Designing New Vanillin Schiff Bases and their Antibacterial Studies

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***Abstract*—The antimicrobial drugs occupy a unique niche in the history of medicine. A series of vanillin substituted Schiff bases (SB-1 to SB-6) were synthesized using vanillin and various aromatic amines in presence of a basic catalyst, triethyl amine. The synthesized compounds were authenticated by Thin Layer Chromatography (TLC), Ultraviolet-Visible, Fourier Transformer-Infrared (FT-IR), Nuclear Magnetic Resonance (NMR) and mass spectroscopic techniques. The Antibacterial activity of the synthesized compounds was studied using disc diffusion method and the concentration was fixed using Minimum inhibitory concentration by test tube dilution method using Gentamicin as standard drug. The antibacterial study revealed that compounds SB-5 and SB-6 showed excellent activity against gram positive bacteria: B.subtilis and S.aureus and gram negative bacteria: P.aeruginosa and K. pneumoniae. All the six Schiff bases showed excellent activity against B. subtilis.**

***Index Terms*—vanillin, aromatic amines, schiff bases, antibacterial activity, test tube dilution method, minimum inhibitory concentration, disc diffusion method**

I. Introduction

Infectious diseases are the major cause of morbidity in the world. The number of multiple drug resistant strains and appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This situation has provided the impetus to the search for new antimicrobial substances. Schiff bases are compounds of containing C=N group. They are often synthesized from amine and aldehyde or ketone. Schiff bases have gained importance due to their application in many pharmacological activities like antibacterial [1], [2], antifungal [3], anti-proliferative [4], antitumor [5], and antipyretic properties. Schiff bases with aryl substituents are more stable and readily synthesized. Whereas those containing alkyl substituent is relatively unstable. Schiff bases of aliphatic aldehydes are unstable and readily polymerizable while those with aromatic aldehydes having effective conjugation are more stable [6]. The antimicrobial drugs occupy a unique niche in the history of medicine. Considering the increased incidences of severe opportunistic bacterial infections in immunological deficient patients together with the development of resistance among pathogenic gram positive and gram negative bacteria, there is a great need in finding new compounds that may be effective against antibiotic resistant bacteria. During the entire preceding history of medicine fewer of drugs had known focus of action and then fewer had been submitted to synthetic investigations.

In the present work a novel series of six Vanillin schiff bases are synthesized (**SB-1** to **SB-6**) and further screened for antibacterial activity against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bactetia (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*). In an attempt to identify a potent and safer antimicrobial agent, we focused our efforts towards the synthesis of novel schiff bases.

II. Materials and Methods

The melting points of the compounds were determined on a Thoshniwal electric Melting point apparatus and the values were uncorrected. The purity of all the compounds was routinely checked by TLC on Silica gel-GF 254 (Merck) coated plates. Spotting was done by iodine in iodine chamber. The UV spectra of the compounds were recorded on double beam Shimadzu UV1800. The I.R spectra of the compounds were recorded on a Thermo Nicolet Nexus 670-FTIR, UKM, Malaysia using KBr disc method. ^{1}H NMR spectra were recorded on Bruker UX-NMR Instrument with TMS as internal standard and CDCl3 as solvent. Chemical shift values were expressed in δ ppm. Mass spectra were recorded on HITACHI RMU GL, UKM, Malaysia. All the solvents and chemicals used were of Analytical grade.

III. Results and Discussion

A. Synthesis

A series of 6 novel vanillin substituted schiff bases were synthesized as per Fig. 1.

2-methoxy-4-((E) (phenylimino)methyl)phenol (**SB-1**).

Vanillin (0.01M) and aniline (0.01M) were dissolved in 50mL of anhydrous ethanol separately. Aniline solution was then added drop-wise into vanillin solution in a conical flask. This mixture was then made up to 150 mL with 95% anhydrous ethanol. 2 to 3 drops of triethylamine (basic catalyst) was added [7], [8]. The mixture was then stirred using magnetic stirrer at 60^{o}C to 70^{o}C for 6 h on water bath. The reaction was monitored by TLC. The sample mixture was evaporated under pressure at 65^{o}C using rotatory evaporator. The solid obtained on concentration of filtrate was crystallized

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from aqueous ethanol. Mol. Formula: $C_{14}H_{13}NO_2$; Mp 100-110 ^oC; UV (λ max): 353 nm; IR (KBr cm^{-1}): 3235 (C-H, Str), 1240 (C-O-C Str), 3055 (Ar-H Str), 1320 (C-N Str), 3645 (Broad O-H Str), 1668 (C=N Str); ^{1}HNMR (CDCl3) (δ ppm): 6.70 - 7.54 (m, 3H, benzylidenimin) , 7.30-7.80 (m, 4H, phenyl), 5.00 (s, 2H, hydroxy), 3.73 (s, 3H,-methoxy), 8.43 (d, 1H, benzylidenimin); MS (m/e) : 227.0.

(E)-4-(4-hydroxy-3-methoxybenzylideneamino)benzaldehyde ***(SB-2)***

Vanillin (0.01M) and p-amino benzaldehyde (0.01M) were dissolved in 50 mL of anhydrous ethanol separately. P-amino benzaldehyde solution was then added drop-wise into vanillin solution in a conical flask. This mixture was then made up to 150 mL with 95% anhydrous ethanol. 2 to 3 drops of triethylamine (basic catalyst) was added [9],[10]. The mixture was then stirred using magnetic stirrer at 60^{o}C to 70^{o}C for 6 h on water bath. The reaction was monitored by TLC. The sample mixture was evaporated under pressure at 65^{o}C using rotatory evaporator. The solid obtained on concentration of filtrate was crystallized from aqueous ethanol. Mol. Formula: $C_{15}H_{13}NO_3$; Mp 130-133 ^oC; UV (λ max): 307 nm; IR (KBr cm^{-1}): 1240 (C-O-C Str), 2620, 2780 (C-H of CHO, Str), 3650 (Broad O-H Str), 1725 (C=O of CHO, Str), 3235 (C-H, Str), 3050 (Ar-H, Str), 1320 (C-N Str), 1540-1620 (C=C, Str), 1658 (C=N Str); ^{1}HNMR (CDCl3) (δ ppm): 9.89 (s, 1H, aldehyde), 7.3-7.8 (m, 4H, phenyl), 8.4 (s, 1H, benzylidenimin), 3.75 (s, 3H, methoxy), 5.00 (s, 1H, hydroxy), 6.6-7.2 (m, 3H, benzylidenimin); MS (m/e) : 255.2.

(E)-4-(4-hydroxy-3-methoxybenzylideneamino)phenol ***(SB-3)***

Vanillin (0.01M) and p-amino phenol (0.01M) were dissolved in 50 mL of anhydrous ethanol separately. P-amino phenol solution was then added drop-wise into vanillin solution in a conical flask. This mixture was then made up to 150 mL with 95% anhydrous ethanol. 2 to 3 drops of triethylamine (basic catalyst) was added [9], [10]. The mixture was then stirred using magnetic stirrer at 60^{o}C to 70^{o}C for 6 h on water bath. The reaction was monitored by TLC. The sample mixture was evaporated under pressure at 65^{o}C using rotatory evaporator. The solid obtained on concentration of filtrate was crystallized from aqueous ethanol. Mol. Formula: $C_{14}H_{13}NO_3$; Mp 140-143 ^oC; UV: 302 nm (λ max): nm; IR (KBr cm^{-1}): 3645 (Broad O-H Str), 3235 (C-H, Str), 1240 (C-O-C, Str), 3055 (Ar-H Str), 1540-1600(C=C, Str) 1320 (C-N, Str), 1668 (C=N, Str); ^{1}HNMR (CDCl3) (δ ppm): 7.23 - 7.83 (m, 4H, phenyl), 5.00 (s, 2H, hydroxy), 3.73 (s, 3H, methoxy), 8.42 (d, 1H, benzylidenimin) 6.6-7.2 (m, 3H, benzylidenimin); MS (m/e) : 243.9.

4-((E)-(4-methoxyphenylimino)methyl)-2-methoxyphenol ***(SB-4)***

Vanillin (0.01M) and p-methoxy aniline (0.01M) were dissolved in 50 mL of anhydrous ethanol separately. P-methoxy aniline solution was then added drop-wise into vanillin solution in a conical flask. This mixture was then made up to 150 mL with 95% anhydrous ethanol. 2 to 3 drops of triethylamine (basic catalyst) was added [9], [10]. The mixture was then stirred using magnetic stirrer at 60^{o}C to 70^{o}C for 6 h on water bath. The reaction was monitored by TLC. The sample mixture was evaporated under pressure at 65^{o}C using rotatory evaporator. The solid obtained on concentration of filtrate was crystallized from aqueous ethanol. Mol. Formula : $C_{14}H_{15}NO_3$; Mp 134-137 ^oC; UV (λ max): 306 nm IR (KBr cm^{-1}): 1255 (C-O-C, Str), 3235 (C-H, Str), 3050 (Ar-H, Str), 1320 (C-N Str), 1540-1620 (C=C, Str), 1658 (C=N Str), 3645 (Broad O-H Str); ^{1}HNMR (CDCl3) (δ ppm): 6.50 – 7.23 (m, 3H, benzylidenimin), 7.30-7.80 (m, 4H, phenyl), 5.00 (s, 1H, hydroxy), 3.73 (s, 6H, methoxy), 8.42 (d, 1H, benzylidenimin); MS (m/e) : 257.2.

(E)-2-(4-hydroxy-3-methoxybenzylideneamino)-4-methoxybenzoic acid ***(SB-5)***

Vanillin (0.01M) and 2-amino-4-methoxy benzoic acid (0.01M) were dissolved in 50 mL of anhydrous ethanol separately. 2-amino -4-methoxy benzoic acid solution was then added drop-wise into vanillin solution in a conical flask. This mixture was then made up to 150 mL with 95% anhydrous ethanol. 2 to 3 drops of tri ethylamine (basic catalyst) was added [9], [10]. The mixture was then stirred using magnetic stirrer at 60^{o}C to 70^{o}C for 6 h on water bath. The reaction was monitored by TLC. The sample mixture was evaporated under pressure at 65^{o}C using rotatory evaporator. The solid obtained on concentration of filtrate was crystallized from aqueous ethanol. Mol. Formula : $C_{16}H_{15}NO_5$; Mp 160-163 ^oC; UV (λ max): 338 nm; IR (KBr cm^{-1}): 3650 (Broad O-H, Str), 2450-2900 (O-H of COOH, Str), 1710 (C=O of COOH, Str), 1275 (C-O of COOH, Str), 1245 (C-O-C, Str), 3235 (C-H, Str), 3050 (Ar-H, Str), 1323 (C-N, Str), 1540-1620 (C=C, Str), 1658 (C=N Str); ^{1}HNMR (CDCl3) (δ ppm): 6.60 – 7.23 (m, 3H, benzylidenimin), 7.35- 8.54 (m, 3H, phenyl), 5.00 (s, 1H, hydroxy), 3.73 (s, 6H,-methoxy), 8.42 (d, 1H, benzylidenimin), 11.00 (S, 1H, carboxylic acid); MS (m/e): 301.1.

(E)-4-(4-hydroxy-3-methoxybenzylideneamino) benzoic acid ***(SB-6)***

Vanillin (0.01M) and p-amino benzoic acid (0.01M) were dissolved in 50 mL of anhydrous ethanol separately. P-amino benzoic acid solution was then added drop-wise into vanillin solution in a conical flask. This mixture was then made up to 150mL with 95% anhydrous ethanol. 2 to 3 drops of tri ethylamine (basic catalyst) was added [9], [10]. The mixture was then stirred using magnetic stirrer at 60^{o}C to 70^{o}C for 6 h on water bath. The reaction was monitored by TLC. The sample mixture was evaporated under pressure at 65^{o}C using rotatory evaporator. The solid obtained on concentration of filtrate was crystallized from aqueous ethanol. Mol. Formula: $C_{15}H_{13}NO_4$; Mp 155-157 ^oC; UV(λ max): 437 nm; IR (KBr cm^{-1}): 3233 (C-H, Str), 3650 (Broad O-H, Str), 1240 (C-O-C Str), 3055 (Ar-H Str), 1540-1600 (C=C, Str), 2900 (O-H of COOH, Str), 1710 (C=O of COOH, Str), 1275 (C-O of COOH, Str), 1320 (C-N Str), 1668 (C=N Str); ^{1}HNMR (CDCl3) (δ ppm): .50 – 7.23 (m, 3H, benzylidenimin), 7.35- 8.54 (m, 4H, phenyl), 5.00 (s, 1H,

hydroxy), 3.73 (s, 3H,-methoxy), 8.42 (d, 1H, benzylidenimin), 11.00 (S, 1H, carboxylic acid); MS (m/e) : 271.6

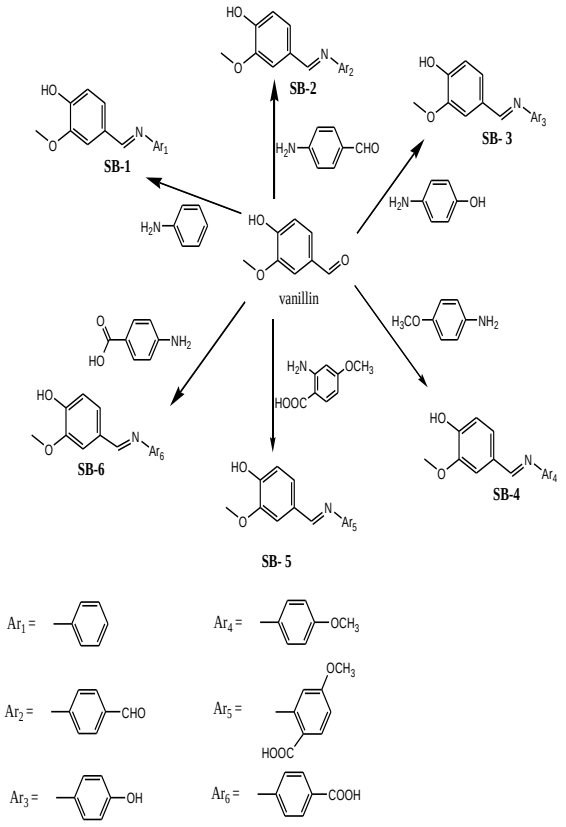

Figure 1. Synthesis of vanillin substituted schiff bases.

B. Antibacterial Activity

The antibacterial studies are done to find the efficacy of the synthesized vanillin Schiff bases. The gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bactetia (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) were used in the antibacterial studies. The antibacterial activity was evaluated by tube dilution method which depends on the inhibition of growth of a microbial culture in a uniform solution of antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic [10]. In this method minimum inhibitory concentration MIC of the test compounds was determined. Test compounds and standard drug were dissolved in dimethyl sulfoxide to give concentration of 2000 µg/mL. Double strength nutrient broth I.P was used. Suspension of each microorganism was made by transferring the organism from culture to 10 mL of sterile normal saline solution.

Determination of minimum inhibitory concentration (MIC).

One mL of sterilized media was poured into sterile test tubes. One mL of 2000 µg/mL test solution was transferred in one tube and serially diluted to give concentrations of 1000, 500, 250 and 125 µg/mL. To all the test tubes 0.1 mL of suspension of bacteria in saline was added and the tubes were incubated at 37°C for 24 h. The growth in the tube was observed visually for turbidity. MIC was determined with the lowest concentration of the sample that retarded the development of turbidity. The activity of the new compounds, control and standard drug Gentamicin [11] is given in Table I. Graphical representation of minimum inhibitory concentration (MIC) of vanillin substituted schiff bases against Gram positive and negative bacterial strains is given in Fig. 2 and Fig. 3, respectively.

TABLE I. MINIMUM INHIBITORY CONCENTRATION OF VANILLIN SCHIFF BASES

Comp.	Minimum inhibitory concentration (MIC) (µg/mL)			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*P.aeruginosa*	*K.pneumoniae*
SB-1	250	250	500	500
SB-2	250	500	250	250
SB-3	250	250	125	250
SB-4	125	125	250	250
SB-5	125	125	125	125
SB-6	125	125	125	125
Control	-	-	-	-
Std*	125	125	125	125

Std* - Gentamici

TABLE II. ANTIBACTERIAL ACTIVITY OF NEW VANILLIN SCHIFF BASES.

Comp.	Zone of inhibition (mm) (250 µg/mL)			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*P.aeruginosa*	*K.pneumoniae*
SB-1	10	6	9	8
SB-2	11	10	8	8
SB-3	11	9	9	8
SB-4	10	8	8	6
SB-5	10	11	10	12
SB-6	12	12	10	12
Std*	10	10	11	12

Std* - Gentamycin.

Disc-diffusion method

The Disc Diffusion method [12], [13] was used to determine the antimicrobial activities of the Schiff bases using standard procedure of 6 mm disc were prepared from whatman's filter paper no. 1. Schiff base solutions of varying concentrations ranging from 125, 250 and 500 µg/ml were prepared. Nutrient agar was prepared, sterilized and used as the growth medium for the culture of microorganisms; 20 ml of the sterilized medium was poured into each sterilized petri dish, covered and allowed to solidify. 16 hour old broth cultures of the specified microorganisms were used for testing antibacterial activity [14].

The sample, control and standard treated discs were air dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 hour so as to allow the maximum diffusion of compounds from the test disc into the agar plate and later incubated at 37 ℃ for 24 h in case bacteria [15], after which the zone of inhibition could be

easily observed. The zone of inhibition of the new compounds, control and standard drug Gentamicin is given in Table II.

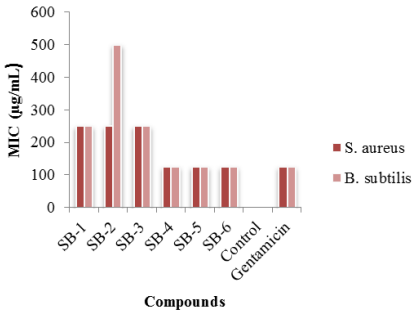

Figure 2. Graphical representation of minimum inhibitory concentration (MIC) of vanillin schiff bases against Gram positive bacterial strains.

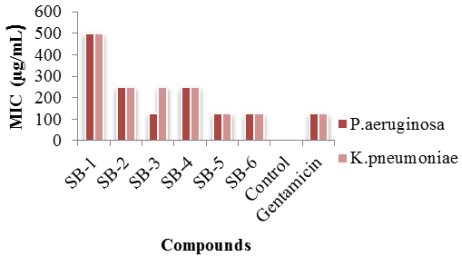

Figure 3. Graphical representation of minimum inhibitory concentration (MIC) of vanillin schiff bases against Gram negative bacterial strains.

IV. Conclusion

A total of six new vanillin schiff bases (**SB-1 to SB-6**) were synthesized. The structure of synthesized compounds was confirmed on the basis of I.R, N.M.R and Mass spectroscopy. The spectroscopic data of IR, ^{1}H-NMR and Mass are in agreement with the structure of synthesized compounds. The Antibacterial activity of the synthesized compounds was studied using disc diffusion method and the concentration was fixed using Minimum inhibitory concentration (MIC) method. The antibacterial study revealed that revealed that all compounds showed little to excellent activity as compared to standard drug Gentamicin. **SB-5** and **SB-6** showed excellent activity against gram positive bacteria: *B.subtilis* and *S.aureus* and gram negative bacteria: *P.aeruginosa* and *K.pneumoniae*. All the six Schiff bases showed excellent activity against *B.subtilis.*

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