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Magnetic and *in-vitro* biochemical study of Ni (II) complexed with piperazinedibiguanide ligand

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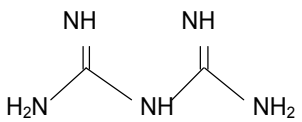
Abstract- Biguanide and substituted biguanides have long been used in biomedical science. A very common example is metformin which is used as oral antihyperglycemic drugs. Similarly, many other substituted biguanides such as polyaminopropyl biguanide (PAPB) and Proguanil used as disinfectant and antimalarial drugs respectively. Biguanide and substituted biguanide also act as very good nitrogen donor ligand for the preparation of coordination complexes with transition metal ions. Since long time synthesis of different substituted biguanides have been done and then its characterization was studied by different researchers. Biochemical studies are also going on for the complexes so that it can be used as either drug or antimicrobial agents. In this research paper, the authors have tried to find out antifungal property of piperazinedibiguanide ligand when complexed with Ni(II) metal ions. The authors have first prepared the ligand and then complexed it with metal ion by reacting with metal salts. Elemental percentage was estimated and magnetic behavior was studied. Further the complexes so obtained tested for fungal growth inhibition by disc dilution test method in both PDA and SDA media. The antifungal activity was done against two ascomycetes. While doing the test, the concentrations of piperazinedibiguanide complexes used were 400, 200 and 100µg/ml. A positive response was obtained against both *Aspergillus niger* and *Aspergillus versicolor*.

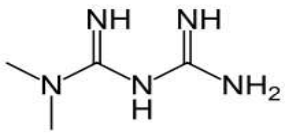
Key words: ***Aspergillus niger, Aspergillus versicolor,*** **PDA, SDA, ascomycetes**

INTRODUCTION

Biguanide[1] and its substituted derivatives are not an unknown name in the field of biomedical sciences. It is a class of drug used to lower sugar level in human blood of diabetes mellitus patients. Substituted biguanides such as proguanil used as antimalarial drugs, phenyl biguanide[2] as 5-HT_3 receptor agonist, phenylaminopropyl biguanide as disinfectant and many more.



Biguanide
(imidodicarbonimidic diamide)



Metformin

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Biguanide and its derivatives are known to have germicidal and antimicrobial activities. Due to these properties many of its substituent is used as components of hair-care products and many day to day personal hygiene products.

These formulations present a tremendous range of product applications such as:

- Chlorine-alternative pool water disinfectants
- Biocidal bandages and wound dressings
- Hair-care products
- Super absorbent gels for baby diapers & personal hygiene products
- Ink-jet printing inks
- Contact lens care solutions
- Biofilms

Malarone a combination of naphthoquinone atovaquone with biguanide proguanil, used as antimalarial drug. It is effective for multidrug resistant parasites[3] mainly for treatment of prophylaxis of *falciparum* malaria.[4]

Besides, the above described biochemical activities of biguanide and substituted biguanides complexes have been made, but the testing of antifungal activity is still missing. An attempt has been made in the present investigation to determine the antifungal activity of these complexes along with study of their magnetic behaviour.

For the present investigation antifungal activity of biguanide and substituted biguanide complexes with transition metals author have taken two fungus of ascomycetes group, *Aspergillus niger* and *Aspergillus versicolor*.

A.niger[5] is a common fungus of genus *Aspergillus*. It causes black mold disease on fruits and vegetables. Mostly found in soil and commonly present indoor environment. *A. versicolor*[6,7] is widely distributed in nature, mostly on substrates exposed to humidity or slow-decaying. Mainly found in cold regions and soil used for cultivation but very rare to be found in forests.

A.versicolor is a toxic and pathogenic fungus for both man and animals. The aflatoxins it synthesizes are responsible for several cancers.

Piperazinedibiguanide is a tetradentate ligand and can form complexes with divalent and trivalent transition metal ions. In this research work, the authors have first prepared the ligand and then complexed with transition metal ions Ni (II) by reacting with metal salts. After forming the complexes, the complexes were tested whether diamagnetic or paramagnetic. For biochemical study complexes were dissolved in respective solvent and its antifungal property was studied by disc dilution method and fungal growth inhibition was calculated. For antifungal study two media were used -PDA (Potato Dextrose Agar) medium & SDA (Sabouraud Dextrose Agar) medium. Both the media were stored in Erlenmeyer flask and autoclaved before use.

MATERIALS & METHODS

The ligand and its complexes[8-11] were prepared by the reported method as described below:

Piperazinedibiguanide sulphate

For the preparation of above mentioned compound, 4.3g of piperazine was heated with 8.4g of dicyandiamide with 75ml of water in a conical flask on water bath for 3 hours. While heating 5ml of aqueous solution of $CuSO_4$ was added in small intervals of 20 minutes. The mixture so obtained was then treated with aqueous solution of 2.8g sodium hydroxide. As a result, the mixture turns reddish (purple). The content was cooled and filtered so that the purple residue gets separated from the filterate. The purple residue was then decomposed with dil. Sulphuric acid (1:3). After cooling, white residue of piperazinedibiguanide sulphate starts depositing in the vessel. It was filtered and the white residue was washed with cold water until free from any copper ion. The white residue was dissolved further with ammonia containing little amount of sodium hydroxide. The basic solution was acidified with dil. Sulphuric acid (1:3). On cooling, white shining crystals of piperazinedibiguanide sulphate starts getting deposited in the beaker. Shining crystals were filtered, washed with cold water and dried in air.

Analysis

Found N = 29.0 %, S = 13.97%

$C_8H_{18}N_{10}\cdot 2H_2SO_4\cdot 1.5H_2O$ requires N=29.35%, S=13.41%

Nickel piperazinedibiguanide nickel hydroxide

It was prepared by adding ammonical solution of nickel sulphate to ammonical solution of piperazine dibiguanide sulphate in presence of excess aqueous solution of sodium hydroxide. The complex was obtained as buff coloured precipitate. It was filtered and washed with cold water and dried in a dessicator to a constant weight when the colour of the complex changes to yellow. The substances are insoluble in water and alcohol & liberate ammonia from ammonium salt solution.

Analysis

Found Ni = 11.00%, H_2O (by loss at 110^0C) = 26.02%

[Ni Pip$(BigH)_2$]$(OH)_2$•Ni $(OH)_2$ requires Ni=11.31%, H_2O=26.00%

Nickel piperazine dibiguanide hydroxide base

This was prepared as an insoluble orange coloured product by keeping the complex nickel chloride (1mol) in contact with a solution of caustic soda (2mol) for 2 days and then heating the mixture on the water bath for some time. The product was washed with cold water and dried in a desiccator to a constant weight.

Analysis

Found, Ni = 15.68%, H_2O (by loss at 105^oC) = 17.00%, N = 37.01%

[Ni Pip $(BigH)_2$]$(OH)_2$•$1.5H_2O$ requires Ni = 15.70% , H_2O = 16.80%, N = 37.46%

Nickel piperazine dibiguanide chloride

This can be prepared by heating the complex nickel base with a solution of NH_4Cl on the water bath until the evolution of ammonia gets ceased. The insoluble orange yellow product so obtained was washed with cold water and dried in air to a constant weight.

Analysis

Found Ni = 11.40%, N = 27.25%, Cl = 13.67%, H_2O (by loss at 110^0C) = 25.90%

[Ni Pip$(BigH)_2$]Cl_2•$7.5H_2O$ requires Ni = 11.31%, N = 27.00%, Cl = 13.60%, H_2O = 26.00%

Antifungal activity[12-24]

Antifungal activity was done by inoculating the above mentioned fungus to PDA (Potato Dextrose Agar) and SDA (Sabouraud and Dextrose Agar) medium. The medium was autoclaved before use and the ratio used for complex solution and medium was 1:10. Further inhibition of mycelium was calculated by disc dilution test method. PDA and SDA was prepared as follows:

Preparation of PDA (Potato Dextrose Agar)[25,26]

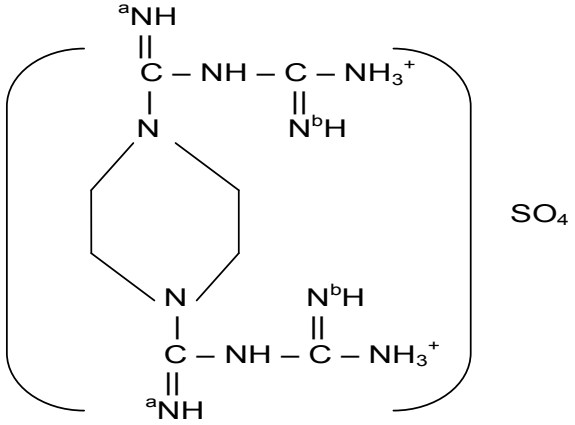
Potato tubers were taken peeled off and weighed 200g. It was chopped into small pieces and transferred to a beaker containing about 100ml of distilled water and boiled for 20 minutes and filtered with muslin cloth. 20g Dextrose, 15g agar and 2g peptone were added into the extract and gently heated. The filterate so obtained was made to 1 litre. The pH of the solution was maintained at 5.6 by using 1N HCl or NaOH and kept in Erlenmeyer flask. This solution so obtained was PDA medium and autoclaved at 121^oC for 20minutes before using.

Preparation of SDA (Sabouraud and Dextrose Agar Medium)[25,26]

It was prepared by combining the ingredients water, dextrose, agar, peptone & antibiotics separately, in many different variations. In the case of using premix, the proper amount (around 70gms) was mixed with one litre of water and heated to dissolve the agar. pH of the medium was adjusted with one molar solution of hydrochloric acid to lower pH. The pH was maintained at 5.5. The medium was then autoclaved and stored at room temperature. The medium can be used to inoculate with fungal spores and mycelium inhibition growth was counted by usual method. After preparation of the media, pure culture of the fungal spores are prepared and isolated by Warcup method.[26] On successive inoculation in new petridish with the medium, pure culture of a particular fungal spore had been obtained. After getting separated, the fungus was identified under microscope and testing was done in UV chamber of the Chemistry Department, Magadh Mahila College, Patna University.

RESULTS & DISCUSSION

Piperazinedibiguanide sulphate is a quadridentate chelating ligand and its complexes with bivalent ligands are known.[8]



Piperazinedibiguanide sulphate

The ligand has been found to be co-ordinate with N^a & N^b of both the biguanide substituents forming usual six membered ring with metal ion. The coordination complexes of a number of biguanide with various metal ions have been investigated for its biochemical activity.

All nickel (II) complexes were diamagnetic except Nickel piperazinedibiguanide nickel hydroxide which was found paramagnetic. This was because of ionic nickel (II)

ion. The diamagnetic behavior of the nickel (II) complexes suggests square planer geometry.

The complexes prepared in laboratory were dissolved in DMSO solvent at different concentrations and was then treated with the fungus isolated in UV chamber. These solutions showed antifungal property against two ascomycetes, *Aspergillus niger* and *Aspergillus versicolor*. Both these fungus had been cultured over PDA and SDA medium. When these had been investigated over PDA and SDA medium they controlled 100% growth of the fungus when the concentration of the complex is increased to 500-800 µg/ml[17]. It was interesting to note that the complexes of biguanide complexes with Ni(II) inhibited the growth to more than 90% at 400µg/ml also. The micelle inhibition was then counted, the data of which are a under.

Table I- Magnetic behaviour of complexes

Compound	Magnetic behaviour
Nickel piperazinedibiguanide nickel hydroxide	paramagnetic
Nickel piperazine dibiguanide hydroxide base	diamagnetic
Nickel piperazine dibiguanide chloride	diamagnetic

Table II- Percentage inhibition of growth in fungus *Aspergillus niger* at indicated dose

Metal complex	Concentration	% Inhibition of fungal growth	
		PDA	SDA
Nickel piperazine dibiguanide nickel hydroxide	400 µg/ml	98.30%	94.70%
	200 µg/ml	88.51%	83.92%
	100 µg/ml	80.17%	78.72%
Nickel piperazine dibiguanide hydroxide base	400 µg/ml	92.60%	89.51%
	200 µg/ml	86.10%	75.44%
	100 µg/ml	69.21%	62.51%
Nickel piperazine dibiguanide chloride	400 µg/ml	97.58%	89.52%
	200 µg/ml	85.91%	80.14%
	100 µg/ml	82.00%	73.59%

Table III- Percentage inhibition of growth in fungus *Aspergillus versicolor* at indicated dose

Metal complex	Concentration	% Inhibition of fungal growth	
		PDA	SDA
Nickel piperazine dibiguanide nickel hydroxide	400 µg/ml	93.53%	90.21%
	200 µg/ml	72.41%	69.63%
	100 µg/ml	62.27%	59.95%
Nickel piperazine dibiguanide hydroxide base	400 µg/ml	85.36%	83.41%
	200 µg/ml	71.92%	68.51%
	100 µg/ml	58.84%	52.87%
Nickel piperazine dibiguanide chloride	400 µg/ml	87.76%	85.04%
	200 µg/ml	65.21%	63.47%
	100 µg/ml	47.27%	45.57%

CONCLUSION

From the data obtained, it was found that all nickel (II) complexes are diamagnetic except one. This is due to free Ni(II) metal ion that contain unpaired electrons. From the biochemical test, the author concludes that in PDA medium, Ni (II) complexes with piperazinedibiguanide can inhibit growth of both the fungi more than 85% at 400 µg/ml. In SDA medium for all the complexes, the growth got reduced by more than 80% for both the fungi.

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