Supporting Information for

Fused pyrazole-phenanthridine based dyads: Synthesis, photo-physical and theoretical studies, and live cell pH imaging

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Figure S1: ^{1}H Spectra of compound 1

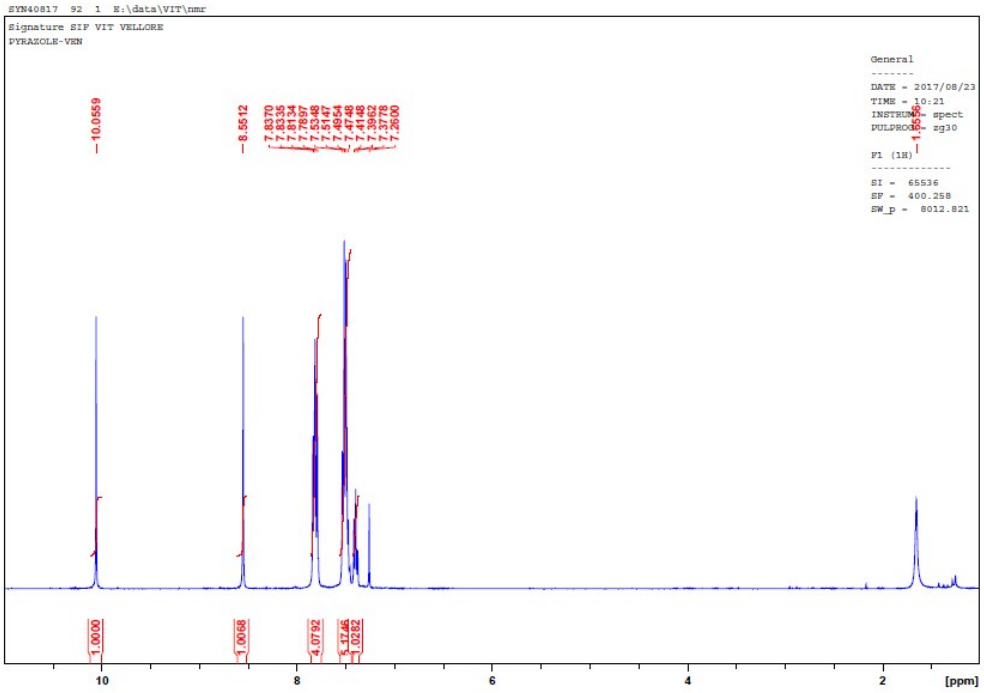


Figure S2: ^{13}C Spectra of compound 1

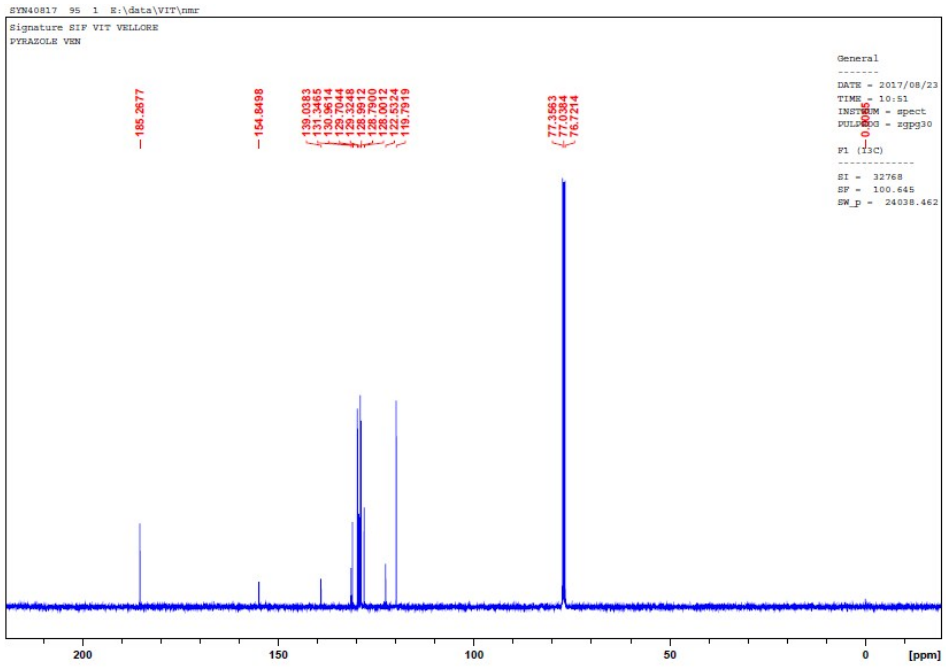


Figure S3: ^{1}H Spectra of compound 1a

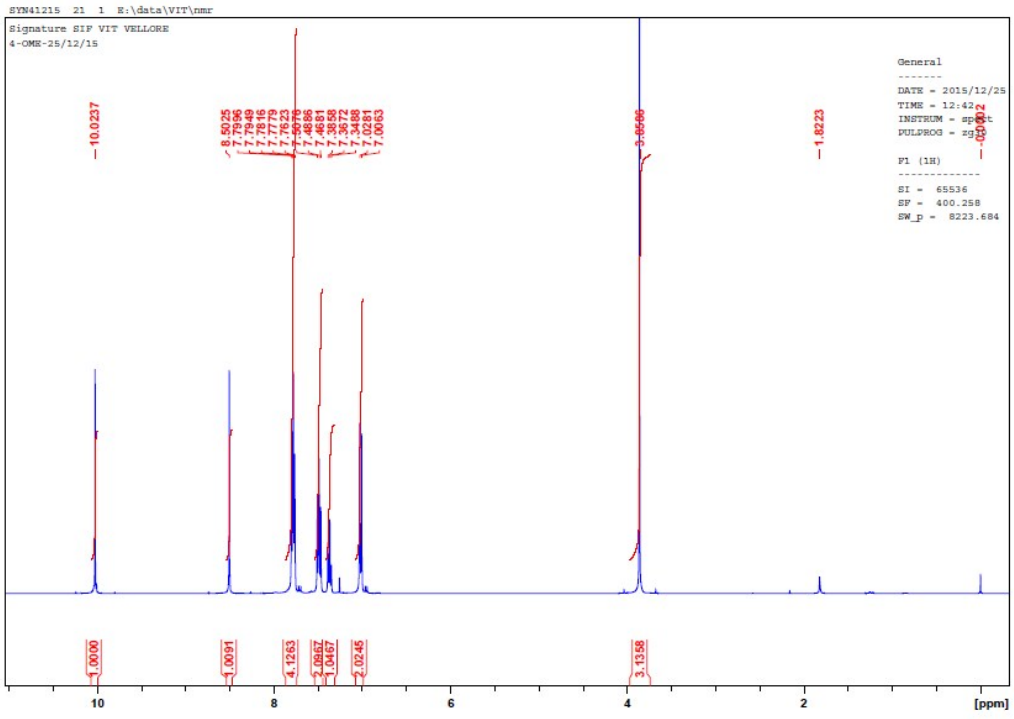


Figure S4: ^{13}C Spectra of compound 1a

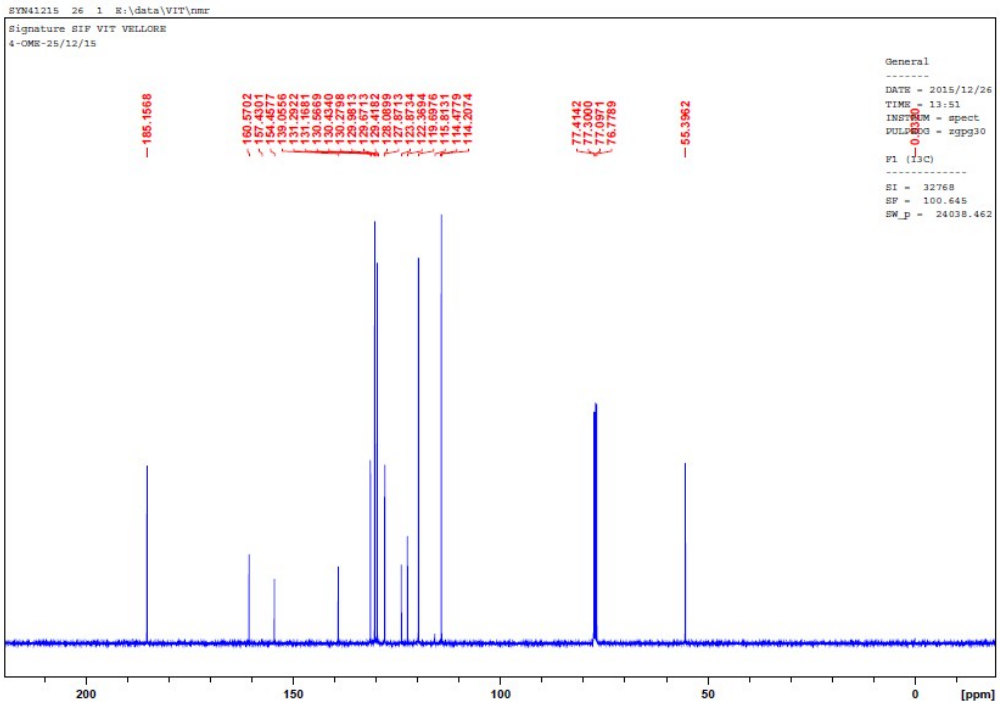


Figure S5:[1]H Spectra of compound 1b

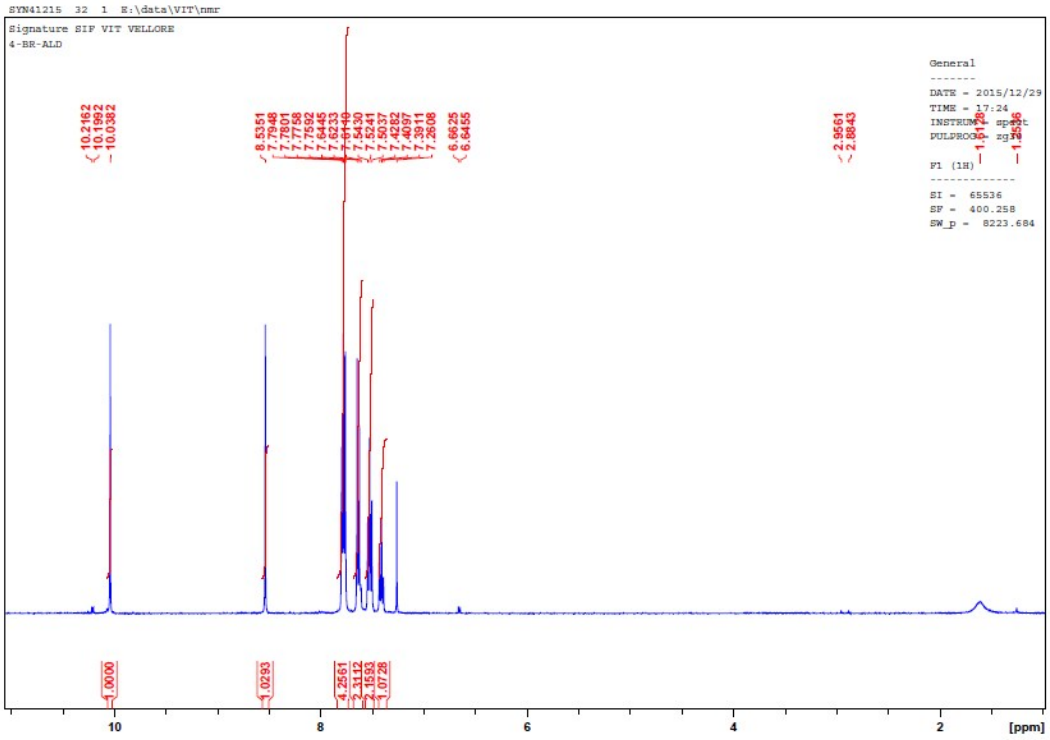


Figure S6:[13]C Spectra of compound 1b

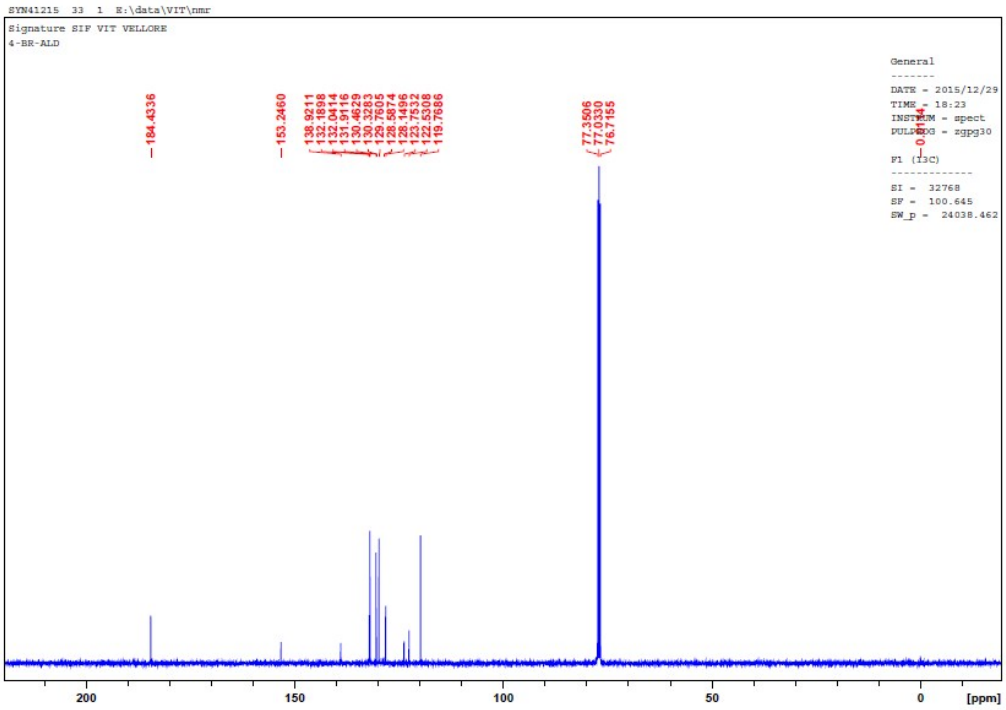


Figure S7: ^{1}H Spectra of compound 1c

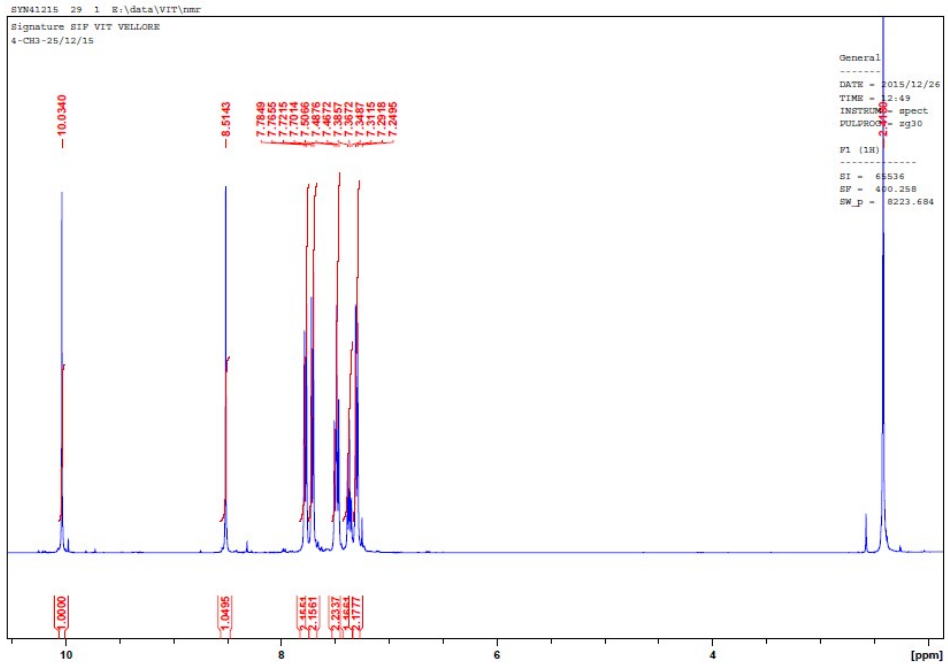


Figure S8: ^{13}C Spectra of compound 1c

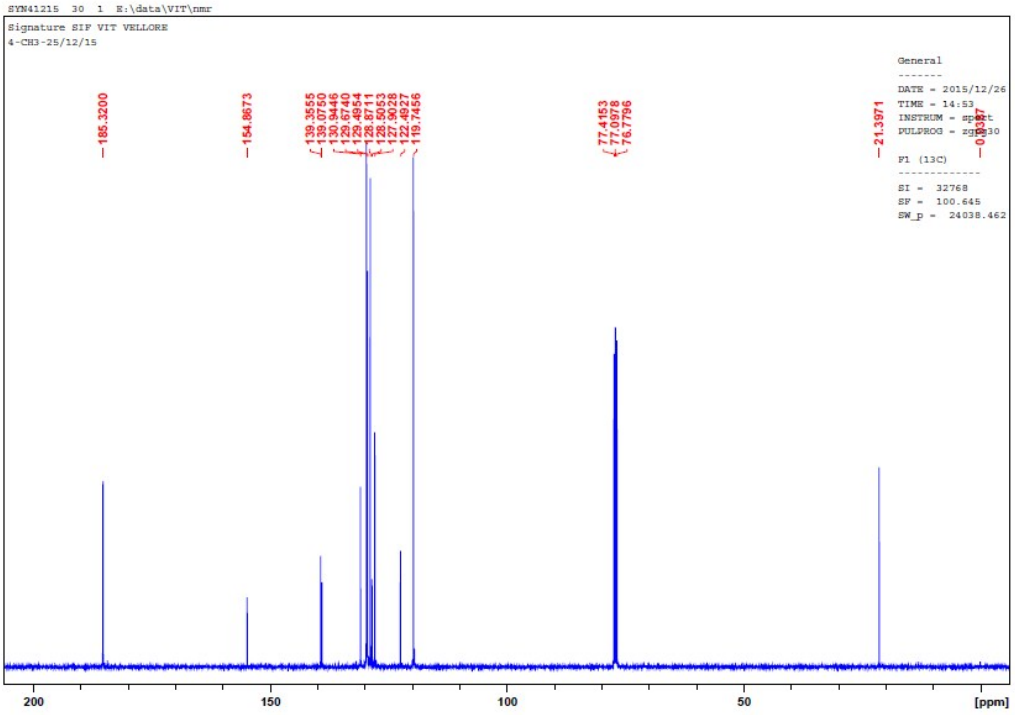


Figure S9: ^{13}C Spectra of compound 2a

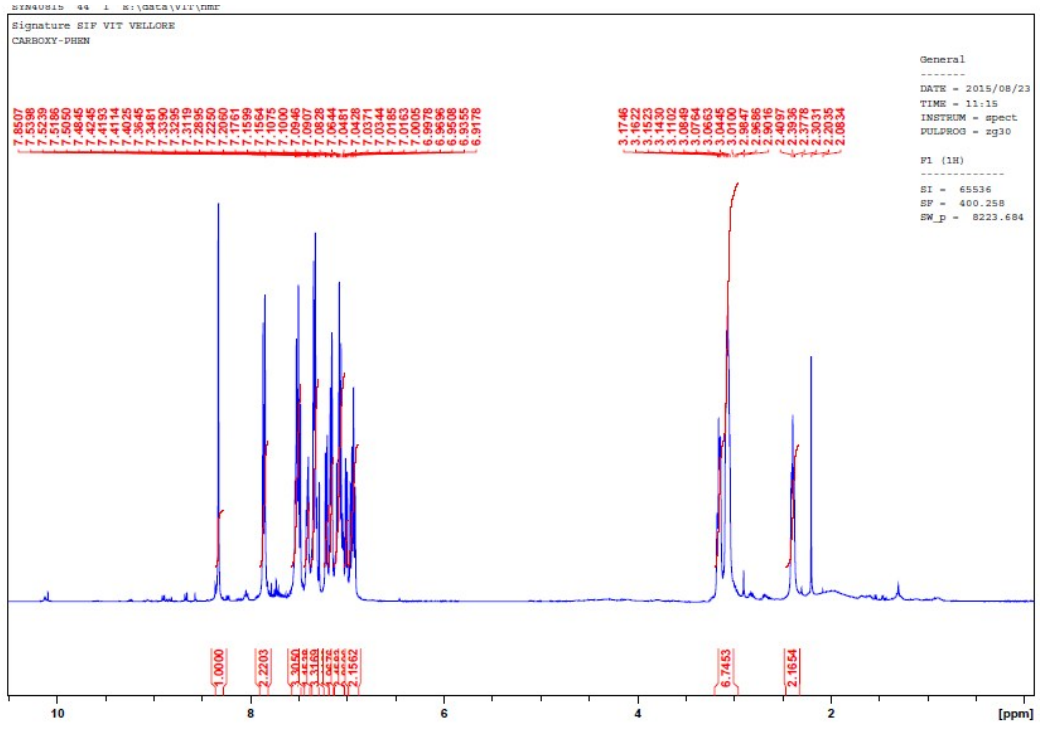


Figure S10: ^{13}C Spectra of compound 2a

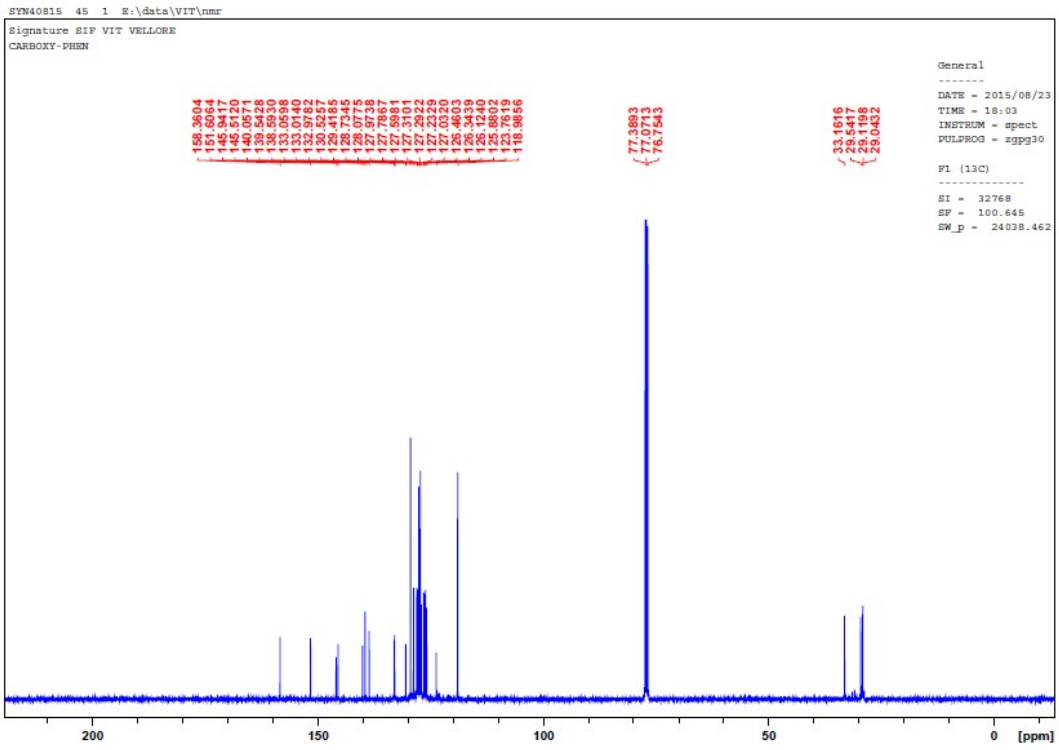


Figure S11: ^{1}H Spectra of compound 2b

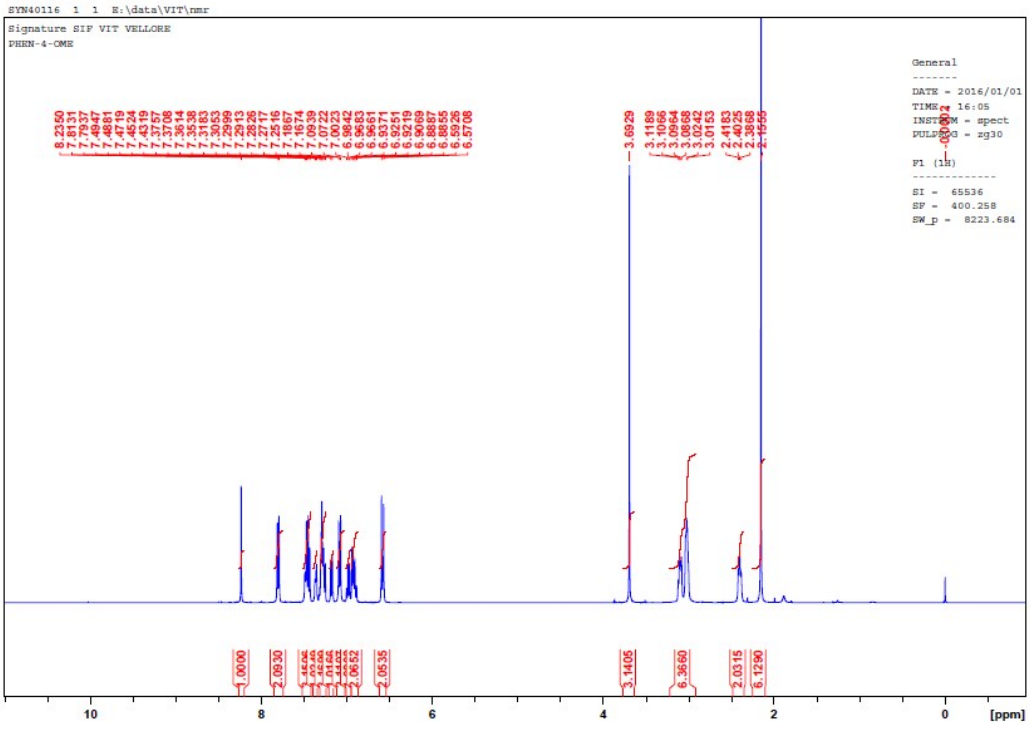


Figure S12: ^{13}C Spectra of compound 2b

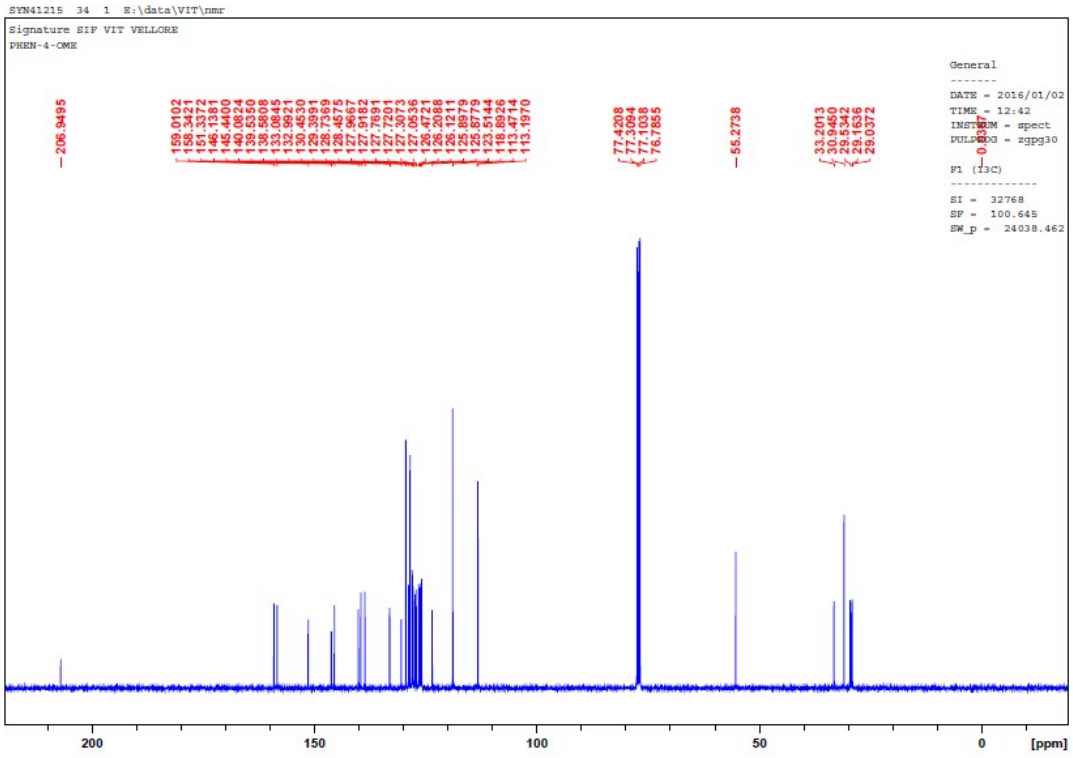


Figure S13: ^{1}H Spectra of compound 2c

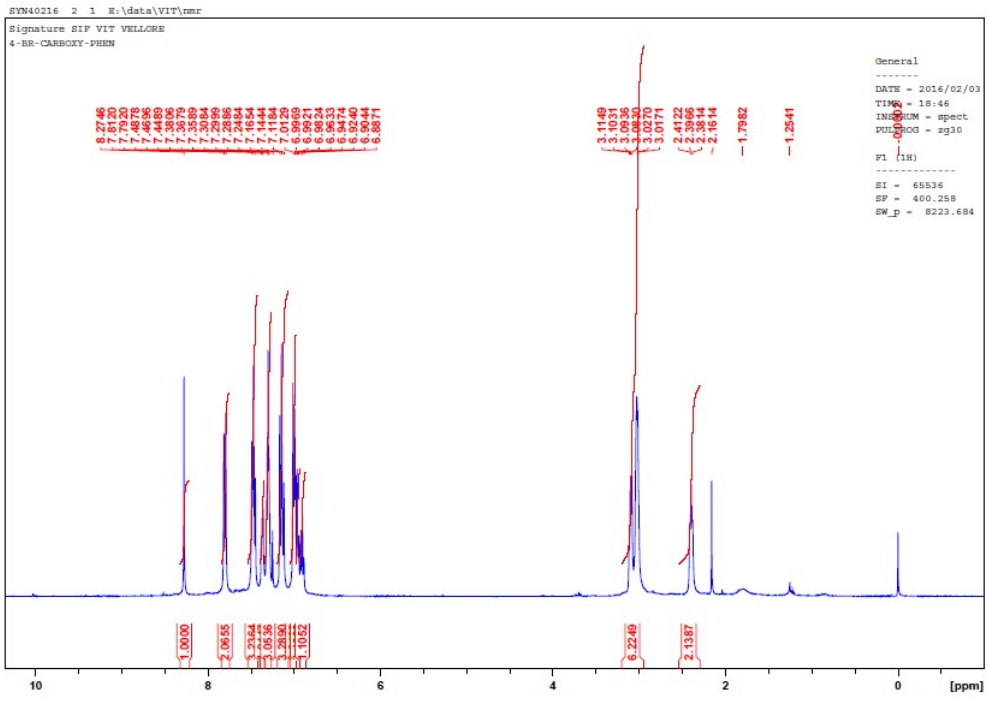


Figure S14: ^{13}C Spectra of compound 2c

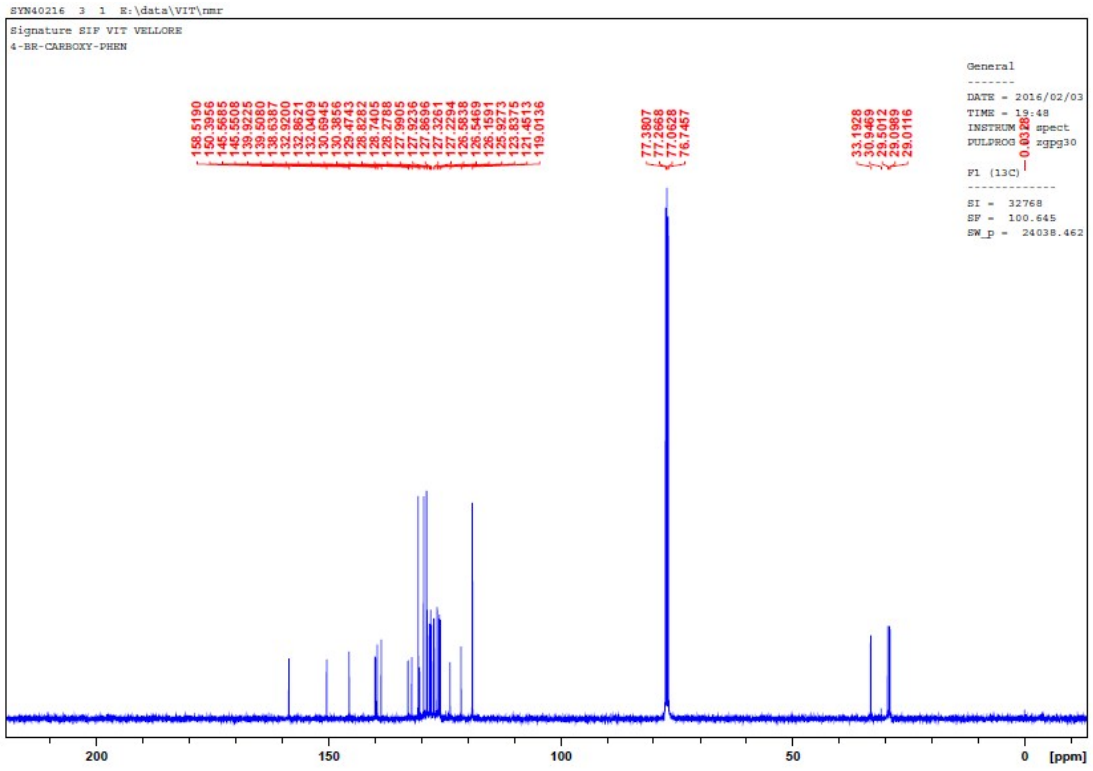


Figure S15:[1]H Spectra of compound 2d

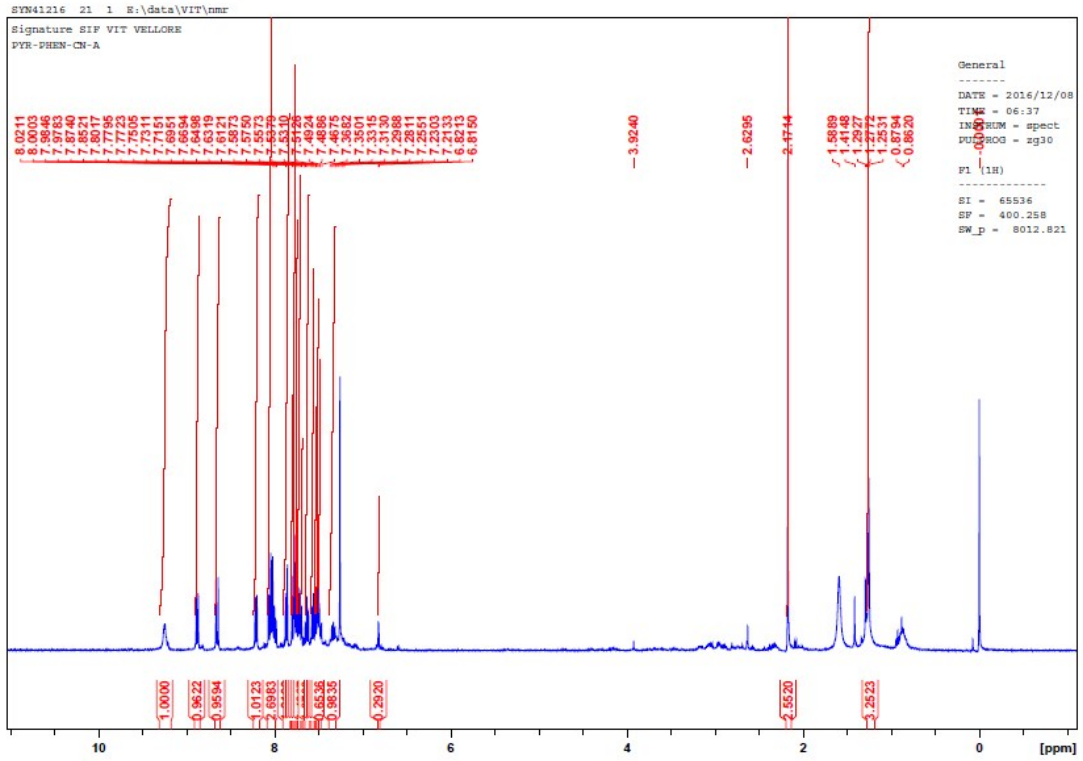


Figure S16:[13]C Spectra of compound 2d

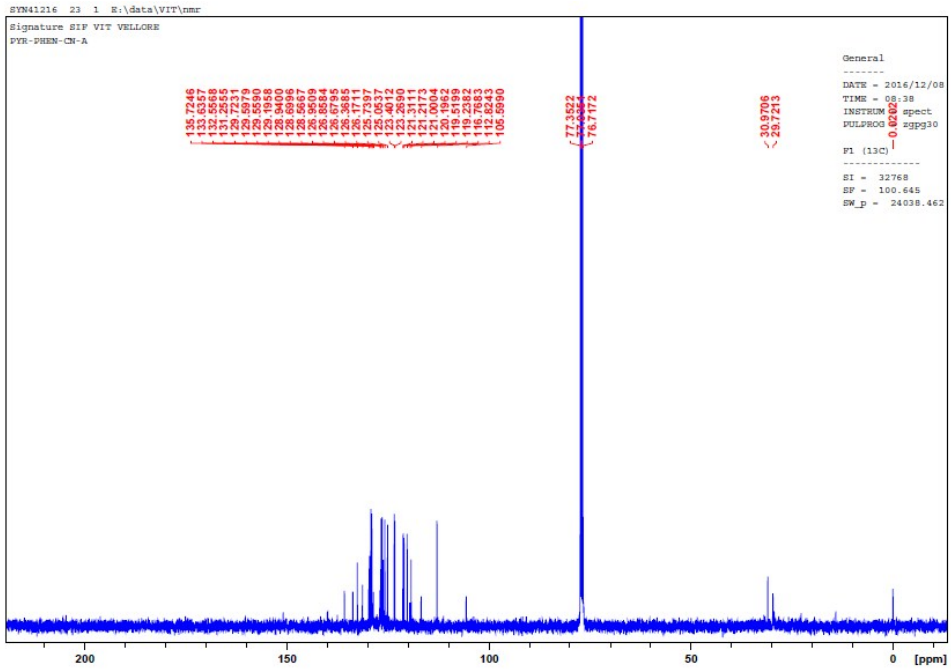


Figure S17: HRMS spectra of compound 2a

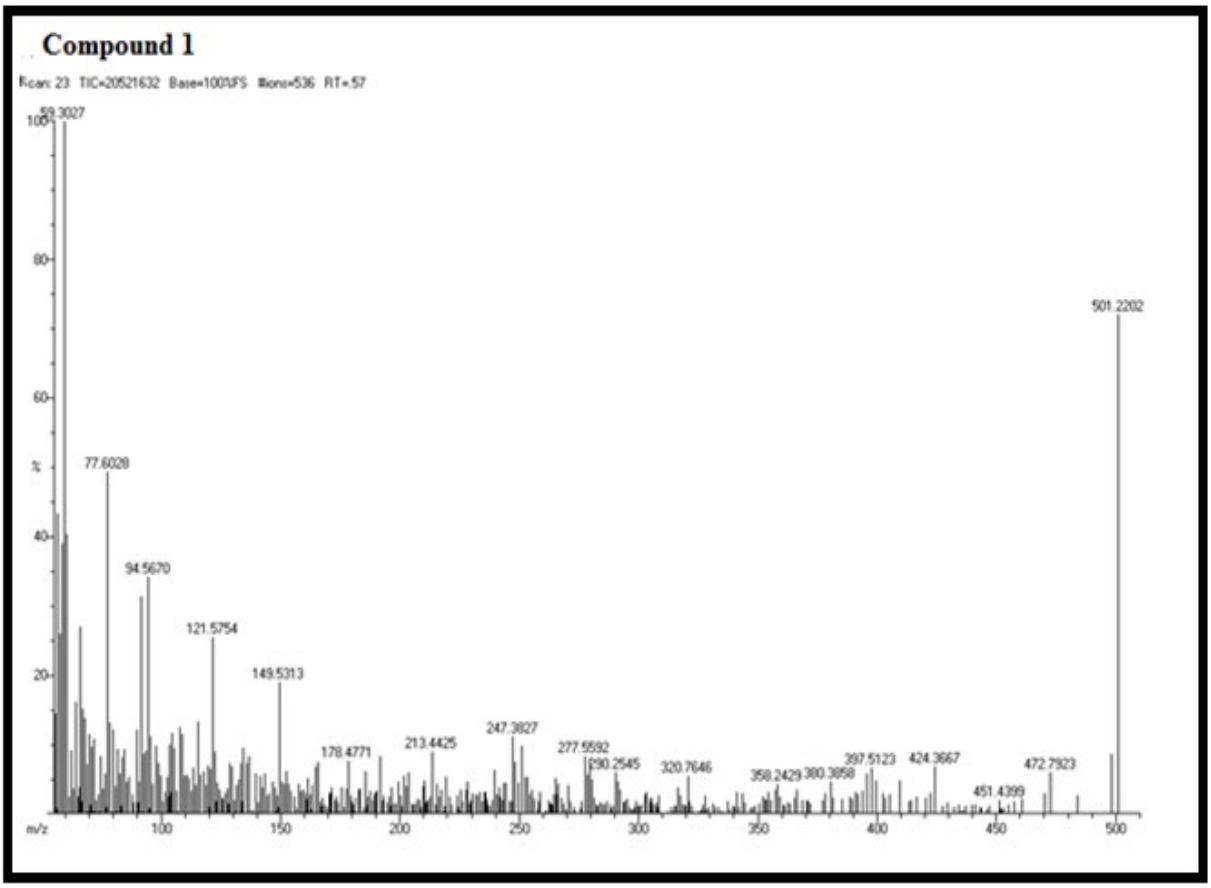


Figure S18: HRMS spectra of compound 2b

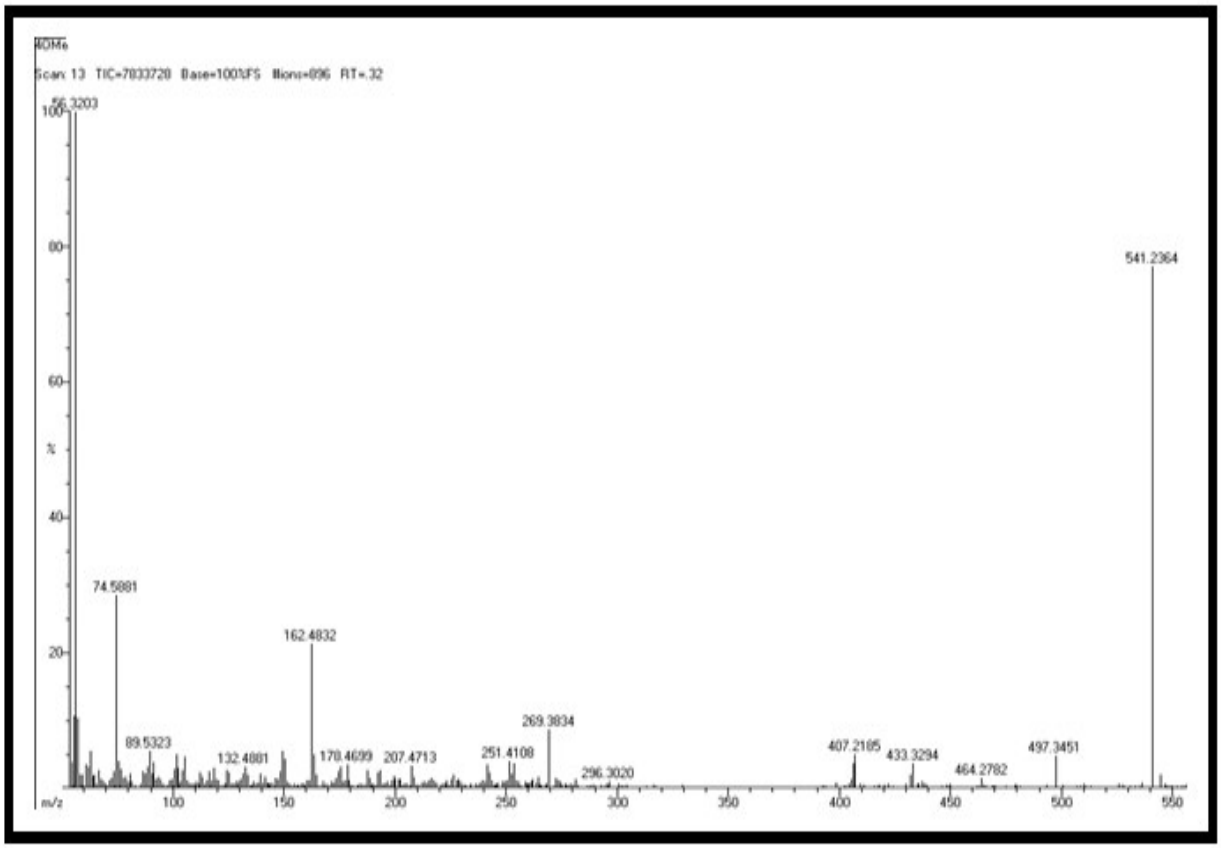

40M6
Scan 13 TIC=7833720 Base=100%FS Ions=696 RT=.32
56.3203
74.5881
89.5323
132.4881
162.4832
170.4699
207.4713
251.4108
269.3834
296.3020
407.2185
433.3294
464.2782
497.3451
541.2364
100
80
60
%
40
20
m/z
100
150
200
250
300
350
400
450
500
550

Figure S19: Normalized absorption spectra of compound 2a-2d (2 x 10^{-5} M) in chloroform solvent.

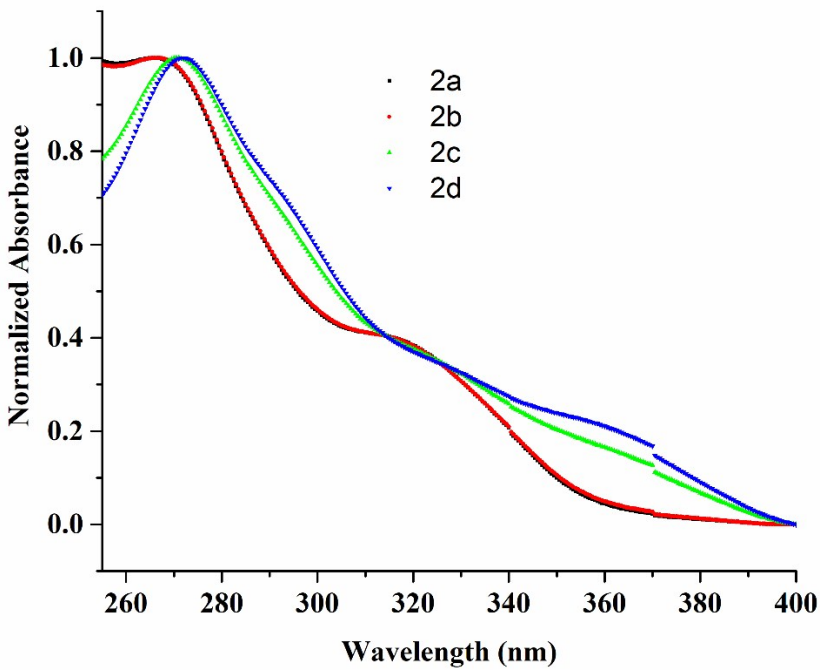


Figure S20: (A) The absorption spectra of the compound 2b (2 x 10^{-5}) in chloroform solvent over the addition of TFA. (B) The absorption spectra of the compound 2b with TFA over the addition of TEA.

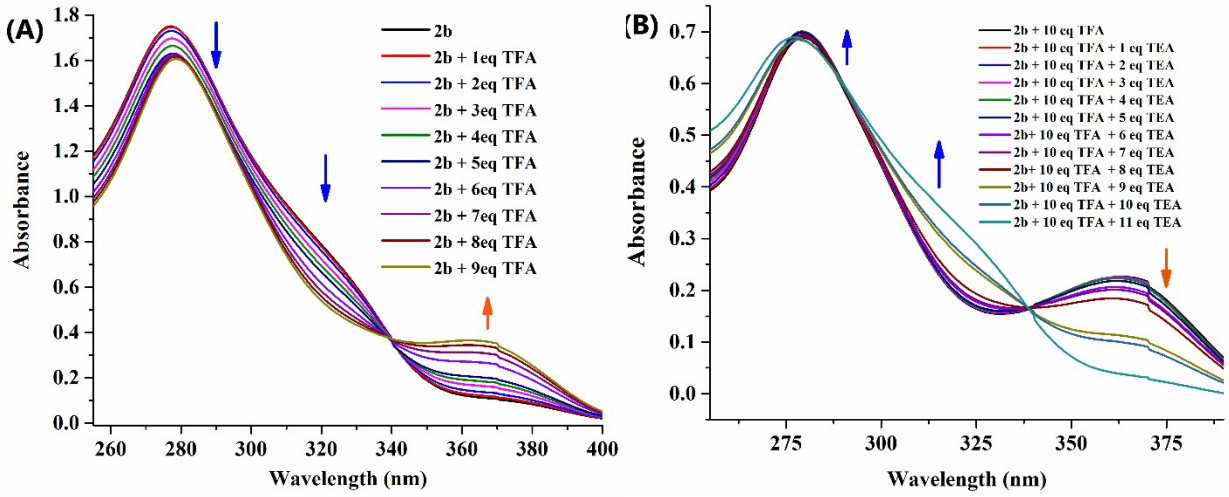


Figure S21: Normalized emission spectra of compound 2a & 2b (2 x 10^{-5} M) in chloroform solvent (λ_{ex} = 267 nm).

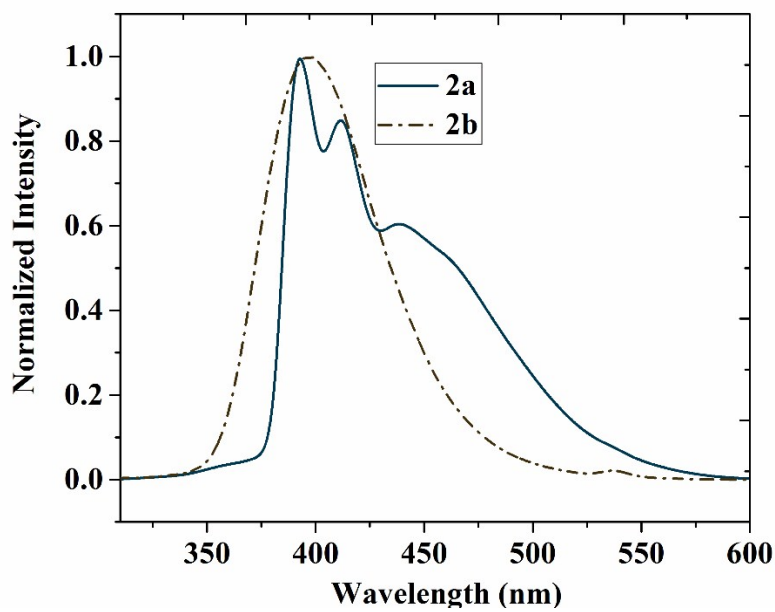


Figure S22: The ratio of the emission intensity I_{392}/I_{457}vs concentration of TFA for 2a (A) and I_{397}/I_{463} for 2bvs concentration of TFA(B).

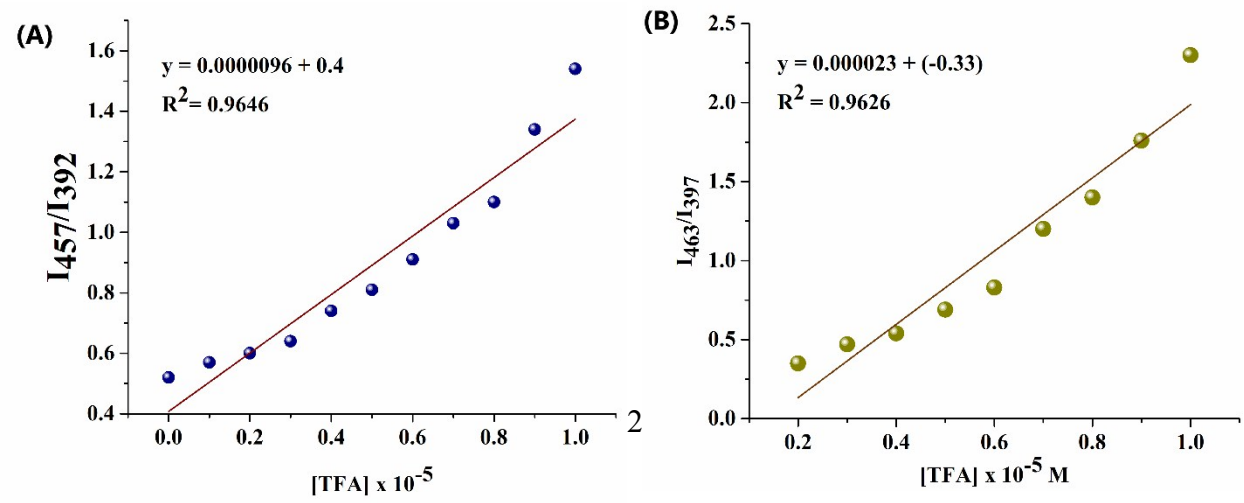


Figure S23: (A)Emission spectra of the compound 2a+ H^+ (2 x 10^{-5}) in chloroform solvent over the addition of TEA (λ_{ex} = 267 nm). (B) Emission spectra of the compound 2b+ H^+ (2 x 10^{-5}) in chloroform solvent over the addition of TEA (λ_{ex} = 267 nm).

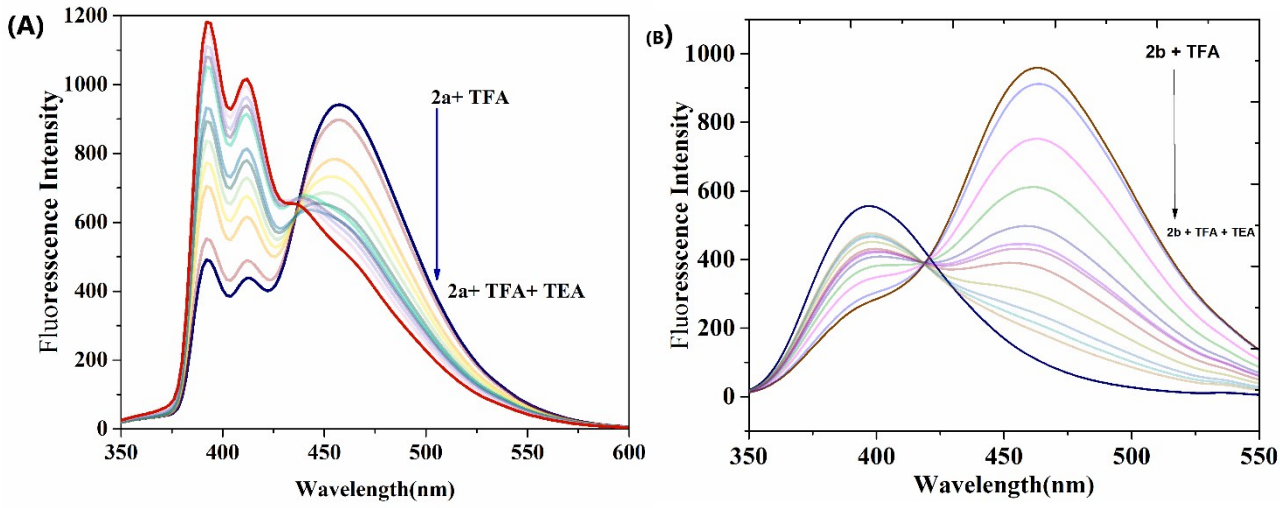


Figure S24: The fluorescence spectra of probe 2b (2 x 10^{-5} M) over the addition of various testing ion (10.0 equiv. for each ion) in 1:1 PBS buffer (pH = 7.4) and acetonitrile solvent. The $\lambda_{ex}$was set to be 267nm.

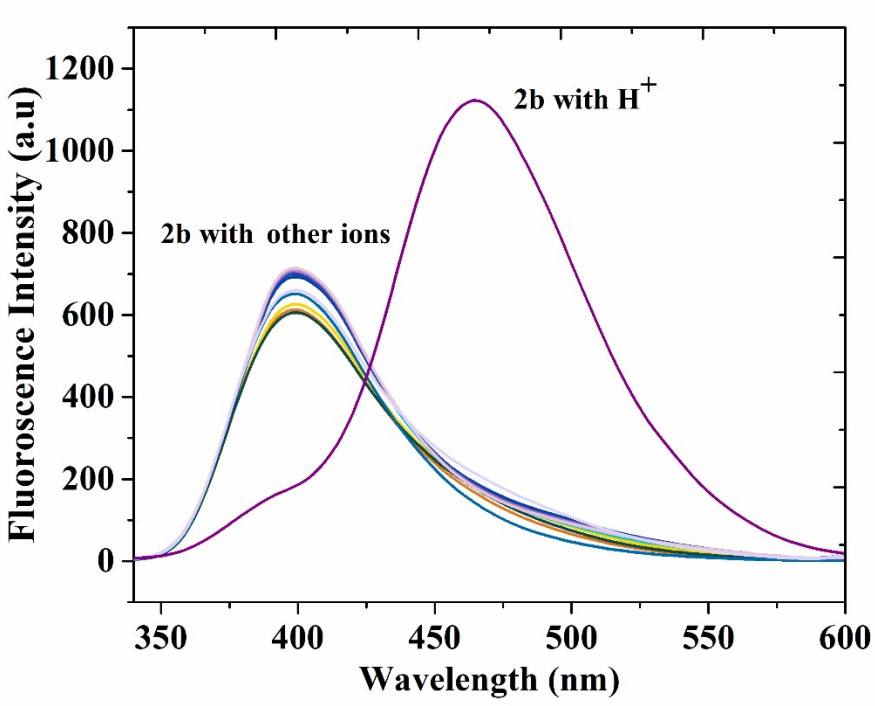


Figure S25: The optimized geometry of 2b at B3LYP/6-31+G* level of theory. The implicit solvation model is used for structure optimization in TFA solvent. All hydrogen atoms are omitted for clarity

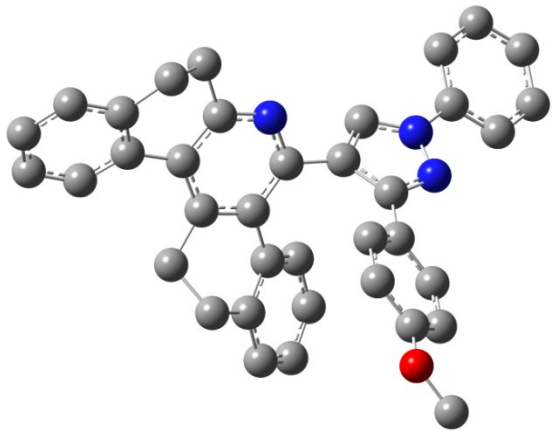


Figure S26: The optimized geometries of 2b with TFA solvent, named as 2b-TFA . The proposed solvent interaction is studied explicitly by considering one TFA molecule. In addition to that, the implicit solvation model is used for structure optimization in TFA solvent at B3LYP/6-31+G* level of theory. All hydrogen atoms are omitted for clarity.

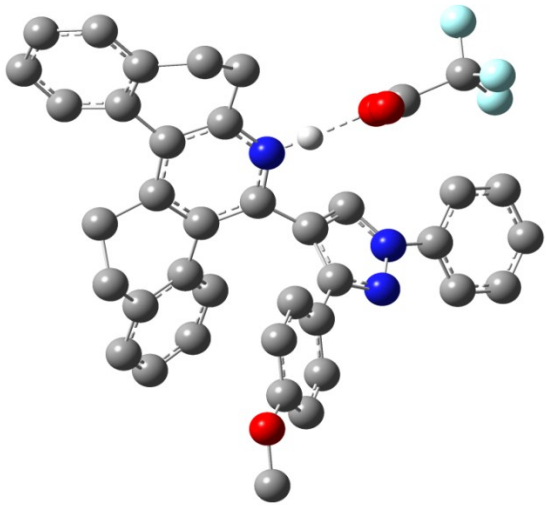
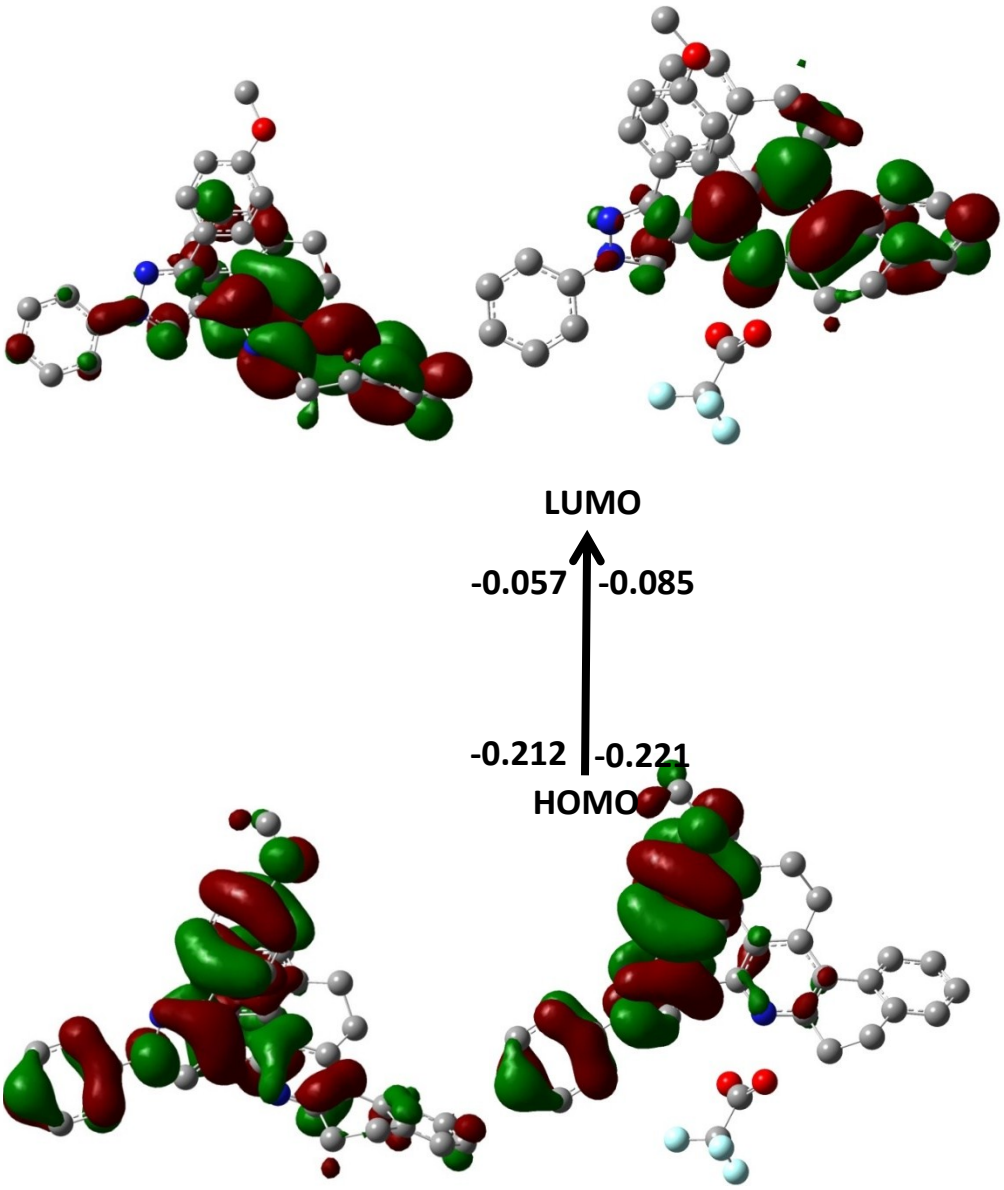


Figure S27: The FMO pairs responsible for the excitation energy in 2b and 2b-TFA. All orbitals are obtained from PCM/B3LYP/6-31+G* single point calculation in TFA solvent. Isovalue for surface = 0.02. All hydrogen atoms are omitted for clarity.



2b 2b-TFA

Figure S28: The MO pairs responsible for UV-Vis wavelength absorption maximum, λ_{max} (nm) in 2b and 2b-TFA. All orbitals are obtained from PCM/B3LYP/6-31+G* single point calculation in TFA solvent. Isovalue for surface = 0.02. All hydrogen atoms are omitted for clarity

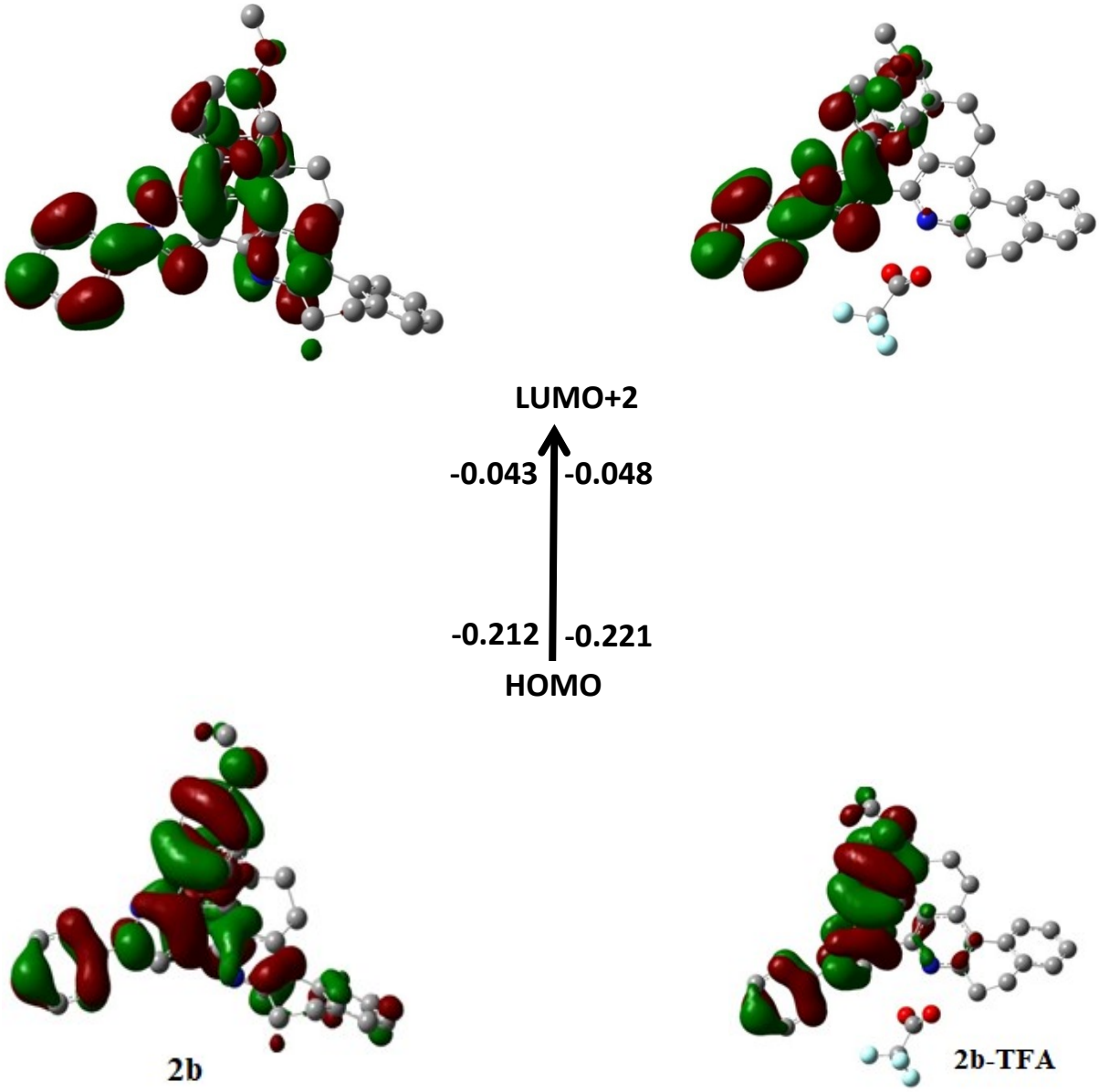


Figure S29: The cytotoxicity of 2a and 2b in *E.coli*

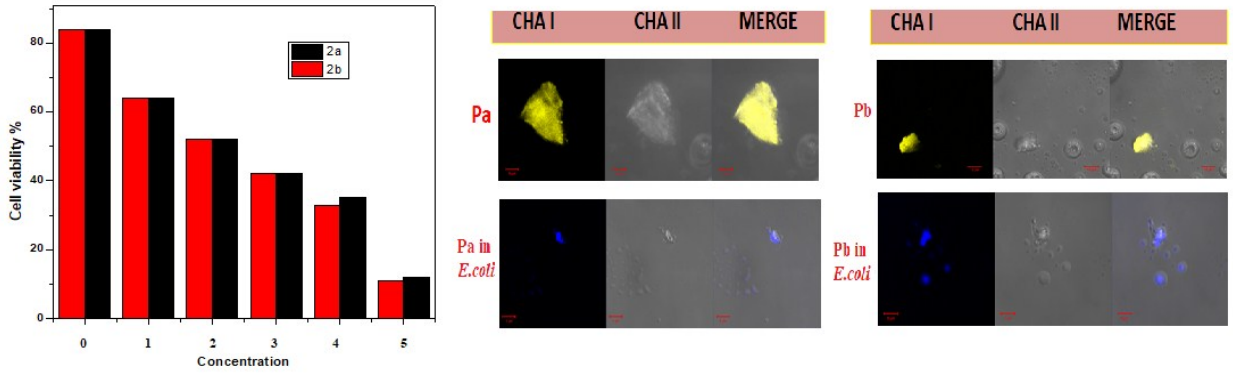


Table S1: Cartesian coordinates (in Å units) of the optimized ground state geometries of **2a**and **2b** in TFA solvent medium at B3LYP/6-31+G* level of theory.

2a (in TFA solvent medium)				2b(in TFA solvent medium)			
C	2.959469	-0.87271	-0.15303	C	-3.09482	-1.05848	0.008711
C	2.111721	-1.84842	0.424445	C	-2.29434	-1.89474	-0.80614
N	0.78695	-1.71543	0.497262	N	-0.96245	-1.83242	-0.83205
C	0.194948	-0.62503	-0.03141	C	-0.3179	-0.96521	-0.02494
C	0.938022	0.406526	-0.64609	C	-1.01211	-0.09005	0.839505
C	2.354735	0.313239	-0.6175	C	-2.43073	-0.08137	0.77819
C	4.416902	-1.17536	-0.20886	C	-4.56855	-1.27244	-0.02496
C	4.965991	-2.05572	0.756557	C	-5.14885	-1.82645	-1.19302
C	4.036601	-2.69708	1.761415	C	-4.239	-2.23254	-2.32983
C	2.721412	-3.07338	1.065513	C	-2.96178	-2.86152	-1.7568
C	0.326725	1.58472	-1.32475	C	-0.34769	0.82194	1.814286
C	1.030255	2.80956	-1.30238	C	-0.97732	2.049418	2.119361
C	2.381263	2.821438	-0.63512	C	-2.30879	2.326575	1.470512
C	3.143475	1.545136	-1.02213	C	-3.15414	1.044349	1.49415
C	-0.87527	1.512194	-2.05086	C	0.829865	0.480999	2.50301
C	-1.39675	2.638465	-2.69059	C	1.400259	1.357365	3.428381
C	-0.72015	3.859788	-2.62253	C	0.797873	2.591395	3.689523
C	0.496262	3.934281	-1.93853	C	-0.39462	2.924348	3.04131
C	5.26103	-0.68635	-1.22312	C	-5.40286	-1.02024	1.080057
C	6.621978	-0.99898	-1.24758	C	-6.78011	-1.24239	1.014591
C	7.166982	-1.82101	-0.25857	C	-7.35235	-1.73494	-0.16053

C	6.331589	-2.35417	0.727339	C	-6.53001	-2.0364	-1.25005
N	-3.39667	-0.00528	0.754468	N	3.321694	-0.38547	-0.58216
C	-2.10913	0.350216	0.835798	C	2.05893	0.057021	-0.58225
C	-1.27791	-0.59236	0.150285	C	1.158367	-0.98053	-0.17836
C	-2.16029	-1.54249	-0.32641	C	1.972288	-2.0746	0.041742
N	-3.41641	-1.15872	0.039173	N	3.258142	-1.68618	-0.19293
C	-1.73063	1.554049	1.602539	C	1.765394	1.440225	-1.00072
C	-4.64472	-1.82099	-0.23341	C	4.441048	-2.46541	-0.0776
C	-0.5601	1.577769	2.380436	C	0.619577	1.749414	-1.75858
C	-0.2251	2.709385	3.128117	C	0.361807	3.051212	-2.17383
C	-1.05518	3.834655	3.114361	C	1.248699	4.087805	-1.84207
C	-2.22566	3.818751	2.347746	C	2.399127	3.799182	-1.09528
C	-2.56022	2.689641	1.598153	C	2.644495	2.48591	-0.68455
C	-5.8313	-1.08012	-0.30275	C	5.668962	-1.83121	0.151879
C	-7.03422	-1.73338	-0.57621	C	6.828297	-2.59911	0.273396
C	-7.06124	-3.11566	-0.79046	C	6.772668	-3.99366	0.177669
C	-5.87165	-3.84678	-0.71849	C	5.543068	-4.6179	-0.05302
C	-4.66328	-3.2076	-0.43215	C	4.377503	-3.86077	-0.18995
H	4.507638	-3.58097	2.20467	O	0.907988	5.333989	-2.28981
H	3.822152	-2.0007	2.586713	C	1.776391	6.427718	-1.99038
H	2.922377	-3.8378	0.298871	H	-4.7525	-2.93514	-2.99479
H	2.000375	-3.49889	1.769607	H	-3.96819	-1.35575	-2.93809
H	2.952263	3.707559	-0.93242	H	-3.22117	-3.7907	-1.22588
H	2.272577	2.858817	0.459109	H	-2.25305	-3.1248	-2.54737
H	3.321497	1.555519	-2.10786	H	-2.83312	3.134593	1.992019
H	4.124489	1.534929	-0.53999	H	-2.17252	2.650058	0.427757
H	-1.40391	0.568928	-2.12866	H	-3.35669	0.774563	2.541446
H	-2.32762	2.557726	-3.24619	H	-4.1223	1.226226	1.020263
H	-1.12431	4.740695	-3.11487	H	1.300467	-0.4795	2.326879
H	1.046886	4.872338	-1.90887	H	2.310616	1.070733	3.948816
H	4.852918	-0.07831	-2.02225	H	1.240149	3.278904	4.406114
H	7.249057	-0.60804	-2.04466	H	-0.88839	3.868417	3.262373
H	8.225751	-2.06675	-0.2681	H	-4.97561	-0.6744	2.0142
H	6.741507	-3.02697	1.477716	H	-7.3993	-1.04013	1.88472
H	-1.98062	-2.4194	-0.93062	H	-8.42378	-1.90762	-0.22126
H	0.080939	0.70184	2.416768	H	-6.96352	-2.45742	-2.15481
H	0.681018	2.70734	3.728881	H	1.724664	-3.06585	0.391371
H	-0.79399	4.71448	3.696823	H	-0.0695	0.959942	-2.04382
H	-2.87682	4.689117	2.328871	H	-0.51998	3.282187	-2.76487
H	-3.46492	2.683193	0.997544	H	3.101865	4.578568	-0.823
H	-5.80222	-0.00802	-0.14511	H	3.533827	2.27339	-0.09858
H	-7.95272	-1.15509	-0.62942	H	5.70474	-0.75108	0.233643
H	-7.99993	-3.61807	-1.00656	H	7.778063	-2.10196	0.451685
H	-5.88054	-4.92268	-0.87002	H	7.67753	-4.58657	0.277332

H	-3.7504	-3.78829	-0.34501	H	5.486621	-5.6994	-0.14179
				H	3.434607	-4.35516	-0.40069
				H	1.313742	7.304754	-2.44489
				H	1.863265	6.578194	-0.90759
				H	2.771826	6.271937	-2.42324

Table S2: Cartesian coordinates (in Å units) of the optimized ground state geometries of **2a-TFA**and **2b-TFA** in TFA solvent medium at B3LYP/6-31+G* level of theory.

2a-TFA (in TFA solvent medium)				**2b-TFA (in TFA solvent medium)**			
C	-3.1222	0.552964	-0.63369	C	3.06928	-1.07576	-0.68547
C	-2.06068	1.462042	-0.48046	C	1.973688	-1.88053	-0.32722
N	-0.80055	1.008561	-0.38387	N	0.734711	-1.36256	-0.32875
C	-0.44725	-0.3044	-0.45587	C	0.434027	-0.08468	-0.69032
C	-1.46227	-1.26053	-0.60884	C	1.485398	0.767358	-1.06065
C	-2.81769	-0.82556	-0.59067	C	2.82204	0.288789	-0.95411
C	-4.48173	1.134221	-0.80275	C	4.401262	-1.73857	-0.72457
C	-4.74397	2.413506	-0.25553	C	4.61831	-2.87122	0.096668
C	-3.6206	3.156185	0.429021	C	3.474463	-3.39155	0.935315
C	-2.30997	2.938234	-0.3415	C	2.163615	-3.29678	0.140131
C	-1.19069	-2.71398	-0.78672	C	1.270799	2.152243	-1.56407
C	-2.12837	-3.63194	-0.26691	C	2.255289	3.122283	-1.27844
C	-3.36532	-3.07014	0.384166	C	3.478915	2.671262	-0.52365
C	-3.88197	-1.8942	-0.45954	C	3.931733	1.311471	-1.07677
C	-0.09091	-3.20069	-1.51464	C	0.180126	2.506964	-2.37692
C	0.104477	-4.57284	-1.67605	C	0.040974	3.811517	-2.85199
C	-0.7999	-5.48008	-1.11611	C	0.992996	4.781653	-2.52547
C	-1.91697	-5.00464	-0.42481	C	2.100349	4.428973	-1.75006
C	-5.49072	0.497297	-1.54756	C	5.425194	-1.33098	-1.59874
C	-6.75156	1.07755	-1.6936	C	6.658638	-1.98369	-1.61536
C	-7.02268	2.313795	-1.10233	C	6.886875	-3.06497	-0.7609
C	-6.01408	2.978827	-0.40024	C	5.861791	-3.50935	0.078276
N	2.982781	-1.40163	0.414723	N	-2.93323	1.324048	-0.05662
C	1.671488	-1.3804	0.66454	C	-1.61805	1.311651	0.17352
C	0.991531	-0.58049	-0.30915	C	-0.98902	0.279682	-0.59671
C	1.993936	-0.11523	-1.14286	C	-2.02559	-0.32745	-1.28479
N	3.164736	-0.63152	-0.68982	N	-3.16687	0.326652	-0.95135
C	1.131252	-2.08972	1.841041	C	-1.0259	2.256627	1.137147

C	4.467538	-0.44176	-1.23534	C	-4.48726	0.068832	-1.41934
C	0.115493	-1.51873	2.626459	C	0.011131	1.867554	2.006493
C	-0.36988	-2.18554	3.753965	C	0.544959	2.758416	2.930498
C	0.155095	-3.42984	4.116241	C	0.051371	4.070359	3.014144
C	1.172265	-4.00228	3.344416	C	-0.98783	4.47329	2.163463
C	1.656863	-3.33857	2.216268	C	-1.51324	3.56755	1.238578
C	5.419557	-1.4607	-1.1162	C	-5.41265	1.11719	-1.48364
C	6.691157	-1.27595	-1.66161	C	-6.70326	0.863633	-1.95123
C	7.011676	-0.08972	-2.33048	C	-7.07062	-0.42207	-2.36234
C	6.053088	0.922008	-2.44134	C	-6.13861	-1.46188	-2.29184
C	4.781648	0.756237	-1.88746	C	-4.84835	-1.22538	-1.81235
H	-3.84655	4.225318	0.483746	H	3.657191	-4.42959	1.228938
H	-3.49432	2.802478	1.462588	H	3.376781	-2.80943	1.863248
H	-2.38857	3.387028	-1.34268	H	2.210952	-3.96251	-0.73424
H	-1.47044	3.413195	0.170845	H	1.312392	-3.60717	0.750182
H	-4.1421	-3.83673	0.468335	H	4.288091	3.401458	-0.62464
H	-3.14567	-2.71952	1.403013	H	3.262485	2.57545	0.550247
H	-4.16833	-2.27083	-1.45235	H	4.219255	1.437032	-2.13085
H	-4.78121	-1.4676	-0.00822	H	4.818604	0.960162	-0.54371
H	0.609731	-2.51009	-1.96966	H	-0.5572	1.76266	-2.65473
H	0.959237	-4.93032	-2.24387	H	-0.8075	4.065859	-3.4815
H	-0.64784	-6.54968	-1.23458	H	0.885414	5.800105	-2.88939
H	-2.64185	-5.70447	-0.01523	H	2.862013	5.170438	-1.51963
H	-5.28931	-0.44352	-2.04601	H	5.256257	-0.51887	-2.29593
H	-7.51374	0.567556	-2.27622	H	7.432971	-1.65212	-2.30163
H	-8.00339	2.76987	-1.20791	H	7.846267	-3.57535	-0.76498
H	-6.20888	3.959164	0.028464	H	6.021525	-4.37469	0.717312
H	1.940276	0.496582	-2.03111	H	-2.01237	-1.13295	-2.00377
H	-0.27854	-0.53838	2.37454	H	0.389116	0.849623	1.982327
H	-1.14966	-1.7257	4.355581	H	1.337377	2.450351	3.606499
H	-0.22168	-3.94712	4.99475	H	-1.38943	5.479369	2.205299
H	1.586883	-4.9688	3.619217	H	-2.31209	3.890254	0.577634
H	2.441612	-3.7868	1.614274	H	-5.11804	2.112479	-1.17065
H	5.159993	-2.38068	-0.60458	H	-7.42081	1.678168	-1.99999
H	7.43017	-2.06706	-1.56873	H	-8.07554	-0.61292	-2.72832
H	8.001862	0.047317	-2.7559	H	-6.41681	-2.46786	-2.59346
H	6.296134	1.853552	-2.94476	H	-4.14291	-2.04543	-1.72408
H	4.054006	1.559714	-1.94348	C	-1.34299	-3.54816	1.505739
C	1.17408	3.541066	1.015339	O	-0.57328	-3.40255	2.468092
O	0.409301	3.503307	1.991227	O	-1.29435	-3.05355	0.344135
O	1.147158	2.882753	-0.06349	H	-0.05941	-1.99016	-0.00943
H	-0.03312	1.722967	-0.21211	C	-2.54873	-4.52749	1.709017
C	2.35485	4.569002	1.072051	F	-3.70703	-4.04003	1.197546
F	3.553236	3.979525	0.822845	F	-2.7875	-4.8197	3.007076

F	2.4671	5.194043	2.264952	F	-2.30873	-5.71328	1.077359
F	2.193601	5.543779	0.132785	O	0.641567	4.872143	3.94878
				C	0.168701	6.21224	4.098461
				H	0.30695	6.785405	3.173967
				H	-0.88816	6.227329	4.389865
				H	0.773143	6.650411	4.893598

Table S3: The absolute energy of the optimized ground state geometries of **2a, 2b, 2a-TFA,** and **2b-TFA**in TFA solvent medium at B3LYP/6-31+G* level of theory

Systems	Absolute Energy (a.u)
2a	-1552.4382982
2b	-1666.9668221
2a-TFA	-2079.290255
2b-TFA	-2193.8190271