675
1.575
1.493
1.474
1.455
1.436
1.417
1.399
0.995
0.978
0.959
Compound 2
O
N
O
Br
2


8.937
8.916
8.492
8.474
8.397
8.377
8.131
8.112
7.848
7.831
7.809
7.546
7.527
7.464
7.424
6.622
6.602
5.582
4.147
4.131
4.113
4.114
3.328
2.506
2.490
2.192
2.072
ADA
N
O
O
NH2
10
9
8
7
6
5
4
3
2
1
-0
ppm
4.43
4.20
4.28
4.45
9.29
9.36
5.25
9.08
8.95
9.18
9.26
2.25


170.682
164.101
163.773
152.059
150.785
142.775
140.773
137.724
136.868
136.641
131.489
131.307
129.987
129.678
129.304
128.804
127.284
127.011
124.963
122.797
122.542
119.729
117.436
117.181
114.887
114.568
114.350
110.372
106.459
101.152
91.167
87.245
74.657
68.049
67.102
57.381
57.163
46.386
46.168
45.958
40.816
40.606
40.406
40.197
39.987
39.778
39.569
38.358
38.176
ADA_C13
O
N
O
N
NH2