Research Article

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Antibacterial Activities of Transition Metal complexes of Mesocyclic Amidine 1,4-diazacycloheptane (DACH)

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Abstract: The titled compound 1,4-diazacycloheptane have vibrational freedom which allows it to coordinate to metal through 1st and 4th positions. Copper (II) and Nickel (II) complexes of DACH were prepared and characterized through UV-Visible, FT-IR, elemental analyses, conductance, and magnetic susceptibilities and compared to the results published in *Inorg. Chem.*, 8(3), 528 (1969). The prepared complexes bearing different coordinating or non-coordinating anions were screened against four different pathogenic bacterial strains to study anionic effect on antibacterial activity. The MIC values of all the compounds suggest that $[\text{Cu}(\text{DACH})_2\text{Br}_2]$ is almost inactive against the tested microbes except *Staph aureus*. Rest of the metal complexes are active at their respective MIC values.

Keywords: 1,4-diazacycloheptane, Coordination complexes, bioassay, minimum inhibitory concentration

1 Introduction

Acyclic molecules containing the linkage $-\text{N}(\text{CH}_2)_n-$ have been exploited as multidentate ligands for transition metal ions where it coordinates as multidentate ligand. Their chemistry offers a wide range of applications in various fields of chemistry [1-9]. The chemistry of the cyclic diamines is still far off. Piperazine is one of the cyclic six-

membered diamines and has already been reported with complexes of palladium (II) and iridium(II) [10,11]. The ring strain plays a crucial role in complex formation reactions, therefore, piperazine cannot form chelated bicyclic complexes with the smaller transition metal ions such as copper(II) and nickel(II). Thereby, ring strain has been decreased by introducing additional methylene groups between the amine functions. Nickel (II) and copper (II) were hence chelated by 1,4-diazacycloheptane (dach) and 1,5-diazacyclooctane (daco) [12], where the former is cyclic seven membered and the latter is eight membered cyclic diamine [13]. Structure of DACH is shown in Figure 1.

The metal complexation of ligands containing oxygen, nitrogen or sulfur have been coordinated with metals like copper, zinc, cobalt and iron, showing enhanced antihypertensive, antimalarial, antimicrobial properties. The complexes of the transition metal ions have been found as efficient electron transfer agents in many biological processes [14-18]. They are also acting as promoters for oxygen transport reaction [19-23]. Here in this paper we are reporting the biological activities of these complexes using minimum inhibitory concentration method in continuation of the work reported earlier [24-27].

Figure 1: Structure of 1,4-diazacycloheptane (DACH).

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2 Experimental

2.1 Instrumentation

PYE UNICAM Infrared Spectrophotometer was used for recording the far IR spectra by making the KBr discs of the sample. The absorption spectra of the complexes were measured by Jasco DEC-1 Spectrophotometer in the range of 200-900 nm. The near IR region i.e. 400-200 cm^{-1} and far IR 400-4000 cm^{-1} has been recorded with FT-IR SHIMADZU. The Molar conductance of the metal complexes was measured by preparing their solutions and recording their conductivity using conductivity meter type HI-8333. Magnetic susceptibilities of the metal complexes were measured by Gouy method at room temperature with $\text{Hg}[\text{Co}(\text{SCN})_4]$ as reference [27]. Elemental analyses were determined with Carloerba Model 1106 CHNS analyzer. The cations and anions were estimated by following different analytical procedures [28]. Melting points of the synthesized samples were recorded using Gallenkamp apparatus and reported as such without further corrections.

2.2 Agar Diffusion method

In this method, bacterial culture suspensions are inoculated on the surface of assay agar medium (base layer). The holes were used as a reservoir for compound or antibiotics. The sample to be tested present in the reservoir come into contact with inoculated medium and after overnight incubation at 37°C, the plates were observed for the zone of inhibition surrounding the reservoir. The zone of inhibition is the clear area around the reservoir, showing the inhibition of the microorganism by the diffused substances through the agar. The diameter of the clear zone around the reservoir (zone of inhibition) was measured. However, if the sample to be tested is ineffective, then no zone of inhibition will develop. The antibiotic effects of the complexes were measured by Agar well diffusion method.

For the biological activities, certain solutions of the complexes were prepared in a solvent. like $[\text{Cu}(\text{DACH})_2]\text{Br}_2$. The $[\text{Cu}(\text{DACH})_2]\text{Br}_2$ was dissolved in DMF and referenced with DMF, $[\text{Cu}(\text{DACH})_2](\text{ClO}_4)_2$ was dissolved in methanol and referenced with methanol, and $[\text{Ni}(\text{DACH})_2]\text{Cl}_2$ and $[\text{Ni}(\text{DACH})_2](\text{NO}_3)_2$ were dissolved in water and referenced with water. The bacterial strains selected include *Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The activities obtained for

the reference and blank solutions were subtracted from the activities of the metal complexes. The reference used were the corresponding metal salts.

In the agar well diffusion method, six wells were dug in sided agar and one at the center, 20 μL solutions of six different concentrations were introduced into these wells. At the center well solution of maxipime was introduced as control drug. After 24 h, diameter of the zone of inhibition was measured in mm. The zone of inhibition of complexes are compared with zone of inhibition for control drug and results were interpreted accordingly.

2.3 Synthesis of piperazine 1,4-diazacycloheptane (DACH)

The solution of dibromopropane (10 mmol) was stirred at room temperature in dry ethanol for 10 min. Ethylene diamine (10 mmol) was added to this solution and continued stirring for an hour.

The reaction mixture was further refluxed after the addition of Solid KOH (0.009mol) till the appearance of white precipitates of KBr, which was then filtered off. The filtrate containing DACH [26], was solidified using rotary evaporator.

2.4 Synthesis of Metal Complexes $\{\text{M}(\text{DACH})_2\text{X}_2$ where $\text{M} = \text{Ni}(\text{II})$ or $\text{Cu}(\text{II})$ and $\text{X}^- = \text{Cl}^-$, Br^- , NO_3^- , or ClO_4^- [31]

Dehydration of the copper (II) or nickel (II) salts was achieved by adding 10-15 ml of 2,2- dimethoxypropane [24-26]. The resulting solution was stirred for 30 min. After complete dehydration the solution was filtered and 10-12 ml of anhydrous ethanol was added to it. Excess of the DACH ligand dissolved in dry diethyl ether was added in drops with vigorous stirring to the metal ion solution. Either the complexes were precipitated immediately or they have been precipitated by suction. The solid product was washed several times with copious ether-ethanol mixture. The compound was dried initially in a vacuum desiccator and kept in vacuum line for 5-6 hrs. set at 60-65°C for complete dryness.

3 Results and Discussion

The ligand was analyzed by elemental analysis, NMR and mass spectrum, whereas, the complexes were

Table 1: Analytical data of metal complexes of DACH.

Complex	Color	Dec. Pt /°C	% C	% H	% N	% Cation	% Anion
[Cu(DACH) ₂] Br ₂	Purple	187	28.47 (28.35)*	5.55 (5.69)	13.03 (13.23)	15.09 (15.00)	37.91 (37.72)
[Cu(DACH) ₂] (ClO ₄) ₂	Maroon	237	25.95 (25.69)	5.29 (5.21)	11.97 (12.11)	13.43 (13.73)	---
[Ni(DACH) ₂] Cl ₂	Yellow	---	35.27 (36.39)	7.29 (7.30)	17.05 (16.98)	17.98 (17.79)	21.20 (21.49)
[Ni(DACH) ₂] (NO ₃) ₂	Yellow	---	31.97 (31.36)	5.84 (6.31)	22.02 (21.94)	15.30 (15.32)	---

*calculated values

Table 2: Molar conductance and magnetic susceptibilities of metal (ii) complexes with DACH.

Complex	Molar Conductance, cm ² ohm ⁻¹ mol ⁻¹	Cor 10 ⁻⁶ χ _M , cgsu	μ _{eff} , BM
[Cu(DACH) ₂]Br ₂	19	1553	1.92
[Cu(DACH) ₂](ClO ₄) ₂	146	1544	1.92
[Ni(DACH) ₂]Cl ₂	123	----	Diamagnetic
[Ni(DACH) ₂](NO ₃) ₂	146	----	Diamagnetic

Table 3: Electronic absorption values for DACH complexes.

Complex	Solvent	λ _{max} , nm	u (cm ⁻¹)	x (dm ³ /mol. cm)	Comments
[Cu(DACH) ₂]Br ₂	CH ₃ NO ₃	610	16,500	214	d-d
[Cu(DACH) ₂](ClO ₄) ₂	CH ₃ NO ₃	505	19,800	304	d-d
[Ni(DACH) ₂] Cl ₂	CH ₃ NO ₃	425	23,500	100	¹ A _{1g} → ¹ A _{2g}
[Ni(DACH) ₂] (NO ₃) ₂	CH ₃ NO ₃	425	23,550	100	¹ A _{1g} → ¹ A _{2g}

characterized using elemental analysis [28], conductance, magnetic susceptibilities, IR, and electronic spectroscopic techniques [11,12]. Elemental analytical data is given in Table 1, which show the composition of the synthesized complexes.

Conductance and magnetic susceptibilities are given in Table 2. Conductance values show electrolytic nature of the complexes except [Cu(DACH)₂]Br₂ which is non electrolyte in nature [30]. Conductance values also confirm the bonding nature of anions in all the synthesized complexes.

The characteristic bands in the infrared spectra (4000-400cm⁻¹) of metal complexes are listed in Table 4. The N-H frequency is altered and moved to higher frequency region suggesting coordination through the two aza-sites of attachment as shown in Figure 2. Metal anion bands are also observed and reported.

Figure 2: Structure of DACH complexes.

Table 4: Assignment of IR bands for complexes of DACH.

Complex	ν (cm ⁻¹) C-H	ν (cm ⁻¹) C-C	ν (cm ⁻¹) C-N	ν (cm ⁻¹) N-H	ν (cm ⁻¹) M-X
[Cu(DACH) ₂] ₂ Br ₂	2930m	1420m	2160	3220m	400
[Cu(DACH) ₂](ClO ₄) ₂	2930m	1420m	2200	3220m	650s
[Ni(DACH) ₂]Cl ₂	2930m	1420m	2140	3250m	400
[Ni(DACH) ₂](NO ₃) ₂	2930m	1420m	2140	3230m	650s

s = small, m = medium

Figure 3: Crystal field splitting diagram for Ni(DACH)₂²⁺.

3.1 Nickel Complexes

The yellow Ni(DACH)₂²⁺ complexes are diamagnetic showing a planar coordination geometry in its solid state. The spectral properties of the nickel complex both in solid and solution states are essentially invariant suggesting the square planar geometry. However, in coordinating solvents the yellow color of the complex fades indicating slow decomposition. From Figure 3 it becomes apparent that the electronic excitation energy is 2.9 eV. Electronic absorption values are given in Table 3, which suggests square planar geometry for nickel complexes.

3.2 Copper Complexes

The behavior of copper (II) can be examined by comparing visible absorption spectra of Cu(daco)₂²⁺ and Cu(DACH)₂²⁺ complexes. The spectrum of [Cu(daco)₂](ClO₄)₂ both in water and nitromethane are similar. It has been observed with two charge-transfer bands in regions 240-255 nm and 280-300 nm which were assigned to

ligand-to-metal charge-transfer transitions. Whereas, the band at 500-510 nm was assigned to the forbidden crystal field transitions occurring in the 3d levels. The band at 500 nm reveals the inseparable individual d-d transitions. The UV-visible spectrum of the [Cu(DACH)₂](ClO₄)₂ complex in weakly coordinating solvent like nitromethane also reveal the same absorption band at 505 nm but the other bands in lower region of the spectrum were not observed owing to the absorption of the solvent. Similarly, the [Cu(DACH)₂Br₂] complex show only an absorption peak at 610nm greater by 105nm from the absorption shown by [Cu(DACH)₂](ClO₄)₂ and hence was assigned octahedral geometry unlike the square planar geometry for the [Cu(DACH)₂](ClO₄)₂ [29]. The data is presented in Table. 3.

3.3 Antimicrobial Activities

Metal complexation impart significant effects on biological activities. The metal complexes of the same nature have been reported with enhanced biological activity [31]. The antibacterial potency have been enhanced upon complexation as compared to neat ligand, thus opening new avenues in the fight against antibiotic resistance [31]. Therefore, the antibacterial activities of complexes were tested against selected pathogenic bacterial strains like *E.coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* and compared with standard drug maxipime.

Table 5 shows zone of inhibition of all the metal complexes against *E. coli*. At lower concentration, no inhibition zone was found, whereas at concentrations like 100 µg/20 µL and 120 µg/20 µL all the synthesized metal complexes shown low inhibitory zones varying in the range 2.0-3.0 mm in comparison to 10.6 mm zone of inhibition for maxipime. Literature reveals that metal ions, geometry around metal ions and the counter ions impart great deal of effect on the inhibition of bacterial activities. The results for *E. coli* show that copper perchlorate and nickel chloride complexes are found to be moderately

Table 5: Results of antibacterial activity of the coordination complexes of DACH against *Escherichia coli*.

Compound	Inhibition zone (mm) for 20 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 40 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 60 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 80 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 100 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 120 $\mu\text{g}/20\mu\text{L}$
$[\text{Cu}(\text{DACH})_2 \text{Br}_2]$	0	0	0	0	0	0
$[\text{Cu}(\text{DACH})_2] (\text{ClO}_4)_2$	0	0	2.0	3.0	3.0	3.0
$[\text{Ni}(\text{DACH})_2] \text{Cl}_2$	0	0	2.0	3.0	3.0	3.0
$[\text{Ni}(\text{DACH})_2] (\text{NO}_3)_2$	0	0	0	0	0	0
Maxipime						21.2

Table 6: Results of antibacterial activity of the coordination complexes of DACH against *Pseudomonas aeruginosa*.

Compound	Inhibition zone (mm) for 20 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 40 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 60 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 80 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 100 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 120 $\mu\text{g}/20\mu\text{L}$
$[\text{Cu}(\text{DACH})_2 \text{Br}_2]$	0	0	0	0	1.0	2.0
$[\text{Cu}(\text{DACH})_2] (\text{ClO}_4)_2$	0	4.0	4.0	4.0	4.0	4.0
$[\text{Ni}(\text{DACH})_2] \text{Cl}_2$	0.0	2.0	2.0	4.0	4.0	5.0
$[\text{Ni}(\text{DACH})_2] (\text{NO}_3)_2$	0.0	1.0	4.0	5.0	5.0	5.0
Maxipime						25.2

active at higher concentrations in comparison to copper bromide and nickel nitrate complexes. The reason may be attributed to geometries and presence of counter ions.

Table 6 shows that complexes show almost no inhibition at lower concentration against *P. aeruginosa*. Intermediate zone of inhibition (1.0-5.0 mm) were observed for copper perchlorate, nickel chloride and nickel nitrate complexes. $[\text{Cu}(\text{DACH})_2 \text{Br}_2]$ show weak activity at higher concentrations. The activities may be due to the geometries of the complexes. $[\text{Cu}(\text{DACH})_2 \text{Br}_2]$ was found octahedral in comparison to planar structures of the other complexes. The standard drug maxipime shown 12.6 mm zone of inhibition.

In case of the activities of all the synthesized metal complexes against *K. pneumoniae*, it has been observed that all the metal complexes show intermediate inhibition zone at higher concentration (Table 7) except the $[\text{Cu}(\text{DACH})_2 \text{Br}_2]$. Zone of inhibition for maxipime as positive control was 9.2 mm. Therefore, it can be assigned that all the metal complexes derived from DACH are behaving similarly when tested for biological activities *P. aeruginosa* and *K. pneumoniae*.

Table 8 shows that at higher concentrations all the complexes showed intermediate inhibitory zones for *S. aureus* (upto 5.0 mm) compared with maxipime as positive control (11.2 mm).

Overall, it may be revealed from the data given in Tables 5-8 that complexes of copper perchlorates and nickel chloride are moderately active against the tested bacterial strains while the complexes in which the anions are either bromide or nitrate show no activity/weak activities against one or more bacterial strains. The results for antibacterial activities show that the complexes inhibit bacterial growth significantly different from each other. These differences may be attributed to anion variation. It was already shown [11,12] that the decomposition behavior and the formation constants of these complexes vary due to the presence of different anions. Also, the behavior of these complexes for the pyramidal geometry formation is varying due to anions presence.

It has been suggested earlier that the oxidation state and apparent charge of the metal ion affects the biological activities. The apparent charge of the metal ion is partially shared with the donor ligands resulting in electron delocalization over the whole chelate system including the counter anions, thereby effectively influencing the solubility of the metal complex [32]. It has been seen earlier that chelation make the ligands more powerful and potent bactericidal agents in comparison to either the metal ion or the uncoordinated ligand. The factors like solubility, conductivity, dipole moment and cell permeability are also responsible for enhancing the biological activity [32].

Table 7: Results of Antibacterial activity of the coordination complexes of DACH against *Klesbiella pneumoniae*.

Compound	Inhibition zone (mm) for 20 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 40 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 60 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 80 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 100 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 120 $\mu\text{g}/20\mu\text{L}$
$[\text{Cu}(\text{DACH})_2 \text{Br}_2]$	0	0	0	0	0	0
$[\text{Cu}(\text{DACH})_2] (\text{ClO}_4)_2$	0	0	2.0	2.0	2.0	4.0
$[\text{Ni}(\text{DACH})_2] \text{Cl}_2$	0.0	2.0	2.0	2.0	2.0	4.0
$[\text{Ni}(\text{DACH})_2] (\text{NO}_3)_2$	0	0	2.0	4.0	4.0	4.0
Maxipime						18.4

Table 8: Results of Antibacterial activity of the coordination complexes of DACH against *Staphylococcus aureus*.

Compound	Inhibition zone (mm) for 20 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 40 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 60 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 80 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 100 $\mu\text{g}/20\mu\text{L}$	Inhibition zone (mm) for 120 $\mu\text{g}/20\mu\text{L}$
$[\text{Cu}(\text{DACH})_2 \text{Br}_2]$	0	0	0	2.0	2.0	3.0
$[\text{Cu}(\text{DACH})_2] (\text{ClO}_4)_2$	0	0	1.0	2.0	2.0	4.0
$[\text{Ni}(\text{DACH})_2] \text{Cl}_2$	0	0	1.0	1.0	2.0	4.0
$[\text{Ni}(\text{DACH})_2] (\text{NO}_3)_2$	0	0	1.0	2.0	2.0	5.0
Maxipime						22.4

In conclusion the copper and nickel complexes containing the perchlorate and chloride counter ions are found to be potentially active as compared to complexes containing bromide and nitrate.

4 Conclusion

It was found that anions presence which may be even non coordinating, play very important role in the metal-based drug identification. Perchlorates complexes of copper are much more active against pathogenic bacteria as compared to bromide complex. Similarly, chloride complexes of diamagnetic planar nickel complexes are active as compared to the nitrate complex.

Conflict of interest: Authors declare no conflict of interest.

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