Evaluation of in Vitro Antimicrobial and in Vivo Cytotoxic Properties of Some Novel Titanium-Based Coordination Complexes

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The aim of the present study was to determine the antimicrobial and cytotoxic activities of eight novel titanium(III) based coordination complexes [Ti(Pht)2(ot-serine)2, S1], [Ti(Pht)2(glycine)2, S2], [Ti(Pht)2(cystine)2, S3], [Ti(Pht)2(i-leucine)2, S4], [Ti(Suc)2(i-leucine)2, S5], [Ti(Suc)2(cystine)2, S6], [Ti(Suc)2(cystein)2, S7] and [Ti(Suc)2(ot-serine)2, S8] against several gram-positive and -negative bacteria, fungi and brine shrimp nauplii. The investigation showed that almost all of the complexes were moderately active against tested bacteria and fungi at high concentration (200 μg/disc) compared with the standard antibiotic, amoxicillin and the antifungal agent, nystatin. In vivo lethality bioassay experiment showed that only S5 and S6 among the complexes had better cytotoxic effect than standard gallic acid. The LC50 values of these two complexes were found to be 1.00 and 1.21 μg/ml, respectively. Thus the results suggest that only two complexes (S5, S6) among the titanium(III) based coordination complexes show the anticancer properties comparable to the standard cytotoxic agent, and further studies of these two complexes may be helpful for their clinical implication.

Key words mixed ligand complex; antimicrobial activity; cytotoxicity activity

Metal coordination complexes had been widely studied for their antimicrobial1,2 and anticancer properties.2–8 Over the past 30 years, platinum-based drugs, notably cisplatin and carboplatin, have dominated the treatment of various cancers by chemical agents. McGowan9 reported the first clinical trials of cisplatin in 1971, with official approval being granted in the US in 1978. By 1983, cisplatin was the US’s biggest selling antitumour drug and is still one of those most widely used. It is one of the most effective drugs for treating testicular, ovarian, bladder and neck cancers. Despite the success of cisplatin, however, it lacks selectivity for tumour tissue, which leads to severe side effects. These include renal impairment, neurotoxicity and ototoxicity (loss of balance/hearing). Various tumor cell lines are now growing resistance to cisplatin, e.g., acquired cisplatin resistance in some (mainly murine) preclinical tumor models.9 Recently there has been considerable interest and increased research activity in developing other transition metal compounds as anticancer drugs, which are less toxic than the platinum-based drugs. Among the other transition metal complexes the titanium complex, titanocene dichloride (TiCp2Cl2) is the only metalloidene-based compound to have entered clinical trials for its potent and broad spectrum activity in mammalian tumors. Compared to standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblasticine, titanocene dichloride was found to exhibit higher cytotoxicity in renal cell carcinoma.10 The titanocene dichloride was found to exhibit more efficacy in a human ovarian cancer xenograft model than cisplatin.11 Recently some derivatives of titanocene dichlorides showed enhanced anti-cancer activity.12 Now scientists all over the world are seeking new titanium-based complexes of potent anti-tumor effects with a different mode of action in the hope of adding new chemotherapeutic agents to the arsenal of weapons used against the world’s most life threatening disease, i.e., cancer. Therefore, it is in our interest to study the antimicrobial and cytotoxic properties of some newly synthesized titanium(III) based complexes.

The aim of this study was to determine the antimicrobial and cytotoxic activities of the newly synthesized titanium(III) complexes to identify their potent cytotoxic principles. We also evaluated the cytotoxicity of the titanium complexes in comparison with the anticancer agents, bleomycin and gallic acid.

MATERIALS AND METHODS

Materials The coordination complexes were obtained from the Inorganic Chemistry Research Laboratory of Rajshahi University, Bangladesh, where these were prepared and characterized.13 The tested bacteria and fungi were obtained from the Microbiology Laboratory of the Institute of Nutrition and Food Sciences (INFs), University of Dhaka, Bangladesh.

Preparation of Mixed Ligand Complexes of Ti(III) with Phthalimide or Succinimide and Amino acids [Ti(Pht/Suc)(AA)2] An aqueous solution of titanium chloride and amino acids containing a minimum amount of KOH (to make it soluble) were mixed in a molar ratio of 1 : 2 and then allowed to stand for about 10 min. Two moles of imide salts (potassium phthalimide or potassium succinimide) were then added. To get the precipitates of complexes, the mixture was then heated at 60 °C for about 25 min and allowed to stand for 10 min. The precipitates formed were removed by filtration, washed several times with distilled water and finally with alcohol and dried in a vacuum desiccator over anhydrous CaCl2.

The prepared complexes were characterized by IR, UV, magnetic moment, melting point, conductivity measurement and literature review.

According to the following equations the complexes of the titanium(III) were obtained.

H-Pht + KOH → K-Pht + H₂O

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Antibacterial Assay

In vitro antibacterial screening is generally performed by the disc diffusion method for primary selection of the compounds as therapeutic agents. The method is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally, the more susceptible the organism, the larger is the zone of inhibition. The method is essentially a qualitative or semi quantitative test indicating sensitivity or resistance of microorganisms to the test materials as well as the bacteriostatic or bactericidal activity of a compound. The antibacterial activity of eight novel titanium(III) based coordination complexes was tested against the three pathogenic fungi and four pathogenic bacteria. The MIC of the complexes was determined against each of the test organisms and noted. From these data, the percentage of mortality of the nauplii was calculated for each concentration and the LC50 values were determined using probit analysis.

RESULTS

Antibacterial Activity

The complexes at the concentration of 30 μg/disc were almost inactive against the tested bacteria but showed activity at the concentration of 200 μg/disc. The titanium complexes S7 and S8 showed slightly higher activity against the tested bacteria than other complexes. The zones of inhibition were found as 18 mm for the complex S7 against S. dysenteriae and 17 mm for the complex S8 against S. β-haemolyticus (Table 1). The results were performed four times to minimize errors.

Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentration (MIC) of a compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. The MIC of the complexes was determined against four pathogenic bacteria B. subtilis, S. β-haemolyticus, E. coli and S. typhi by serial dilution technique. The results were compared with the standard amoxicillin.

Antifungal Assay

The antifungal activity of the complexes was tested against the three pathogenic fungi Candida albicans, Aspergillus niger and Aspergillus fumigatus at a concentration of 200 μg/disc−1 for each. The media used in this respect was potato dextrose agar (PDA). The activity was determined after 72 h of incubation at room temperature (30 °C).

Cytotoxicity Bioassay

Brine shrimp lethality bioassay is a recent development in the assay procedure of bioactive compounds which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g., anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds. Here, in vivo lethality test was carried out using brine shrimp nauplii eggs (Artemia salina L.). Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In the other side of the tank was placed a light source to attract the nauplii. After 2 d of hatching period the nauplii were ready for the experiment. Ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. After 24 h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. From these data, the percentage of mortality of the nauplii was calculated for each concentration and the LC50 values were determined using probit analysis.

Table 1. In vitro Antibacterial Activity of the Coordination Complexes S1, S2, S3, S4, S5, S6, S7, S8 and Standard Amoxicillin

<table>
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<tr>
<th>Diameter of zone of inhibition (in mm)</th>
<th>S1 (μg/disc)</th>
<th>S2 (μg/disc)</th>
<th>S3 (μg/disc)</th>
<th>S4 (μg/disc)</th>
<th>S5 (μg/disc)</th>
<th>S6 (μg/disc)</th>
<th>S7 (μg/disc)</th>
<th>S8 (μg/disc)</th>
<th>Amoxicillin (μg/disc)</th>
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<tr>
<td><strong>Gram positive bacteria</strong></td>
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<td>Bacillus subtilis</td>
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<td>Streptococcus β-haemolyticus</td>
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<td><strong>Gram negative bacteria</strong></td>
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<td>Shigella dysenteriae</td>
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<td>Pseudomonas aeruginosa</td>
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32 and 64 μg/ml, respectively; and for the compound S₈ 32, 64, 32 and 64 μg/ml, respectively against the same tested bacteria. The MIC values of these four complexes (S₅, S₆, S₇, S₈) were lower than that of the standard amoxicillin (4—8 μg/ml).

**Antifungal Activity**  Table 2 showed that the complexes were moderately active against the tested fungi at high concentration in comparison with the standard nystatin except complex S₄. The zone of inhibition against *C. albicans* and *A. niger* was found to be 16 and 14 mm for complex S₇. Complex S₇ showed maximum activity against the tested *A. fumigatus*.

**Cytotoxic Activity**  The mortality rate of brine shrimp napuli was found to increase with increase in the concentration of complexes. Table 3 summarizes the LC₅₀ values of the complexes: S₁, S₂, S₃, S₄, S₅, S₆, S₇, and S₈ were found to be 20.26, 3.96, 2.90, 4.36, 3.31, 4.86, 1.21, and 4.33 μg/ml, respectively. The standard anticancer drug bleomycin gave its LC₅₀ value at 0.41 μg/ml. The lowest LC₅₀ values were found for complexes S₁ and S₈ with the values of 1.00 and 1.21 μg/ml, respectively. The maximum LC₅₀ value (20.26 μg/ml) was shown by complex S₁.

### DISCUSSION

Compared to the standard antineoplastic agents cisplatin, doxorubicin, mitoxantrone and vinblastine, titanocene dichloride (titanium complex) was found to exhibit higher cytotoxicity in renal cell carcinoma. The titanium based complexes were also found to exhibit more efficacy in the mammalian cancer model than cisplatin. Therefore, it is of interest to explore some novel titanium based complexes as potent cytotoxic agents which might act as potent anticancer agents in a clinical trial.

In the present investigation, we synthesized eight novel titanium(III) based coordination complexes and studied their cytotoxicity as well as antimicrobial potency. The newly synthesized titanium(III) based complexes displayed poor antibacterial activity, but gave moderate activity with the increase of concentrations. The MIC values (32—64 μg/ml) of the complexes against the tested bacteria indicated poor antibacterial activity compared with the standard antibiotic, amoxicillin. This is probably the first report of poor antibacterial activity of titanium complexes. Therefore, our findings are interesting as we did not find any reports to support the antibacterial activity of these complexes.

All the titanium complexes except S₄ showed moderate antifungal activity compared with standard nystatin. This is an interesting finding as very few reports have been made on titanium complexes as antifungal agents. Our present investigations show how these complexes can be used as antifungal agents. As different ligands modify the antifungal activity of the complexes, so proper ligand selection may reveal titanium complexes to be potent antifungal agents. Therefore, the present findings may also open a new search for these complexes for use in fungal diseases.

Among the eight new complexes of titanium used in the present investigation, only S₁ and S₈ showed potent cytotoxic effect. The cytotoxicity data of these two complexes are very near that of standard bleomycin and gallic acid. Therefore, we may suggest that complexes S₁ and S₈ are the most potent cytotoxic agents among the complexes. The cytotoxic properties of titanium complexes were previously reported and our present findings displayed a similar type of properties for these newly synthesized complexes. Cytotoxicity of cisplatin was found to be higher (LD₅₀ = 0.02 μg/ml) in previous literature against the L1210 tumor cells. In this present investigation of brine shrimp lethality bioassay we obtained higher cytotoxicity data for the two new titanium based complexes S₁ and S₈, and further investigations are required to evaluate
their cytotoxic effects on cancer cell lines.

In conclusion, we propose the probable use of complexes S7 and S8 as potent cytotoxic agents. Further studies are required to explore their potent anticancer activity in the mammalian cancer cell model for its clinical implications.

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REFERENCES