INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 1, p. 111-118, 2018

RESEARCH PAPER OPEN ACCESS

Antifungal activity of Vanillin, Benzophenone, Acetophenone Thiosemicarbazones and their Cobalt (II) and Nickel (II) complexes

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Key words: Antifungal activity, Minimal fungicidal concentration, Schiff bases, Schiff base complexes

http://dx.doi.org/10.12692/ijb/12.1.111-118 Article published on January 12, 2018

Abstract

Since the prehistoric times fungal infections are one of the most common diseases known to humanity and mortality due to these infections even with antifungal therapy is still unacceptably high. Therefore, the development of new antifungal agents targeting specific fungal structures or functions is being actively pursued. The antifungal activity of three schiff bases (vanillin thiosemicarbazone, benzophenone thiosemicarbazone and acetophenone thiosemicarbazone) and two schiff base complexes vanillin thiosemicarbazone with Ni(II) and Co(II) were studied against some fungus (*Candida albicans, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates* and *Mucor* sp.) by disc diffusion method. The antifungal activity was compared with the standard drug *nystatin*. Both schiff bases and schiff base complexes showed significant antifungal activity against all test organisms. But among all the experimental compounds the activity of vanillin thiosemicarbazone with Ni (II) against all test organisms was quite comparable with that of standard drug *nystatin* at dose 100µg/disc. Other four compounds vanillin thiosemicarbazone, benzophenone thiosemicarbazone, acetophenone thiosemicarbazone and vanillin thiosemicarbazone with Co (II) showed moderate activity. Among all schiff bases, VTS showed highest zone of inhibition (27±1.2 mm) against *Candida albicans* at dose 200µg/disc. For schiff base complexes, the highest zone of inhibition was found for NVTS (23±0.9 mm) against *Mucor* sp. at dose 200µg/disc. Better results were obtained with high doses. All these synthesized compounds were found to possess cytotoxic effect. Minimal fungicidal concentration of these compounds was also determined. It is concluded that these compounds are biologically active and can be primarily considered as potent antifungal agents.

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Introduction

Schiff bases and their metal complexes have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like anti-inflammatory (Sathe *et al.*, 2011; Ali *et al.*, 2012; Chandramouli *et al.*, 2012), analgesic (Chinnasamy *et al.*, 2010; Ali *et al.*, 2012; Chandramouli *et al.*, 2012), antimicrobial (Venkatesh, 2011; Islam *et al.*, 2013), anticonvulsant (Chaubey and Pandeya, 2012), antitubercular (Cimerman *et al.*, 2000; Aboul-Fadl *et al.*, 2003), anticancer (Ali *et al.*, 2012; Miri *et al.*, 2013), antioxidant (Schiff, 1864; Wei *et al.*, 2006), anthelmintic (Avaji, 2009), and so forth. The nitrogen atom of azomethine may be involved in the formation of a hydrogen bond with the active centers of cell constituents and interferes in normal cell processes (Venugopala and Jayashree, 2003; Vashi and Naik, 2004). Schiff bases derived from isatin derivatives and N[4-(4′-chlorophenyl) thiozole-2-yl] thiosemicarbazide, have already proved to be potent antimicrobial agents (Pandeya *et al.*, 1999). The synthesis and characterization of a number of new schiff bases derived from metronidazole have been undertaken and their antigiardial and antimicrobial activities were evaluated (Sadeh *et al.*, 2011). Metal-based drugs represent a novel group of antifungal agents with potential applications for the control of fungal infections. This inspires synthetic chemists to search for new metal complexes for bioactive compounds. The field of macrocylic chemistry of metals is developing very rapidly because of its applications and importance in the area of coordination chemistry (Chaudhary *et al.*, 2003). In the present paper, the antifungal activity of three schiff bases namely vanillin thiosemicarbazone (VTS), benzophenone thiosemicarbazone (BTS), acetophenone thiosemicarbazone (ATS) and two schiff base complexes namely vanillin thiosemicarbazone with Ni (II)-(NVTS) and vanillin thiosemicarbazone with Co (II)-(CVTS) have been studied.

Materials and methods

Chemicals

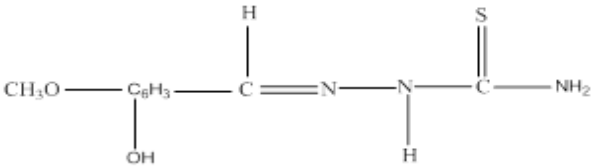
All chemicals and reagents used to carry out the research work were of reagent grade and purchased from BDH (England). All chemicals and reagents were used without further purification.

Synthesis of the compounds

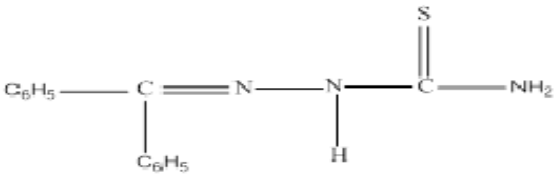
The schiff bases were synthesized by the method in the same way as described in literature (Quraishi *et al.*, 2002; Glinma *et al.*, 2011). The schiff bases were verified by taking melting points (Barton and Ollis, 1979; Quraishi *et al.*, 2002; Glinma *et al.*, 2011), elemental analysis and conducting infrared spectral (IR) studies (as KBr disc by a Shimadzu FTIR, Japan). The new bond > C = N- (azomethine) formed during the synthesis in all the cases was confirmed from IR spectrum of > C = N- bond at around 1630 cm^{-1} which was in this accordance with the literature (Barton and Ollis, 1979). The characteristics data of the synthesized schiff bases are given in the Table 1. The schiff base complexes were synthesized according to previously described method (Aravindakshan and Nair, 1984).

The formation of the synthesized schiff base complexes were verified by measuring melting points and conducting infrared spectral studies (Aravindakshan and Nair, 1984). The disappearance of peaks at 775 cm^{-1} (for γc=s) and appearance at 660 cm^{-1} (for γc-s) confirmed the formation of M–S bond in the complexes. Furthermore, the appearance of peak at 550 cm^{-1} in both the complexes might be related to M-N where M stand for metal *viz.* Co (II) or Ni(II), which is given bellow. The shifting of the peak for C=N (at 1620 cm^{-1}) to a lower wave number (~ 1580 cm^{-1}) supported the participation of N (of the ligand) in the complex formation. The characteristics data of the synthesized schiff base complexes are given in the Table 2.

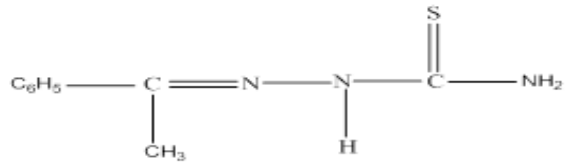
The structures of synthesized compounds are shown below:



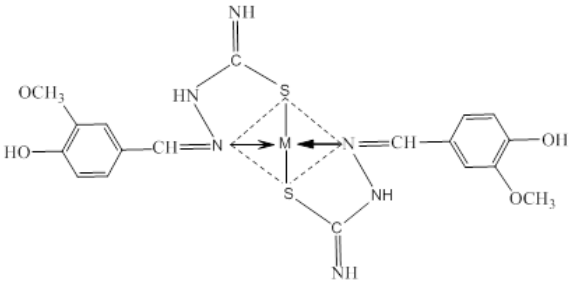
Vanillin thiosemicarbazone (VTS)



Benzophenone thiosemicarbazone (BTS)



Acetophenone thiosemicarbazone (ATS)



Square planar structure of the complex where M stands for Co/Ni

Antifungal activity test

Antifungal activity was performed by disc diffusion assay method (Srivastava, 1984). Nystatin (100µg/disc) was used as standard.

Test organisms

For antifungal activity test, five experimental fungi (*Candida albicans, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates and Mucor sp.*) were collected from the Institute of Biological Science, University of Rajshahi, Bangladesh.

Preparation of the media

Potato dextrose agar (PDA) media was used to perform the antifungal activity test and for subculture of the test organisms. Accurately weighed 20.0 g potato, 2.0 g dextrose and 2g agar were dispersed in a conical flask with 100 ml distilled water. It was heated in a water bath to dissolve the ingredients until a transparent solution was obtained. The pH of the media was adjusted to 5.6. The volume was adjusted by adding distilled water and sterilized in an autoclave.

Preparation of inoculum

The spore of isolated pure fungi were inoculated in screw capped tube containing equal amount of PDA media and incubated at 28°C for 5-7 days for the development of new pure culture that was used as inoculum.

Preparation of test sample

Sample solutions of VTS, BTS, ATS, NVTS and CVTS at concentration of 100 µg/disc and 200 µg/disc were prepared separately (by dissolving 10mg and 20mg in 1mL DMSO of each test compound). Desired amounts of the sample solutions were applied on the discs with the help of a micropipette in an aseptic condition. The discs were left for a few minutes in the same condition for complete removal of solvents.

Procedure

Distilled water (10mL) was poured in several clean test tubes and plugged with cotton. The test tubes, petridishes, glass rods, cottons and the media were sterilized by autoclave and then transferred to the laminar air flow cabinet. Media (6mL) was poured carefully in the medium sized petridishes in each. The petridishes were rotated several times, first clockwise and then anticlockwise for homogenous thickness. Then the media was allowed to cool and solidified at about 30°C.The test tubes containing distilled water were inoculated with fresh culture of the test fungi and shaken gently to form a uniform suspension of the organisms because of their high prevalence sporulation process. A piece of cotton was immerged in the test tubes with the help of individual glass rod and then gently rubbed with the media. The cotton was then discarded. Finally, the plates were stored in a refrigerator (4°C) for overnight. Preparation and placement of the discs, diffusion, incubation and measurement of zone of inhibition were almost the same as those of the antibacterial screening. The incubation period was adjusted for 48-72 hours at room temperature.

Minimum fungicidal concentration (MFC)

The Minimal Fungicidal Concentration (MFC) is defined as the lowest concentration of the compounds that is required to kill a particular fungal species.

The *in vitro* MFC was determined as described in the literature (Espinel-Ingroff *et al.*, 2002). After 72 h of incubation, 20 µL was subcultured from each well that showed no visible growth (growth inhibition of over 98%), from the last positive well (growth similar to that for the growth control well), and from the growth control (extract-free medium) onto PDA media. The media were incubated at 27 °C until growth was seen in the growth control subculture. The minimum fungicidal concentration was regarded as the lowest extract concentration that did not yield any fungal growth on the solid medium used.

Statistical analysis

The experimental results have been expressed as the mean ± S.E.M. Data have been calculated by one way ANOVA followed by Dunnett "t" test using SPSS software of 16 version.

Results and discussion

The results for antifungal activity of these schiff bases and schiff base complexes are shown in Table 3 and 4 respectively. The antifungal activity was compared with the standard drug *nystatin*. Both schiff bases and schiff base complexes showed significant antifungal activities against all test organisms. Better results were obtained with high doses.

Table 1. Characteristics data of the synthesized schiff bases.

Synthesized compounds	Characteristics					
	% Yield	Physical form	Color	Solubility	Melting point °C	IR spectra, cm^{-1}
VTS	83	Crystalline	White	DMSO, Ethanol, Methanol.	136-138	1630sh (>C=N-) 1586s (C_6H_5) 775w (C=S) 2840s (OCH_3) 3530s (phenolic–OH) 3156s (NH_2)
BTS	76	Crystalline	White	DMSO, Ethanol, Methanol.	169-170	1625w (>C=N-) 1532s, 3182-3070w (C_6H_5) 775s (>C=S) 3200w (NH_2)
ATS	86	Crystalline	White	DMSO, Ethanol, Methanol.	114-116	1620s (>C=N-) 1570s (C_6H_5) 775s (>C=S) 3200w (NH_2)

The activity of NVTS against all test organisms was quite comparable with that of standard drug *nystatin* at dose 100µg/disc. Other four compounds (VTS, BTS, ATS and CVTS) showed moderate activity. The solvent DMSO showed no activity against any fungal strain. MFC values of the synthesized compounds were determined as µg/mL and are shown in Table 5. Antifungal activity was studied of two schiff bases derived from glycylglycine with imidazole-2-carboxaldehyde and indole-3-carboxaldehyde and their complexes with Zn (II) (Joseyphus and Nair, 2008).

Table 2. Characteristics data of the synthesized schiff base complexes.

Synthesized compounds	Characteristics					
	% Yield	Physical form	Color	Solubility	Melting point °C	IR spectra cm^{-1}
NVTS	49	Crystalline	Grey	DMSO, Ethanol, Methanol.	236-240	3150m (-OH), 1598s (>C=N), 660m (C-S), 555m (Ni-N), 350m (Ni-S)
CVTS	46	Crystalline	Black	DMSO, Ethanol, Methanol.	More than 250	3150m (-OH), 1595s (>C=N), 660m (C-S), 550m (Co-N), 348m (Co-S)

They found the schiff base ligands and their complexes indicates that the complexes exhibit higher antifungal activity than the free ligands. It was found that the antifungal activity of schiff base derived from glycine using 2,3-butanedione, 5-methyl-2,6-pyrimidine-dione and its metal complexes with Cu(II), Ni(II), Co(II) and Zn(II) (Srivastva *et al.,* 2016).

Table 3. Antifungal activity of the schiff bases.

Test organisms	VTS (µg/disc)		BTS (µg/disc)		ATS (µg/disc)		*Nystatin* (µg/disc)
	100	200	100	200	100	200	100
Diameter of zone of inhibition (in mm)							
Aspergillus flavus	15±0.6	17±0.5	14±0.4	15±0.6	R	11±0.4	24
Aspergillus fumigatus	R	R	15±0.5	17±0.8	18±0.4	22±1.1	22±0.4
Aspergillus niger	R	12±0.5	15±0.8	17±0.8	17±0.8	21±0.8	25±0.5
Candida albicans	22±1.0	27±1.2	14±0.8	18±0.7	8±1.2	12±1.3	31±0.8
Mucor sp.	19±0.6	24±0.6	18±0.8	23±1.1	10±1.0	13±1.0	29±0.4

R = Resistance.

They found that both schiff base and metal complexes possess antifungal activity and metal complexes exhibit more activity than free schiff base against fungi. Schiff base complexes of Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II), Hg (II) and Tin (II) with 3-methyl-2-(pyridine-2-yl-methylene hydrazinocarbonyl)quinoxaline-4-oxide and 2-(2-hydroxy benzylidene-hydrazinocarbonyl)-3-methylquinoxaline-4-oxide were prepared and antifungal activity was studied (Mahal *et al.,* 2015). They investigated that the antifungal activity was enhanced by chelation.

Table 4. Antifungal activity of the schiff base complexes.

Test organisms	NVTS (µg/disc)		CVTS (µg/disc)		*Nystatin* (µg/disc)
	100	200	100	200	100
Diameter of zone of inhibition (in mm)					
Aspergillus flavus	12±0.8	17±1.0	13±0.6	R	24
Aspergillus fumigatus	14±0.6	R	15±1.1	R	22±0.4
Aspergillus niger	15±0.8	19±1.2	09±0.5	14±0.8	25±0.5
Candida albicans	20±1.1	R	11±0.6	17±0.8	31±0.8
Mucor sp.	14±0.7	23±0.9	15±1.0	R	29±0.4

R = Resistance.

The Cu(II) complexes has the highest inhibition zone against *A. niger*. Six schiff bases were prepared by reacting 3,3′-diaminodipropylamine with different benzaldehyde derivatives and their antifungal activity was studied. It was found that, these compounds showed antifungal activity and considered as a promising and potential antifungal agent (Matar *et al.,* 2015).

The mode of action of these compounds should be done to elucidate the structure–function relationship. Based on previously published study of some schiff base complexes (Joseyphus and Nair, 2008), it was suggested that the mode of action may involve various targets in microorganisms.

These mechanisms can be classified into four points: (1) the interference with the cell wall synthesis as a result the cell permeability may be altered or they may disorganize the lipoproteins leading to cell death, (2) Deactivate various cellular enzymes which are important in the microorganism's metabolic

pathways, (3) Formation of a hydrogen bond through the azomethine group with the active centres of cell constituents resulting in interfering with the normal cell processes, (4) Denaturation of one or more proteins of the cell, as a result of which the normal cellular processes are impaired. Finally, from the results discussed above it is clear that the synthesized schiff bases and schiff base complexes are biologically active.

Table 5. Minimal Fungicidal Concentration (MFC) of schiff bases and schiff base complexes.

Test organisms	VTS (µg/mL)	BTS (µg/mL)	ATS (µg/mL)	NVTS (µg/mL)	CVTS (µg/mL)
Aspergillus flavus	48	96	48	48	24
Aspergillus fumigatus	24	48	24	24	48
Aspergillus niger	48	24	24	24	24
Candida albicans	12	48	96	48	12
Mucor sp.	24	24	48	24	24

Conclusion

All the synthesized schiff bases and schiff base complexes have been investigated for their antifungal activity. With our synthesized compounds, it is evident that they showed significant antifungal activity against test organisms. Therefore, these compounds may be used as new antifungal drugs after performing further research works with advanced technology.

Acknowledgement

The authors are thankful to the authorities of the Institute of Biological Science (IBS), University of Rajshahi, Rajshahi-6205, Bangladesh for kindly providing test microorganisms.

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