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ORIGINAL ARTICLE

Biological evaluation of potent antioxidant, lipoxygenase inhibitor and antibacterial: A comparative study

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Abstract Three biologically active new Schiff bases, 2-[(3-hydroxybenzylidene)amino]phenol **5**, 2-[(4-hydroxybenzylidene)amino]phenol **6** and 4-[(2-hydroxyphenylimino)methyl]benzene-1,3-diol **7**, were synthesized by the reaction of 2-aminophenol **1** with three different hydroxyl-benzaldehydes **2**–**4**. They were characterized by spectroscopic analysis (IR, ^{1}H NMR, EI-MS) along with elemental analyses. The products were biological screened out for antioxidant, lipoxygenase inhibition, antibacterial and urease inhibition activities. The compounds **5** and **6** showed potent while **7** showed moderate antioxidant activity. Compound **6** showed potent whereas **5** and **7** showed significant lipoxygenase inhibition activity. All the target compounds showed excellent activities against *Staphylococcus intermedius*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* bacteria. All the compounds showed non-significant activity against urease enzyme.

1. Introduction

Schiff bases are azomethine compounds, after the name of Hugo Schiff (Da Silva et al., 2011), and are prepared by the condensation of primary amines with aldehydes or ketones in acid or base catalyst (Dueke-Eze et al., 2011). In organic, Schiff bases are important intermediates in the synthesis (Rana et al., 2012). Due to their azomethine moiety, they possess a remarkable anticancer (Shkawat et al., 1973), antibacterial

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(More et al., 2001), antifungal (Chaitanya et al., 2010), anti-tumor (Hodnett and Dunn, 1970), herbicides (Samadhiya and Halve, 2001), anti-inflammatory (Sathe et al., 2011) and analgesic (Chinnasamy et al., 2010) activities. They are also used as pigments, dyes, catalysts, and as polymer stabilizers (Taggi et al., 2002). Due to the attractive biological activities of Schiff bases, we have reported the synthesis and biological activities of various Schiff bases (Aslam et al., 2012).

We herein report the syntheses and characterizations of three Schiff bases, named 2-[(3-hydroxybenzylidene)amino]-phenol **5**, 2-[(4-hydroxybenzylidene)amino]phenol **6** and 4-[(2-hydroxyphenylimino)methyl]benzene-1,3-diol **7**, derived from 2-aminophenol **1** and hydroxyl-benzaldehydes **2**–**4**, along with their antioxidant, lipoxygenase inhibition, antibacterial and urease inhibition activities.

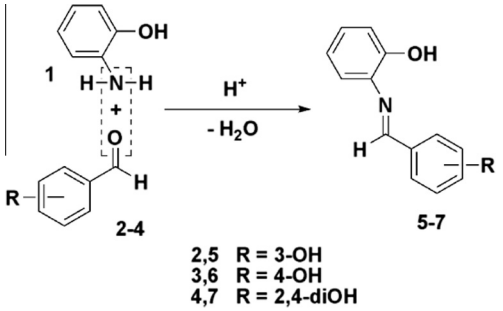
2. Experimental

2.1. Material and methods

All the solvents and chemicals were purchased from Merck. The melting points were determined by Gallenkamp apparatus and are uncorrected. Elemental analyses were carried out by Perkin–Elmer 2400 Series II elemental analyzer. IR spectra were measured on Thermo Nicolet Avatar 320 FT-IR spectrometer by using KBr pellets. Mass spectra on electron impact mode were measured on Finnigan MAT-112 spectrometer and ions are given in m/z. TLC was performed on pre-coated silica gel G-25-UV_{254} plates to check the purity. The ^{1}H NMR spectra were performed on Bruker AMX-400 spectrometer in DMSO-d_6 solvent and TMS used as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) are reported in Hertz.

2.2. General procedure for the synthesis of Schiff bases 5–7

The reaction mixture of 2-aminophenol **1** (0.01 mol in 50 mL EtOH) and hydroxybenzaldehydes **2**–**4** (0.01 mol in 50 mL EtOH) followed by 3–4 drops of conc. H_2SO_4, was refluxed for 3 h at 70 °C with constant stirring. The mixture was concentrated to one-third of its volume by using rotary evaporator. The conc. mixture was placed at ambient temperature to obtain the solid products. The products were filtered, washed with cold methanol and recrystallized with absolute methanol. The final products were dried on anhydrous calcium hydroxide at reduced pressure. The completion of reaction was monitored by TLC from time to time (Scheme 1).



Scheme 1 Synthetic scheme of the Schiff bases **5**–**7**.

2.3. 2-[(3-Hydroxybenzylidene)amino]phenol 5

Chocolate solid; yield 75.02%; m.p. 119 °C; Anal. Calcd. for $C_{13}H_{11}NO_2$: C, 73.23, H, 5.20, N, 6.57; found C, 73.41, H, 5.31, N, 6.51; IR (KBr) ν_{max} cm^{-1}: 1680 (C═N); ^{1}H NMR (DMSO-$_6d$, 400 MHz, ppm) δ: 8.56 (1H, s, ─N═CH, azomethine); EI-MS: m/z [M]$^+$ 213.3.

2.4. 2-[(4-Hydroxybenzylidene)amino]phenol 6

Fire brick solid; yield 81.07%; m.p. 123 °C; Anal. Calcd. for $C_{13}H_{11}NO_2$: C, 73.23, H, 5.20, N, 6.57; found C, 73.33, H, 5.39, N, 6.63; IR (KBr) ν_{max} cm^{-1}: 1671 (C═N); ^{1}H NMR (DMSO-$_6d$, 400 MHz, ppm) δ: 8.53 (1H, s, ─N═CH, azomethine); EI-MS: m/z [M]$^+$ 213.7.

2.5. 4-[(2-Hydroxyphenylimino)methyl]benzene-1,3-diol 7

Chocolate solid; yield 73.21%; m.p. 132 °C; Anal. Calcd. for $C_{13}H_{11}NO_3$: C, 68.11, H, 4.84, N, 6.11; found C, 68.29, H, 4.97, N, 6.26; IR (KBr) ν_{max} cm^{-1}: 1625 (C═N); ^{1}H NMR (DMSO-$_6d$, 400 MHz, ppm) δ: 8.76 (1H, s, ─N═CH, azomethine); EI-MS: m/z [M]$^+$ 229.1.

2.6. Biological assays

2.6.1. Antioxidant: DPPH radical scavenging assay

The free radical scavenging activity was carried out by 1,1-diphenyl-2-picryl-hydrazil (DPPH) (Ferheen et al., 2009). The solution of DPPH (0.3 μM) was prepared in ethanol. The solution of each sample was prepared in methanol. Five microliters of solution of each sample (with concentration range 5–500 μg) was added to 95 μL of DPPH solution, 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 by elisa plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was assessed in contrast to methanol treated control (DMSO). BHA (butylated hydroxylanisole) used as standard.

$$\text{DPPH scavenging effect } (\%) = \text{Ac} - \text{As}/\text{Ac} \times 100$$

where Ac, absorbance of control (DMSO treated); As, absorbance of sample.

IC_{50} values were checked by observing the effect of different concentrations (1–1000 μM) and were calculated using EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, USA).

2.6.2. Urease inhibition assay

The urease enzyme solution was prepared by taking 0.125 units in each well in phosphate buffer ($K_2HPO_4{\cdot}3H_2O$, 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 sample (concentration range 5–500 μM), contents were mixed and incubated for 15 min at 30 °C. Forty microliters 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