In this chapter, the materials employed at different stages of the investigation and procedures in the purification of chemicals/solvents are given. A brief description of the physicochemical and biochemical techniques employed in this study is also given in this chapter.

The following biological experiments are carried out in the present study.

Section (i): Purification of solvents and chemicals used in the present study

The following solvents and chemicals are purified [1] and used in the present study. The make and quality of chemicals are given in Table 4.1.

SOLVENTS

Acetic acid (Glacial)

It was purified by adding some acetic anhydride and heated for one hour to just below its boiling point in the presence of CrO_3 and then fractionally distilled.

Chloroform

Commercially available $CHCl_3$ was washed with water to remove ethanol. Dried with $CaCl_2$ and distilled. The distilled $CHCl_3$ was stored in dark.

Dimethylformamide

It was dried over $CaSO_4$ followed by distillation under reduced pressure.

Dimethyl sulphoxide

Dried over $CaSO_4$ and then fractionally distilled at low pressure.

Ethanol

Rectified spirit (95% ethanol) converted to absolute (99.5% ethanol) by refluxing with freshly ignited CaO (250 g/L) for 6 hrs. Allowed to stand overnight and distilled.

Methanol

It was purified by adding NaOH solution containing iodine. After standing for 24 hrs, the solution was poured slowly into about a quarter of its volume of 10% $AgNO_3$, shaken for several hours and then distilled.

CHEMICALS

Lanthanum(III) nitrate hexahydrate, Cerium(III) nitrate hexahydrate, Praseodymium(III) nitrate hexahydrate, Neodymium(III) nitrate hexahydrate, Samarium(III) nitrate hexahydrate, 2-formylpyridine, 2-acetylpyridine, 2-benzoylpyridine, acetylhydrazide, benzhydrazide are used as supplied without further purification.

Table 2.1: Metal salts and organic compounds used in the present study

Inorganic/ Organic compounds	Formulae	Make and Quality
Lanthanum(III) nitrate hexahydrate	$La(NO_3)_3.6H_2O$	Aldrich
Cerium(III) nitrate hexahydrate	$Ce(NO_3)_3.6H_2O$	Aldrich
Praseodymium(III) nitrate hexahydrate	$Pr(NO_3)_3.6H_2O$	Aldrich
Neodymium(III) nitrate hexahydrate	$Nd(NO_3)_3.6H_2O$	Aldrich
Samarium(III) nitrate hexahydrate	$Sm(NO_3)_3.6H_2O$	Aldrich
2-formylpyridine	$(C_6H_4N)CHO$	Aldrich
2-acetylpyridine	$(C_6H_4N)COCH_3$	Aldrich
2-benzoylpyridine	$(C_6H_4N)COC_6H_5$	Aldrich
acetylhydrazide	$CH_3CONHNH_2$	Aldrich
benzhydrazide	$C_6H_5CONHNH_2$	Aldrich
Sodium hydroxide	NaOH	Merck
Sodium chloride	NaCl	Merck
Tris(hydroxymethyl)aminomethane	Tris	Merck
Sucrose		Merck
Ethylenedimine tetraaceticacid disodium salt	EDTA-Na_2	SRL
Agarose		Aldrich
Calf-thymus DNA	CT DNA	Genie, Bangalore
pBR 322 DNA (CsCl purified)		Genie, Bangalore

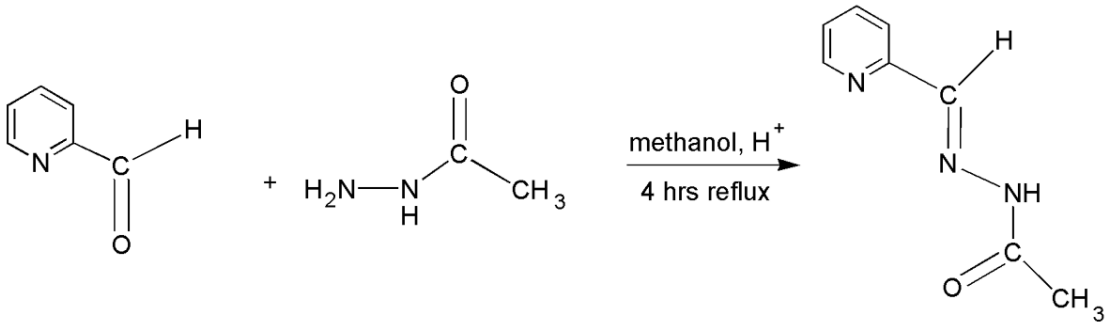
Section (ii): Synthesis of ligands and their lanthanide complexes

Synthesis of 2-Formylpyridiene Acetyl hydrazone

A methanolic solution of acetyl hydrazide (0.370 g, 5 mmol) was refluxed with 2-formylpyridiene (0.475 ml, 5 mmol) continuously for 4h. After adding a few drops of glacial acetic acid. There was no immediate formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 1day white crystalline product is obtained. The compound was collected by filtration washed with hot water and recrystallized from methanol and dried in vacuum. Analytical data of FPAH are given in Table 1. The ^{1}H-NMR spectra (CD_3OH, ppm); δ 2.36 (singlet, 3H), δ 5.01 (singlet, 3H), δ 8.13 (singlet, 1H), δ 7.89-8.65 (multiplet, 4H), are respectively assigned to $–CH_3$ (carbonyl), C -H(hydrazine), NH- and pyridine protons. LC-MS spectrum of FPAH shows molecular ion peaks at (*m/z*) 163.

Yield: 73%

M.P: 88-89º C

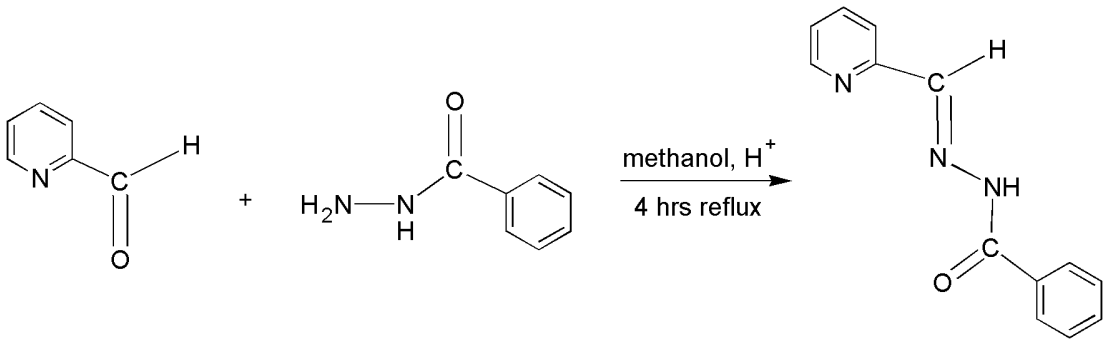


Synthesis of 2-Formylpyridiene benzhydrazone

A methanolic solution of Benzhydrazide (0.68gm, 5 mmol) was refluxed with 2-Formylpyridiene (0.475 ml, 5 mmol) continuously for 4h. After adding a few drops of glacial acetic acid. There was no immediate formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 1day white crystalline product is obtained. The compound was collected by filtration washed with hot water and recrystallized from methanol and dried in vacuum. Analytical data of FPAH are given in Table 1. The ^{1}H-NMR spectra (CD_3OH, ppm); δ 2.36 (singlet, 3H), δ 5.01 (singlet, 3H), δ 8.13 (singlet, 1H), δ 7.89-8.65 (multiplet, 4H), are respectively assigned to $–CH_3$ (carbonyl), C -H(hydrazine), NH- and pyridine + pyridine protons. LC-MS spectrum of FPBH shows molecular ion peaks at (*m/z*) 225.

Yield: 75%

M.P: 103-105º C

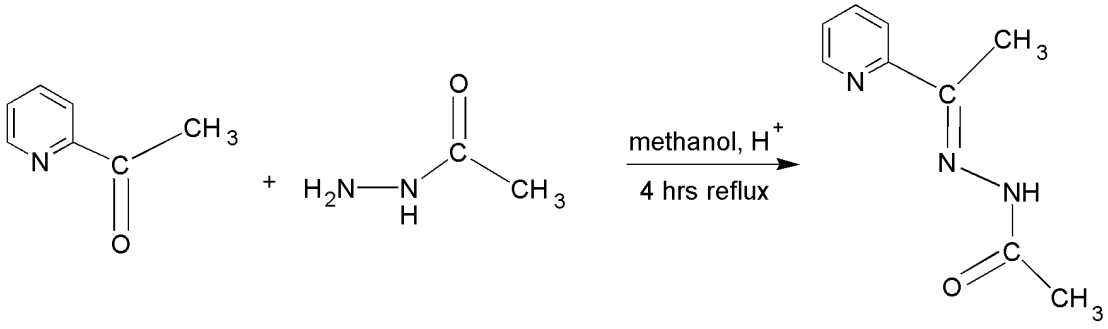


Synthesis of 2-acetylpyridine acetylhydrazone (APAH)

A methanolic solution of acetyl hydrazide (0.370 g, 5 mmol) was refluxed with 2-Acetylpyridiene (0.56 ml, 5 mmol) continuously for 4h. After adding a few drops of glacial acetic acid. There was no immediate formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 1day white crystalline product is obtained. The compound was collected by filtration washed with hot water and recrystallized from methanol and dried in vacuum. Analytical data of APAH are given in Table 1. The ^{1}H-NMR spectra ($CDCl_3$, ppm); δ 2.4 (singlet, 3H), δ 2.5 (singlet, 3H), δ 8.82 (singlet, 1H), δ 7.75-8.8 (multiplet, 4H), are respectively assigned to $–CH_3$ (carbonyl), $–CH_3$ (hydrazine), NH- and pyridine protons. LC-MS spectrum of APAH shows molecular ion peaks at (*m/z*) 177.

Yield: 74%

M.P: 90-92^{o} C

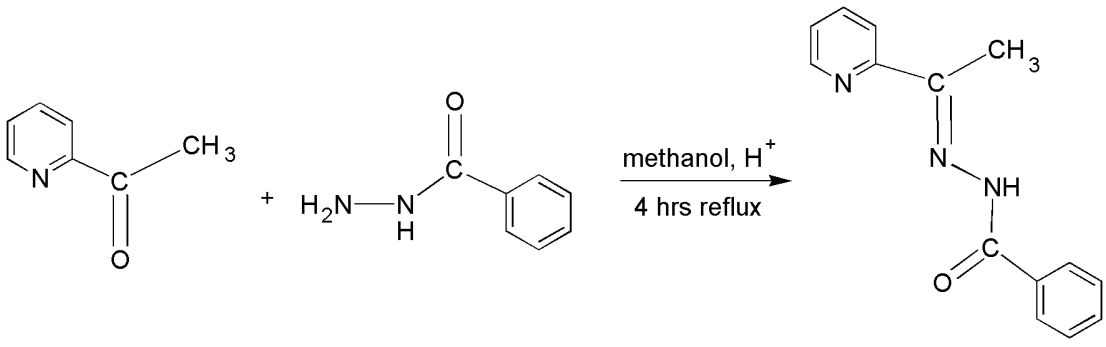


Synthesis of 2-acetylpyridine benzhydrazone (APBH)

A methanolic solution of Benzhydrazide (0.68gm, 5 mmol) was refluxed with 2-Acetylpyridiene (0.560 ml, 5 mmol) continuously for 4h. After adding a few drops of glacial acetic acid. There was no immediate formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 1day white crystalline product is obtained. The compound was collected by filtration washed with hot water and recrystallized from methanol and dried in vacuum. Analytical data of APAH are given in Table 1. The ^{1}H-NMR spectra ($CDCl_3$, ppm); δ 2.5 (singlet, 3H), δ 7.21 (singlet, 1H), δ 7.45-8.6 (multiplet, 9H), are respectively assigned to $–CH_3$ (carbonyl), NH- and pyridine + phenyl protons. LC-MS spectrum of APBH shows molecular ion peaks at (*m/z*) 239.

Yield: 73%

M.P: 158-160º C

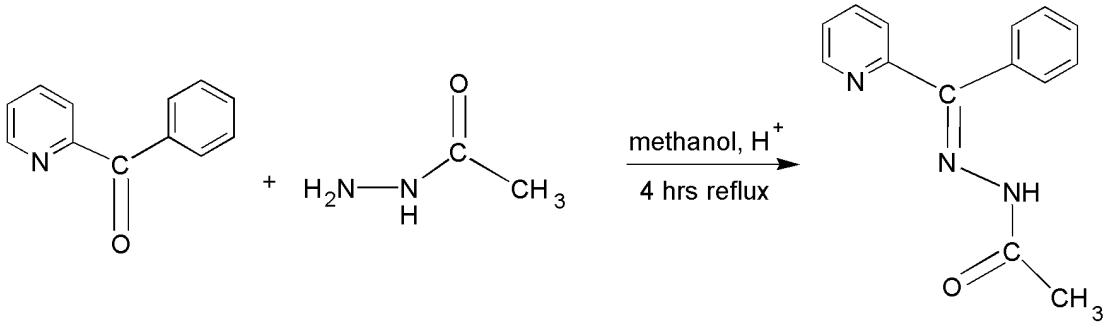


Synthesis of 2-Benzoyl pyridine acetylhydrazone

A methanolic solution of acetyl hydrazide (0.37gm, 5 mmol) was refluxed with 2-Benzoyl pyridine (0.916 gm, 5 mmol) continuously for 4h. After adding a few drops of glacial acetic acid. There was no immediate formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 1day white crystalline product is obtained. The compound was collected by filtration washed with hot water and recrystallized from methanol and dried in vacuum. Analytical data of BPBH are given in Table 1. The ^{1}H-NMR spectra ($CDCl_3$, ppm); δ1.80 (singlet, 3H), δ 7.40 (singlet, 1H), δ(7.42- 8.80) (multiplet 9H), are respectively assigned to $–CH_3$, >NH and aromatic (pyridine + phenyl ring) protons. LC-MS spectrum of BPBH shows molecular ion peaks at (*m/z*) 239.

Yield: 76%

M.P: 80-82º C

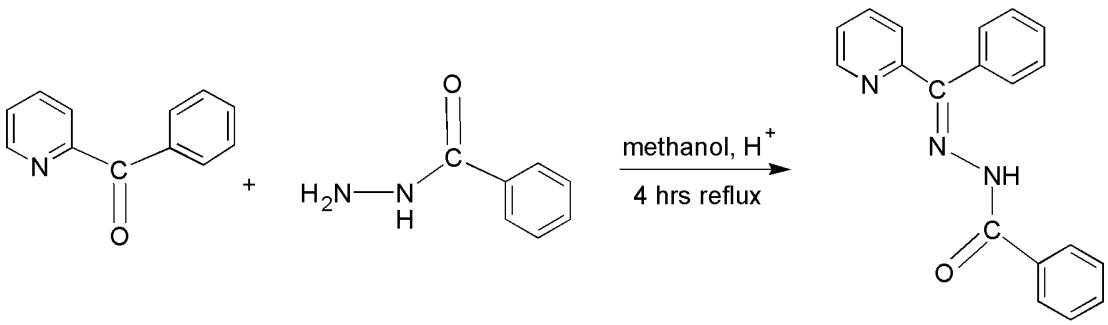


Synthesis of 2-Benzoyl pyridine benzhydrazone

A methanolic solution of Benzhydrazide (0.68gm, 5 mmol) was refluxed with 2-Benzoyl pyridine (0.916 gm, 5 mmol) continuously for 4h. After adding a few drops of glacial acetic acid. There was no immediate formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 1day white crystalline product is obtained. The compound was collected by filtration washed with hot water and recrystallized from methanol and dried in vacuum. Analytical data of BPBH are given in Table 1. The ^{1}H-NMR spectra ($CDCl_3$, ppm); δ 7.40 (singlet, 1H), δ(7.42- 8.80) (multiplet 14H), are respectively assigned to >NH and aromatic (pyridine + phenyl ring) protons. LC-MS spectrum of BPBH shows molecular ion peaks at (*m/z*) 301.

Yield: 71%

M.P: 140-145° C



Preparation of $[La(FPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPAH (2 mmol, 0.326 mg) and $La(NO_3)_3.6H_2O$ (0.433gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h white product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Ce(FPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPAH (2 mmol, 0.326 mg) and $Ce(NO_3)_3.6H_2O$ (1 mmol, 0.434mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h yellow crystalline product is obtained. The yellow coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Pr(NO_3)_3(FPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPAH (2 mmol, 0.326 gm) and $Pr(NO_3)_3.6H_2O$ (1 mmol, 0.435 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1day green product is obtained. The green coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Nd(FPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPAH (2 mmol, 0.326 gm) and $Nd(NO_3)_3.6H_2O$ (1 mmol, 0.437 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside at room temperature. After 2 day, baby pink color product is obtained. The pink coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Sm(FPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPAH (2 mmol, 0.326 mg) and $Sm(NO_3)_3.6H_2O$ (1 mmol, 0.447 mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 3-4 days, white crystalline product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[La(FPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPBH (2 mmol, 0.45 mg) and $La(NO_3)_3.6H_2O$ (0.433gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h white product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Ce(FPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPBH (2 mmol, 0.45 mg) and $Ce(NO_3)_3.6H_2O$ (1 mmol, 0.434mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h yellow crystalline product is obtained. The yellow coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Pr(FPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPBH (2 mmol, 0.45 gm) and $Pr(NO_3)_3.6H_2O$ (1 mmol, 0.435 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1day green product is obtained. The green coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Nd(FPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPBH (2 mmol, 0.45 gm) and $Nd(NO_3)_3.6H_2O$ (1 mmol, 0.437 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside at room temperature. After 2 day, baby pink color product is obtained. The pink coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Sm(FPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of FPBH (2 mmol, 0.45 mg) and $Sm(NO_3)_3.6H_2O$ (1 mmol, 0.447 mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 3-4 days, white crystalline product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[La(APAH)_2(NO_3)_3(H_2O)_2].2NO_3.3H_2O$

The complex was prepared by mixing hot ethanolic solution of APAH (2 mmol, 0.354 mg) and $La(NO_3)_3.6H_2O$ (0.433gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h white product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Ce(APAH)_2(NO_3)_3(H_2O)_2].2NO_3.H_2O$

The complex was prepared by mixing hot ethanolic solution of APAH (2 mmol, 0.354 mg) and $Ce(NO_3)_3.6H_2O$ (1 mmol, 0.434mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h yellow crystalline product is obtained. The yellow coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Pr(APAH)_2(NO_3)_3(H_2O)_2].2NO_3.3H_2O$

The complex was prepared by mixing hot ethanolic solution of APAH (2 mmol, 0.354 gm) and $Pr(NO_3)_3.6H_2O$ (1 mmol, 0.435 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1day green product is obtained. The green coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Nd(APAH)_2(NO_3)_3(H_2O)_2].2NO_3.3H_2O$

The complex was prepared by mixing hot ethanolic solution of APAH (2 mmol, 0.354 gm) and $Nd(NO_3)_3.6H_2O$ (1 mmol, 0.437 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside at room temperature. After 2 day, baby pink color product is obtained. The pink coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Sm(APAH)_2(NO_3)_3(H_2O)_2].2NO_3.2H_2O$

The complex was prepared by mixing hot ethanolic solution of APAH (2 mmol, 0.354 mg) and $Sm(NO_3)_3.6H_2O$ (1 mmol, 0.447 mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 3-4 days, whie crystalline product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[La(APBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of APBH (2 mmol, 0.478 mg) and $La(NO_3)_3.6H_2O$ (0.433gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h white product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Ce(APBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of APBH (2 mmol, 0.478 mg) and $Ce(NO_3)_3.6H_2O$ (1 mmol, 0.434mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h yellow crystalline product is obtained. The yellow coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Pr(APBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of APBH (2 mmol, 0.478 gm) and $Pr(NO_3)_3.6H_2O$ (1 mmol, 0.435 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1day green product is obtained. The green coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Nd(APBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of APBH (2 mmol, 0.478 gm) and $Nd(NO_3)_3.6H_2O$ (1 mmol, 0.437 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside at room temperature. After 2 day, baby pink color product is obtained. The pink coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Sm(APBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of APBH (2 mmol, 0.478 mg) and $Sm(NO_3)_3.6H_2O$ (1 mmol, 0.447 mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 3-4 days, white crystalline product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[La(BPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of BPAH (2 mmol, 0.478 mg) and $La(NO_3)_3.6H_2O$ (0.433gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h white

product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Ce(BPAH)_2(NO_3)_3(H_2O)_2].2NO_3$

The complex was prepared by mixing hot ethanolic solution of BPAH (2 mmol, 0.478 mg) and $Ce(NO_3)_3.6H_2O$ (1 mmol, 0.434mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h yellow crystalline product is obtained. The yellow coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Pr(BPAH)_2(NO_3)(H_2O)_2].2NO_3$

The complex was prepared by mixing hot ethanolic solution of BPAH (2 mmol, 0.478 gm) and $Pr(NO_3)_3.6H_2O$ (1 mmol, 0.435 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1day green product is obtained. The green coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Nd(BPAH)_2(NO_3)_3(H_2O)_2].2NO_3$

The complex was prepared by mixing hot ethanolic solution of BPAH (2 mmol, 0.478 gm) and $Nd(NO_3)_3.6H_2O$ (1 mmol, 0.437 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside at room temperature. After 2 day, baby pink color product is obtained. The

pink coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Sm(BPAH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of BPAH (2 mmol, 0.478 mg) and $Sm(NO_3)_3.6H_2O$ (1 mmol, 0.447 mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside for slow evaporation at room temperature. After 3-4 days, white crystalline product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[La (BPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of BPBH (2 mmol, 0.602 mg) and $La(NO_3)_3.6H_2O$ (0.433gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1h white product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Ce(BPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of BPBH (2 mmol, 0.602 mg) and $Ce(NO_3)_3.6H_2O$ (1 mmol, 0.434mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water

bath for 1h yellow crystalline product is obtained. The yellow coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Pr(BPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of BPBH (2 mmol, 0.602 gm) and $Pr(NO_3)_3.6H_2O$ (1 mmol, 0.435 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 1day green product is obtained. The green coloured complex was collected by filtration, washed with small quantity of ether.

Preparation of $[Nd(BPBH)_2(NO_3)_3]$

The complex was prepared by mixing hot ethanolic solution of BPBH (2 mmol, 0.602 gm) and $Nd(NO_3)_3.6H_2O$ (1 mmol, 0.437 gm) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was kept aside at room temperature. After 2 day, baby pink color product is obtained. The pink coloured complex was collected by filtration, washed with small quantity of ether

Preparation of $[Sm\ (BPBH)_2(NO_3)].2NO_3$

The complex was prepared by mixing hot ethanolic solution of BPBH (2 mmol, 0.602 mg) and $Sm(NO_3)_3.6H_2O$ (1 mmol, 0.447 mg) dissolved in ethanol in 2:1 ratio in a clean 100-ml round bottom flask and the contents were refluxed on water bath for 6h. There was no formation of the product. Then the reaction mixture was

kept aside for slow evaporation at room temperature. After 3-4 days, white crystalline product is obtained. The white coloured complex was collected by filtration, washed with small quantity of ether.

Section (iii): A brief introduction to physicochemical and spectral techniques employed in the present study

The characterization of metal complexes is usually carried out by a combination of spectroscopic methods, magnetic susceptibility measurements and molecular weight determination. Spectroscopic technique provides the principal means of elucidating the molecular structure. Spectrum is generally produced by the interaction between the electromagnetic radiation and the matter is used in any of the spectroscopic techniques for identifying the materials and studying the molecular structure. Depending on the processes and the magnitude of energy changes in which different spectroscopic techniques operate over different limited frequency ranges within the broad spectrum o the electromagnetic radiation.

Physico chemical and spectral techniques used to elucidate the structures of ligands and the complexes are

a. Mass Spectra
b. Conductivity data
c. UV-Visible spectroscopy
d. Infrared spectroscopy
e. ^{1}H NMR spectroscopy
f. Cyclic votammetry and

g. Single crystal X-ray diffraction studies

A brief description of these techniques, their signicicance in the characterization and details of the instruments used are described as follows.

(a) Mass spectra

Mass spectra of the ligands and complexes were recorded JEOL GCMATE II GC-MS Mass spectrometer.

(b) Conductivity data

The conductance measurements at 298±2 K in dry and purified Dimethyl formamide were carried out on CM model 162 Conductivity cell (ELICO). The present complexes are easily soluble in dimethylformamide (DMF). Therefore, solid complexes are dissolved in DMF to perform conductivity measurements. A 20 mg of complex was transferred into different 25 ml standard flasks and dissolved in DMF and diluted upto mark with DMF. The solutions were transferred into a 100 ml beaker to measure the conductance of the solution. Specific and molar conductance values are calculated using the following equation.

Specific conductance (k) = Cell constant x Conductance

= (l/a) x Conductance

= 1.0969 x Conductance

$$\text{Molar conductance } (\mu) = \frac{K \times 1000}{C}$$

Where

l = distance between two electrodes

a = area of two electrodes

c = concentration of conducting materials

(d) Ultraviolet and Visible spectroscopy

Electronic spectroscopy gives the most clear-cut evidence for the electronic energy levels of transition metal complexes is a powerful tool in the hands of structural and theoretical chemists. It can provide a convenient means for the analysis of numerous organic and inorganic species. Electronic spectra of transition metal ions and complexes are observed in the ultraviolet (UV) and visible regions which bridge the gap between Far-ultraviolet and near infrared regions in the electromagnetic spectrum.

Electronic transitions occur when electrons within the molecule or ion move from one energy level to another. These transition are of $\sigma \rightarrow \sigma^*$; $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ type in the ligand part and occur in the UV regions. Whereas d-d and f-f type of transitions in the metal part of the complex molecule occur in the visible region [2, 3]. The electronic spectra of metal complexes have been utilized by many workers to predict the structure of the complexes.

Different types of direct reading spectrophotometers are available for UV-Visible studies. The record of UV-Vis spectrum obtained directly from the instrument is simply a plot of absorption intensity of UV-Vis rays against the wavelength. In the present study, the electronic spectra of the complexes in solution state are recorded on UV lamda50 (Perkin Elmer) double beam spectrophotometer in the range 200-1100 nm.

Perkin Elmer UV lamda50 double beam spectrophotometer

(e) Infrared spectroscopy

Infrared radiation is capable of affecting both rotational and vibrational energy levels in the molecules [4,5]. Some molecular vibrations are characteristic of the entire molecule, where as others are associated with certain functional groups. The effects of these vibrations on chemical bonds between the atoms are classified into two major types, stretching (*ν***)** and bending (δ) and are further described by such terms as scissoring (σ), rocking (ρ), wagging (W) and twisting (t). Frequencies of stretching and bending vibration depend largely on the vibrating atomic masses and on the bond orders.

IR spectra of solids are usually complex with a considerable number of peaks, each corresponding to a particular vibrational transition. Since a complete assignment of all the peaks to the specific vibrational modes is possible, straight forward identification of specific functional groups, covalently boded linkage such as hydroxyl groups, trapped water, oxy anions, carbonates, nitrates, sulphates etc. and the modes of binding of the ligand to the metal ion can be made. As such, infrared

spectroscopy has become an important technique in the elucidation of the structure of organic compounds and metal complexes.

Plots of IR spectra show the frequency or wave number of incident radiation on the x-axis and transmittance on the y-axis. The wave number unit is used more often since it is directly proportional to the energy of vibration and such modern IR instruments are linear in cm^{-1} scale.

In the present study, the infrared spectra of all the complexes/lignads in solid state in the range of 4000-300cm^{-1} were recorded on a Perkin-Elmer spectrum 100 Fourier Transform Infrared spectrometer (used resolution 4 cm^{-1}; minimum resolution 0.125 cm^{-1}) as KBr pellets. About 10 mg of finely powdered sample, thoroughly mixed with even dried spectral grade KBr and pressed into a thin pellet under anhydrous conditions is used for recording IR spectra.

Perkin-Elmer spectrum 100 Fourier Transform Infrared spectrometer

(f) NMR spectrometer

Nuclear magnetic resonance (NMR) spectroscopy which is based on the magnetic properties of nucleus [6] is basically another form of spectroscopy akin to

that of infrared and ultraviolet. Atomic nuclei having non-zero spin when placed in a magnetic field orient themselves at different angles with respect to the static field due to their spin magnetic moment and also process about the direction of the applied field due to their spin angular momentum. The energy of interaction between the applied and nuclear magnetic fields is quantized in such a way that only certain orientations of the nucleus relative to the applied field are possible. Change in orientation of nuclear spin from lower energy to higher energy state occurs by the absorption of electromagnetic radiation. The frequency of which is equal to that of nuclear precession with the magnetic field the magnitude of energy changes involved is usually small and the frequencies fall in the radio frequency region. Another magnetic field, which rotates in phase with nuclear precession, is applied at right angles to the first, in order to induce transitions from one energy level to another. NMR spectroscopy thus consists of observing the point of resonance at which such transitions are induced.

The recorder gives plot of the intensity of resonance signal on y-axis and the strength of magnetic field on x-axis. The area of the peak or signal indicates the number of resonating nuclei at any particular field strength and for this reason; all instruments are equipped with automatic integrator which can record peak areas in the form of superimposed integration trace on the chart as per as x-axis is concerned. Since accurate measurement of the strength of the sweeping magnetic field posses considerable problem it is difficult to assign positions on the absolute scale. Hence the position of resonance peaks is recorded always in the relation the position of an arbitrary standard. Tetramethylsilane (TMS) is generally used as internal reference to

locate the resonance frequency of most of the protons. The difference between the absorption position of a particular proton and the absorption of reference proton is called the chemical shift of the proton. Protons in different chemical environments exhibit different chemical shifts owing to the diamagnetic shielding effects produced by circulation of bonding and non-bonding electrons in the neighborhood of the nucleus. These are very sensitive even to minor changes in the chemical environments. Chemical shifts provide valuable information and made NMR an invaluable technique for elucidation of the structure of the molecules. It is customary to express chemical shifts conveniently in terms of a frequency independent dimensionless parameter called δ, according to which the resonance peaks owing to TMS, occupy 0.0 positions on the right hand edge of the chart.

The *sensitivity* of an NMR spectrometer is a measure of the minimum number of spins detectable by the spectrometer. Since the NMR signal increased as the population difference between the energy levels increases, the sensitivity improves as the field strength increases [7].

NMR samples were prepared by dissolving compound in a deuterated lock solvent. Some of the solvents (for ex: DMSO – d_6) will readily absorb moisture from the atmosphere and give water signal in the spectrum.

Samples for ^{1}H NMR are usually dissolved in DMSO or $CDCl_3$ and the ^{1}H peak of tetramethylsilane (TMS) is used as the internal reference for ^{1}H NMR spectra. ^{1}H NMR spectra were recorded at 300.00 MHz on a Avance-300 Bruker, Switchzerland NMR spectrometer.

(h) Cyclic voltammetry

Cyclic voltammetry (CV) is a versatile electro analytical technique for the study of electro active species [8]. An increasing number of inorganic chemists have been using cyclic voltammetry to evaluate the effect of ligands on the oxidation-reduction potential of the central metal ion in the complexes and multi-nuclear clusters. It is also a rapid method for obtaining good estimates of formal reduction potential and formation constant [9]. The effectiveness CV results from its capacity for rapidly observing the redox behaviour over a wide range.

CV consists of cyclic the potential of an electrode, which is immersed in an unstirred solution and measuring the resulting current. The potential of this working electrode is measured against a reference electrode such as saturated calomel electrode (SCE) or a silver/silver chloride electrode (Ag/AgCl). The controlling potential which is applied across these two electrodes can be considered the response signal to the potential excitation signal. The voltammogram is a display of current (vertical axis) versus potential (horizontal axis). Because the potential varies linearly with time, the horizontal axis can also be thought of as a time axis.

The important parameters of cyclic voltammogram are the magnitudes of the anodic peak current (i_{pa}), cathodic peak current (i_{pc}), the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). A redox couple in which both species rapidly exchange electrons with the working electrode is termed as electrochemically reversible couple. The half-wave potential ($E_{1/2}$) for a reversible couple is centered between E_{pa} and E_{pc} [10, 11].

$$E_{1/2} = \frac{E_{pa} + E_{pc}}{2}$$

The values of i_{pa} and i_{pc} should be identical for a simple reversible (fast) couple. However, the ratio of peak currents can be significantly influenced by chemical reactions coupled to the electrode process. Electrochemical irreversibility is caused by low electron exchange of the redox species with the working electrode.

In the present study, cyclic voltammetry was performed with a BAS model CV-27 controller and a conventional time electrode, Ag/AgCl reference electrode, glassy carbon working electrode and platinum counter electrode. Nitrogen was used as purge gas and all solutions were 0.1 M concentration in tetrabutylammonium hexafloro phosphate ($TBAPF_6$).

CH Instruments 660C Electrochemical Analyzer

(i) Single crystal X-ray diffraction studies

Crystal data were collected by using the Enraf Nonius CAD-MV31 single crystal X-ray diffractometer, Indian Institute of technology-Madras, Chennai. Enraf NoniusCAD4MV31 single crystal X-ray diffractometer is a fully automated four circle instrument controlled by a computer. It consists of an FR 590 generator, a

goniometer, CAD4F interface and a micro V AX3100 equipped with a printer and plotter. The detector is a scintillation counter. A single crystal is mounted on a thin glass fiber fixed on the goniometer head. The unit cell dimensions and orientation matrix are determined using 25 reflections and then the intensity data of a given set of reflection are collected automatically by the computer. An IBM compatible PC/AT 486 is attached to MicroVAX facilitating the data transfer on to a DOS floppy of 5.25" or 3.5". Maximum X-ray powder is 40 mA x50 KV.

The monochromatic X-rays incident on a plane of single crystal at angle θ are diffracted according to Bragg's relation 2d sin(q) = nl where d is the interplanar spacing of the incident plane, l is the wave length of X-rays and n is a positive integer. The intensity of the diffracted rays depends on the arrangement and nature of atoms in the crystal. Collection of intensities of a full set of planes in the crystal contains the complete structural information about the molecule. fourier transformation techniques are used to determine the exact coordinates of atoms in the unit cell from this data.

The data collected was reduced using SAINT program [12]. the trial structure was obtained by direct method [13] using SHELXS-86, wchich revealed the position of all non hydrogen atoms and reined by full-matrix least square on F^2 (SHELXS-97) [14] and graphic tool was DIAMOND for windows [15]. All non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were treated with a mixture of independent and constrained refinements.

Section (iv): Experiments in DNA binding and cleavage activities of metal complexes

The following **biological experiments** are carried out in the present study.

(a) DNA binding experiments (Absorption titration)

(b) Gel electrophoresis

(a) DNA binding experiments (Absorption titration)

Stock solution of CT DNA was made by dissolving in appropriate buffers and kept overnight at 4^oC for complete dissolution and was used after no more than four days. Solution of CT-DNA in 5mM Tris-HCl/50mM NaCl (pH 7.0) gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{270}) 0f 1.8-1.9, indicating that the DNA is sufficiently free of protein [16]. Concentrated stock solution of DNA was prepared in 5mM Tris-HCl/50mM NaCl in water at pH 7.0 and the concentration of CT-DNA was determined by UV absorbance at 260nm after 1:100 dilutions. The molar absorption coefficient was taken as 6600 cm^{-1}[17]. Doubly distilled water was used to prepare buffer solutions. Solutions were prepared with the appropriate metal complexes (20 µM of 1.0 mM solution in DMF), CT DNA (diluted from a 1 mg/ml solution), NaCl (final concentration 50mM) and Tris – HCl (pH 7.0, final concentration 50mM) and diluted with H_2O to a total volume of 1 ml. Spectra were recorded against an analogous blank solution containing the same concentration of DNA / NaCl and Tris – HCl buffer. After addition of DNA to metal complex, the resulting solution was allowed to equilibrate for 5-10 min, at room temperature. The absorption readings

(usually corresponding to the changes at maximum absorption) were noted. The data were then fit to the following equation to obtain the intrinsic binding constant K_b [18].

$$[DNA] / (\varepsilon_a-\varepsilon_f) = [DNA] / (\varepsilon_b-\varepsilon_f) + 1/ K_b(\varepsilon_b-\varepsilon_f) \quad (1)$$

Where [DNA] is the concentration of DNA in base pairs, εa, ε_b and ε_f are apparent extinction coefficient ($A_{obs}/[M]$), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively. A plot of $[DNA] / (\varepsilon_a-\varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_b-\varepsilon_f)$ and Y-intercept equal to $1/ K_b(\varepsilon_b-\varepsilon_f)$; K_b is the ratio of the intercept.

UV-Visible spectroscopic tool for studying interaction of complexes with DNA

Metal complexes often have intense optical transitions in visible spectrum and many are capable of fluorescing under experimental (ambient) conditions. These optical transitions of metal complexes are commonly affected by their microenvironment thereby making controlled sensitive spectroscopic handling possible with the fitting of the molecular surroundings. For example polypyridyl complexes of Ru(II) have optical properties that could strongly be tuned by varying the extent and type of binding with DNA. Thus for a metal complex to act as DNA probe, its spectroscopic properties should change upon binding with DNA. This variation in spectroscopic properties should change upon binding with DNA. These variations in spectroscopic properties provide an excellent means to obtain solution state data about DNA conformation and structure. Among the spectroscopic techniques available for probing interactions between metal complexes and DNA, UV-Visible spectroscopy is widely used.

UV-Vis. spectroscopy is one of the important techniques for the study of complex-DNA interactions [19, 20]. The binding of interactive drugs to DNA has been characterized using absorption titrations.

The hypochromism and associated red-shift reflect the binding of the complexes with DNA through intercalation mode [21]. An example, upon addition of calf-thymus DNA to the solution of $[Ru(Phen)_2(Phi)]^{2+}$ complex, hypochromism and red shift were observed, which are associated with charge-transfer band of the complex. Such spectral characteristics are attributable to a mode of binding that involves a strong stacking interaction between aromatic chromophore and the base pairs of DNA. The magnitudes of red-shift and hypochromism are commonly correlated with the strength of the intercalative interactions.

The degree of hypochromism generally correlates well with the overall binding strength. In some cases, on the addition of DNA to the complex solution hyperchromic shift was also observed. Allen J Bard and his group have observed [22] a similar hyperchromic effect upon addition of successive amounts of calf-thymus DNA to the manganese complex. Therefore, change in absorbance upon addition of DNA has been considered as an indication of the binding of the complexes with DNA. Cationic complexes may also bind with anionic (PO_4^{3-}) part of DNA electrostatically which could easily be monitored through UV/Vis spectroscopy. Red-shift and hyperchromic effect observed in the region (194-196 nm) assigned to phosphate regions of DNA through electrostatic interaction [23].

(c) Gel electrophoresis

Electrophoresis through agarose is the standard method used to separate, identify or purify DNA fragments [24-27]. The technique is simple, easy to perform and capable of resolving fragments of DNA that cannot be determined directly by staining with low concentration of the fluorescent, intercalating dye ethidium bromide. Using this method, bands can be detected even DNA is present in 1-10 ng by direct examination of the gel in the Ultraviolet-visible light. Agarose gels are usually run in a horizontal configuration in an electric field of constant strength and direction. When an electric field is applied across the gel, DNA, which is negatively charged at neutral pH, migrates towards anode. This method has been used to identify the products of the DNA cleavage which was carried out in present study. Gel electrophoresis experiments were carried out on UVI-tech-UK gel documentation system.

UVI-tech gel documentation system

(i) Preparation of the sample solution: About 10 mg of the sample was accurately weighed using electrical balance and made the solution with dimethyl formamide. The solution was diluted suitably to 10 µM concentration.

(ii) Buffer solution

Tris-borate ethylenediaminetetraacetate (TBE) electrolyte buffer (5X stock)

54 g of tris base (MW 121.14) was dissolved in 100 ml of water. A 27.5 g of boric acid was added to it, and then 20 ml of 0.5 M EDTA (pH 8.0) solution was added and diluted to 1 lit. with water and resulting buffer was stored at 4 ^{o}C.

(iii) Loading buffer (6X)

A 0.25% bromophenol blue in 40% sucrose/water was used as the loading buffer. This buffer was prepared by first dissolving a 2 g of sucrose in 3 ml of water and by then adding a 12.5 mg of bromophenol blue to this solution. The volume was made upto 5 ml. The resulting buffer was stored at 4 ^{o}C.

(iv) Ethidium bromide stock solution (10 mg/ml)

A 100 mg of ethidium bromide was dissolved in 10 ml of water by stirring in dark for several hours. The resulting solution was stored in an alluminium foiled bottle at ambient temperature. A working concentration of 0.5 µg/ml was used for staining the gels after electrophoresis.

(v) 0.8% agarose gel casting: About 0.8 gm of low met (molecular biology grade, Gelrose) agarose was added to 100 ml of TBE buffer. The slurry was then heated on boiling -water bath until the agarose dissolved completely. The resulting clear solution was poured onto the gel mold and immediately the comb was clamped into position near one end of the gel. The teeth of the comb formed the sample wells. Care was taken to see that at least 0.5 – 1.0 mm of agarose was left between the bottom of the teeth and base of the gel, so that the sample wells are completely sealed. After 30 – 45 min. the comb and auto clamped tape were removed carefully and gel was

mounted in the tank. A 1.5 litre of working buffer (TBE) was transferred into gel until the gel was covered to a depth of about 1.5 mm.

(vi) Assay of nuclease activity

The solution containing metal complexes was taken in a clean eppendroff tube and 1µg of plasmid pBR322 DNA was added. The contents were incubated for 30 minutes at 37^0C and loaded on 0.8% agarose gel after mixing 3µl of loading buffer (0.25% bromophenol blue + 0.25% Xylene cynaol + 30% glycerol sterilized distilled water). Electrophoresis was performed at constant voltage till the bromophenol blue reached to the 3/4 of the gel. Further gel was stained for 10 min by immersing it in ethidium bromide solution (5 µg/ml of water). The gel was then de-stained for 10 min by keeping it in sterile distilled water and the plasmid bands were visualized by photographing the gel under a UV Transilluminator.

The efficiency of DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its super coiled (SC) form. The reactions were carried out under oxidative and/or hydrolytic conditions.

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