

Synthesis, Characterization and Biocidal Activities of Some Acyldihydrazones

Parul Gupta

Basic Engineering Department, Indira Institute of Engineering & Management, Parandwadi, Pune-410506, India

Abstract

Schiff base Ligands viz. diacetyl salicylaldehyde oxalic acid dihydrazone (Hdsodh), diacetyl salicylaldehyde malonic acid dihydrazone (Hdsmdh), diacetyl salicylaldehyde succinic acid dihydrazone (Hdssdh), diacetyl salicylaldehyde phthalic acid dihydrazone (Hdspdh), diacetyl benzaldehyde oxalic acid dihydrazone (dbodh), diacetyl benzaldehyde malonic acid dihydrazone (dbmdh), diacetyl benzaldehyde succinic acid dihydrazone (dbsdh), benzil salicylaldehyde oxalic acid dihydrazone (Hbsodh), benzil salicylaldehyde malonic acid dihydrazone (Hbsmdh), benzil salicylaldehyde succinic acid dihydrazone (Hbssdh), glyoxal salicylaldehyde succinic acid dihydrazone (Hgssdh), glyoxal salicylaldehyde phthalic acid dihydrazone (Hgspdh) were synthesized and characterized by elemental analyses and infrared spectra and also been screened for their antifungal and antibacterial activities. The compounds show a significant antifungal activity against a number of pathogenic fungi viz. *Stemphylium*, *Myrothecium*, *Alternaria*, *Fusarium*, *Curvularia*, *Pseudocercospora* and *Colletotrichum* species. The antibacterial activity was studied against *Escherichia coli*, *Pseudomonas fluorescens* (gram -ve) and *Clostridium thermocellum*, *Bacillus subtilis* (gram +ve) species.

Keywords: Acyldihydrazones, Transition Metal Complexes, Antifungal Activity, Antibacterial Activity

I. INTRODUCTION

Schiff bases constitute an interesting class of chelating agents capable of coordination with one or more metal ions giving mononuclear as well as polynuclear metal complexes, which serve as model for metallo-proteins [1]. A number of papers have been appeared [2-7] highlighting the flexible nature of Schiff base ligands, their analytical and biological properties.

Acyldihydrazines (or organic acid dihydrazides) such as oxalic acid dihydrazide, $(\text{CONHNH}_2)_2$, malonic acid dihydrazide, $\text{CH}_2(\text{CONHNH}_2)_2$ etc. having two terminal amino groups may react either with two molecules of mono keto group containing compounds [8], one molecule of diketone compounds [9] or one molecule each of two different keto group containing compounds under suitable conditions. The acyldihydrazones thus obtained have two $\text{C}=\text{N}$ groups besides several other potential donor sites for bonding with metal ions [10-12]. Since acyldihydrazones are strong biologically active compounds [13-15], a study of the acyldihydrazones had been undertaken. Accordingly a number of ligands diacetyl salicylaldehyde oxalic acid dihydrazone (Hdsodh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCO CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, diacetyl salicylaldehyde malonic acid dihydrazone (Hdsmdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOCH}_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, diacetyl salicylaldehyde succinic acid dihydrazone (Hdssdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, diacetyl salicylaldehyde phthalic acid dihydrazone (Hdspdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOC}_6\text{H}_4\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, diacetyl benzaldehyde oxalic

acid dihydrazone (dbodh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNH COCONHN}=\text{CHC}_6\text{H}_5$, diacetyl benzaldehyde malonic acid dihydrazone (dbmdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOCH}_2\text{CONHN}=\text{CHC}_6\text{H}_5$, diacetyl benzaldehyde succinic acid dihydrazone (dbsdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNH CO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_5$, benzil salicylaldehyde oxalic acid dihydrazone (Hbsodh), $\text{C}_6\text{H}_5\text{COC}(\text{C}_6\text{H}_5)=\text{NNHCOCONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, benzil salicylaldehyde malonic acid dihydrazone (Hbsmdh), $\text{C}_6\text{H}_5\text{COC}(\text{C}_6\text{H}_5)=\text{N NHCOCH}_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, benzil salicylaldehyde succinic acid dihydrazone (Hbssdh), $\text{C}_6\text{H}_5\text{COC}(\text{C}_6\text{H}_5)=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CH C}_6\text{H}_4(\text{OH})$, glyoxal salicylaldehyde succinic acid dihydrazone (Hgssdh), $\text{HCOCH}=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, glyoxal salicylaldehyde phthalic acid dihydrazone (Hgspdh), $\text{HCOCH}=\text{NNHCOC}_6\text{H}_4\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ were synthesized.

II. EXPERIMENTAL

A. Materials

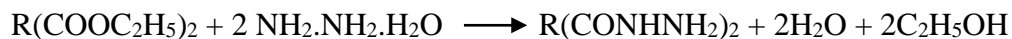
Hydrazine hydrate, $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (90–95 %) and organic chemicals such as diethyl oxalate $(\text{COOC}_2\text{H}_5)_2$, diethyl malonate $(\text{CH}_2(\text{COOC}_2\text{H}_5)_2)$, diethyl succinate $(\text{CH}_2\text{CH}_2(\text{COOC}_2\text{H}_5)_2)$, diethyl phthalate $(\text{C}_6\text{H}_4(\text{COOC}_2\text{H}_5)_2)$, benzaldehyde $(\text{C}_6\text{H}_5\text{CHO})$, salicylaldehyde $(\text{HO})\text{C}_6\text{H}_4\text{CHO}$, benzil $(\text{C}_6\text{H}_5\text{COCOC}_6\text{H}_5)$, diacetyl $(\text{CH}_3\text{COCOCH}_3)$, glyoxal $(\text{CHO})_2$ were of BDH (AR) or equivalent grade.

The organic solvents viz. ethanol, methanol, chloroform, benzene, diethyl ether, ethyl acetate, acetone, dimethyl sulfoxide and dimethyl formamide etc. were purified before use.

B. Synthesis of the ligands

Preparation of acyldihydrazines

Oxalic acid dihydrazide (odh), $\text{H}_2\text{NNHCOCONHNH}_2$, was prepared by adding hydrazine hydrate (10 mL) to ice cold dilute solution of diethyl oxalate (14.6 mL) in absolute ethanol (50 mL) in 1:2 molar ratio. The reaction was exothermic and the product was immediately obtained. The compound was filtered by suction, washed first with distilled water and then with dilute ethanol. The pure odh was recrystallized from hot water at 110 °C. Malonic acid dihydrazide (mdh), $\text{H}_2\text{NNHCOCH}_2\text{CONHNH}_2$ and succinic acid dihydrazide (sdh), $\text{H}_2\text{NNHCO}(\text{CH}_2)_2\text{CONHNH}_2$ were prepared by refluxing diethyl malonate (16 mL) and diethyl succinate (17.4 mL) and hydrazine hydrate (10 mL) in about 1:2 molar ratio and recrystallized from hot aqueous ethanol. Phthalic acid dihydrazide (pdh) was obtained by refluxing diethyl phthalate and hydrazine hydrate in 1:2 molar ratio for 3 hours. The solid product obtained was filtered and washed with ethanol, recrystallized from hot ethanol.



where R = nil for odh, CH_2 for mdh, $(\text{CH}_2)_2$ for sdh and C_6H_4 for pdh.

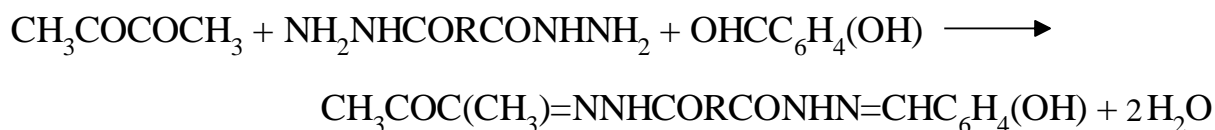
odh m.p. 232 °C (lit 232 °C); mdh m.p. 150 °C (152 °C).

Preparation of acyldihydrazones

A. Diacetyl salicylaldehyde acyldihydrazones

The ligands diacetyl salicylaldehyde oxalic acid dihydrazone (Hdsodh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOCONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, diacetyl salicylaldehyde malonic acid dihydrazone (Hdsmdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOCH}_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ and diacetyl salicylaldehyde succinic acid dihydrazone (Hdssdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ were prepared by reacting a mixture of 50 mL ethanolic solution of diacetyl (100 mmol, 8.6 mL) and salicylaldehyde (100 mmol, 12.2 mL) with an aqueous solution (50 mL) each of oxalic acid dihydrazide (100 mmol, 11.8 g), malonic acid dihydrazide (100 mmol, 13.2 g) and succinic acid dihydrazide (100 mmol, 14.4 g) in a RB flask separately. The reaction mixture was stirred for 1–2 hours on a magnetic stirrer at room temperature and the product was filtered by suction. Since the ligands were sparingly soluble in common organic solvents, they were purified by washing several times with distilled water and then with ethanol. The pure ligands were dried in a dessicator over anhydrous calcium chloride.

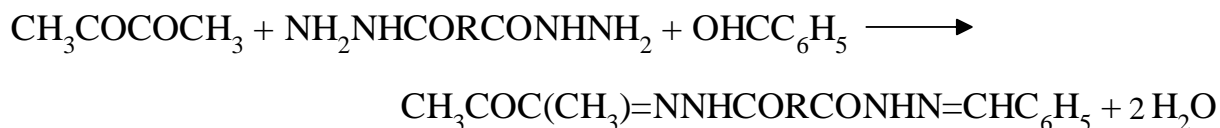
Diacetyl salicylaldehyde phthalic acid dihydrazone (Hdspdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOC}_6\text{H}_4\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ was prepared by reacting 50 mL aqueous ethanolic solution (v/v, 1:1) of phthalic acid dihydrazide (100 mmol, 19.4 g) with a mixed solution of diacetyl (100 mmol, 8.6 mL) and salicylaldehyde (100 mmol, 12.2 mL) in 50 mL ethanol. The reacting solution mixture was stirred for ~3 hours at room temperature. The product was filtered, washed several times with water and aqueous ethanol and dried in a desiccator.



where R = nil, CH_2 , $(\text{CH}_2)_2$ and C_6H_4 for Hdsodh, Hdsmdh, Hdssdh and Hdspdh respectively.

B. Diacetyl benzaldehyde acyldihydrazones

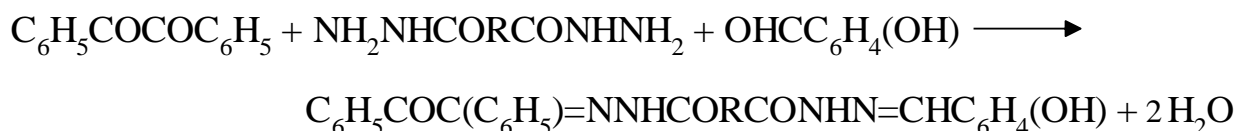
For the synthesis of diacetyl benzaldehyde oxalic acid dihydrazone (dbodh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOCONHN}=\text{CHC}_6\text{H}_5$, diacetyl benzaldehyde malonic acid dihydrazone (dbmdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCOCH}_2\text{CONHN}=\text{CHC}_6\text{H}_5$ and diacetyl benzaldehyde succinic acid dihydrazone (dbsdh), $\text{CH}_3\text{COC}(\text{CH}_3)=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_5$, a mixture of ethanolic solution (50 mL) of diacetyl (100 mmol, 8.6 mL) and benzaldehyde (100 mmol, 10.6 mL) was reacted with a hot aqueous solution (50 mL) of each of oxalic acid dihydrazide (100 mmol, 11.8 g) and malonic acid dihydrazide (100 mmol, 13.2 g) and succinic acid dihydrazide (100 mmol, 14.4 g) separately in a RB flask. The reaction mixtures were stirred continuously for 1–2 hours on a magnetic stirrer at room temperature with shaking at regular interval to complete the reaction. The products were filtered by suction, purified by washing several times with hot water and then with ethanol. The pure ligands, thus obtained were dried in a desiccator over anhydrous CaCl_2 .



where R = nil for dbodh, CH_2 for dbmdh and $(\text{CH}_2)_2$ for dbsdh.

C. Benzil salicylaldehyde acyldihydrazones

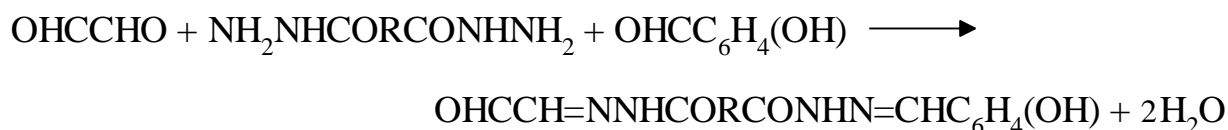
For the synthesis of benzil salicylaldehyde oxalic acid dihydrazone (Hbsodh), $\text{C}_6\text{H}_5\text{COC}(\text{C}_6\text{H}_5)=\text{NNHCOCONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, benzil salicylaldehyde malonic acid dihydrazone (Hbsmdh), $\text{C}_6\text{H}_5\text{COC}(\text{C}_6\text{H}_5)=\text{NNHCOCH}_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ and benzil salicylaldehyde succinic acid dihydrazone (Hbssdh), $\text{C}_6\text{H}_5\text{COC}(\text{C}_6\text{H}_5)=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$, benzil (100 mmol, 21.0 g) and salicylaldehyde (100 mmol, 12.2 mL) were taken in a beaker containing 50 mL ethanol. Above solution mixture was reacted separately with 50 mL aqueous solution each of oxalic acid dihydrazide (100 mmol, 11.8 g), malonic acid dihydrazide (100 mmol, 13.2 g) and succinic acid dihydrazide (100 mmol, 14.4 g). The reactants were stirred continuously for ~ 2 hours on a magnetic stirrer at room temperature. The product was filtered by suction and purified by washing several times with hot water and then with ethanol to remove unreacted components. The pure ligands were dried in a desiccator over anhydrous calcium chloride.



where R = nil for Hbsodh, CH_2 for Hbsmdh and $(\text{CH}_2)_2$ for Hbssdh.

D. Glyoxal salicylaldehyde acyldihydrazones

The ligands glyoxal salicylaldehyde succinic acid dihydrazone (Hgssdh), $\text{HCOCH}=\text{NNHCO}(\text{CH}_2)_2\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ and glyoxal salicylaldehyde phthalic acid dihydrazone (Hgspdh), $\text{HCOCH}=\text{NNHCOC}_6\text{H}_4\text{CONHN}=\text{CHC}_6\text{H}_4(\text{OH})$ were synthesized by reacting 50 mL aqueous solution of succinic acid dihydrazide (100 mmol, 14.4 g) or aqueous ethanolic solution (v/v, 1:1) of phthalic acid dihydrazide (100 mmol, 19.4 g) into 50 mL aqueous ethanolic solution of glyoxal (100 mmol, 5.8 mL) mixed with salicylaldehyde (100 mmol, 12.2 mL). The reactants were continuously stirred on a magnetic stirrer at room temperature for an hour to insure complete precipitation. The product was filtered by suction and purified by washing several times with water and aqueous ethanol. The pure ligands were dried in a desiccator over anhydrous CaCl_2 .



where R = $(\text{CH}_2)_2$ for Hgssdh and C_6H_4 for Hgspdh.

III. ANALYSES AND INSTRUMENTATION

C, H, N data were determined on an Elementar Vario EL model elemental analyzer from 'Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow'. IR spectra were recorded in KBr medium on Vector-22 Spectrophotometer in the range 4000–500 cm^{-1} . The analytical and physicochemical data are given in tables 1-3.

Table I. Analytical data and other general characteristics of ligands

Compound (Colour)	Empirical formula (Formula weight)	M.P./ Decomp.(° C)	Analysis found (calc)%			Yield (%)
			C	H	N	
dsodh (Light yellow)	C ₁₃ H ₁₄ N ₄ O ₄ (290)	264	53.78 (53.79)	4.68 (4.83)	19.10 (19.31)	83
dsmhdh (Creamy white)	C ₁₄ H ₁₆ N ₄ O ₄ (304)	206	55.04 (55.26)	5.16 (5.26)	18.13 (18.42)	82
Hbsodh (Light yellow)	C ₂₃ H ₁₈ N ₄ O ₄ (414)	208	65.70 (66.67)	4.18 (4.35)	12.32 (13.52)	85
Hbsmdh (Cream yellow)	C ₂₄ H ₂₀ N ₄ O ₄ (428)	178	67.22 (67.29)	4.52 (4.67)	12.80 (13.08)	80
dbodh (Creamy white)	C ₁₃ H ₁₄ N ₄ O ₃ (274)	280	56.71 (56.93)	5.06 (5.11)	20.32 (20.44)	79
dbmdh (White)	C ₁₄ H ₁₆ N ₄ O ₃ (288)	210	58.10 (58.33)	5.47 (5.55)	19.25 (19.44)	86
Hdssdh (White)	C ₁₅ H ₁₈ N ₄ O ₄ (318)	222	56.42 (56.60)	5.57 (5.66)	17.53 (17.61)	86
dbsdh (Cream)	C ₁₅ H ₁₈ N ₄ O ₃ (302)	240	59.44 (59.60)	5.88 (5.96)	18.41 (18.54)	84
Hgssdh (White)	C ₁₃ H ₁₄ N ₄ O ₄ (290)	>300	53.63 (53.79)	4.79 (4.83)	19.22 (19.31)	80
Hgspdh (Yellow)	C ₁₇ H ₁₄ N ₄ O ₄ (338)	>300	60.24 (60.36)	4.16 (4.14)	16.48 (16.57)	78
Hbssdh (Light yellow)	C ₂₅ H ₂₂ N ₄ O ₄ (442)	206	67.65 (67.87)	4.92 (4.98)	12.60 (12.67)	82
Hdspdh (Cream yellow)	C ₁₉ H ₁₈ N ₄ O ₄ (366)	180	62.10 (62.29)	4.89 (4.92)	15.28 (15.30)	64

IV. BIOCIDAL SCREENING

A. Antifungal activity

The ligands as well as their complexes were screened for their antifungal activity against several fungi viz. *Stemphylium*, *Myrothecium*, *Curvularia*, *Fusarium Colletotrichum*, *Pseudocercospora* and *Alternaria* species. These species were isolated from the infected organs of the host plants on potato dextrose agar (potato 250g + dextrose 20g + agar 20g) medium. The cultures of the fungi were purified by single spore isolation technique.

The solutions in different concentrations 0.5, 1, 2 mg/mL of each compound in DMSO were prepared for testing against spore germination. A drop of the solution of each concentration was kept separately on glass slides. The conidia, fungal reproducing spores (approx. 200) lifted with the help of an inoculating needle, were mixed in every drop of each compound separately.

Each treatment was replicated thrice and a parallel DMSO solvent control set was run concurrently on separate glass slides. All the slides were incubated in humid chambers at 25 ± 2 °C for 24 hours. Each slide was observed under the microscope for spore germination and percent germination was finally calculated.

B. Antibacterial activity

The antibacterial activity of the ligands and their complexes were studied against *Pseudomonas fluorescence*, *Escherichia coli* (gram -ve) and *Clostridium thermocellum*, *Bacillus subtilis* (gram +ve) bacteria. Each of the compounds was dissolved in DMSO and solutions of the concentration 1.0 and 2.0 mg/mL were prepared separately. Paper discs of whatman filter paper (No.42) of uniform diameter (8 mm) were cut and sterilized on an autoclave. The paper disc soaked in the desired concentration of the complex solution was placed aseptically in the petri dishes containing nutrient agar media (agar 15 g + beef extract 3 g + peptone 5 g) seeded with above bacterial species separately. The Petridishes were incubated at 32 °C and the inhibition zones were recorded after 24 hours of incubation. Each treatment was replicated 9 times.

A common standard antibiotic Ampicillin was also screened for antibacterial activity in the same solvent and at the same concentration. The percent Activity Index data for the metal complexes were calculated as follows:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

V. RESULTS AND DISCUSSION

A. Infrared spectra

The ligands dsodh and dsmdh show two broad bands centered at 3545, 3250 cm^{-1} respectively due to -OH and >NH groups. A broad $\nu(\text{C}=\text{O})$ band is observed at 1665 cm^{-1} in dsodh and at 1661 cm^{-1} in dsmdh may be due to the presence of three >C=O groups in the ligands. The ligands Hbsodh and Hbsmdh show two broad bands due to -OH and >NH groups in the region 3200-3350 cm^{-1} . The amide I band, $\nu(\text{C}=\text{O})$ in the ligands Hbsodh and Hbsmdh appears as a broad band at 1673 cm^{-1} and 1688 cm^{-1} respectively due to presence of three >C=O groups in the ligands. The ligands dbodh and dbmdh show a medium intensity band at 3245 cm^{-1} and 3214 cm^{-1} , respectively due to >NH groups. $\nu(\text{C}=\text{O})$ in the ligands appears as a broad band at 1660 cm^{-1} and 1656 cm^{-1} respectively, in dbodh and dbmdh due to presence of three >C=O groups in each ligand. The ligand Hdssdh shows bands at 3372 cm^{-1} due to phenolic -OH and at 3197 cm^{-1} due to >NH groups in the ligand. The amide I, $\nu(\text{C}=\text{O})$ in the ligands, Hdssdh and dbsdh appears at 1665 cm^{-1} and 1671 cm^{-1} , respectively as a broad band due to presence of three >C=O groups. The ligands Hgssdh and Hgspdh show $\nu(\text{OH})$ and $\nu(\text{NH})$ bands at 3398, 3477 cm^{-1} and 3207, 3167 cm^{-1} , respectively. A broad $\nu(\text{C}=\text{O})$ band is observed at 1683 cm^{-1} in Hgssdh and at 1660 cm^{-1} in Hgspdh due to presence of three >C=O groups in the ligands.

The ligands Hbssdh and Hdspdh show $\nu(\text{NH})$ at 3207 cm^{-1} and 3162 cm^{-1} respectively due to presence of two >NH groups in each ligand. Both the ligands also show a broad band due to

phenolic OH group at 3294 cm^{-1} and 3392 cm^{-1} respectively in Hbssdh and Hdspdh. $\nu(\text{C}=\text{O})$ in the ligands Hbssdh and Hdspdh appears as a broad band at 1672 cm^{-1} and 1686 cm^{-1} , respectively due to presence of three $>\text{C}=\text{O}$ groups in each ligand.

Table II. IR spectral data (cm^{-1}) and assignment of important bands

Compound	$\nu(\text{OH}+\text{NH})$	Amide I $\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	Amide II	Amide III	$\nu(\text{N}-\text{N})$
dsodh	3245b 3250b	1665b	1625s	1571s	1354s	964s
dsmdh	3480b 3217b	1661b	1620s	1569s	1365s	960s
Hbsodh	3327b 3233s	1673b	1630s	1530m	1371s	983s
Hbsmdh	3280b 3204s	1688b	1615s	1535s	1370s	992w
Hdssdh	3372b, 3197s	1665b	1618s	1569s	1378s	958m
dbsdh	3201b	1671b	1610s	1552s	1380s	998w
Hgssdh	3398b, 3207s	1683b	1619s	1546s	1370s	985w
Hgspdh	3477b, 3167s	1660b	1617s	1573s	1377m	971w
Hbssdh	3294b, 3207s	1672b	1619s	1587s	1379m	960w
Hdspdh	3392b, 3162s	1686b	1635s	1572s	1361m	959m
Compoud	$\nu(\text{NH})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	Amide II	Amide III	$\nu(\text{N}-\text{N})$
dbodh	3245m	1660b	1617s	1526s	1363s	966m
dbmdh	3214m	1656b	1619s	1562s	1372s	957w

s = strong, w = weak, b = broad, m = medium

B. Antifungal activity

The ligands as well as their complexes exhibit an appreciable inhibitory effect on the growth of *Alternaria* sp., *Stemphylium* sp., *Myrothecium* sp., *Fusarium* sp., *Curvularia* sp. and *Pseudocercospora* sp. at 0.5, 1.0 and 1.5 mg/mL concentrations. In all the cases, the activity is greatly enhanced at higher concentration. The dsodh ligand shows better activity than dsmdh against *Stemphylium* and *Alternaria* sp., but lower activity against

Myrothecium sp. Among dbmdh and dbodh ligands, dbmdh shows slightly better activity than dbodh. The ligand Hdssdh shows highest activity against *Curvularia* sp..

Table III A. Antifungal activity of the complexes and their components

Compound	Inhibition of spore germination								
	<i>Stemphylium</i> sp.(mg mL ⁻¹) 1)			<i>Myrothecium</i> sp.(mg mL ⁻¹) 1)			<i>Alternaria</i> sp.(mg mL ⁻¹)		
	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0
dsodh	39.06	39.56	45.83	53.13	54.67	57.29	50.53	56.15	64.67
dsmdh	14.67	19.05	43.95	53.34	56.24	60.61	43.88	51.01	60.65
Compound	% Inhibition of spore germination								
	<i>Alternaria</i> sp.(mg mL ⁻¹)			<i>Curvularia</i> sp.(mg mL ⁻¹)			<i>Colletotrichum</i> sp.(mg mL ⁻¹)		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
dbodh	32	45	60	41	52	61	40	53	58
dbmdh	35	47	58	43	56	63	42	58	65
Compound	% Inhibition of spore germination								
	<i>Alternaria</i> sp.(mg mL ⁻¹)			<i>Curvularia</i> sp.(mg mL ⁻¹)			<i>Pseudocercospora</i> sp.(mg mL ⁻¹)		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Hdssdh	35	52	65	43	57	72	40	53	69
dbdsdh	32	50	60	41	55	68	38	52	66
Compound	% Inhibition of spore germination								
	<i>Alternaria</i> sp.(mg mL ⁻¹)			<i>Pseudocercospora</i> sp.(mg mL ⁻¹)			<i>Colletotrichum</i> sp.(mg mL ⁻¹)		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Hgssdh	36	50	58	35	48	55	41	52	60
Hgspdh	42	55	66	40	54	62	38	52	60
Compound	% Inhibition of spore germination								
	<i>Alternaria</i> sp.(mg mL ⁻¹)			<i>Pseudocercospora</i> sp.(mg mL ⁻¹)			<i>Colletotrichum</i> sp.(mg mL ⁻¹)		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Hgssdh	36	50	58	35	48	55	41	52	60
Hgspdh	42	55	66	40	54	62	38	52	60

Compound	<i>Curvularia</i> sp.(mg mL ⁻¹)			<i>Fusarium</i> sp. (mg mL ⁻¹)			<i>Colletotrichum</i> sp.(mg mL ⁻¹)		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Hbssdh	42	48	55	47	54	60	43	50	56
Hdspdh	38	43	52	40	52	58	39	46	55

Antibacterial activity

The antibacterial screening data of the ligands indicate that dsmdh is more active ligand than dsodh . The ligands (Hbsodh) and (Hbsmdh) are screened against *Bacillus subtilis* (Gram +ve) and *Pseudomonas fluorescens* (Gram-ve) bacteria and generally show significant antibacterial activity. Hbsmdh ligand shows better activity than Hbsodh. Hbsmdh shows highest activity against *Pseudomonas Fluorescence* (mg mL⁻¹) at higher concentration.

The activity increases at higher concentration [16]. The percent activity index data also suggest a fair degree of antibacterial activity for the ligands as compare to the common standard antibiotic Ampicillin.

The variation in the effectiveness of different compounds against different organism depends either on the impermeability of the cells of microbes or differences in ribosomes of the microbial cells.

Table III B. Antibacterial activity of the complexes and their components

Compound	Diameter of inhibition zone				% Activity index			
	<i>Pseudomonas fluorescens</i> (mg mL ⁻¹)		<i>Clostridium thermocellum</i> (mg mL ⁻¹)		<i>Pseudomonas fluorescens</i> (mg mL ⁻¹)		<i>Clostridium thermocellum</i> (mg mL ⁻¹)	
	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
dsodh	1.5	2.0	1.5	2.0	8.33	11.11	10.71	12.5
dsmdh	4.5	6.0	3.5	4.5	25.00	33.33	25.00	28.13
Hdssdh	4	5	4	5	31	36	36	42
dbsdh	5	6	4	5	38	43	36	42
Ampicillin	18.0	18.0	14.0	16.0	100.00	100.00	100.00	100.00
Compound	Diameter of inhibition zone (in mm)				% Activity Index			
	<i>Pseudomonas fluorescens</i> (mg mL ⁻¹)		<i>Bacillus subtilis</i> (mg mL ⁻¹)		<i>Pseudomonas Fluorescence</i> (mg mL ⁻¹)		<i>Bacillus subtilis</i> (mg mL ⁻¹)	
	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
Hbsodh	2.5	3.0	1.0	2.0	20	21	9	15

Hbsmdh	6.0	8.0	2.5	4.0	48	57	23	31
dbodh	3	4	3	4	23	29	27	31
dbmdh	4	5	3	5	31	36	27	38
Hbssdh	3	4	4	5	23	29	36	38
Hdspdh	3	4	3	4	23	29	27	31
Compounds	Diameter of inhibition zone (in mm)				% Activity Index			
	<i>Escherichia coli</i> (mg mL ⁻¹)		<i>Bacillus subtilis</i> (mg mL ⁻¹)		<i>Escherichia coli</i> (mg mL ⁻¹)		<i>Bacillus subtilis</i> (mg mL ⁻¹)	
	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
Hgssdh	4	5	4	6	40	45	36	46
Hgspdh	4	5	4	5	40	45	36	38

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