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Synthesis, antibacterial, lipoxygenase and urease inhibitory activities of 2-aminophenol derivatives

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Abstract

In oder to discover potentially active antibacterial compounds, three Schiff bases: *2-[(4-methoxybenzylidene)-amino]phenol* **(5)**, *2-[(3,4-dimethoxybenzylidene)-amino]phenol* **(6)**, *2-[(3,4,5-trimethoxybenzylidene)-amino]phenol* **(7)**, were synthesized by the condensation of 2-aminophenol **(1)** with aldehydes **(2-4)** in methanol along with H_2SO_4. The synthesized Schiff bases **(5-7)** were characterized by ^{1}H-NMR, IR, EIMS and elemental analysis. The compound with greater $-OCH_3$ groups was found to be more biologically active than others. The antibacterial activity was excellent against *S. aureus* and *B. subtilis*, while LOX was excellent for **7** with IC_{50} 19.3 μM.

Keywords: Schiff base; antibacterial; urease inhibition; lipoxygenase inhibition

Introduction

Schiff bases are bioactive compounds based on azomethine (-HC=N-) linkage, which are obtained by the reaction of amines with aldehydes or ketones in acidic or basic medium. Schiff bases are medicinally important compounds due to their broad range of biological and industrial applications. A number of significant curative compounds have been obtained from the Schiff base reactions (Vasoya et al., 2005). These are also used as intermediates in polymer chemistry and organic synthesis as well (Vasoya et al., 2005). Literature reveals that these compounds have shown a broad range of activities such as antimalarial (Li et al., 2003), anticancer (Villar et al., 2004; Shi et al., 2012), antitumor (Hu et al., 2008), antibacterial (Venugopal et al., 2008; Pandey et al., 2000), antifungal (Pandey et al., 2000), antitubercular (Hearn et al., 2009), anti-HIV (Pandey et al., 1999), antimicrobial (Wadher et al., 2009) and antiviral (Karthikeyan et al., 2006). Some of them have been used as powerful corrosion inhi-

bitors (Agarwal et al., 1992) and also as complexing agents (Kulkarni et al., 2012). Medicinal importance and significance of Schiff bases (Patole et al., 2006) in various areas has developed our interest to synthesize such compounds (Aslam et al., 2012). In this article, we report the synthesis and spectroscopic study of Schiff bases and evaluation of their antibacterial, urease and lipoxygenase inhibition activities.

Experimental

Chemistry

Materials and methods

Reagent grade chemicals were employed for the synthesis which were purchased from Merck and were used without any purification or alteration. The glassware was dried in an oven at 100 ^{o}C after washing with distilled water.

Physical measurements

The balance used for weighing was an electric Mettler Toledo balance, model AL 204. The melting points are reported uncorrected and were recorded on Gallenkamp apparatus. Perkin-Elmer 2400 Series II elemental analyzer was used for analysis of elements. Thermo Nicolet Avatar 320 FT-IR spectrometer was used for recording IR spectra within the range of 400-4000 cm^{-1} by employing KBr disc method. The measurement of electrical conductivity was accomplished by making use of conductivity meter model Jenway 4010. The recording of El-MS spectra was conducted by electron impact mode on Finnigan MAT-112 spectrometer (Finnigan, Waltham, MA, USA) and *m/z* (%) of $[M]^+$ ions reported. The precoated silica gel G-25-UV_{254} plates (E-Merck) were used for determining the purity of compounds by taking TLC. The proton NMR spectra were recorded on a Bruker AMX-400 spectrometer and DMSO-d_6 was used as solvent. The scalar coupling constants (*J*) are reported in Hertz while chemical shift δ values are reported in ppm.

General procedure for the synthesis of Schiff bases (**5-7**)

The addition of 3-4 drops of conc. H_2SO_4 was made to a mixture of 2-aminophenol **(1)** (0.01 mol in 50 mL EtOH) and aldehydes **(2-4)** (0.01 mol in 50 ml EtOH) and it was refluxed at 70 °C on water bath with stirring for 3 h. The resulting solution was concentrated by using rotary evaporator to one third of its volume and was cooled in an ice bath. The concentrated reaction mixture was kept at room temperature and solid products were obtained. The products were washed with methanol at ambient temperature and were recrystallized with methanol as well. The product thus recrystallized was dried under reduced pressure over anhydrous calcium chloride. The reaction was monitored by TLC after lapse of small intervals of time (Figure 1).

2-[(4-Methoxybenzylidene)-amino]phenol (5)**:** Yield 72.62 %; m.p: 93 ºC; IR (KBr, ν_{max} cm^{-1}): 3339 (C-OH), 2969 (C-H), 1685 (C=N), 1594 (C=C); ^{1}H-NMR (DMSO-$_6d$, 400 MHz) δ: 8.60 (1H, s, H-9), 7.96 (2H, d, *J* = 8.4 Hz, H-4, -6), 7.16 (1H, d, *J* = 8.0 Hz, H-15), 7.34 (2H, dd, *J* = 6.0, 3.2 Hz, H-16, -19), 7.04 (1H, dd, *J* = 8.0, 7.6 Hz, H-13), 6.86 (1H, d, *J* = 8.0 Hz,

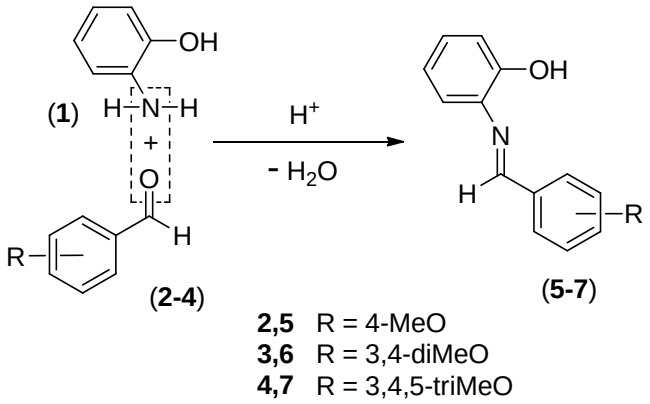

Figure 1. Synthesis of Schiff bases**.**

H-12), 6.81 (2H, dd, *J* = 8.0, 7.6 Hz, H-14), 3.83 (3H, s, H-18); EI-MS: *m/z* 227.06 $[M]^+$ (calcd. 227.09 for $C_{14}H_{13}NO_2$); Elemental analysis: Found (Calcd. %); C: 74.12 (73.99), H: 5.80 (5.77), N: 6.19 (6.16).

2-[(3,4-Dimethoxybenzylidene)-amino]phenol (6): Yield 81.16 %; m.p: 97 ºC; IR (KBr, ν_{max} cm^{-1}): 3376 (C-OH), 2967 (C-H), 1679 (C=N), 1583 (C=C); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 8.87 (1H, s, H-6), 7.74 (1H, br. s, H-3), 7.44 (1H, dd, *J* = 8.0, 1.6 Hz, H-5), 7.17 (1H. d, *J* = 8.0 Hz, H-8), 7.03-7.07 (2H, m, H-14, -12), 6.87 (1H, d, *J* = 7.6 Hz, H-11), 6.81 (1H, t, *J* = 7.6 Hz, H-13), 3.85 (3H, s, H-17), 3.83 (3H, s, H-19); EI-MS: *m/z* 257.14 $[M]^+$ (calcd. 257.10 for $C_{15}H_{15}NO_3$); Elemental analysis: Found (Calcd. %); C: 70.19 (70.02), H: 5.93 (5.88), N: 5.57 (5.44).

2-[(3,4,5-Trimethoxybenzylidene)-amino]phenol (7): Yield 77.91 %; m.p: 158 ºC; IR (KBr, ν_{max} cm^{-1}): 3376 (C-OH), 2958 (C-H), 1687 (C=N), 1601 (C=C); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 8.61 (1H, s, H-18), 7.38 (2H, s, H-10, -12), 7.18 (1H, d, *J* = 7.6 Hz, H-3), 7.08 (1H, t, *J* = 8.0 Hz, H-5), 6.87 (1H, *J* = 8.0 Hz, H-6), 6.82 (1H, t, *J* = 7.6 Hz, H-4), 3.85 (6H, s, H-20, -22), 3.72 (3H, s, H-16); EI-MS: *m/z* 287.16 $[M]^+$ (calcd. 287.11 for $C_{16}H_{17}NO_4$); Elemental analysis: Found (Calcd. %); C: 66.74 (66.89), H: 6.22 (5.96), N: 4.94 (4.88).

Biological activities

Antibacterial activity

The bacterial strains for determining the antibacterial activity of Schiff bases **(5-7)** 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 **(5-7)** (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 **(5-7)** (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 was calculated to determine the antibacterial activity (Bibi et al. 2011).

Urease inhibitory activity

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 0.01 M $LiCl_2$). Each well was filled with 80 µL of 0.05 M potassium phosphate buffer (pH 8.2), 10 µL of the Schiff bases **(5-7)** (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 **(5-7)** at which inhibition is 50 %) was determined by monitoring the effect of increasing concentrations of Schiff bases **(5-7)** on extent of inhibition (Ferheen et al. 2009).

Lipoxygenase inhibitory activity

160 µl of 100 mM sodium phosphate buffer (pH 8.0) and 10 µl of Schiff bases **(5-7)** 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 (Ali et al. 2009).

Results and Discussion

Chemistry

The Schiff bases **(5-7)** were synthesized by condensing 2-aminophenol **(1)** with aldehyde **(2-4)** as illustrated in figure 1. It was found that the Schiff bases **(5-7)** showed a sharp melting point and is fairly stable in air which indicates that the Schiff bases are pure. The synthesis of Schiff bases **(5-7)** was determined by a direct comparison of spots of product with that of reactants on TLC. The data for the Schiff bases **(5-7)** is entirely in concord with the structure of respective Schiff base. The structural aspects were additionally established and confirmed by IR, ^{1}H-NMR and EI-Mass spectroscopy. The bands at 1685, 1679 and 1687cm^{-1} correspond to azomethine (-C=N-) linkage in the Schiff bases respectively and also the absence of aldehydic bands at 2800-2700 cm^{-1} were further observed in the IR spectrum of Schiff bases **(5-7)**. The ^{1}H-NMR of Schiff bases **(5-7)** showed the presence of protons of azomethine group (-CH=N-) at 8.60, 8.87 and 8.61 ppm respectively, which is a strong indication for the development of azomethine group. The EI-MS Mass spectral data for molecular ion peaks $[M]^+$ was observed at *m/z* 227.06, 257.14 and and 287.16, which is also

Table 1. Antibacterial activity of the tested compounds.

Bacteria	Zone inhibition (mm)			
	Gentamicin (0.3%)	5	6	7
B. subtilis	29	26	24	26
S. aureus	30	16	22	27
E. coli	25	16	16	24
S. typhi	28	17	16	12
P. aeruginosa	30	10	13	16

a supportive aspect with the molecular weight of Schiff bases **(5-7)** respectively. The data for elemental analyses of Schiff bases **(5-7)** was also found in complete harmony with the assumed molecular mass.

Biological activities

Antibacterial activity

The results of antibacterial activity are summarized in in Table 1. Gentamicin was taken as a standard drug.. The Schiff base **(5)** was significantly active against *Bacillus subtilis* and showed moterate activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi.* While Schiff base **(6)** was significantly active against *Bacillus subtilis, Staphylococcus aure-us* and moderate for *Escherichia coli, Salmonella typhi.* The Schiff base **(7)** was found to be extraordinary active against *Escherichia coli* and significantly active for *Bacillus subtilis, Staphylococcus aureus.* In case of *Pseudomonas aeruginosa,* all Schiff bases (**5-7**) were found to be non-significant. Among these Schiff bases **(5-7)**, compound bearing trimethoxy group **(7)**, has shown excellent activity against all the tested bacteria. The Schiff base **(7)** was also non-signifucant towards *Salmonella typhi.* The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the microbial cells or on differences in the ribosomes of the cells. Although the exact biochemical mechanism is not completely understood, the mode of action of antimicrobials may involve various targets in the microorganisms. These targets include the following: (i) Interference with the synthesis of cellular walls, causing damage that can lead to altered cell permeability characteristics or disorganized lipoprotein arrangements, ultimately resulting in cell death. (ii) Deactivation of various cellular enzymes that play a vital role in the metabolic pathways of these microorganisms. (iii) Denaturation of one or more cellular proteins, causing the normal cellular processes to be impaired. (iv) Formation of a hydrogen bond through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (-C=N-) with the active centers of cell constituents, resulting in interferences with the normal cell process.

Urease inhibitory activity

Urease (EC 3.5.1.5) is an enzyme, which is found in bacteria, yeast, higher plants with special reference a pathogenic bacterium i.e. *Helicobacter pylori*. Many gastrointestinal or urinary tract pathogens also produce urease. It is a nickel-enzyme, which catalyzes the hydrolysis of urea to ammonia and carbamate, which decomposes to ammonia and carbonic acid (Figure 2), consequently pH is increased. It causes the gastric ulceration, urinary stone

Table 1. Urease and lipoxygenase inhibitory activities of the compounds.

Compound	Urease inhibitory activity (IC_{50} (μM)	Lipoxygenase inhibitory activity (IC_{50} (μM)
5	> 220	43.8
6	> 220	39.0
7	> 220	19.3
Baicalein	-	22.6
Thiourea	21.6	-

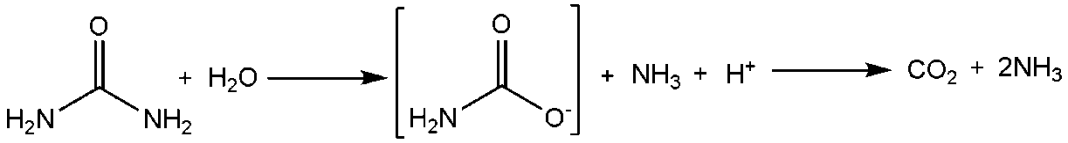

Figure 2. The breakdown of urea to ammonia and carbamate.

formation, pyelonephritis and other dysfunctions. Schiff bases **(5-7)** showed non-significant IC_{50} values (Table 2) for urease inhibition.

Lipoxygenase inhibitory activity

Lipoxygenase (EC 1.13.11.12) is a non-heme iron-based enzyme that catalysis many reactions of xenobiotic metabolism and also catalyze the polyunsaturated fatty acid or lipids into leukotrienes (Figure 3), which plays an important role in the pathophysiology of several inflammatory diseases. Many compounds have been identified which inhibit the biosynthesis of leukotrienes. Lipoxygenases (LOX's) are sensitive to antioxidants and the most of their actions may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipid-oxy or lipidperoxy-radicals formed in course of enzymic peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. In case of LOX, the IC_{50} values were found to be moderate for Schiff base **(5)**, significant for Schiff base **(6)** and potent for Schiff base **(7)**. The order was **7**>**6**>**5**, on the basis of IC_{50} values. LOX inhibitory effects of the synthesized compounds showed their potential to as new anti-inflammatory lead compounds. The present study guided us to the inference that the biological activities depend on the number of substituted methoxy groups. The schiff base (**7**) was found to be extraordinary active against *Escherichia coli*. LOX was found to be potent for Schiff base **(7)**. Extensive studies are required to explore lead compounds based on compou-

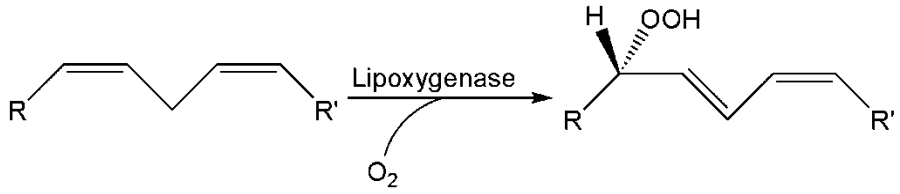

Figure 3. General reaction catalyzed by lipoxygenase.

nds based on compounds investigated in current study.

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Conflict of interest statement

There is no conflict of interest associated with the authors of this paper.

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