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A ONE-POT SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL INVESTIGATIONS: SCHIFF BASES

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ABSTRACT:
Two biologically active Schiff bases, $C_{13}H_{10}BrON$ **(4)**, $C_{13}H_9Br_2O_2N$ **(5)**, were synthesized by the reaction of 2-aminophenol **(1)** with 3-bromobenzaldehyde **(2)** or 3,5-dibromo-4-hydroxybenzaldehyde **(3)** in the presence of conc. H_2SO_4. The characterization of Schiff bases were carried out by using spectroscopic techniques including IR, ^{1}H-NMR, FAB-MS along with elemental analyses. The Schiff bases were checked for biological screening and Schiff base **(4)** was found to be potent antioxidant agent while Schiff base **(5)** showed non-significant antioxidant activity. In enzyme inhibition, the lipoxygenase inhibition activity was found to be moderate for Schiff base **(4)** and significant for Schiff base **(5)**. The Schiff bases **(4-5)** also have excellent antibacterial activity for strains; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*, using gentamicin as standard drug.
Keyword: Schiff base, lipoxygenase inhibition activity, urease inhibition activity

1. Introduction

The Schiff bases are the compounds having azomethine linkage (Figure 1) and can be synthesized from an amino and a carbonyl compound. These are significant chelating ligands in co-ordination chemistry to co-ordinate metals ions through azomethine nitrogen and have been considered broadly[1]. The imine or azomethine moieties are present in a range of natural, natural-derived, and non-natural compounds[2]. The biological activities of Schiff bases have been revealed due to the imine moiety present in these compounds[3-6]. The Schiff bases have broad applications in food and dye industries, and in analytical chemistry, catalysis and also in the field of agrochemical[7]. These have played an influential part in the improvement of modern coordination chemistry, but also they can also be found at key points in the development of inorganic biochemistry, catalysis and also in optical materials[8]. Keeping the above facts in mind and as part of ongoing efforts on Schiff bases[9], in the present paper the synthesis, characterization and biological activities of the Schiff bases **(4-5)** were investigated and reported.

2. Experimental

2.1 Materials and methods: Reagent grade solvents and chemicals were purchased from Merck which were used without further purification. Washing of all the glassware during the reaction was accomplished by using distilled water and drying was carried out at 110 ^oC.

2.2 Physical measurements: The melting points of the synthesized compounds were determined by Gallenkamp melting point apparatus and are uncorrected. Elemental composition was determined by Perkin-Elmer 2400 Series II elemental analyzer. For IR spectra, Thermo Nicolet Avatar 320 FT-IR spectrometer within 400-4000 cm^{-1} range was used by employing KBr disc method. The matrix used during the FAB (Fast atom bombardment) mass spectra was glycerol and ions in *m/z* (%) were recorded on JEOL SX102/DA-6000 mass spectrometer. Pre-coated silica gel G-25-UV_{254} plates (E-Merck) were utilized for checking the purity of compounds by TLC method. The compounds were dissolved in DMSO-d_6 for the measurement of ^{1}H-NMR spectra on Bruker AMX-400 spectrometer. The values for chemical shift (δ) are given in ppm, while employing TMS as internal standard, and the

data of scalar coupling constants (*J*) is presented in Hertz.

2.3 General procedure for the synthesis of Schiff bases (4-5): 2-Aminophenol **(1)** (0.01 mol in 50 mL EtOH) was mixed with 3-bromobenzaldehyde **(2)** (0.01 mol in 50 mL EtOH) or 3,5-dibromo-4-hydroxybenzaldehyde **(3)** (0.01 mol in 50 mL EtOH) and the reaction mixture was refluxed for 3 h with stirring at 70 ^{o}C after adding 3-4 drops of conc. H_2SO_4. Then the mixture was concentrated and excess solvent was evaporated for diminution of its volume to one third, by using rotary evaporator. The solid products were obtained by placing the reaction mixture at ambient temperature. The products, thus obtained, were washed with cooled methanol after filtration and recrystallized with absolute methanol after drying. Anhydrous calcium hydroxide at reduced pressure was used for drying purpose. The completion of reaction was monitored by taking TLC after certain intervals of time.

2.4 2-[(3-Bromobenzylidene)-amino]phenol (4): Saddle brown solid; yield, 61.03 %; mp, 173 ºC; IR (KBr) ν_{max} (cm^{-1}): 3379 (C-OH), 3053 (C-H), 1688 (C=N), 1602 (C=C), 846 (C-Br); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 8.89 (1H, s, -N=CH-); MS *m/z*: 275.3 $[M+H]^+$; Anal. Calcd. for $C_{13}H_{10}BrON$: C, 56.55; H, 3.65; N, 5.07. Found: C, 56.45; H, 3.71; N, 5.13.

2.5 2,6-Dibromo-4-[(2-hydroxyphenylimino)methyl]phenol (5): Peru solid; yield, 77.18 %; mp, 169 ºC; IR (KBr) ν_{max} (cm^{-1}): 3305, 3376 (C-OH), 3013 (C-H), 1634 (C=N), 1580 (C=C), 758, 801 (C-Br); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 8.51 (1H, s, -N=CH-); MS *m/z*: 369.7 $[M+H]^+$; Anal. Calcd. for $C_{13}H_9Br_2O_2N$: C, 42.08; H, 2.44; N, 3.78. Found: C, 42.22; H, 2.51; N, 4.02.

2.6 Pharmacological assays

2.6.1 Urease inhibition assay. The solution of urease enzyme was prepared by taking 0.125 units in each well in phosphate buffer ($K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01M $LiCl_2$). Each well was filled with 80 µL of 0.05 M potassium phosphate buffer (pH 8.2), 10 µL of the Schiff bases **(4-5)** (with concentration range 5 µM- 500 µM) was poured in each labeled test well, contents were mixed and incubated for 15 min at 30 °C. 40 µL of substrate solution (urea) (50 mM) was poured in each well for initiating reaction. Then, 70 µL alkaline reagent (0.5 % NaOH and 0.1 % active NaOCl) and 40 µL of phenol reagent (1 % phenol and 0.005 % w/v sodium nitroprusside) were introduced to each well. The well plate, containing reaction mixture, was incubated for 50 minutes and absorbance was recorded at 630 nm. IC_{50} (*i.e.* the concentration of Schiff bases **(4-5)** at which inhibition is 50 %) was determined by monitoring the effect of increasing concentrations of Schiff bases **(4-5)** on extent of inhibition[10].

2.6.2 Antioxidant: DPPH radical scavenging assay. The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH)[10, 11]. The solution of DPPH (0.3 µM) was prepared in ethanol. The solution of each sample was prepared in methanol. 5 µL solution of each sample (with concentration range 5-500 µg) was added to 95 µL of DPPH solution in ethanol, the mixture was then dispersed in 96 well plates and placed for 30 min into the incubator at 37 °C, then absorbance was recorded at 515 nm with the aid of elisa plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was assessed in contrast to methanol treated control. The standard used for this experiment was BHA.

DPPH scavenging effect (%) = Ac – As/Ac × 100

Where, Ac = Absorbance of control (DMSO treated); As = Absorbance of sample.

IC50 (i.e. the concentration of the compound at which 50% radicals are scavenged or inhibited by the test compound) of Schiff bases **(4-5)** was checked by observing the effect of different concentrations (1-1000 µM). IC_{50} values were calculated using EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, U.S.A).

2.6.3 Lipoxygenase inhibition assay. 160 µL of 100 mM sodium phosphate buffer (pH 8.0) and 10 µL of Schiff bases **(4-5)** in methanol (of various concentrations 5-500 µM) was added in each well labeled as test. 20 µL of lipoxygenase (LOX) solution (enzyme 130 units per well) was added, mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 µL substrate solution (linoleic acid, 0.5 mM, 0.12 %w/v tween 20 in ratio of 1:2) in each well and the absorbance was measured after 15 min at 234 nm[12].

2.6.4 Antibacterial assay. The bacterial employed for determining the antibacterial activity of Schiff bases **(4-5)** were *Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Salmonella typhi (S. typhi)* and *Pseudomonas aeruginosa (P. aeruginosa)*. Agar well diffusion method was used for antibacterial assay and Mueller Hinton

agar medium was used for this purpose. Schiff bases **(4-5)** (200 mg) were dissolved in 10 ml (99.9 %) DMSO and final concentration was made up to 20 mg/mL. The growth of microorganism was carried out overnight individually in tryptic Soya broth and finally it was mixed with physiological saline until colony formation was achieved in accordance with turbidity standard. The agar medium used for individual organisms was Molton Mueller Hinton agar medium and was used with 10 mL of prepared inoculum (inoculum size was 10^8 cells/ml as per McFarland standard). It was transferred to 20×100 mm petri dishes after proper homogenization. The required numbers of wells were adjusted in the seeded plates with help of a sterile crock-borer (8.0 mm) after solidification. The Schiff bases **(4-5)** (100 µl) were poured to respective wells. All the plates were incubated at 37 ˚C for 24 h after making positive (gentamicin 0.3 %) and negative control (DMSO) plates ready. The diameter of the zone of inhibition and percentage of growth inhibition was calculated to determine the antibacterial activity[13, 14].

3. Results and Discussion

3.1 Chemistry and Characterization. The plan for condensation of 2-aminophenol **(1)** with 3-bromobenzaldehyde **(2)** or 3,5-dibromo-4-hydroxybenzaldehyde **(3)** is presented in Figure 2 which shows that reaction was performed to synthesize Schiff bases **(4-5)**. The synthesized Schiff bases **(4-5)** thus obtained were very pure because these showed sharp melting point and are stable in air. TLC was used for direct comparison of spots of product with that of reactants in order to monitor the completion of reaction. Elemental analysis of compound showed that the data was in coincidence with that of structure of Schiff bases **(4-5)**. IR, ^{1}H-NMR and FAB-MS data was used for establishing the structure of Schiff bases **(4-5)**.

3.1.1 Infrared spectra. The absence of aldehydic band at 2800-2700 cm^{-1} and appearance of azomethine (-C=N-) band at 1688 and 1634 cm^{-1} in the Schiff bases **(4-5)** were observed in the IR spectrum, confirmed the formation of the said Schiff bases.

3.1.2 ^{1}H-NMR spectra. The protons of azomethine group (CH=N) in ^{1}H-NMR were observed as singlet at 8.89 and 8.51 ppm which is a clear evidence for the formation of azomethine linkage, a characteristic of Schiff bases **(4-5)**.

3.1.3 FAB-MS spectra. FAB-MS (+ve) of Schiff bases **(4-5)** showed a peak at *m/z* 275.3 and 369.7 representing the molecular ion of the Schiff bases, which was in complete synchronization with the theoretical mass *i.e.* 274.9 and 368.9 of the synthesized Schiff bases **(4-5)**, respectively. The data of elemental analysis further supported the molecular weight of Schiff bases **(4-5)**.

3.2 Pharmacological activities. The Schiff bases were checked for antioxidant, urease and lipoxygenase inhibition activities. The results for these biological activities are shown in Table 1. The urease inhibition activity was found to be non-significant for Schiff bases **(4-5).** Schiff base **(4)** was found to be potent antioxidant agent while Schiff base **(5)** showed non-significant antioxidant activity. The lipoxygenase inhibition activity for Schiff base **(4)** was found to be moderate and significant for Schiff base **(5)**. The Schiff bases **(4-5)** were also checked for antibacterial activity using the bacterial strains; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*, against gentamicin as standard drug (Table 2). These Schiff bases have greater activity because of the hydroxyl group[15], which may bind with the bacteria leading to excellent biological activity. Furthermore, the mode of action of the Schiff bases may involve formation of a hydrogen bond through the azomethine group with the active centre of cell constitutes resulting in interference with the normal cell process.

Conclusion

From the abovementioned discussions, the Schiff base **(4)** is a potent antioxidant agent and non-significant for Schiff base **(5)**. In enzyme inhibition, the lipoxygenase inhibition activity was found to be moderate for Schiff base **(4)** and significant for Schiff base **(5)**. The Schiff bases **(4-5)** also have excellent antibacterial activity for strains; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*, using gentamicin as standard drug.

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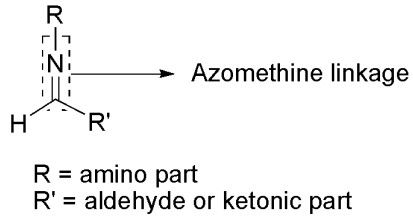

Figure. 1 General representation of the structure of a Schiff base.

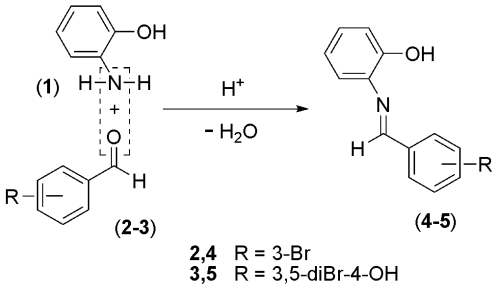

Figure. 2 The condensation reaction for synthesis of Schiff bases (4-5).

Table 1. IC_{50} (µM) values for Schiff bases (4-5) in the urease inhibition, antioxidant and lipoxygenase inhibition assays.

Compound	Urease Inhibition Activity IC_{50} (µM)	DPPH Scavenging Activity IC_{50} (µM)	Lipoxygenase Inhibition Activity IC_{50} (µM)
4	+	38.4	+
5	+	69.1	109.6
BHA	-	44.2	-
Baicalein	-	-	22.6
Thiourea	21.6	-	-

(+, Non-significant)

Table 2. % Inhibition values for Schiff base (4-5) in the antibacterial assay.

Bacteria	Gentamicin (0.3%)	4		Gentamicin (0.3%)	5	
	Zone inhibition (mm)	Zone inhibition (mm)	Inhibition (%)	Zone inhibition (mm)	Zone inhibition (mm)	Inhibition (%)
B. subtilis	**33**	31	94	**28**	28	100
S. aureus	**27**	20	74	**30**	29	97
E. coli	**25**	18	72	**25**	18	72
S. typhi	**28**	17	61	**28**	00	00
P. aeruginosa	**25**	10	40	**30**	00	00