International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 4 Issue 6, June 2017

ISSN: 2394-4404

Syntheses, Characterization And Antimicrobial Studies Of Some P-Vanillin Schiff Bases

Umofia, E

Omuaru, V.O.T

Achugasim, O.

Department of Pure and Industrial Chemistry, University of Port Harcourt

Eruteya, O.C.

Department of Microbiology, University of Port Harcourt

Abstract: ***Schiff bases are a group of organic compounds which have been found to possess numerous biological properties ranging from antimicrobial to anticancer properties. They play an important role in the pharmaceutical and agrochemical industry, through the development of more potent drugs. This research was focused on the synthesis of some p-vanillin-derived Schiff bases using an eco-friendly method as well as investigate their antimicrobial properties against three pathogens; Escherichia coli, Staphylococcus aureus and Saccharomyces cerevisiae. The results obtained showed that only one of the compounds exhibited a broad spectrum of activity against all the pathogens tested. The active Schiff base had a chloro-group in its molecular scaffold, thus, its effect was related to its structure. The inactivity of the other compounds was attributed to the fact that these compounds may be pathogen-specific.***

Keywords: ***Schiff base, p-vanillin, toluidine, o-chloroaniline, Staphylococcus aureus, Saccharomyces cerevisiae, green solvent.***

I. INTRODUCTION

There are numerous pathogens, both known and unknown, most of which have been found to be extremely harmful to humans, plants and animals. With the discovery of new diseases and increase in mortality rate caused by popular disease-causing pathogens, there is urgent need to find better and more effective means of fighting these pathogens. Over the years, new drugs have been synthesized with the purpose of preventing and/or curing different illnesses. The research and discovery of different compounds which possess antimicrobial properties forms a whole new world of possibilities in combating pathogens as well as curbing death rate.

One of such compounds that have been discovered is the Schiff base. Schiff bases are a group of organic compounds, discovered in 1864 by a German Organic Chemist, Hugo Schiff by the condensation of primary amines with carbonyl compounds [1]. These compounds are characterized by a distinct functional group called the imino group which is a carbon-nitrogen double bond (-C=N-). Schiff bases are also called imines, azomethines or azils [2]. There is a possibility that the imino group found in these compounds is responsible for their biological activities as well as their chemical reactivity [3]. These compounds are known to have a wide range of biological properties which includes: antibacterial, antifungal, antiviral, antimalarial, antidiabetic, antioxidant, antitumor, antitubercular, anticonvulsant, anti-inflammatory [3-12] activities.

N-(Salicylidene)-2-hydroxyaniline has antibacterial properties against *Mycobacterium tuberculosis* [13]. It has been reported that a 5-nitroisoquinoline-derived Schiff base acts as a very effective antimalarial agent against a chlorine-resistant *P. Falciparum* strain [14]. Schiff bases containing a 2,4-dichloro-5-fluorophenyl moiety are known to inhibit the growth of *Aspergillus flavus, Aspergillus fumigatus, Trichophyton menta agrophyte* and *Penicillum marneffei* [15]. These biological properties of Schiff bases make them

valuable in the pharmaceutical and agrochemical industries [16] to warrant the synthesis of new drugs which are active against different microbial strains.

This research work was aimed at synthesizing and characterizing some *p*-vanillin-derived Schiff bases using a solvent-mixture (ethanol-water, 1:1) and finally investigating their antimicrobial properties on two strains of bacteria, *Staphylococcus aureus* and *Escherichia coli*, and a fungus, *Saccharomyces cerevisiae*, as well as determining the possible effect of chemical structure on the biological activity of the compounds. The concept called Structure-Activity Relationship (SAR) has been used in the study of the antimicrobial properties of Schiff bases [5, 17-20].

II. MATERIALS AND METHODS

The chemicals were from Hopkin and Williams, May and Baker and Sigma Aldrich brands. The melting points were determined with a melting point apparatus and were uncorrected, thin Layer Chromatography (TLC) was carried out using a Merck pre-coated silica gel plate (10x10 cm) and the Rf values were obtained using ethyl acetate as solvent and the spots located and visualized using an ultraviolet lamp at 256 nm. ^{1}H NMR and ^{13}C NMR spectra of the samples were recorded in DMSO-D_6 and $CDCl_3$ by employing TMS as an internal standard with a Bruker AVANCE III 500 at 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C. The IR spectra of the samples were recorded on a Perkin-Elmer Spectrum 400 FT-IR/FT-NIR spectrometer in the range of 400-4000 cm^{-1} using KBr pellets.

A. EXPERIMENTAL

SYNTHESIS OF 2-METHOXY-4-{(E)-[(2-METHYLPHENYL)IMINO]METHYL}PHENOL (1)

o-Toluidine (1.07g, 0.01 mol) was introduced into 20 ml ethanol-water (1:1) mixture in a 150 ml flat bottom flask. *p*-Vanillin (1.52g, 0.01 mol) was added to the mixture in the flask followed by a few drops of 10% KOH. The reaction mixture was stirred with a magnetic stirrer for 15-20 min at about 40^{O}C. The reaction was monitored using thin layer chromatography (ethyl acetate). At the end of the reaction, the mixture was poured into a 50 ml beaker and allowed to cool. The crude product was collected and purified using ethanol.

SYNTHESIS OF 2-METHOXY-4-{(E)-[(4-METHYLPHENYL)IMINO]METHYL}PHENOL (2)

Same procedure as compound (1) but with *p*-toluidine (1.07g, 0.01 mol) and *p*-vanillin (1.52g, 0.01 mol).

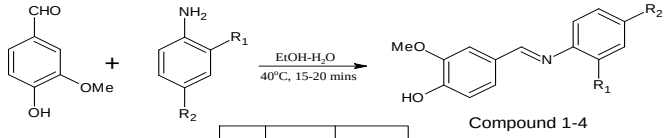
SYNTHESIS OF 2-METHOXY-4-[(E)-(PHENYLIMINO)METHYL]PHENOL (3)

Same procedure as compound (1) but with aniline (0.93g, 0.01 mol) and *p*-vanillin (1.52g, 0.01 mol).

SYNTHESIS OF 4-[(E)-(CHLOROPHENYL)IMINOMETHYL]-2-METHOXY-PHENOL (4)

Same procedure as compound (1) but with *o*-chloroaniline (1.28g, 0.01 mol) and *p*-vanillin (1.52g, 0.01 mol).

Scheme 1 illustrates the synthetic route for compound 1-4.



Compound 1-4

	R_1	R_2
1	CH_3	H
2	H	CH_3
3	H	H
4	Cl	H

Scheme 1: Synthetic route for compound 1-4

B. BIOLOGICAL ACTIVITY

The susceptibility test of the Schiff bases was carried out using the agar diffusion method [21]. The compounds were tested against gram positive bacteria, *Staphylococcus aureus*, gram negative bacteria, *Escherichia coli*, and yeast, *Saccharomyces cerevisiae.* The purity and viability of the isolates was confirmed using selective media, mannitol salt and eosine methylene blue agar for the *S. aureus* and *E. coli* respectively and potato dextrose agar (PDA) for *S. cerevisiae*. The Petri dishes were incubated at 37^{o}C for 24 hours. Slants were prepared using nutrient agar medium for bacteria and PDA for yeast and incubated again to ensure growth and purity of the organisms.

The compounds (1-4) were dissolved in 30% dimethyl sulphoxide (DMSO) to obtain 150mg/ml, 100mg/ml and 50mg/ml. They were stored overnight in a refrigerator at 15^{o}C [22]. The agar diffusion method was employed using Mueller-Hinton agar medium for the bacteria and PDA medium for the yeast. From an overnight broth culture, a 1x 10^{8} cell/ml McFarland standard was prepared and 0.1ml of the isolates aseptically transferred to sterile Petri dishes before adding 20ml of the prepared molten agar. The content was thoroughly mixed and allowed to solidify. Three holes (5.0mm) were made in each plate using a cup-borer and 0.2ml of the various Schiff base concentrations transferred to each hole aseptically using a pipette. The plates were allowed to stand for pre-diffusion for 1 hour before incubation at 37^{o}C for 24 hours. Similar Petri dishes were prepared with DMSO to serve as the control for bacteria and fungus. The zones of growth inhibition were measured and recorded in millimetres. The compound activities at all concentrations are presented in tables 3 and 4.

III. RESULT AND DISCUSSION

A. SPECTRAL DATA OF COMPOUNDS

2-METHOXY-4-{(E)-[(2-METHYLPHENYL)IMINO] METHYL}PHENOL (1)

ISSN: 2394-4404

IR-cm^{-1} (KBr): 1622 (C=N), 1586, 3001, 2962, 1153, 3552.

^{1}H NMR (DMSO-d_6) δ ppm: 2.28 (s, 3H, -CH_3); 3.85 (s, 3H, -OCH_3); 6.89-7.55 (m, 6H, Ar-H); 8.32 (s, 1H, Ar-H); 9.71 (s, 1H, H-C=N); 11.98 (O-H, exchangeable in D_2O).

^{13}C NMR (DMSO-d_6) δ ppm: 17.59 (s, CH_3); 55.55 (s, OCH_3); 110.70-150.89 (m, aromatic); 159.35 (s, C=N, azomethine); 115.37 (s, C-CH_3, aromatic); 147.95 (s, C-N, Aromatic); 150.11 (s, C-OCH_3, aromatic); 150.89 (s, C-OH, phenolic).

2-METHOXY-4-{(E)-[(4-METHYLPHENYL)IMINO] METHYL}PHENOL (2)

IR-cm^{-1} (KBr): 1621 (C=N), 1583, 3034, 2962, 1154, 3558.

^{1}H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, -CH_3); 3.84 (s, 3H, -OCH_3); 6.88-7.51 (m, 6H, Ar-H); 8.44 (s, 1H, Ar-H); 9.70 (s, 1H, H-C=N); 12.10 (O-H, exchangeable in D_2O).

^{13}C NMR (DMSO-d_6) δ ppm: 20.54 (s, CH_3); 55.53 (s, OCH_3); 110.26-150.03 (m, aromatic); 159.31 (s, C=N, azomethine); 115.30 (s, C-CH_3, aromatic); 147.95 (s, C-N, Aromatic); 149.28 (s, C-OCH_3, aromatic); 150.03 (s, C-OH, phenolic).

2-METHOXY-4-[(E)-(PHENYLIMINO)METHYL] PHENOL (3)

IR-cm^{-1} (KBr): 1624 (C=N), 1531, 3255, 1142, 3442.

^{1}H NMR ($CDCl_3$): δ3.95 (s, 3H, -OCH_3); 6.40-7.70 (m, 6H, Ar-H); 8.40 (s, 1H, Ar-H); 9.85 (s, 1H, H-C=N); 12.10 (O-H, exchangeable in D_2O).

^{13}C NMR ($CDCl_3$): δ56.00 (s, OCH_3); 108.50-152.01 (m, aromatic); 160.50 (s, C=N, azomethine); 147.00 (s, C-N, Aromatic); 149.05 (s, C-OCH3, aromatic); 152.10 (s, C-OH, phenolic).

4-[(E)-(CHLOROPHENYL)IMINOMETHYL]-2-METHOXY-PHENOL (4)

IR-cm^{-1} (KBr): 1631 (C=N), 1514, 2925, 1111, 3437.

^{1}H NMR ($CDCl_3$): δ3.96 (s, 3H, -OCH_3); 6.30-7.70 (m, 6H, Ar-H); 8.30 (s, 1H, Ar-H); 9.86 (s, 1H, H-C=N); 13.15 (O-H, exchangeable in D_2O).

^{13}C NMR ($CDCl_3$): δ56.01 (s, OCH_3); 109.02-152.05 (m, aromatic); 162.03 (s, C=N, azomethine); 143.02 (s, C-N, aromatic); 147.01 (s, C-Cl, aromatic); 149.05 (s, C-OCH_3, aromatic); 152.05 (s, C-OH, phenolic).

The green method used for the synthesis of the Schiff bases gave the products in high yields, was cheap, easy and had a straight-forward work-up procedure. The physicochemical properties of compounds 1-4 are illustrated in table 1 and 2. The spectral studies of the compounds proved that the structures were as expected.

COMPOUND	PHYSICAL STATE/ COLOUR	Rf VALUE	MELTING POINT (^{O}C)	YIELD (%)
1	Cream flakes	0.79	110-112	93.9
2	Pale yellow flakes	0.82	114-116	91.7
3	Pale yellow powder	0.84	140-142	91.4
4	Orange oil	0.83	-	87.4

Table 1: Physicochemical properties of Schiff bases 1-4

COMPOUND	WATER	ETHANOL	METHANOL	DMSO	DCM
1	Insoluble	Sparingly soluble	Soluble	Soluble	Soluble
2	Insoluble	Soluble	Soluble	Soluble	Soluble
3	insoluble	Sparingly soluble	Sparingly soluble	soluble	soluble
4	insoluble	Sparingly soluble	soluble	soluble	soluble

DMSO- Dimethyl sulfoxide; DCM- Dichloromethane

Table 2: Solubility test of Schiff bases 1-4

Compounds 1, 2 and 3 were found to be inactive against all the pathogens used for the susceptibility test. Only compound 4, the *o*-chloroaniline-*p*-vanillin Schiff base showed activity against the test organisms as observed in table 3 and 4, and figure 1, 2 and 3. DMSO was used as a control and showed no inhibition to the growth of the organisms.

Compounds	Gram positive bacteria			Gram negative bacteria		
	S. aureus			*E. coli*		
	Concentration (mg/ml)					
	50	100	150	50	100	150
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	11mm	12mm	13mm	8mm	13mm	14mm
DMSO (30%) (control)	-			-		

-, not active.

Table 3: Susceptibility Test Result of Schiff Bases on the Bacteria

Compounds	Yeast		
	S. cerevisiae		
	Concentration (mg/ml)		
	50	100	150
1	-	-	-
2	-	-	-
3	-	-	-
4	16mm	17mm	18mm
DMSO (30%) (control)	-		

-, not active.

Table 4: Susceptibility Test Result of Schiff Bases on the Yeast

Figure 1: Susceptibility test of Schiff base (4) on E. coli

ISSN: 2394-4404

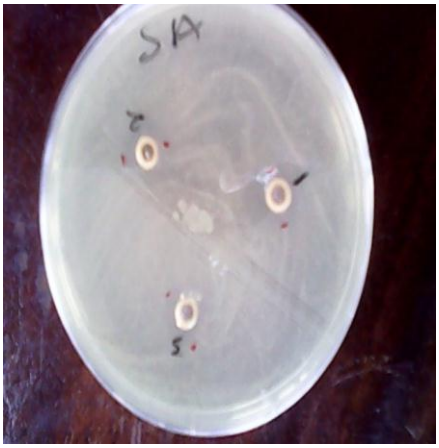


Figure 2: Susceptibility test of Schiff base (4) on S. aureus

Figure 3: Susceptibility test of Schiff base (4) on S. cerevisiae

The measured values for compound 4 were observed to increase with increase in concentration and this was the trend for all the organisms. Sobola *et al.*[23] reported the average diameter of the zones of inhibition of the Schiff bases of *o*-vanillin and *o*-chloroaniline against *S. aureus* and *E. coli* as 12mm and 8.5mm respectively. When compared to compound 4, the value was observed to be similar for *S. aureus* but less for *E. coli*, which was observed to have an average diameter of 12mm.

Compound 4 is believed to have its activity due to the presence of the chloro-group. Chlorine-containing compounds are known for their bactericidal and fungicidal activities and thus are observed to demonstrate a broad spectrum of antimicrobial activity [24]**.** Therefore chloro-substituted compounds have been found useful in the production of bactericides, fungicides, disinfectants and some antibiotics [25]. It must be emphasized that although compounds 1-3 showed no activity, a general conclusion cannot be drawn about their biological activity because while most Schiff bases are active on a broad spectrum, others tend to be more selective or pathogen-specific. This is explained in a report on the Schiff base of 2-hydroxy-1-naphthaldehyde and 8-aminoquinoline [17] which showed selectivity against only one gram-positive bacterium, *Staphylococcus epidermidis*, out of four gram-positive and three gram-negative bacteria strains, and four fungi which were tested. Thus, the inactive Schiff bases in this work, compounds 1-3, may be active against other organisms.

IV. CONCLUSION

This research proved the efficiency of using a green solvent-mixture (ethanol-water, 1:1) for the synthesis of Schiff bases. The results obtained from the biological studies of the compounds showed that not all Schiff bases are active on a broad spectrum, thus, some may be selective or pathogen-specific. The broad spectrum biological activity observed in compound 4 was clearly related to its structure. Therefore the concept of structure-activity relationships in the biological studies of Schiff bases will prove helpful in the design of new and more potent drugs.

ACKNOWLEDGEMENT

The authors would like to thank the analysts and technologists at the Department of Chemistry, Vaal University of Technology, Vanderbijlpark, and School of Chemistry, University of Witwatersrand, Johannesburg, South Africa, for their assistance in carrying out the spectral analyses of the Schiff bases.

REFERENCES

[1] Cimerman, Z.; Miljanic,S. and Galic,N. (2000). Schiff Bases Derived from Aminopyridines as Spectrofluorimetric Analytical Reagents. Croatica Chemica Acta, 73(1), 81-95.

[2] Savalia, R.V; Patel A.P; Trivedi; P.T; Gohel, H.R. and Khetani, D.B. (2013). Rapid and Economic Synthesis of Schiff Base of Salicylaldehyde by Microwave Irradiation. Research Journal of Chemical Sciences, 3(10), 97-99.

[3] da Silva, C.M.; da Silva, D.L.; Modolo, L.V.; Alves, R.B.; de Resende, M.A.; Martins, C.V.B. and de Fatima, A. (2011). Schiff Bases: A Short Review of their Antimicrobial Activities. Journal of Advanced Research, 2, 1-8

[4] Arun, N.T.; and Gowramma, B. (2014). Synthesis and Biological Evaluation of Some Novel Schiff Bases of Cephalexin. International Journal of Pharmaceutical Sciences and Research, 5(3), 1008-1014.

[5] Saini, L.; Jain, A. and Bhadoriya, U. (2011). Synthesis and Antimicrobial Activities of Some Novel Schiff Base Derivatives of 5-nitroisatin. Asian Journal of Pharmacy and Life Science, 1(3), 232-238.

[6] Sriram, D.; Yogeeswari, P.; Myneedu, N.S. and Saraswat, V. (2006). Abacavir Prodrugs: Microwave-Assisted Synthesis and their Evaluation of Anti-HIV Activities. Bioorganic and Medicinal Chemistry Letters, 16(8), 2127–2129.

[7] Pattan, S.R.; Suresh, C.H.; Pujar, V.D. (2005). Reddy, V.V.K.; Rasal, V.P. and Koti, B.C.: Synthesis and Antidiabetic Activity of 2-Amino-[5-(4-Sulphonylbenzylidine)-2,4-Thiazolidinedione]-7-Chloro-6-Fluorobenzothiazole. Indian Journal of Chemistry, 44, 2404-2408.

[8] Sashidhara, K. V.; Rosaiah, J. N.; Bhatia, G. and Saxena, J. K.. (2008). Novel Keto-enamine Schiff Bases from7-

hydroxy-4-methyl-2-oxo-2H-benzo[h]chromene-8,10-dicarbaldehyde as Potential Antidyslipidemic and Antioxidant Agents. European Journal of Medicinal Chemistry, 43(11), 2592–2596.

[9] Hu, G.; Wang, G.; Duan, N.; Wen, X.; Cao, T.; Xie, S. and Huang, W. (2012). Design, Synthesis and Antitumor Activities of fluoroquinolone C-3 heterocycles (IV): s-triazole Schiff-Mannich Bases Derived from Ofloxacin. Acta Pharmaceutica Sinica B, 2(3), 312–317

[10] Khokra, S.L.; Arora, K.; Mehta, H.; Aggarwal, A. and Yadav, M. (2011). Common Methods to Synthesize Benzothiazole Derivatives and their Medicinal Significance: A Review. International Journal of Pharmaceutical Sciences and Research, 2(6), 1356-1377.

[11] Bhat, M.A. and Al-Omar, M.A. (2011). Synthesis, Characterization and in vivo Anticonvulsant and Neurotoxicity Screening of Schiff Bases of Phthalimide. Acta Poloniae Pharmaceutica-Drug Research, 68(3), 375-380

[12] Nithinchandra, K.B.; Aamir, S. and Shabaraya, A.R. (2012). Regioselective Reaction: Synthesis, Characterization and Pharmacological Activity of some New Mannich and Schiff Bases Containing Sydnone. European Journal of Medicinal Chemistry, 54, 597–604

[13] de Souza, A.O.; Galetti, F.C.S.; Silva, C.L.; Bicalho, B.; Parma, M.M.; Fonsesca, S.F.; Marsaioli, A.J.; Trindade, A.C.L.B.; Gil, R.P.F.; Bezerra, F.S.; Andrade-Neto, M. and de Oliveira, M.C.F. (2007). Antimycobacterial and Cytotoxicity Activity of Synthetic and Natural Compounds. Quim Nova, 30(7), 1563-1566

[14] Rathelot, P.; Vanelle, P.; Gasquet, M.; Delmas, F.; Crozet, M.P.; Timon-David, P. and Maldonado, J. (1995). Synthesis of Novel Functionalized 5-Nitroisoquinolines and Evaluation of In Vitro Antimalarial Activity. European Journal of Medicinal Chemistry, 30(6), 503–508.

[15] Karthikeyan, M.S.; Prasad, D.J.; Poojary, B.; Bhat, K.S.; Holla, B.S. and Kumari, N.S. (2006). Synthesis and Biological activity of Mannich Bases Bearing 2,4-dichloro-5-fluorophenyl moiety. Bioorganic Medicinal Chemistry, 14(22), 7482-9.

[16] Mangamamba, T.; Ganorkar, M.C. and Swarnabala, G. (2014). Characterization of Complexes Synthesized using Schiff Base Ligands and their Screening for Toxicity in Two Fungal and One Bacterial Species in Rice Pathogens. International Journal of Inorganic Chemistry, Article ID 736538, 1-22.

[17] Hisaindee, S.; Al-kaabi, L.; Ajeb, S., Torky, Y.; Iratni, R.; Saleh, N. and AbuQamar, S.F. (2013). Antipathogenic Effects of Structurally-Related Schiff Base Derivatives: Structural-Activity Relationship. Arabian Journal of Chemistry, 4(1), 15.

[18] Ashraf, M.A.; Mahmood, K. and Wajid, A. (2011). Synthesis, Characterization and Biological Activity of Schiff Bases. International Proceedings of Chemical, Biological and Environmental Engineering, 10, 1-7

[19] Bhardwaj, S.; Kumar, L.; Verma, R. and Singh, U.K. (2010). Synthesis, Characterization and Antimicrobial Activities of Schiff Bases of Isatin and Isatin Derivatives. Journal of Pharmacy Research, 3(12), 2983 – 2985.

[20] Vinita, G.; Sanchita S. and Gupta, Y.K., (2013). Synthesis and Antimicrobial Activity of Some Salicylaldehyde Schiff Bases of 2-aminopyridine. Research Journal of Chemical Sciences, 3(9), 26 – 29.

[21] Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. The American Journal of Clinical Pathology. 45(4), 493-496.

[22] Akujobi, C.; Anyanwu, B.N.; Onyeze, C. and Ibekwe, V.I. (2004). Antibacterial and Preliminary Phytochemical Screening of Four Medicinal Plants'. Journal of Applied Sciences, 7, 4328-4338.

[23] Sobola, A.O.; Watkins G.M. and Brecht, B.V. (2014). Synthesis, Characterization and Antimicrobial Activity of Copper (II) Complexes of Some Ortho-substituted Aniline Schiff Bases; Crystal structure of Bis(2–methoxy-6-imino)methylphenol Copper (II) Complex. South African Journal of Chemistry, 67, 45-51.

[24] McDonnell, G. and Russell, A.D. (1999). Antiseptics and Disinfectants: Activity, Action, and Resistance. American Society of Microbiology; Clinical Microbiology Reviews, 12(1), 147-179.

[25] Rutala, W.A. and Weber, D.J. (2008). Guideline for Disinfection and Sterilization in Healthcare Facilities. Centers for Disease Control and Prevention, (CDC), 1-158.