Supplemantary

Synthesis of metal-based biologically active agents from ONO-donor Schiff base ligand

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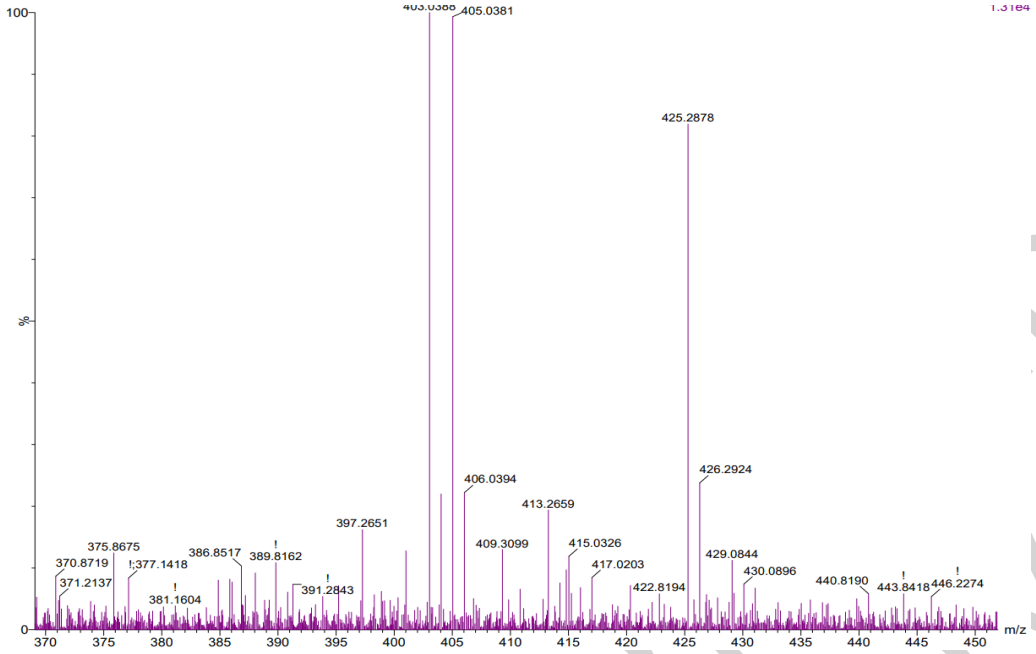


Fig. S1. Mass spectrum of azo-Schiff base ligand

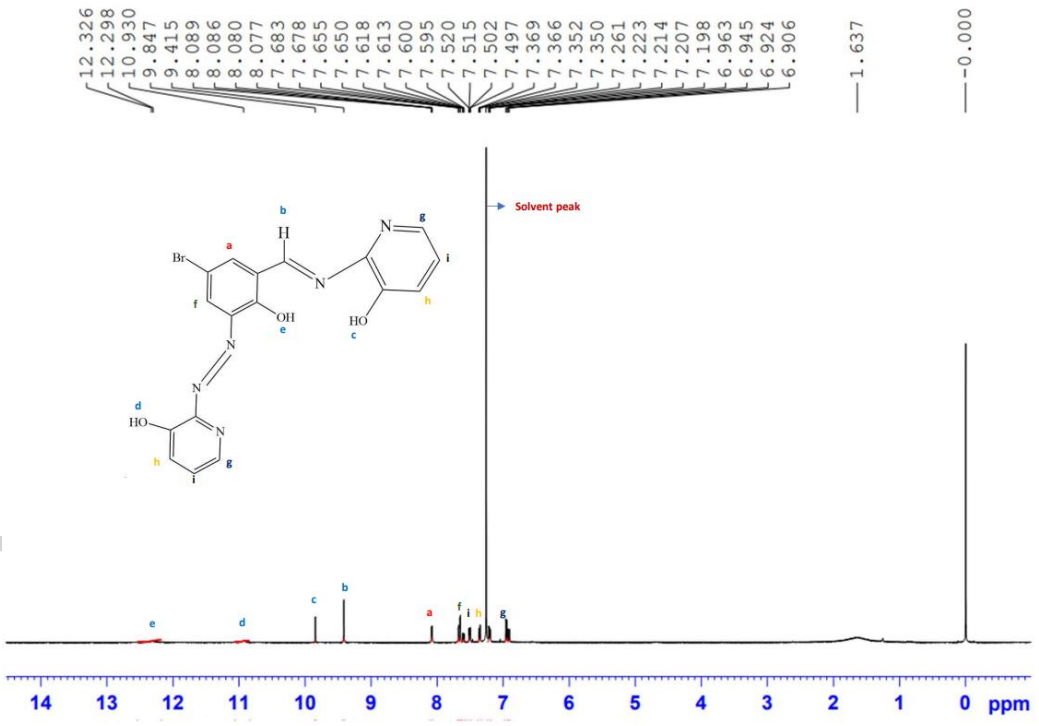


Fig. S2. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 12.32 (s, 1H, -OH), 10.93 (s, 1H, -OH), 9.84 (s, 1H, -OH), 9.41 (s, 1H, N=CH), 8.08(d,1H, Ar-H), 7.06 (d, 1H, Ar-H) J=2.5Hz, 6.90-6.96 (dd, 2H, Ar-H),7.65-7.68 (dd, 2H, Ar-H), 7.49-7.52(dd, 2H, Ar-H)

FT-IR Spectrum of Ligand and Metal complexes

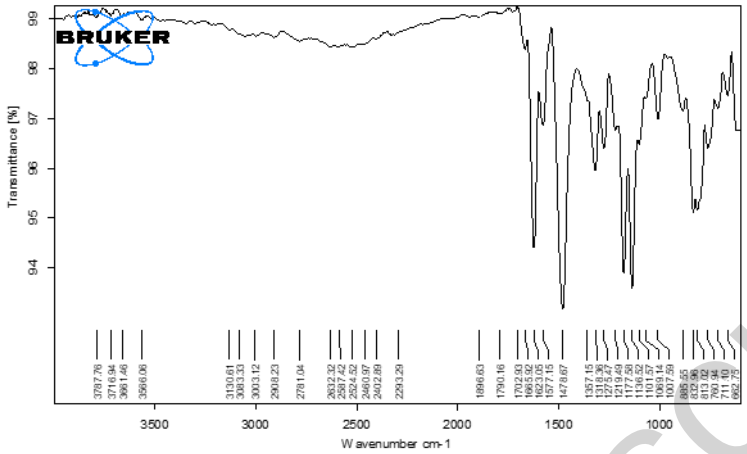


Fig.S3. FTIR of Ligand

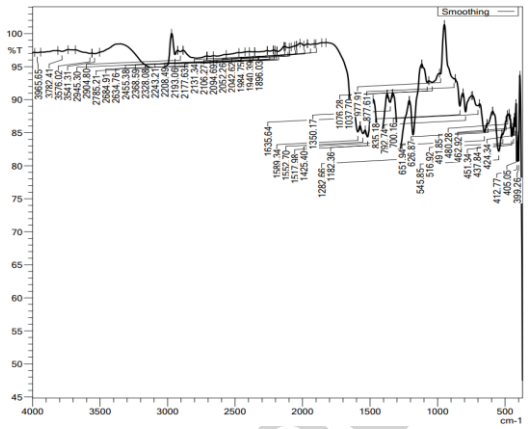


Fig.S4. FTIR of Mn(II) complex

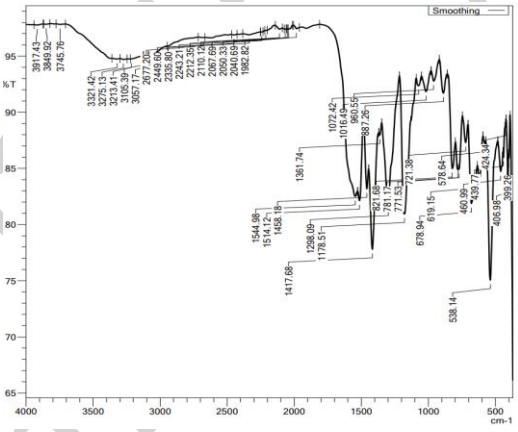


Fig. S5. FTIR of Co(II) complex

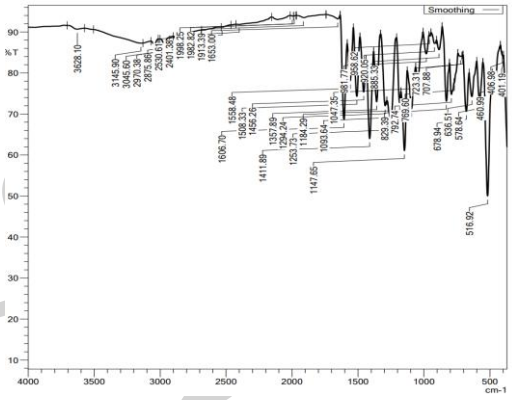


Fig. S6. FTIR of Ni(II) complex

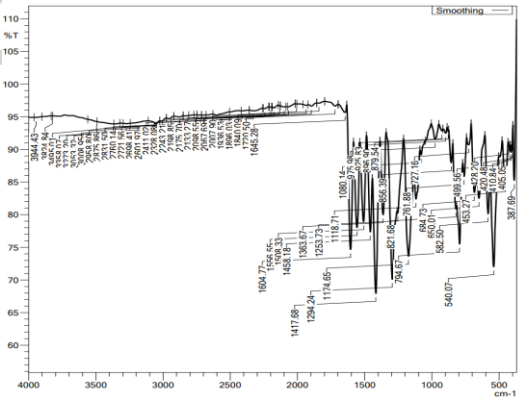


Fig.S7. FTIR of Cu(II) complex

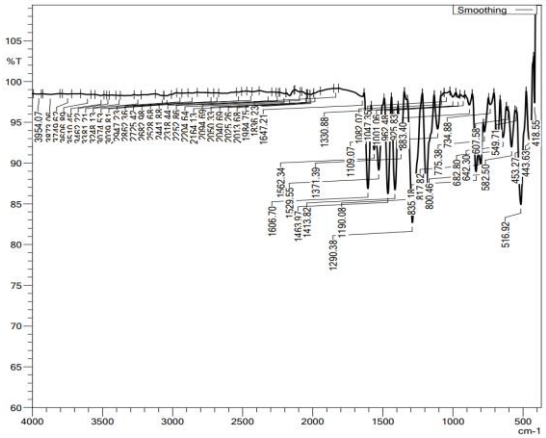


Fig.S8. FTIR of Zn(II) complex

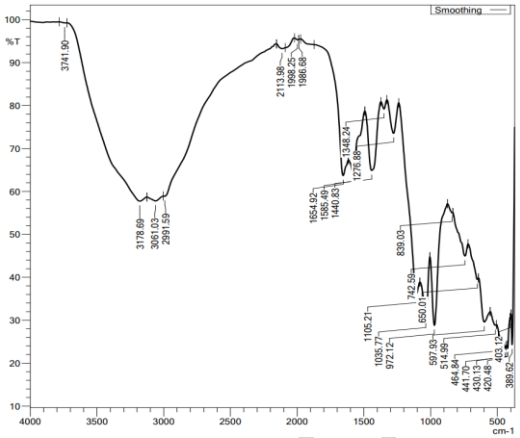


Fig. S9. FTIR of VO(II) complex

Electronic Spectra of Ligand and metal complexes

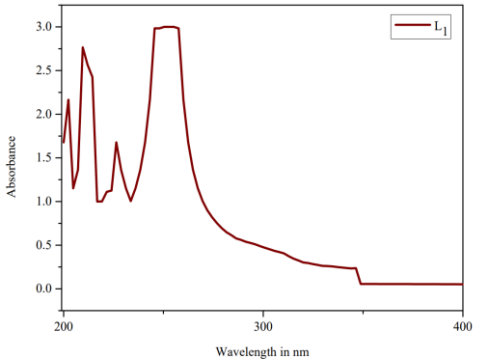


Fig. S10. UV spectrum of Ligand

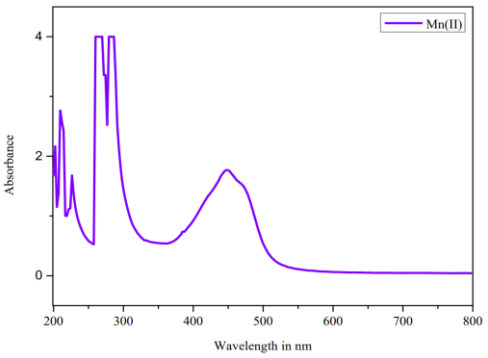


Fig.S11. UV spectrum of Mn(II) complex

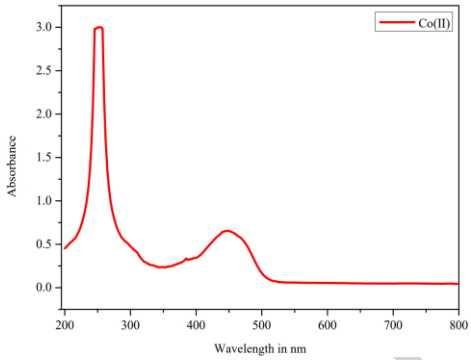


Fig. S12. UV spectrum of Co(II) complex

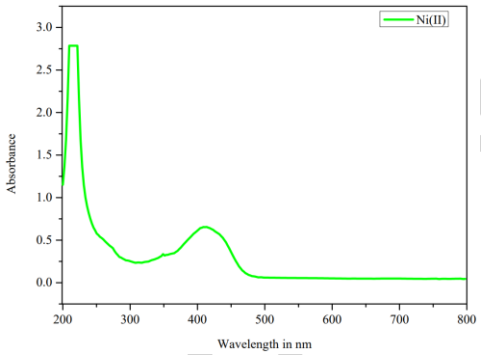


Fig. S13. UV spectrum of Ni(II) complex

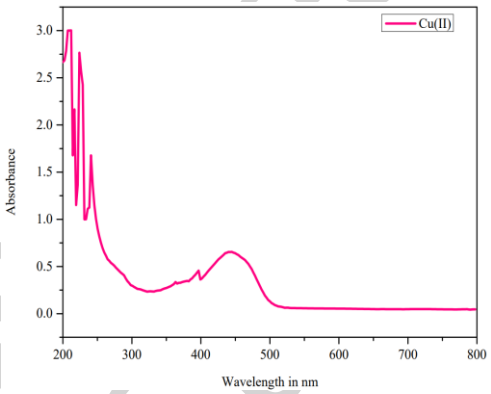


Fig.S14. UV spectrum of Cu(II) complex

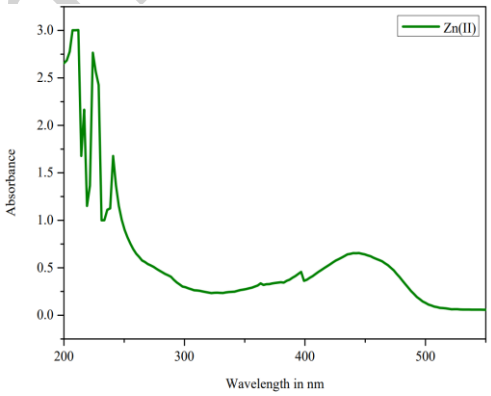


Fig.S15. UV spectrum of Zn(II) complex

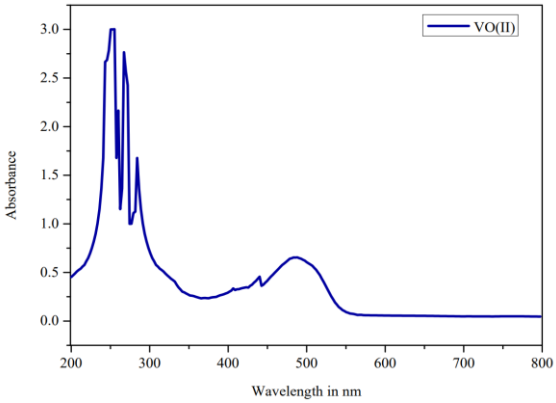


Fig.S16. UV spectrum of VO(II) complex

TGA of metal complexes

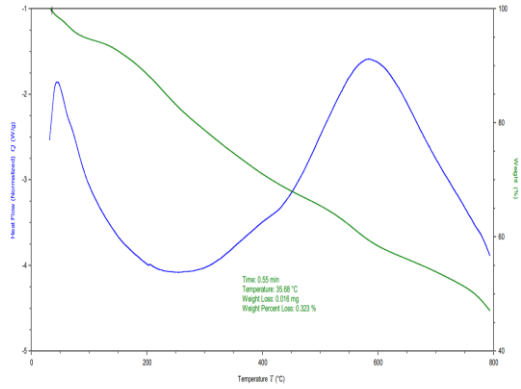


Fig.S17. TGA of Mn(II) complex

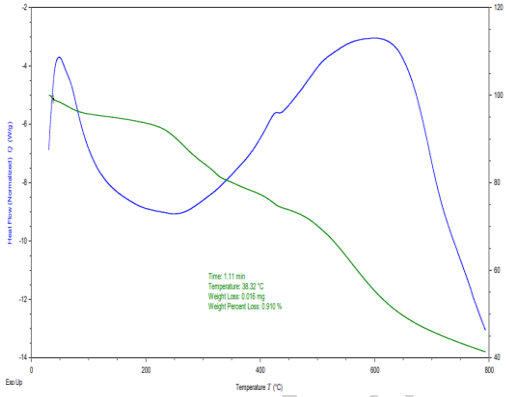


Fig.S18. TGA of Co(II) complex

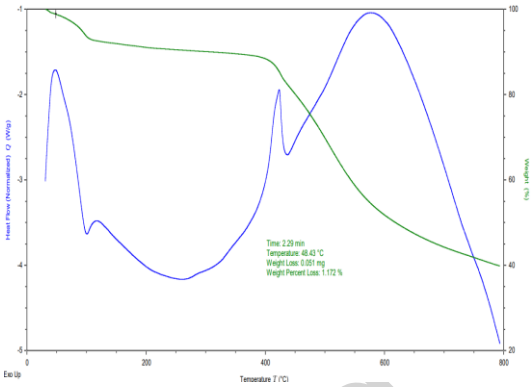


Fig.S19. TGA of Ni(II) complex

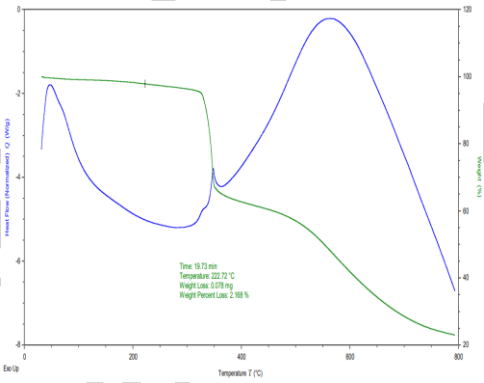


Fig.S20. TGA of Cu(II) complex

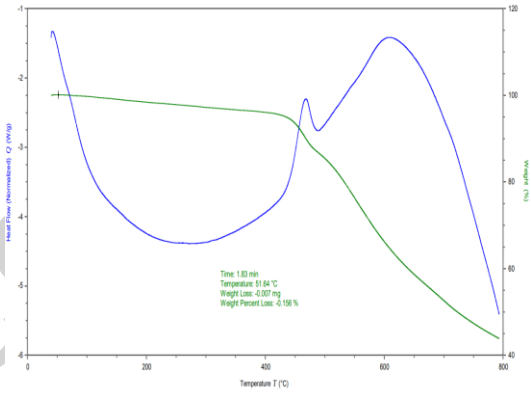


Fig.S21. TGA of Zn(II) complex

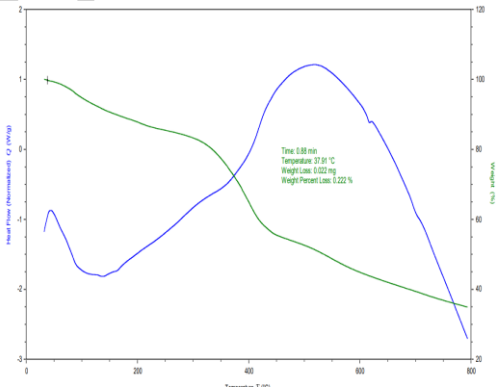


Fig.S22. TGA of VO(II) complex

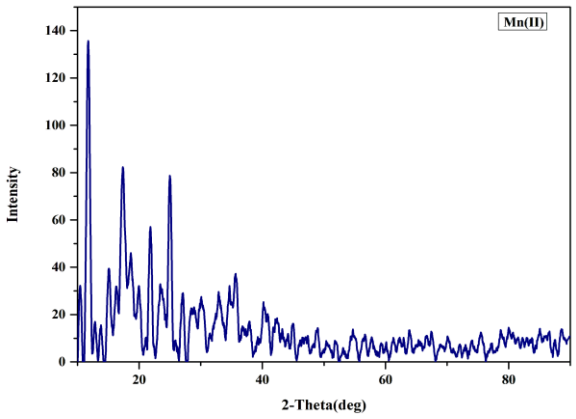


Fig.S23. P-XRD of Mn(II) complex

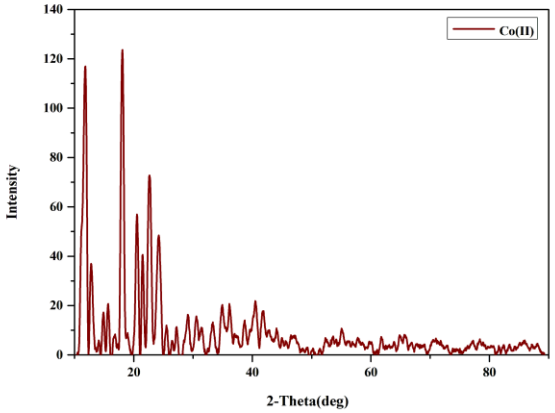


Fig. S24. P-XRD of Co(II) complex

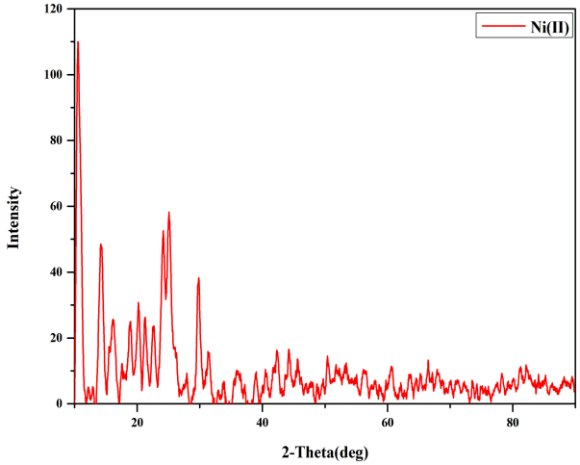


Fig.S25. P-XRD of Ni(II) complex

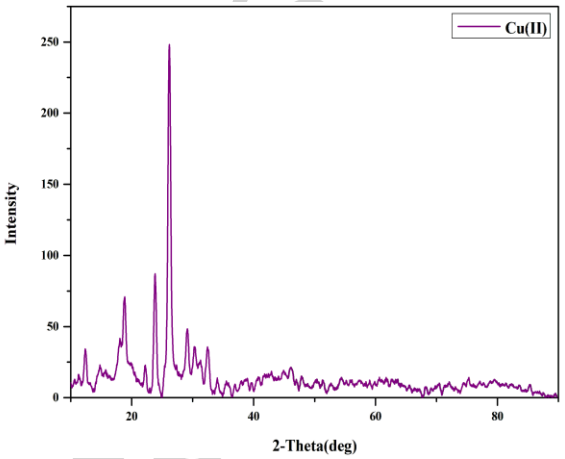


Fig. S26. P-XRD of Cu(II) complex

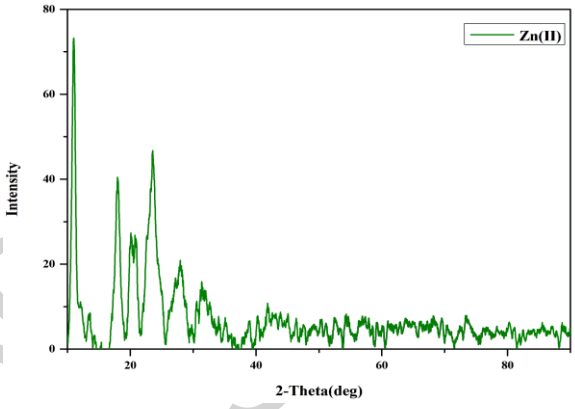


Fig.S27. P-XRD of Zn(II) complex

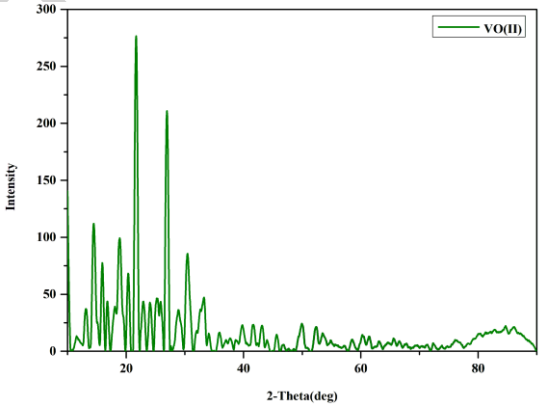


Fig.S28. P-XRD of VO(II) complex

Experimental procedure of neuroprotective activity:

1) SHSY-5Y Neuroblastoma Cell Line was procured from National centre for cell sciences (NCCS), Pune maintained in DMEM medium supplemented with 10 % fetal bovine serum.

2) Cells were incubated at a concentration of 1 × 10^4 cells/ml in culture medium for 24 h at 37 ˚C and 5 % CO_2.

3) Cells were seeded at a concentration (70 µl) 10^4 cells/well in 100 µl culture medium and 100 µl Sample (100 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells).

4) Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.

5) Cell cultures were incubated for 24 h at 37 ˚C and 5 % CO_2 in CO_2 incubator (Thermo scientific BB150)

6) After incubation, the medium was completely removed and Added 20 µl of MTT reagent (5mg/min PBS).

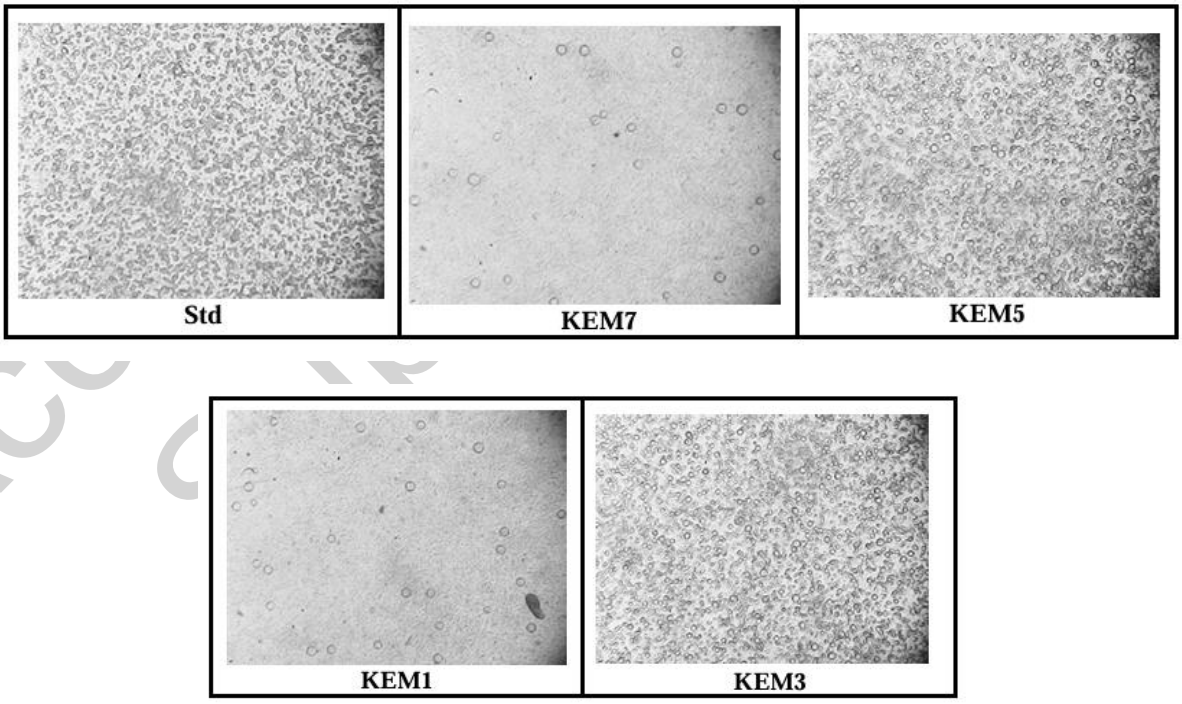
7) After addition of MTT, cells incubated for 4 hrs at 37 ˚C in CO_2 incubator.

8) Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only.

9) After removing the medium completely. Added 200µl of DMSO (kept for 10 min) and incubate at 37^0C (wrapped with aluminium foil).

10) Triplicate samples were analysed by measuring the absorbance of each sample by a Elisa microplate reader (Benesphera E21) at a wavelength of 570 nm and calculated by using following formula:

$$\text{Viability percentage} = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells} \times 100$$



Photos of Neuroprotective activity

KEM1-Co(II) Complex, KEM3-Cu(II) Complex, KEM5-Zn(II) Complex, KEM7-Ligand

Experimental procedure of anticancer activity

Anticancer activity (Sulforhodamine B assay) anticancer activity of the synthesized compounds was tested by using SRB assay on MCF-7 human breast cancer cell line. The medium used for growing the cell line contains 2 mM L-glutamine and 10 % fetal bovine serum. The experiment was carried out in 96 well microtiter plates in 100 µL at plating densities, depending on the double time of separate cell line. An inoculated plate was incubated at a temperature of 37 ˚C, 5 % carbon dioxide and 95 % air and 100 % relative humidity for a time period of 24 hours.

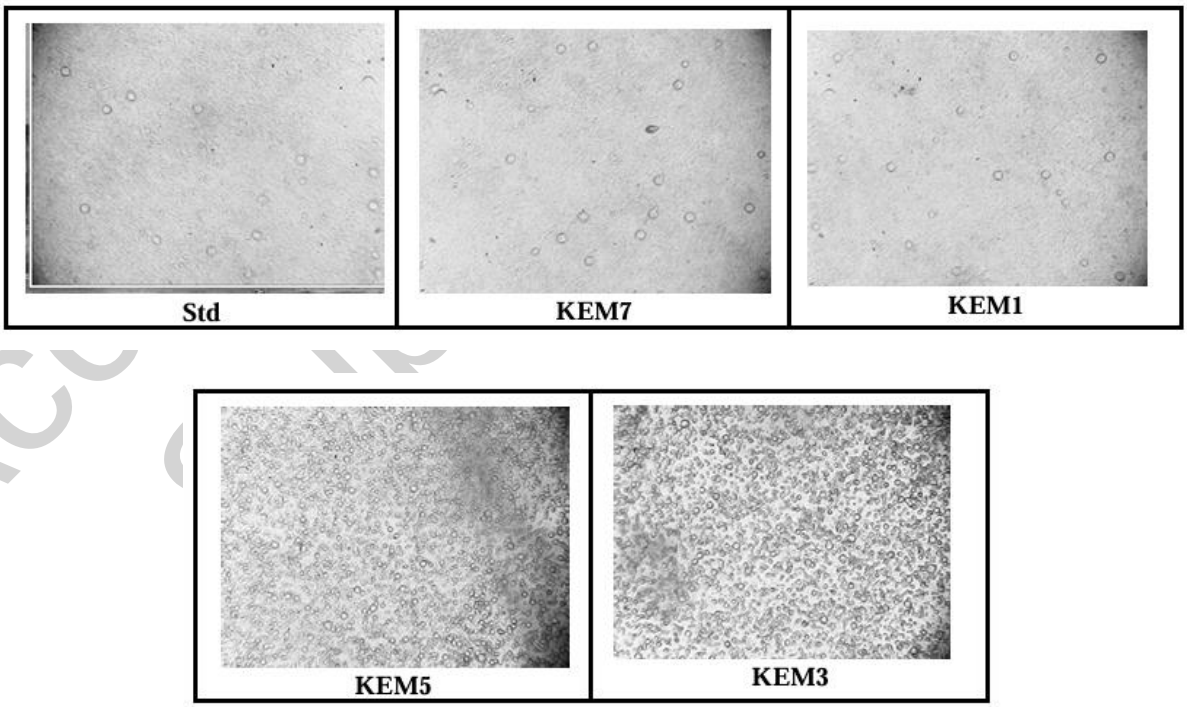
The synthesized compounds were not added in the plates at that time. The synthesized compound was dissolved in dimethyl sulfoxide (DMSO) and solution with a concentration of 1 mg/ml was prepared. The prepared solution diluted up to 100 µg /ml by using water. Aliquots of 100 µl of dilutions of the synthesized compounds was added into the microtiter wells. The microtiter plate was incubated in the standard condition for 48 hours and the process was finished by addition of cold trichloroacetic acid (TCA). Trichloroacetic acid (TCA) was added for the washing of stain.

The cell line was fixed in situ by slow addition in 50 µl of trichloroacetic acid (30 % w/v) and incubated at 4 ˚C for 60 minutes. The upper layer of the solution was discarded and plate was washed five times with water and finally air dried.

The 50 µl sulforhodamine B (SRB) solutions at 0.4 % w/v were prepared. After that 1 % acetic acid was added to well for removal of unbound dye. Microtiter plates were incubated at room temperature for 20 minutes. The staining unbound dye was removed by five times washing with the water. All plates were air dried. Bound dye stain was eluted with 10 mM trizma base.

Absorbance was measured at a wavelength of 540 nm and reference wavelength of 690 nm (Benesphera E21). The percent of inhibition is calculated by formula given below:

$$Inhibition\ percentage = \frac{Control\ OD - Sample\ OD}{Control\ OD} \times 100$$



Photos of Anticancer activity

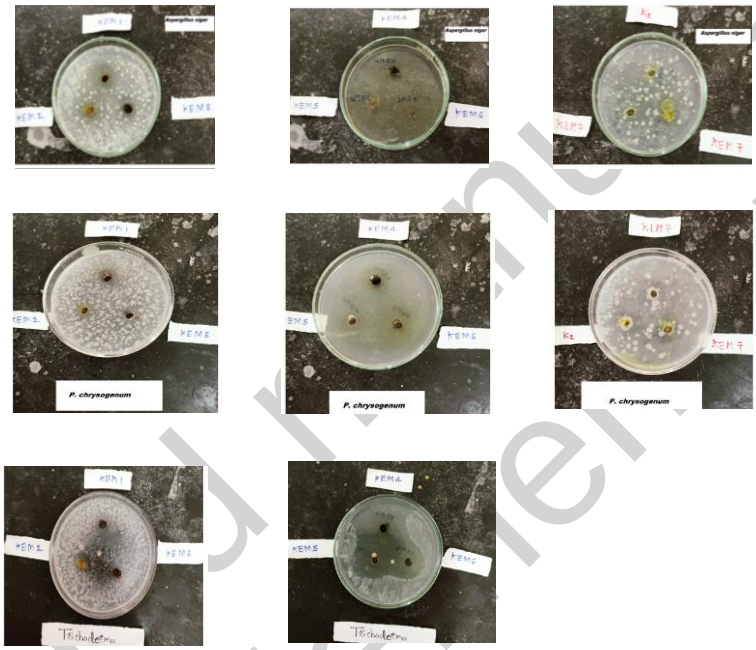
KEM1-Co(II) Complex, KEM3-Cu(II) Complex, KEM5-Zn(II) Complex, KEM7-Ligand

Experimental procedure of Antimicrobial activity

The antimicrobial susceptibility testing was done by the agar Disk Diffusion Method as described by NCCLS 2002. To begin, Petri dishes were prepared using Mueller-Hinton agar for bacterial cultures and Potato Dextrose agar for yeast cultures.

Preparation of McFarland 0.5 turbidity standards by using solution of 0.5 ml $BaCl_2 \times 2H_2O$ + 99.5 ml of H_2SO_4 with constant stirring to maintain the suspension and stored in dark room at 22 to 25 $^{\circ}C$. Following the incubation, the application of L and the metal complexes was made to agar plates that had been previously inoculated. Each plate underwent a visual examination.

The resulting zones of inhibition displayed a uniform circular pattern within the growth lawn. The diameters of these inhibition zones, along with the diameter of the well in millimetres, were measured using sliding callipers and recorded.



Photos of Antifungal activity

KEM1-Co(II) Complex, KEM2-Ni(II) Complex, KEM3-Cu(II) Complex, KEM4-Mn(II) Complex, KEM5-Zn(II) Complex, KEM6-VO(II) Complex, KEM7-Ligand

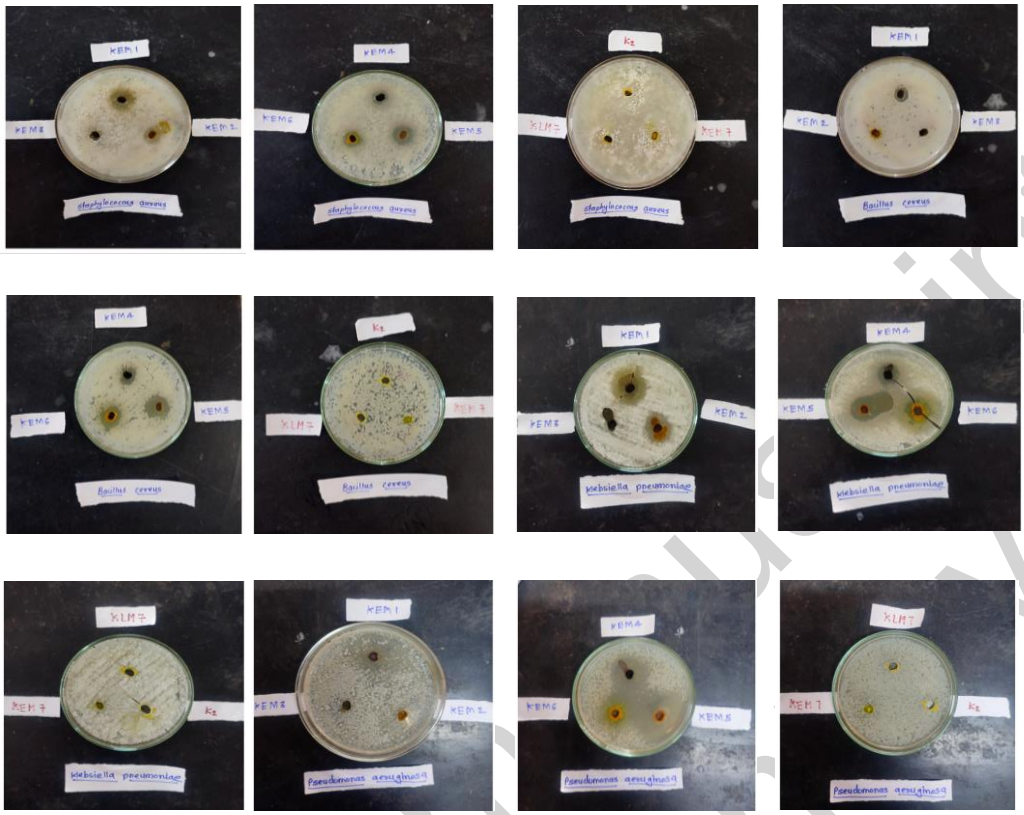


Photo of Antibacterial activity

KEM 1-Co(II) Complex, KEM 2-Ni(II) Complex, KEM 3-Cu(II) Complex, KEM 4-Mn(II) Complex, KEM 5-Zn(II) Complex, KEM 6-VO(II) Complex, KEM 7-Ligand