African Journal of Biotechnology Vol. 10 (2), pp. 209-213, 10 January, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1085
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Cytotoxic, antibacterial activity and physico-chemical properties of some acid catalyzed Schiff bases

Malik Amanullah[1], Sher Khan Sadozai[1], Wajid Rehman[2]*, Zonera Hassan[3] Abdur Rauf[1] and Muhammad Iqbal[1]

[1]Department of Chemistry Gomal University Dera Ismail Khan, Pakistan.
[2]Department of Chemistry, Hazara University, Mansehra, Pakistan.
[3]Department of Biochemistry, Hazara University, Mansehra, Pakistan.

Accepted 30 September, 2010

Some novel Schiff bases have been derived using aromatic aldehydes and aromatic amine in ethanol under acidic conditions. The geometry of these Schiff bases was established using various spectroscopic methods like infrared (IR), nuclear magnetic resonance (NMR) and mass spectrometry. The conductivity studies reveal the non electrolytic behavior of Schiff bases. The synthesized Schiff bases were tested *in vitro* with the aim of identifying novel lead compounds active against various types of bacteria and shrimps.

Key words: Acid catalyzed, Schiff bases, NMR, antibacterial activity, brine shrimp.

INTRODUCTION

In chemistry, Schiff bases finds versatile use (Sheehan and Grenda, 1962); some of them are the basic units in certain dyes, whereas, some are used as liquid crystals. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds. Schiff bases appear to be important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate (Liimatainen et al., 2000). Many biological important Schiff bases ligands have been reported which posses antibacterial (Shi et al., 2010), antifungal (Zhong et al., 2009), antimicrobial (Raman et al., 2003), anticonvulsant (Verma et Al., 2004), antiHIV (Pandeya et al., 1999), anti-inflammatory (Janos et al, 2009), and antitumor activity (Billman and Schmidgall, 2006). The antitumor activity of the bases towards ascetic tumors increases considerably with a slight increase in water solubility.

Previously a number of biologically important complexes have been reported by our group (Rehman et. al., 2008, 2009; Jamil et al., 2010). In order to broaden the scale of investigations on the Schiff bases, we have now synthesized, structurally characterized and determined cytotoxic and antibacterial activity of a number of Schiff bases, derived from various aromatic aldehydes and aromatic amines and the results are reported herein.

*Corresponding author. E-mail: sono_waj@yahoo.com.

Abbreviations: FTIR, Fourier transform infrared; **IR,** infrared; **NMR,** nuclear magnetic resonance; **TLC,** thin layer chromatography; **DMSO,** dimethyl sulfoxide; **GC-MS,** gas chromatography-mass spectrometry; **MIC,** minimum inhibitory concentration.

MATERIALS AND METHODS

All the glassware used were cleaned with chromic mixture, detergent and finally with distilled water and then dried in oven at 110°C. All the solvents used in the experiment were purified and dried according to standard procedures (Perrin and Armengo, 1988).

All the chemicals used were of analytical grade and used as such without further purification. Thin layer chromatography (TLC) was performed on precoated silica gel (E-Merck) 60F-254 analytical plates (0.25 mm). The solvent system used was n-hexane/ethyl acetate (1:9).

Melting points were determined on Gallen Kamp digital (England) melting point apparatus. Fourier transform infrared (FTIR) spectra were recorded on Bruker FTIR Tensor 27 spectrophotometer; nuclear magnetic resonance (NMR) spectra were recorded on Bruker 300MHZ FTNMR spectrophotometer, mass spectra were recorded on gas chromatography-mass spectrometry (GC- MS) model MAT 312.

Synthesis of Schiff bases

General procedure

10 m moles of aromatic amine were dissolved in a 15 ml of ethanol in a 100 ml 2-neck round bottom flask equipped with a reflux condenser protected by a calcium chloride drying tube and a quick fit thermometer, then 10 m mole of aromatic aldehyde were dissolved with constant stirring; to this reaction mixture 0.2 to 0.4ml HCl were added with cooling on an ice bath. Yellow color solid separates just after the addition of HCl. The mixture was stirred for 1 - 2 h at room temperature. The reaction mixture was refluxed on a water bath at 90 to 95°C for 3 to 4 h, the reaction was examined by TLC with time to time till completion. The excess of solvent was removed under reduced pressure; the product was washed with water and 95% ethanol, respectively. The product was purified by column chromatography using hexane-ethyl acetate (3:7) system.

3-methoxy-benzylidene-p-tolyl-amine (Schiff base 1)

This compound was obtained as yellow solid, 75% yield, m.p 112°C. IR (KBr) Cm^{-1} 1588 (C=N), 3044 (C–H Aro), 1590 (C=C Aro) ^{1}H-NMR 2.3 (s,3H,CH_3), 3.8 (s, 3H, OCH_3), 8.4 (s, 1H, N=CH), 7.1 to 7.4 (m, 8H, Aro) ^{13}C-NMR 18.8 (CH_3), 55.5 (OCH_3), 115 to 135 (Aro), 155.5 (NCH), 160.9, (HC=N), 167.1 (OC) M/z 225,118, 91.

3,4-Dimethoxy-benzylidene-p-tolyl-amine (Schiff base 2)

This compound was obtained as yellow solid, 75% yield, m.p 132°C. IR (KBr) Cm^{-1} 1576 (C=N), 3090 (C–H Aro), 1601 (C=C Aro) ^{1}H-NMR 2.39 (s,3H,CH_3), 3.95 (s, 6H, 2-OCH_3), 8.41 (s, 1H, N=CH), 7.0 to 7.7 (m, 7H, Aro) ^{13}C-NMR 18.2 (CH_3), 55.3 (*p*-OCH_3), 50.2 (*o*-OCH_3), 119 to 145 (Aro), 153.9 (NCH), 160.0, (HC=N), 165.9 (OC) M/z 255, 118,91.

3,4,5-Trimethoxy-benzylidene-p-tolyl-amine (Schiff base 3)

This compound was obtained as yellow solid, 75% yield, m.p 170°C. IR (KBr) Cm^{-1} 1580 (C=N), 3078 (C –H Aro), 1610(C=C Aro)^{1}H-NMR 2.35 (s,3H,CH_3), 3.92 (s, 9H, 3- OCH_3),8.34 (s, 1H, N=CH), 7.1-7.24 (m, 7H, Aro) ^{13}C-NMR 18.5 (CH_3), 55.5 (*p*-OCH_3), 50.0 (*o*-OCH_3), 44.8 (*m*-OCH_3), 114-141 (Aro), 152.9 (NCH), 160.4, (HC=N), 165.2 (OC) M/z 285,270,118,91.

2-Methoxy-4-(p-tolylimino-methyl)-phenol (Schiff base 4)

This compound was obtained as yellow solid, 75% yield, m.p 159°C. IR (KBr) Cm^{-1} 1577 (C=N), 3065 (C–H Aro), 1605 (C=C Aro) ^{1}H-NMR 2.40 (s,3H,CH_3), 3.71 (s, 3H, O-CH_3),8.37 (s, 1H, N=CH), 7.1-7.7 (m, 7H, Aro) 4.31 (s. 1H,OH) ^{13}C-NMR 18.7 (CH_3), 55.0 (OCH_3), 114-135 (Aro), 148 (COH Aro), 153.5 (NCH), 160.4, (HC=N), 165.5 (OC) M/z 241,118,91.

4-Flouro-benzylidene-p-tolyl-amine (Schiff base 5)

This compound was obtained as yellow solid, 75% yield, m.p. 110°C. IR (KBr) Cm^{-1} 1587 (C=N), 3077 (C–H Aro), 1612(C =C Aro) ^{1}H-NMR 2.36 (s,3H,CH_3), 8.41 (s, 1H, N=CH),7.1-7.8 (m, 8H, Aro), ^{13}C-NMR 18.6 (CH_3), 119-140 (Aro), 155.0 (NCH), 160.8, (HC=N), 163.4 (CF), 167.4 (OC) M/z 213, 118, 91.

4-Chloro-benzylidene-p-tolyl-amine (Schiff base 6)

This compound was obtained as yellow solid, 75% yield, m.p 144°C. IR (KBr) Cm^{-1} 1570 (C=N), 3055 (C–H Aro), 1600 (C=C Aro) ^{1}H-NMR 2.36 (s,3H,CH_3), 8.41 (s, 1H, N=CH),7.1-7.8 (m, 8H, Aro) ^{13}C-NMR 18.5 (CH_3), 117-130 (Aro), 155.1 (NCH), 160.3, 159.1 (CCl), (HC=N), 167.0 (OC) M/z 229,118,91.

4-Bromo-benzylidene-p-tolyl-amine (Schiff base 7)

This compound was obtained as yellow solid, 75% yield, m.p. 129°C. IR (KBr) Cm^{-1} 1579 (C=N), 3076 (C–H Aro), 1597(C=C Aro) ^{1}H-NMR 2.36 (s,3H,CH_3), 8.40 (s, 1H, N=CH),7.0-7.7 (m, 8H, Aro), ^{13}C-NMR 18.9 (CH_3), 121-140 (Aro), 155.9 (NCH), 160.8, 151.1 (CBr), (HC=N), 167.0 (OC) M/z 273,118,91.

4-Iodo-benzylidene-p-tolyl-amine (Schiff base 8)

This compound was obtained as yellow solid, 75% yield, m.p. 121°C. IR (KBr) Cm^{-1} 1585 (C=N), 3066(C–H Aro), 1595(C=C Aro) ^{1}H-NMR 2.36 (s,3H,CH_3), 8.39 (s, 1H, N=CH),7.1-7.7 (m, 7H, Aro) ^{13}C-NMR 18.5 (CH_3), 115-130 (Aro), 155.0 (NCH), 160.8, 149.0 (CI), (HC=N), 167.5 (OC) M/z 321, 118, 91.

Biological studies

All the bases were tested against various organisms for the following activities:

Brine shrimp lethality bioassay

Artificial sea water was prepared by dissolving 3.8 g sea salt per liter of double distilled water and filtered. Sea water was placed in a small tank and shrimp eggs (Artemia Salina) (1 mg) were added to the large compartment of the tank, which was darkened by covering it with aluminum foil. The illuminated compartment attracted shrimps larvae through perforation in the dam. It was allowed to stand for 24 h at 25°C for the shrimps to hatch and mature. Different concentrations (150,100 and 150 µg/ml) of the test samples were prepared in dimethyl sulfoxide (DMSO). Three (3) replicates were prepared for each concentration making a total of 24 vials. After 2 days when shrimp larvae matured, 5 ml sea water and 12 shrimps per vial were added, allowed to stand for 24 h under illumination. After 24 h, the number of surviving shrimps were counted and recorded. Data were analyzed with a Finney computer program to determine the LD_{50} values (Solis et al., 1993; Finney 1979).

Antibacterial activity

The antibacterial activity was determined by using the agar well diffusion method (Rahman et al., 2001). The wells were dug in the media with a sterile borer and eight-hour-old bacterial inoculums containing ca 10^4 - 10^6 colony-forming units (CFU)/ml was spread on the surface of the nutrient agar using a sterile cotton swab. The recommended concentration of the test sample (2 mg/ml in DMSO) was introduced in the respective wells. Other wells containing DMSO and the reference antibacterial drug served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 20 h. The activity was determined by using the diameter of the inhibition zone (in mm) showing complete inhibition. Growth inhibition was calculated with reference to the positive control.

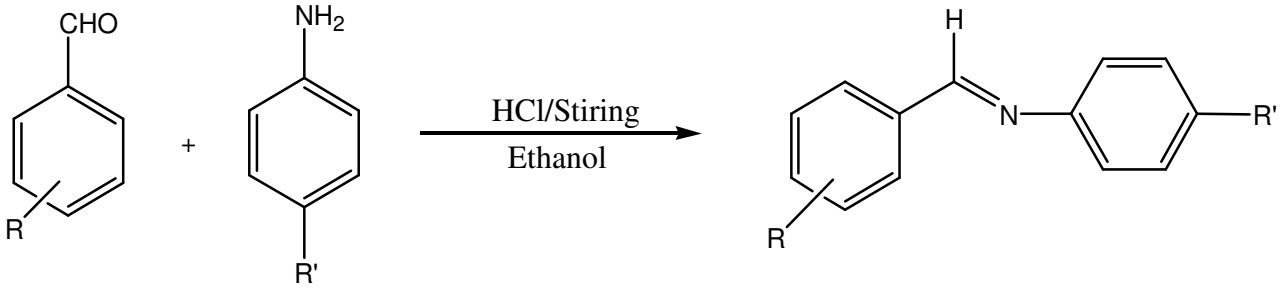

Figure 1. R and R´ as described in 1.

Table 1. Synthesis of Schiff bases.

Schiff base	R	R´	Mol. formula	Mol. wt
1	3- OCH_3	4-CH_3	$C_{15}H_{15}NO$	225
2	3, 4- OCH_3	4-CH_3	$C_{16}H_{17}NO_2$	255
3	3, 4, 5 -OCH_3	4-CH_3	$C_{17}H_{19}NO_3$	285
4	3-OCH_3, 4-OH	4-CH_3	$C_{15}H_{15}NO_2$	241
5	4-F	4-CH_3	$C_{14}H_{12}FNO$	213
6	4-Cl	4-CH_3	$C_{14}H_{12}$ Cl N	229
7	4-Br	4-CH_3	$C_{14}H_{12}BrN$	273
8	4-I	4-CH_3	$C_{14}H_{12}$I N	321

RESULTS AND DISCUSSION

Synthesis of Schiff bases

Schiff bases have been synthesized by reacting aromatic aldehydes and primary aromatic amine under acidic conditions using ethanol as solvent. This is depicted in Figure 1 and Table 1.

Spectroscopic characterization

Several Schiff bases have been synthesized with structures as characterized by NMR, mass and IR data (see experimental section). IR spectroscopy is a constructive tool in structural determination of Schiff base compounds (Rehman et al., 2005) The FTIR spectrum of Schiff base showed stretching frequencies at 1580, 3078 and 1610 cm^{-1} which are characteristics of C=N, C-H and C=C (Aro), respectively. The ^{1}H NMR spectrum showed signals at 2.3, 3.9 and 8.3 ppm (s) which are characteristics of methyl, methoxy and N=CH groups, respectively present in the structure. The signals of aromatic protons appeared at the range of 7.1 - 7.2 ppm (m). The main peaks in ^{13}C-NMR spectra are successfully assigned and these results are supported by literature (Rehman et al., 2009). The mass spectrum showed molecular ion peak at m/z which is the molecular weight of the product. The remaining fragments appeared at m/z 270, 118 and 91, respectively.

Biological activities

Brine shrimp bio-assay

Bioactive compounds are often toxic to shrimp larvae, hence *in vivo* lethality to shrimp larvae can be used as simple and convenient preliminary monitor for new bio-active synthetic products (Wikipedia, 2006). This is rapid, inexpensive, in-house general bioassay that may serve as an intermediate test before further *in vivo* animal experiments on large scale.

All the synthesized Schiff bases were investigated for their cytotoxic property by brine shrimp lethality bioassay. Most of them showed significant activity against brine shrimp nauplii. The LD_{50} values of the synthesized bases are presented in Table 2.

The Schiff base 4 was found to be highly active with LD_{50} value of 0.053 µ/ml. The compounds 1, 2 and 6 also showed good activity with LD_{50} of 45.02, 25.52 and 1.26 µ/ml, respectively; the rest of the synthesized Schiff bases also exhibited significant activity. The activity order is as 4 < 6 < 2< 1 < 7 < 3 < 8 < 5.

Antibacterial studies

The resulting synthesized Schiff bases were checked against various microorganisms such as *Salmonella typhi, Schigella flexenari, Escherichia coli, Staphylococcus aureus* and *Bacillus subtills* in order to establish their

Table 2. Brine shrimp bioassay of Schiff bases.

Schiff base	% Age of mortality at doses			LD $_{50}$
	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	
1	4	9	11	45.02
2	75	80	98	25.52
3	8	7	5	51.04
4	85	15	20	0.053
5	34	52	75	111.20
6	37	95	24	1.26
7	4	8	11	45.12
8	10	20	60	61.77

Table 3. Anti bacterial activity results of Schiff bases.

Bacteria	High concentration samples								
	1	2	3	4	5	6	7	8	*Standard drug
Bacillus subtills	++	++	++	+++	+	n.a	+	n.c	++++
Staphylococcus aureus	+++	+	+	++	+	++	n.c	n.a	++++
Escherichia coli	+	+++	+	+++	+	+	n.a	+	++++
Schigella flexenari	n.a	++	+	+++	++	+++	+	+	++++
Pseudomonas aeruginosa	++	++	++	++	+	++	n.a	+	++++
Salmonella typih	++	++	+	+++	+	+	+	++	++++

* Impinium; ++++, highly active; +++, good activity; ++, moderate activity; +, significant activity; n.a, no activity; n.c, not checked.

Table 4. Minimum inhibitory concentration (MIC) values for the Schiff bases.

Microorganism	MIC (µg/ml)							
	1	2	3	4	5	6	7	8
Bacillus subtilis	18	16	40	9	60	32	68	128
Staphylococcus aureus	20	14	34	9	58	28	72	120
Escherichia coli	22	18	36	12	64	26	64	124
Salmonella typhi	18	16	40	8	60	30	64	120
Pseudomonas aeruginosa	22	15	38	8	64	32	68	128

bioactivities. In order to compare the results obtained, the impinem was used as a standard drug. The result obtained showed that synthesized bases have significant activity but lower than the standard drug. The result indicate that the base 1, 2 and 4 showed promising activity because it is proposed that oxygen of methoxy group may bind with bacteria and hence play an important role in enhancing the activity. Among these three, compound 4 has greater activity because of the hydroxyl group, which enhances the biological activity (Rehman et. al., 2005); base 6 was found to be of moderate activity. However Schiff base 3, 5, 7 and 8 showed somewhat less inhibition than others. The anti-bacterial activity results of these bases are presented in Table 3.

Minimum inhibitory concentration (MIC)

The MIC values for Schiff bases against *B. subtilis, S. aureus, E. coli, S. typhi and P. aeruginosa* were 18, 20, 22, 18 and 2 µg/ml for Schiff base 1; 16, 14, 18, 16 and 15 µg/ml for Schiff base 2; 40, 34, 36, 40 and 38 µg/ml for Schiff base 3; 9, 9, 12, 8 and 8 µg/ml , for Schiff base 4; 60, 58, 64, 60 and 64 µg/ml for Schiff base 5; 32, 28, 26, 30 and 32 µg/ml for Schiff base 6; 68, 72, 64, 64 and 68 µg/ml for Schiff base 7; 128, 120, 124, 120 and 128 µg/ml for Schiff base 8, respectively (Table 4). The values of MIC showed the following order: 4 > 2 > 1 > 6 > 3 > 5 > 7 > 8, among these Schiff bases, the Schiff base 4 exhibited excellent MIC result as it is proposed that the

oxygen of methoxy may bind with the bacteria leading to excellent activity.

ACKNOWLEDGEMENTS

This work was jointly supported by Gomal University Dera Ismail Khan, Pakistan and Hazara University Mansehra, Pakistan. The authors are grateful to Dr Raza Shah HEJRIC, University of Karachi, Pakistan for spectral studies.

REFERENCES

Billman JH, Schmidgall RL (2006). Preparation and antitumor activity of some Schiff bases of 2-Amino-4, 5-dichlorobenzenesulfonanilide and 2-Amino-*p*-toluenesulfonanilide. J. Pharm. Sci. 59: 1191-1194.

Finney (1979). Probit analysis, 3rd ed., Cambridge University Press, Cambridge.

Jamil K, Rehman W, Muhammad B, Danish M (2010) Biologically Active Organotin (IV) Schiff Base Complexes. J. Iran. Chem. Soc. 7: 495-499.

Janos G, Tamas L (2009). Macrophage Migration Inhibitory Factor (MIF) Tautomerase Inhibitors as Potential Novel Anti-Inflammatory Agents: Current Developments. Curr. Med. Chem. 16: 1091-1114.

Liimatainen J, Lehtonen A, Sillanpää R (2000). *cis*-Dioxomolybdenum(VI) complexes with tridentate and tetradentate Schiff base ligands. Preparation, structures and inhibition of aerial oxidation of aldehydes. Polyhedron. 19: 1133-1138.

Pandeya S N, Sriram D, Nath G, DeClercq E (1999) Synthesis, antibacterial, antifungal l and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and *N*-[4-(4′-chlorophenyl)thiazol-2-yl] thiosemicarbazide. Eur. J. Pharm. Sci. 9: 25-31.

Rahman A, Choudhary MI, Thomsen WJ (2001). Bioassay Techniques for Drug Development, Harwood Academic Publishers, the Netherlands. 16: p. 1.

Raman N, Kulandaisamy A, Thangaraja C (2003) Redox and antimicrobial studies of transition metal (II) tetradentate Schiff base complexes. Trans. Met. Chem. 28: 29-36.

Rehman W, Badshah A, Baloch MK (2008). Synthesis, spectral characterization and bio-analysis of some organotin (IV) complexes. Eur. J. Med. Chem. 43: 2380-2385.

Rehman W, Badshah A, Khan S, Ahmad I (2009). Synthesis, Structural Investigations and Biological Screening of Diorganotin (IV) Complexes Derived from 5,5-Diethyl Sodium Barbital. Chin. J. Str. Chem. 28: 419-426.

Rehman W, Badshah A, Khan S, Tuyet LTA (2009). Synthesis, characterization, antimicrobial and antitumor screening of some diorganotin (IV) complexes of 2-[(9H-Purin-6-ylimino)]-phenol. Eur. J. Med. Chem. 44: 3981-3985.

Rehman W, Baloch MK, Badshah (2005). A Comparative study of structure- activity relationship of di and triorganotin (IV) complexes of monomethyl glutarate. J. Braz. Chem. Soc. 16: 827-834.

Rehman W, Muhammad B, Ali F, Atif S (2008). Syntheses, Spectral Studies and Bioanalyses of Some Triorganotin (IV) Complexes of Cephlaxine. Chin. J. Str. Chem. 27: 846-852.

Rehman W, Rehman S, Muhammad B, Shah ST, Tauseef I, Guo CY (2009). Synthesis, Characterization and Anti-in flammatory Activity of Some Organotin (IV) Complexes. Pol. J. Chem. 83: 2043-2049.

Rehman W, Saman F, Ahmad I (2008). Synthesis, Characterization, and Biological Study of Some Biologically Potent Schiff Base Transition Metal Complexes. Russ. J. Coord. Chem. 34: 678-682.

Sheehan JC, Grenda V (1962). The N-(2-Hydroxyarylidene) Protecting Group in Peptide Synthesis. J. Am. Chem. Soc. 84: 2417-2420.

Shi L, Fang RQ, Zhu ZW, Yang Y, Cheng K, Zhu HL (2010). Design and Synthesis of Potent Inhibitors of *β*-Ketoacyl-Acyl Carrier Protein Synthase III (FabH) as Potential Antibacterial Agents. Eur. J. Med. Chem (in press) doi:10.1016/j.ejmech.2010.05.033.

Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD (1993). A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Medica. 59: 250-252.

Verma M, Pandeya SN, Singh KN, Stables JP (2004). Anticonvulsant activity of Schiff bases of isatin derivatives. Acta. Pharm. 54: 49-56.

Wikipedia Free encyclopedia, US registered 501 (2006).

Zhong Z, Li P, Xing R, Chen X, Liu S (2009). Preparation, characterization and antifungal properties of 2-(α-arylamino phosphonate)-chitosan. Int. J. Biol. Mol. 45: 255-259.