Synthesis, structural interpretation and biological activity of a transition metal-guanidine complex

Shahzara Changez 1, Faiza Asghar 1, Zaheer Ahmed Nizami 1

1 Department of Chemistry, University of Wah. Wah Cantt
zarachangez@yahoo.com, dr.faiza.asghar@uow.edu.pk, dr.zaheer.ahmad@uow.edu.pk

ABSTRACT

The aim of present research work is to synthesize, characterize and to study the biocidal activities of transition metal complex of guanidine which follows three basic steps. Firstly, thiourea was synthesized by reacting potassium thiocyanate with an acid chloride and substituted amine. In the second step, guanidine was prepared by reacting the synthesized thiourea with substituted amine and triethylamine in DMF as solvent, followed by addition of HgCl2. Finally, transition metal complex of guanidine was synthesized by reacting guanidine ligand with metal salt in a suitable solvent at room temperature. The purified products were characterized by FTIR spectroscopy, atomic absorption spectroscopy and elemental analysis. These complexes were also screened for antibacterial and antifungal activities, which validated that they can serve as efficient antimicrobial agents.

Keywords: Guanidine, Transition metal complex, Antibacterial, Antifungal.
1. Introduction

Guanidines are the compounds which contain \( \text{CN}_3 \) unit, and were first produced by the oxidative degradation of guanine in 1861.[1] Due to the presence of three nitrogen atoms and resonance stabilization of its conjugated acids these are considered as strongest super bases among amine derivatives.[2] Two Y-shaped symmetry-independent molecules were interconnected through hydrogen bonding network in the unit cell.[3,4] Substituted guanidines are a field of principal investigation as different substituents on nitrogen atoms can be introduced, thus modifying guanidines. These substituents can be aryl/alkyl groups which may comprise additional e–donating atoms which play potent role in attachment of ligand to the metal center.

Guanidines exist in natural products and are the main unit in biological, agrochemical and in drugs. They comprise a significant class of heteroatoms of organic compounds and act as ancillary ligands for stabilizing different metal complexes and also act as base catalysts.[5] Guanidines possesses a huge range of applications, such as bioinorganic chemistry, medicinal chemistry, polymerization catalysis [6], as preparatory materials for the synthesis of useful drugs and other preparations. Guanidine and its derivatives have been applied to cure diseases such as hypertension and diabetes,[7] and also possess potent biocidal activities. Important antibiotic drugs include: Streptomycin, Trimethoprim’s Polyhexamethylene bis-guanidine, and others, are used in curing urinary tract infections and as a sterilizer for water pools, and in wound healing.[8] Commercially available guanidine fungicides are dodine [1-dodecylguanidinium acetate], and guazatine [Bis (8- guanidine-octyl) amine].[9] One of the guanidine based drug Melarsoprol is used to treat sleeping sickness.[10] The structure activity relationship studies of guanidines have shown that the pharmacodynamics properties of the compounds strongly depend on the substituent present on the nitrogen atoms. Guanidine derivatives having extended conjugation and electron withdrawing groups exhibited a notable increase in lipophilicity. Synthesis of more lipophilic guanidines is of particular interest due to their migration through the cell membrane.[11]

Guanidines form variety of complexes with transition metals mainly with Cu, Ni, Zn, Fe, Pt, Pd, Mo etc. and these guanidine metal complexes display certain pharmacological characteristics like antiseptic and antitumor activities. The N-donor stabilized cobalt complexes acts as promoter for ethene polymerization and commercially beneficial oxidation processes e.g. oxygenation of unsaturated hydrocarbons and oxidation of saturated hydrocarbons.[12]

Due to the synthesis of variety of metal complexes the biologically active guanidine complexes have captivated considerable attention. Ethylenediamine and antiulcer drug famotidine cobalt (III) complexes were prepared and examined with the help of XRD. Antiseptic and fungicidal activities of produced blend display more refinement and well growth inhibition as compare to original product.[13] Diiron (III) guanidine complexes were described to increase the speed of DNA breakage.[14] Complexes of Cu (II) with ligand such as fluorinated \( \alpha \)-hydroxycarboxylates are stated as powerful antileishmanial compounds.[15] In this paper, we have described the design, preparation, structural elucidation, and biocidal behavior of a new substituted guanidine and its metal complex owing to its unique properties.
2. EXPERIMENTAL

All the chemicals were purchased from Merck/Sigma Aldrich. Solvents were distilled applying established methods. Melting points were noted using digital melting point apparatus model BIBBY Scientific Limited. FTIR data was obtained on BRUKER model ALPHA FT-IR Spectrophotometer.

2.1 Procedure for the synthesis of Guanidine

Firstly, the formation of thiourea was performed by adopting the scheme stated formerly by our group with some variations. [16] Synthesized thiourea was mixed with substituted amine in DMF in equimolar ratio with two equivalents of triethylamine maintaining temperature below 5°C using ice bath with continuous stirring for 30-45 min. Afterwards, one equivalent of HgCl₂ was added with vigorous stirring for 30 min in ice bath. After 30 mins ice bath was removed and the reaction mixture was left for 48 h stirring after which it turned black. Next day filter it and pour the filtrate onto ice and again filter. The product obtained on the filter paper was guanidine.

2.1.1 N,N-methyl phenyl-N′-phenyl acetyl-N''-(3-bromophenyl) guanidine

Quantities used were 5.12 g (20 mmol) thioureas, 2.0 ml (20 mmol) 3-bromoaniline, 5.6 ml (40 mmol) Et₃N, 5.44 g (20 mmol) HgCl₂, Yield: 72%, (4.54 g). m.p. 94-96 °C, FTIR data : 3299, 3145 cm⁻¹(N-H), 1594 cm⁻¹ (C=O), 1571 cm⁻¹ (C=N). Elemental analysis data: Calculated: C, 62.57; H, 4.77; N, 9.95. Found: C, 62.71; H, 4.89; N, 10.04.

2.2 Procedure for Guanidine Metal Complex

The solution of transition metal salt (Cu (CH₃COO)₂·H₂O) in methanol was mixed with the synthesized guanidine ligand (2 mmol) in 15 ml methanol at ambient conditions. Stirring was done for 4 h. The precipitates obtained were filtered off and washed with methanol and were dissolved in chloroform /n-hexane mixture (1:1) and kept for drying to furnish crystals of desirable metal complex and these were then separated from the mother liquor.

2.2.1 Bis [N,N-methyl phenyl-N′-phenyl acetyl-N''-(3-bromophenyl) guanidato]Cu(II) 

Quantities used were 1.26 g (4 mmol) N,N,N,N-methylphenyl-N′-phenyl acetyl-N''-(3-bromophenyl) guanidine, 0.4 g (2 mmol) Cu (CH₃COO)₂·H₂O, Yield: 80%. (1.11 g). m.p. 224-226 °C, FTIR data, 3425 cm⁻¹(N-H), 1571 cm⁻¹ (C=O), 1553 cm⁻¹ (C=N). Elemental analysis data: Calculated: C, 58.32; H, 4.23; Cu, 7.01; N, 9.27. Found: C, 58.45; H, 4.10; Cu, 7.13; N, 9.18.

2.3 Antibacterial activity

By using disc diffusion method, antibacterial activities of synthesized compounds were studied for five typical gram positive (Staphylococcus aureus, Salmonella typhimurium, Micrococcus luteus) and gram negative (Bordetella bronchiseptica and Enterobacter aerogenes) bacterial strains. Before using, the microbial isolates in examination were initially cultured for 18h in a nutrient broth and consistent to 0.5 M McFarland turbidity measurements (106cfuml⁻¹). 2.3 g of supplement agar were dissolved in100ml distilled H₂O (pH=7) to prepare sterile medium. This sterile medium was autoclaved and chilled to 45 °C and seeded. By dissolving 75mL, three nutrient medium dishes were prepared. By using sterile 6mm diameter cork borer, wells were bored into agar. For 2 h, approx. 100 µL of every investigation sample was injected into wells, allowed to settle at ambient conditions and nurtured at 37 °C. Through panels fixed in parallel the corresponding diluents were
castoff to seal the well. Following, culturing of plate for 24 h at 37°C, the width of region of prohibition was calculated. At a concentration of 1mg/ml, properties were matched with penicillium (+ive control). The comparative %age of verified complex was determined using formula given bellow:

Relative percentage inhibition of tested complex

\[ = 100 \times \frac{(A-B)}{(C-B)} \]

Where:

A = to total zone of probation of specimen.
B = to total zone of inhibition of solvent.
C = to total zone of inhibition of original drug.

2.4 Antifungal activity

Three antifungal strains, specifically Aspergillus fumigatus, Fusarium moniliforme and Aspergillus flavus, were used to check the sensitivity of prepared compounds by using agar tube dilution method. In 1ml Dimethylsulfoxide, 0.002g of compound was added to prepare sample. Similarly for preparing culture, 6.5g of dextrose agar was mixed for each 100ml distilled H2O (pH=5.6). Afterwards, in screw capped tubes, 10 ml of glucose resin was mixed tubes and sterilized at 121°C for 21 min. Tubing’s were aerated at 50°C and to the compound obtained from standard solution, 70 µL of glucose agar was added. The tubes packed with media were afterwards hardened in inclined position at ambient temperature. Considering individual sample, three slants of test compounds were made. Tubing containing hardened culture and investigation sample were packed in a four millimeter fraction of inoculant, obtained from 7 days previous fungal culture. Single test tube of individual sample was arranged for positive control. The test tubes were incubated for a week at 28°C. During culturing, the cultures were observed two times in seven days. By evaluating the straight length (mm) of fungus in stand, calculations were noted and growth incubation was determined w.r.t control. %age inhibition was calculated as following:

Percentage Inhibition = 100 - linear progress in test (cm)/linear progress in control (cm)

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic route for the production of substituted thiourea is outlined in Scheme1. The compounds were assembled as stated in the experimental section.

![Scheme1. Synthesis of Thiourea](image)

Similarly, the pathways adopted for guanidine and its metal complex is sketched in scheme 2 and 3, respectively. The compounds have been fabricated as described in the experimental section.
Structural interpretation of compounds was done by adopting different analytical techniques like FTIR, elemental analysis and atomic absorption spectroscopy (AAS).

3.2 Spectral Study

3.2.1 FT-IR Study

In the FT-IR spectra of ligand, there are two N-H bands appearing at 3299-3145 cm\(^{-1}\) while the C=O band appears at 1594 cm\(^{-1}\). The relatively lower frequency value of C=O than normal amides (1690-1630 cm\(^{-1}\)) is due to the intramolecular N'...H...O interaction that weakens the C=O band. In case of complex, the N-H peak appears at relatively higher frequency 3425 cm\(^{-1}\) implying that this N-H is not involved in intramolecular hydrogen bonding. This single N-H peak in complex also depicts the deprotonation of ligand and subsequent coordination of ligand with metal center through nitrogen atom. The lower frequency value of C=O (1571 cm\(^{-1}\)) in complex also verifies the co-ordination of oxygen atom with metal ion which reduces the double bond character of C=O.

3.2.2 Elemental Analysis

The percentage of C, N, and H were analyzed by CHNS analyzer whereas copper was determined through AAS. From the values given in experimental section it can be noted that the results obtained are in
close agreement with the calculated ones. The elemental analysis being a good characterization tool confirms that the metal to ligand ratio in complex is 1:2.

3.2.3 Biological activities

Antibacterial and antifungal activities of all compounds were performed. The zone of inhibition shows average values of three reading with standard deviation. Synthesized compounds exhibit good activity against microbial and fungal strains but less than that of standard drug. From the values given in Table 1 and 2, it can be noted that guanidines show less activity (antibacterial as well as antifungal) than that of the complex.

Table 1. Bactericidal activity of synthesized samples and standard drug.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Micrococcus luteus</th>
<th>Salmonella typhimurium</th>
<th>Staphylococcus aureus</th>
<th>Enterobacter aerogenes</th>
<th>Bordetella bronchiseptica</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>14.2±0.58</td>
<td>16.6±0.58</td>
<td>14.5±0.33</td>
<td>10.7±0.33</td>
<td>12.8±0.33</td>
</tr>
<tr>
<td>M1</td>
<td>18.9±0.60</td>
<td>22.3±0.55</td>
<td>19.1±0.03</td>
<td>17.4±0.33</td>
<td>17.0±0.70</td>
</tr>
<tr>
<td>PC</td>
<td>27.3±0.33</td>
<td>26.3±0.33</td>
<td>26.7±0.33</td>
<td>27.3±0.33</td>
<td>27.3±0.33</td>
</tr>
</tbody>
</table>

PC = penicillin (1mg/mL) was used as original drug (positive control), while DMSO as negative control. a = Region of inhibition in mm.

Table 2. Antifungal activity of synthesized samples and standard drug.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Aspergillus flavius</th>
<th>Aspergillus fumigatus</th>
<th>Fusarium moniliforme</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>4.15±0.33</td>
<td>3.71±0.33</td>
<td>4.63±0.03</td>
</tr>
<tr>
<td>M1</td>
<td>1.86±0.33</td>
<td>3.12±0.06</td>
<td>2.20±0.07</td>
</tr>
<tr>
<td>PC</td>
<td>0.83±0.01</td>
<td>0.89 ± 0.03</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>NC</td>
<td>10.3±0.3</td>
<td>10.7 ± 0.03</td>
<td>10.3 ± 0.3</td>
</tr>
</tbody>
</table>

PC = Terbinafin (1mg/mL) was used as model drug (positive control), while Dimethyl sulfoxide was used as negative control (NC).
4. CONCLUSION

In this paper, guanidine-metal complex was synthesized and structurally characterized by FT-IR spectroscopy, which confirms the purity of complex. Moreover, the synthesized complex was explored for biological activities, which indicates the complex is promising biocidal (antifungal, antibacterial) agent showing greater activity as compared to the parent ligand.

5. ACKNOWLEDGEMENT

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6. REFERENCES


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