



# Fluorescence, DNA Interaction and Cytotoxicity Studies of 4,5-Dihydro-1H-Pyrazol-1-Yl Moiety Based Os(IV) Compounds: Synthesis, Characterization and Biological Evaluation

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## Abstract

Osmium(IV) pyrazole compounds and ligands were synthesized and well characterised. Ligands were characterized by heteronuclear NMR spectroscopy (<sup>1</sup>H & <sup>13</sup>C), elemental analysis, IR spectroscopy and liquid crystal mass spectroscopy. Os(IV) complexes were characterized by ESI-MS, ICP-OES, IR spectroscopy, conductance measurements, magnetic measurements and electronic spectroscopy. Binding of compounds with HS-DNA were evaluated using viscosity measurements, absorption titration, fluorescence quenching, and molecular docking, which show effective intercalation mode exhibited by compounds. Binding constant of Os(IV) complexes are found to be 8.1 to 9.2 × 10<sup>4</sup> M<sup>-1</sup>. Bacteriostatic and cytotoxic activities were carried out to evaluate MIC, LC<sub>50</sub>, and IC<sub>50</sub>. The compounds have been undergone bacteriostatic screening using three sets of Gram<sup>+ve</sup> and two sets of Gram<sup>-ve</sup> bacteria. MIC of complexes are found to be 72.5–100 μM, whereas that of ligands fall at about 122.5–150 μM. LC<sub>50</sub> count of ligands fall in the range of 16.22–17.28 μg/mL whereas that of complexes of Os(IV) fall in the range of 4.87–5.87 μg/mL. IC<sub>50</sub> of osmium compounds were evaluated using HCT-116 cell line. All the Os(IV) compounds show moderate IC<sub>50</sub>.

**Keywords** Binding · Absorption titration · Molecular docking · HCT-116

## Introduction

The field of medicinal inorganic chemistry were explored widely [1]. Metal complexes with biologically active heterocyclic ligands enhance its applications [2]. Design and synthesis of such compounds having less side effects and more potentiality has been a primary step towards exploring its application in biological medicinal chemistry [3–5]. Transition metals have been studied widely since they often alter and bind with the DNA nucleic bases [6, 7]. The key area of interest for the chemist have been developing drugs which provide resistance to diseases, more effective and less toxic or having less side effects [8, 9]. Design of heterocyclic ligands which fit with metal

ions geometrically is equally important [10]. Substitution on heterocyclic rings enhance biological potentiality of ligand as well as its metal ion based complex [11]. The substituents having ability to enhance lipophilicity, stabilize the oxidation state of metal ion based complex and stabilize the complex making it kinetically inert and thermodynamically stable in important aspect of designing such compounds concluded by structural and activity relationship [12].

DNA has been a primary target of biomolecules to cure diseases genetically by interaction with base pairs via electrostatic, Vander Waal and hydrophobic forces of attractions. Complexes with octahedral geometry have been found to be kinetically more inert since releasing of bioactive ligand on specific DNA site is of prime importance. Quinoline based heterocycles have been proved to have good binding affinity than ciprofloxacin drug [13]. Binding affinity of quinoline heterocycles are highly stabilized by hydrogen bonding interactions concluded by docking studies [14, 15]. Quinoline class of heterocycles have been proved to possess greater cytotoxicity activities when explored to study on wide cancer cell lines [16, 17].

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Quinoline nucleus-based complexes have been synthesized and studied and their biological assessment have been proved to be impactful and superior than ligands [18]. Osmium metal ion readily bind with -N, O donor ligands [19–22]. Osmium metal ion-based complexes have been explored to in cytotoxic assays and varied cell lines [23]. These have been also in doing interaction with DNA and adhering its mechanism which puts it into plethora of vast medicinal synthetic chemistry [24, 25]. Osmium metal ion complexes often prefer to bind via intercalation mode with DNA macromolecule Osmium metal ion-based complexes have been also proved effective in playing role in DNA binding and cleavage agent [26].

Present report includes, synthesis of six different substituted pyrazole nucleus-based -N,O donor ligands and Os(IV) complexes. The compounds have been well characterized and justified using various techniques. All the compounds have undergone cytotoxicity assays, bacteriostatic study, DNA binding and cleavage activities.

## Experimental

### Materials and Reagents

Phosphorous oxychloride, dimethyl formamide used in reaction were purchased from SDFCL, SD-Fine chemicals ltd. 4-hydroxide benzhydrazide, potassium tertiary butoxide, osmium tetroxide (Crystals), hydrobromic acid, ammonium bromide was purchased from Sigma Aldrich, Artemia cyst eggs were purchased from local aquarium. Luria broth, ethidium bromide and TAE buffer solution were purchased from Himedia(India). Bacterial culture was borrowed from MTCC, Chandigarh (India). Herring sperm DNA solution was purchased from Sigma Aldrich.

### Physical Measurement

Liquid crystal mass spectroscopy, waters model with micromass technology were used for mass analysis. Electronic spectra were taken using UV (160A) spectrophotometer. Elemental analysis were carried out using Euro elemental analyzer. Melting point of compounds were taken using Thermo Cal<sub>10</sub>. Melting point apparatus. Magnetic property of complexes were measured using Guoy's method. The smeared images of cytotoxicity assays were visualized by AlphaDigDoc software (California). Heteronuclear spectra were taken using Bruker Avance NMR spectrophotometer (400 MHz). Infra-red spectra were taken using Shimadzu instrument (400–4000 cm<sup>-1</sup>).

## Characterization

### General Method for Synthesis of Ligands

2-Chloro-6-methoxyquinoline-3-carbaldehyde have been synthesized using Vilsmeier–Haack reaction (Scheme 1) [27].

### Synthesis and Characterization

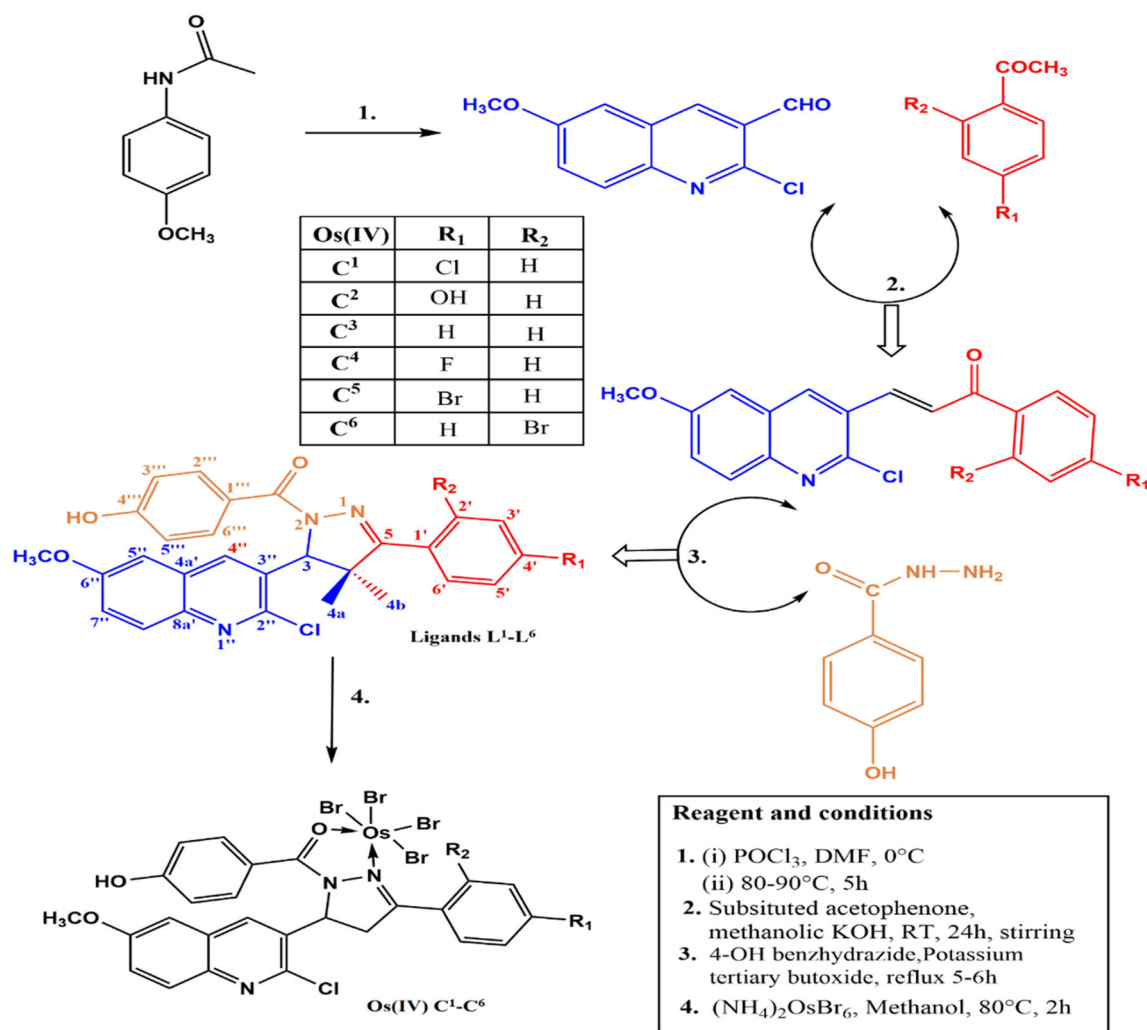
#### of (5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-(4-Chlorophenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone (L<sup>1</sup>)

Ligand L<sup>1</sup> have been synthesized by Claisen Schmidt condensation reaction between 2-chloro-6-methoxyquinoline-3-carbaldehyde (2 g, 9 mmol) and 4-chloro acetophenone (1.39 g, 9 mmol) in presence of potassium hydroxide (0.2 g, 4 mmol) as base. The chalcone formed (2 mmol, 0.42 g) have been cyclized with (1 mmol, 0.15 g) in presence of potassium tertiary butoxide (4 mmol, 0.22 g) to form ligand L<sup>1</sup>. Empirical formula: C<sub>26</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>, Color: Yellow, yield: 90%, mol. Wt: 492.36 g/mol, m.p.: 285 °C; anal. Cal. (%) for: C, 63.43; H, 3.89, N, 8.53; Found (%), C, 63.22; H, 3.80, N, 8.20; Mass m/z(%): 425[M]<sup>+</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ/ppm: 2.404 (s, 3H, OCH<sub>3</sub>), 3.248(dd, 1H, H<sup>4</sup>a, <sup>2</sup>J<sub>1</sub> = 5.2 Hz, <sup>3</sup>J<sub>2</sub> = 5.2 Hz), 4.005(dd, 1H, H<sup>4</sup>b, <sup>2</sup>J<sub>1</sub> = 11.6 Hz, <sup>3</sup>J<sub>2</sub> = 11.6 Hz), 6.269(t, 1H, H<sup>5</sup>), 7.244–8.175(m, 14H, Ar-H), 9.644(s, 1H, OH) <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ/ppm: 189.65, (C=O, C<sub>quat</sub>); 152.35, (C5, C<sub>quat</sub>); 146.35, (C1', C<sub>quat</sub>); 145.06, (C4', C<sub>quat</sub>); 142.32, (C2'', C<sub>quat</sub>); 132.36, (C3'', C<sub>quat</sub>); 132.35, (C2'', C6'', -CH); 132.05 (C3', C5', -CH); 131.43 (C4'', CH); 131.38, (C5'', CH); 131.37 (C7'', CH); 131.36 (C4a', C<sub>quat</sub>); 131.12, (C8'', C<sub>quat</sub>); 130.88, (C8a', C<sub>quat</sub>); 128.67, (C2''', C6'', CH); 128.27 (C6''', C<sub>quat</sub>); 126.76, (C3'', C5'', CH); 126.43, (C1'', C<sub>quat</sub>); 126.42, (C4'', C<sub>quat</sub>); 55.67, (methoxy, CH<sub>3</sub>); 44.59 (C4, CH<sub>2</sub>); 36.46 (C3, CH). (UV-Vis (DMSO, ε = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 232.00 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup>cm<sup>-1</sup>). IR (KBr, cm<sup>-1</sup>): 3099 ν(C-H); 1540 ν(C-N); 1312 ν(C-H); 1666 ν(C=C); 1610 ν(C=O).

### Synthesis and Characterization

#### of (5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-(4-Hydroxyphenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone (L<sup>2</sup>)

It was synthesized using 4-hydroxy acetophenone (9 mmol, 1.22 g) using procedure mentioned in the synthesis of ligand L<sup>1</sup>. Empirical formula: C<sub>26</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>4</sub>, Color: Yellow, yield: 88%, mol. Wt: 473.91 g/mol, m.p.: 281 °C; anal. Cal. (%) for: C, 65.90; H, 4.25, N, 8.87; Found (%), C, 65.85; H, 4.12, N, 8.80; Mass m/z(%): 473.00 [M]<sup>+</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ/ppm: 2.412 (s, 3H, OCH<sub>3</sub>), 3.260(dd, 1H, H<sup>4</sup>a, <sup>2</sup>J<sub>1</sub> = 5.6 Hz, <sup>3</sup>J<sub>2</sub> = 2.8 Hz), 4.017(dd, 1H, H<sup>4</sup>b, <sup>2</sup>J<sub>1</sub> = 7.6 Hz, <sup>3</sup>J<sub>2</sub> = 12.8 Hz), 6.276(t, 1H, H<sup>5</sup>), 7.489–8.183(m, 14H, Ar-H),



**Scheme 1** General reaction scheme for synthesis of ligands L<sup>1</sup>–L<sup>6</sup> and complexes C<sup>1</sup>–C<sup>6</sup>

9.743(s, 1H, OH) <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ/ppm: 189.70, (C=O, C<sub>quat</sub>); 152.40 (C5, C<sub>quat</sub>); 146.40 (C1', C<sub>quat</sub>); 145.11, (C4', C<sub>quat</sub>); 142.37, (C2'', C<sub>quat</sub>); 132.41, (C3'', C<sub>quat</sub>); 131.84, (C2', C6', CH); 131.83, (C3', C5', CH); 131.53, (C4'', CH); 130.91, (C5'', CH); 130.86, (C7'', CH); 130.85, (C4a', C<sub>quat</sub>); 129.37, (C8'', CH); 129.13, (C8a', C<sub>quat</sub>); 126.92 (C6'', C<sub>quat</sub>); 126.52 (C2''', C6''', CH); 124.01 (C1'', C<sub>quat</sub>); 124.84, (C3''', C5'''); 124.67, (C4''', C<sub>quat</sub>); 54.52, (methoxy, OCH<sub>3</sub>); 42.84 (C4, CH<sub>2</sub>); 34.71 (C3, CH). (UV-Vis (DMSO, *c* = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 230.50 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup> cm<sup>-1</sup>). IR (KBr, cm<sup>-1</sup>): 3067 ν(C-H); 1521 ν(C-N); 1320 ν(C-H); 1678 ν(C=C); 1625 ν(C=O).

### Synthesis and Characterization of (5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-Phenyl-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl) Methanone (L<sup>3</sup>)

It was synthesized using acetophenone (9 mmol, 1.08 g) using procedure mentioned in the synthesis of ligand L<sup>1</sup>. Empirical

formula: C<sub>26</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>3</sub>, Color: Yellow, yield: 82%, mol. Wt: 457.91 g/mol, m.p.: 283 °C; anal. Cal. (%) for: C, 68.20; H, 4.40; N, 9.18; Found (%), C, 68.08; H, 4.30; N, 9.11; Mass m/z(%): 457.00 [M]<sup>+</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ/ppm: 2.418 (s, 3H, OCH<sub>3</sub>), 3.265 (dd, 1H, H<sup>4a</sup>, <sup>2</sup>J<sub>1</sub> = 5.6 Hz, <sup>3</sup>J<sub>2</sub> = 8.4 Hz), 4.027 (dd, 1H, H<sup>4b</sup>, <sup>2</sup>J<sub>1</sub> = 10.4 Hz, <sup>3</sup>J<sub>2</sub> = 9.2 Hz), 6.278 (t, 1H, H<sup>5</sup>), 7.489–8.188 (m, 14H, Ar-H), 9.883 (s, 1H, OH) <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ/ppm: 189.75, (C=O, C<sub>quat</sub>); 152.45, (C5, C<sub>quat</sub>); 146.45, (C1', C<sub>quat</sub>); 145.16, (C4', C<sub>quat</sub>); 142.42, (C2'', C<sub>quat</sub>); 132.46, (C3'', C<sub>quat</sub>); 131.89, (C2', C6', CH); 131.88, (C3', C5', CH); 131.58, (C4'', CH); 130.96, (C5'', CH); 130.91, (C7'', CH); 130.90, (C4a', C<sub>quat</sub>); 129.42, (C8'', CH); 128.18, (C8a', C<sub>quat</sub>); 126.97, (C2''', C6''', CH), 126.57, (C6'', CH); 126.06, (C1'', CH); 124.89, (C3''', C5''', CH); 124.72, (C4''', C<sub>quat</sub>); 56.20, (methoxy, OCH<sub>3</sub>); 42.89, (C4, CH<sub>2</sub>); 34.46, (C4, CH). (UV-Vis (DMSO, *c* = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 238.50 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup> cm<sup>-1</sup>). IR (KBr, cm<sup>-1</sup>): 3055 ν(C-H); 1543 ν(C-N); 1335 ν(C-H); 1680 ν(C=C); 1632 ν(C=O).

**Synthesis and Characterization of (5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-(4-Fluorophenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone (L<sup>4</sup>)**

It was synthesized using 4-floro acetophenone (9 mmol, 1.24 g) using procedure mentioned in the synthesis of ligand L<sup>1</sup>. Empirical formula: C<sub>26</sub>H<sub>19</sub>ClFN<sub>3</sub>O<sub>3</sub>, Color: Yellow, yield: 84%, mol. Wt: 475.90 g/mol, m.p.: 280 °C; Mass m/z(%):475.00 [M]<sup>+</sup>. (UV-Vis (DMSO, *c* = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 267.50 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup> cm<sup>-1</sup>). <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ/ppm: 2.422(S, 3H, OCH<sub>3</sub>), 3.271(dd, 1H, H<sup>4</sup>a, <sup>2</sup>J<sub>1</sub> = 5.2 Hz, <sup>3</sup>J<sub>2</sub> = 4.8 Hz), 4.029(dd, 1H, H<sup>4</sup>b, <sup>2</sup>J<sub>1</sub> = 11.6 Hz, <sup>3</sup>J<sub>2</sub> = 10.4 Hz), 6.286(t, 1H, H<sup>5</sup>), 7.494–8.195(m, 14H, Ar-H), 9.443(s, 1H, OH) <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ/ppm: 189.81, (C=O, C<sub>quat</sub>); 152.51, (C5, C<sub>quat</sub>); 146.50, (C1', C<sub>quat</sub>); 144.22, (C4', C<sub>quat</sub>); 142.48, (C2'', C<sub>quat</sub>); 132.52, (C3'', C<sub>quat</sub>); 131.95, (C2', C6', CH); 131.94, (C3', C5', CH); 131.64, (C4'', CH); 130.02, (C5'', CH); 130.97, (C7'', CH); 130.96, (C8'', CH); 129.48, (C2''', C6'''); 128.24, (C4a', C<sub>quat</sub>); 126.63, (C8a', C<sub>quat</sub>); 126.03, (C8a'', C<sub>quat</sub>); 125.12, (C1'', C<sub>quat</sub>); 124.95, (C3''', C5''', CH); 124.78, (C4''', CH); 56.43, (Methoxy, OCH<sub>3</sub>); 42.95, (C4, CH<sub>2</sub>); 34.82, (C3, CH). (UV-Vis (DMSO, *c* = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 239.50 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup> cm<sup>-1</sup>). IR (KBr, cm<sup>-1</sup>): 3046 ν(=C-H); 1533 ν(C-N); 1342 ν(C-H); 1687 ν(C=C); 1646 ν(C=O).

**Synthesis and Characterization of 7(3-(4-Bromophenyl)-5-(2-Chloro-6-Methoxyquinolin-3-Yl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone (L<sup>5</sup>)**

It was synthesized using 4-bromo acetophenone (9 mmol, 1.79 g) using procedure mentioned in the synthesis of ligand L<sup>1</sup>. Empirical formula: C<sub>26</sub>H<sub>19</sub>BrClN<sub>3</sub>O<sub>3</sub>, Color: Yellow, yield: 80%, mol. Wt: 536.81 g/mol, m.p.: 292 °C; anal. Cal. (%) for: C, 58.17; H, 3.57, N, 7.83; Found (%),C, 58.10; H, 3.45, N, 7.75; Mass m/z(%): 536.00[M]<sup>+</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ/ppm: 2.435 (S, 3H, OCH<sub>3</sub>), 3.277(dd, 1H, H<sup>4</sup>a, <sup>2</sup>J<sub>1</sub> = 5.2 Hz, <sup>3</sup>J<sub>2</sub> = 5.6 Hz), 4.032(dd, 1H, H<sup>4</sup>b, <sup>2</sup>J<sub>1</sub> = 8.8 Hz, <sup>3</sup>J<sub>2</sub> = 12.4 Hz), 6.288(t, 1H, H<sup>5</sup>), 7.511–8.210(m, 14H, Ar-H), 9.646(s, 1H, OH). <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ/ppm: 189.56, (C=O, C<sub>quat</sub>); 152.26, (C5, C<sub>quat</sub>); 146.26, (C1', C<sub>quat</sub>); 144.97, (C4', C<sub>quat</sub>); 142.23, (C2'', C<sub>quat</sub>); 132.27, (C3'', C<sub>quat</sub>); 131.70, (C2', C6', CH); 131.69, (C3', C5', CH); 131.39, (C4'', C<sub>quat</sub>); 130.77, (C5'', CH); 130.72, (C7'', CH); 130.71, (C4a', C<sub>quat</sub>); 129.23, (C8'', CH); 128.99, (C8a', C<sub>quat</sub>); 126.78, (C2''', C6''', CH); 126.38, (C6'', C<sub>quat</sub>); 124.87, (C1'', C<sub>quat</sub>); 124.70, (C3''', C5''', CH); 124.53, (C4'', C<sub>quat</sub>); 54.60, (Methoxy, OCH<sub>3</sub>); 42.70, (C4, CH<sub>2</sub>); 34.57, (C3, CH). (UV-Vis (DMSO, *c* = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 230.50 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup>

cm<sup>-1</sup>). IR (KBr, cm<sup>-1</sup>): 3032 ν(=C-H); 1520 ν(C-N); 1352 ν(C-H); 1653ν(C=C); 1648ν(C=O).

**Synthesis and Characterization of (3-(2-Bromophenyl)-5-(2-Chloro-6-Methoxyquinolin-3-Yl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone (L<sup>6</sup>)**

It was synthesized using 3-bromo acetophenone (9 mmol, 1.79 g) using procedure mentioned in the synthesis of ligand L<sup>1</sup>. Empirical formula: C<sub>26</sub>H<sub>19</sub>BrClN<sub>3</sub>O<sub>3</sub>, Color: Yellow, yield: 85%, mol. Wt: 536.81 g/mol, m.p.: 280 °C; anal. Cal. (%) for: C, 58.17; H, 3.57, N, 7.83; Found (%),C, 58.10; H, 3.10, N, 7.70. Mass m/z(%): 536.00 [M]<sup>+</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ/ppm: 2.438 (S, 3H, OCH<sub>3</sub>), 3.281(dd, 1H, H<sup>4</sup>a, <sup>2</sup>J<sub>1</sub> = 5.2 Hz, <sup>3</sup>J<sub>2</sub> = 6.8 Hz), 4.042(dd, 1H, H<sup>4</sup>b, <sup>2</sup>J<sub>1</sub> = 8.4 Hz, <sup>3</sup>J<sub>2</sub> = 12.8 Hz), 6.298(t, 1H, H<sup>5</sup>), 7.519–8.218(m, 14H, Ar-H), 9.748(s, 1H, OH) <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ/ppm: 189.61, (C=O, C<sub>quat</sub>); 152.31, (C5, C<sub>quat</sub>); 146.31, (C1', C<sub>quat</sub>); 145.02, (C2', C<sub>quat</sub>); 142.28, (C2'', C<sub>quat</sub>); 132.32, (C3'', C<sub>quat</sub>); 131.75, (C3', CH); 131.74, (C4', CH); 131.44, (C5', CH); 130.39, (C6', CH); 130.37, (C4'', CH); 130.33, (C4a', C<sub>quat</sub>); 129.85, (C5'', CH); 128.61, (C8a', C<sub>quat</sub>); 126.46, (C6'', C<sub>quat</sub>); 126.00 (C1'', C<sub>quat</sub>); 124.49, (C7'', CH); 124.32, (C8'', CH); 124.18, (C2''', C6''', CH); 124.15, (C4'', C<sub>quat</sub>); 119.80, (C3''', C5''', CH); 53.12, (Methoxy, OCH<sub>3</sub>); 42.32, (C4, CH<sub>2</sub>); 34.19, (C3, CH). (UV-Vis (DMSO, *c* = 10<sup>-4</sup> mol dm<sup>-3</sup>): λ<sub>max</sub> (ε) = 231.50 nm (29,350) mol<sup>-1</sup>dm<sup>3</sup> cm<sup>-1</sup>). IR (KBr, cm<sup>-1</sup>): 3024 ν(=C-H); 1517 ν(C-N); 1361 ν(C-H); 1690 ν(C=C); 1628 ν(C=O).

**Synthesis and Characterization of Metal Salt and Complexes**

**Synthesis of Ammonium Hexabromoosmate(IV)**

(NH<sub>4</sub>)<sub>2</sub>OsBr<sub>6</sub> have been synthesized using osmium tetroxide and hydrobromic acid as starting reactants (Scheme 1) [28, 29].

**Synthesis and Characterization of Tetrabromido(5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-(4-Chlorophenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone)Osmium(IV) (C<sup>1</sup>)**

Tetrabromido(5-(2-chloro-6-methoxyquinolin-3-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(4-hydroxyphenyl)methanone)osmium(IV) have been synthesized using (NH<sub>4</sub>)<sub>2</sub>OsBr<sub>6</sub> (0.60 g, 0.085 mmol) and ligand L<sup>1</sup> (0.41 g, 0.085 mmol). Color: Brownish black, yield: 60%, mol. Wt: 1000.63 g/mol, m.p.: >300 °c. Cal. (%) for: Os,18.98; Found (%), Os,18.68;. ESI-MS



(70 eV): 1002.20, 922.30, 842.39, 762.49, 682.59, 492.59 m/z; Magnetic Moment: 2.70 B.M. (UV-Vis (DMSO,  $c = 10^{-4}$  mol dm $^{-3}$ ):  $\lambda_{\max}$  ( $\epsilon$ ) = 284.00 nm (29,350) mol $^{-1}$ dm $^3$  cm $^{-1}$ ). Conductance: 36  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . IR (KBr, cm $^{-1}$ ): 3112  $\nu$ (=C-H); 1562  $\nu$ (C-N); 1318  $\nu$ (C-H); 1680  $\nu$ (C=C); 1618  $\nu$ (C=O); 556  $\nu$ (N-Os); 611  $\nu$ (N-Os-Br).

**Synthesis and Characterization of Tetrabromido(of 5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-(4-Hydroxyphenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone Osmium(IV) (C $^2$ )**

It was synthesized using (NH $_4$ ) $_2$ OsBr $_6$  (0.60 g, 0.085 mmol) and ligand L $^2$  (0.40 g, 0.085 mmol). Color: Brownish black, yield: 60%, mol. Wt: 983.76 g/mol, m.p.: >300 °c. Cal. (%) for: Os,19.34; Found (%),Os,19.20; (UV-Vis (DMSO,  $c = 10^{-4}$  mol dm $^{-3}$ ):  $\lambda_{\max}$  ( $\epsilon$ ) = 239.50 nm (29,350) mol $^{-1}$ dm $^3$  cm $^{-1}$ ). Conductance: 32  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . IR (KBr, cm $^{-1}$ ): 3098  $\nu$ (=C-H); 1534  $\nu$ (C-N); 1342  $\nu$ (C-H); 1686  $\nu$ (C=C); 1634  $\nu$ (C=O); 562  $\nu$ (N-Os); 580  $\nu$ (N-Os-Br).

**Synthesis and Characterization of Tetrabromido(5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-Phenyl-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone)Osmium(IV) (C $^3$ )**

It was synthesized using (NH $_4$ ) $_2$ OsBr $_6$  (0.60 g, 0.085 mmol) and ligand L $^3$  (0.38 g, 0.085 mmol). Color: Brownish black, yield: 60%, mol. Wt: 967.76 g/mol, m.p.: >300 °c. Cal. (%) for: Os,19.66; Found (%),Os,19.48; (UV-Vis (DMSO,  $c = 10^{-4}$  mol dm $^{-3}$ ):  $\lambda_{\max}$  ( $\epsilon$ ) = 237.50 nm (29,350) mol $^{-1}$ dm $^3$  cm $^{-1}$ ). Conductance: 28  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . IR (KBr, cm $^{-1}$ ): 3068  $\nu$ (=C-H); 1558  $\nu$ (C-N); 1344  $\nu$ (C-H); 1710  $\nu$ (C=C); 1667  $\nu$ (C=O); 575  $\nu$ (N-Os); 624  $\nu$ (N-Os-Br).

**Synthesis and Characterization of Tetrabromido(5-(2-Chloro-6-Methoxyquinolin-3-Yl)-3-(4-Fluorophenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone)Osmium(IV) (C $^4$ )**

It was synthesized using (NH $_4$ ) $_2$ OsBr $_6$  (0.60 g, 0.085 mmol) and ligand L $^4$  (0.40 g, 0.085 mmol). Color: Brownish black, yield: 60%, mol. Wt: 985.75 g/mol, m.p.: >300 °c. Cal. (%) for: Os,19.30; Found (%),Os,19.13; (UV-Vis (DMSO,  $c = 10^{-4}$  mol dm $^{-3}$ ):  $\lambda_{\max}$  ( $\epsilon$ ) = 256.50 nm (29,350) mol $^{-1}$ dm $^3$  cm $^{-1}$ ). Conductance: 26  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . IR (KBr, cm $^{-1}$ ): 3057  $\nu$ (=C-H); 1543  $\nu$ (C-N); 1348  $\nu$ (C-H); 1693  $\nu$ (C=C); 1653  $\nu$ (C=O); 586  $\nu$ (N-Os); 617  $\nu$ (N-Os-Br).

**Synthesis and Characterization of Tetrabromido(3-(4-Bromophenyl)-5-(2-Chloro-6-Methoxyquinolin-3-Yl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone)Osmium(IV) (C $^5$ )**

It was synthesized using (NH $_4$ ) $_2$ OsBr $_6$  (0.60 g, 0.085 mmol) and ligand L $^5$  (0.45 g, 0.085 mmol). Color: Brownish black, yield: 60%, mol. Wt: 1046.66 g/mol, m.p.: >300 °c. Cal. (%) for: Os,18.18; Found (%),Os,18.02; (UV-Vis (DMSO,  $c = 10^{-4}$  mol dm $^{-3}$ ):  $\lambda_{\max}$  ( $\epsilon$ ) = 225.50 nm (29,350) mol $^{-1}$ dm $^3$  cm $^{-1}$ ). Conductance: 24  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . IR (KBr, cm $^{-1}$ ): 3047  $\nu$ (=C-H); 1538  $\nu$ (C-N); 1362  $\nu$ (C-H); 1661  $\nu$ (C=C); 1653  $\nu$ (C=O); 592  $\nu$ (N-Os); 627  $\nu$ (N-Os-Br).

**Synthesis and Characterization of Tetrabromido(3-(2-Bromophenyl)-5-(2-Chloro-6-Methoxyquinolin-3-Yl)-4,5-Dihydro-1H-Pyrazol-1-Yl)(4-Hydroxyphenyl)Methanone)Osmium(IV) (C $^6$ )**

It was synthesized using (NH $_4$ ) $_2$ OsBr $_6$  (0.60 g, 0.085 mmol) and ligand L $^5$  (0.45 g, 0.085 mmol). Color: Brownish black, yield: 60%, mol. Wt: 1046.66 g/mol, m.p.: >300 °c. Cal. (%) for: Os,18.18; Found (%),Os,17.98; Conductance: 28  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . (UV-Vis (DMSO,  $c = 10^{-4}$  mol dm $^{-3}$ ):  $\lambda_{\max}$  ( $\epsilon$ ) = 223.00 nm (29,350) mol $^{-1}$ dm $^3$  cm $^{-1}$ ). Conductance: 36  $\bar{\Omega}$  mol $^{-1}$  cm $^2$ . IR (KBr, cm $^{-1}$ ): 3044  $\nu$ (=C-H); 1526  $\nu$ (C-N); 1377  $\nu$ (C-H); 1706  $\nu$ (C=C); 1634  $\nu$ (C=O); 565  $\nu$ (N-Os); 610  $\nu$ (N-Os-Br).

**Biological Studies**

**Hydrodynamic Volume Measurement for Complex to HS-DNA Interaction**

Mode of intercalation between compounds and DNA solution have been determined with the help of hydrodynamic volume measurement study. Different concentration of compounds have been mixed with DNA solution keeping its concentration at 200  $\mu$ M. The flow time of incubated solution have been calculated in triplicate manner keeping it in static environment at temperature 27  $\pm$  0.5 °C, and phosphate buffer pH 7.2 in digital viscometric bath [30].

**Molecular Docking with B-DNA**

The PDB files of compounds formatted with the help of CHIMERA 1.5.1 software have been interacted with B-DNA PDB file using HEX 6.0 software by activating docking process [31].

### Absorption Titration Study of Complex to HS- DNA

HS-DNA solution have been dissolved in phosphate buffer solution keeping pH 7.2 in dimethyl sulfoxide at low temperature of 4 °C. With the fixed concentration of compounds and varying concentration of DNA solution, titration study have been carried out [32].

### Fluorescence Quenching Study

The binding ability and the number of active sites on biomacromolecules on which compounds are effective have been determined with the help of fluorescence study. Depending upon the ability of Os(IV) complexes to displace EB-DNA, relative change in intensity is observed [33].

### Gel Electrophoresis

The photolytic cleavage property of Os(IV) complexes have been determined by gel electrophoresis technique [34].

### Bacteriostatic Activity

MIC activity have been carried out by growing bacterial culture in Luria broth solution. The bacterial culture have been allowed to grow keeping it in orbital shaker at optimum revolution and temperature [35].

### In-Vitro Cytotoxicity

The varying concentration of compounds have been treated with pre-cultured *S. Pombe* cell's DNA previously grown in liquid yeast media to calculate percentage cell viability of compounds [36].

### In-Vivo Cytotoxicity

Brine shrimp lethality assay have been carried out by using protocol of Mayer et al., to calculate LC<sub>50</sub> values of compounds [37].

### Cytotoxicity Using HCT-116 Cell Line

MTT assay have been used to carry out screening of test compounds using HCT-116 cell line. DMSO have been used to solubilize the test compounds with different concentration ranging from 10 to 1000 µg/mL with 1% DMSO as vehicle control [38].

## Results and Discussion

### Characterization

#### <sup>1</sup>H NMR and <sup>13</sup>C-APT Characterization of Compounds

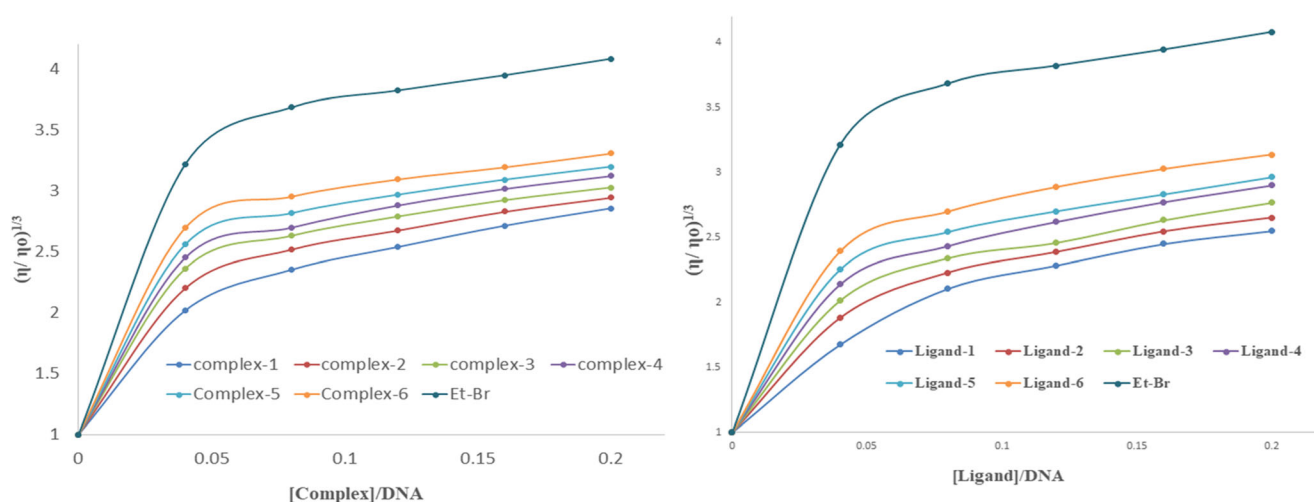
All the ligands were well characterized using <sup>1</sup>H-NMR and <sup>13</sup>C-APT spectroscopy. In ligand L<sup>1</sup>, H<sub>4a</sub> proton represents in dd pattern at 3.248 δ/ppm whereas its analogues proton H<sub>4b</sub> shows same pattern at 4.005 δ/ppm. Further, H<sub>3</sub> proton falls at 6.269 δ/ppm as triplet. Aromatic region is justified between 7.244–8.175 δ/ppm. Hydroxy proton and methoxy proton are obtained as singlet at 9.644 and 2.404 δ/ppm respectively. Carbon skeleton of ligand L<sup>1</sup> were justified using <sup>13</sup>C-APT. Carbonyl carbon shows its presence at 189.65 δ/ppm whereas methoxy carbon shows its primary carbon peak at 55.67 δ/ppm. <sup>1</sup>H NMR and <sup>13</sup>C-APT characterization of all compounds were given in Supplementary Material 1 and 2.

#### LC-MS and ESI-MS Characterization of Compounds

Compounds were well justified using LC-MS and ESI-MS spectroscopy. Base peak of ligand L<sup>1</sup> is obtained at 358 m/z as highly intense peak whereas molecular ion peak is obtained at 492 m/z with lower intensity. Complex C<sup>1</sup> were well characterized using ESI-MS spectroscopy. Molecular ion of C<sup>1</sup> represents at 1002.20 m/z with [M+], [M + 2], [M + 4], [M + 6] and [M + 8] peaks whereas ligand detached from molecule shows its present at 492.59 m/z. Relative isotopic abundance pattern of bromine atoms were reflected at 922.30, 842.39, 762.49 and 682.59 m/z showing quintet, quartet, triplet and doublet pattern with relative intensity of 1:3:5:3:1, 1:2:2:1, 1:3:1 and 1:3 respectively. LC-MS of ligands L<sup>1</sup>-L<sup>6</sup>, ESI-MS spectrum and ICP-OES plot of complex C<sup>1</sup> are given in Supplementary Material 3 and 4.

#### IR Spectra, Electronic Spectra, Magnetic and Conductance Measurement of Compounds

IR spectroscopy justify the formation of ligand and metal complexation. ν(=C-H) stretching band marks its presence at 3099 cm<sup>-1</sup> in ligand L<sup>1</sup>. Consequently, ν(C-N), ν(C-H), ν(C=C), and ν(=C-O) stretching bands are observed at 1340, 1312, 1666 and 1610 cm<sup>-1</sup>, respectively. Upon the complexation in case of Os(IV) pyrazole compound C<sup>1</sup>, disturbance in vibrational bands caused marks presence of bands viz. ν(=C-H), ν(C-N), ν(C-H), ν(C=C), and ν(=C-O) at 3112, 1562, 1318, 1680, and 1618 cm<sup>-1</sup>, respectively. The shift in frequency is observed due to the complexation of osmium metal ion with relative coordinating sites of pyrazole nucleus ligand L<sup>1</sup>. The band at 556 cm<sup>-1</sup> represents the coordinate covalent bond formed between nitrogen atom of ligand and osmium metal ion. The complex has octahedral geometry,



**Fig. 1** Plots represent relative viscosity of HS-DNA upon increasing concentration of compounds

consequently the expected  $\nu(\text{N-Os-Br})$  linkage vibrational stretching band is observed at  $611\text{ cm}^{-1}$ . Electronic spectra of ligands fall in wavelength of  $223.00\text{--}284.00\text{ nm}$  whereas that of Os(IV) complexes shows peaks at  $230.50\text{--}267.50$ . Complex  $\text{C}^1$  shows bands at  $284.00$ ,  $339.50$  and  $458.50\text{ nm}$  justifying  $n\text{-}\pi^*$ ,  $\pi\text{-}\pi^*$  and  $d\text{-}d$  transition bands accounting for  $(t_{2g})^4(e_g)^0$  electronic configuration representing octahedral behavior. Magnetic measurement of all the complexes justify paramagnetic behavior of Os(IV) complexes. All the complexes show non-electrolytic behavior confirmed by conductance measurements. IR spectrum of all the compounds are given in Supplementary Material 5. CHNS data of all the ligands have been given in Supplementary Material 6.

## Biological Studies

### Viscosity Measurement

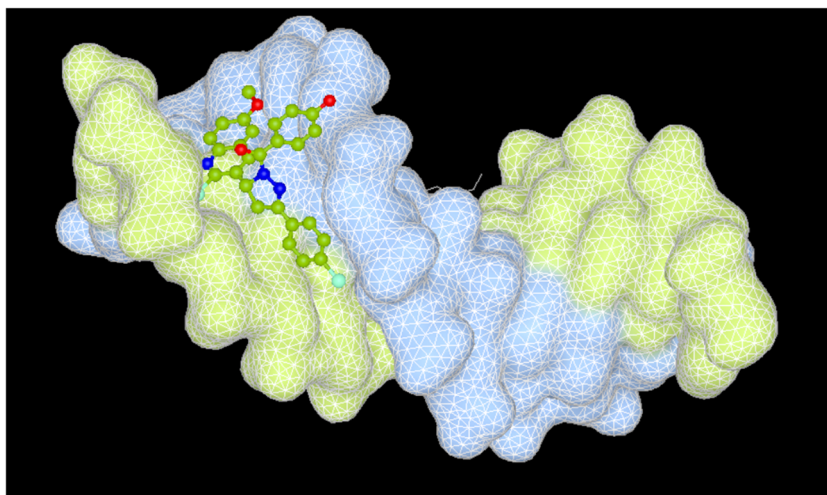
Mode of interaction of compounds were well concluded with the help of hydrodynamic volume measurement study. The

HS-DNA solution were incubated prior to run with the compound's concentration. The solution was poured into viscometer held in digital viscometric bath in static environment. Flow time of solution were measured in triplicate manner in order to get the accurate results. All the compounds show intercalative mode of binding [39]. Figure shows increase in viscosity of compounds kept w.r.t. ethidium bromide taken as reference material. The viscosity measurement plot of compounds is given in Fig. 1.

### Molecular Docking

Molecular docking is phenomenon of calculating effective docking energy upon interaction of molecules with the macro biomolecules. The molecules were allowed to run in ChemBio3D software to refine the molecule according to most stable conformational arrangement. The molecules were formatted as PDB files and were allowed to interact with B-DNA in HEX 6.0 software. The molecules and specific sites of DNA tend to refine, adjust according to conformational

**Fig. 2** Docking of Os(IV) compound  $\text{C}^1$  with B-DNA



**Table 1** Intrinsic binding constant ( $K_b$ ) values and percentage hypochromism shift values of all the synthesized compounds

Compound	$K_b$ Values $\times 10^5$	% Hypochromism	Compound	$K_b$ Values $\times 10^5$	% Hypochromism
L <sup>1</sup>	0.136 $\pm$ 0.015	31.12 $\pm$ 0.18	C <sup>1</sup>	0.822 $\pm$ 0.022	36.20 $\pm$ 0.17
L <sup>2</sup>	0.145 $\pm$ 0.011	11.86 $\pm$ 0.17	C <sup>2</sup>	0.910 $\pm$ 0.020	20.18 $\pm$ 0.18
L <sup>3</sup>	0.189 $\pm$ 0.012	12.89 $\pm$ 0.15	C <sup>3</sup>	0.856 $\pm$ 0.018	17.52 $\pm$ 0.22
L <sup>4</sup>	0.170 $\pm$ 0.013	21.23 $\pm$ 0.12	C <sup>4</sup>	0.811 $\pm$ 0.016	26.24 $\pm$ 0.25
L <sup>5</sup>	0.192 $\pm$ 0.014	24.42 $\pm$ 0.18	C <sup>5</sup>	0.924 $\pm$ 0.014	32.88 $\pm$ 0.21
L <sup>6</sup>	0.201 $\pm$ 0.012	31.90 $\pm$ 0.10	C <sup>6</sup>	0.887 $\pm$ 0.012	40.81 $\pm$ 0.18

changes and interact with each other by activating docking process. The effective energy of interaction in which molecule befit with DNA were counted as its docking energy.. Docking energy of complexes C<sup>1</sup>, C<sup>2</sup>, C<sup>3</sup>, C<sup>4</sup>, C<sup>5</sup> and C<sup>6</sup> are  $-326.49$ ,  $-318.71$ ,  $-312.94$ ,  $-292.30$ ,  $-292.30$  and  $-292.30$  kJ/mol whereas, their respective ligands show docking interaction energy of  $-295.49$ ,  $-300.25$ ,  $-289.39$ ,  $-287.06$ ,  $-290.79$  and  $-293.84$  kJ/mol respectively showing effective intercalation [40, 41]. Docked structure of complex C<sup>1</sup> is given in Fig. 2. Docked images of all the compounds are given in Supplementary Material 7.

#### Absorption Titration Study of Complex to HS- DNA

Absorption titration is UV-visible spectrophotometer-based activity to calculate binding constant of compounds upon interaction with HS-DNA. Compounds were titrated in absence and presence of DNA solution. With fixed concentration of compound and varying concentration of DNA solution, the experiment was carried out. The relative shifts in DNA treated and untreated compounds helps in predicting percentage hypochromism shift. The effective binding constant were calculated with the help of equation given by Wolfe et al.,

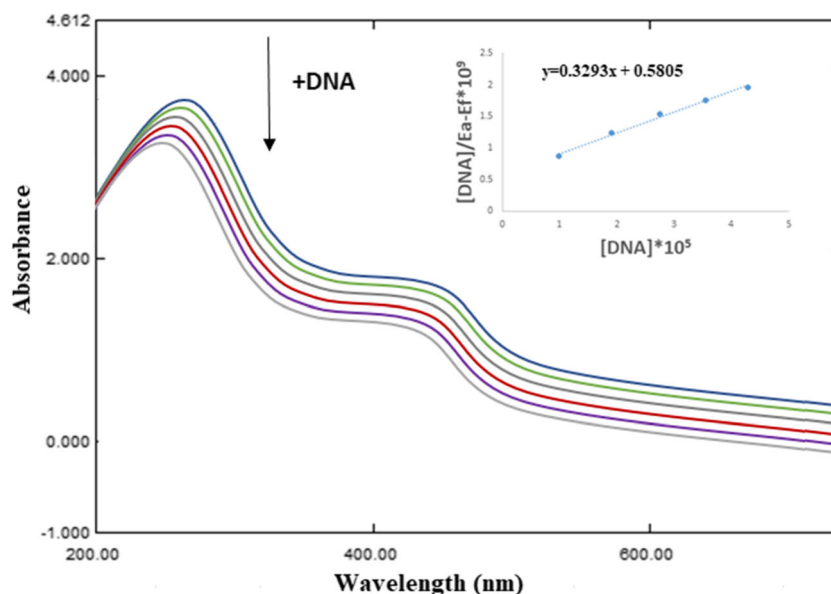
**Fig. 3** Absorption titration plot of varying concentration of HS-DNA with Os(IV) compounds. Incribed graph represents change in absorption

Table 1. shows binding constant and percentage hypochromism shift of compounds. Absorption titration curve of complex C<sup>1</sup> were represented in Fig. 3. All Os(IV) complexes have better binding constant compared to reported some Co(III) complexes with different 2-oxo-1,2-dihydrobenzo[h]quinoline-3-carbaldehyde moiety based ligands. While Os(IV) complexes C<sup>2</sup>, C<sup>5</sup> and C<sup>6</sup> show selectively better strength compare to Co(III) complex with 2-Oxo-1,2-dihydro-benzo[h]quinoline-3-carbaldehyde N-phenylthiosemicarbazone ligand [42].

#### Fluorescence Quenching Study

Phenomenon of quenching deals with decrease in intensity of compound in presence of suitable quenchers. Chemically, bromide ion was used as chemical quenching agent taking ethidium bromide. Ethidium bromide was used as fluorophore material since its an effective intercalating agent. Intense emission bands were observed at 610 nm when allowed to excite at wavelength of 470 nm due to formation of EB-DNA bond. Upon addition of varying complex concentration, the ethidium bromide slowly gets displaced from bonding with macromolecule resulting in decrease in fluorescence



**Table 2** Stern-Volmer quenching constant ( $K_{sv}$ ), binding sites ( $n$ ), and binding constant ( $K_f$ ) values

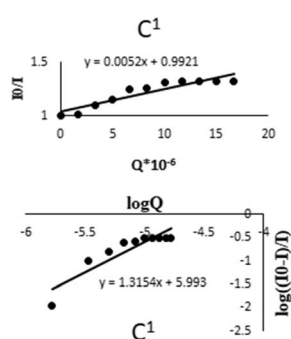
Complexes	$K_{sv} M^{-1} \times 10^4$	Binding Sites( $n$ )	$K_f M^{-1} \times 10^4$
C <sup>1</sup>	0.521 ± 0.022	0.9920	0.179 ± 0.023
C <sup>2</sup>	0.812 ± 0.021	0.8693	0.149 ± 0.021
C <sup>3</sup>	0.183 ± 0.032	1.024	0.107 ± 0.020
C <sup>4</sup>	0.522 ± 0.035	0.8898	0.168 ± 0.018
C <sup>5</sup>	0.564 ± 0.028	1.079	0.127 ± 0.017
C <sup>6</sup>	0.112 ± 0.018	0.8692	0.211 ± 0.015

intensity. Binding constant, effective binding sites and quenching constant were evaluated from fluorometric titration and are given in Table 2. The data were in good agreement with the absorption titration concluding effectiveness of intercalation mode of binding. Plots have been represented in Fig. 4 and mechanism of fluorescence quenching were presented in Fig. 5. Complexes C<sup>2</sup> and C<sup>5</sup> have quenching constant greater than reported complexes Pd(II) and their respective ligand [43].

### Gel Electrophoresis

Gel electrophoresis is a well-known technique for separation of charged particles based on difference in polarity to attract towards opposite poles of gel electrophoresis plate. 1% agarose gel were used in carrying out photolytic cleavage study. The solutions of compounds were run over casting apparatus set at optimum voltage required to run the gel. Upon addition of varying concentration of compounds on the solution of *S. Pombe* cell's DNA. The degradation of DNA were observed resulting in the unwinding of its helix by rupturing

**Fig. 4** Plot of fluorescence titration whereas, graphs represent Stern-Volmer equation parameters to calculate quenching constant ( $K_{sv}$ ), binding sites ( $n$ ) and binding constant ( $K_f$ )



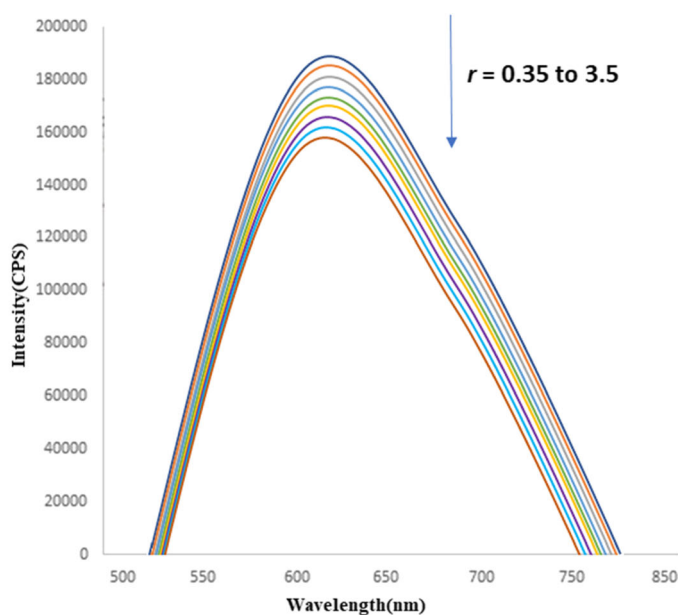
the bonds formed between its base pairs. DNA converts to single strand from double stranded structure. The unwinded helical structure were allowed to move over mobile phase of gel under specific voltage which stretch the helix more compactly resulting in smeared image when observed under spectrophotometer. The RNA remains intact, whereas DNA gets as smeared forming intense band. Intensity of smearing depends upon the potency of compound at specific concentration to cleave the macromolecule. Photographed image of treated compounds with *S. Pombe* cell's DNA under gel electrophoresis are represented in Supplementary Material 8.

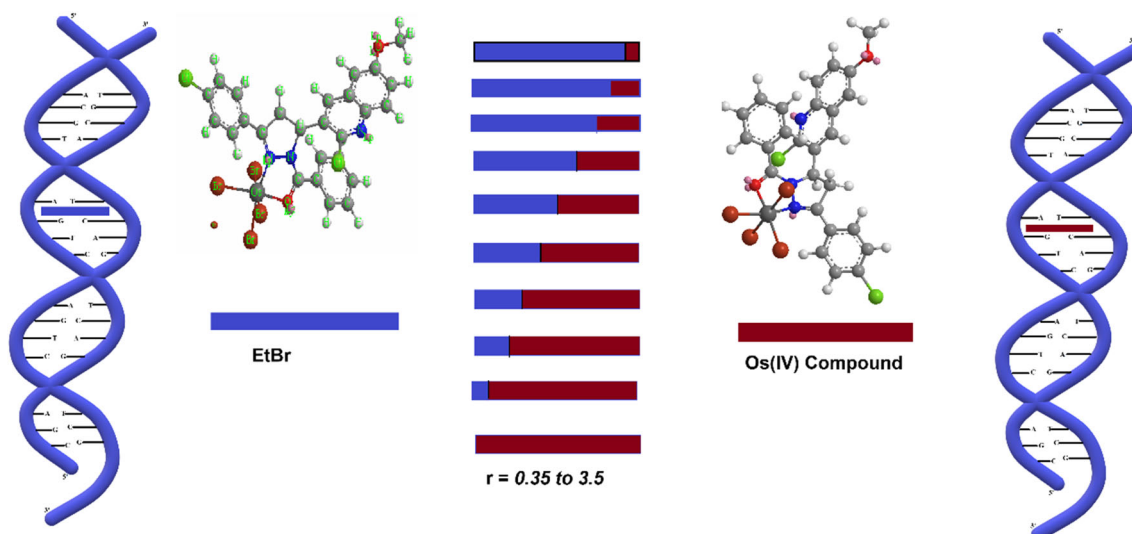
### Bacteriostatic Activity

This activity was carried out using Gram<sup>+ve</sup> and Gram<sup>-ve</sup> bacteria. All the compounds in different concentration range were treated with pre-grown bacterial culture. The compounds having substituents with electron delocalizing tendency tend to show better MIC values since they rupture the cell walls of bacteria there by resisting the growth. The minimal effective dosage of DMSO solubilized compound which resist the growth of bacteria is noted as its MIC value. Os(IV) complexes have better bacteriostatic activity compare to their respective ligands. MIC of all the compounds were represented in Fig. 6. MIC of complexes are found to be 72.5–100 μM, whereas that of ligands fall at about 122.5–150 μM. MIC of Os(IV) complexes are better than reported quinoline based quinazolinone-4-thiazolidinone heterocycles (5a-5 k) [44].

### Cellular Level in-Vivo Cytotoxicity

This assay were carried out using *S. Pombe* cell's DNA. Cell culture have been pre-grown 17 h before treatment with the





**Fig. 5** Fluorescence quenching observed with Os(IV) compound

test compounds. Varying concentration of compounds have been treated with the cell culture keeping it in conical flask in laminar air flow cabinet in presence of FV light. The solutions were kept for 24 h overnight before taking readings. The treated solutions were centrifuged to collect the culture. The collected culture was kept in incubation with tryptan blue dye and dead cells were counted under digital microscope. The dye allows dead cells to penetrate into it whereas it resists live cells. Percentage cell viability of Os(IV) complexes were found better than that of respective ligands. Table 3 shows percentage cell viability of compounds. Percentage cell viability of Os(IV) complexes is more effective compare to reported oxovanadium(IV) complexes [45].

#### *In-Vitro* Cytotoxicity against Artemia Cyst Lethality Bioassay

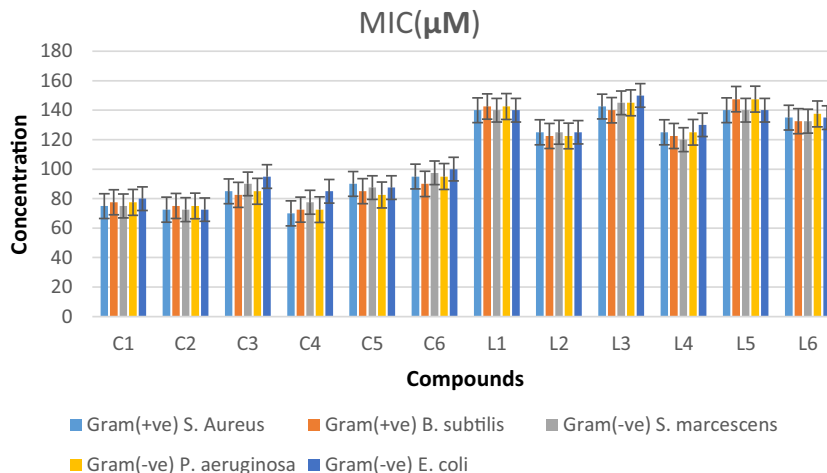
Meyer et al.'s protocol was implemented to carry out the assay. This is primary assay to judge molecule for the pharmaceutical

importance. Nauplii hatched from brine shrimp eggs have been treated with different concentration of compounds. The death of larvae is noted as lethality result. It is concluded that with increase in concentration of compounds, percentage mortality was increased. Cytotoxic value of ligands and Os(IV) complexes are respectively. Os(IV) complexes possess better  $LC_{50}$  than synthesized ligands.  $LC_{50}$  count of ligands fall in the range of 16.22–17.28  $\mu\text{g/mL}$  whereas that of Os(IV) fall in the range of 4.87–5.87  $\mu\text{g/mL}$ .  $LC_{50}$  of Os(IV) compounds show better value compare to reported oxovanadium (IV) complexes with 2-thiophene carboxylic acid hydrazide ligand [46].  $LC_{50}$  of compounds are represented in Fig. 7.

#### Cytotoxicity Using HCT-116 Cell Line

Cytotoxicity of complexes were screened over HCT-116 cell line. Figure 8 Show plot of percentage proliferation vs. concentration ( $\mu\text{g/mL}$ ) with different concentration of test

**Fig. 6** MIC values of compounds in terms of  $\mu\text{M}$  upon exposure to series of Gram(+ve) and Gram(-ve) bacteria



**Table 3** Percentage viability on *S. Poble* cell's DNA presented with standard deviation for three independent experiment

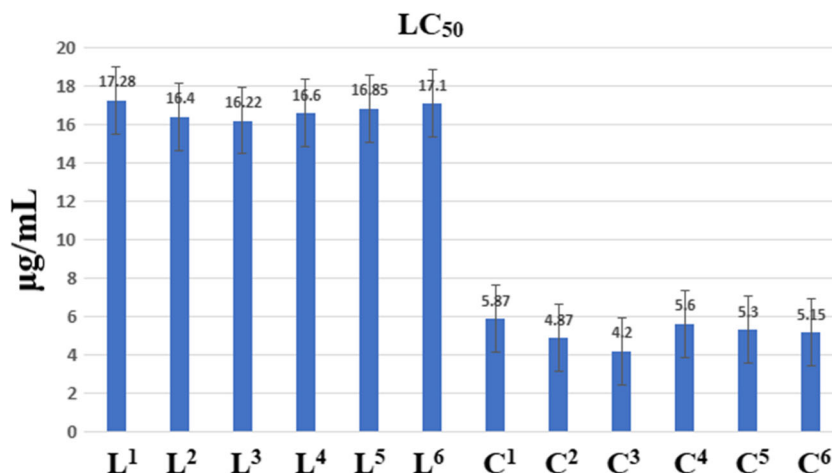
Ligands						
Percentage viability per concentration ( $\mu\text{g/mL}$ )	L <sup>1</sup>	L <sup>2</sup>	L <sup>3</sup>	L <sup>4</sup>	L <sup>5</sup>	L <sup>6</sup>
2	42 ± 1.5	35 ± 2.2	37 ± 2.5	44 ± 2.4	41 ± 2.5	39 ± 1.5
4	40 ± 1.6	31 ± 2.0	34 ± 1.4	42 ± 2.2	38 ± 1.5	36 ± 1.2
6	38 ± 1.5	29 ± 1.8	33 ± 1.6	40 ± 1.4	36 ± 1.0	34 ± 1.8
8	36 ± 2.0	27 ± 1.6	29 ± 1.8	38 ± 1.6	34 ± 1.2	31 ± 1.6
10	34 ± 2.1	24 ± 1.5	26 ± 2.0	35 ± 1.8	32 ± 1.3	29 ± 2.0
Complexes						
Percentage viability per concentration ( $\mu\text{g/mL}$ )	C <sup>1</sup>	C <sup>2</sup>	C <sup>3</sup>	C <sup>4</sup>	C <sup>5</sup>	C <sup>6</sup>
2	36 ± 1.6	29 ± 1.4	31 ± 1.2	38 ± 1.7	35 ± 1.6	33 ± 2.2
4	32 ± 1.5	26 ± 1.6	28 ± 1.5	34 ± 1.7	31 ± 1.5	29 ± 2.1
6	30 ± 1.5	24 ± 1.8	26 ± 1.8	32 ± 1.8	29 ± 1.5	27 ± 2.0
8	28 ± 2.0	21 ± 2.1	24 ± 2.0	29 ± 2.0	26 ± 1.5	25 ± 1.8
10	26 ± 2.0	19 ± 2.5	20 ± 2.5	28 ± 2.0	25 ± 2.0	22 ± 2.2

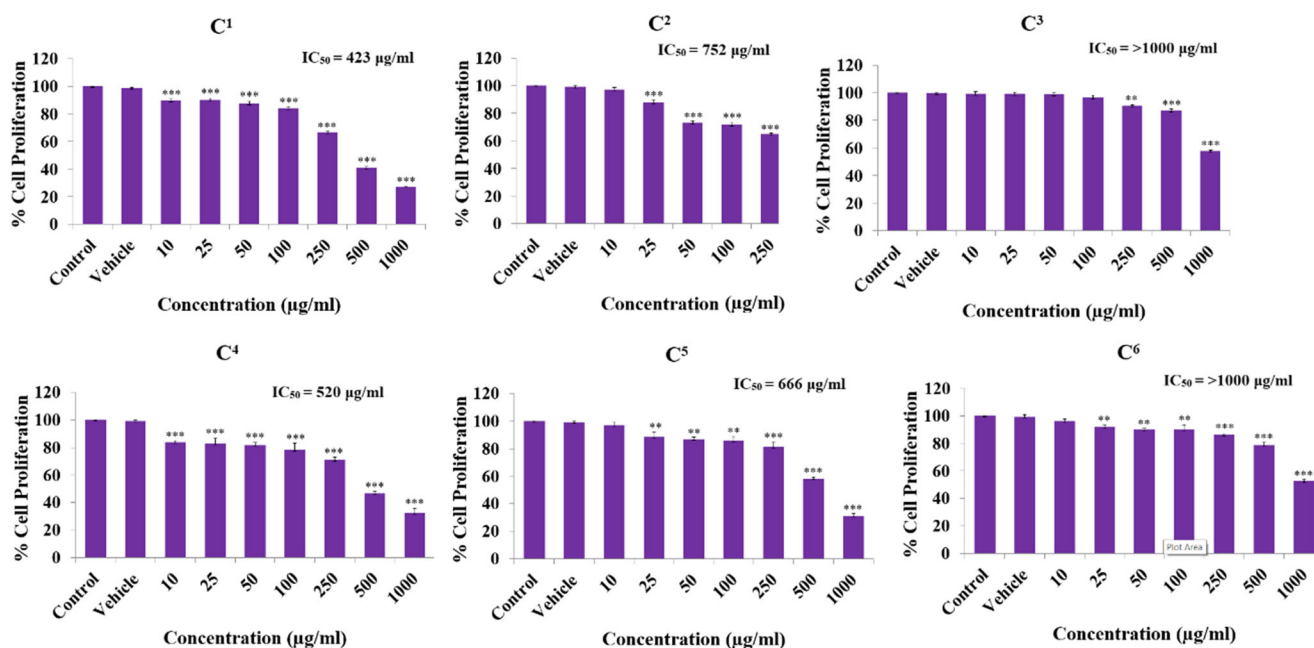
compounds taken with respect to vehicle and control media. Complexes C<sup>1</sup>, C<sup>2</sup>, C<sup>4</sup> and C<sup>5</sup> have IC<sub>50</sub> of 423, 752, 520 and 666  $\mu\text{g/mL}$  while others show moderate impact. IC<sub>50</sub> of Os(IV) C<sup>1</sup> dominate over reported Re(I) with 5-(4-Chlorophenyl)-1-(4-nitrophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole ligand [47]. Complex C<sub>1</sub> represents least IC<sub>50</sub> amongst the complexes since it possess chlorine functionality which put an inductive effect in dominance.

## Conclusion

Various pyrazole nucleus-based ligands and their Os(IV) complexes were synthesized and well characterized using different spectroscopic techniques viz. <sup>1</sup>H-NMR, <sup>13</sup>C-APT, LC-MS, ESI-MS, ICP-OES, IR spectroscopy, electronic spectroscopy, conductance and magnetic measurements. The synthesized compounds were subjected for

biological screening. All the compounds show intercalation mode of binding when interacted with HS-DNA justified with docking energy and DNA interaction study. Docking energy of ligands fall at about  $-287.06$  to  $-300.25$  kJ/mol. Whereas, complexes fall at about  $-292.30$  to  $-326.49$  kJ/mol showing effective intercalation. Binding constant of Os(IV) complexes are found to be  $8.1$  to  $9.2 \times 10^4 \text{ M}^{-1}$ . All Os(IV) complexes have better binding constant compared to reported Co(III) complexes with different substituted 2-oxo-1,2-dihydrobenzo[h]quinoline-3-carbaldehyde moiety based ligands. Binding constant have been further justified with fluorescence quenching study. Quenching constant of complexes fall at about  $1.1$  to  $8.1 \times 10^3 \text{ M}^{-1}$ . Complexes C<sup>2</sup> and C<sup>5</sup> have quenching constant better than reported Pd(II) complexes. MIC of complexes are found to be  $72.5$ – $100 \mu\text{M}$ , whereas that of ligands fall at about  $122.5$ – $150 \mu\text{M}$ . MIC of Os(IV) complexes are better than

**Fig. 7** Bar graph represents LC<sub>50</sub> of osmium(IV) compounds and ligands



**Fig. 8** Plots of % cell proliferation vs. concentration represents IC<sub>50</sub> of Os(IV) compounds

reported quinoline based quinazolinone-4-thiazolidinone heterocycles. Percentage cell viability of Os(IV) complexes is more effective compare to reported oxovanadium(IV) complexes. LC<sub>50</sub> of Os(IV) compounds show better value compare to reported oxovanadium (IV) complexes with 2-thiophene carboxylic acid hydrazide ligand. Os(IV) complexes showed intense and impactful smearing of *S. Pombe* cell's DNA when allowed to run over gel electrophoresis. IC<sub>50</sub> of Os(IV) C<sup>1</sup> dominate over reported Re(I) with 5-(4-chlorophenyl)-1-(4-nitrophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole ligand.

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**Data Availability** Not applicable.

## Compliance with Ethical Standards

**Competing Interests** Not applicable

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