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Novel Nickel Cyclam Complexes with Potent Antimicrobial and Cytotoxic Properties

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Abstract: In the course of present investigation, nine new cyclam based coordination complexes: [Ni(cyclam)(2-napthoate)$_2$], [Ni(cyclam)(4-aminobenzoate)$_2$], [Ni(cyclam)(dinitrobenzoate)$_2$], [Ni(cyclam)(3-hydroxybenzoate)$_2$], [Ni(cyclam)(4-hydroxybenzoate)$_2$], [Ni(cyclam)(3-nitrobenzoate)$_2$], [Ni(cyclam)(4-nitrobenzoate)$_2$],[Ni(cyclam)(1-adamentanedicarboxylate)$_2$], [Ni(cyclam)(1,3-adamentanedicarboxylate)$_2$] (T_1-T_9) respectively were tested for their therapeutic effects as the antimicrobial and cytotoxic activities of against Gram-positive and Gram-negative bacteria, fungi, and brine shrimp nauplii. The complex T_2 and T_3 showed very high antibacterial activity at the concentration of 100 mg disc^{-1} and gave its MIC values between 1-4mg mL^{-1} and 16-64 mg mL^{-1} compared to that of 1-4mg mL^{-1} for standard, kanamycin against the tested microorganisms, respectively. The complexes gave comparatively better antibacterial activity against the Gram-positives than the Gram-negatives. All the complexes showed toxicity against brine shrimp nauplii (*Artemia salina* L.) but titanium based complex T_5 exhibited potent toxicity with the LD_{50} value of 0.3970498 μg/mL compared with the reference standard gallic acid and bleomycin. These new complexes also exhibited promising antifungal activities in which the complexes T_1 and T_6 showed the most potent activities against the tested fungi. Our data shows that all the pathogenic microorganisms (Gram positive & negative bacteria and fungi) showed very high sensitivity with minimal cytotoxicity towards the complex T_1, T_2, T_3 and T_6. But further work is necessary in order to explore the exact mechanism of their antimicrobial and cytotoxic properties.

Keywords: Cyclam complexes, antimicrobial properties, cytotoxic activities

INTRODUCTION

Coordination complexes of transition metal had been widely studied for their antimicrobial[40,36] and anticancer properties[73,1,41,57,63,9]. Macrocyclic ligands are prominent in naturally occurring ligands in biology, such as the 16-membered inner ring in the porphyrin ligand of heme proteins and the 15-membered ring in the corrin ligand of vitamin B_{12}[34]. The man-made ligand cyclam (1,4,8,11-tetraazacyclotetradecane) is a 14-membered heterocycle and it, along with its derivatives which have been studies extensively owing to its capacity to bind, via various conformational isomers[30], a wide range of transition metal ions, forming complexes of considerable thermodynamic stability and kinetic inertness[65], is of interest in diverse fields[46], such as catalysis[10], selective metal recovery, anti-HIV activity[34], antibiotics[77], antitumor agents[30] Receptor for Phosphate Dianions[42] and diagnosis[55]. When the cyclam ligand is condensed with various azomycin-containing synthons, it produces chemical compounds that can chelate with radioactive metals. It is expected that these radiolabeled markers would become bound selectively to hypoxic cells on the bioreduction of their azomycin substituent[24].

The currently available, most potent, and specific CXCR4 antagonists are the nonpeptide bicyclam derivatives, which are composed of two cyclam moieties connected by a conformationally constraining linker[27]. These were originally developed as antiviral agents blocking the cell entry of T cell tropic HIV strains[74]. The prototype bicyclam, AMD3100, is a highly specific CXCR4 antagonist that inhibits binding and function of the natural chemokine ligand SDF -1α (stromal cell derived factor-1 α) with high affinity and potency[46].

Nickel compounds are known to be carcinogenic in humans and experimental animals[14,44,47]. Although the mechanisms leading to tumor formation are not clear, it is confirmed that nickel compounds can enhance the

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cytotoxicity and genotoxicity[28,70] and accumulate in genetic material[1]. But the lipophilic chelating agents, cyclam compounds exhibited a higher order of effectiveness in alleviating nickel-induced alterations compared to ethylenediamine tetraacetic acid (EDTA), cyclohexanediamine tetraacetic acid (CDTA), diethylene triamine pentaacetic acid (DTPA), and hydroxyethylenediamine triacetic acid (HEDTA), the hydrophilic chelating agents[32]. The higher efficacy of lipophilic agents may be due to their ability to bind to nickel present in extracellular fluid as well as in intracellular fluid[58]. Cyclam and cyclam derivatives revealed a higher order of efficacy against the lethal response of nickel even at a lower dose. These drugs significantly enhanced the urinary and biliary excretion of nickel and restored the altered levels of trace metals (viz., Cu, Zn, Fe, Mn) compared to triethylenetetraamine (TETA) and a tripeptide, glutathione (GSH)[3,58].

With the considerable success of coordination complexes in modern scientific era scientists all over the world are engaged to discover new bioactive complexes of potent anti-tumor[51], Anti-HIV and antimicrobial effects with the 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 cancer and other infectious diseases like AIDS, SARS etc.[33]. Therefore, it is of our interest to study the cytotoxic and antimicrobial properties of some novel nickel cyclam complexes to assess their biological potency. We have found promising cytotoxic and antimicrobial activities of these novel complexes and further studies on mammalian cancer cell lines may explore their valuable cytotoxicity which may come as potent anticancer agent(s) in the modern clinical trials.

MATERIAL AND METHODS

Preparation of Compounds: Synthesis of Cyclam base Cyclam (1,4,8,11-tetraazacyclotetradecane), nickel acetate, sodium-2-napthoate, sodium-4-aminobenzoate, sodium dinitrobenzoate, sodium-3-hydroxybenzoate, sodium-4-hydroxybenzoate, sodium-3-nitrobenzoate, sodium-4-nitrobenzoate, sodium-1-adamentanedicarboxylate and sodium-1,3-adamentanedicarboxylate were purchased from Aldrich Chemical Company. Unless otherwise noted, all chemicals and starting materials were obtained commercially and used without further purification. Nickelcyclamperchlorate was prepared according to following method[34,18].

$Ni(OAC)_2$ (2 mmoles) was stirred in water (100ml) under N_2 to give a off-white suspension. Cyclam(401.3 mg, 2 mmole) was dissolved in water (100ml) under N_2 with stirring and this solution was added dropwise to the $Ni(OAc)_2$ solution over a period of 1 hour to give a cloudy, ash solution. The mixture was left to stir overnight. The resulting cloudy, ash solution was filtered to remove the ash precipitate. This gave a pale white solution which was rotary evaporated to dryness to give a pale white powder which was dried in vacuo. $[Ni(cyclam)](OAc)_2$ (0.15 mmole) was dissolved in 1 M $HClO_4$ (2 mole equivalent, 150µl) to give a clear, pale white solution which was left in a refrigerator at 4°C to allow slow evaporation.

Synthesis of $[Ni(cyclam)(2\text{-napthoate})_2]$, T_1: A solution of sodium-2-napthoate (194.1mg, 1 mmole) was dissolved in 5 ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (228.9mg, 0.5 mmole) was made in 50 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-2-napthoate = 1:2), stirred for 45 minutes at room temperature and allowed to stand 8 days for slow evaporation. A reddish brown crystal was observed. The crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$. The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 237°C and yield value 0.2132g (70%).

Synthesis of $[Ni(cyclam)(4\text{-aminobenzoate})_2]$, T_2.: A solution of sodium-4-aminobenzoate (159.2mg, 1 mmole) was dissolved in 5 ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (228.9mg, 0.5 mmole) was made in 50 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium 4-aminobenzoate = 1:2), stirred for 45 minutes at room temperature and allowed to stand 9 days for slow evaporation. A light pink crystal was observed. The crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials, which was dissolved in CH_2Cl_2, melting point 272°C and yield value 0.1615g (60%).

Synthesis of $[Ni(cyclam)(dinitrobenzoate)_2]$, T_3.: A solution of sodium dinitrobenzoate (117.0mg, 0.5 mmole) was dissolved in 4 ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (114.5mg, 0.25 mmole) was made in 35 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium dinitrobenzoate = 1:2), stirred for 35 minutes at room temperature and allowed to stand 7 days for slow evaporation. A red crystal was observed. The crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials, which was dissolved in CH_2Cl_2, melting point 280°C and yield value 0.1202g (70%).

Synthesis of [Ni(cyclam)(3-hydroxybenzoate)$_2$], T$_4$.: A solution of sodium-3-hydroxybenzoate (80.1mg, 0.5 mmole) was dissolved in 4 ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (114.5mg, 0.25 mmole) was made in 35 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-3-hydroxybenzoate = 1:2), stirred for 35 minutes at room temperature and allowed to stand 7 days for slow evaporation. A light pink crystal was observed. The crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 271°C and yield value 0.0805g (62%).

Synthesis of [Ni(cyclam)(4-hydroxybenzoate)$_2$], T$_5$.: A solution of sodium-4-hydroxybenzoate (161.2mg, 1 mmole) was dissolved in 4 ml methanol in a 50 ml conical flask. A solution of of nickelcyclamperchlorate (228.9mg, 0.5 mmole) was made in 45 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-4-hydroxybenzoate = 1:2), stirred for 45 minutes at room temperature and allowed to stand 8 days for slow evaporation. A light pink crystal was observed. The crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 265°C and yield value 0.2215g (74%).

Synthesis of [Ni(cyclam)(3-nitrobenzoate)$_2$], T$_6$.: A solution of sodium-3-nitrobenzoate (188.1mg, 1 mmole) was dissolved in 5ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (228.9mg, 0.5 mmole) was made in 50 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-3-nitroenzoate = 1:2), stirred for 45 minutes at room temperature and allowed to stand 7 days for slow evaporation. A red crystal was observed and the crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 225°C and yield value 0.1725g (58%).

Synthesis of [Ni(cyclam)(4-nitrobenzoate)$_2$], T$_7$.: A solution of sodium-4-nitrobenzoate (188.0mg, 1 mmole) was dissolved in 5ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (228.9mg, 0.5 mmole) was made in 40 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-4-nitroenzoate = 1:2), stirred for 45 minutes at room temperature and allowed to stand 7 days for slow evaporation. A yellow crystal was observed and the crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 222°C and yield value 0.1745g (60%).

Synthesis of [Ni(cyclam)(1-adamentanedicarboxylate)$_2$], T$_8$.: A solution of sodium-1-adamentanedicarboxylate (101.1mg, 0.5 mmole) was dissolved in 4 ml methanol in a 50ml conical flask. A solution of nickelcyclamperchlorate (114.5mg, 0.25 mmole) was made in 45 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-1-adamentanedicarboxylate = 1:2), stirred for 40 minutes at room temperature and allowed to stand 9 days for slow evaporation. A yellow granulated crystal was observed and then the crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 290°C and yield value 0.1405g (75%).

Synthesis of [Ni(cyclam)(1,3-adamentanedicarboxylate)$_2$], T$_9$.: A solution of sodium-1,3-adamentanedicarboxylate (159.2mg, 1 mmole) was dissolved in 5 ml methanol in a 50 ml conical flask. A solution of nickelcyclamperchlorate (228.9mg, 0.5 mmole) was made in 55 ml methanol in a 200 ml beaker. Two solution were mixed in a calculated ratio (nickelcyclamperchlorate: sodium-1,3-adamentanedicarboxylate = 1:2), stirred for 40 minutes at room temperature and allowed to stand 9 days for slow evaporation. An off-white crystal was observed and then the crystal was washed and dried in vacuum desiccators over anhydrous $CaCl_2$.The product was free from starting materials which was dissolved in CH_2Cl_2, melting point 298°C and yield value 0.1605g (75%).

These prepared complexes were characterized on the basis of elemental analysis, magnetic moment, melting point, conductivity measurement, nuclear magnetic resonance (NMR), infrared (IR), ultraviolet (UV) spectral studies[15].

Antibacterial Screening: *In vitro* antibacterial screening is generally performed by disc diffusion method[7,6,64] for primary selection of the compounds as therapeutic agent. Disc diffusion method is equally suited to screening of antibiotics or the products of plant evaluation[38] and 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 bigger is the zone of inhibition. In this method the compounds are applied to the agar medium by using paper discs[11,21]. The method is

essentially a qualitative or semi quantitative test which allows classification of microorganisms as susceptible, intermediate or resistance to the test materials as well as bacteriostatic or bactericidal activity of a compound[62].

The antibacterial activity of the complexes T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9 was determined at a concentration of 30 µg/disc and 100 µg/disc against four gram-positive (*Staphylococcus aureus, Streptococcus b-haemolyticus, Bacillus megaterium* and *Bacillus subtilis*) and six gram-negative (*Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae, Shigella sonnei* and *Shigella shiga*) bacteria. The diameters of the zone of inhibition produced by the compounds were compared with the standard antibiotic kanamycin 30 µg/disc (Oxoid Ltd. England). The experiments were performed at four times to minimize the error.

Growth Media and Conditions: Nutrient agar was used to culture the pathogenic bacteria. Nutrient broth was used as liquid culture of all the tested bacteria and is used in the minimum inhibitory concentration determination experiments. In every case of bacterial culture the temperature was maintained at 37 ºC. Potato dextrose agar (PDA) media was prepared in the lab to maintain the fungal growth. Antifungal activity of the complexes was done of PDA petridishes spreaded with fungal spores and kept at 28 ºC for about 72 hours. For PDA preparation 20 gm Potato was extracted with distilled water 100 ml at 100 ºC for 1 hour and it was then filtered off by cotton filter. The potato juice (100 ml) was then mixed with 2 gm Dextrose and 1.5 gm agar and finally the P^H of the prepared media (PDA) was adjusted at 7.00.

MIC Measurements: A current definition of the Minimum Inhibitory Concentration, MIC, is ``the lowest concentration of test agent that inhibited visible growth of bacteria after 18 h incubation at 37°C[13,78]. The determination of the MIC involves a semi quantitative test procedure, which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. The method displays tubes of growth broth containing a test level of preservative, into which an inoculum of microbes was added. The end result of the test was the minimum concentration of antimicrobial (test materials) which gave a clear solution, i.e., no visual growth[16,19]. The MIC of each agent was determined by the serial dilution technique[72,4,48,23] using Nutrient broth medium with an inoculum size $5x10^5$ colony-forming units (CFU)/mL. Six bacterial species were used: *Bacillus megaterium, Bacillus subtilis,* Streptococcus -â-haemolyticus, *Eschericia coli Shigella sonnei* and *Shigella dysenteriae*. DMSO was used for our experiments made dilutions of the coordination complexes under test in MIC determination. Bacteria were incubated on nutrient broth slants for 18 h at 37°C. Final adjustment was made using optical density measurement for bacteria (absorbance 0.05 at a wavelength of 660 nm)[59].

Inoculum: The method of inoculation has been followed by Tanaka *et al.,*[71] and Inagaki *et al.,*[35]. Briefly, the pathogenic bacteria were cultured on a trypticase soy agar-based sheep blood agar plate for 24 h at 37°C. Bacteria are suspended in endotoxin-free sterile saline and harvested by centrifugation (3000x g, 4°C, 10 min). Organisms were resuspended in cold sterile saline and diluted to $2x10^9$ to $4x10^9$ CFU/mL, as estimated by turbidimetry. The suspension was warmed to 45C and then 10mL of the suspension was mixed with 10 of 4% (w/v) molten noble agar (Difco Laboratories, Detroit, MI) at 45°C. The agar/bacterial suspension (1.0 mL) was placed in a 1.0-mL syringe and the suspension was rapidly injected via a 26-gause needle into 49 mL of rapidly stirred ice-cooled sterile saline, resulting in solidification of the agar droplets into beads of ca. 200 µm diameter. The final concentration of agar was 0.04% (w/v) and the final number of bacteria was 2×10^7 to 4×10^7 CFU/mL.

Antifungal Screening: The antifungal activity of the complexes were tested by disc diffusion method[7,6,64] against the six pathogenic fungi *Candida albicans*, *Aspergillus niger, Aspergillus flavus, Epidermophyton floccusum, Trichoderma* species and *Penicillium* speices at a concentration of 100 µg/disc for each. The media used in this respect was potato dextrose agar (PDA). The activity was determined after 72 hours of incubation at room temperature (30 ºC).

Collection of Test Organisms: The bacterial species used in this experiment were *Streptyococcus β-haemolyticus* (ATCC-12873), *Staphylococcus aureus* (ATCC-25933), *Bacillus megaterium* (QL-38), *Bacillus subtilis* (QL-40), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922), *Salmonella typhi* (ATCC-26853), *Shigella dysenteriae* (AL-35587), *Shigella shiga* (ATCC-26107) and *Shigella sonnei* (AJ-8992) all of which were collected from the Institute of Nutrition and Food Sciences (INFS), Dhaka University and International Center for Diarrhea Disease and Research, Bangladesh (ICDDRB) Dhaka, Bangladesh. Tested fungi *Candida albicans* (ATCC 10231), *Aspergillus flavus* and *Aspergillus niger* (CCRC 31494), *Epidermophyton floccosum*, *Trichoderma* species and *Penicillium* species were collected from the Institute of Biological Sciences

(IBSc) and Pathology Laboratory, Department of Botany, Rajshahi University, Bangladesh, from their stock culture.

Brine Shrimp Lethality Bioassay: Brine shrimp lethality bioassay[61,50,53,52,37] 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. The brine shrimp assay was proposed by Michael *et al.,*[56] and later developed by Vanhaecke *et al.,*[75] and Sleet and Brendel[67]. It is based on the ability to kill laboratory-cultured brine shrimp (*Artemia* nauplii). The assay is considered a useful tool for preliminary assessment of toxicity[68] and it has been used for the detection of fungal toxins[29], plant extract toxicity[52], heavy metals[49], cyanobacterial toxins[37], pesticides[5], and cytotoxicity testing of dental materials[60].

In the present study, in-vivo lethality test was carried out using brine shrimp nauplii eggs (*A. salina* L.). Eggs were placed in one side of a small tank divided by a net containing 3.8 % NaCl solution for hatching. In other side of the tank, a light source was placed in order to attrack the nauplii. After two days of hatching period the nauplii were ready for the experiment. Three mg of the complexes were accurately measured and dissolved in 600 µL of DMSO to get a concentration of 5 mg/ml. From the stock solutions 1, 2, 5, 10, 20, 40 and 80 µL were placed in 7 different vials making the volume up to 5 ml by NaCl solution. The final concentration of the samples, in the vials became 1, 2, 5, 10, 20, 40 and 80 µg/mL respectively.

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 hours of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. The resulting data were transformed to the probit analysis[25] for the determination of LC_{50} values for the complexes.

Study Design: The nine novel complexes were collected from the inorganic research laboratory, department of chemistry, Rajshahi University, Bangladesh. In the present study the complexes were coded as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9. To determine the cytotoxicity of these complexes, we studied the brine shrimp lethality bioassay and measured the LD_{50} values after probit transformation to assess their potency. Here a simple zoological organism *Artemia salina* L. was used. Antibacterial activity was done by disc diffusion method[7,6,64] and then we determined the minimum inhibitory concentration values for the complexes to estimate their potentiality against the tested pathogenic bacteria. We also determined the antifungal activity of the new complexes against some pathogenic fungi to evaluate their antifungal property.

Statistical Analysis: Statistical analyses of the antibacterial and antifungal activities of nine novel cyclam complexes with different concentrations of each (30 and 100 µg/disc) was performed using Kruskal-Wallis test[20]. Individual antibacterial and antifungal activity differences of the tested complexes (T_1-T_9) was examined using post hoc Nemenyi's test following Kruskal-Wallis test. A significance level of 5 % was considered as significance ($P< 0.05$) in all cases. Probit analysis[25] was used to determine the LD_{50} values from the mortality data using Probit software. The cytotoxicity of the novel cyclam coordination complexes was compared with the standard gallic acid and also with the anticancer agent bleomycin. Determination of LD_{50} by probit analysis allowed the ranking of these coordination complexes with respect to their biocidal activity.

RESULTS AND DISCUSSIONS

Results:

Antibacterial Activity: At concentration of 30 µg/disc the complexes T_1, T_4, T_5 T_6 T_7 T_8 and T_9 did not show remarkable antibacterial activity whereas the complexes T_2 and T_3 showed modest antibacterial activity against the tested Gram positive and Gram negative bacteria (Table 1). The complex T_2 showed significant antibacterial activity at concentration of 30 µg/disc in comparison with the standard kanamycin. The complex T_2 showed maximum zone of inhibition of 23 mm and 21 mm against the Gram-positive *Bacillus megaterium* and *Bacillus subtilis* at the concentration of 30µg/disc whereas that of the standard, kanamycin 28 mm and 25 mm in diameter. However, complexes T_2, T_3, T_4, T_6 and T_7 showed promising antibacterial activity against the Gram positive bacteria at concentration of 100 µg/disc but T_1, T_5, T_8 and T_9 were inactive against some Gram negative bacteria (Table 1). From the data it is shown that at concentration of 100 µg/disc the complex T_2 showed remarkable antibacterial activity against the tested bacteria in comparison with the standard kanamycin. In the present investigation we found that the complex T_2 showed comparatively better antibacterial activity among the cyclam complexes against all the pathogenic test bacteria.

Minimum Inhibitory Concentration (MIC): The MIC values of the complexes against *Bacillus megaterium, Bacillus subtilis, Streptococcus -â- haemolyticus, Escherichia coli, Shigella sonnei* and *Shigella dysenteriae* were shown in Table-2. The MIC values of

Table 1: In vitro antibacterial activity of the coordination complexes T_1-T_9 (30 and 100 µg/disc) and standard kanamycin by the disc-diffusion method

Diameter of zone of inhibition (in mm)

Compounds	T_1		T_2		T_3		T_4		T_5		T_6		T_7		T_8		T_9		Kanamycin
µg/disc	30	100	30	100	30	100	30	100	30	100	30	100	30	100	30	100	30	100	30
Gram positive bacteria																			
Staphylococcus aureus	7	15	16	28	12	21	7	17	9	19	8	17	8	16	9	17	9	16	26
Streptococcus -b-haemolyticus	9	16	18	30	13	23	8	18	10	20	8	16	9	17	7	15	10	16	25
Bacillus subtilis	7	14	21	32	14	22	7	17	0	12	0	14	8	15	8	15	9	17	25
Bacillus megaterium	8	16	23	34	11	18	8	16	0	11	9	17	8	15	10	16	0	15	28
Gram negative Bacteria																			
Salmonella typhi	7	14	16	24	0	14	7	16	0	14	9	16	8	15	7	14	11	17	28
Pseudomonas aeruginosa	0	13	17	26	7	15	9	15	0	13	0	14	8	14	9	17	9	15	29
Escherichia coli	7	14	19	30	0	13	9	17	8	16	0	13	8	14	0	14	0	13	26
Shigella dysenteriae	0	15	16	27	8	16	8	16	7	14	0	13	0	13	0	13	8	14	25
Shigella sonnei	0	12	16	29	0	11	8	16	9	19	8	14	7	15	7	15	0	10	30
Shigella shiga	7	15	15	25	9	16	0	12	11	20	0	0	0	11	0	12	0	12	27

Table 2: Minimum Inhibitory Concentration (MIC) values of the complexes T_1-T_9 and standard kanamycin by the macro-dilution method

Test organisms	Minimum inhibitory concentration (µg/ ml)									
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Kanamycin
Bacillus megaterium	32	1	32	64	256	128	128	128	128	2
Bacillus subtilis	64	2	16	64	128	128	128	64	64	4
Streptococcus-b-haemolyticus	32	2	16	64	64	128	64	128	64	4
Escherichia coli	32	1	64	64	128	128	128	128	256	2
Shigella sonnei	64	4	32	64	32	128	128	128	256	4
Shigella dysenteriae	128	2	64	128	64	128	128	128	128	1

the complex T_2 against all the tested bacteria were very small, ranging from 1-4 µg/ml for tested bacteria which indicated that the complex T_2 was most active against the bacteria. For T_1 and T_3 the MIC values were found to 32, 64, 32, 32, 64 & 128 µg/ml and 32, 16, 16, 64, 32 and 64 µg/ml respectively against the bacteria; whereas the complexes T_4, T_5, T_6, T_7, T_8 and T_9 showed MIC values between 32-256 µg/ml which was indicative of their much less antibacterial properties than the other complexes. From the MIC results it was indicated that all the complexes were more active against the Gram positive bacteria than the Gram negative bacteria.

Antifungal Activity: Table-3 showed that the complexes T_1 and T_7 were noticeable active against the tested fungi at concentration of 100µg/disc with comparing the standard fluconazole. The maximum zone of inhibition against *Aspergillus flavus* and *Candida albicans* were found to be each 26mm respectively, for the complex T_2 which were near to the zone of inhibition of 13 and 15 mm respectively. The other complexes showed almost inactive against the tested fungi.

Cytotoxicity: The mortality rate of brine shrimp nauplii was found to increase with increasing the concentration of complexes. Table-4 summarizes that the LD_{50} values of the complexes T_1, T_2, T_3, T_4, T_5, T_6, T_7 T_8 and T_9 were found at 0.876, 1.906, 1.922, 1.056, 0.397, 0.766, 1.725, 0.926, and 1.297 µg/ml (ppm), respectively. The standard anticancer drug bleomycin gave its LD_{50} value at 0.41 µg/ml. The lowest LD_{50} value at 0.397 ppm was found in case of complex T_5 which was indicative of its potent cytotoxicity than the other coordination complexes in this experiment. Cyclam and nickel based

Table 3: In vitro antifungal activity of the complexes T_1-T_9 (100 µg/disc) and standard nystatin by the disc-diffusion method

Compound	Diameter of zone of inhibition (in mm)									
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Fluconazole
µg/disc	100	100	100	100	100	100	100	100	100	100
Candida albicans	26	13	8	0	0	22	16	8	0	18
Aspergillus flavus	26	15	12	8	10	22	17	8	0	24
Aspergillus niger	24	15	8	0	0	19	15	0	0	22
Epidermophyton floccosum	20	12	9	8	0	18	14	0	0	27
Trichoderma species	19	13	0	0	8	15	12	9	0	24
Penicillium species	28	14	8	8	0	20	14	8	0	22

Table 4: The results of cytotoxic effect of the complexes T_1-T_9, standard bleomycin and gallic acid

Test complexes	LD_{50} (ppm)	95% confidence limit (ppm)		Regression equation	χ^2 (with 4df)
		lower	upper		
T_1	0.876	0.484	1.585	Y = 3.935225 + 1.12946 X	1.178288
T_2	1.906	1.325	2.743	Y = 3.234488 + 1.378975 X	2.215286
T_3	1.922	1.302	2.837	Y = 3.351594 + 1.283975 X	0.787674
T_4	1.056	0.631	1.767	Y = 3.774363 + 1.196986 X	1.304117
T_5	0.397	0.157	1.002	Y = 4.365093 + 1.06022 X	0.1719799
T_6	0.766	0.360	1.629	Y = 4.172973 +.9351931 X	0.2034779
T_7	1.725	1.113	2.674	Y = 3.55393 + 1.169079 X	0.3919106
T_8	0.926	0.455	1.886	Y = 4.119807 + 0.910341 X	0.49543
T_9	1.297	0.674	2.498	Y = 4.047564 + 0.855740 X	0.2299423
Bleomycin	0.41	0.27	0.62	Y = 3.163 + 2.989 X	0.62*
Gallic Acid	4.53	3.33	6.15	Y = 3.933 + 1.626 X	1.25*

*Data collected from Hossain et al 2004.

cyclam complexes have been reported previously for their potent cytotoxic properties[3,69] and our present findings also support the previous investigations as the complex T_5 (LD_{50}=0.397 ppm) showed much cytotoxicity than any other complexes in our experiment. The complexes T_2 (the best antibacterial active) and T_1 (the best antifungal active) showed more cytotoxicity compared with the control DMSO and Gallic acid, used as standard agent[66]. In our present investigations the most active complexes T_1 (LD_{50} = 0.876ppm) and T_2 (LD_{50} = 1.906 ppm) also showed toxicity against *Artemia*.

Discussion: Compared to standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine, transition metal complex was found to exhibit higher cytotoxicity in renal cell carcinoma[43]. The titanium based complexes was also found to exhibit more effective in mammalian cancer model than cisplatin[26]. Therefore it is of our interest to explore some novel transition metal based complexes as potent cytotoxic agents which might come as potent anticancer agent in clinical trials. In the present investigations we found the complex T_5 with potent cytotoxicity. Among the other complexes only T_2, T_3 and T_9 showed promisng cytotoxic effect compared with the reference standard gallic acid. Cytotoxic properties of coordination complexes had been previously reported by many authors[73,1,41,57,63,9,8,12,17] and our present findings also displayed the similar type of properties for the newly synthesized cyclam complexes. The different LC_{50} values for the cyclam complexes indicated the different mode of actions of their cytotoxicity. Further investigations are required to explore the exact mechanism of their cytotoxic properties which may be helpful for to explore new

type of potent cytotoxic agent(s) with the hope of adding new and alternative chemotherapeutic agent(s) in clinical implications.

The newly synthesized complex T_2 displayed a fair antibacterial activity at the concentration of 30 µg/disc, but gave promising activity at concentrations of 100µg/disc. The complex T_1 showed substantial antifungal activity but the complex T_2 showed poor antifungal activity at 30 µg/disc, which may be indicative of their use as either antifungal agent or antibacterial agent. The MIC values of the complexes T_1 and T_2 against the tested organisms indicated their noticeable antibacterial and antifungal potencies compared with standard antibiotic, kanamycin and fluconazole respectively. The mechanism of biocidal activity of these coordination complexes may be due to oxidative DNA damage as the previous reports[76,39]. The different antibacterial activity of the complexes indicated their different mechanism of biocidal property and further studies are required to explore the exact mechanism of antibacterial potency[22]. It was concluded that among the tested complexes, the complexes T_1 and T_2 possesses substantial antimicrobial activity with a minimum inhibitory concentration and moderate cytotoxicity. Further, acute toxicity and other pharmacological tests are necessary to utilize the complex T_1 and T_2 as potential chemotherapeutic agents.

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