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Synthesis, characterization and antibacterial activities of two new Schiff bases devired from *(E)-2-(((2-aminoethyl) imino) methyl) phenol (E)-2-(4-aminobut-1-enyl)-5-methylbenzamine*

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In the present studies two new Schiff bases ligands (E)-2-(4-aminobut-1-enyl) phenol and (E)-2-(4-aminobut-1-enyl)-5-methylaniline were synthesized. The compounds were properly characterized through FT-IR and elemental analysis and its antibacterial activities were checked against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus aureus*. The Schiff base ligands were screened o for their antibacterial inhibiting potentials through Disc-diffusion method, *in vitro*. The results indicated that as compared to control i.e. Ciprofloxacin and Erythromycin the Schiff base have significant biological activity against *E. coli,* while no effective inhibition was noted for other tested bacterial strains. This research highlighted Schiff bases an effective drug against the *E. coli*.

Keywords: Schiff base, Antibacterial, FT-IR, chemical synthesis

INTRODUCTION

In the last decades, the misuse, overuse and uncontrollable prescriptions of the antimicrobial drugs by population led to the increase of antimicrobial resistance to conventional drugs, worldwide. Therefore, this alarming a serious threat to toward the present approached utilized for the treatment of such infectious complications (WHO, 2014). Among all, a resistant bacterial strain like *S. aureus* are the main causal agents of staphylococcal infection that led to increase the mortality among the patients, globally. The rate of this disease is noted to increasing at the rate of 10-25% with 11,000 fatal victims/annually and this rate may vary depending on the immunological status toward the bacterial strain (Strywski et al. 2008 and Mohmmad et al. 2015). Besides, bacteria are also economically very important being responsible for the production of bovine mastitis that leads to the losses of cattle's milk production (Cardozo et al. 2014).

To cope this global health challenge, there is need for the isolation and development of effective and novel antimicrobial strategies. Consequently, the achievements and designing of new antimicrobial drug quite distinct from the classical antibiotics is of key importance for the treatment of such infectious diseases well known for their chronic infections and could not be cured with conventional antibiotics that eventually leads to death (Khan et al. 2021). However, the synthetic molecules have emerged as potential substitutes for the conventional drugs or even as an adjuvant to them (Gosh et al. 2015). Based on such assumptions, Schiff's bases have emerged as an important antimicrobial molecule. Recently, the Schiff bases have extensive used as a food stuff manufacturing, pigment production, diagnostic chemistry, catalysis, antifungal, herbicides and insecticides, via; pharmacological activity or biological activities. At the moment, the contemplation of Schiff bases along with their metal complexes is escalating outstanding to their marvelous biological and pharmacological bioassays. Schiff bases played an important role as an effective storage space procedure, sensors, electrodes, planetary cells as well as biological sensor apart from its biological effectiveness (Venugopala et al. 2008 and Wang et al. 2008). Concerning the antimicrobial action, it´s been reported that Schiff bases present significant antibacterial potential (Villar et al. 2004 and Bhat et al. 2005). Presently, scientists have expended expressive effort to

use Schiff bases to target a condensing protein ketoacyl-acyl synthase (KAS) from multi drug resistant bacterial strains (Cheng et al. 2009; Lee et al. 2012). In addition, the ability of Schiff bases to bind oxygen in redox systems and also oxidize DNA are also quite important to antibacterial activity (Sheheryar et al. 2017).

Recently, our research group, showed that Schiff bases complexes with cobalt causes significant antibacterial activities against *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus* at a concentration of 32.2µg (Sheheryar et al. 2017). Therefore, the aim of the current research was to reinforce the importance of Schiff bases as possible antibiotic agent to treat bacterial infections.

MATERIALS AND METHODS

General experimental condition

Chemicals like, salicyldehyde (Fluka), 4-dimethyl amino benzaldehyde (Fluka) and Ethylene-di-amine (Fluka) were utilized. The whole set of solvents library used in the current study were got from the Merck deprived of any sort of purification and interference. Similarly, the Infra-Red spectra of the synthesized compounds were recorded on the KBr pellets in the wave number that ranges from f 4000-400 cm^{-1} (PCSIR laboratories, Peshawar). The Elemental analysis were also taken under consideration for the carbon, hydrogen and nitrogen. The compounds were also checked through TLC (Thin layer chromatography) analysis. The TLC apparatus consist of pre-coated silica gel plates (DC-Alugram 60 UV254 of Merck) and reading was noted before UV at 254nm. Same after the staining with cerium (IV) sulfate and sprayed reagents and allow to heat up till to coloration.

Synthesis of Schiff bases procedure

(E)-2-(((2-aminoethyl) imino) methyl)phenol (SBI)

The pure solution of Salicyldehyde 5mM (6.11g) in 15 mL ethyl-acetate was mixed to hot stirred solution of Ethylene-di-amine 5mM (3.0g) in 15mL ethyl-acetate. The whole reaction mixture was then refluxed in the RC (Reflux condenser) for 5 hours at 150°C. The solid product obtained was properly filtered and simultaneously washed with ethanol and then dried on $CaCl_2$ in desiccator. Similarly, the pure Schiff bases were obtained in the form of yellowish crystalline solid. These ligands were noted to insoluble in ordinary used organic solvents, like, n-hexane, benzene and dimethyl-ether, however completely soluble in DMSO (Dimethyl-sulfide) and DCM (Dichloromethane).

(E)-2-(4-aminobut-1-enyl)-5-methylbenzamine (SBII)

The solution of 4-dimethylamino benzaldehyde 5 mM (3.72 g) in 18mL ethyl acetate were mixed to already stirred 1.50g solution of Ethylene-di-amine 5mM in the 20mL of ethyl-acetate. The whole reaction mixture was properly refluxed at 150°C for 5 hours. After this time a solid product was obtained, filtered, simultaneously washed with ethanol and later dried on anhydrous calcium chlorides ($Cacl_3$) in desiccator. Similarly, pure schiff bases were isolated with yellow color in solid form. This ligand was noted to be in soluble in ordinary organic solvents like, n-hexanes, di-ethylether and benzenes, while found to be soluble in polar solvents like, DMSO and DCM.

Biological activity

Antibacterial activities of the isolated compounds against the *Escherichia coli, Bacillus cereus, Klebsiella pneumoniae, Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were taken under considerations following disc-diffusion approach (Sheheryar et al. 2017). Nutrient ager media was used as a culture for the antibacterial activity and for the desolations of compounds DMSO was utilized, respectively.

For the preparation of nutrients agar media 100- mL distal water was taken in the conical flask, adding the media slowly and warm it, while shaking, in order to dissolve the media completely. After this the whole mixture was sterilized in autoclave at 121°C, 15 pascal pressure for 15 minutes. The prepared media was cool down and stored for the analysis.

Antibacterial activities by diffusion method

Sheheryar et al. (2017), was followed for the determination of Schiff bases's antibacterial abilities *via*; disc-diffusion approach, *in vitro*. Prepared the bacterial inoculum from the mother cultures being grown overnight (24 hours) in the nutrient broth and adjust turbidity up to 0.06 OD at 600 nm. All plates were incubated at 37°C for the growth. The solidified media i.e. agar was stacked through sterilized cotton swab and reference bacterial strain (10µL) with the help of double autoclaved filter paper and 100µL treatment solution of each drug were given a the concentrated of 6 and 12µL in DMSO. Similarly, the plates were properly incubated for 24 hours at 37°C. After incubation the inhibition zones were measured in 'mm' and later on converted in to percentage and compared to the control, respectively.

RESULTS AND DISCUSSION

In this study, Schiff base were obtained from the reaction ethylene-di-amine with different phenolic compounds. Among all, the reaction between salicyldehye and ethylene-di-amine produced Schiff base 1 (SB-1), whereas the reaction between 4-dimethylamino benzaldehyde results in the Schiff base 2 (SB-2) production (Figure. 1). These compounds were properly labeled as SB-1 and SB-2, respectively and its properties are presented in the Table 1. Similarly, the chemical structures of these properly characterized by using elemental and IR analysis. It was noted that SB-1 and SB-2 showed strong bands at 1600 and 1681 cm^{-1} and assigned to *v*(C=N) stretching mode. Similarly, at the absence of a *v*(C=O) peak at near 1700 cm^{-1} and considered to be a strong indication of Schiff base

condensation. These bands also showed strong bands at 1505 and 1502 cm^{-1} and are assigned to v(C=C) stretching mode in the SB-1 and SB-2, respectively. These bands also showed the presence of benzene ring. However, the medium band equivalent to the phenolic oxygen (C-O) was also noted at 1239 and 1251 cm^{-1} for the SB-1 and SB-2, respectively.

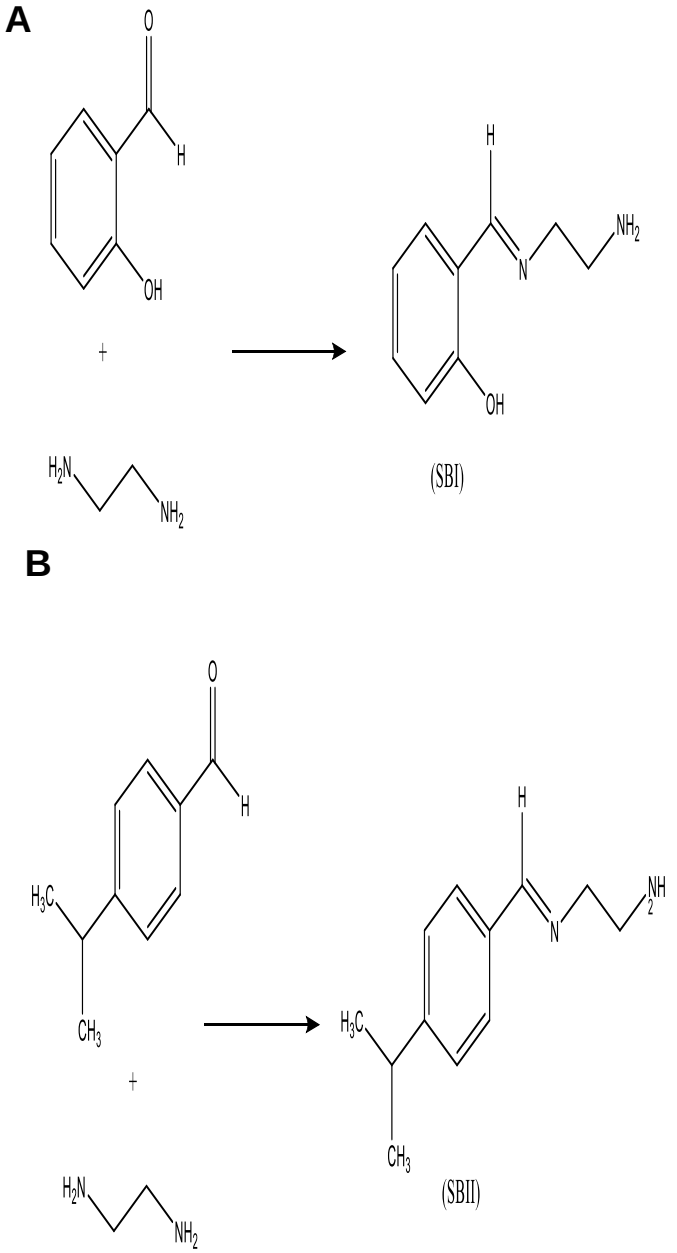

Figure 1: Synthesis of Schiff bases. The scheme shows the synthesis of Schiff base I (A) by the combination of Salicyldehye 5 mM and Ethylene-di-amine 5 Mm and the Schiff base II (B) is synthesized by the addition of 4-dimethylamino benzaldehyde 5 mM with Ethylene-di-amine 5mM and the mixture was refluxed at 150 °C for 5 h to obtain the Schiff basses I and II.

Antibacterial activities

By using the disc-diffusion approach the antibacterial activates of the synthesized compounds were carried out against the *B. cereus, S. aureus, E. coli, K. pneumonia, P. aeruginosa* and *S. typhimurium***,** *in vitro* (Tables 2 and 3). Erythromycin was used as a positive control. The Schiff base SB-2 showed no antibacterial activity against any bacterial strains by applying the 6 µL (0.7 µM) and 12 µL (1.4 µM). Whereas, SB-I showed significant inhibitory effect against the *E. coli* even at very low concentration. The measured zone of inhibition of SB-I against the *E. coli* was 8.5 and 13 mm at 6 µL (0.7 µM) and 12 µL (1.4 µM), respectively. The present results are in line with the previous investigations of Sheheryar et al. (2017) using the Schiff bases against *E. coli*.

Table 1: The results of molecular formula and formula weight derived for Schiff base I and II

S.No	Molecular formula	Formula weight	Elemental analysis			
			% of C	% of H	% of O	% of N
1	$C_9H_{12}ON_2$	164	73.61	7.97	9.81	8.58
2	$C_{12}H_{18}N2$	190	73.17	9.75	--	17.03

Among all, the *E. coli* strain causes severe stubborn and dangerous inflammations in the human cells/tissues. This strain has the abilities of permeability to the cells of red blood cells where it causes and shifting from one tissue to another for example, it causes inflammation n septicemia, meningitis and other sever urinary tract dysfunctions. The findings showed that the Schiff bases have the potential to actively inhabit the growth and development of *E.coli*, our results are strongly agree with the previous investigations of Sheheryar et al. (2017).

Table 2: The inhibition of bacterial colonies growth by the application of 6µL of Schiff base I and II, respectively.

Samples	*E. coli* (-ve)	*B. cereus* (+ve)	*S. typhimurium* (-ve)	*K. pneumonia* (-ve)	*P. aeruginosa* (-ve)	*S. aureus* (+ve)
Schiff base I	28.3% 8.5 mm	--	--	--	--	--
Schiff base II	--	--	--	--	--	--
Positive control (Ciprofloxacin)	100% 31 mm	100% 30 mm	100% 29 mm	100% 18 mm	100% 26.5 mm	
Positive control (erythromycin)	--	--	--	--	--	100% 26.5 mm
Negative control (DMSO)	0%	0%	0%	0%	0%	0%

Table 3: Bacterial growth inhibition shown by applying 12 µL of Schiff base and II

Samples	*E. coli* (-ve)	*B. cereus* (+ve)	*S. typhimurium* (-ve)	*K. pneumoniae* (-ve)	*P. aeruginosa* (-ve)	*S. aureus* (+ve)
Schiff base I	13 mm 43.33%	--	--	--	--	--
Schiff base II	--	--	--	--	--	--
Positive control (Ciprofloxacin)	30 mm 100%	30 mm 100%	35 mm 100%	19.5 mm 100%	35 mm 100%	--
Positive control (Erythromycin)	--	--	--	--	--	31 mm 100%
Negative control (DMSO)	--	--	--	--	--	--

CONCLUSION

The current findings showed synthesis, characterization of two new 'Schiff bases' and checked via; *in vitro* for the antibacterial activities. These Schiff bases were physically and chemically characterized with the help of IR and elemental analysis. The antibacterial activities of the Schiff bases were properly tested on the bacterial strains, including, E. *coli, P. aeruginosa, B. cereus, S. typhimurium*, *S. aureus* and *K. pneumoniae,* accordingly and 'Erythromycin' was used as standard. Interestingly, the tested compound i.e. SB-I showed significant biological activity against two bacterial strains *E. coli* and, while compound SB-2 showed no remarkable activity.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example, WEP designed and performleed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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