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TRANSITION METAL BASED BIOLOGICALLY ACTIVE COMPOUNDS AS SELECTIVE ANTIFUNGAL AGENTS

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ABSTRACT

The *in vitro* antifungal activities of few novel 4-aminoantipyrine derived Schiff bases and their metal complexes were tested against fungi such as *Aspergillus niger, Fusarium solani, Culvularia lunata, Rhizoctonia bataicola* and *Candida albicans*. All the metal complexes showed stronger antifungal activities than the free ligands. The synthesized compounds were subjected to molecular docking studies for the inhibition of the enzyme glucosamine-6-phosphate synthase which is a new target for antifungals

KEYWORDS

Antifungal agents, Schiff base, 4-aminoantipyrine, Transition metal complexes

INTRODUCTION

Fungi are very versatile organisms that live in and on animals as part of their natural flora, but they can also be the cause of numerous infections. Fungal infections are a growing problem in contemporary medicine[1]. The emergence of new diseases and infections, such as Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), and the re-emergences of old ones, like Tuberculosis (T.B.), have led to an increase in the incidence of fungal infections. Also, the emergence of fungi which are resistant to the current prescription drugs is a matter of urgent concern, and it is this challenge that is driving the demand for new drugs, yet only a few antifungal agents are used in clinical practice. Although several antifungal agents, such as amphotericinB and the azole class of drugs are currently available there is clearly a critical need for the development of new specific antifungal agents. Studies of new kinds of chemotherapeutic Schiff base are now attracting the attention of biochemists. Schiff bases are characterized by the –N=CH-(imine) group which is important in elucidating the mechanism of transamination and racemisation reaction in biological systems [2]. The transition metal complexes of 4-aminoantipyrine and its derivatives have been extensively examined due to their applications in various fields like biological, analytical and therapeutical. Further, they have been investigated due to their diverse biological properties as antifungal, antibacterial, analgesic, sedative, antipyretic, anti-inflammatory and DNA binding agent [3-5]. Bearing these facts in mind, we have synthesised 4-aminoantipyrine based Schiff bases having oxygen and nitrogen donors, derived from 4-aminoantipyrine, 2-hydroxy benzaldehyde/2-hydroxy-3-methoxybenzalde-hyde and 2-amino benzoic acid and their complexes with Cu(II), Ni(II), Zn(II) metal ions [6]. Herein, it is planned to test the both *in vitro* and *in silico* antifungal activity. The enzyme, namely glucosamine-6-phosphate synthase is a new target for antifungals[7,8]. In spite of the fact that glucosamine-6-phosphate synthase is present in all kinds of cells, it may be exploited as a target for potential antifungal drugs. The objective is to develop new, metal based Schiff bases and their metal complexes as antifungal drugs that would become the next generation that fight against fungal infections.

MATERIALS AND METHODS

All reagents, 4-aminoantipyrine, 2-hydroxybenzaldehyde/2-hydroxy-3-methoxybenzaldehyde, 2-aminobenzoic acid and various metal(II) chlorides, were procured from Merck products. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. Microanalyses (C, H and N) were performed in Carlo Erba 1108 analyzer at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow, India. Molar conductivities in DMSO (10^{-3} M) at room temperature were measured using systronic model-304 digital conductivity meter. Magnetic susceptibility measurements of the complexes were carried out by Gouy balance using copper sulphate pentahydrate as the calibrant. IR

spectra were recorded with Shimadzu spectrophotometer in the 4000-400 cm^{-1} range using KBr pellets. NMR spectra were recorded on a Bruker Avance Dry 300 FT-NMR spectrometer in $CDCl_3$ with TMS as the internal reference. FAB-MS spectra were recorded with a VGZAB-HS spectrometer at room temperature in a 3-nitrobenzylalcohol matrix. Electron paramagnetic resonance spectra of the copper complexes were recorded on a Varian E 112 EPR spectrometer in DMSO solution both at room temperature (300 K) and liquid nitrogen temperature (77 K) using TCNE (tetracyanoethylene) as the g-marker The absorption spectra were recorded using Shimadzu model UV-1601 spectrophotometer at room temperature.

i) Synthesis of Schiff base ligands and their metal complexes

Synthesis of 2-hydroxy-benzylidene-4-aminoantipyrine /2-hydroxy-3-methoxy benzylidene-4-aminoantipyrine

This ligand has been synthesized by us, already published in the literature[6] . An ethanolic solution of 4-aminoantipyrine (0.02 mol) was added to an ethnolic solution of 2-hydroxybenzaldehyde/2-hydroxy-3-methoxybenzaldehyde (0.02 mol). The resultant mixture was refluxed for *ca*. 3 h. The solid product formed was filtered and recrystallized from ethanol.

ii) Synthesis of Schiff bases **(L^1)/ (L^2)**

These Schiff bases have been synthesized following the procedure adopted by us[6]. An ethanolic solution of 2-hydroxy-benzylidene-4-aminoantipyrine/2-hydroxy-3-methoxybenzylidene-4-aminoantipyrine (0.01 mol) was added to an ethanolic solution of 2-aminobenzoic acid (1.37 g, 0.01 mol) and the resultant mixture was refluxed for *ca*. 10 h after the addition of anhydrous potassium carbonate. The potassium carbonate was filtered off from the reaction mixture, and the solvent was evaporated. The orange color solid separated was filtered and recrystallized from ethanol.

iii) Synthesis of metal complexes

The metal complexes of the Schiff bases have been synthesized following the procedure adopted by us[6]. A solution of metal(II) chloride in ethanol (2 mmol) was stirred with an ethanolic solution of the Schiff base (4 mmol), for *ca*. 2 h on a magnetic stirrer at room temperature. Then the solution was reduced to one-third on a water bath. The solid complex precipitated was filtered off and washed thoroughly with ethanol and dried in *vacuo.* The proposed structure of the complexes is presented in Fig.1.

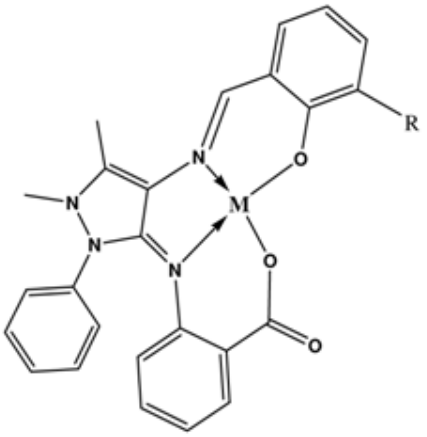

Figure . 1
The proposed structure of the complexes
M=Cu(II), Ni(II) and Zn(II). R=H; OCH_3

iv) Molecular docking using HEX 4.2.

Macromolecular Docking was done using HEX 4.2 – software using Spherical Polar Fourier Correlations. In Hex's docking calculations, each molecule is modeled using 3D parametric functions, which are used to encode both surface shape and electrostatic charge and potential distributions. With suitable scaling factors, this docking score can be interpreted as interaction energy. Hex reads protein and glucosamine-6-phosphate synthase molecular structures from PDB- format files. These are treated as receptor. In order to run a docking calculation in *Hex*, first we have to load a *receptor(*glucosamine-6-phosphate synthase*)* and a *complex* in *pdb* file format structure using the *File* pull-down menu. Then docking can be carried out using the options.

Controls → Docking → Activate.

To save the Docking Results:

The current docking orientation can be written to a single pdb

file by selecting

File → Save → Both.

v) Docking of metal complexes

Structure of the metal complexes was drawn using the drawing tools in CD/ChemSketch. The structure of the receptor (glucosamine-6-phosphate synthase) was obtained from *Protein Data Bank.* Docking menu was clicked to carryout Docking process. The results obtained after the docking completion, were shown in Fig. 2 and Fig.3.as examples. We have calculated the E-Total value of metal complexes against glucosamine-6-phosphate synthase using Hex 4.2 docking software, and observed that our metal complexes found to have higher binding energy value.

vi) In vitro Antifungal activity

The *in vitro* antifungal activity of the ligands and their complexes were tested against the fungi *Aspergillus niger, Fusarium solani, Culvularia lunata, Rhizoctonia bataicola* and *Candida albicans* by the paper disk method using nutrient agar as the medium. The stock solution was prepared by dissolving the 1 mg of sample in 10 mL of DMSO to give the concentration of 100 µg/mL and the solution

was serially diluted in order to find the Minimum Inhibitory Concentration (MIC) value. The nutrient agar medium was inoculated with the test organisms. The standard solutions of streptomycin were prepared in DMSO to give concentration of 100 µg/mL. The sterilized blank paper disks of 6 mm diameter were impregnated with tested compounds and placed on the surface of the agar plates previously spread with 0.1 mL of overnight culture of microorganisms. The incubation was carried out for 72 h at 30 ^{o}C. During this period, the test solution diffused and the growth of the inoculated microorganisms were affected. The inhibition zone was developed, at which the concentration was noted.

RESULTS AND DISCUSSION

The Schiff base ligands and their Cu(II), Ni(II), and Zn(II) complexes were synthesized and characterized by spectral and elemental analysis data following the literature[6]. The complexes were found to be air stable. The ligands were soluble in common organic solvents and all the complexes were freely soluble in $CHCl_3$, DMF and DMSO.

1. Antifungal activity

To provide in the field of bioinorganic chemistry, consequently, the metal complexes synthesized have been investigated for their antifungal actions. The antifungal tests were carried out using the disk diffusion method. The Schiff base ligand and its metal complexes also screened *in vitro* in order to find out the antifungal activity against *Aspergillus niger, Fusarium solani, Culvularia lunata, Rhizoctonia bataicola* and *Candida albicans.* The results of the antifungal studies are presented in Table 1 which revealed that the metal complexes are toxic than the free ligand against the same organisms. Such increased activity on metal chelation can be explained on the basis of Tweedy's chelation theory [9]. According to this, chelation reduces the polarity of the metal ion considerably because of the partial sharing of its positive charge with the donor groups and also due to π-electron delocalization on the whole chelating ring. The lipids and polysaccharides are some important constituents of the cell wall and membranes which are preferred for metal ion interaction. Apart from this, the cell wall also contains many phosphates, carbonyl and cystenyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Furthermore, increased lipophilicity enhances the penetration of the complexes into lipid membrane and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins which restricts further growth of the organism.

Table 1
Minimum inhibitory concentration values of the synthesized compounds against the growth of five fungi (mg/mL)

Compound	*A. niger*	*F. solani*	*C. lunatai*	*R. bataicola*	*C. albicans*
L^1	++	++	+	+	++
L^2	+	+	++	++	++
$[CuL^1]$	+++	++	+++	+++	++++
$[NiL^1]$	++	++	+++	+++	++
$[ZnL^1]$	+++	++	+++	+++	+++
$[CuL^2]$	++++	+++	++++	+++	++++
$[NiL^2]$	++	++	+++	+++	+++
$[ZnL^2]$	+++	+++	+++	++	+++
Nystatin	+++	++++	+++	++++	++++

2. Molecular docking using HEX.

With *in vitro* antimicrobial results in hand it is thought worth-while to do in silico studies to support the *in vitro* activity. The docking was used to determine the orientation of inhibitors bound in the active site of glucosamine-6-phosphate synthase. The interaction of the metal complexes with DNA was also studied by molecular modeling with special reference to docking. Computer docking technique plays an important role in the drug design and discovery, as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion and to predict the correct binding geometry for each ligand at the active site. The Schiff base and its metal complexes were docked by using the HEX docking software package. All the metal complexes were made to undergo docking with the selected microorganism and their E-Total were recorded. The docking of $[CuL^1]$ and $[CuL^2]$ complexes with glucosamine-6-phosphate synthase reveals that all the inhibitor compounds are exhibiting the strongly bonding with active site of enzyme which is showed in Fig. 2 and Fig.3. Prior to docking, the structures of the metal complexes were constructed using ChemDrawUltra11.0 and geometry optimized by MM2 force field and saved as Pdb format. The crystal structure of the complex of glucosamine-6-phosphate synthase (PDB ID 1jxa) was downloaded from Protein Data Bank. Crystallographic water molecules were removed from the protein. The structural analysis of docked structures gave significant details about the binding pattern of these complexes. E-total values of docked metal complexes CuL^1, NiL^1, ZnL^1, CuL^2, NiL^2 and ZnL^2 with DNA were found

to be -126.56, -126.87.3, -126.75.4, -163.74, -163.66 and -163. 51 kJmol^{-1} respectively. The more negative the relative binding, the more potent the binding between fructosamine-6-phosphate to glucosamine-6-phosphate and target molecules. Among all the complexes, [CuL^1] has higher binding value and it may be considered as good inhibitor of GlcN-6-Psynthase, so it can be predicted as the activity may be due to inhibition of enzymeGlcN-6-Psynthase,which catalyses a complex reaction involving ammonia transfer from L-glutaminetoFru-6-P, followed by isomerisation of the formed fructosamine-6-phosphate to glucosamine-6-phosphate. Hence, this work has widened the scope of developing the 4-aminoantipyrine derived Schiff bases and their metal complexes as promising DNA antifungal agents.

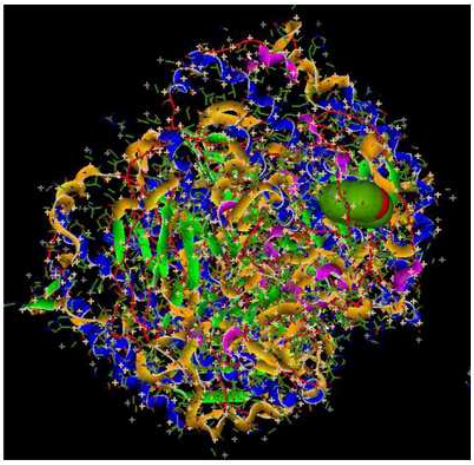

Figure .2
The binding model of [CuL^1] in the enzyme active site of glucosamine-6-phosphate synthase

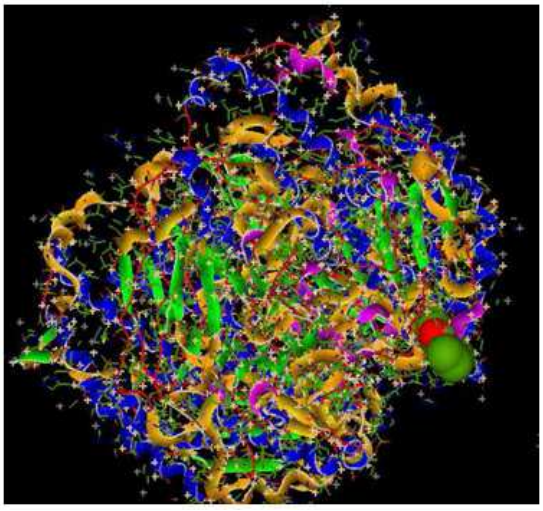

Figure .3
The binding model of [CuL^2] in the enzyme active site of glucosamine-6-phosphate synthase

CONCLUSION

Six new metal complexes containing 4-aminoantipyrine derivatives were synthesized and characterized following the literature method and tested for antifungal activities. The results of the antifungal studies show that the metal complexes are toxic than the free ligands against all organisms. Molecular docking studies also reveal that metal complexes act as good inhibitors of glucosamine-6-phosphate synthase. Hence, this study has widened the scope of developing these 4-aminoantipyrine derived Schiff base metal complexes as promising antifungal agents.

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