Synthesis, spectral and evaluation of biological activity of Ni(II) mixed ligand complex containing 2-aminothiazole and triphenylphosphine


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Keywords: Triphenylphosphine, Amino thiazole, Ni(II) mixed ligand complex, Antimicrobial activities, Molecular docking studies, MIC level, Antioxidant activity.

A mixed ligand Ni(II) complex has been synthesized using 2-aminothiazole and triphenylphosphine in good yield. The structure was characterized by physico-chemical. The antimicrobial activity of the synthesized compound was evaluated against six bacteria (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Pseudomonas aeruginosa, Vibrio cholerae and E. coli) and two phytopathogens fungi (Aspergillus aureus and Aspergillus fumigates) using standard method at MIC level. Further the compounds are subject to in silico molecular docking studies on antibacterial receptor 1STE, the lowest docking results concludes that the compounds showing good interactions with amino acids at active sites of the receptor this evidence that the compounds binds to active sites of the receptor and suggesting that it can be a good antimicrobial agent. The complex showed significant antioxidant activity.

Introduction

The drug resistance property of bacteria and fungi becoming a major worldwide problem. It is therefore need to design a suitable potent drug that overcome this resistance has become one of the most important area of research today [1]. During recent years coordination compounds of biologically active ligands [2 - 4] have received much attention. The presence of nitrogen, oxygen and sulphur in these complexes can enhance antitumor, antibacterial and antifungal activities of transition metal complexes [5]. The coordination geometries of such complexes depend on the nature of the ligand, number and type of coordinating atoms and the chain length between the coordinating atoms [6]. Metal complexes of biologically vital ligands are often more active than the free ligands [7]. The interaction phases and the geometric position of the transition metal in ligand chelation environment serve as models to enzyme containing metal ion [8].

Phosphine based ligands have widespread pharmacological applications including antiviral, antioxidant, antifungal, anticancerogenic, antibacterial and antitumor [9]. Particularly phosphine based nickel(II) complex has been reported to possess significant bioactivities [10]. These metals play vital role in controlling gene expression, inhibiting cell division and hence are used as valuable anticancer drugs. However, problem associated with such complexes is their ready dissociation in solution leading to very reactive species that are unable to reach their pharmacological targets such as DNA. This rapid aquation and formation of very reactive species could be overcome if nickel(II) complex are stabilized by bulky ligands such as triphenylphosphine [11]. On considering above the presence of the amino group shows more potent biological activity and anthelmintic potential [12, 13]. In this context, an attempt has been made to synthesize a pharmacologically active new mixed ligand Ni(II) metal complex. The antimicrobial activity, molecular docking and the In-vitro antioxidant scavenging activity of the metal complex have been evaluated.

Fig. 1 Proposed structure of metal complex
2. MATERIALS AND METHODS

2.1. General Experiments

2-Aminothiazole, Triphenylphosphine, Nickel chloride, LR grade methanol, LR grade were procured from Sigma-Aldrich (INDIA), Himedia (INDIA), Labo Chemicals (INDIA) (Commercially available from local sources) were used as received without further purification. Freshly distilled ethanol and methanol solvents were employed for all synthetic purposes. Spectroscopic grade solvents were employed for spectral works. Other chemicals were of AR grade. The progress of the reaction was monitored by TLC. Yields refer to isolated yields after purification of compound that have a purity of ≥97%.

The products of this reaction was authenticated by matching spectroscopic data of the products obtained with those of the reported in the literature. 1H NMR spectrum recorded on Bruker 400 MHz spectrometer at Sophisticated Analytical Instruments Facility, Cochin University, Cochin, Kerala, IISc, Bengaluru, Karnataka, India. The chemical shifts have been proven in δ values (ppm) with tetramethylsilane (TMS) as an internal standard. The signals are designated as follows: s, singlet; d, doublet; t, triplet and m, multiplet. An elemental analysis was carried out with a Perkin-Elmer 2400 Series II C, H, N analyzer. Molecular weights of unknown compounds were characterized by LC-MS spectroscopy, Centralized instrumentation facility, Mysore University, Karnataka, India. UV-vis spectra recorded on varian, Cary 5000. The Fourier transform infrared (FT-IR) spectrum of the compound was taken as KBr pellet (100 mg) the usage of a Shimadzu Fourier Transform Infrared (FT-IR) spectrometer.

Melting point was determined in an electrically heated apparatus using the Crystallization Apparatus, Shimadzu, fourier transform infrared (FT-IR) spectrometer. the compound was taken as KBr pellet (100 mg) the usage of a Cary 5000. The Fourier transform infrared (FT-IR) spectrum of the test stock solution compounds were added and the plates were allowed to cool for an hour to facilitate the diffusion. The plates were then incubated at 37 ºC for 48 h. At the end of the incubation period, the diameter of the zone of inhibition around the wells was measured using vernier callipers.

2.2. Synthesis of [Ni(PPh3)2(ATH)Cl]2 (Ni(II) complex)

An ethanolic solution of NiCl2 (0.5g, 3.6 mmol) was mixed with a hot stirring ethanolic solution of the 2-aminothiazole (0.36g, 3.6 mmol) and triphenylphosphate (0.94g, 3.6 mmol) (Scheme). The mixture was stirred with heating for 6 h, when the solid precipitated. The excess solvent was removed by filtration. The solid product was recrystallized from methanol and the obtained complex was kept in a vacuum desiccator. The melting point of the greenish coloured solid product was 190 - 196 ºC. Yield: 65 % . Elemental analysis (%) found (Calculated) for C55H64Cl2Ni3P3S4: C - 51.81 (51.22), H - 6.13 (3.89), N - 5.69 (5.01), Ni - 11.92 (11.99). C55H64Cl2Ni3P3S4 = 941.987 g/mol.

2.3. Antimicrobial activity

2.3.1. Antibacterial screening

The antibacterial activity of the metal complex was tested against five different bacteria namely Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Pseudomonas aeruginosa, Vibrio cholerae and Escherichia coli by agar well diffusion method. Twenty four old Muller-Hinton broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 9mm with the help of sterile cork borer. The standard drug (chloramphenicol, 100 μg/mL of sterile distilled water), three different concentrations (100, 50 and 25 μg/mL in 10% DMSO) and control (10% DMSO) were added to respective labelled wells. The plates were allowed to stand for 30 min. and were incubated at 37 ºC for 24 h in upright position and the zone of inhibition was recorded [13]. During this period, the test solution diffused and zone of inhibition were recorded using vernier callipers.

2.3.2. Antifungal screening

Antifungal activity of the metal complex was evaluated against Aspergillus aureus and Aspergillus fumigates fungus, using the sabouraud dextrose agar diffusion method [14]. Wells were made (9 mm diameter) with a sterile cork borer. The standard drug (Fluconazole, 100 μg/mL of sterile distilled water) and control (10% DMSO) were added to respectively labelled wells. To these wells 140 μl from each (100, 50 and 25 μg/mL in 10% DMSO) of the test stock solution compounds were added and the plates were allowed to cool for an hour to facilitate the diffusion. The plates were then incubated at 37 ºC for 48 h. At the end of the incubation period, the diameter of the zone of inhibition around the wells was measured using vernier callipers.

2.4. Molecular docking studies

The molecular docking study was done by following the procedure reported [15]. The insilico molecular docking has been carried out on the antibacterial receptor on PDB code: 1STE, the crystal structure of the receptor has been obtained from the protine data bank and the all the water molecules and heteroatoms are removed before screened for docking studies.

2.6. Antioxidant activity

This activity for the synthesized Ni(II) complex was performed using DPPH method as per literature [15]. The compounds of different concentrations were dissolved in methanol and were introduced to each vials of 5mL. To this test vials 3 mL of 0.004% DPPH in methanol was added and the mixtures were incubated in dark condition at ambient temperature for 30 min. Ascorbic acid is used as the standard. The absorbance reduced while the DPPH is scavenged by way of an antioxidant, through contribution of hydrogen to shape a strong DPPH molecule. DPPH scavenging activity calculated by the use of the following equation and absorbancemeasured at 517 nm.

\[
\text{Scavenging ratio (\%)} = \left( \frac{\text{Ai} - \text{Ao}}{\text{Ac} - \text{Ao}} \right) \times 100\%
\]

Where

\( \text{Ai} \) is the absorbance within the presence of the check compound.

\( \text{Ao} \) is absorbance of the clean inside the absence of the check compound.

\( \text{Ac} \) is the absorbance within the absence of the test compound.
3. RESULTS AND DISCUSSION

3.1. Chemistry

Synthesis of mononuclear mixed ligand Ni(II) complex was achieved by mixing stoichiometric amounts of 2-aminothiazole and triphenylphosphine (Fig 1). The metal complex is amorphous in nature and soluble in DMSO and DMF. The composition of the complex was confirmed by spectroscopic analysis. The analytical data of the compound are consistent with their proposed molecular formula. The molar conductivities of 10^{-3} M of the complex (dissolved in DMF) at room temperature was measured and it was found that the value 4.29 Ω^{-1} mol^{-1} cm^2. Melting point found was 190 - 196 ºC. Yield: 65 %. The elemental analyses of the Ni(II) complex was consistent with the calculated results from the empirical formula. Elemental analysis (%) found (Calculated) C_{61}H_{59}Cl_{12}N_{14}P_{6}S_{4}: C - 51.81 (51.22), H - 4.13 (3.89), N - 5.69 (5.01), Ni -11.92 (11.99). C_{61}H_{59}Cl_{12}N_{14}P_{6}S_{4} = 491.987 g/mol.

The presence of planar aromatic and heterocyclic group enhances the biological property of the Ni(II) metal complex. Particular attention has been devoted to transition metal complexes endowed with planar aromatic side groups, which can bind with DNA of host cell by both metal ion coordination and intercalation of the aromatic moiety, this requirement is met when the metal complex has empty coordination sites available or labile ligands, which can be replaced by O or N donor atoms of the DNA bases.

IR spectral studies

The infrared spectral data of the [NiCl2(pph3)(Ath)] represented in fig 2. The metal complex displayed a characteristic (νcm) band at 3378 cm^{-1}, a medium intensity band at 1674 assigned to (νcm) the thiazole moiety. A broad band at 1645 cm^{-1} is the aromatic νC=C stretching. The band due to νC=S appears at 720 cm^{-1}, the bands 620 and 480 cm^{-1} less intense absorption bands indicating νC=S and νC=S, respectively.

Elecronic absorption studies

The electronic spectrum (fig 3) of the square planar nickel complex shows the two bands at 16,657, 18518 and 22,222 cm^{-1} which are attributed to ^2A_{1g}→T_{2g}(v_3), ^2A_{1g}→T_{2g}(v_2) and ^2A_{1g}→T_{2g}(P) (v_3) transitions. These transitions, as well as the measured value of the magnetic moment (eff = 0) suggest a square-planar stereochemistry of the compound.

NMR spectral studies

The ^1H NMR spectrum of the [NiCl2(pph3)(Ath)] (fig 4) was obtained in DMSO-d6 at room temperature. The spectrum of the Ni(II) complex showed a singlet due to the proton of thiazole-NH at 9.79 ppm. The multiplets appeared in the range 7.68 - 7.53 ppm for the aromatic ring protons of the triphenylphosphine and another two multiplets in the range 7.19 - 7.16 ppm represents the ring protons of the thiazole.

3.2. Biological activity studies

3.2.1. In vitro antibacterial and antifungal activity

The data showed that the Ni(II) complex have the capacity of inhibiting the metabolic growth of the investigated bacterial and fungal strains. The results of antimicrobial activity in different concentrations metal complex is collected in Table 1. The inhibitory activity of complex is related to the cell wall structure of the microbes, which is essential to the survival of bacteria. Some antibiotics are able to kill the bacteria by inhibiting the synthesis of peptidoglycan [16]. A possible explanation for the poor activity of these complex may be attributed to their inability to chelate metals, which is essential for the metabolism of microorganisms and/or to form hydrogen bonds with the active centres of cell structure, resulting in an interference with the normal cell cycle. Furthermore, the low activity of the complex may be due to their low lipophilicity, because of which penetration of the complex through the lipid membrane may decrease and hence, they could neither block nor inhibit the growth of the microorganism. Therefore, we confirm that the toxicity of the complex can be related to the strengths of the M–L bond, size of the cation and...
receptor sites [16-18]. The size of the inhibition zone depends upon culture medium, incubation conditions, rate of diffusion and the concentration of the antimicrobial agent (the activity increases as the concentration increases). In the present study, the Ni(II) metal complex are active against the bacteria and fungi, which may indicate broad-spectrum properties. The remarkable activity of the complex may be arising from the 2-aminothiazole and triphenylphosphine ring, which may play an important role in the antibacterial activity. The mode of action may involve the formation of a hydrogen bond through the tertiary nitrogen of the thiazole and phosphate of triphenylphosphine of rings with the active centers of the cell constituents, resulting in interference with the normal cell process.

As a result of this, the primary screening against the bacterial strains in different concentrations showed good zone of inhibition as shown in Fig 5 and Fig 6. The Ni(II) complex showed good antibacterial activity towards B. cereus, S. aureus and E. coli respectively [18] and the Ni(II) complex performs highest antifungal activity against A. aureus and A. fumigates, the primary screening against the fungal strains in different concentrations showed good zone of inhibition as shown in Fig 4 and Fig 5. The MIC study of metal complex against bacterial and fungal strains at different concentrations i.e., 1, 10, 25, 50, and 100 μg/mL was evaluated. The MIC data of antimicrobial activity of the metal complex are reported in Table 2. The Fig 7 and 8 represents the MIC activity against bacterial and fungal strains. The Ni(II) complex showed potential MIC values against bacterial and fungal strains.

Table 1 Antimicrobial activity of Ni(II) complex

<table>
<thead>
<tr>
<th>Concentration in μg/ml</th>
<th>Growth inhibition against bacteria in mm</th>
<th>Growth inhibition against fungicides in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. aeruginos</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>e</td>
</tr>
<tr>
<td>25</td>
<td>18.06±0.05</td>
<td>12.03±0.02</td>
</tr>
<tr>
<td>25</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

[NI(PPh3)2(AtCl)]

<table>
<thead>
<tr>
<th>Concentration in μg/ml</th>
<th>Growth inhibition against bacteria in mm</th>
<th>Growth inhibition against fungicides in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. aeruginos</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>e</td>
</tr>
<tr>
<td>50</td>
<td>21.19±0.05</td>
<td>15.00±0.02</td>
</tr>
<tr>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>22.06±0.04</td>
<td>17.06±0.02</td>
</tr>
<tr>
<td>04</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>30.00±0.01</td>
<td>18.12±0.02</td>
</tr>
<tr>
<td>01</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

*Each value is expressed as mean ± SD of three replicates for the zone of inhibition
*Stnd*: Ciprofloxacin and Stnd*: Fluconazole
3.3.2. Molecular docking studies

The results of the molecular docking revealed that the complex showed good binding interactions with the anti-microbial receptor 1STE, which evident that they showed excellent docking score -333.25 kcal mol\(^{-1}\). The lowest binding scores indicates the best docking interactions with the selected anti-microbial receptor and it supports for the wet analysis which is to be carried out on the different bacterial strains. The complex showed their best docking interactions with different amino acid residues as shown in Table 3 and indexed in the Fig 9.

**Table 3. Interaction of complex with amino acids residues of receptor and binding score values**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Docking receptor</th>
<th>Binding energy (kcal/mol)</th>
<th>Amino acid residues</th>
<th>Receptor PDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(II)</td>
<td>A. aerogenes, A. baumannii, S. aureus, V. cholerae, L. monocytogenes, E. coli, A. niger, A. fumigatus</td>
<td>-333.25</td>
<td>Asp88, His31, Tyr32, Lys56, Leu56, Leu58, His79, Glu81, Pro749</td>
<td>1STE in Staphylococcus aureus</td>
</tr>
</tbody>
</table>

4.3. Antioxidant activity

**Free radical scavenging activity using the DPPH method.**

The DPPH radical scavenging activity data represented in Table 4 and Fig 10. DPPH solution in methanol gives strong absorbance at 517 nm. If DPPH abstracts a hydrogen radical from an external source, the absorption decreases stoichiometrically.
Table 4: Radical scavenging activity of complex

<table>
<thead>
<tr>
<th>Concentration (µL)</th>
<th>[Ni(pphp)(Adh)Cl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>114.24 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>84.75 ± 0.39</td>
</tr>
<tr>
<td>15</td>
<td>40.95 ± 0.14</td>
</tr>
<tr>
<td>20</td>
<td>29.60 ± 0.02</td>
</tr>
<tr>
<td>25</td>
<td>29.25 ± 0.18</td>
</tr>
</tbody>
</table>

Fig 10. Antioxidant activity of complex

5. CONCLUSION

In the present work, we successfully designed and developed a mixed ligand Ni(II) mixed ligand complex. The Ni(II) complex has been characterized by various analytical techniques. The metal complex was further studied on its pharmacology by exposing to antimicrobial and antioxidant activity in different concentration. The Ni(II) mixed ligand complex performs good activity against both bacterial and fungal strains. The MIC studies of metal complex against bacterial and fungal strains at serial dilution were evaluated. Molecular docking studies reveals that Ni(II) complex have comparatively good binding interactions at amino acid residues presents at the active core of the receptor which evident that they are more biological potent this computational study is further supported from the invitro biological studies on bacterial and fungal strains.

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References


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