Supporting Information

Recognition and Mechanistic Investigation of Anion Sensing by Ruthenium (II) Arene Complexes and Bio-Imaging application

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EXPERIMENTAL SECTION

Stability of complexes

Since the stock solutions of complexes **1**, **2, 3** and **4** were prepared in DMSO, therefore all the biological studies were carried out with 1% DMSO solution in the media. As it becomes imperative to evaluate the stability of the complexes in DMSO it was evaluated through ^{1}H and ^{13}C NMR in DMSO-d_6 at a time interval of 0 h, 12 h, 24 h.

For determining the stability of the complexes through UV spectroscopy, 4×10^{-5} M solution of all the complexes was prepared by adding 16 µL from 5 mM stock solution to 1.984 mL of DMSO in cuvette and UV spectra were recorded at 0 and 24 h.

Calculation of Excited-State Lifetimes

The temperature-dependent fluorescence lifetime was investigated on a TCSPC system (model: Fluorescence-01-NL) equipped with a temperature-controlled cell holder. For analysing the lifetime decay data, IBH DAS 6.0 software was used. The iterative reconvoluation method was used to measure the fluorescence lifetime decay, and the fit value was judged by the reduced w-square (w2) value.

Equations S(iii) and S(iv) were fitted to get an idea about the time-resolved emission decays where the decays of the probe, L1 + CO_3^{2-} and L1 + SO_4^{2-} were evaluated using a tri, bi and mono-exponential function:[1]

$$F(t) = \sum_{i=1}^{2} a_i \exp\left(-\frac{t}{\tau_i}\right) \qquad \dots \qquad \dots \; S(i)$$

Where, F(t) is the PL decay at normalized condition, α_i is the pre-exponential factor, and a_1 and a_2 denotes the normalized amplitude having decay component τ_1 and τ_2 respectively. Eqn. (iv) gives the value of average lifetime (in ns):

$$\langle \tau \rangle = \sum_{i=1}^{2} a_i \tau_i \qquad \dots \qquad \dots \; S(ii)$$

Where, a_i is the contribution of the i^{th} decay component, and $a_i = \alpha_i/\Sigma\alpha_i$.

Fluorescence Quantum Yield

Quantum yield has been calculated according to the procedure suggested in manual "A Guide to Recording Fluorescence Quantum Yields" by HORIBA Jobin Yvon IBH pvt. Ltd[2] and also followed the published procedure[3]. 2-Aminopyridine, which was dissolved in 0.1 M H_2SO_4 (Φ_R = 0.60) was used as reference to estimate the quantum yield (QY) values of receptor L1, L1 + CO_3^{2-} and L1 + SO_4^{2-}in DMSO[4] by using the eqn. S(v):[5]

$$\Phi_s = \frac{S_S}{S_R} \times \frac{A_R}{A_S} \times \frac{\eta_S^{\,2}}{\eta_R^{\,2}} \times \Phi_R \qquad \ldots \qquad \ldots \ \text{S(iii)}$$

Where 'Φ' denotes the fluorescence QY, 'Area' terms denote the integration of the fluorescence curve, 'A' denotes optical density and the refractive index as η (η = 1.479 for DMSO solvent medium). While S are the slope from the plot of the integrated fluorescence intensity vs. the absorbance of the standard and the sample at different concentration respectively. Subscripts R and S stand for the respective parameters belonging to the experimental reference as well as the sample.

Effect of pH

The stability of complex **3** was also evaluated at different pH. The fluorescence spectra of complexes were taken after addition of 16 µL from 5 mM complex 3 stock solution to different pH solutions prepared in milli-Q water.

Cell culture

Breast carcinoma cells (MCF7), Human squamous carcinoma (A431), human melanoma (A375), were purchased from NCCS (National Centre for Cell Science), Pune. All the cell lines were cultured in Dulbecco minimum essential medium (DMEM). The growth and maintenance procedure of the cell lines and materials used were as per our previous publication.[6]

In vitro cytotoxicity assay

The cells were seeded in 96-well flat-bottomed culture plate in 100 µl cell suspension and were incubated overnight at 37 °C in a 5% CO_2 incubator for attachment. Complex treatment was done by making 5 mM stock solution of the complexes **1**, **2, 3** and **4** in DMSO and then this stock solution was further diluted to 160 µg/mL, 80 µg/mL, 40 µg/mL, 20 µg/mL. After 72 h treatment, the MTT experiment was carried out as per our previous publication.[6]

Bio-Imaging

Complexes were evaluated for their fluorescence properties in the cells. The 5×10^4 A375 cells were placed in confocal dishes and were incubated overnight in the CO_2 incubator for attachment. The cells in confocal dishes having 2 mL of media were treated with 200 µL of 10 mM stock solution of complexes **1** for 4 h and untreated cells were taken as control. The fluorescence was viewed by the help of Fluoview FV100 (OLYMPUS, Tokyo, Japan) fluorescence microscope using DAPI filter. The excitation wavelength of complex **1** was 330 nm and emission wavelength was 370 nm.

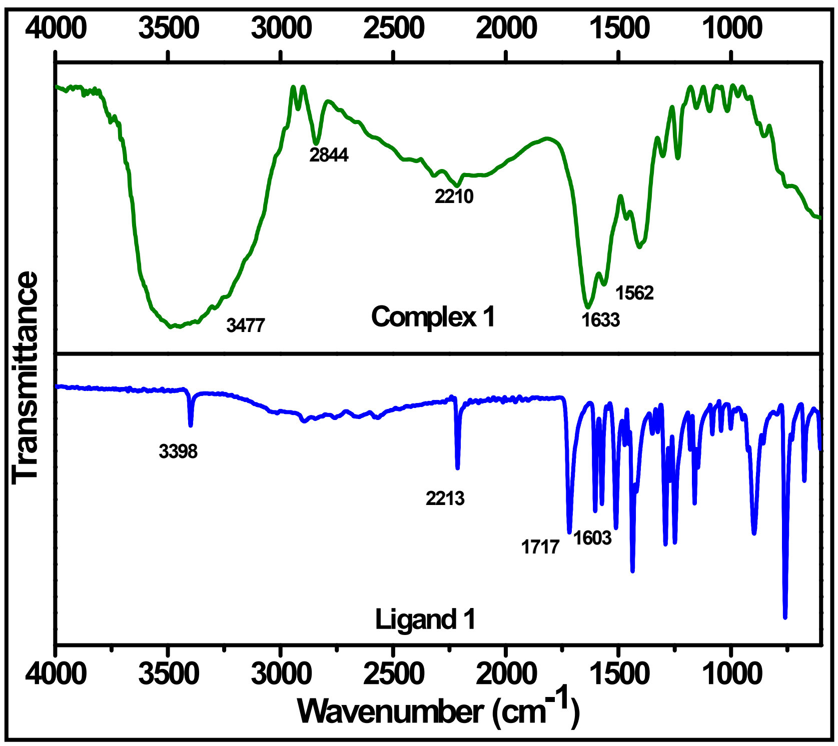


Figure S1. IR spectra of **1**.

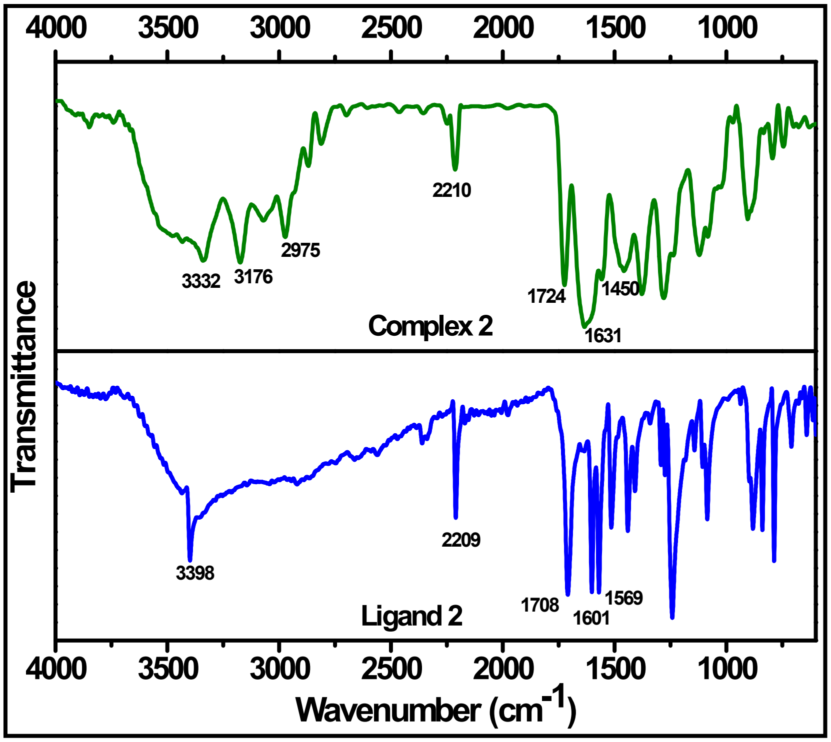


Figure S2. IR spectra of **2**.

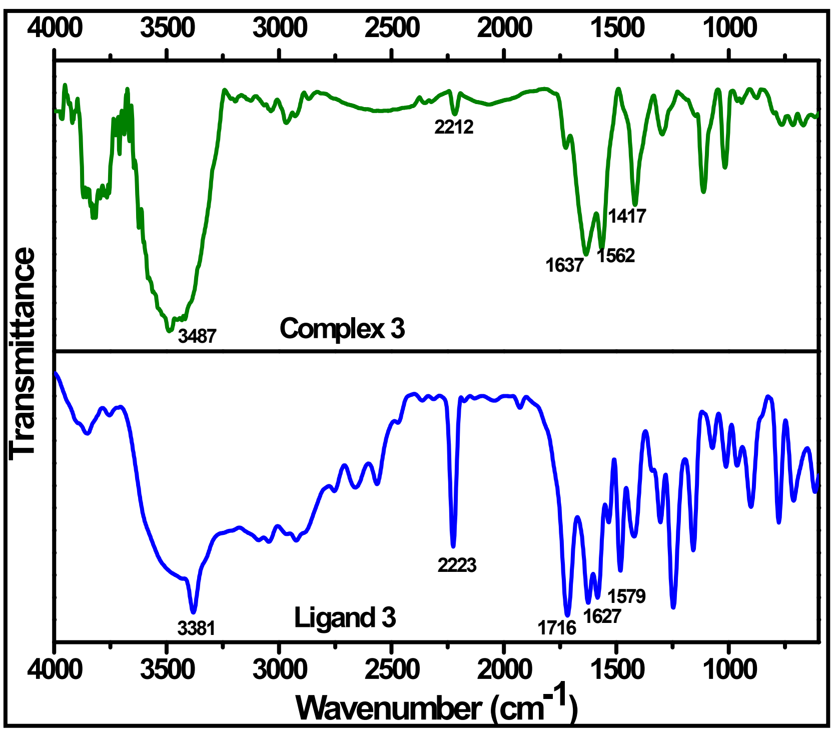


Figure S3. IR spectra of **3**.

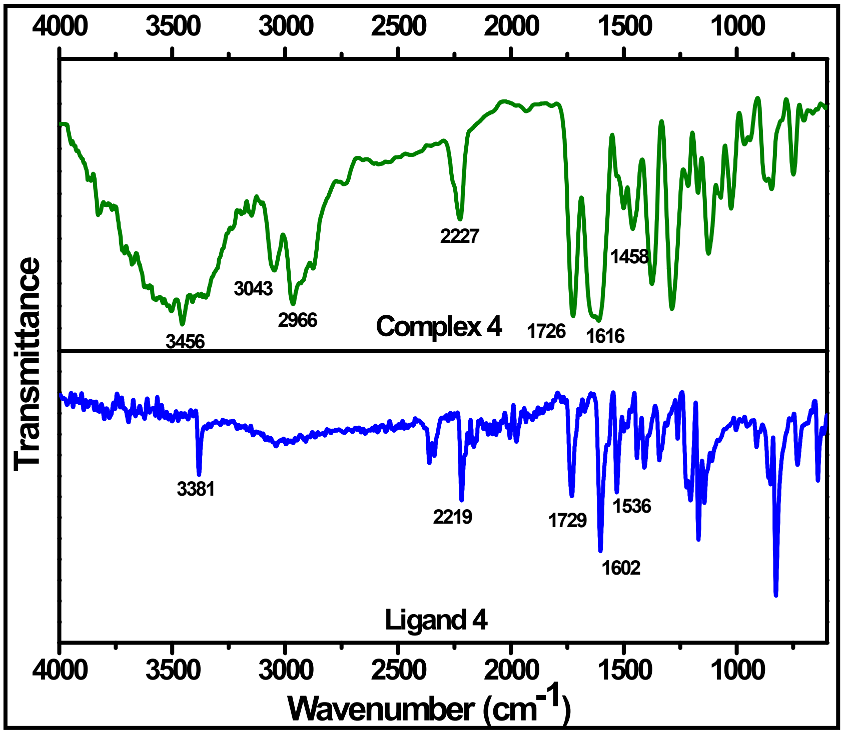
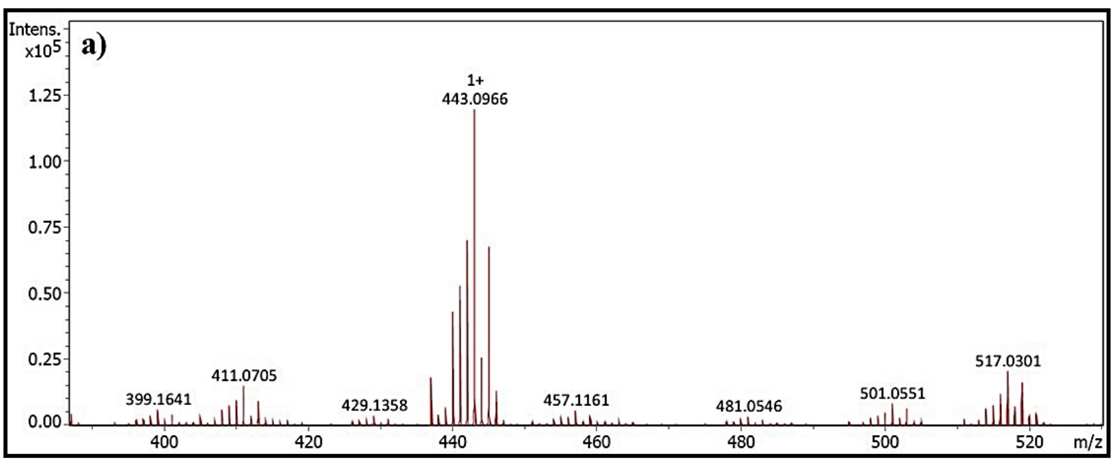


Figure S4. IR spectra of **4**.



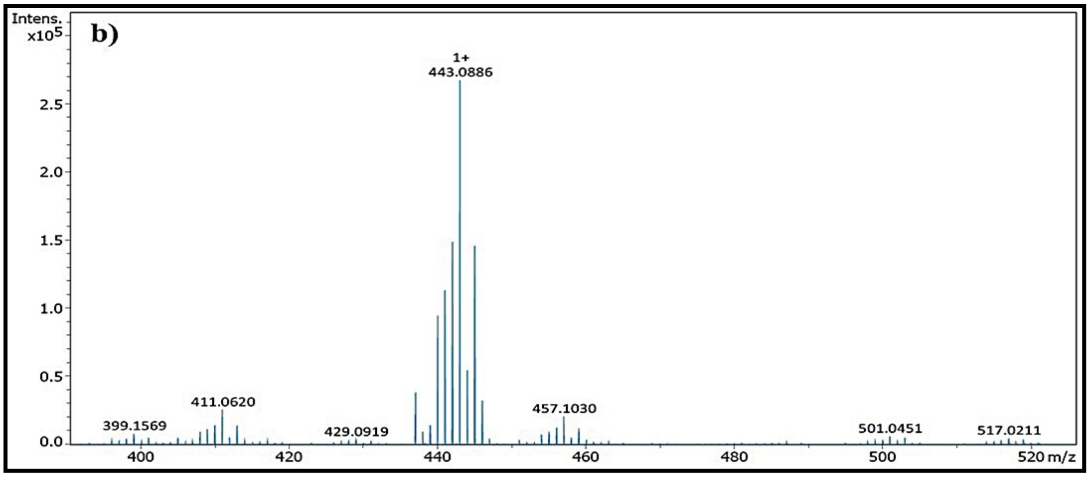


Figure S5. ESI-MS of **1** recorded after dissolving in a) MeOH, b) acetonitrile at room temperature.

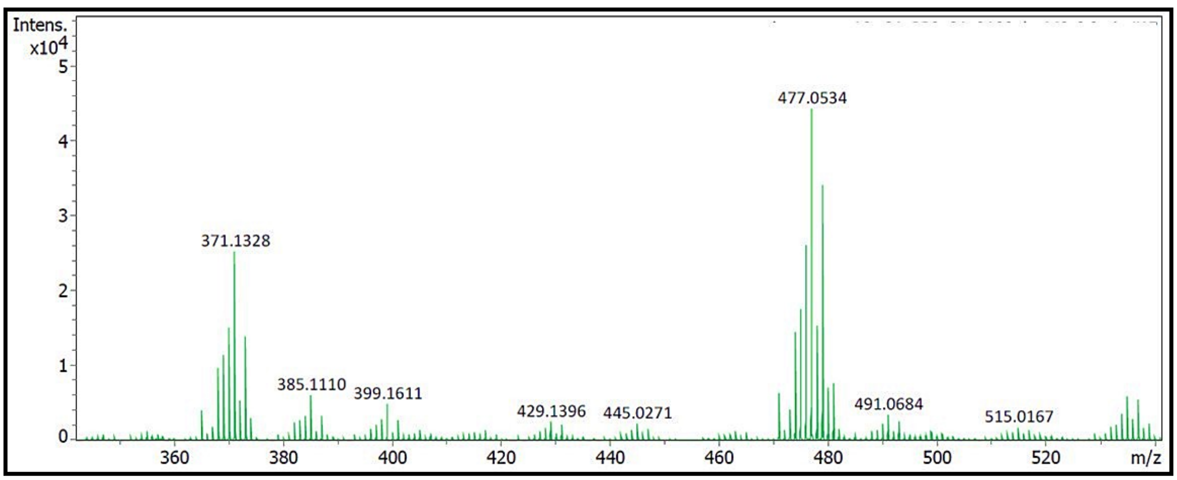


Figure S6. ESI-MS of **2** recorded after dissolving in MeOH at room temperature.

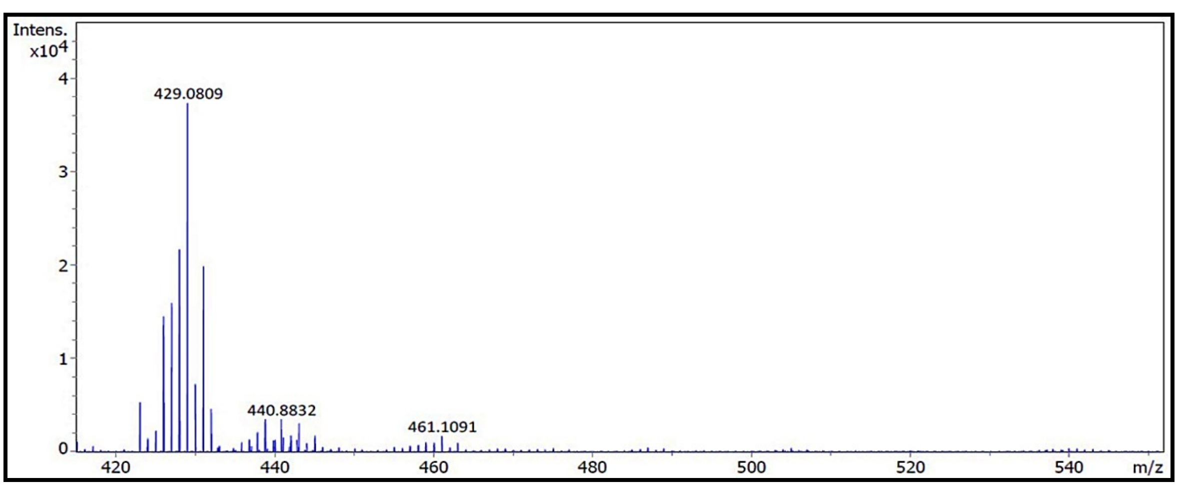


Figure S7. ESI-MS of **3** recorded after dissolving in MeOH at room temperature.

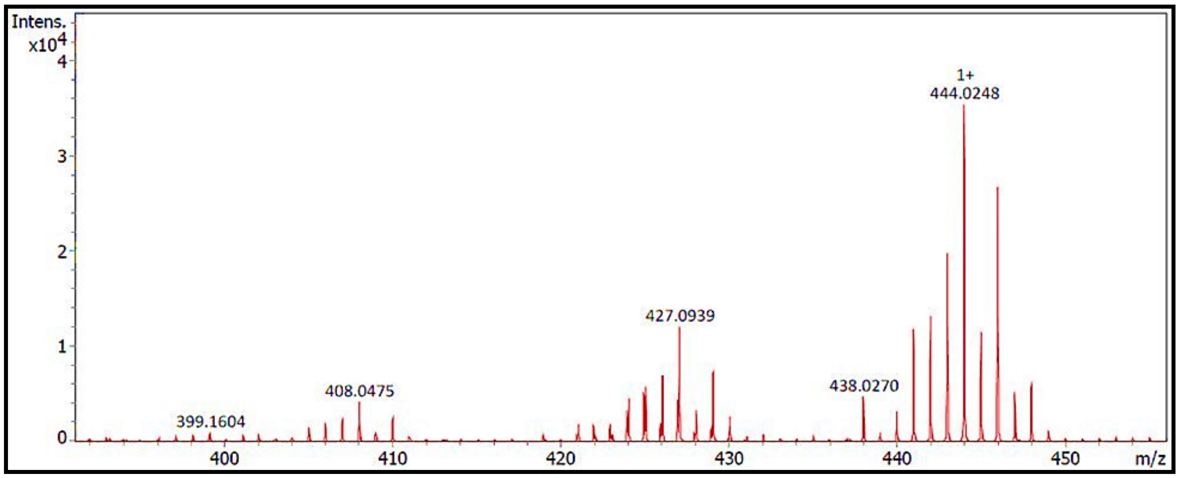


Figure S8. ESI-MS of **4** recorded after dissolving in MeOH at room temperature.

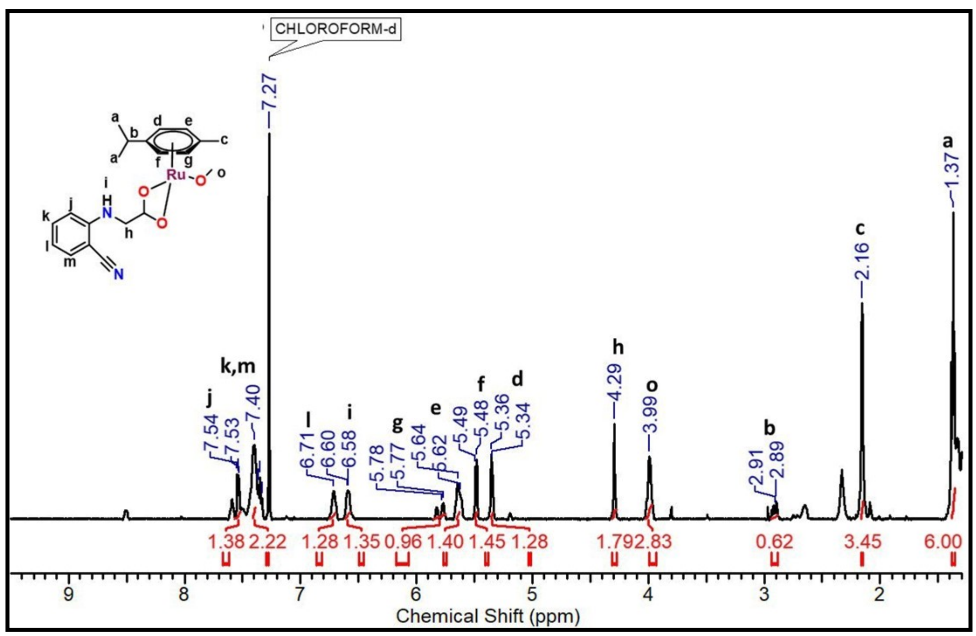


Figure S9. ^{1}H NMR of **1** in $CDCl_3$ at room temperature.

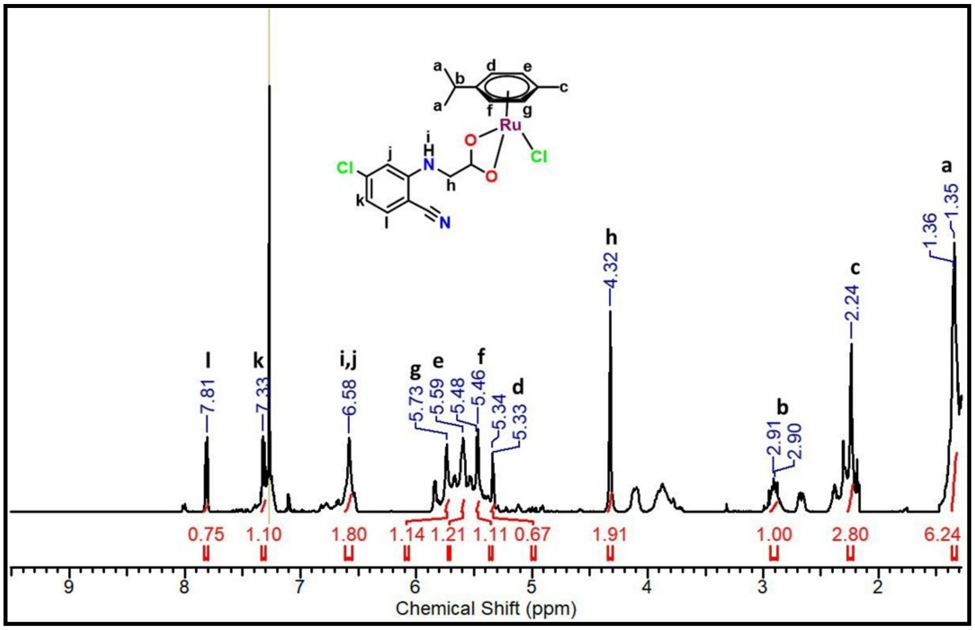


Figure S10. **¹H NMR** of **2** in $CDCl_3$ at room temperature.

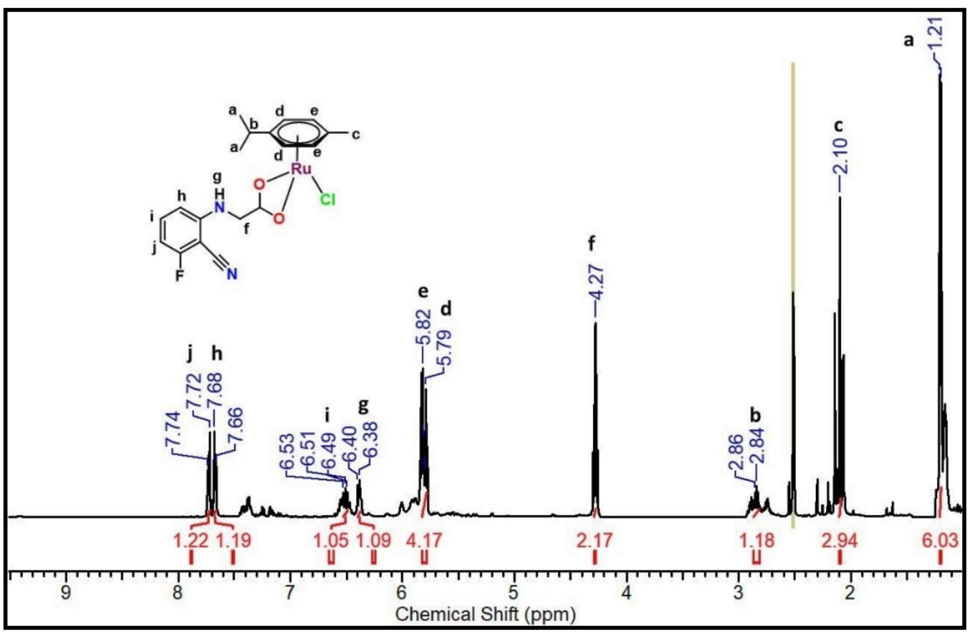


Figure S11. **¹H NMR** of **3** in DMSO-d_6 at room temperature.

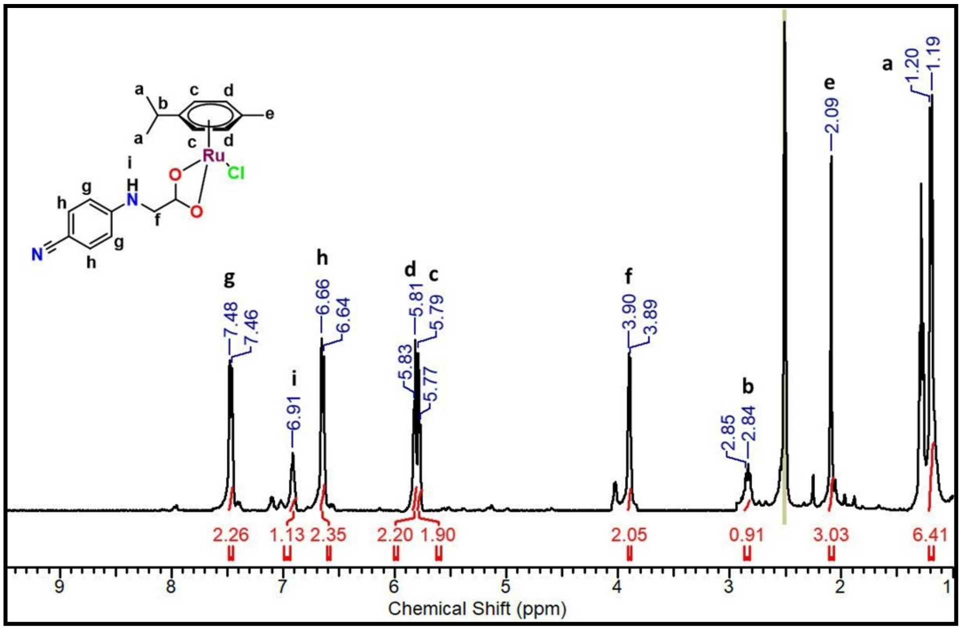


Figure S12. ^{1}H NMR of **4** in DMSO-d_6 at room temperature.

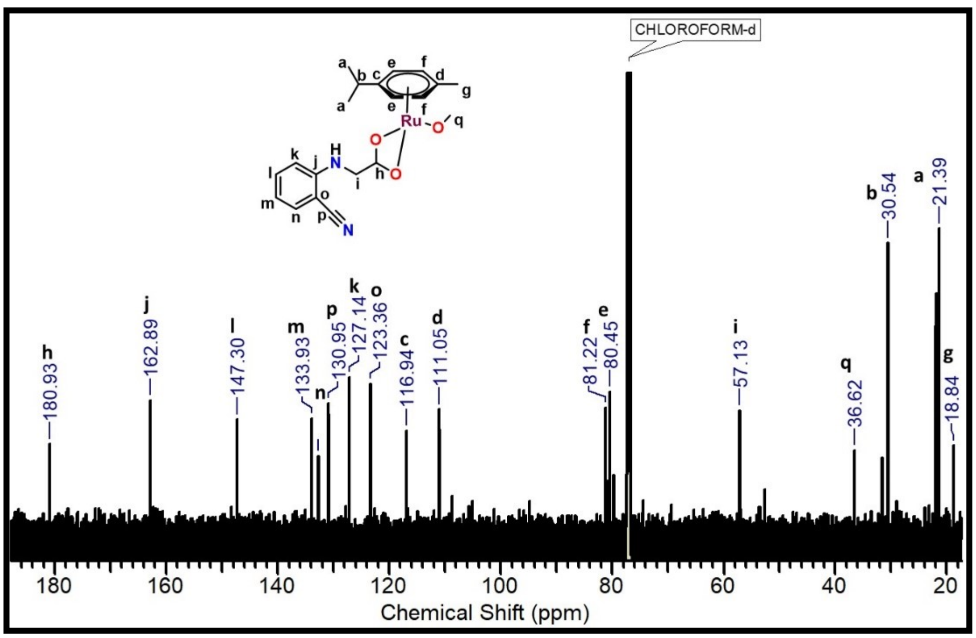


Figure S13. ^{13}C NMR of **1** in $CDCl_3$ at room temperature.

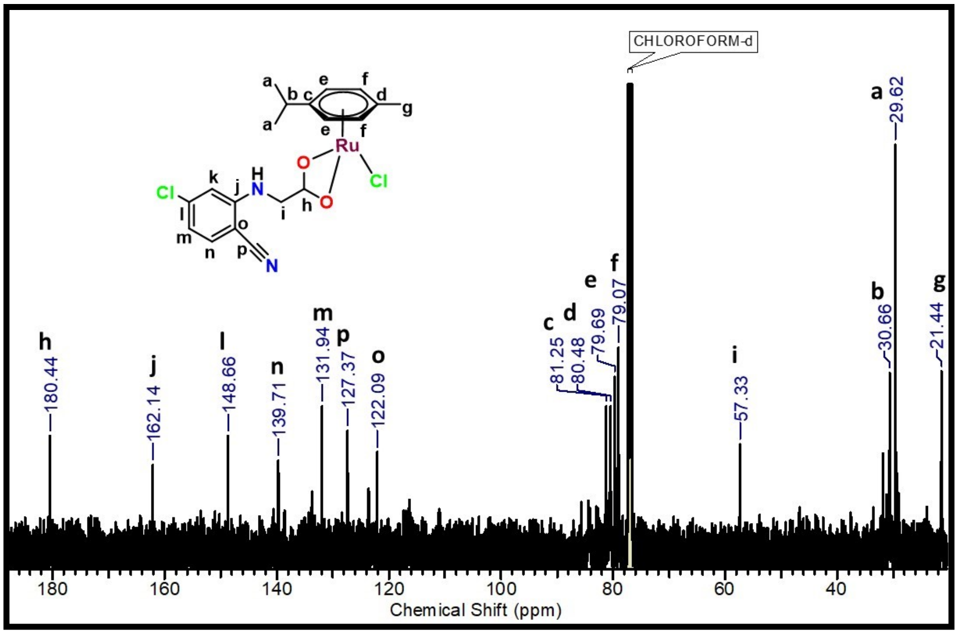


Figure S14. **^{13}C NMR** of **2** in $CDCl_3$ at room temperature.

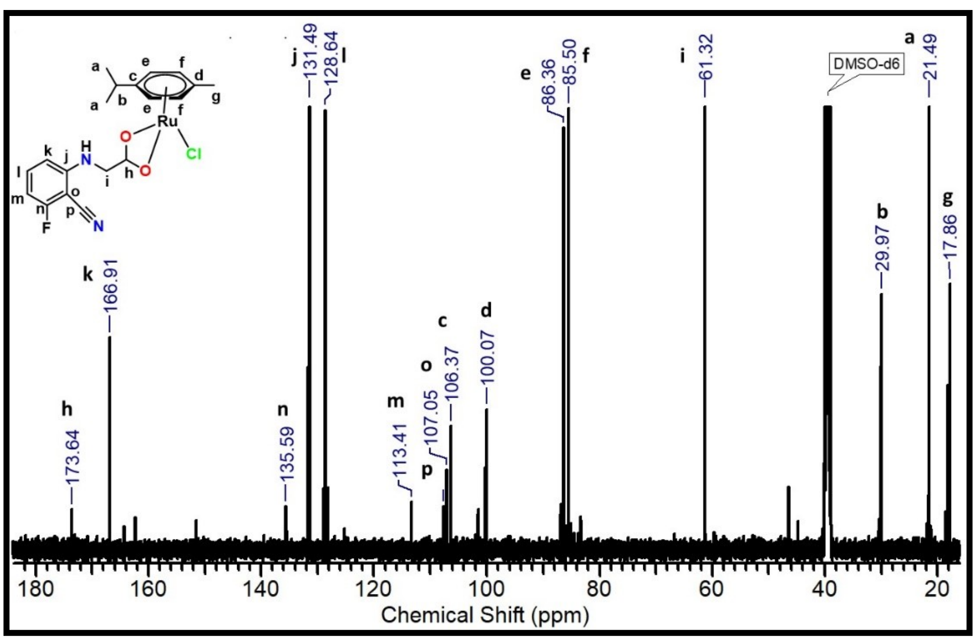


Figure S15. **^{13}C NMR** of **3** in DMSO-d_6 at room temperature.

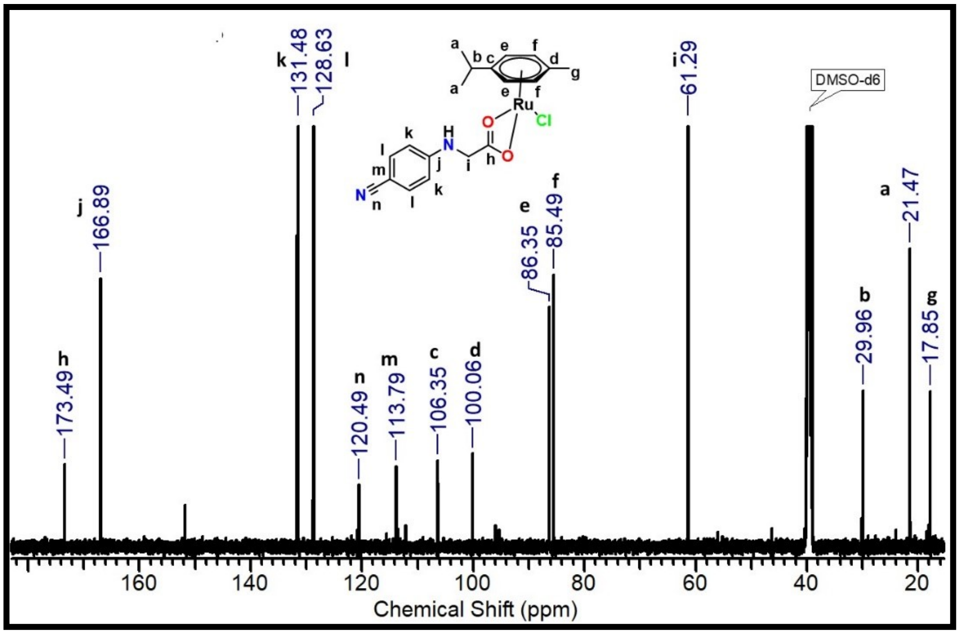


Figure S16. ¹³C NMR of **4** in DMSO-d_6 at room temperature.

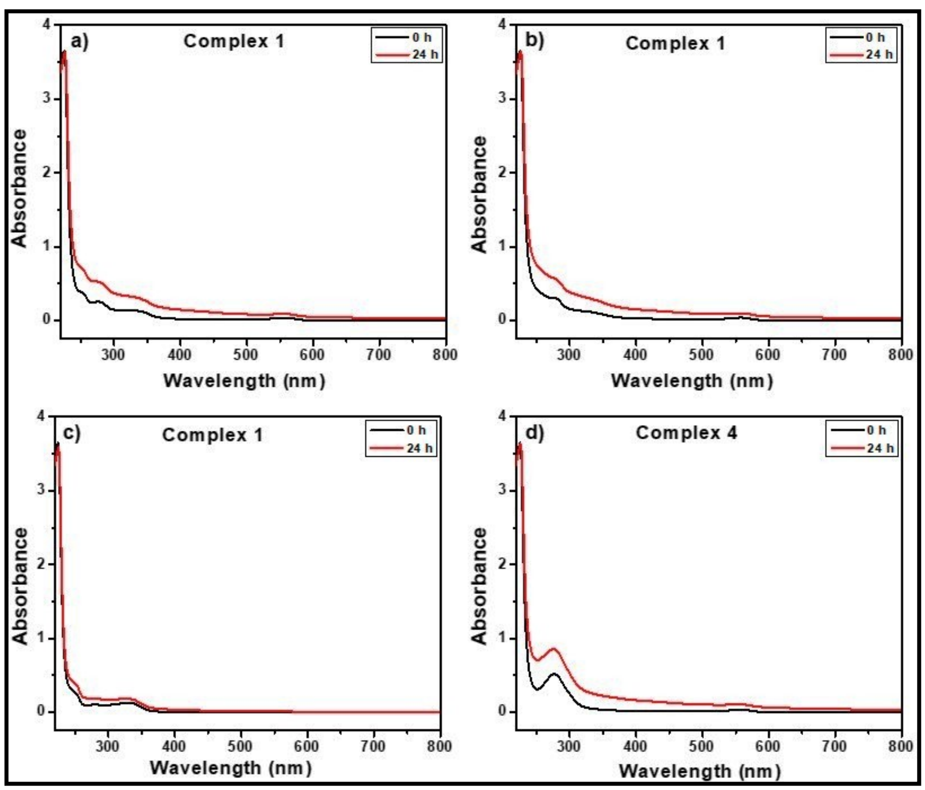


Figure S17. Stability of **1**, **2**, **3** and **4** in DMSO-d_6 at room temperature at 0h and 24 h.

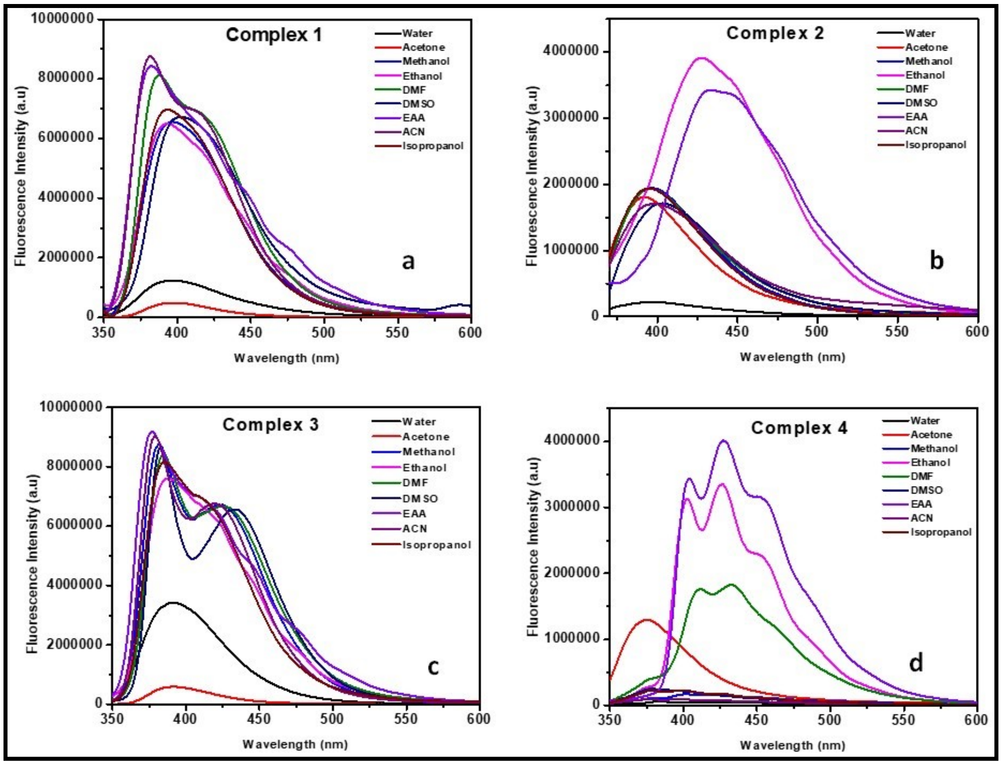


Figure S18. Effect of solvents on the fluorescence of **1**, **2**, **3** and **4** in DMSO-d_6 at room temperature

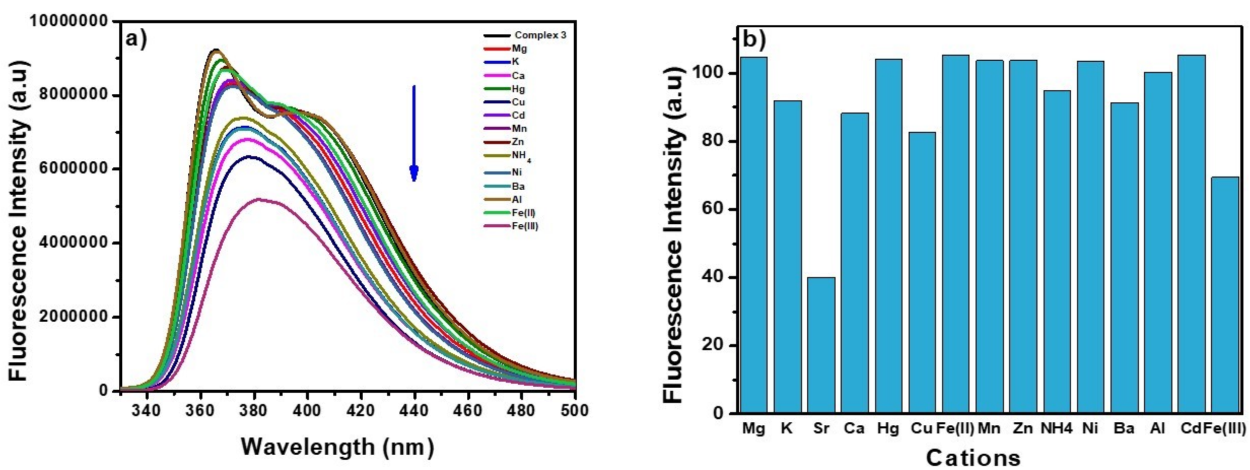


Figure S19. Effect of cations on the fluorescence of **3** in DMSO-d_6 at room temperature.

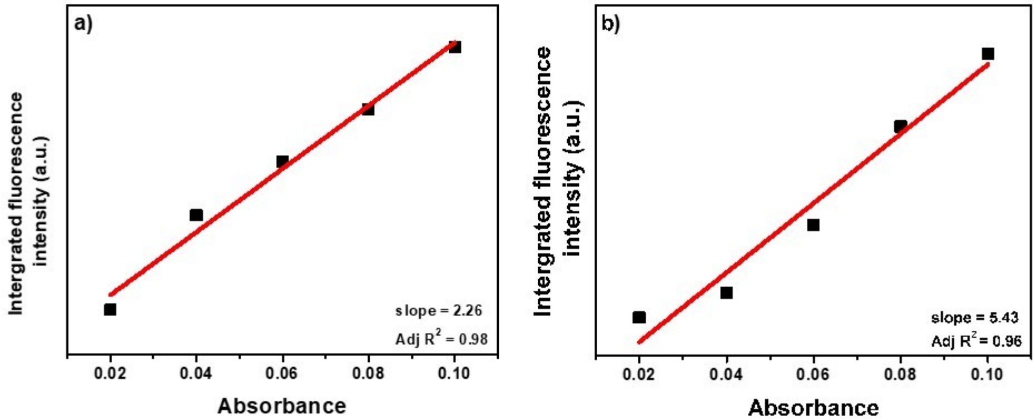


Figure S20. Quantum Yield of **3** with respect to 2 amino pyridine.

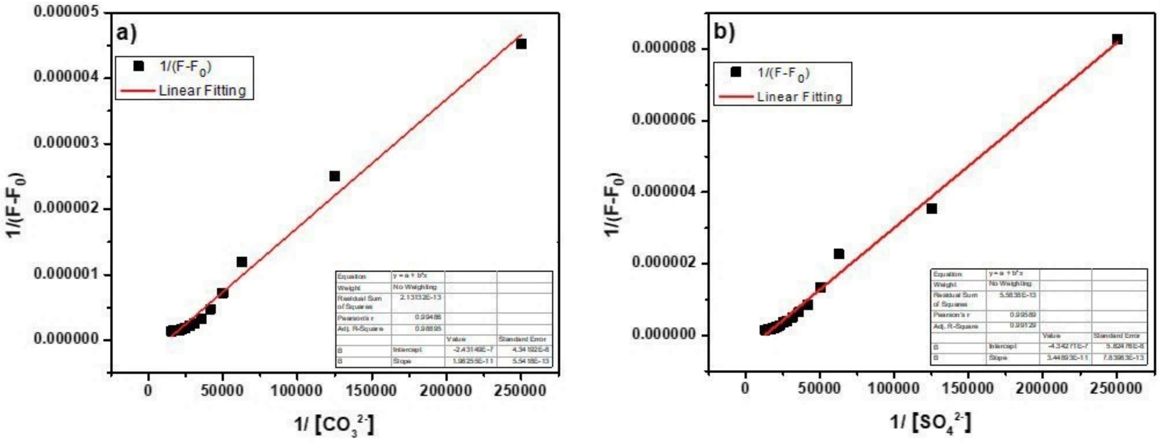


Figure S21. B-H plot of **3** obtained by titration with CO_3^{2-} and SO_4^{2-} anions.

Table S1. Quantum yield (ϕ) of **3, 3 + CO_3^{2-}** and **3 + SO_4^{2-}** anions in DMSO in comparison with the reference taken as Amino pyridine (∫$_S$ represents the area integral of sample and reference).

Complexes	λmax (nm)	Abs (a.u.)	∫ Area	Φ values
3	380	0.10	227315719	0.468
3 + CO_3^{2-}	380	0.099	65364296	0.134
3 + SO_4^{2-}	380	0.098	62404914	0.128
Amino pyridine (reference)	-	0.10	313005188.6	0.60

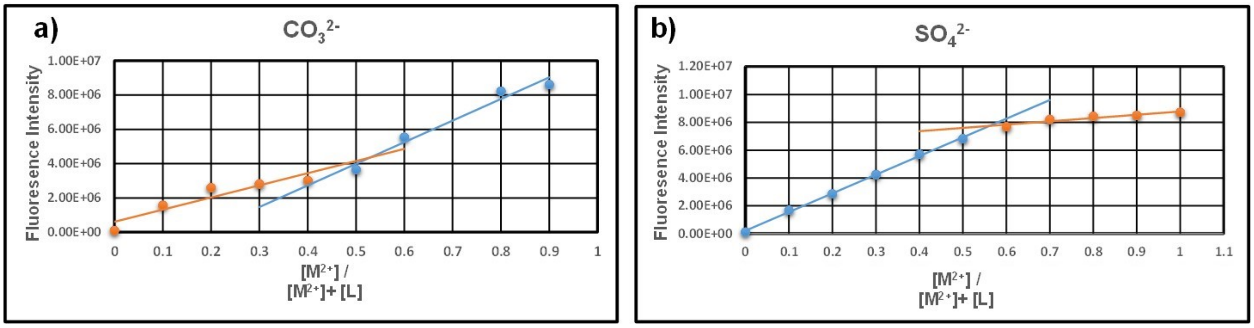


Figure S22. JOB's plot of **3** on serial dilution with CO_3^{2-} and SO_4^{2-} anions.

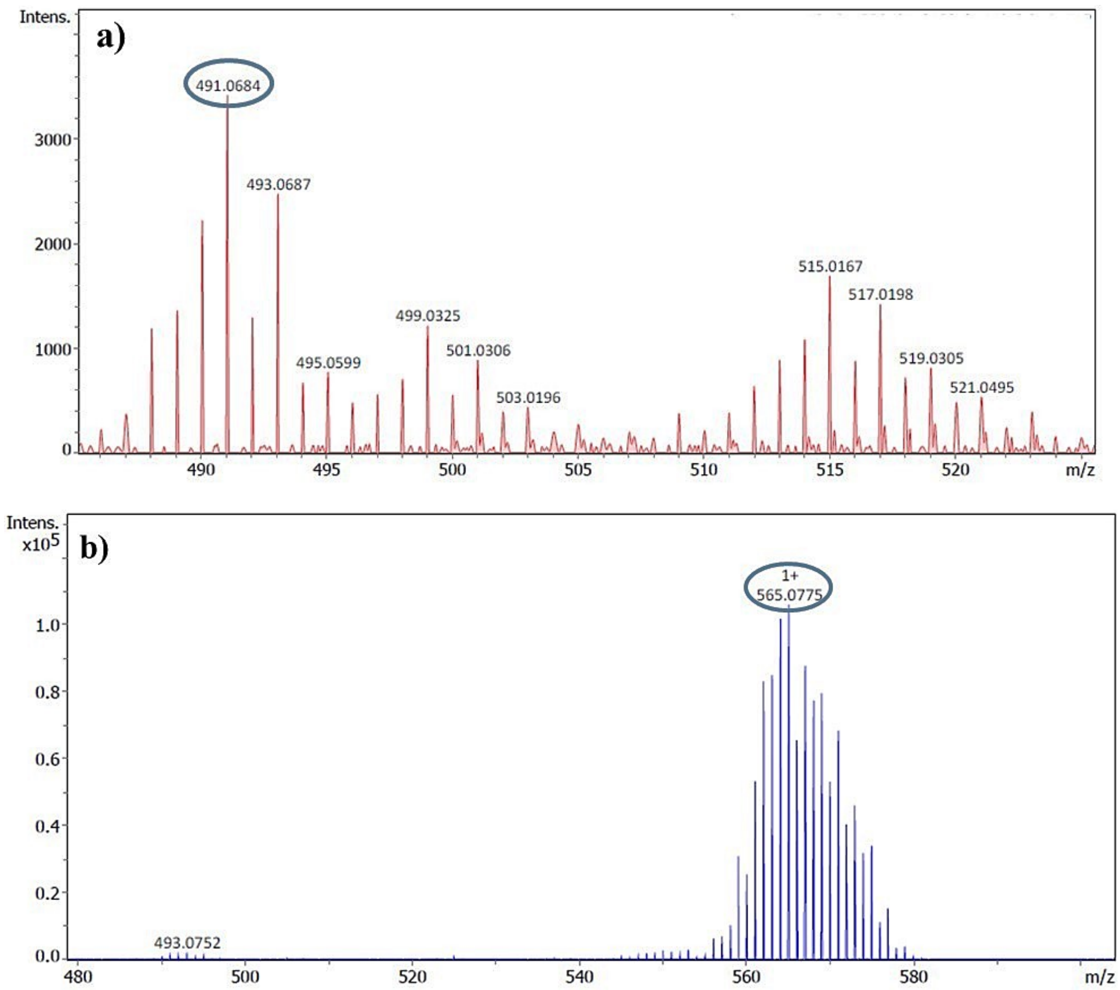


Figure S23. ESI spectra for the probable interaction of **3** with a) CO_3^{2-}, b) SO_4^{2-} anions, respectively.

Calculations of the interaction energies of the individual interactions

Interaction energies of the interactions "NH···O(3) + CH···O(4)", "O(1)···Na(1) + Cl···Na(1)" and "O(3)···Na(1) + O(5)···Na(1)" in **3**•Na_2CO_3 were calculated using the equations S1–S3:

$E_{int}[NH\cdots O(3) + CH\cdots O(4)] = [E_{int}(\mathbf{3}\cdots Na_2CO_3) + E_{int}(Na(2)CO_3^{-}\cdots \mathbf{3}\bullet Na(1)^{+}) - E_{int}(Na(1)^{+}\cdots \mathbf{3}\bullet Na(2)CO_3^{-})]/2$ (S1)

$E_{int}[O(1)\cdots Na(1) + Cl\cdots Na(1)] = [E_{int}(\mathbf{3}\cdots Na_2CO_3) + E_{int}(Na(1)^{+}\cdots \mathbf{3}\bullet Na(2)CO_3^{-}) - E_{int}(Na(2)CO_3^{-}\cdots \mathbf{3}\bullet Na(1)^{+})]/2$ (S2)

$E_{int}[O(3)\cdots Na(1) + O(5)\cdots Na(1)] = [E_{int}(Na(1)^{+}\cdots \mathbf{3}\bullet Na(2)CO_3^{-}) + E_{int}(Na(2)CO_3^{-}\cdots \mathbf{3}\bullet Na(1)^{+}) - E_{int}(\mathbf{3}\cdots Na_2CO_3)]/2$ (S3)

where $E_{int}(\mathbf{3}\cdots Na_2CO_3) = E(\mathbf{3}\bullet Na_2CO_3) - E\{\mathbf{3}\} - E\{Na_2CO_3\} + BSSE$
$E_{int}(Na(2)CO_3^{-}\cdots \mathbf{3}\bullet Na(1)^{+}) = E(\mathbf{3}\bullet Na_2CO_3) - E\{Na(2)CO_3^{-}\} - E\{\mathbf{3}\bullet Na(1)^{+}\} + BSSE$
$E_{int}(Na(1)^{+}\cdots \mathbf{3}\bullet Na(2)CO_3^{-}) = E(\mathbf{3}\bullet Na_2CO_3) - E\{Na(1)^{+}\} - E\{\mathbf{3}\bullet Na(2)CO_3^{-}\} + BSSE$.
$E(\mathbf{3}\bullet Na_2CO_3)$ is total energy of the adduct **3**•Na_2CO_3 and E{**3**}, $E\{Na_2CO_3\}$, $E\{Na(2)CO_3^{-}\}$ – $E\{\mathbf{3}\bullet Na(1)^{+}\}$, $E\{Na(1)^{+}\}$ – $E\{\mathbf{3}\bullet Na(2)CO_3^{-}\}$ are total energies of the fragments with unrelaxed geometries corresponding to those in **3**•Na_2CO_3.

Table S2. Cartesian atomic coordinates of the equilibrium structures (nuclear charges of elements are indicated in the first column).

1 (gas)

44	-1.553492	-0.409645	-0.120527
8	-0.140254	-0.037783	-1.681081
8	0.227193	0.694206	0.328950
8	-0.287658	-2.114213	0.353171
6	0.642676	0.533273	-0.861659
6	2.016067	0.969815	-1.277808
7	2.810375	1.410503	-0.140258
6	0.194681	-2.964945	-0.696909
6	-2.980390	1.215089	0.139012
6	-3.217429	0.540015	-1.083639
1	-3.136692	1.098743	-2.016544
6	-3.414913	-0.872197	-1.132986
1	-3.497252	-1.369634	-2.098497
6	-3.371181	-1.650659	0.045515
6	-3.114604	-0.980445	1.281503
1	-2.954868	-1.571675	2.182282
6	-2.928625	0.416559	1.326247
1	-2.621028	0.874060	2.264991

6	-3.480853	-3.138817	0.003273
1	-4.491451	-3.441164	0.310001
1	-2.773392	-3.613628	0.693416
1	-3.311444	-3.531924	-1.005528
6	-2.729175	2.702375	0.138100
1	-2.185701	2.928281	-0.795650
6	-4.082964	3.416870	0.095007
1	-4.677115	3.129926	-0.782355
1	-3.937362	4.503475	0.063820
1	-4.671955	3.184784	0.994071
6	-1.892941	3.190803	1.312651
1	-2.446914	3.119911	2.259720
1	-1.646206	4.249806	1.172758
1	-0.951694	2.635010	1.410120
1	0.488155	-1.893258	0.927844
1	0.568830	-3.895933	-0.257698
1	0.989381	-2.473516	-1.270280
1	-0.648037	-3.177810	-1.360773
1	2.480084	0.114897	-1.798730
1	1.900928	1.759504	-2.038012
1	2.274189	1.311763	0.719976
6	6.708605	-0.189581	0.202433
6	5.646546	-0.858430	0.791847
6	6.475930	0.980378	-0.521004
6	4.350643	-0.343654	0.655895
6	5.191804	1.506417	-0.633064
6	4.111968	0.865630	-0.034354
1	7.717793	-0.582514	0.300404
1	5.804745	-1.783789	1.342222
6	3.214544	-1.065765	1.124913
1	5.010648	2.440332	-1.162375
1	7.309668	1.499998	-0.989341
7	2.235878	-1.619835	1.422996

169 **2** (gas)

44	0.912073	-0.017152	1.973366
17	-0.649064	0.804796	3.569575
8	-0.609417	-1.522770	1.511074
8	-0.655545	0.418536	0.511881
6	2.476230	1.360730	1.447354
6	2.555606	0.212974	0.585325
6	2.594593	-1.092825	1.104504
6	2.537761	-1.313615	2.521029
6	2.523023	-0.187476	3.377461
6	2.493410	1.136838	2.842610
6	2.386029	-2.708551	3.059021

1	1.706963	-3.290587	2.429969
1	3.359751	-3.209886	3.077046
1	1.982565	-2.691767	4.073801
6	2.293079	2.733208	0.834390
6	1.531539	3.701818	1.738939
6	3.666185	3.292374	0.437650
1	4.196821	2.620254	-0.245359
1	3.550949	4.260907	-0.058773
1	4.291750	3.437342	1.326014
6	-1.202063	-0.689329	0.767528
6	-2.576078	-0.998899	0.223665
7	-2.862693	-2.399857	0.395505
1	-3.285257	-0.355903	0.768542
1	-2.604364	-0.706179	-0.832920
1	2.373612	-0.328978	4.442730
1	2.307965	1.961757	3.521741
1	2.511577	-1.941661	0.432543
1	2.440449	0.355907	-0.485655
1	1.698568	2.584624	-0.077089
1	2.134062	4.000366	2.604695
1	1.295030	4.612469	1.180617
1	0.594777	3.262286	2.098473
1	-2.230442	-2.901833	1.012314
6	-6.523835	-4.179587	-0.642194
6	-6.262857	-2.837252	-0.916205
6	-5.533311	-4.913303	-0.005237
6	-5.067944	-2.220451	-0.578820
6	-4.316081	-4.326817	0.346822
6	-4.058537	-2.958946	0.066927
1	-7.470995	-4.626526	-0.919267
17	-7.489506	-1.887424	-1.710581
1	-4.923865	-1.172086	-0.811625
6	-3.293871	-5.095751	0.991284
1	-5.694327	-5.961480	0.226809
7	-2.433546	-5.675275	1.509180

3 (gas)

44	3.134985	0.815431	-0.469155
17	3.013781	-0.266536	-2.586096
8	1.003723	0.361845	-0.304101
8	2.479105	-1.114394	0.336326
6	5.212264	1.114969	0.053141
6	4.381975	1.314305	1.207163
6	3.292984	2.209075	1.201030
6	2.946838	2.899305	-0.002292
6	3.781526	2.746022	-1.142173

6	4.899899	1.864694	-1.104770
6	1.664908	3.678469	-0.084036
1	0.875541	3.181854	0.485698
1	1.814140	4.683680	0.324736
1	1.330944	3.768733	-1.120064
6	6.314323	0.077663	0.102882
6	6.662699	-0.502919	-1.267338
6	7.548533	0.687950	0.781469
1	7.317345	1.069692	1.781685
1	8.337263	-0.064814	0.877704
1	7.941972	1.517983	0.183295
6	1.282420	-0.810915	0.079628
6	0.194674	-1.852218	0.188550
7	-1.095646	-1.217677	0.107241
1	0.360139	-2.571527	-0.628695
1	0.323598	-2.389083	1.136214
1	3.483220	3.186277	-2.087535
1	5.405480	1.638995	-2.037624
1	2.621761	2.250294	2.052872
1	4.527526	0.666245	2.067813
1	5.938446	-0.739933	0.732744
1	7.163658	0.240228	-1.899174
1	7.356327	-1.339899	-1.142880
1	5.770732	-0.863797	-1.790366
1	-1.085296	-0.249705	-0.201470
6	-4.736751	-3.319237	0.048651
6	-3.524096	-3.984184	0.240274
6	-4.684515	-1.949916	-0.124925
6	-2.309037	-3.312694	0.257011
6	-3.490897	-1.229579	-0.114352
6	-2.262542	-1.916144	0.077939
1	-5.690696	-3.831958	0.031376
1	-3.529474	-5.061546	0.377277
1	-1.389728	-3.867705	0.403737
6	-3.485201	0.188810	-0.286734
9	-5.821998	-1.266207	-0.310483
7	-3.399134	1.337289	-0.416026

4 (gas)

44	3.719819	0.413234	0.146439
17	3.023039	0.319272	2.420852
8	2.066928	-0.985648	-0.220367
8	1.715746	1.168534	-0.241774
6	4.831967	2.081362	-0.596257
6	4.616212	1.168616	-1.683254
6	4.926062	-0.196338	-1.547088

6	5.504455	-0.710206	-0.336751
6	5.784695	0.204439	0.701151
6	5.451719	1.587934	0.577746
6	5.700480	-2.189315	-0.156444
1	4.884644	-2.746073	-0.625477
1	6.643299	-2.502330	-0.618166
1	5.728059	-2.449553	0.904118
6	4.316231	3.500770	-0.703595
6	3.813149	4.060624	0.627977
6	5.413662	4.385194	-1.310932
1	5.750859	4.006045	-2.281469
1	5.041636	5.404615	-1.452859
1	6.281485	4.430026	-0.642724
6	1.261489	-0.010655	-0.245273
6	-0.229996	-0.237869	-0.228716
7	-0.522662	-1.637204	-0.399902
1	-0.598448	0.157818	0.731324
1	-0.675788	0.368759	-1.026631
1	6.101627	-0.172347	1.668464
1	5.528452	2.219498	1.455623
1	4.605096	-0.895252	-2.314147
1	4.063534	1.507259	-2.554637
1	3.466258	3.467478	-1.397224
1	4.638972	4.251441	1.322640
1	3.310946	5.016684	0.452903
1	3.102810	3.377815	1.105974
1	0.263456	-2.252015	-0.222200
6	-4.367681	-3.239644	0.007626
6	-4.187177	-1.854163	-0.049292
6	-3.243627	-4.078877	-0.061345
6	-2.916997	-1.309361	-0.172895
6	-1.977385	-3.542722	-0.186490
6	-1.786639	-2.145525	-0.245983
6	-5.683837	-3.797800	0.135952
1	-5.051639	-1.199729	0.007824
1	-2.804192	-0.231415	-0.213115
1	-1.112737	-4.198753	-0.242455
1	-3.375971	-5.155356	-0.013732
7	-6.746037	-4.250300	0.238133

3 (SMD)

44	3.112799	0.844568	-0.465605
17	2.834906	-0.174436	-2.677818
8	0.960278	0.393218	-0.230962
8	2.448429	-1.114951	0.298258
6	5.218413	1.087985	0.045169

6	4.400542	1.275414	1.207088
6	3.332697	2.193418	1.223692
6	3.013219	2.942357	0.049101
6	3.825174	2.780478	-1.101126
6	4.914430	1.864819	-1.095296
6	1.783554	3.798444	0.003207
1	1.039478	3.451655	0.724198
1	2.053401	4.829860	0.257168
1	1.345009	3.794502	-0.998074
6	6.319624	0.050619	0.075511
6	6.652939	-0.530895	-1.295983
6	7.560224	0.679782	0.722782
1	7.338919	1.070225	1.722137
1	8.354963	-0.067847	0.816921
1	7.936735	1.504758	0.106179
6	1.242419	-0.790224	0.114508
6	0.156617	-1.825966	0.277807
7	-1.142113	-1.212401	0.128082
1	0.329609	-2.603655	-0.480350
1	0.268037	-2.295025	1.262803
1	3.544896	3.273228	-2.026733
1	5.425802	1.668064	-2.031868
1	2.672028	2.238423	2.083846
1	4.541294	0.612225	2.056800
1	5.967519	-0.761368	0.724589
1	7.118582	0.217956	-1.947176
1	7.366040	-1.353272	-1.178834
1	5.760053	-0.920525	-1.797826
1	-1.138575	-0.247717	-0.189911
6	-4.739426	-3.398247	0.006330
6	-3.516776	-4.019714	0.290432
6	-4.704770	-2.043549	-0.233129
6	-2.320764	-3.319444	0.330860
6	-3.528901	-1.288889	-0.203515
6	-2.292489	-1.929858	0.082168
1	-5.677413	-3.940615	-0.027800
1	-3.503889	-5.088636	0.483789
1	-1.398715	-3.844520	0.553034
6	-3.576157	0.111195	-0.463813
9	-5.846853	-1.386554	-0.513752
7	-3.574075	1.252333	-0.670719

173 **3**•Na_2CO_3 (SMD)

44	-1.909827	-0.398340	0.198724
17	-1.354441	-2.728374	-0.453765
8	0.205833	-0.069835	-0.299079

8	-1.312535	0.185846	-1.846800
6	-3.959636	0.327028	0.294292
6	-3.049705	1.399377	0.576844
6	-2.055067	1.267413	1.562233
6	-1.925392	0.056005	2.318557
6	-2.838736	-0.988168	2.058229
6	-3.846241	-0.855489	1.056892
6	-0.779859	-0.124692	3.268868
1	0.120954	0.366800	2.890231
1	-1.033630	0.331295	4.232660
1	-0.572084	-1.185190	3.432201
6	-4.949196	0.478047	-0.839422
6	-5.295917	-0.837712	-1.531888
6	-6.207537	1.160967	-0.287035
1	-5.971760	2.127162	0.172104
1	-6.926203	1.332397	-1.095205
1	-6.685190	0.526931	0.469429
6	-0.102982	0.211215	-1.505031
6	0.977494	0.568883	-2.506413
7	2.315935	0.516183	-1.976757
1	0.914380	-0.152181	-3.327676
1	0.742937	1.549082	-2.934218
1	-2.693739	-1.950490	2.539345
1	-4.443320	-1.724946	0.803000
1	-1.301364	2.042939	1.667600
1	-3.050813	2.277521	-0.063233
1	-4.486310	1.149468	-1.573191
1	-5.880936	-1.495444	-0.879348
1	-5.903286	-0.630225	-2.418708
1	-4.397155	-1.376091	-1.853360
1	2.718316	-0.434116	-1.959870
6	4.179887	2.923545	0.952813
6	4.766132	1.758791	0.427609
6	2.928979	3.240000	0.491403
6	4.125334	0.987394	-0.519053
6	2.222654	2.483186	-0.463256
6	2.836876	1.325900	-1.009389
1	4.670335	3.547866	1.691124
1	5.752986	1.461909	0.772196
1	4.603875	0.091231	-0.909074
6	0.889612	2.911892	-0.745183
9	2.302597	4.332331	0.978004
7	-0.197443	3.278514	-0.918712
8	3.007834	-2.127140	-1.437648
6	3.817272	-2.295368	-0.446429

8	5.089664	-2.280884	-0.591804
8	3.311471	-2.472757	0.751118
11	1.250361	-2.126129	-0.015447
11	5.341015	-2.544012	1.583288

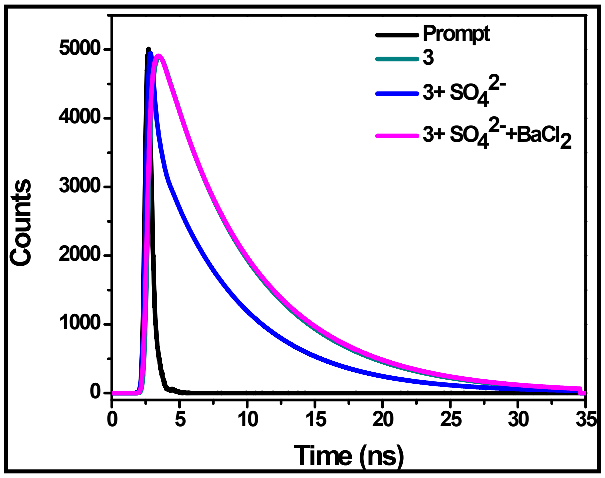
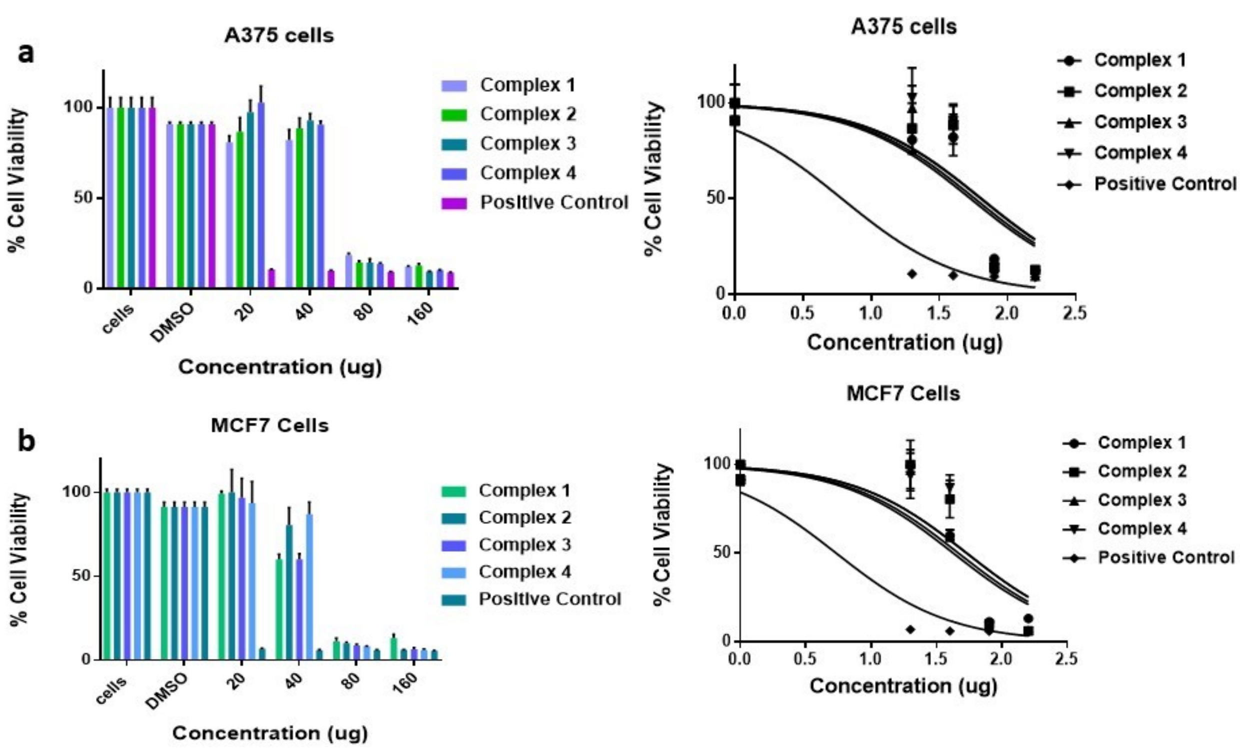


Figure S24. Fluorescence life time decay of **3** with 4×10^{-5} M resultant solution in DMSO on addition of 4×10^{-4} M concentration of anions (SO_4^{2-}), followed by further addition of 4×10^{-4} M concentration of $BaCl_2$.



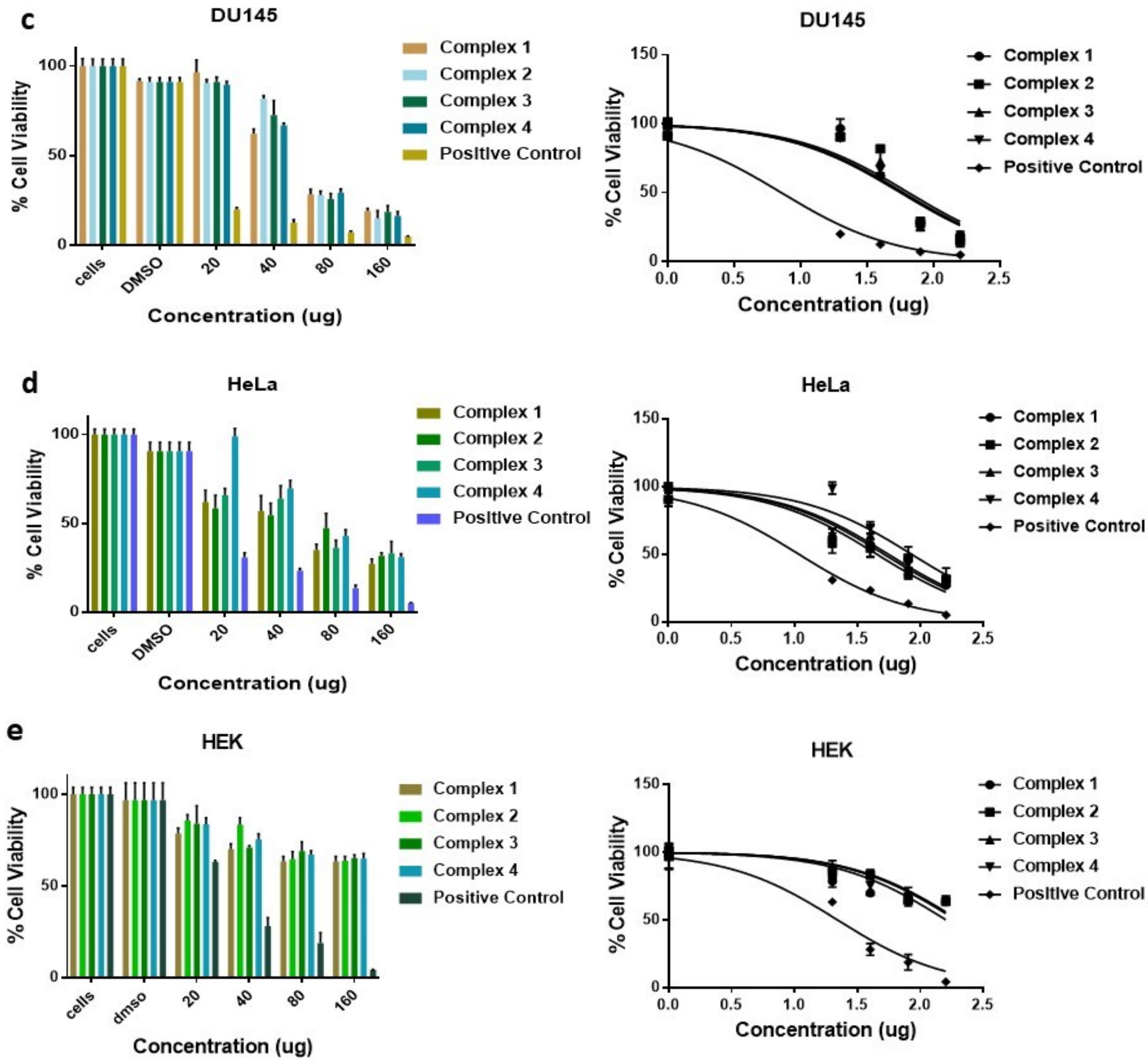


Figure S25. Biocompatibility of **1**, **2**, **3** and **4** on a) A375 cell line, b) MCF7 cell line, c) DU145 cell line, d) HeLa cell line, e) HEK cell line.

References

1 A. Bhattacharya, R. Prajapati, S. Chatterjee and T. K. Mukherjee, *Langmuir*, 2014, **30**, 14894–14904.

2 6.

3 L. Bao, Z.-L. Zhang, Z.-Q. Tian, L. Zhang, C. Liu, Y. Lin, B. Qi and D.-W. Pang, *Advanced Materials*, 2011, **23**, 5801–5806.

4 P. Dahiya, M. Kumbhakar, T. Mukherjee and H. Pal, *Chemical Physics Letters*, 2005, **414**, 148–154.

5 Substituent dependent sensing behavior of Schiff base chemosensors in detecting Zn2+and Al3+ ions: Drug sample analysis and living cell imaging - ScienceDirect, https://www.sciencedirect.com/science/article/pii/S092540051832029X?via%3Dihub, (accessed October 6, 2021).

6 N. Malviya, C. Sonkar, R. Ganguly, D. Bhattacherjee, K. P. Bhabak and S. Mukhopadhyay, *ACS Appl. Mater. Interfaces*, 2019, **11**, 47606–47618.