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ORIGINAL RESEARCH

Synthesis, Characterization and Antimicrobial Activity of *p*-Vanillin and Vanillin Schiff Bases

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Abstract:

Introduction: The unending interest in the study of Schiff base ligands arise from the ease of their preparation and their versatility in several fields of Chemistry and Biochemistry. Some of the major biochemical processes such as trans-amination and glycosylation involve the formation of Schiff base intermediates. In addition, compounds containing the azomethine group have been found to often possess different biological activity such as anti-microbial, anti-viral and anti-inflammatory activity. In most cases, the extent of the potency depends on the nature of the constituent amino and or aldehyde moiety.

Aims: The aim of this study was to synthesize, characterize and evaluate the antimicrobial activity of Schiff base ligands derived from *p*-vanillin and vanillin.

Materials and Methods: The Schiff base ligands were prepared by condensing *p*-vanillin and vanillin with substituted aniline, aminonaphthalene and 3-aminopyridine respectively. The ligands have been characterized by elemental analysis, ^{1}H- & ^{13}C-NMR, infrared, Raman and electronic spectral data. The antimicrobial study was carried out by screening the prepared ligands against *Staphylococcus aureus subsp. aureus* ATCC® 6538™, *Bacillus substillis subsp. spizizenii* ATCC® 6633™*, *Escherichia coli* ATCC® 8739™* and *Candida albicans* ATCC® 2091™* using agar diffusion technique.

Results: The azomethine, HC=N, ^{1}H- & ^{13}C-NMR signals were observed at 8.66-8.30 ppm and 164.42-157.99 ppm respectively. The infrared $\nu_{C=N}$ band appeared at 1622-1607 cm^{-1}. The *p*-vanillin ligands exhibited higher activity than the vanillin based ligands. The *p*-hydroxyl and 3-aminopyridine ligands possess significant antimicrobial activity especially, antifungal activity.

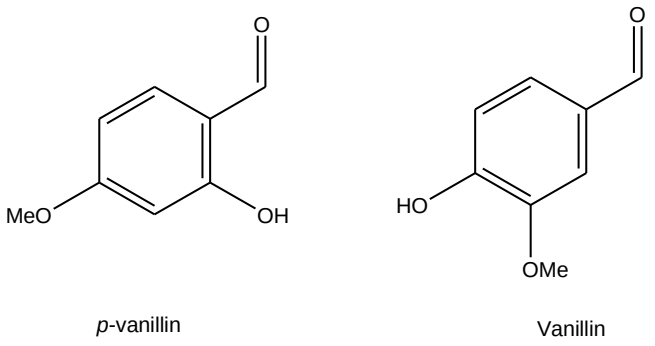
Conclusion: The study shows that the antimicrobial activity of the Schiff base ligands depends largely on the nature of the aldehyde moiety of the synthesized compounds.

Keywords: Salicylaldehyde, Schiff bases, anti-fungal agents, vanillin

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

The unending interest in the study of Schiff base ligands arise from the ease of their preparation and their versatility in several fields of Chemistry and Biochemistry. The first Schiff base compound was prepared by Hugo Schiff in 1861[1]. Schiff bases are prepared form one pot condensation reaction between primary amine and an aldehyde or ketone (ref.). Schiff bases play significant biological roles [2] in living systems, for example, in vision and in determining the flexibility of the wall of the veins, etc. Schiff base structures are involved in a number of biochemical processes such as transamination [3] and non-enzymatic glycosylation [4]. Furthermore, compounds containing the azomethine group have been found to often possess different biological activity such as anti-microbial [5-9], anti-viral [10] and anti-inflammatory activity [11-14]. In most cases, the extent of the potency depends on the nature of the substituents on the amino and or the aldehyde moiety. Study on Schiff base ligands is most often related to the salicylaldimine compounds due to their versatility in terms of synthesis and applications.



p-vanillin Vanillin

Fig. 1: Structures of p-vanillin and Vanillin

Although, several studies have been reported on the synthesis and applications of Schiff bases compounds containing 2-hydroxylbenzaldehyde (salicylaldehyde) [15-19] and 2-hydroxyl-4-methoxylbenzaldehye (o-vanillin) [20-23], similar attention has not been given to their closest analogues (*p*-vanillin and vanillin) [24] . Para-vanillin is 2-hydroxy -4-methoxylbenzaldehyde while vanillin is 4-hydroxyl-3-methoxylbenzaldehyde as shown in figure 1. The aim of this study, therefore, was to synthesize, characterize and evaluate the antimicrobial activity of Schiff base ligands derived from *p*-vanillin and vanillin

2. MATERIAL AND METHODS

All the chemicals and reagents were of reagent grade and used without further purification. The proton and carbon-13 NMR spectra were obtained from Bruker Avance NMR equipment operating at 400 MHz. The samples were dissolved in deuterated chloroform, d_1, with TMS as internal standard. The FT-IR absorption spectra were recorded on PerkinElmer Spectrum 100 FT-IR equipped with universal attenuated total reflectance (ATR) accessory, while Raman spectra were recorded on a Bruker Vertex 70 RAM 11 spectrophotometer using a 1064 nm ND-YAG laser. The electronic spectra were obtained from the PerkinElmer Lambda 25 spectrophotometer. The elemental analysis, CHN, was done on Vario MICRO V1.6.2 elemental analysen systeme GmbH, while the melting points (uncorrected) of the compounds were determined using the Galenkemp melting point apparatus. All the micro-organisms were obtained from Microbiologics, Cape Town.

2.1 Synthesis of the Schiff base ligands

The Schiff base ligands were prepared according to the general procedure as reported in the literature [25-28]. The ligands were prepared by condensing equimolar amounts of *p*-vanillin or vanillin with *p*-substituted aniline, 1-aminonaphthalene, 3-aminopyridine and 3-aminomethylpyridine respectively. Synthesis of the *p*-substituted aniline ligands has earlier been reported [28].

Ligand L1

10 mmol (1.522 g) of *p*-vanillin in hot 10 ml ethanol was mixed with 10 mmol (0.91 ml) of aniline in hot ethanol and refluxed in 100 ml round bottom flask for 1 hr to obtain yellow precipitate. The precipitate was filtered under suction, washed with ethanol and dried in a desiccator. The same procedure was repeated for all the syntheses to obtain ligands L1 – L9 by condensing *p*-vanillin with aniline, *p*-chloroaniline, *p*-bromoaniline, *p*-methylaniline, *p*-methoxylaniline, *p*-nitroaniline, *p*-hydroxylaniline, 1-aminonaphthalene and 3-aminopyridine respectively.
In the same vein, condensation of vanillin with the various amine compounds yielded ligands L10 – L17 under the same condition. The analytical and some selected spectral data for the compounds are presented in tables 1 – 3.

3. RESULTS AND DISCUSSION

3.1 Analytical and Elemental analysis

The Schiff base ligands were obtained in good yield with relatively high purity as indicated by the elemental analysis result in table 1. The experimental values of the microanalysis results were in close agreement with the calculated values. All the *p*-vanillin derived compounds have bright yellow colour while the vanillin analogues are mostly cream in colour. The variation in colour may be due to the position of the methoxy and the hydroxyl groups on the aldehyde moiety of the ligands.

3.2 ^{1}H-NMR and ^{13}C-NMR Study

The selected ^{1}H-NMR and ^{13}C-NMR spectral data for the compounds are presented in table 2. The characteristic azomethine proton, HC=N, for the Schiff base ligands were observed as a strong 1 H singlet at 8.66 – 8.30 ppm. This was further confirmed by the appearance of a strong carbon signal at 164.42 – 157.99 ppm corresponding to the azomethine carbon, C=N, of the Schiff base ligands. The signal due to the methoxyl group on the vanillin and *p*-vanillin moiety of the ligands was observed as 3 H singlet at 4.02 - 3.88 ppm. In addition, the methyl protons of the *p*-toluidine Schiff base ligands appeared as 3 H singlet at 2.41 ppm in the ^{1}H-NMR spectra of the compounds. The methoxyl and the methyl carbon signals were observed at 56.58 – 55.86 ppm and 21.45 ppm respectively.

Table 1: Analytical data for the Schiff base ligands

Ligands	Molecular Formula	%C	%H	% N
L1	$C_{14}H_{13}NO_2$	73.17 (73.98)	5.66 (5.77)	6.01 (6.16)
L2	$C_{14}H_{12}NO_2Cl$	63.12 (64.27)	4.58 (4.62)	5.24 (5.35)
L3	$C_{14}H_{12}NO_2Br$	54.13 (54.92)	3.78 (3.95)	4.45 (4.57)
L4	$C_{15}H_{15}NO_2$	73.83 (74.66)	6.18 (6.27)	5.69 (5.80)
L5	$C_{15}H_{15}NO_3$	69.21 (70.02)	5.83 (5.88)	5.35 (5.44)
L6	$C_{14}H_{12}N_2O_4$	61.42 (61.76)	4.40 (4.44)	10.28 (10.29)
L7	$C_{14}H_{13}NO_2$	68.71 (69.12)	5.46 (5.39)	5.79 (5.76)
L8	$C_{18}H_{15}NO_2$	77.28 (77.95)	5.36 (5.45)	4.90 (5.05)
L9	$C_{13}H_{12}N_2O_2$	67.63 (68.40)	5.29 (5.29)	12.09 (12.27)
L10	$C_{14}H_{13}NO_2$	74.01 (73.98)	5.87 (5.73)	6.16 (6.16)
L11	$C_{14}H_{12}NO_2Cl$	64.68 (64.27)	5.01 (4.62)	5.34 (5.35)
L12	$C_{14}H_{12}NO_2Br$	54.94 (54.92)	3.93 (3.95)	4.52 (4.57)
L13	$C_{15}H_{15}NO_2$	74.64 (74.66)	6.24 (6.27)	5.56 (5.80)
L14	$C_{15}H_{15}NO_3$	70.76 (70.02)	6.41 (5.88)	5.47 (5.44)
L15	$C_{14}H_{13}NO_2$	68.38 (69.12)	5.72 (5.39)	5.57 (5.76)
L16	$C_{18}H_{15}NO_2$	77.95 (77.95)	5.54 (5.45)	5.04 (5.05)
L17	$C_{13}H_{12}N_2O_2$	68.35 (68.40)	5.48 (5.29)	12.03 (12.27)

Furthermore, the hydroxyl, OH, proton of the p-vanillin compounds was observed far downfield as 1 H broad signal at 13.97 – 12.27 ppm due to a strong intra-molecular hydrogen bonding [15] within the Schiff base ligands. However, the hydroxyl signal for the vanillin based ligands resonated at 7.02 – 6.06 ppm except for the *p*-hydroxyl Schiff base ligand, which occurred at 9.53 ppm

3.3 Infrared and Raman Study

The infrared spectral data for the compounds are presented in table 3. The stretching vibration band for the phenolic hydroxyl group was nearly non-evident in the spectra of the *p*-vanillin Schiff base ligands due to strong intra-molecular hydrogen bonding typical of the ortho-hydroxylbenzaldimines [17, 29]. The presence of the hydroxyl group was substantiated by the appearance of a prominent band at 1286 -1271 cm^{-1} corresponding to ν_{C-O} stretch of the hydroxyl group [16, 29]. The free phenolic OH group of the vanillin ligands was, however, observed as a strong and broad band at 3453 – 3362 cm^{-1}. In addition, the formation of the Schiff base ligands was inferred from the appearance of the $\nu_{C=N}$ band at about 1622 cm^{-1} and 1614-1607 cm^{-1} [15, 16] for the vanillin and the *p*-vanillin ligands respectively. This was further confirmed with the Raman spectral data for the ligands.

Some selected Raman spectral data for the compounds are presented in table 3. The data further substantiates the formation of the Schiff base ligands. The imine functional group, C=N, was observed as a strong peak at 1632 – 1611 cm^{-1} very close to the ring stretch peak [30]. The ring stretch, C=C, appeared as intense doublets at 1609 – 1567 cm^{-1} in the spectra of some of the compounds. The medium peaks at 3072 cm^{-1} and 1228-1118 cm^{-1} correspond to the aromatic C-H stretch and deformations respectively [30].

Table 2: Selected ^{1}H- and ^{13}C-NMR spectral data

Ligands	^{1}H-NMR (δ) ppm				^{13}C-NMR (δ) ppm
	HC=N	OH	OCH_3	CH_3	HC=N
L1	8.57	13.84	3.88	-------	161.90
L2	8.53	13.54	3.88	-------	162.30
L3	8.53	13.52	3.88	------	162.33
L4	8.56	13.96	3.88	2.41	161.01
L5	8.54	13.97	3.87	------	160.03
L6	8.58	13.10	3.90	-------	164.42
L7	8.43	13.97, 9.63	3.80	------	160.48
L8	8.66	13.90	3.87	-------	162.99
L9	8.56	12.27	3.87	-------	163.81
L10	8.38	6.15	4.00	--------	160.43
L11	8.35	6.10	4.02	--------	160.68
L12	8.30	6.10	4.00	--------	160.71
L13	8.40	6.10	3.88	2.41	159.87
L14	8.40	6.10	3.86, 4.01	--------	158.64
L15	8.43	9.53, 9.53	3.84	--------	157.99
L16	8.47	6.06	4.00	-------	160.43
L17	8.38	7.02	4.00	-------	162.16

3.4 Electronic spectral study

The electronic study of the Schiff base compounds was carried out in methanol and the selected spectral data for the compounds are presented in table 3. The Schiff base ligands exhibited two high energy absorption bands at 228 – 213 nm and 243 – 231 nm due to the π→π* transition of the benzene rings [16]. In addition, two other bands were observed at 295 – 282 nm and 381 – 330 nm corresponding to the π→π* and n→π* transitions of the azomethine group, HC=N [31]. The appearance of a low energy band at 441 - 412 nm indicates the existence of keto-enol tautomerism in the Schiff base ligands [32, 33].

Table 3: Selected Infrared, Raman and UV/Visible Spectral data for the Schiff bases

Ligands	IR (cm^{-1})		Raman (cm^{-1})		λ_{max} (nm)
	$\nu_{C=N}$	ν_{C-O}	$\nu_{C=N}$	$\nu_{C=C}$	
L1	1613	1287	1613	1592, 1575	224, 236, 285, 334, 412
L2	1607	1286	1615	1591, 1572	228, 237, 291, 339, 415
L3	1607	1288	1614	1584, 1570	227, 237, 292, 340, 415
L4	1613	1288	1628	1605, 1573	224, 238, 283, 336,414
L5	1614	1286	--------	1595, 1571	213, 236, 286, 293, 343, 420
L6	1614	1286	--------	1585, 1558	359
L7	1607	1286	1632	1609	244, 283, 295, 342, 342, 419
L8	1607	1287	1613	1567	228, 342, 381
L9	1608	1285	1611	1565	235, 288, 331, 415
L10	1622	1282	1623	1586, 1515	227, 284, 326, 441
L11	1623	1276	1624	1601, 1584	231, 285, 330, 442
L12	1622	1279	1622	1599, 1579	230, 285, 331, 444
L13	1623	1277	1624	1592	227, 283, 329, 430
L14	1623	1285	1623	1594	227, 284, 336, 441
L15	1623	1275	1624	1588	243, 282, 337
L16	1622	1283	1621	1596, 1572	210, 231, 320, 337
L17	1620	1271	1622	1598, 1579	239, 289, 328

3.5 Antimicrobial Study

The Schiff base ligands were screened for their in vitro antimicrobial activity against three bacterial strains (Staphylococcus aureus subsp. aureus ATCC® 6538™*, Bacillus subtilis subsp. spizizenii ATCC® 6633™* and Escherichia coli ATCC® 8739™*) and one fungal strain (Candida albicans ATCC® 2091™*) using disc diffusion method [34].

The test organisms were cultured on nutrient agar medium. Each test organism was inoculated onto a nutrient agar plate and incubated at 37 ℃ for 24 hr to obtain the primary culture. Several discrete colonies of the culture were dissolved in 10 mL saline water to obtain bacterial suspension corresponding to 106 – 108

CFUs. The turbidity of the suspension was compared with 0.5 McFarland standard. 0.1 mL of the bacterial suspension was inoculated onto Mueller Hinton plate and sterile discs containing the test compounds were firmly placed on the plate. The assay was incubated at 37 ℃ for 16 hr and the zone of inhibition was measured as millimetres diameter. The control solvent for the assay was dimethylformamide (DMF) while ampicillin was used as the standard antibacterial drug. The antifungal study was conducted in similar manner by replacing Mueller Hinton with potato disc assay and using ketoconazole as the standard antifungal agent. The test was repeated two more times for those compounds that showed activity of more than 6.5 mm; and their activity was recorded as average zone of inhibition as presented in figures 2 and 3.

The *p*-vanillin ligands exhibited higher antimicrobial activity than the vanillin based ligands, probably due to the electronic effect of the *p*-methoxyl group on the p-vanillin ligands. Similar ligands with the o-methoxyl group (2-hydroxyl-3-methoxylbenzaldimines), however, exhibited higher activity, while the un-substituted salicylaldimine (2-hydroxylbenzaldimines) ligands were nearly non-active against the tested organisms [34]. Furthermore, the *p*-hydroxyl and the 3-aminopyridine ligands possess higher activity than the aniline derived Schiff base ligands. The compounds exhibited significant antifungal activity against Candida albicans and slight activity against E. coli. The gram positive bacterial strains were more susceptible to the test compounds than Escherichia coli ATCC® 8739™*. The cell wall of Gram negative bacteria such as E. coli, is made up of thick peptidoglycan membrane and this reduces the permeation of the test compounds into the bacteria. Consequently, gram negative bacteria exhibit higher resistance to antimicrobial agents.

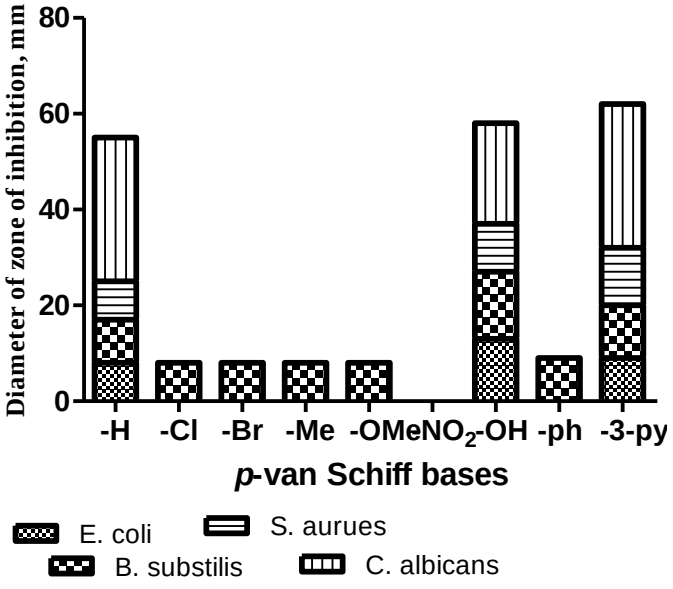


Fig. 2: Disc diffusion result for the p-vanillin Ligands

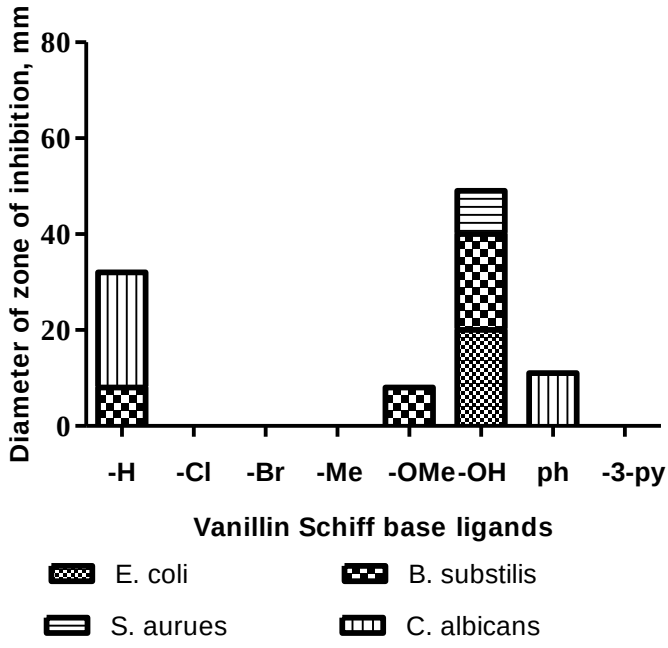


Fig. 3: Disc diffusion result for the vanillin ligands

4. CONCLUSION

The *p*-vanillin and vanillin Schiff base ligands were successfully prepared. The antimicrobial activity of the compounds depends on the nature of the aldehyde moiety of the Schiff base ligands. The *p*-hydroxyl and 3-amainpoyridine ligands exhibited promising antifungal activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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