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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SCHIFF BASE DERIVATIVES

DEMIR MÜLAZIMOĞLU AYŞEN[A]*, ÖZKALP BIROL[B] AND MERCIMEK BEDRETTIN[A]

[a]Department of Chemistry, Ahmet Keleşoğlu Education Faculty, Selçuk University, 42090, Konya, Turkey

[b]Vocational School of Health Services, Selçuk University, 42003, Konya, Turkey

ABSTRACT

In this study, Schiff base ligands were synthesized, characterized and investigated of antibacterial activities on five bacteria. 2-((4-benzyloxy-benzylidene)amino)phenol derived from 4-benzyloxybenzaldehyde and 2-aminophenol. (4-benzyloxy-benzylidene)pyridine-2-yl-amine derived from 4-benzyloxy-benzaldehyde and 2-aminopyridine. The Schiff bases were characterized by elemental analysis, FT-IR and ^{1}H-NMR. The Schiff bases were screened for in-vitro antibacterial activities by disc diffusion and broth microdilution techniques. The minimum inhibitory concentration (MIC) values were calculated by microplate reader at 620 nm. It was found compounds showed mild to moderate antibacterial activity by disc diffusion technique.

Keywords: *Schiff base, synthesis, antibacterial activity, disc diffusion, microdilution*

Introduction

In coordination chemistry, Schiff bases have a significant role as ligands still a century after their discovery[1, 2]. The importance of Schiff bases and their metal complexes are important as biochemical[3], electrochemical[4], analytical[5], antifungal and antibacterial activities[6-9], redox catalysts[10,11]. Schiff bases with donors (N, O, S, etc.) have structural similarities with natural biological systems and imports in elucidating the mechanism of transformation and rasemination reaction in biological systems due to presence of imine (-N=CH-) group[12].

In the present work, we have synthesized two Schiff base ligands. The antibacterial activities of the synthesized Schiff bases were reported in vitro using disc diffusion and microdilution tecnique against the bacteria: *Bacillus cereus*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus mutans*, *Staphylococcus aureus*

Experimental

4-benzyloxybenzaldehyde (Fluka), 2-aminophenol (Fluka) and 2-aminopyridine (Fluka) were used as received. All solvents used in this study were obtained from Merck and used without further purification. IR spectra were taken on a Perkin Elmer 1600 Series FTIR spectrophotometer on KBr pellets in the wave number range of 4000-400 cm^{-1}. The ^{1}H NMR spectra were recorded on Bruker DPX-400, in $CDCl_3$ as solvent and TMS as internal reference. The elemental analysis (C, H and N) were performed on a Leco CHNS-932 elemental analyzer. Melting point was determined on a Electrothermal 9100.

Corresponding Author:

e-mail:aysendemir79@gmail.com

Tel: *+90-332-3238220: +90 3323238225*

Synthesis of Schiff Base Ligands

2-((4-benzyloxy-benzylidene) amino) phenol (HL'): A solution of 4-benzyloxybenzaldehyde 5 mmol (1.061 g) in 10 mL absolute ethanol was added to a stirred solution of 2-aminophenol 5 mmol (0.546 g) in 10 mL warm absolute ethanol. The reaction mixture was refluxed for 3 h. The solid product was filtered off and washed with ethanol and subsequently dried over anhydrous $CaCl_2$ in a desiccator. The pure Schiff base was isolated as a white crystalline solid. This ligand is insoluble in common organic solvents such as diethylether, n-hexane, benzene, etc and soluble in polar solvents such as DMSO, $CHCl_3$ and DMF. FT-IR (cm^{-1}): 1600 (CH=N), 1505 (C=C), 2899 (alif.-CH), 1239 (C-O). ^{1}H-NMR ($CDCl_3$): 8.62 (s, 1H) HC=N; 6.88-7.88 (m, 13H) Ar-H; 5,15 (s, 2H) O-CH_2 ppm.

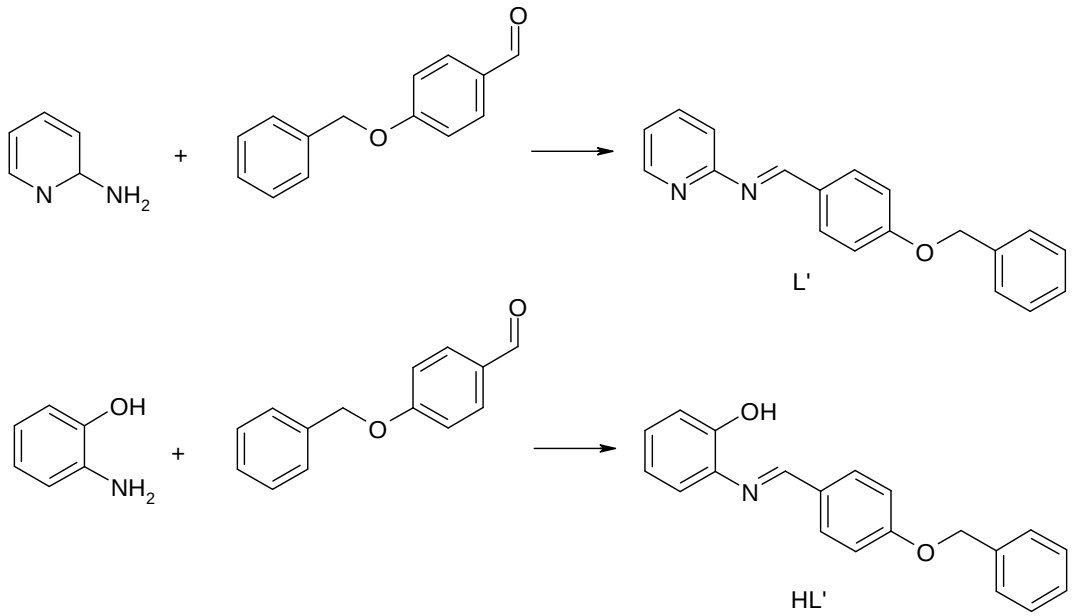

Figure 1: Synthesis of Schiff base derivatives

(4-benzyloxy-benzylidene)pyridin-2-yl-amine (L'): A solution of 4-benzyloxy-benzaldehyde 5 mmol (1.061 g) in 10 mL absolute ethanol was added to a stirred solution of 2-aminopyridine 5 mmol (0.471 g) in 10 mL warm absolute ethanol. The reaction mixture was refluxed for 2 h. The solid product was filtered off and washed with ethanol and subsequently dried over anhydrous $CaCl_2$ in a desiccator. The pure Schiff base was isolated as a white crystalline solid. This ligand is insoluble in common organic solvents such as diethylether, n-hexane, benzene, etc and soluble in polar solvents such as DMSO and DMF. FT-IR (cm^{-1}): 1681 (CH=N), 1502 (C=C), 2815 (alif.-CH), 1590 (C=N py), 1251 (C-O). ^{1}H-NMR ($CDCl_3$): 9.88 (s, 1H) HC=N; 7.09-7.83 (m, 13H) Ar-H; 5.15 (s, 2H) O-CH_2 ppm.

Biological Activity

The test microorganisms *Bacillus cereus* RSKK (1122), *Escherichia coli* RSKK (340), *Bacillus subtilis* RSKK (246), *Streptococcus mutans* RSKK (676), *Staphylococcus aureus* RSKK (96090) were obtained from the Refik Saydam National Public Health Agency. The organisms were maintained on agar slope at 4 ºC and sub-cultured for 24 h before use. Cephazoline (CZ 30, Bio-Disc) was used as positive control for disc diffusion tests. DMSO (Merck) was used as negative control. 96-well microtiter plates (TPP 92096) were used for microdilution tests. Nutrient Broth (Difco) and Mueller Hinton Agar (Difco) were used.

Antibacterial Testing by Disc Diffusion

The antibacterial activities of the Schiff bases were tested in vitro using disc diffusion method[13]. Bacterial inoculums were prepared from overnight grown cultures (24 h) in Nutrient broth (Difco) and turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 cfu/mL). Aliquots (100 µL) of inoculums were spread over the surface of Mueller Hinton Agar (Difco) plates with a sterile glass spreader. Sterilized paper discs (Oxoid, 6mm diameter) were wetted with 10 µL of a solution of each compound to be tested, in the concentration of 0,02 g/mL (200 µg per disc) in DMSO. The plates were then incubated 24 h at 37 °C. The formed inhibition zones were measured in mm. The values are reported at Table 2 as a mean of three replicates.

Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) was determined by broth microdilution method[14]. For MIC determination, the inoculums was prepared using a 4–6 h broth culture of each bacterial strains adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted in Nutrient broth media to give concentration of $\approx 10^6$ cfu/mL for bacteria. Two-fold serial dilutions of compounds were prepared in Nutrienth broth in 96-well plates starting from a stock solution of compounds (2.00 mg/mL DMSO). DMSO had no effect on the microorganism in the concentrations studied. An equal volume of bacterial inoculum was added to each well on the microtitre plate. In this manner final concentration of compounds range 500-0.49 µg/mL and $5x10^5$ cfu/mL bacteria in each well (last wells are broth only control well). The inoculated microtiter plates were then incubated at 37 °C for 24 h, and the growth was recorded spectrophotometrically at 620 nm using a microplate reader. The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculum). The MIC values are reported at Table 3 as the mean of three replicates.

Results and Discussion

In this study, Schiff base ligands were synthesized following the preparative route illustrated in Figure 1. The compounds were labeled as L' and HL'. All structures were characterized by FT-IR, ^{1}H-NMR and elemental analysis. Some properties of the Schiff bases are given in Table 1.

Table 1: Some properties of the Schiff bases

Compound	M. F.	F.W. (g/mol)	m.p. (°C)	Elemental analysis % calculated (found)		
				C	H	N
L'	$C_{19}H_{16}N_2O$	288.35	111-112	79.14 (79.04)	5.59 (5.48)	9.71 (9.64)
HL'	$C_{20}H_{17}NO_2$	303.36	130-131	79.19 (79.11)	5.65 (5.62)	4.62 (4.60)

The important IR absorption bands for the synthesized Schiff bases are listed. In the HL' and L', the strong bands show at 1600 cm^{-1} and 1681 cm^{-1} are assigned to the ν(C=N) streching mode. The absence of a ν(C=O) peak at around 1700 cm^{-1} is indicative of Schiff's base condensation. The bands show at 1505 cm^{-1} and 1502 cm^{-1} are assigned to the ν(C=C) stretching mode in the HL' and L'. These bands prove the presence benzene. A medium band corresponding to phenolic oxygen ν(C–O) is observed at 1239 and 1251 cm^{-1} for HL' and L', respectively[15,16].

The ^{1}H-NMR spectrum of the HL' and L' exhibits a singlet at 8.62 ppm and 9.88 ppm due to

the imine protons, respectively. The ^{1}H-NMR spectrum of the HL' shows multiplet in the range 6.88-7.88 ppm and L' shows multiplet in the range 7.09-7.83 ppm due to the aromatic protons. The O-CH_2 protons of HL' and L' exhibit singlet in the region 5.15 ppm. [16,17]. The results of elemental analysis were found in good agreement with the calculated values.

Antibacterial Activities

The antibacterial activities of the synthesized compounds were evaluated in vitro using disc diffusion method and broth microdilution techniques. The antibacterial activities data are given in Table 2-3.

Table 2: Antibacterial activities of the Schiff base ligands

Compound	*E. coli*	*B. subtilis*	*S. mutans*	*B. cereus*	*S. aureus*
	Zone of inhibition (mm)				
L'	14	12	10	10	14
HL'	10	12	8	10	12
Control (DMSO)	-	-	-	-	-
Standard (Cephazoline)	16	19	22	18	22

The antibacterial activities of the Schiff bases were tested in vitro using disc diffusion method[13]. Cephazoline was used as standard and DMSO was used as control.. DMSO had no effect on the bacterias in the concentrations studied. HL' showed moderate activity against *E. coli*, *B. subtilis*, *S. aureus* and *B.cereus* because of the presence of phenyl rings and -OH groups. L' showed moderate activity against *E. coli*, *B. subtilis*, *S. mutans*, *S. aureus* and *B.cereus* because of the presence of phenyl rings. Inhibition values of the compounds are summarized in Table 2.

Table 3: Minimum concentration inhibitory (MIC, µg/mL) of the Schiff base ligands against some bacterias

Compound	*E. coli*	*B. subtilis*	*S. mutans*	*S.aureus*	*B.cereus*
	MIC (µg/mL)				
L'	250	250	125	125	250
HL'	125	62.5	250	62.5	125

As it can be seen in Table 3, the compounds inhibited both the gram-pozitive and gram-negative bacterias with MIC's between 62.5 and 250 µg/mL. Most of them demonstrate weak activity against gram-positive and gram-negative bacterias. Absorbans-concentration curve of HL' against *B. subtilis* is given in Figure 2.

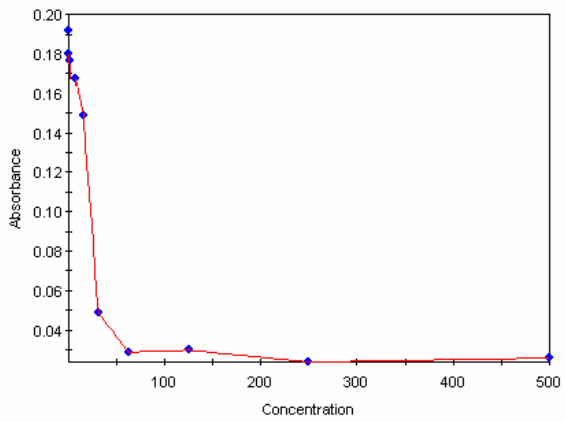

Figure 2: Absorbans-concentration curve of HL' against *B. subtilis*

In studies on Schiff bases and their metal complexes, metal complexes are more effective on bacterias compared to free Schiff bases[18, 19]. However the effects of Schiff bases vary among themselves as well[20]. The results at present the study have shown that free Schiff bases might also effective on bacterias by disc diffusion method.

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Author Information: Dr. DEMIR MÜLAZIMOĞLU AYŞEN Department of Chemistry, Ahmet Keleşoğlu Education Faculty, Selçuk University, 42090, Konya, Turkey

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