Supporting Information

Investigating the Photosensitivity of Koneramines for Cell Imaging and Therapeutic Applications

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Experimental Section

Materials

Pyridine-2-carboxaldehyde (Sigma Aldrich), 9-Anthracenecarboxaldehyde (Sigma Aldrich), ethylenediamine (Merck), sodium borohydride (SDFCL), anhydrous $CdCl_2$ (SDFCL), anhydrous $ZnCl_2$ (NICE), $CuCl_2 \cdot 2H_2O$ (Merck), $CDCl_3$ (Merck), anhydrous $MgSO_4$ (HIMEDIA), anhydrous Na_2SO_4 (FINAR), DMEM (Sigma Aldrich), FBS (gibco), Human Recombinant Insulin (HIMEDIA), MTT (Sigma Aldrich), Penicillin Streptomycin antibiotic (gibco), thiazolyl blue tetrazolium bromide (Sigma Aldrich), Formaldehyde solution (Merck), DMSO (Thermo Fisher Scientific India Pvt. Ltd.) were used as received from commercial sources. MCF-7 cells were purchased from NCCS Pune.

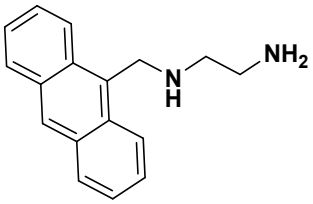
Methods

Elemental analyses were carried out on a Perkin-Elmer CHNS/O analyser. NMR spectra were recorded on JEOL 500 MHz and JEOL 400 MHz spectrometers. Temperature was kept constant using a variable temperature unit within the error limit of ±1 K. The software MestReNova was used for the processing of the NMR spectra. Tetramethylsilane (TMS) or the deuterated solvent residual peaks were used for calibration. Mass spectrometry experiments were performed on Agilent 6546 LC/Q-TOF equipped with an electrospray interface. Spectra were collected by a constant infusion of the sample dissolved in methanol or acetonitrile with 0.1% formic acid. The freeware mMass was used to simulate the calculated isotopic distributions. UV-Vis spectra were recorded on Shimadzu UV-2450. Fluorescence spectra were recorded on Edinburgh Instruments FLSP920.

Crystal Structure Determinations Single-crystal

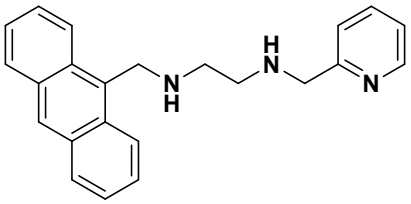
Single-crystal X-ray data were collected at either 293 K or 123 K on a Bruker SMART APEX CCD diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The linear absorption coefficients, the scattering factors for the atoms, and the anomalous dispersion corrections were taken from International Tables for X-ray Crystallography. Data integration and reduction were conducted with SAINT. An empirical absorption correction was applied to the collected reflections with SADABS using XPREP. Structures were determined by a direct method using SHELXTL and refined on F2 by a full-matrix least-squares technique using the SHELXL-97 program package. The lattice parameters and structural data are listed at the end of this Supporting Information.

Synthesis of N^1-(anthracen-9-ylmethyl)ethane-1,2-diamine:



In a 100 mL round-bottom flask 9-Anthracenecarboxaldehyde (0.5 g, 2.42 mmol) was dissolved in 30 mL 2:1 mixture of $CHCl_3$ and CH_3OH and ethylenediamine (0.728 g, 12.12 mmol) was added to it. The solution was continued to stir for 10h at room temperature. To this stirring solution sodium borohydride (0.915 g, 24.2 mmol) was added in small portions. The reaction mixture was further continued to stir for 4h at room temperature. The orange solution was evaporated under reduced pressure to give a sticky residue. This residue was washed several times with water to remove the excess of sodium borohydride and ethylenediamine. The organic compound was extracted in $CHCl_3$ (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain a brownish oily compound. Yield: 0.560 g (92%).^{1}H NMR (500 MHz, 25 °C, $CDCl_3$) δ = 8.40 (s, 1H, An), 8.36 (d, *J* = 8.7 Hz, 2H, An), 8.01 (d, *J* = 8.5 Hz, 2H, An), 7.56 – 7.52 (t, 2H, An), 7.48 – 7.45 (t, 2H, An), 4.76 (s, 2H), 2.96 – 2.92 (t, 2H, En), 2.90 – 2.85 (t, 2H, En). ^{13}C NMR (500 MHz, $CDCl_3$) δ = 131.78, 131.64, 130.36, 129.26, 127.29, 126.20, 125.03, 124.22, 53.00, 45.75, 41.87.

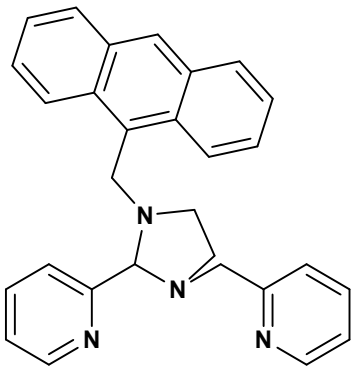
Synthesis of N-(anthracen-9-ylmethyl)-N'-(pyridin-2-ylmethyl)ethane-1,2-diamine:



In a 100 mL round-bottom flask N-(9-Methylanthracenyl)ethylenediamine (0.548 g, 2.19 mmol) was dissolved in 15 mL CH_3OH. Pyridine-2-carboxaldehyde (0.234 g, 2.19 mmol) was added to the solution and stirring was continued for 5h at room temperature. To this stirring solution sodium borohydride (0.828 g, 21.9 mmol) was added in small portions. The reaction mixture was further continued to stir for 4h at room temperature. The orange solution was evaporated under reduced pressure to give a sticky residue. It was washed with 30 mL of water, and the compound was extracted in CH_2Cl_2 (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain a brownish oily compound. Yield: 0.697 g (93%). ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$) δ = 8.53 (d, *J* = 4.2 Hz, 1H, Py), 8.39 (s, 1H, An), 8.36 (d, *J* = 8.7 Hz, 2H, An), 8.00 (d, *J* = 8.2 Hz, 2H, An), 7.59 (t, *J* = 7.6 Hz, 1H, Py), 7.54 – 7.49 (m, 2H, An), 7.49 – 7.43 (m, 2H, An), 7.23 (d, *J* = 7.7 Hz, 1H, Py), 7.15 – 7.10 (m, 1H, Py), 4.74 (s, 2H, CH_2-An),

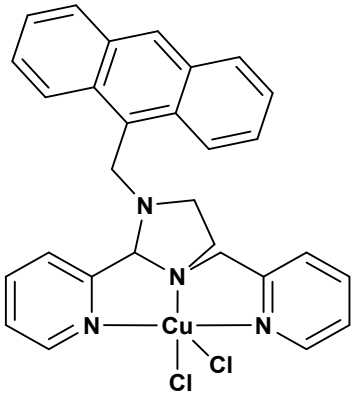
3.89 (s, 2H, CH_2-Py), 3.04 – 3.01 (t, *J* = 5.9 Hz, 2H, CH_2-En), 2.85 (t, *J* = 5.9 Hz, 2H, CH_2-En). ^{13}C NMR (500 MHz, $CDCl_3$) δ = 160.11, 149.36, 136.49, 131.95, 131.67, 130.43, 129.24, 127.24, 126.15, 125.01, 124.35, 122.28, 121.95, 55.30, 49.93, 49.17, 45.78.

Synthesis of 2-((3-(anthracen-9-ylmethyl)-2-(pyridin-2-yl)imidazolidin-1-yl)methyl)pyridine:



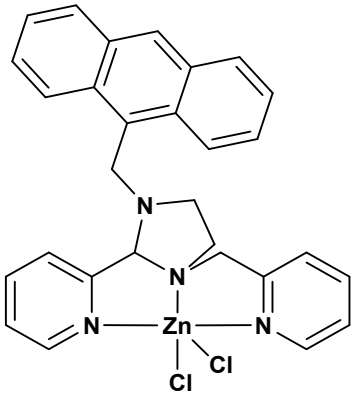
In a 100 mL round-bottom flask N-(anthracen-9-ylmethyl)-N'-(pyridin-2-ylmethyl)ethane-1,2-diamine (0.697 g, 2.04 mmol) was dissolved in 15 mL CH_3OH. Pyridine-2-carboxaldehyde (0.218 g, 2.04 mmol) was added to the solution and stirring was continued for 4h at room temperature. The solution was evaporated under reduced pressure to give a brown oily compound. Yield: 0.834 g (95%).^{1}H NMR (500 MHz, 25 °C, $CDCl_3$) δ 8.53 (d, *J* = 5.4 Hz, 1H, Py), 8.46 (d, *J* = 4.4 Hz, 1H, Py), 8.32 (s, 1H, An), 8.16 (d, *J* = 8.8 Hz, 2H, An), 7.96 – 7.89 (m, 3H, An), 7.74 (t, *J* = 7.7 Hz, 1H, Py), 7.55 (t, *J* = 7.7 Hz, 1H, Py), 7.38 (m, *J* = 14.6, 7.4 Hz, 5H, Py, An), 7.24 (d, *J* = 4.7 Hz, 1H, Py), 7.09 – 7.05 (m, 1H, Py), 4.66 (d, *J* = 12.9 Hz, 1H, CH_2-Py), 4.54 (d, *J* = 13.0 Hz, 1H, CH_2-Py), 4.48 (s, 1H, C*H), 3.96 (d, *J* = 14.4 Hz, 1H, CH_2-An), 3.76 (d, *J* = 14.4 Hz, 1H, CH_2-An), 3.20 (m, *J* = 16.1, 7.0 Hz, 1H, CH_2-Im), 2.95 – 2.84 (m, 2H, CH_2-Im), 2.71 (m, *J* = 9.0, 4.7 Hz, 1H, CH_2-Im). ^{13}C NMR (500 MHz, $CDCl_3$) δ 161.25, 159.37, 148.86, 148.28, 136.43, 136.37, 131.39, 130.85, 129.99, 128.94, 127.41, 125.43, 124.90, 124.75, 123.47, 123.36, 122.78, 121.86, 90.23, 59.19, 51.17, 51.03, 48.19.

Synthesis of $[Cu(L^{An})Cl_2]$:



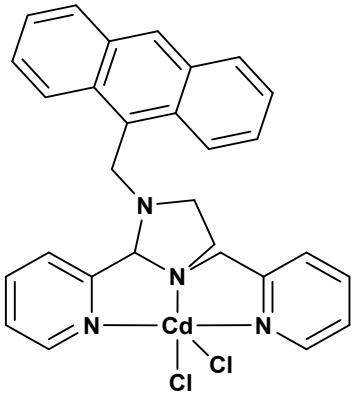
In a 100 mL round-bottom flask, 2-((3-(anthracen-9-ylmethyl)-2-(pyridin-2-yl)imidazolidin-1-yl)methyl)pyridine (0.834 g, 1.94 mmol) was dissolved in 20 mL CH_3OH and stirred for 5 mins.to get a yellowish brown solution. Addition of solid $CuCl_2 \cdot 2H_2O$ (0.331 g, 1.94 mmol) changed the colour of the solution to green and stirring was continued for 3 h. The solution was then concentrated to dryness to give a solid green compound which was washed with diethylether and redissolved in methanol to give rod-like green SCXRD quality crystals at room temperature in 5 days. Yield: 0.476 g (43%). Anal Calcd.(%) for ($C_{29}H_{26}N_4CuCl_2 \cdot 0.8CH_2Cl_2$): C 56.55, H 4.40, N 8.85; found: C 56.89, H 4.66, N 8.43. ESI-MS: *m/z* = 528.1142 (calcd. 528.1137) = $[Cu(L^{An})Cl]^+$.

Synthesis of $[Zn(L^{An})Cl_2]$:



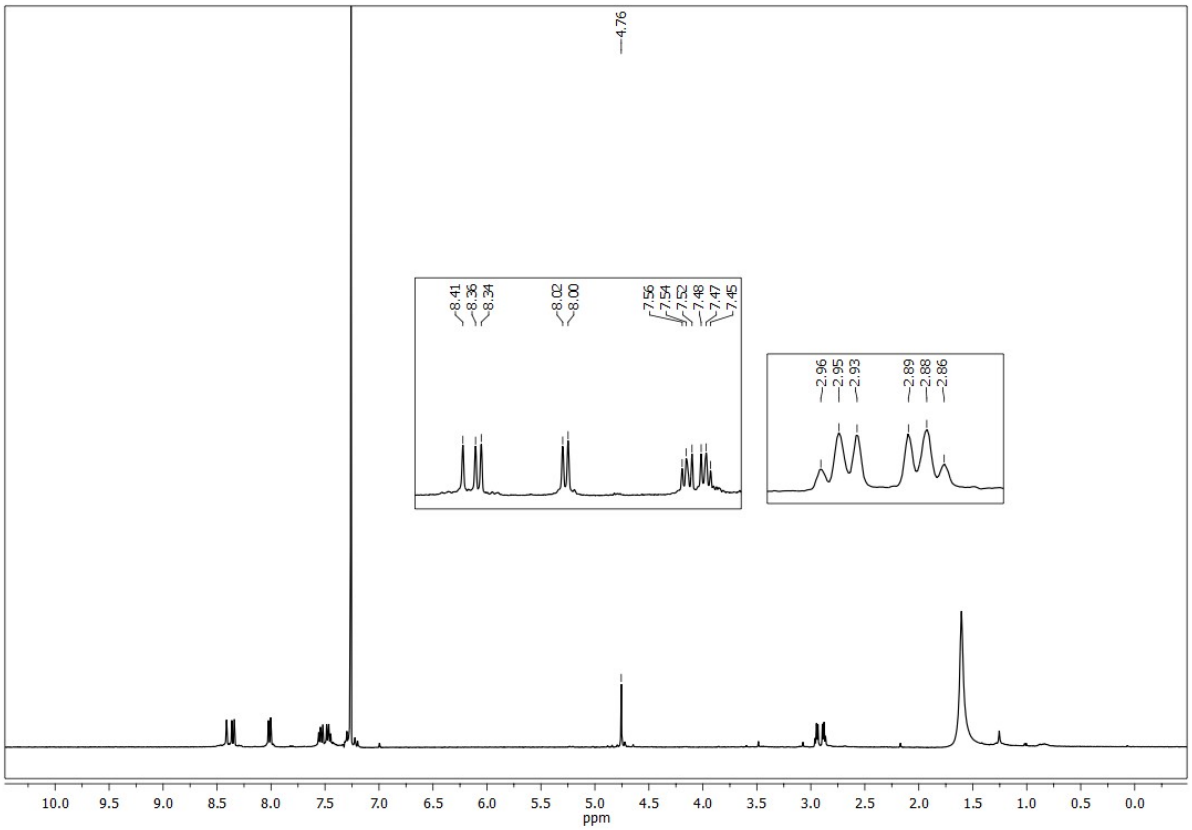
In a 50 mL round-bottom flask 2-((3-(anthracen-9-ylmethyl)-2-(pyridin-2-yl)imidazolidin-1-yl)methyl)pyridine (0.341 g, 0.79 mmol) was dissolved in 20 mL CH_3OH and stirred for 5 mins.to get a yellowish-brown solution. An off-white precipitate started forming within 5 mins of addition of solid anhydrous $ZnCl_2$ (0.108 g, 0.79). The solution was continued to stir for 2 h. The solution was filtered, and the precipitate was washed with 0.5 mL cold methanol. The precipitate was redissolved in methanol to give prisms of SCXRD quality crystals at room temperature in 3 days. Yield: 0.300 g (66 %). Anal Calcd.(%) for ($C_{29}H_{26}N_4ZnCl_2 \cdot H_2O$): C 59.56, H 4.83, N 9.58; found: C 59.38, H 4.40, N 9.22. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$) δ 9.22 (d, *J* = 4.8 Hz, 1H, Py), 8.99 (d, *J* = 5.0 Hz, 1H, Py), 8.49 (s, 1H, An), 8.47 (d, *J* = 9.0 Hz, 2H, An), 8.06 (d, *J* = 8.4 Hz, 2H, An), 7.94 (t, *J* = 7.7 Hz, 1H, Py), 7.62 – 7.57 (m, 2H, An), 7.55 – 7.49 (m, 3H, An, Py), 7.41 (m, *J* = 11.6, 7.8 Hz, 2H, Py, An), 7.25 – 7.21 (m, 1H, Py), 6.83 (d, *J* = 7.9 Hz, 1H, Py), 5.32 (d, *J* = 13.3 Hz, 1H, CH_2-Py), 5.10 (d, *J* = 13.3 Hz, 2H, C*H-Im, CH_2-An), 4.69 (d, *J* = 14.5 Hz, 1H, CH_2-An), 4.48 (d, *J* = 14.5 Hz, 1H, , CH_2-Py), 3.48 – 3.37 (m, 1H, CH_2-Im), 3.32 – 3.24 (m, 2H, CH_2-Im), 3.03 (t, *J* = 7.3 Hz, 1H, CH_2-Im). ESI-MS: *m/z* = 529.1130 (calcd. 529.1137) = $[Zn(L^{An})Cl]^+$.

Synthesis of [Cd(L^{An})Cl_2]:



In a 50 mL round-bottom flask 2-((3-(anthracen-9-ylmethyl)-2-(pyridin-2-yl)imidazolidin-1-yl)methyl)pyridine (0.774 g, 1.8 mmol) was dissolved in 20 mL CH_3OH and stirred for 5 mins.to get a yellowish-brown solution. A light-yellow precipitate started forming within 15 mins of addition of solid anhydrous $CdCl_2$ (0.330 g, 1.8 mmol). The solution was continued to stir for 2 h. and it was filtered, and the precipitate was washed with 0.5 mL cold methanol. The precipitate was dissolved in DCM and to give yellow prisms of SCXRD quality crystals overnight at room temperature. Yield: 0.497 g (45%). Anal Calcd.(%) for ($C_{29}H_{26}N_4CdCl_2$): C 56.74, H 4.27, N 9.13; found: C 56.59, H 4.15, N 8.99. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$) δ 8.96 (d, *J* = 4.8 Hz, 1H, Py), 8.48 (d, *J* = 5.2 Hz, 1H, Py), 8.46 (d, *J* = 9.1 Hz, 2H, An), 8.16 (s, 1H, An), 7.85 (d, *J* = 8.4 Hz, 2H, An), 7.76 (t, *J* = 6.9 Hz, 1H, Py), 7.64 – 7.59 (m, 2H, An), 7.49 – 7.43 (m, 2H, An), 7.42 – 7.36 (m, 1H, Py), 7.16 (d, *J* = 7.7 Hz, 1H, Py), 6.86 – 6.76 (m, 2H, Py), 6.22 (d, *J* = 7.0 Hz, 1H, Py), 4.95 (d, *J* = 13.5 Hz, 1H, CH_2-Py), 4.86 (d, *J* = 13.6 Hz, 1H, CH_2-Py), 4.52 (s, 1H, C*H-Im), 4.07 (dd, *J* = 14.5, 8.6 Hz, 1H, CH_2-Im), 3.77 – 3.70 (m, 3H, CH_2-Im, CH_2-An), 3.19 (td, *J* = 9.6, 5.6 Hz, 1H, CH_2-Im), 2.85 (td, *J* = 9.8, 5.5 Hz, 1H, CH_2-Im). ESI-MS: *m/z* = 579.0866 (calcd. 579.0878) = [Cd(L^{An})Cl]$^+$.

Figure S 1. ^{1}H NMR of N^{1}-(anthracen-9-ylmethyl)ethane-1,2-diamine.



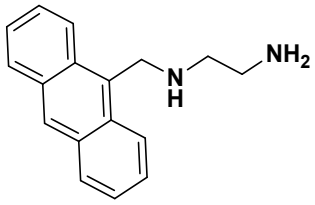


Figure S 2. ^{13}C NMR of N^1-(anthracen-9-ylmethyl)ethane-1,2-diamine.

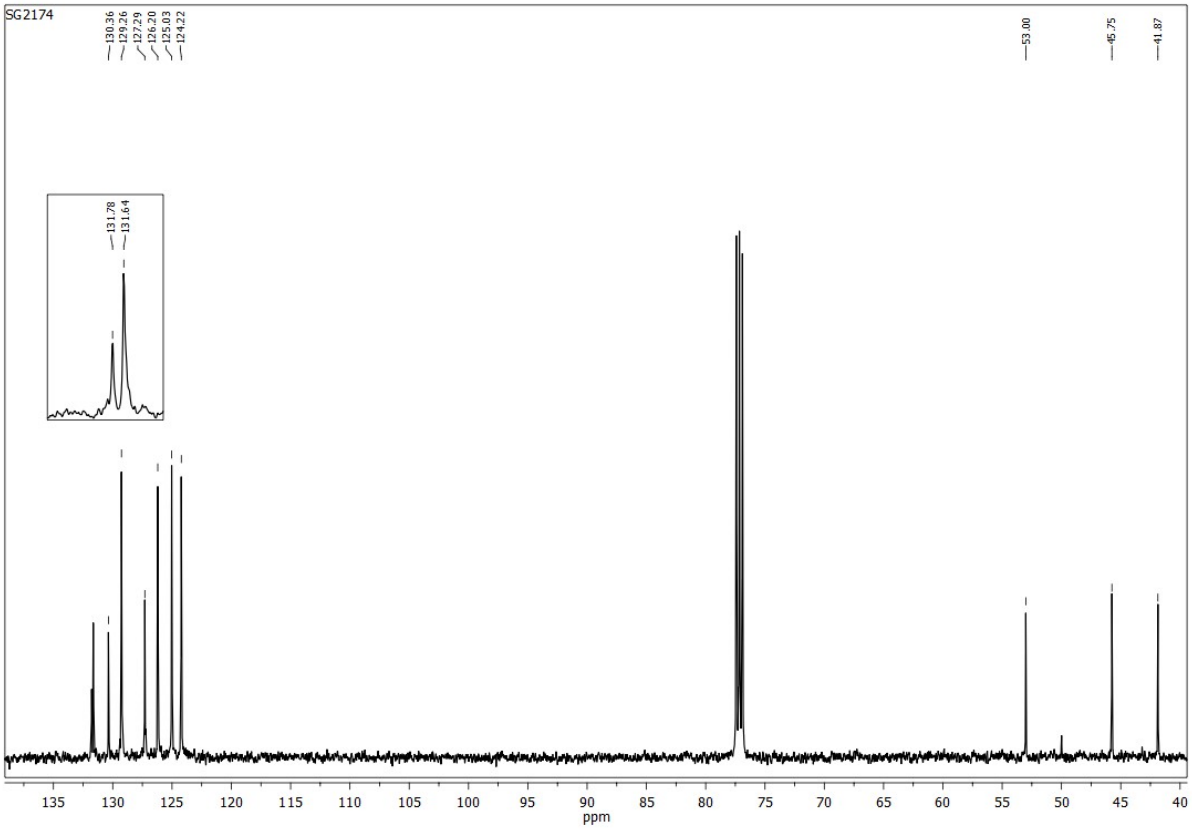
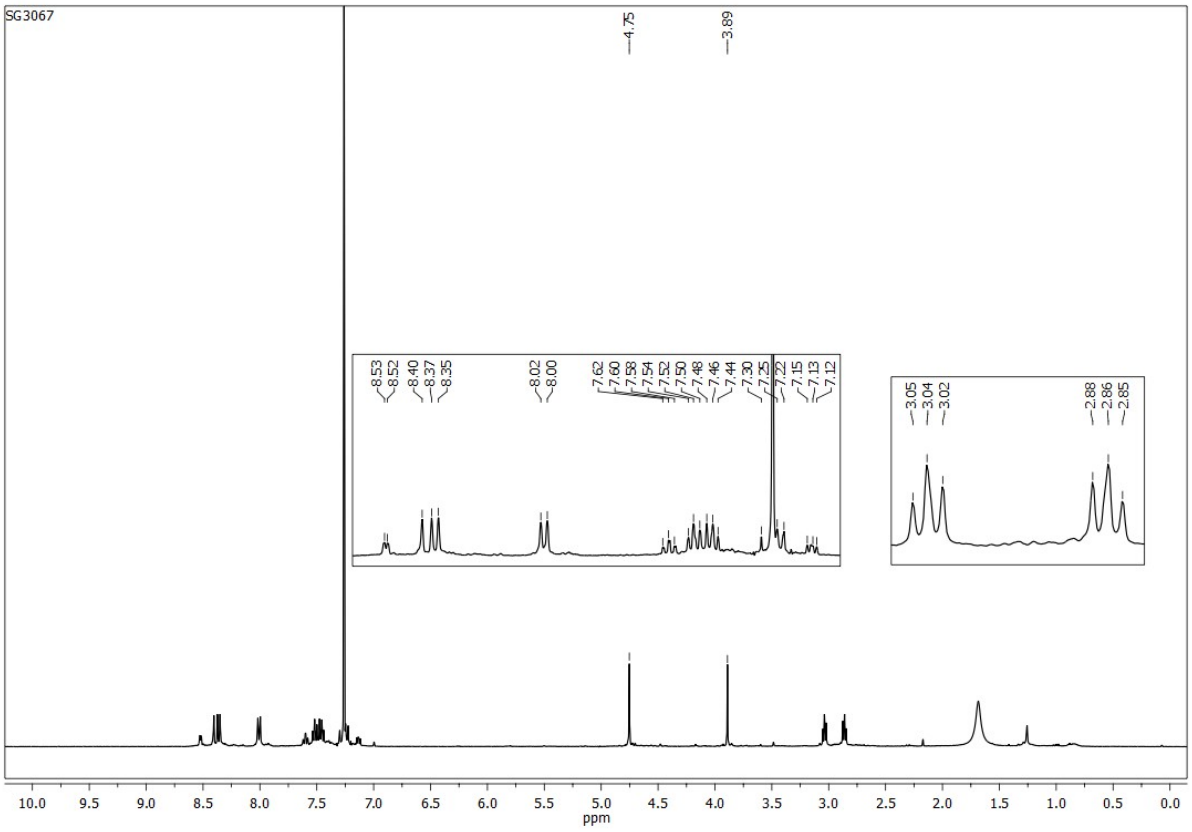


Figure S 3. ^{1}H NMR of N-(anthracen-9-ylmethyl)-N'-(pyridin-2-ylmethyl)ethane-1,2-diamine



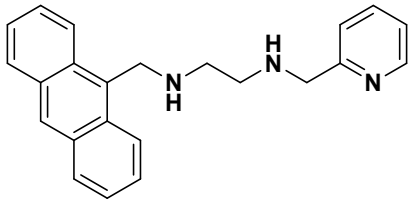


Figure S 4. ^{13}C NMR of N-(anthracen-9-ylmethyl)-N'-(pyridin-2-ylmethyl)ethane-1,2-diamine

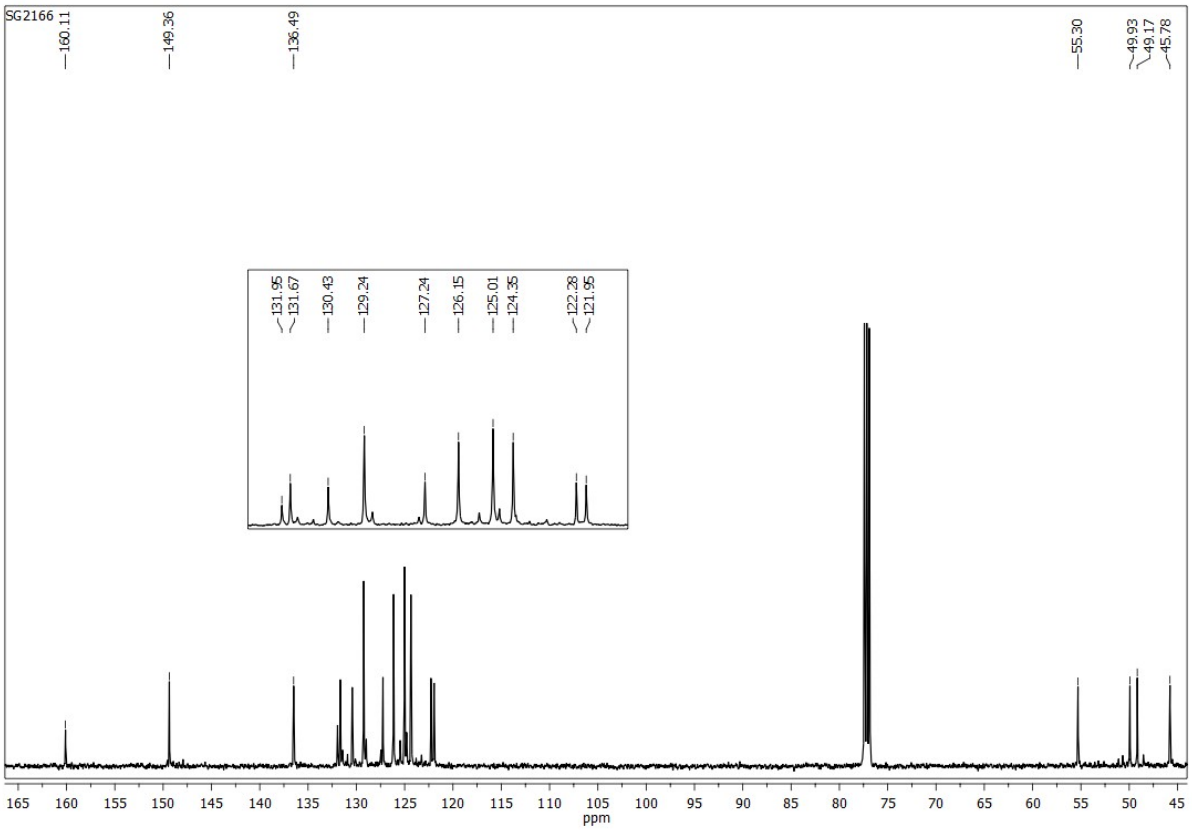
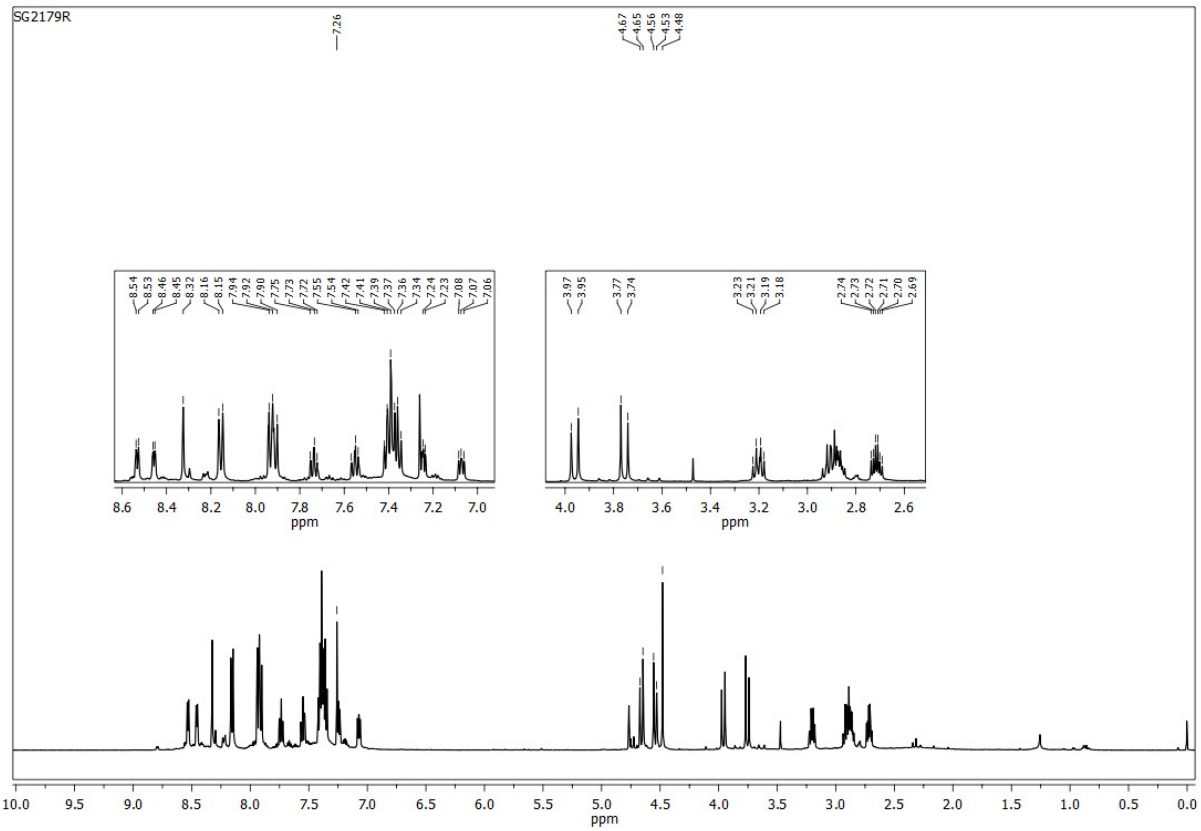


Figure S 5. ^{1}H NMR of 2-((3-(anthracen-9-ylmethyl)-2-(pyridin-2-yl)imidazolidin-1-yl)methyl)pyridine



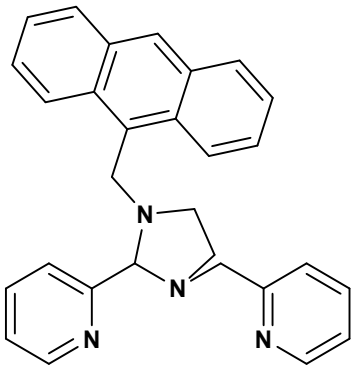


Figure S 6. ^{13}C NMR of 2-((3-(anthracen-9-ylmethyl)-2-(pyridin-2-yl)imidazolidin-1-yl)methyl)pyridine

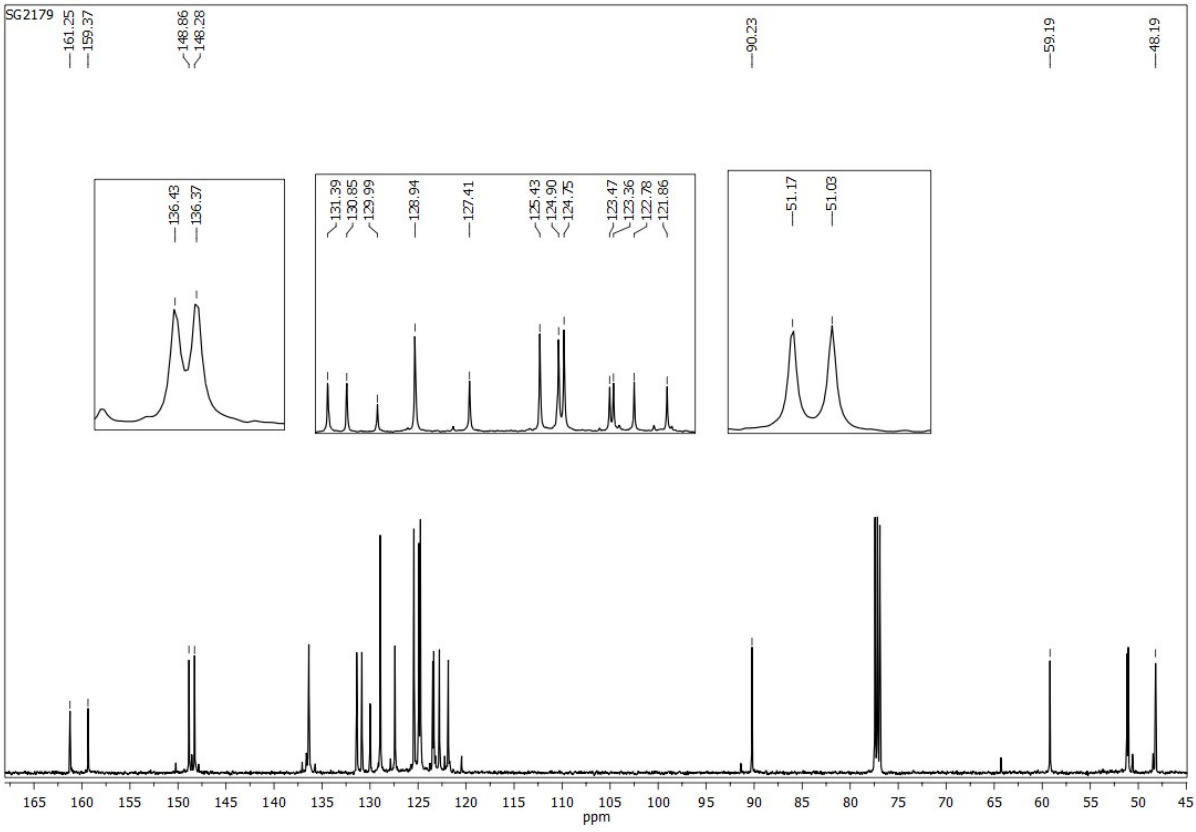
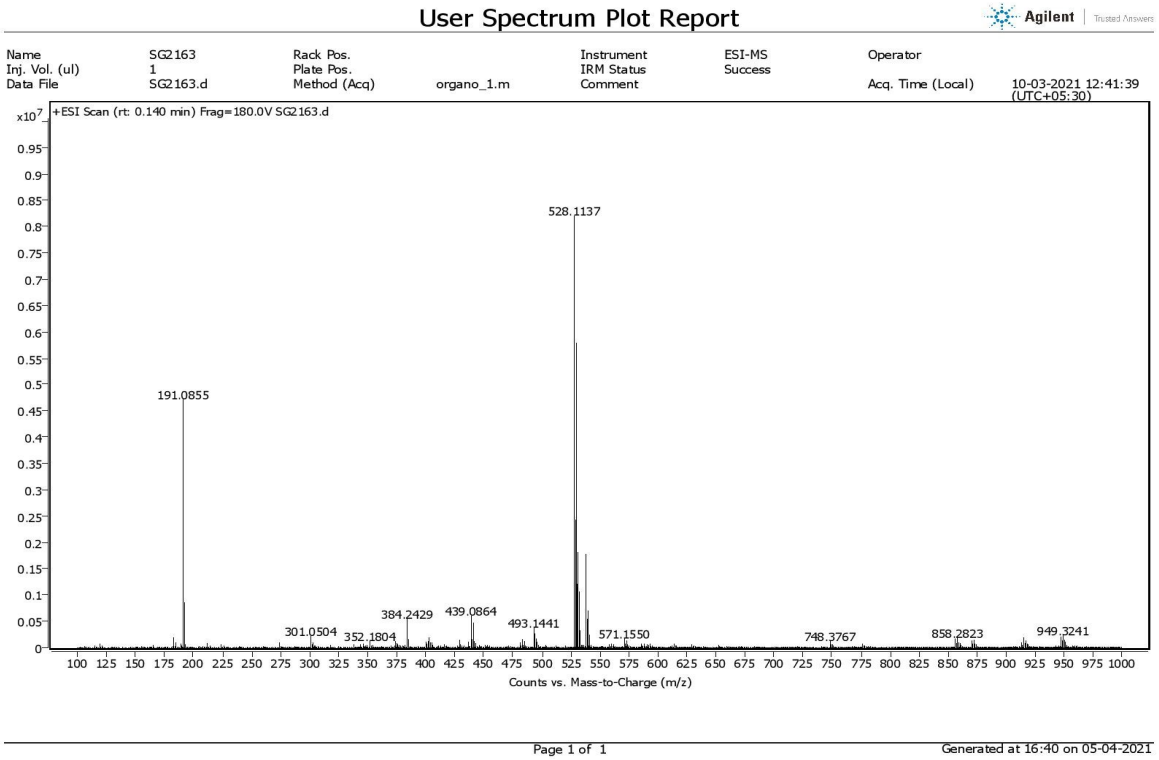


Figure S 7. ESI-MS of $[Cu(L^{An})Cl_2]$



Calculated *m/z* for $[Cu(L^{An})Cl]^+$ = 528.1142; observed *m/z* = 528.1137 and calculated *m/z* for $[Cu(L^{An})HCOO]^+$ = 538.1430; observed *m/z* = 538.1421. Formate must have originated from the acetonitrile solution (with 0.1% HCOOH) used to infuse the sample.

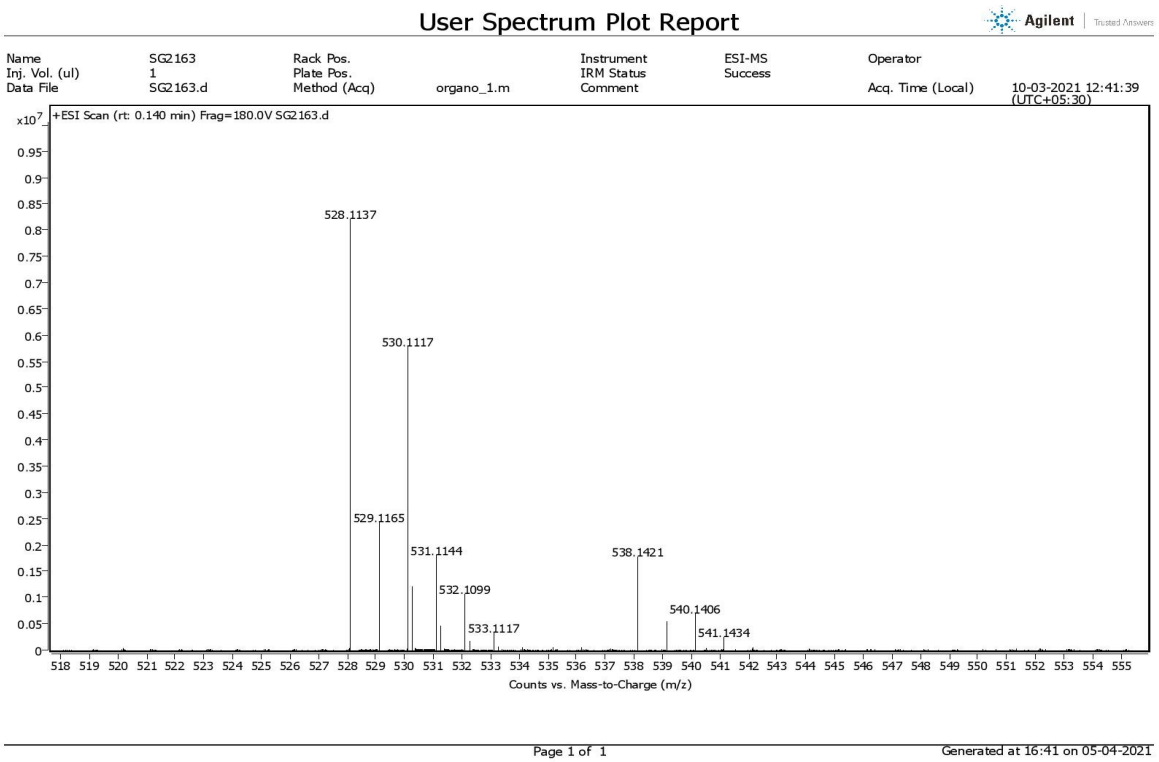
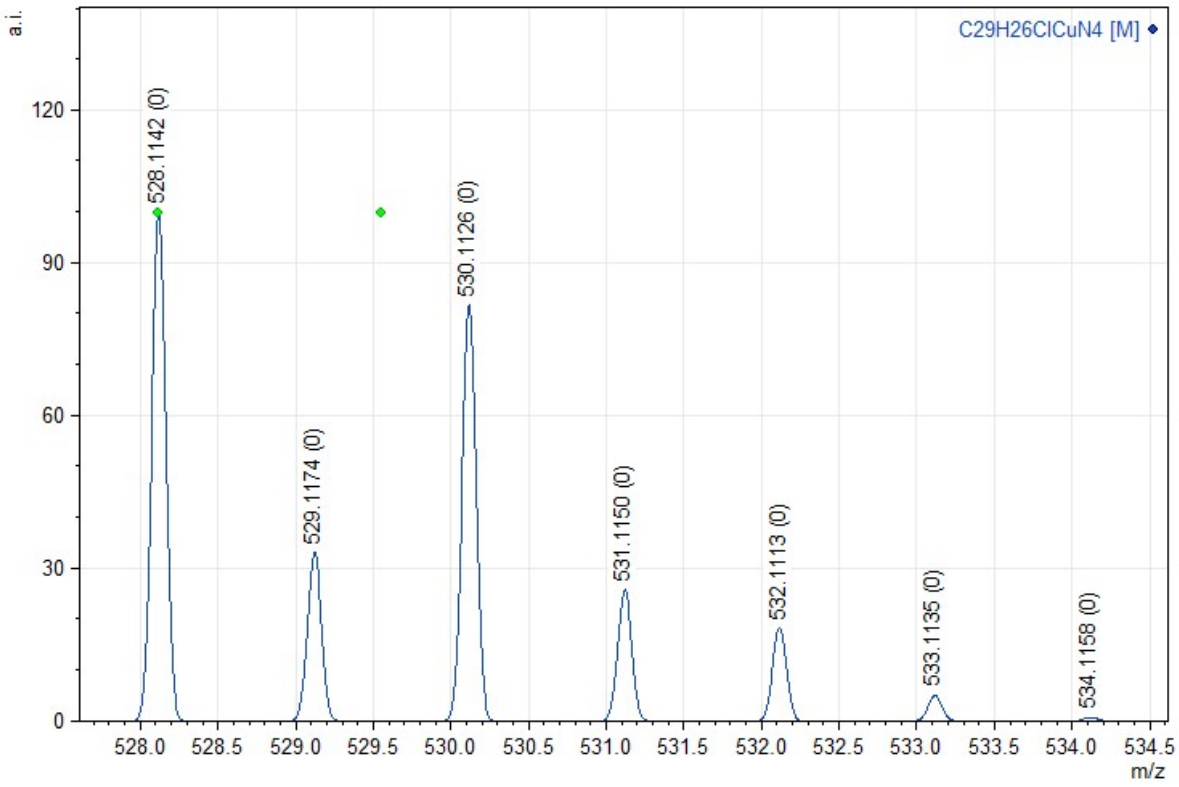
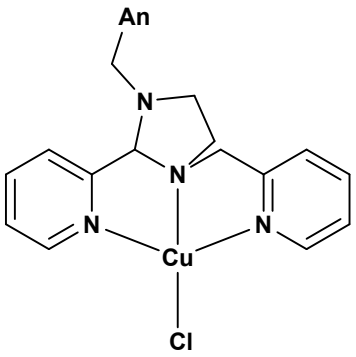


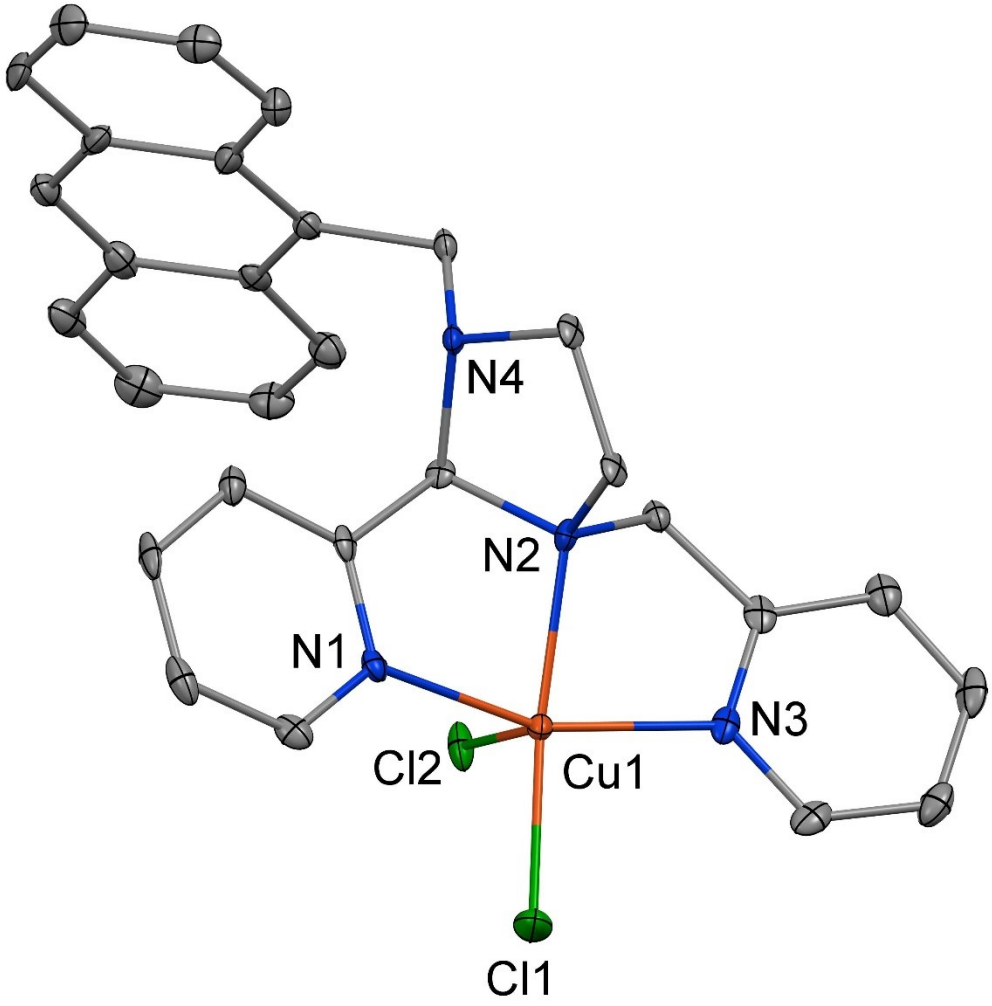
Figure S 8. Simulated ESI-MS of $[Cu(L^{An})Cl_2]$





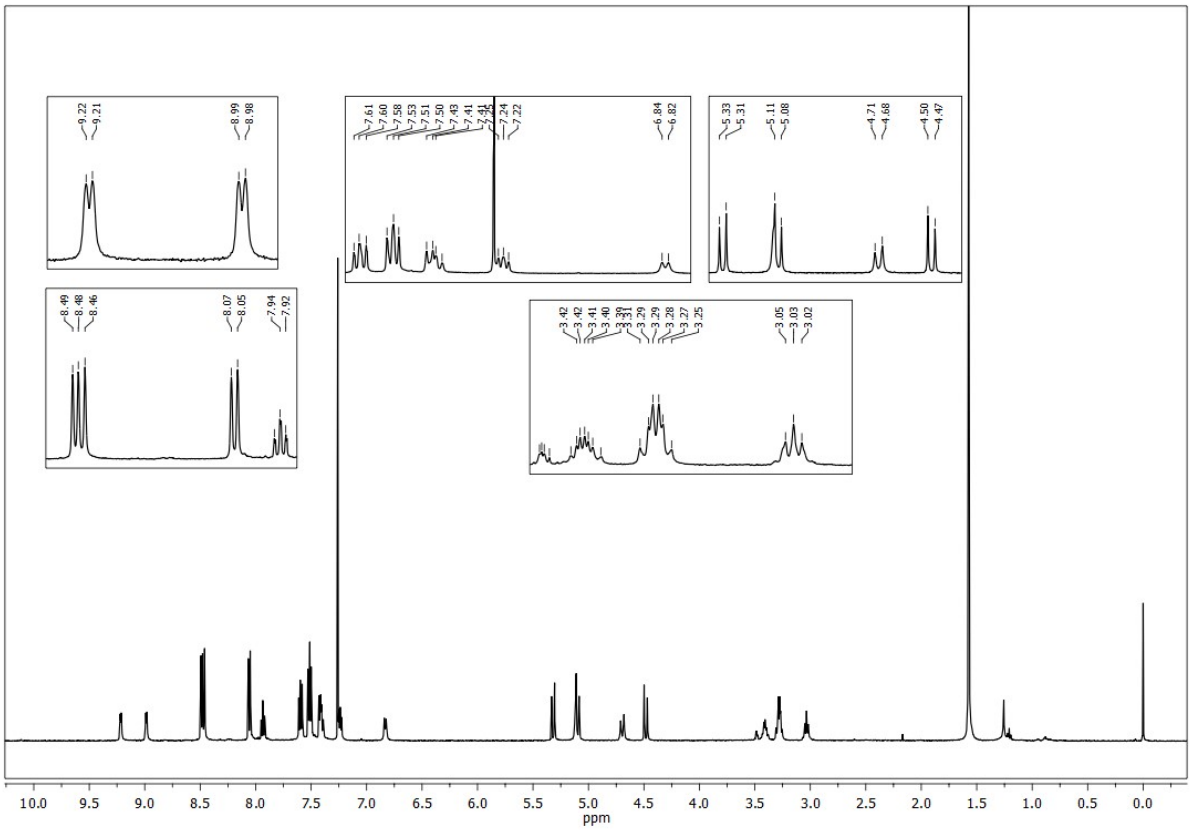
$C_{29}H_{26}N_4CuCl$

Figure S 9. ORTEP of $[Cu(L^{An})Cl_2]$ drawn at 50% probability



Selected bond distances (Å): Cu(1)-N(1), 2.026(3); Cu(1)-N(2), 2.031(4); Cu(1)-N(3), 2.027(3); Cu(1)-Cl(1), 2.2443(12); Cu(1)-Cl(2), 2.4821(12); Selected bond angles (°): Cl(1)-Cu(1)-Cl(2), 105.35(5); N(1)-Cu(1)-Cl(2), 96.93(10); N(1)-Cu(1)-Cl(1), 96.58(10); N(1)-Cu(1)-N(3), 159.00(14) = α; N(1)-Cu(1)-N(2), 80.51(14); N(3)-Cu(1)-Cl(2), 93.16(10); N(3)-Cu(1)-Cl(1), 98.44(11); N(3)-Cu(1)-N(2), 80.66(14); N(2)-Cu(1)-Cl(2), 92.69(10); N(2)-Cu(1)-Cl(1), 161.95(10) = β; ($\tau = 0.049$)

Figure S 10. ^{1}H NMR of $[Zn(L^{An})Cl_2]$



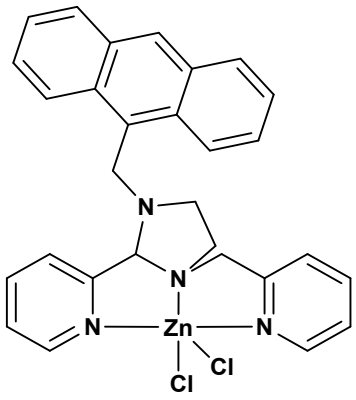
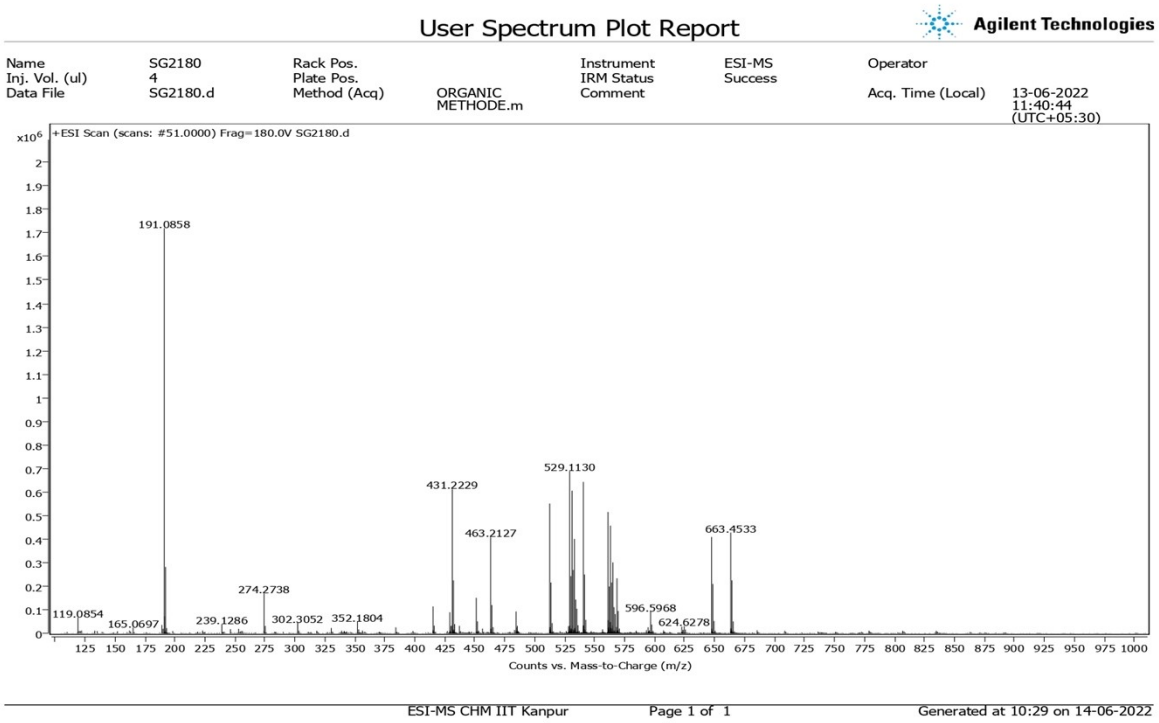


Figure S 11. ESI-MS of $[Zn(L^{An})Cl_2]$



Calculated *m/z* for $[Zn(L^{An})Cl]^+$ = 529.1137; observed *m/z* = 529.1130.

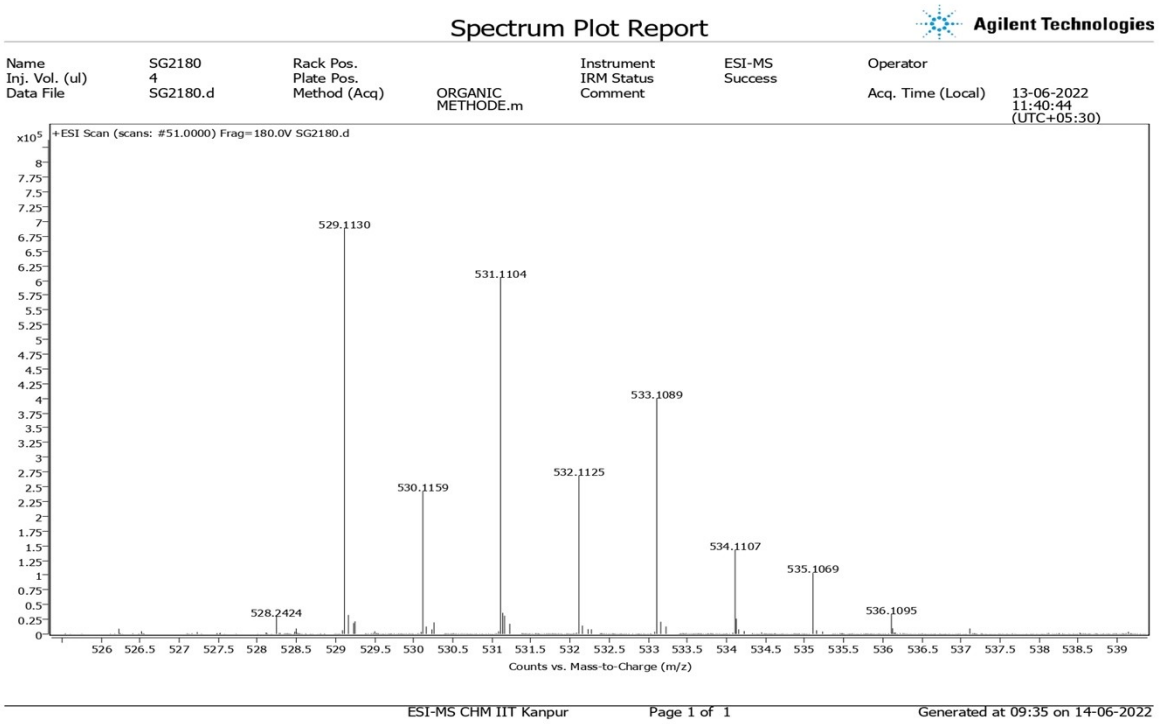
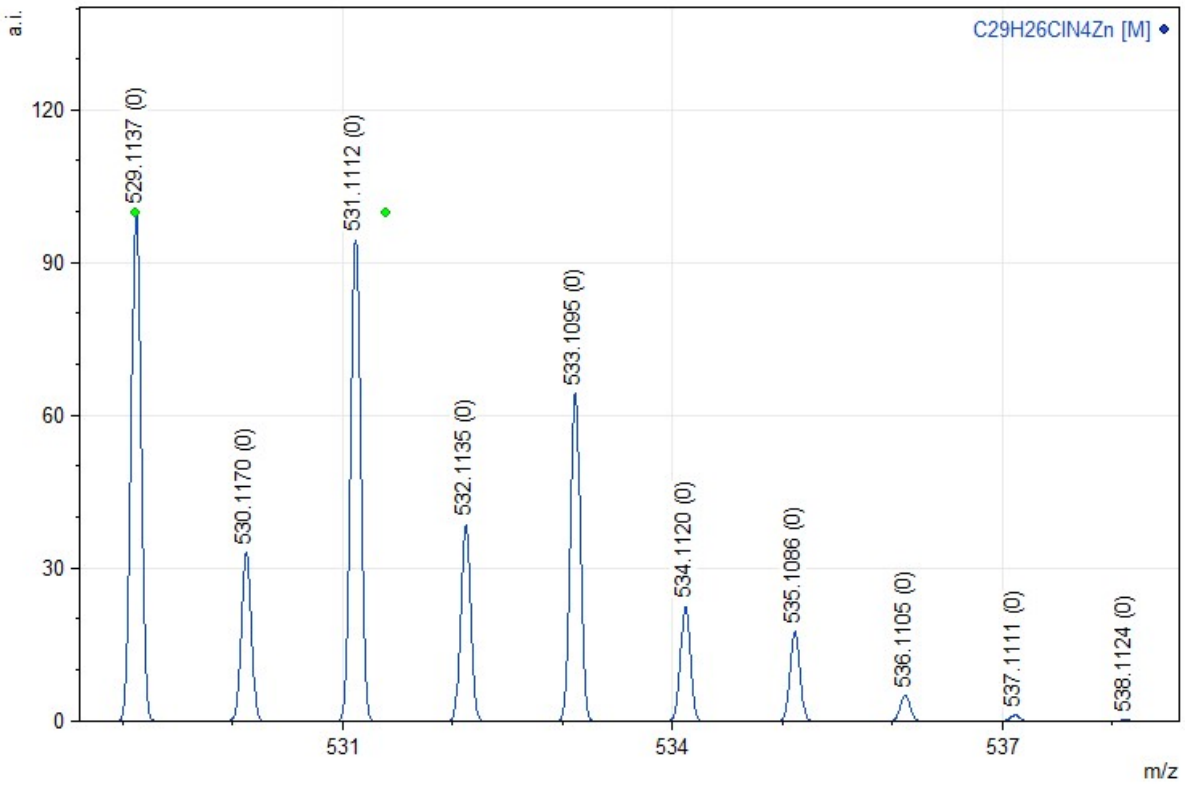
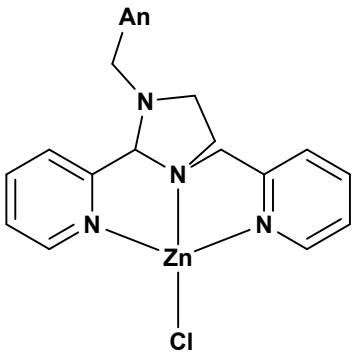


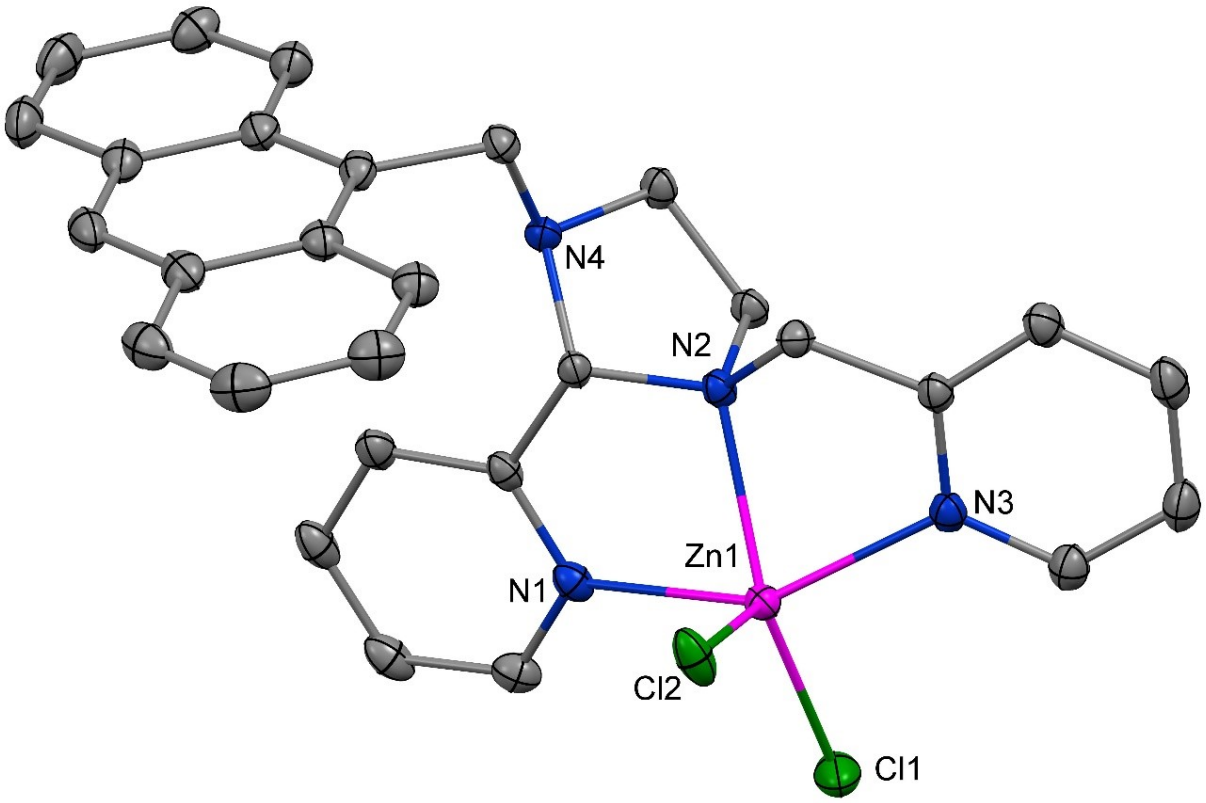
Figure S 12. Simulated ESI-MS of $[Zn(L^{An})Cl_2]$





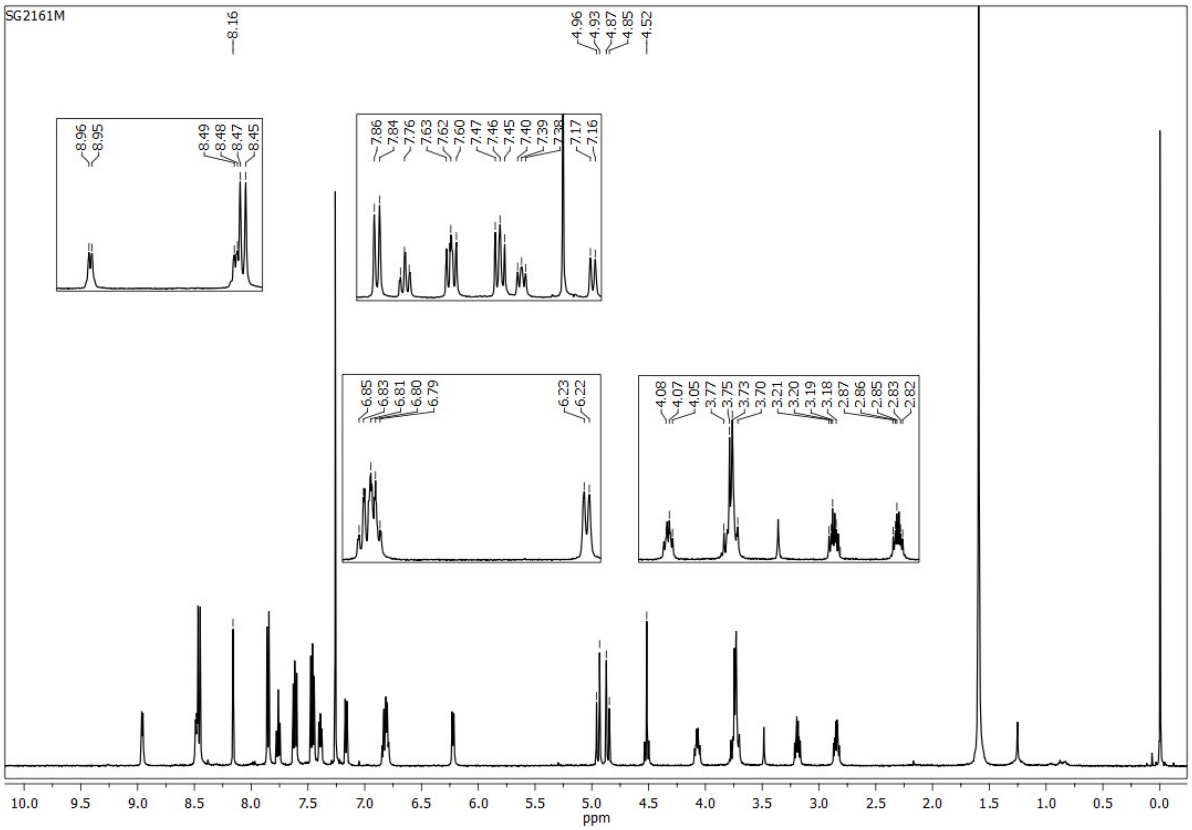
$C_{29}H_{26}N_4ZnCl$

Figure S 13. ORTEP of $[Zn(L^{An})Cl_2]$ drawn at 50% probability



Selected bond distances (Å): Zn(1)-N(1), 2.1632(19); Zn(1)-N(2), 2.1945(18); Zn(1)-N(3), 2.1508(18); Zn(1)-Cl(1), 2.2561(7); Zn(1)-Cl(2), 2.2820(6); Selected bond angles (°): Cl(1)-Zn(1)-Cl(2), 114.84(3); N(3)-Zn(1)-Cl(2), 96.90(5); N(3)-Zn(1)-Cl(1), 100.31(5); N(3)-Zn(1)-N(1), 147.75(7) = β; N(3)-Zn(1)-N(2), 75.50(7), N(1)-Zn(1)-Cl(2), 100.60(5); N(1)-Zn(1)-Cl(1), 96.62(5); N(1)-Zn(1)-N(2), 75.29(7); N(2)-Zn(1)-Cl(2), 98.40(5); N(2)-Zn(1)-Cl(1), 146.74(5) = α; (τ = 0.016)

Figure S 14. ^{1}H NMR of $[Cd(L^{An})Cl_2]$



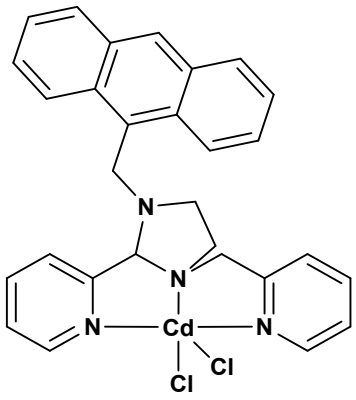
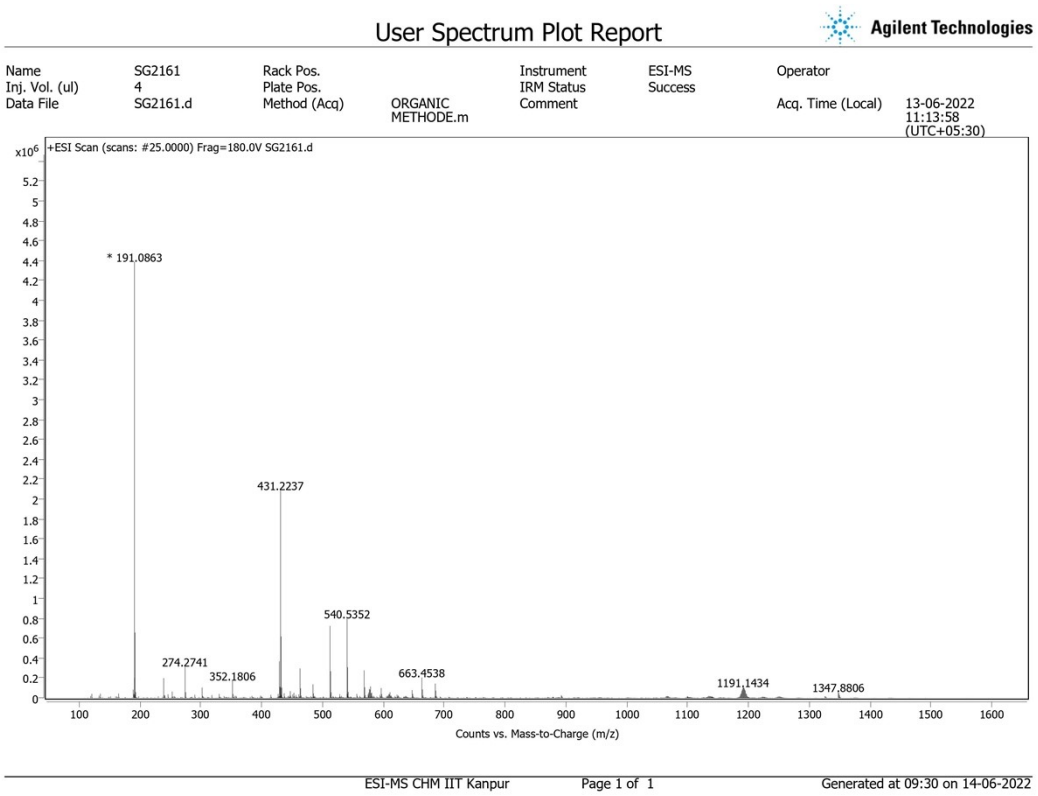


Figure S 15. ESI-MS of $[Cd(L^{An})Cl_2]$



Calculated *m/z* for $[Cd(L^{An})Cl]^+$ = 579.0878; observed *m/z* = 579.0866.

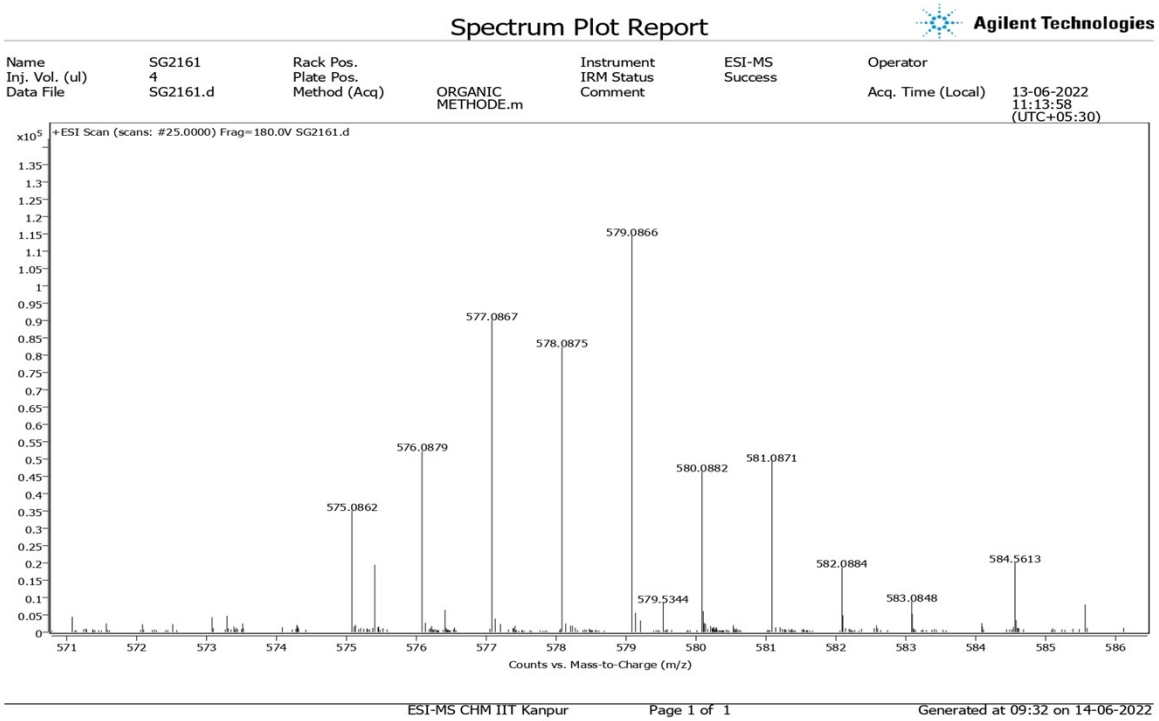
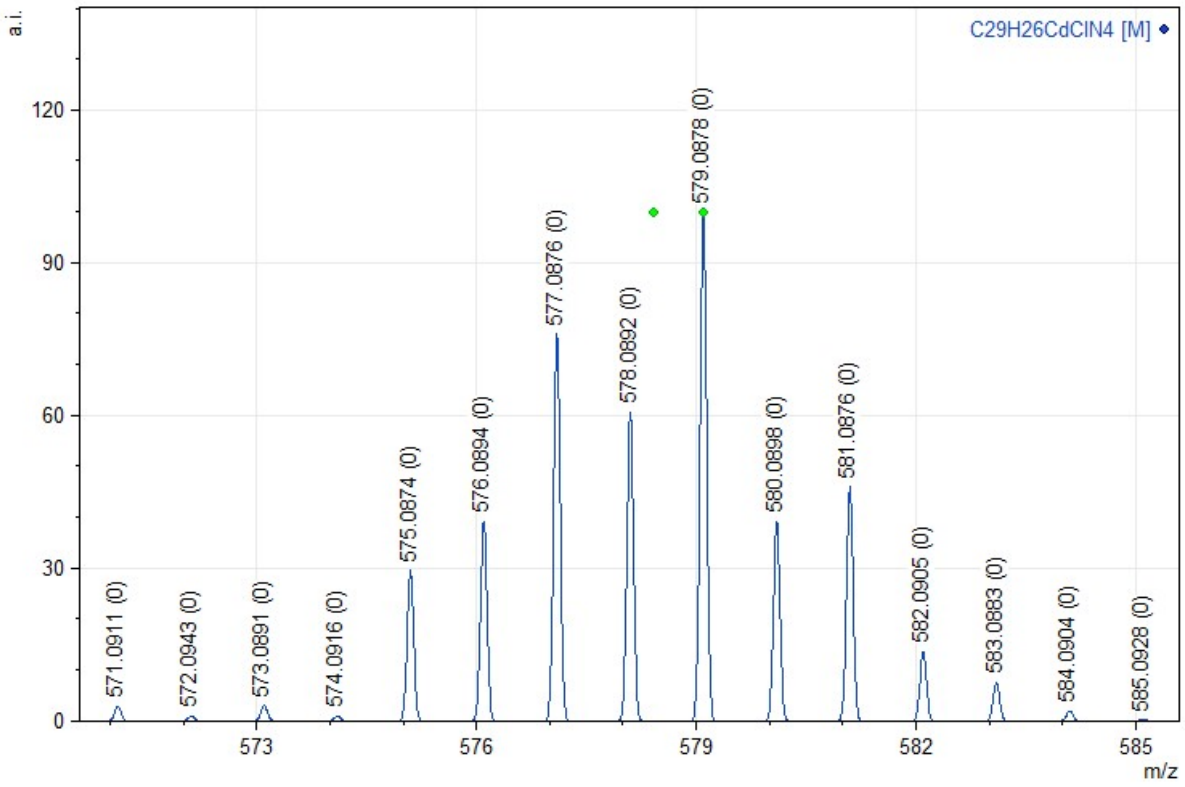
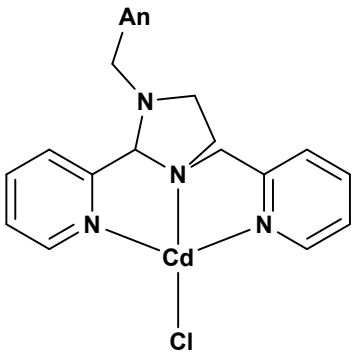


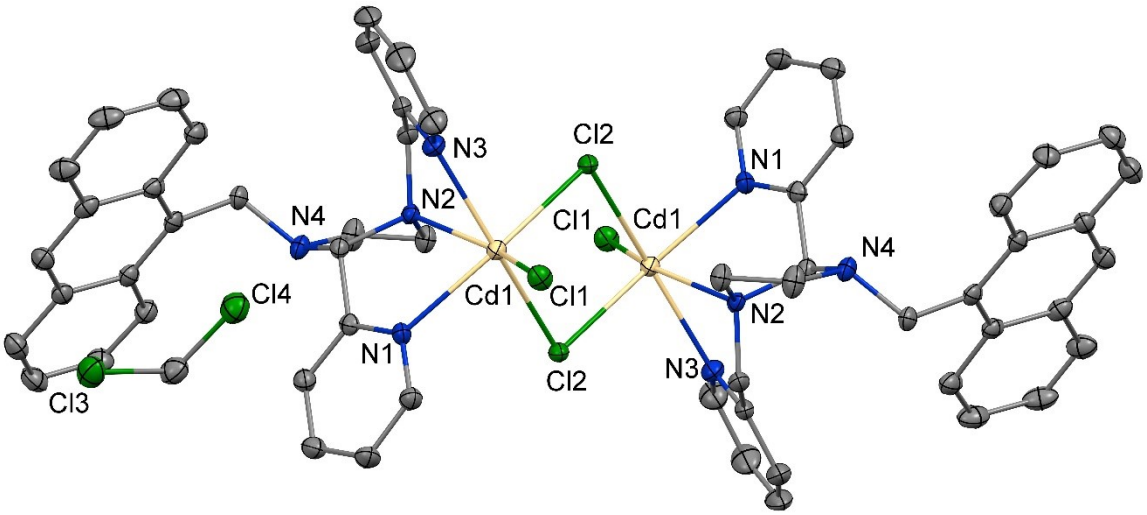
Figure S 16. Simulated ESI-MS of $[Cd(L^{An})Cl_2]$





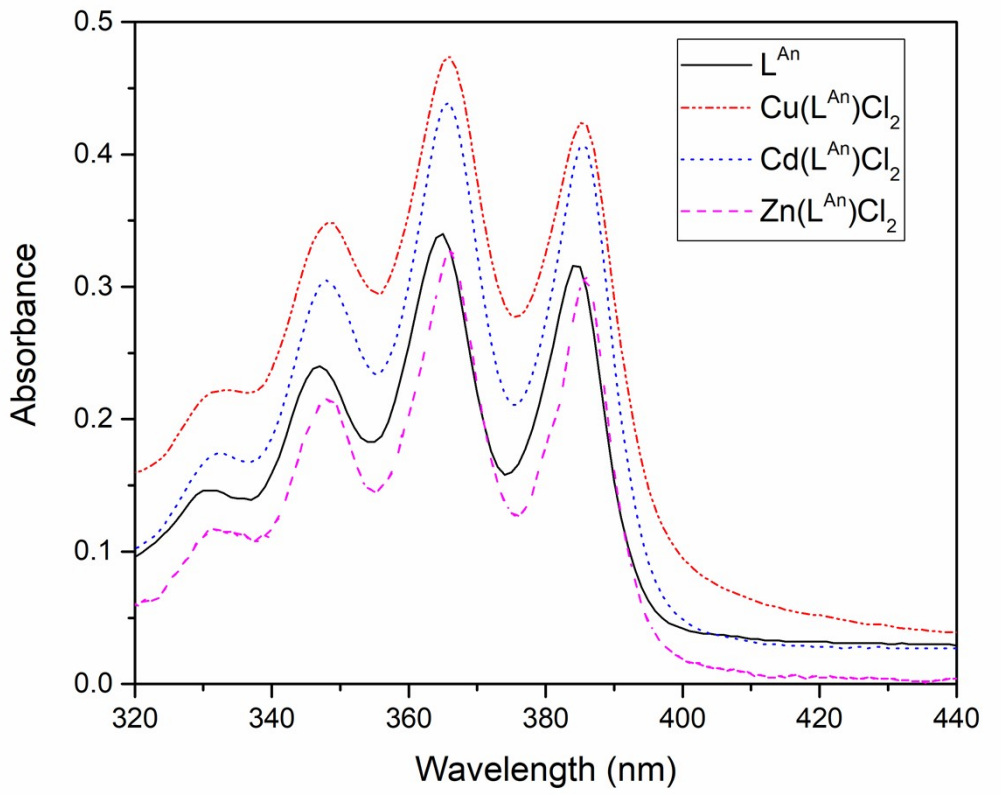
$C_{29}H_{26}N_4CdCl$

Figure S 17. ORTEP of $[Cd(L^{An})Cl_2]$ drawn at 50% probability



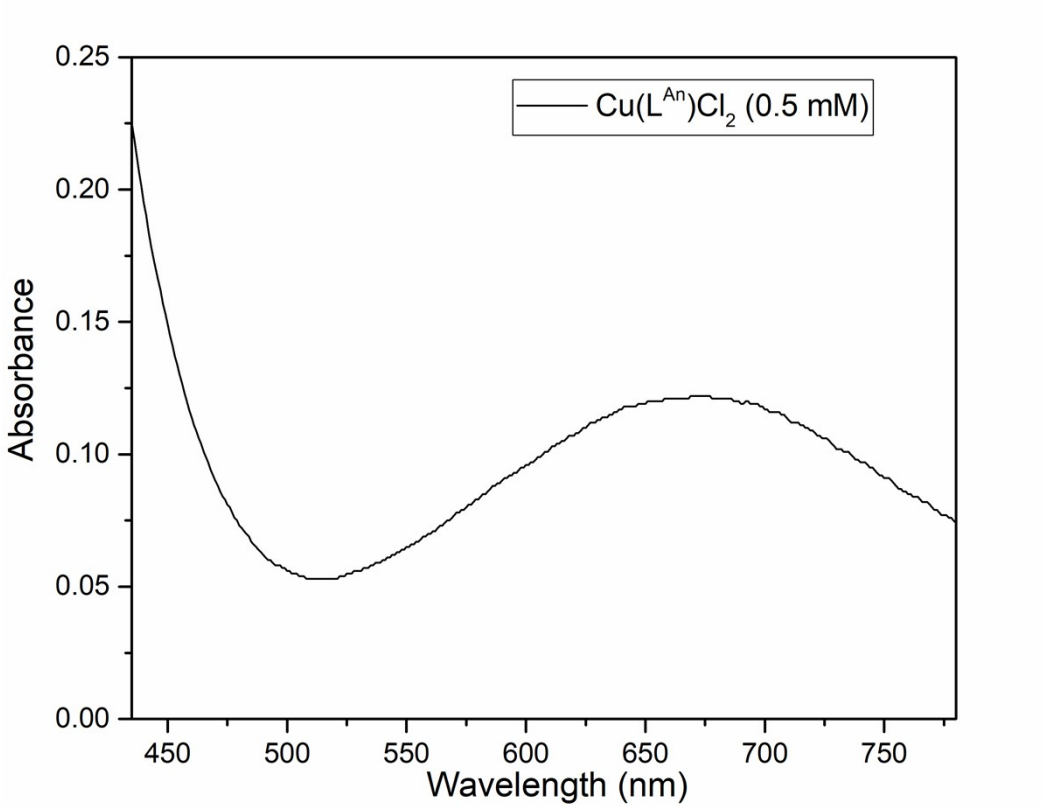
Selected bond distances (Å): Cd(1)-N(1), 2.358(2); Cd(1)-N(2), 2.444(2); Cd(1)-N(3), 2.373(2); Cd(1)-Cl(1), 2.4934(7); Cd(1)-Cl(2), 2.6076(7); Selected bond angles (°): Cl(2)-Cd(1)-Cl(2^1), 85.03(2); Cl(1)-Cd(1)-Cl(2^1), 101.95(2); Cl(1)-Cd(1)-Cl(2), 109.16(2); N(2)-Cd(1)-Cl(2^1), 96.05(5); N(2)-Cd(1)-Cl(2), 86.93(5); N(2)-Cd(1)-Cl(1), 156.67(5); N(3)-Cd(1)-Cl(2), 87.41(6); N(3)-Cd(1)-Cl(2^1), 164.80(6); N(3)-Cd(1)-Cl(1), 92.99(6); N(3)-Cd(1)-N(2), 70.35(8); N(1)-Cd(1)-Cl(2^1), 81.11(5); N(1)-Cd(1)-Cl(2), 152.36(6); N(1)-Cd(1)-Cl(1), 97.09(6); N(1)-Cd(1)-N(2), 71.00(7); N(1)-Cd(1)-N(3), 100.02(8)

Figure S 18. Absorption spectra of ligand and metal complexes



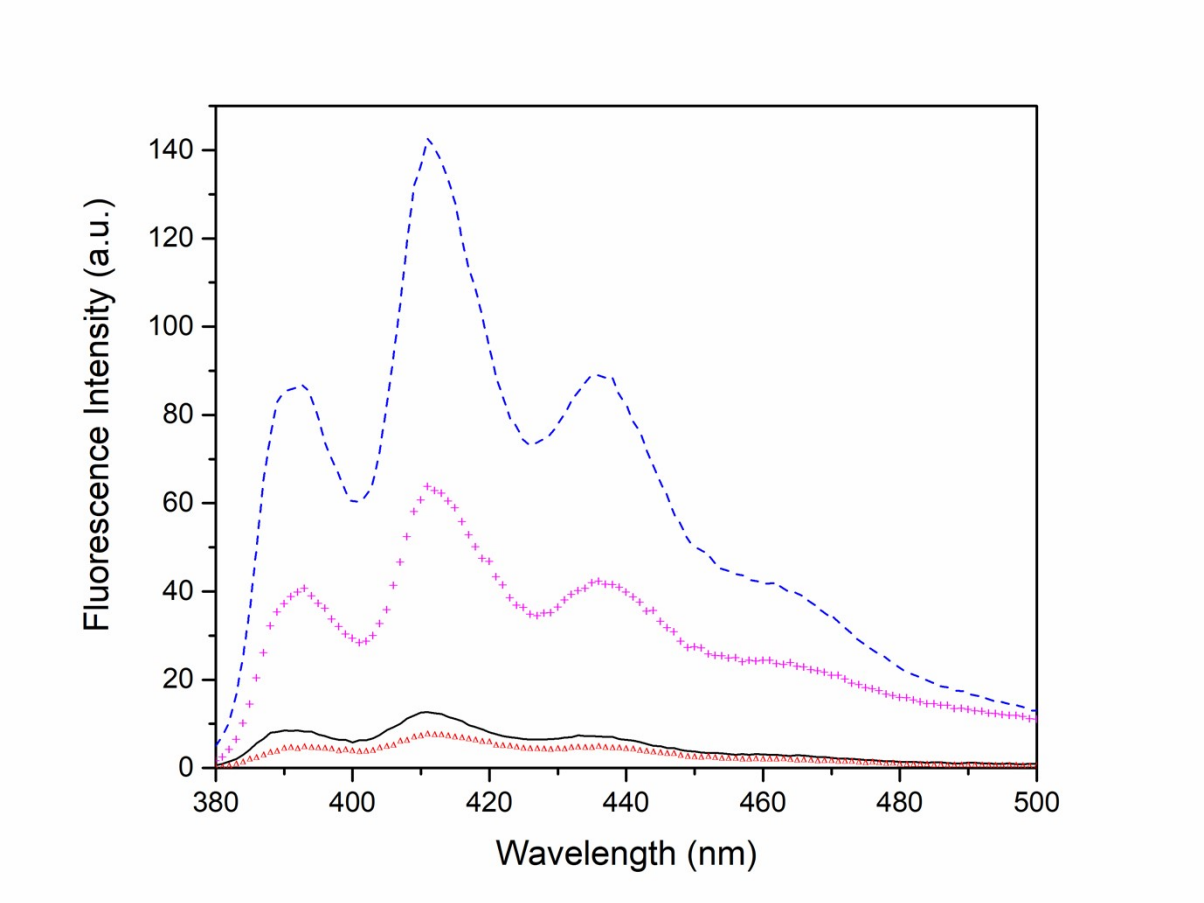
Absorption spectra of L^{An}, $[Cu(L^{An})Cl_2]$, $[Cd(L^{An})Cl_2]$ and $[Zn(L^{An})Cl_2]$. in CH_3OH at 0.05 mM concentration.

Figure S 19. Absorption spectra of [Cu(L^{An})Cl_2] at higher concentration



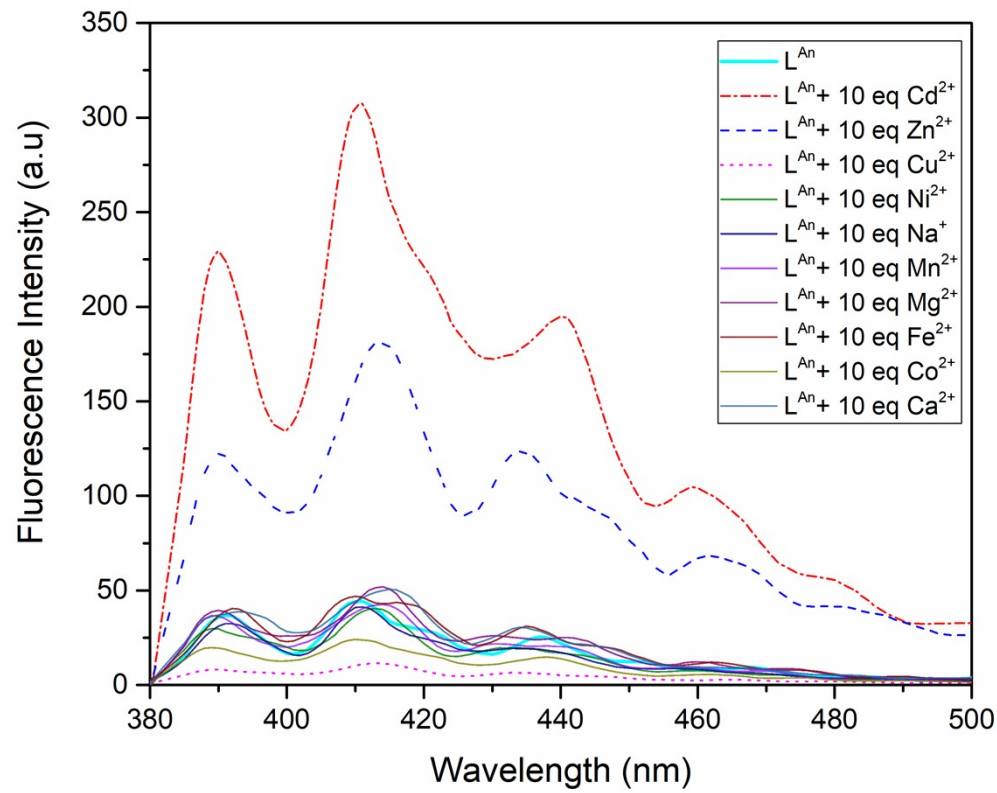
Absorption spectra of [Cu(L^{An})Cl_2] in CH_3OH at 0.5 mM concentration. Absorbance between 550-750 nm due to d-d transition is observed at higher concentration.

Figure S 20. Emission spectra of ligand and metal complexes



Emission spectra of L^{An} (—), $[Cu(L^{An})Cl_2]$ (Δ Δ Δ), $[Cd(L^{An})Cl_2]$ (- - -) and $[Zn(L^{An})Cl_2]$ (+ + +) in CH_3OH at 0.05 mM concentration (λ_{ex} = 366 nm).

Figure S 21. Emission spectra of ligand in the presence of different metal ions



Emission spectra of L^{An} (0.025 mM) and upon addition of 10 Eq. of different metal ions (10 mM) show selective enhancement in the presence of Zn^{2+} and Cd^{2+}

Fluorescence enhancement factor calculation

$Z = F/F^0[(v+v^0)/ v^0]$ (equation S1)

F^0 = Fluorescence intensity before addition of guest

F = Fluorescence intensity after addition of guest

v^0 = Volume of host/ receptor before addition of guest

v = Volume of guest added.

Figure S 22. Absorption and emission profile of L^{An}

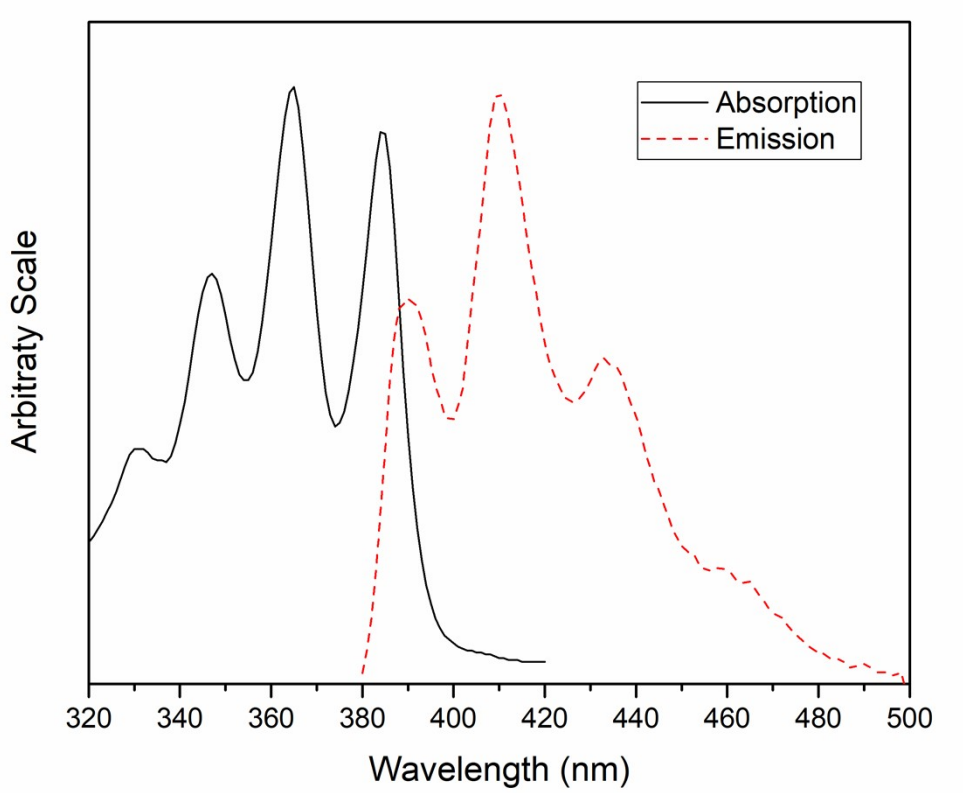


Table S 1. Crystal data for $[Cu(L^{An})Cl_2]$

Identification code	25febc_1_0m_a	CCDC-2203047
Empirical formula	$C_{29}H_{26}Cl_2CuN_4$	
Formula weight	564.98	
Temperature	100 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a = 8.9490(9) Å	α = 90°
	b = 21.114(2) Å	β = 93.251(3)°
	c = 13.2684(13) Å	γ = 90°
Volume	2503.0(4) $Å^3$	
Z	4	
Density (calculated)	1.499 g/cm^3	
Absorption coefficient	1.113 mm^{-1}	
F(000)	1164.0	
Crystal size	0.26 × 0.26 × 0.24 mm^3	
Theta range for data collection	5.964 to 50.096°	
Index ranges	$-10 \leq h \leq 10$, $-25 \leq k \leq 25$, $-15 \leq l \leq 15$	
Reflections collected	29678	
Independent reflections	4439 [R_{int} = 0.0564, R_{sigma} = 0.0360]	
Completeness to theta = 25.048°	99.9%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.746 and 0.664	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	4439/0/319	
Goodness-of-fit on F^2	1.283	
Final R indices [I>=2sigma (I)]	R_1 = 0.0628, wR_2 = 0.1173	
R indices [all data]	R_1 = 0.0681, wR_2 = 0.1194	
Largest diff. peak and hole	0.90 and -0.67 e $Å^{-3}$	

Table S 2. Crystal data for $[Zn(L^{An})Cl_2]$

Identification code	28octc_0m	CCDC-2203046
Empirical formula	$C_{29}H_{26}Cl_2N_4Zn$	
Formula weight	567.81	
Temperature	100 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a = 8.9812(9) Å	α = 90°
	b = 21.205(3) Å	β = 93.512(3)°
	c = 13.4228(15) Å	γ = 90°
Volume	2551.6(5) $Å^3$	
Z	4	
Density (calculated)	1.478 g/cm^3	
Absorption coefficient	1.198 mm^{-1}	
F(000)	1172.0	
Crystal size	0.24 × 0.24 × 0.22 mm^3	
Theta range for data collection	5.648 to 56.642°	
Index ranges	$-11 \leq h \leq 11$, $-28 \leq k \leq 28$, $-17 \leq l \leq 17$	
Reflections collected	40043	
Independent reflections	6331 [R_{int} = 0.0663, R_{sigma} = 0.0434]	
Completeness to theta = 28.321°	99.7%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.744 and 0.700	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	6331/0/325	
Goodness-of-fit on F^2	1.045	
Final R indices [I>=2Sigma (I)]	R_1 = 0.0359, wR_2 = 0.0829	
R indices [all data]	R_1 = 0.0546, wR_2 = 0.0968	
Largest diff. peak and hole	0.52 and -0.52 e $Å^{-3}$	

Table S 3. Crystal data for $[Cd(L^{An})Cl_2]$

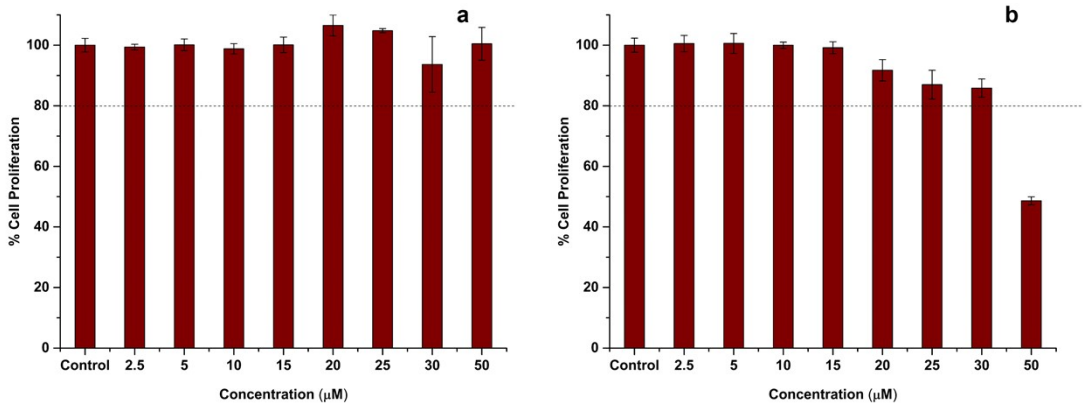
Identification code	18febc_1_0m	CCDC-2203048
Empirical formula	$C_{30}H_{28}CdCl_4N_4$	
Formula weight	698.76	
Temperature	100 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 11.1251(8) Å	α = 113.459(2)°
	b = 11.3839(9) Å	β = 94.795(2)°
	c = 13.3920(10) Å	γ = 93.261(2)°
Volume	1542.9(2) $Å^3$	
Z	2	
Density (calculated)	1.504 g/cm^3	
Absorption coefficient	1.080 mm^{-1}	
F(000)	704.0	
Crystal size	0.26 × 0.24 × 0.24 mm^3	
Theta range for data collection	5.258 to 56.604°	
Index ranges	$-14 \leq h \leq 14$, $-15 \leq k \leq 15$, $-17 \leq l \leq 17$	
Reflections collected	24451	
Independent reflections	7648 [R_{int} = 0.0402, R_{sigma} = 0.0437]	
Completeness to theta = 28.302°	99.8%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.746 and 0.635	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	7648/0/352	
Goodness-of-fit on F^2	1.054	
Final R indices [I>=2Sigma (I)]	R_1 = 0.0335, wR_2 = 0.0831	
R indices [all data]	R_1 = 0.0423, wR_2 = 0.0895	
Largest diff. peak and hole	0.99 and -0.97 e $Å^{-3}$	

Cytotoxicity Study on MCF-7 cells:

MTT assay was performed on MCF-7 cells to find the cytotoxicity of chemosensor L^{An}. Briefly, the cells were cultured in a DMEM medium containing 10% FBS, 1% penicillin-streptomycin, and 0.01 mg/mL insulin at 37 °C and in an atmosphere of 5% CO_2. 5000 cells were seeded in 96 well plates and incubated for 18 h in a CO_2 incubator. The media was removed, and different concentrations of ligand L were added (2.5, 5, 10, 15, 20, 25, 30, and 50 μM) to the respective well and incubated for 24 h. Untreated cells were taken as control. After 24 h of incubation, media was removed from each well, and 200 μL MTT dye (0.5 mg/mL) was added and incubated for 4 hrs. After incubation, MTT dye was removed, followed by the addition of 200 μL DMSO for 30 min. The purple-coloured formazan was measured at 570 nm by UV-vis spectrophotometry. Parallelly, the cytotoxicity analysis of $ZnCl_2$ and $CdCl_2$ at different concentrations (2.5–50 μM) was performed in MCF-7 cells following a similar method as for the chemosensor L^{An}. All readings were taken in triplicate. The percentage cell viability was calculated by the following formula.

$$\text{Cell viability} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Figure S 23. Cytotoxicity study of metal salts after 24 h incubation in MCF-7 cells

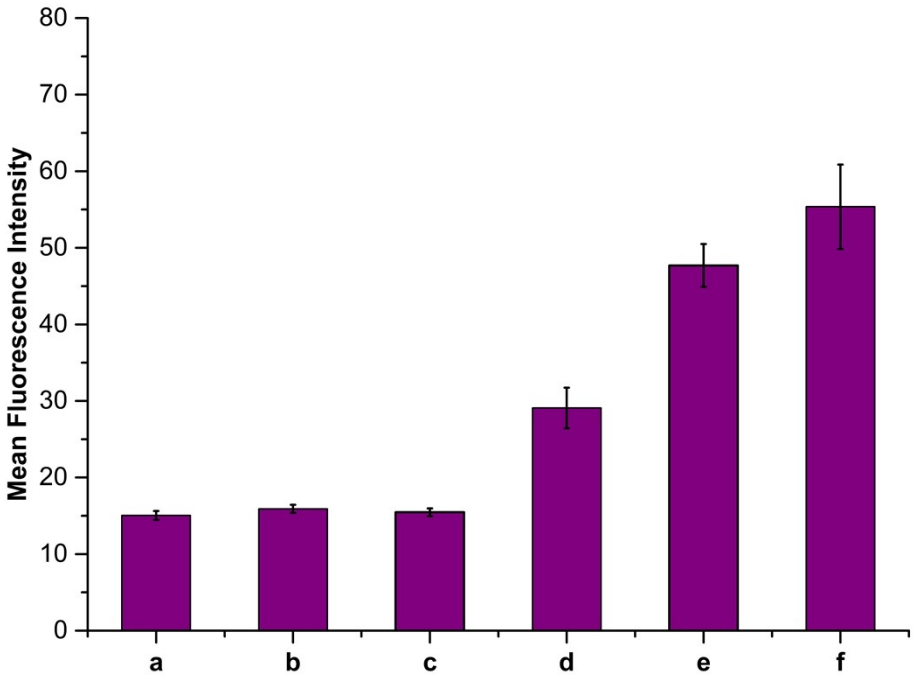


Cell viability percentage of MCF-7 cells after treatment with different concentrations of (a)$ZnCl_2$ (non-toxic up to 50 μM), (b) $CdCl_2$ (non-toxic up to 30 μM).

Cell imaging:

To establish the chemosensing abilities of L^{An} in cells, confocal imaging studies have been carried out in the presence of Zn^{2+} and Cd^{2+} salts. For this purpose, 50,000 MCF-7 cells were cultured in 24 well plates and incubated for 18 h in a CO_2 incubator. After 18 h, the cells were incubated with $ZnCl_2$ and $CdCl_2$ salts (30 μM) for 12 h. After incubation, cells were washed with PBS, followed by the addition of L^{An} (30 μM) for the next 12 h. Further, cells were washed with PBS and fixed with 4% formaldehyde solution for 20 min. Confocal microscopy images were obtained to understand the metal-ligand binding inside the cells. Also, cells individually treated with bare L^{An} (30 μM), $Zn^{2+,}$ and Cd^{2+} salts were used as a negative control.

Figure S 24. Mean Fluorescence Intensity per cell



(a) Untreated cells, (b) 30 μM $ZnCl_2$, (c) 30 μM $CdCl_2$, (d) 30 μM L^{An}, (e) 30 μM $ZnCl_2$ followed by 30 μM L^{An}, (f) 30 μM $CdCl_2$ followed by 30 μM L^{An}.

Anti-microbial evaluation

Growth media and Reagents

All bacterial media and supplements including Mueller-Hinton cation supplemented broth II (MHBII), Mueller-Hinton agar (MHA) and Tryptic soy broth (TSB) were purchased from Becton-Dickinson (Franklin Lakes, NJ, USA). All other chemicals and antibiotics were procured from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute Medium (RPMI) and Fetal Bovine Serum (FBS) were purchased from Lonza (Lonza, USA). All methods were performed in accordance with the relevant guidelines and regulations.

Bacterial strains

The molecules were screened against a bacterial panel consisting of ESKAPE pathogens, namely *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* BAA-1705, *Acinetobacter baumannii* BAA-1605, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus* sp. The panel was further expanded to include drug-resistant clinical *S. aureus* and *Enterococci* strains including those resistant to Vancomycin and other clinically utilized antibiotics. These strains were procured from Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection (BEI/NARSA/ATCC, USA) and routinely cultivated on MHA and MHBII. Before starting the experiment, a single colony was picked from MHA plate, inoculated in MHBII, and incubated overnight at 37 °C with shaking for 18–24 h to get the starter culture.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted according to the CLSI guidelines using the broth microdilution assay.[1] 10 mg/mL stock solutions of test compounds were prepared in DMSO. Bacterial cultures were inoculated in MHBII, and optical density (OD) was measured at 600nm, followed by dilution to achieve ~10^6 CFU/mL. The compounds were tested from 64–0.5 mg/L in two-fold serial diluted fashion with 2.5 µL of each concentration added to well of a 96-well round bottom microtiter plate. Later, 97.5 µL of bacterial suspension was added to each well containing either test compound or appropriate controls. The plates were incubated at 37 °C for 18-24 h following which the MIC was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

Table S 4. Minimum inhibitory concentration (MIC) in µg/mL and selectivity index (SI) values

Compound	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*K. pneumoniae* BAA 1705	*A. baumannii* BAA 1605	*P. aeruginosa* ATCC 27853	Selectivity Index (CC_{50}/MIC)
L^{An}	>64	4	>64	>64	>64	20
$L^{An}CuCl_2$	>64	4	>64	>64	>64	<10
$L^{An}CdCl_2$	64	1	>64	>64	64	40
$L^{An}ZnCl_2$	64	2	>64	>64	>64	10
Levofloxacin	0.0078	0.125	64	4	1	ND

Cell cytotoxicity

Cell toxicity was performed against Vero cells using the MTT assay.[2] ~10^3 cells/well were seeded in 96 well plate and incubated at 37 °C in an 5% CO_2 atmosphere. After 24 h, compound was added ranging from 100-12.5 µg/mL concentration and incubated for 72 h. After the incubation was over, MTT was added in each well, incubated at 37 °C for further 4 h, residual medium was discarded, 0.1 mL of DMSO was added to solubilise the formazan crystals and OD was taken at 540 nm for the calculation of CC_{50}. CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as positive control and each experiment was repeated in triplicate.

Bacterial time kill kinetics

The presence or absence of bactericidal activity was assessed by the time-kill method as described earlier.[3] Briefly, *S. aureus* ATCC 29213 were diluted ~10^6 CFU/mL in MHBII and treated with 1x and 10x MIC the hit and vancomycin and incubated at 37 °C with shaking for 24 h. 100 µL samples were collected at 0, 1, 6 and 24 h, serially diluted in PBS and plated on MHA followed by incubation at 37 °C for 18–20 h. The kill curves were constructed by counting the colonies from plates and plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of compound. Each experiment was repeated three times in duplicate and the mean data were plotted.

Drug interaction with FDA approved drugs

Interaction of the hit with FDA approved drugs namely Vancomycin, Levofloxacin, Ceftazidime, Linezolid and Gentamycin was tested by the checkerboard method. Serial two-fold dilutions of each drug were freshly prepared prior to testing. The hit was two-fold diluted along the abscissa while the antibiotics were serially diluted along the ordinate (8 dilutions) in 96 well microtiter plate. 95 µL of ~10^5 CFU/mL was added to each well and plates were incubated at 37 °C for 24 h. After the incubation, the ΣFICs (fractional inhibitory concentrations) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone and FIC B is the MIC of drug B in the combination/MIC of drug B alone. The combination is considered synergistic when the ∑FIC is ≤0.5, indifferent when the ∑FIC is >0.5 to 4, and antagonistic when the ∑FIC is >4.[4]

Table S 5. Synergy studies of $L^{An}CdCl_2$ with FDA-approved standards drugs

Compound/Drug	**MIC (µg/mL)**	**MIC (µg/ml) of $L^{An}CdCl_2$ in presence of drug "A"**	**MIC (µg/ml) of drug in presence of $L^{An}CdCl_2$ "B"**	**FIC A**	**FIC B**	**FIC (FIC A + FIC B)**	**Inference**
$L^{An}CdCl_2$	1						
Ceftazidime	16	1	16	1	1	2	No interaction
Daptomycin	1	1	1	1	1	2	No interaction
Gentamycin	0.25	1	0.25	1	1	2	No interaction
Levofloxacin	0.25	1	0.25	1	1	2	No interaction
Linezolid	2	1	2	1	1	2	No interaction
Meropenem	0.125	1	0.125	1	1	2	No interaction
Minocycline	0.25	1	0.25	1	1	2	No interaction
Rifampicin	0.0078	1	0.0078	1	1	2	No interaction
Vancomycin	1	1	1	1	1	2	No interaction

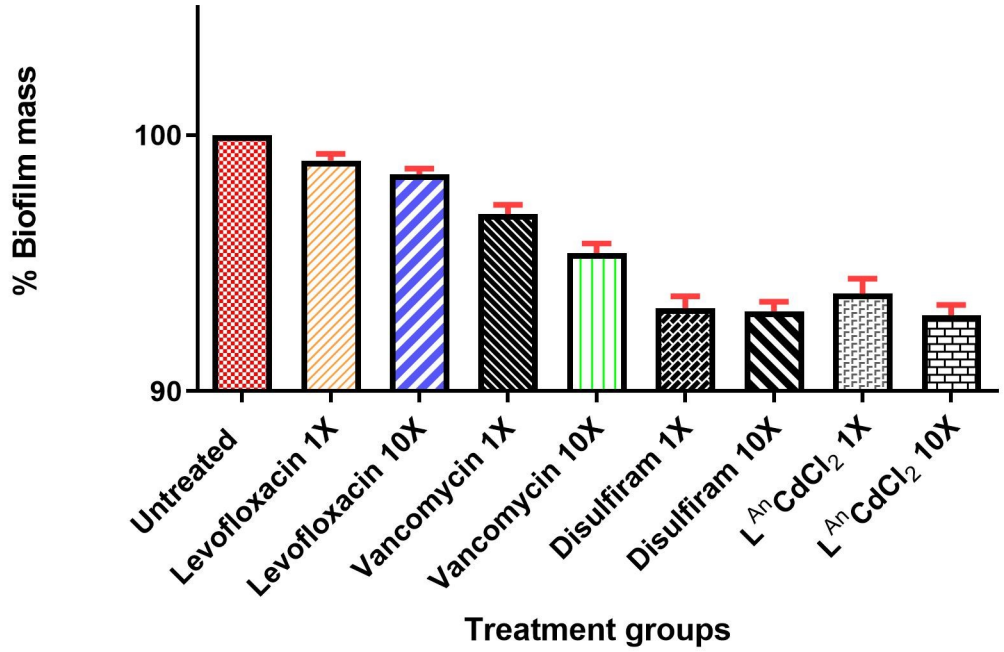
Determination of Post antibiotic effect (PAE)

To determine the PAE, overnight culture of *S. aureus* ATCC 29213 was diluted in MHBII ~10^5 CFU/mL and exposed to 1x and 5x MIC of Vancomycin, Levofloxacin, the hit and vancomycin were incubated at 37 °C for 1 h. Following the incubation period, culture was centrifuged and washed 2 times with pre-warmed MHBII to remove any traces of antibiotics. Finally, cells were resuspended in drug free MHBII and incubated further at 37 °C. Samples were taken after every 1h, serially diluted and plated on TSA for enumeration of CFU. The PAE was calculated as PAE = T – C; where, T is referred to the difference in time required for 1 Log_{10} increase in CFU versus CFU observed immediately after the removal of drug and C in a similarly treated drug free control.[5]

Determination of activity against *S. aureus* biofilm

The determination of anti-biofilm activity was performed as described earlier.[6] Briefly, *S. aureus* ATCC 29213 were grown overnight in 1% TSB with shaking (180 RPM) at 37 °C. The overnight culture was diluted in fresh TSB broth (1:100) and 0.2 mL of freshly diluted culture was transferred into 96 well polystyrene flat bottom plate, covered with adhesive foil lid for maintaining low oxygen and incubated in static condition for 48 h at 37 °C. After incubation, media was decanted, and plate was rinsed gently 3 times with the 1x PBS (pH 7.4) to remove the planktonic bacteria. Plates were refilled with TSB with different drug concentration and incubated for 24 h at 37 °C. After drug treatment, the media was decanted, washed 3 times with 1x PBS (pH 7.4) and biofilm was fixed by incubating the plate at 60 °C for 1 h. After fixing, the biofilm is stained by 0.06% crystal violet for 10 min, rinsed with PBS and dried at room temperature. For quantification of biofilm, the bound crystal violet was eluted by 30% acetic acid (0.2 mL). Absorbance was taken on microtiter plate reader at 600nm for biofilm quantification.

Figure S 25. Biofilm (*S. aureus* ATCC 29213) eradication by $L^{An}CdCl_2$



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