DFT Calculations, Docking, Antioxidant and Anticancer Activity of Mononuclear Nickel(II) Complexes

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ABSTRACT

A series of mononuclear nickel(II) complexes (1–6) have been synthesized from substituted salicylaldehydes containing Schiff bases and characterized by varies spectral methods. The structural parameters of the complexes were evaluated by UV-Vis and DFT calculations, and results indicates square pyramidal (1–3) and octahedral (4–6) geometry around nickel(II) ion. Antimicrobial activity of the complexes exhibits moderate activity, which also exhibit better scavenging activity against ABTS and DPPH methods. In the molecular docking studies, the complexes are showed π−π, σ−π, hydrogen bonding, van der Waals and electrostatic interactions with EGFR kinase receptor. The nickel(II) complexes 2 and 5 show better anticancer activity against MDA-MB-231 cell lines compared to cisplatin and other complexes.

1. Introduction

The ‘privileged ligands’ (Schiff base ligands) play a vital role in coordination chemistry due to their willingness in coordination with many transition metal ions and easily form a highly stable complexes. Notably, the complexes of Schiff base ligands show good antibacterial, antifungal, antioxidant, anti-inflammatory, antimalarial, antiviral, antitubercular, hypothermic, hypertensive and anticancer activities [1–4]. Recently, a verity of tridentate Schiff base complexes display potential application in the area of industrial and analytical fields such as dyes, pigments and as catalysts in various reaction such as reduction reaction of ketones, polymerization reaction, oxidation of organic compounds, reduction of thionyl chloride, Henry reaction, aldol reaction, epoxidation of alkenes, hydroxylation of ketones, synthesis of bis(indolyl)methanes and Diels-Alder reaction [5–7]. Compare to organic ligands, the corresponding complexes show better activity in various fields due to their chaleting behaviour and structural aspects [8].

The first metal-based anticancer drug cisplatin was discovered in the year of 1965, which cures 95% ovarian and testicular cancers. And also, other platinum-based anticancer drugs such as carboplatin, oxaliplatin, and nedaplatin have been developed and cure variety of cancers. However, the uses of these platinum based drugs are associated with severe toxicity, side effects, low water solubility and drug resistance. To overcome these drawbacks, many researchers focus to develop other platinum based transition metal complexes with improved anticancer activity and less toxicity. In this connection, a variety of nickel(II) complexes were designed and developed, and tested against various cancer cell lines [9–11]. Based on the above facts, we have opted to synthesis salicylaldehydes containing Schiff base ligands and their mononuclear nickel(II) complexes and studied their spectral, DFT calculation, antibacterial, antioxidant, docking and anticancer properties.

2. Experimental Methods

2.1 Materials

Diethylenetriamine, triethylenetetramine, salicylaldehyde, 5-methylsalicylaldehyde and 5-bromosalicylaldehyde were purchased from Sigma-Aldrich (USA). Analytical grade of solvents were purchased from AVRA and E. Merck, and used as received without further purification. Tetra(n-butyl)ammonium perchlorate (TBAP) was purchased from Fluka (Switzerland), which used as the supporting electrolyte in the electrochemical measurements. The NiCl₂·6H₂O salt was purchased from Merck. The ligands (L¹–6) were synthesized from previously reported procedure [12].

2.2 Physical Measurements

Elemental analysis (CHN) of the complexes was carried out with a Perkin-Elmer 240 elemental analyser. Vibrational spectra were recorded on a Shimadzu model IR-Affinity-1 spectrophotometer using KBr disc technique in the range of 4000– 400 cm⁻¹. Electronic absorption spectra were recorded using a Perkin-Elmer Lambda 35 double beam spectrophotometer. Electro spray ionization (ESI) mass spectra were recorded on Q-Tof mass spectrometer using acetonitrile as a medium. Cyclic voltammograms were recorded on a CHI-660D (CH Instruments Co., USA) electrochemical analyzer using a three-electrode cell in which a glassy carbon electrode was the working electrode, a saturated Ag/AgCl electrode was the reference electrode and platinum wire was used as the auxiliary electrode. TBAP was used as the supporting electrolyte (0.1 M) and all complex solutions were around 10⁻¹ M concentration. X-band EPR spectra of complexes were recorded on Varian EPR-E 112 spectrometer at room temperature.

2.3 General Procedure for Synthesis of Mononuclear Nickel(II) Complexes (1–6)

All the nickel(II) complexes (1–6) were synthesized using the similar procedure as given below: A methanolic solution (30 mL) of Schiff base ligand (L¹–6, 1 mmol) and NiCl₂·6H₂O (0.13 g, 1 mmol) was stirred at room temperature for 2 h, and the reaction was carried out for 6 h under reflux and the reaction mixture was filtered hot and kept aside for slow evaporation. The resulting product was washed with diethyl ether and dried in vacuum. Attempts taken to obtain single crystal but for all the complexes went unsuccessful.

2.3.1 [Ni(L)]⁺ (1)

Yield: 0.33 g (89.66%). Colour: Yellowish brown. Anal. Calc. for: C₅₉H₅₄N₆O₄Ni (368.06): C, 58.74; H, 5.20; N, 11.42. Found: C, 58.80; H, 5.16; N, 11.36%. Selected IR data (KBr, ν/cm⁻¹): 1618 v(C=N), 1385 v(μ=μ), 1189 v(C–O), 758 v(C=O), 509 v(C–C).

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3.2.2 [Ni(U)] (2)

Yield: 0.35 g (88.35%). Colour: Yellowish brown. Anal. Calc. for: C_{28}H_{32}N_{2}O_{6} (525.85): C, 61.67; H, 5.08; N, 13.65. Found: C, 61.83; H, 5.12; N, 13.68. Selected IR data (KBr, 𝜈/cm⁻¹): 1630 (v(C=N)), 1386 (v(AR-O)), 1594 (v(N-H)). UV-Vis (DMF): 𝜆/λ (nm): 271 (v(π*) (320.20), 359 (v(π*)) (11,000), 641 (d-d) (315), 921 (d-d) (905). ESIMS (m/z): 438.16 [Ni(L)]⁺.

3.2.3 [Ni(U)] (3)

Yield: 0.47 g (90.38%). Colour: Yellowish brown. Anal. Calc. for: C_{28}H_{32}N_{2}O_{6} (525.85): C, 61.67; H, 5.08; N, 13.65. Selected IR data (KBr, 𝜈/cm⁻¹): 1630 (v(C=N)), 1386 (v(AR-O)), 1594 (v(N-H)). UV-Vis (DMF): 𝜆/λ (nm): 264 (v(π*) (321.10), 356 (v(π*)) (10,710), 638 (d-d) (320), 924 (d-d) (975). ESIMS (m/z): 522.90 [Ni(L)]⁺.

3.2.4 [Ni(U)] (4)

Yield: 0.39 g (94.68%). Colour: Yellowish brown. Anal. Calc. for: C_{28}H_{32}N_{2}O_{6} (525.85): C, 61.67; H, 5.08; N, 13.65. Found: C, 61.83; H, 5.12; N, 13.68. Selected IR data (KBr, 𝜈/cm⁻¹): 1630 (v(C=N)), 1386 (v(AR-O)), 1594 (v(N-H)). UV-Vis (DMF): 𝜆/λ (nm): 264 (v(π*) (321.10), 356 (v(π*)) (10,710), 638 (d-d) (320), 924 (d-d) (975). ESIMS (m/z): 410.13 [Ni(L)]⁺.

3.2.5 [Ni(U)] (5)

Yield: 0.39 g (98.80%). Colour: Yellowish brown. Anal. Calc. for: C_{28}H_{32}N_{2}O_{6} (525.85): C, 61.67; H, 5.08; N, 13.65. Found: C, 61.83; H, 5.12; N, 13.68. Selected IR data (KBr, 𝜈/cm⁻¹): 1630 (v(C=N)), 1386 (v(AR-O)), 1594 (v(N-H)). UV-Vis (DMF): 𝜆/λ (nm): 264 (v(π*) (321.10), 356 (v(π*)) (10,710), 638 (d-d) (320), 924 (d-d) (975). ESIMS (m/z): 410.13 [Ni(L)]⁺.

3.2.6 [Ni(U)] (6)

Yield: 0.48 g (84.37%). Colour: Yellowish brown. Anal. Calc. for: C_{28}H_{32}N_{2}O_{6} (525.85): C, 61.67; H, 5.08; N, 13.65. Found: C, 61.83; H, 5.12; N, 13.68. Selected IR data (KBr, 𝜈/cm⁻¹): 1630 (v(C=N)), 1386 (v(AR-O)), 1594 (v(N-H)). UV-Vis (DMF): 𝜆/λ (nm): 279 (v(π*) (320.20), 360 (v(π*)) (11,430), 647 (d-d) (345), 917 (d-d) (80)). ESIMS (m/z): 565.95 [Ni(L)]⁺.

2.4 Computational Details

The complexes were optimized by using Gaussian 03 software package [13]. The quantum chemical calculations were performed applying DFT method with Becke-3-Lee-Yang-Parr (B3LYP) supplemented with the standard 6-31G(d) and LANL2DZ basis set [14]. The optimized geometry corresponding to the minimum on the potential energy surface has been obtained by solving self-consistent field equation iteratively.

2.5 Antibacterial Activity

Antibacterial activity of Schiff base ligands and their mononuclear nickel(II) complexes [1–6] were tested against Gram negative (Escherichia coli, Klebsiella pneumoniae and Vibrio cholera) and Gram positive (Staphylococcus aureus) bacterial strains by agar well diffusion method [15]. Ciprofloxacin and Streptomycin (100 μg/mL) were used as standards. All discs and materials were sterilized in an autoclave before the experiments. Nutrient agar was used as sources for culturing bacteria at 37 °C on a rotary platform in an incubator. The ingredients were dissolved in distilled water and sterilized at 121 °C at 15 lbs for 15 min. Nutrient agar medium was prepared and plated aseptically into the sterile plates. Bacterial inoculums were prepared by growing a single colony overnight in nutrient broth and then made a lawn culture using sterile swab over the nutrient medium plates. After the lawn preparation, discs impregnated with the ligand and complexes at different concentrations (25–100 μg/mL) and DMSO (control) was placed on the petriplates using sterile forceps and sonicated to ensure optimum nanoparticle dispersion using a sonicator bath at room temperature for 15 minutes to avoid the nanoparticles agglomeration. After incubation for 24 h at 37 °C, a clear zone around the disc was an evidence of antibacterial activity. From this clear zone, the inhibition zone was measured.

2.6 In Vitro Antioxidant Activity

The antioxidant activity of the synthesized mononuclear nickel(II) complexes were studied using standard methods. The concentration of the complexes, and standard solutions used were 1.56–100 μg/mL. The absorbance was measured spectrophotometrically against the corresponding blank solutions. The percentage of inhibition was calculated by using the following formula,

Radical scavenging activity (%) = (OD control – OD sample/OD control) × 100

2.6.1 DPPH Free Radical

A molecular docking study was carried out by the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5 docking programs. The structure of present complexes [1–6] was converted into PDB format from mol format using OPENBABEL. The crystal structure of the BSA (PDB ID: 3V03) was downloaded from the protein data bank (http://www.wwpdb.org/pdb). Receptor (BSA) and ligand (complexes) files were set using AutoDock Tools. Initially, all the heteroatoms with water molecules were deleted and polar hydrogen atoms and Kollman charges were additional to receptor molecule, then rotatable bonds in complexes were assigned. Remaining bonds were allowed to rotate. The BSA was covered in a box with number of grid points in x × y × z directions, 110 × 110 × 110 and a grid spacing of 0.4 Å. Lamarckian genetic algorithm, as implemented in AutoDock, was working to carry out docking calculations. Residual parameters were defaulting settings. For every docking case, the lowest energy docked conformation, according to the AutoDock scoring function, was selected as the binding mode. Visualization of the docked position has been done by using PyMOL molecular graphics program.

2.8 Anticancer Activity

2.8.1 MTT Assay

The anticancer activity of complexes [1–6] were tested against human breast cancer cell line (MDA-MB-231) and normal human dermal fibroblast cell line (NHD) by MTT assay after 48 h treatment [18]. The MDA-MB-231 cells were grown in DMEM medium containing 10% FBS. For screening experiments, the cells (1 × 10⁴ cells/well) were plated in 96-well plates with the medium containing 10% FBS and incubated for 24 h under CO₂ at 37 °C. Later, the medium was replaced with DMEM containing 1% FBS and the complexes (10–250 μM) dissolved in 0.05% DMSO were added to the cells incubated at 37 °C in 5% CO₂. After treatment, the plates were incubated for 24 h in order to perform cytotoxic analysis using MTT assay. 10 μL of MTT (5 mg/mL) was added to each well and incubated for 4 h. Purple color formazine crystals formed were then dissolved in dimethylsulfoxide (100 μL). These crystals were observed at 570 nm in a multiwell ELISA plate reader. The cell viability (%) was evaluated using the following equation:

Cell viability (%) = (A₅⁷⁰ nm of treated cells / A₅⁷⁰ nm of control cells) × 100
3. Results and Discussion

3.1 Synthesis and Spectral Characterization

The Schiff base ligands (L1–6) and their mononuclear nickel(II) complexes (1–6) have been synthesized using general procedure and obtained in good yield (Scheme 1). The authenticities of complexes were determined by elemental analysis, FT IR, UV-Vis, CV, EPR and ESI mass spectroscopy.

The obtained molecular ion peaks are comparable with their molecular weight. The observed mass data clearly demonstrate the proposed molecular formulæ of the complexes.

3.3 Electrochemical Properties

The electrochemical properties of redox active nickel(II) complexes (1–6) were evaluated by cyclic voltammetry in the potential range +1.4 to –1.4 V in DMF containing 0.1 M tetrabutylammonium perchlorate.

3.3.1 Reduction Process

All the complexes display an irreversible one electron transfer process in between −0.82 and −0.99 V, which can be assigned to the reduction of the metal(II) ion centre (Fig. 2). The reduction capability of complexes 3 and 6 is less negative with respect to other complexes, which demonstrates that the +1 oxidation state is more balanced out in complexes 3 and 6 than other complexes. The reduction process can be assigned as follows:

Ni(II) ↔ Ni(I)

3.3.2 Oxidation Process

All the complexes display an irreversible one electron transfer process in between +1.15 to +1.4 V for oxidation process, which can be assigned to the oxidation of the metal(II) ion centre (Fig. 3). The oxidation capability of complexes 2 and 5 is more positive with respect to other complexes. The oxidation process can be assigned as follows:

Ni(II) ↔ Ni(III)

3.4 Geometry Optimization

DFT calculation is a tool of expanding significance for the structural investigation of coordination and organometallic complexes. In the absence of crystal data, DFT calculations provide more suitable information about their geometrical properties. Taking into consideration, the mononuclear nickel(II) complexes (1–6) were optimized at B3LYP/LANL2DZ levels in gas phase and the optimized ground state geometries structures are shown in Fig. 4 and 5. The calculated bond lengths and angles of the complexes are listed in Table 1. The five coordinated complexes (1–3), coordinate through two phenolic oxygen, two azomethine nitrogen and one primary –NH atoms. The observed Ni–O and Ni–N bond lengths of the complexes (1–3) observed in the range from 2.198 to 2.0601 Å and 2.3743 to 2.0543 Å, respectively, which is more consistent with previously reported work [21, 22]. The combined B3LYP method and LANL2DZ basis gives a tremendous evaluation of Ni(II) to N and O bond distances, Ni–N1, Ni–N2, Ni–O1and Ni–O2. Molecular geometries can be predicted by calculating τ values using the equation [23].
where $\alpha$ and $\beta$ are the equatorial and axial bond angles, respectively. Generally, if the molecular geometry is square pyramidal, $\tau$ value is close to 0, while if $\tau$ value is close to 1 then the geometry is similar to trigonal bipyramidal. For complexes (1–3) $\alpha$ (Ni–N1–O2) values are 109.14, 110.36 and 112.13, respectively and $\beta$ (Ni(N2)–N1–O1) values are 108.12, 109.09 and 110.09, respectively. The observed results indicate the complexes (1–3) as their $\tau$ values is equal to 0, which indicate the distorted square pyramidal geometry around nickel(II) center. The six coordinated complexes (4–6) coordinate through two phenolic oxygen, two azomethine nitrogen and two primary –NH atoms.

**5.3.2 Antioxidant Activities**

The *In vitro* antioxidant activity of the complexes (1–6) was tested by ABTS and DPPH free radical scavenging assay method using ascorbic acid and butylated hydroxyl toluene (BHT) as standard. Generally, ABTS is a long-life ABTS+–radical cation and DPPH is more stable radical, which react with any compound that supply an electron or hydrogen atom, resulting in a colour change from purple to yellow. The antioxidant activity of the complexes was tested at different concentrations (25–100 µg/mL). $IC_{50}$ values has observed from the plots of percentage of inhibition with an increase in concentration of the complexes (Fig. 6), and observed $IC_{50}$ values are listed in Table 1. As seen from results, the complexes 2 and 5 show higher scavenging ability than standard drugs such as ascorbic acid and BHT (Table 2). The antioxidant activity follows the order $5 > 2 >$ ascorbic acid > BHT $> 4 > 2 = 6 > 3$. Importantly, the complexes 2 and 5 show better antioxidant activity over to other complexes, which may be attributed to the significant contribution of the methyl substituent [24]. The observed higher antioxidant activity of the metal complexes depend on the nature of geometry, ionization potential, reactivity, reducing capacity and including conjugation [25].

**3.5 Biological Evolutions**

### 3.5.1 Antibacterial Activity

Generally, metal complexes are more dynamic than ligands as they may serve as central cytotoxic species. In this way, they exhibit wide range nature and can be further utilized as a part of pharmaceutical industry for humankind, as an antimicrobial agent, in the wake of testing its toxicity to people. In this regard, we are interested to evaluate the *In vitro* antibacterial activity of mononuclear nickel(II) complexes (1–6) were tested against three Gram negative (Escherichia coli, Klebsiella pneumoniae and Vibrio cholera) and one Gram positive (Staphylococcus aureus) strains by agar well diffusion method. The antibacterial activity of the complexes has been found to be significant efficiency than the corresponding ligands at the measured concentrations. The positive control (Ciprofloxacin) produces remarkable sized inhibition zones against the tested bacteria however negative control (DMSO) creates no discernible inhibitory impact against any of the test organisms. Tested complexes demonstrated zone of inhibition ranging 10–19 mm against the Gram positive microorganisms and between 9 to 18 mm against Gram-negative microorganisms. The Schiff base ligands (1–4) show zone of inhibition ranging 6.1–15.8 mm against Gram positive microorganisms and 5.7–14.8 mm against Gram negative microorganisms. It has been noticed that the metal complexes established expanded zone of inhibition against the bacterial strains when contrasted with their corresponding ligands ranging 10.4–21.2 mm. From the zone of inhibition, all the complexes show higher activity against Gram positive than Gram negative bacteria. Complex 5 was observed to be best active agent against E. coli, K. pneumoniae, V. cholera and S. aureus with the zone of inhibition of 19, 21.5, 16.9 and 17.4 mm, respectively.

**Table 1** B3LYP/LANLDZ bond lengths (Å) and bond angles (°) of nickel(II) complexes (1–6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Calculated B3LYP/LANLDZ</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond length (Å)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni–N(1)</td>
<td>2.112</td>
<td>2.034</td>
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<tr>
<td>Ni–N(2)</td>
<td>2.374</td>
<td>2.367</td>
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<td>Ni–O(2)</td>
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<tr>
<td>Ni–NH(1)</td>
<td>2.211</td>
<td>2.214</td>
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<tr>
<td>Ni–NH(2)</td>
<td>2.321</td>
<td>2.307</td>
</tr>
<tr>
<td>Bond angle (°)</td>
<td></td>
<td></td>
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<tr>
<td>O1–Ni–N1</td>
<td>108.54</td>
<td>107.11</td>
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<td>111.03</td>
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<td>O1–Ni–N1</td>
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<td>161.926</td>
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<td>O1–Ni–N2</td>
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<td>Ni–Ni–N1</td>
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<td>N1–Ni–N1</td>
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<td>110.46</td>
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<td>N2–Ni–N1</td>
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<td>N2–Ni–N1</td>
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<tr>
<td>N2–Ni–N1</td>
<td>96.22</td>
<td>97.27</td>
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</table>

**Table 2** In vitro antioxidant and anticancer activity of complexes (1–6)

<table>
<thead>
<tr>
<th>Complexes</th>
<th>IC$_{50}$ value (µg/mL)</th>
</tr>
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<tr>
<td>Standards</td>
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<tr>
<td>ABTS</td>
<td>22.17 ± 0.20</td>
</tr>
<tr>
<td>DPPH</td>
<td>22.16 ± 0.15</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>24.22 ± 0.43</td>
</tr>
<tr>
<td>NHDF</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1</td>
<td>16.09 ± 0.11</td>
</tr>
<tr>
<td>2</td>
<td>12.47 ± 0.72</td>
</tr>
<tr>
<td>3</td>
<td>28.23 ± 1.03</td>
</tr>
<tr>
<td>4</td>
<td>19.10 ± 0.21</td>
</tr>
<tr>
<td>5</td>
<td>10.17 ± 0.74</td>
</tr>
<tr>
<td>6</td>
<td>15.42 ± 0.21</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>&gt;100</td>
</tr>
<tr>
<td>BHT</td>
<td>17.16 ± 0.29</td>
</tr>
<tr>
<td>Carcillin</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

**3.5.3 Molecular Docking with EGFR Kinase Receptor**

All the complexes are mostly located in active site of the EGFR kinase receptor as shown in Fig. 7, and the observed free binding energy values are listed in Table 3. The complexes 2 and 5 effectively bind to EGFR kinase receptor via $\pi$–$\pi$, $\sigma$–$\pi$ hydrogen bonding, electrostatic and van der Waals interactions.
Complex 2 was stabilized by one hydrogen bond interaction formed between hydrogen atom of Asp1046 and azomethine nitrogen atom (bond length: H∙∙∙N = 3.6 Å), which also show two π–π interaction formed between the two phenol ring and Lys868 (bond length: 3.7 and 3.6 Å). The electrostatic interaction formed between the complex and residues Glu885 and Asp1046, and van der Waals interaction formed between complex and residues Lys868, Ile899, Leu899, Leu899, Val914, Val914, Ile1044 and Asp1046. Complex 5 shows one π–π interaction formed between the phenol ring and Lys868 (bond length: 3.6 Å), which also shows one σ–π interaction formed between the phenol ring and Val914 (bond length: 3.6 Å). The electrostatic interaction formed between the complex and residues Lys868, Glu885 and Asp1046, and van der Waals interaction formed between complex and residues Val914, Ala946, Leu982, Leu986, Leu989, Val914, Val914, Ile1045 and Phe1047. The observed data indicate EGFR kinase receptor as the significant inhibitor for all the complexes, because all the complexes were retained tightly by the binding pocket of EGFR.

Table 3 Molecular docking parameters of the complexes (1‒6) with EGFR kinase receptor

<table>
<thead>
<tr>
<th>Complex</th>
<th>Final intermolecular energy (kcal mol⁻¹)</th>
<th>Final total internal energy (kcal mol⁻¹)</th>
<th>Torsional free energy (kcal mol⁻¹)</th>
<th>Unbound system energy (kcal mol⁻¹)</th>
<th>Estimate free energy of binding (kcal mol⁻¹)</th>
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<td>-5.07</td>
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</table>

3.5.4 MTT Assay

In vitro anticancer activity of complexes (1‒6) was tested against human breast cancer cell line (MDA-MB-231) and normal human dermal fibroblast cell line (NHDF) by MTT assay after 48 h treatment. The potential anticancer drug (cisplatin) was used as the standard positive control. The IC₅₀ values were listed in Table 2. The cell viability was concentration dependent, with increasing complex concentrations and decrease in cell viability. All the complexes show superior potency to cancer cell line (MDA-MB-231), and all the complexes (1‒6) exhibit less toxic in normal cell line (NHDF). The observed data reveal good anticancer activity of the complexes 2 and 5 against MDA-MB-231 cancer cell lines, compared to cisplatin. The higher activity of these complexes is due to electron releasing groups in para position, which increase in lipophilicity and hydrophobic interactions.

3.5.5 Apoptosis by Hoechst 33258 Staining Assay

Apoptosis induction is one of the conceptions in the development of drug; many anticancer drugs in present use were showed to induce apoptosis in susceptible cells [26]. In order to understand the type of cell death induced by Ni(II) complexes was tested on MDA-MB-231 cells stained with Hoechst 33258. After the treatment of complexes for 48 h, the results show the morphological changes such as fragmentation of chromatin, cell shrinkage, plasma membrane blebbing, cytoplasmic vacuolation, nuclear fragmentation and nuclear swelling as compared to control cells (Fig. 8). These morphological changes indicate that the complexes induced either apoptosis or necrosis.

3.5.6 Apoptosis by AO/EB Staining Assay

Further to sustain the morphological changes of nickel(II) complexes (1‒6) with MDA-MB-231 cells was stained with acridine orange (AO) and ethidium bromide (EB) after treatment with the complexes for 48 h (Fig. 9). The control cells show bright green spot, whereas, the complexes (25 μM) treatment with MDA-MB-231 cells after stained with AO/EB the morphological was changed, the many cells become shrinkage, cell blebbing and chromatin condensation were observed. These data show that all the complexes induce apoptosis in the MDA-MB-231 cells.

4. Conclusion

A series of mononuclear nickel(II) complexes derived from Schiff base ligands have been successfully synthesized and fully characterized. The geometry of the complexes 1‒3 show square pyramidal and the other three complexes 4‒6 show distorted octahedral geometry around Ni(II) ion. DFT calculations used to predict the molecular geometry of the complexes. All the complexes show pronounced anti bacterial activity and also they display significant antioxidant activity with respect to standard drugs. The complexes strongly interact with epidermal growth factor receptor (EGFR). In vitro anticancer activity of the complexes shows comparable activity to the cisplatin against the same cancer cell lines. The methyl substituted complexes 2 and 5 show higher anticancer activity when compared to other complexes. The morphological assessment data observed from Hoechst 33258 and AO/EB staining assays exhibit that the complexes induce the cancer cell death through apoptosis.

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References


