SYNTHESIS, X-RAY CRYSTALLOGRAPHY, MOLECULAR DOCKING AND BIOLOGICAL SCREENING OF 2-AMINOPHENOL BASED SCHIFF BASES

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ABSTRACT

Schiff bases, 2-[(2-hydroxyphenyl)methylidene]amine)phenol (4) and 2-[(3-4-(dimethylamino)phenyl-2-propenylidene]amine)phenol (5), have been synthesized by the condensation of 2-aminophenol (1) with 2-hydroxybenzaldehyde (2) and 4-dimethylaminocinnamaldehyde (3), respectively. They showed good lipoxigenase inhibition and antioxidant activities, as compound 4 showed potent antioxidant activity. The products also showed good activities against Gram-positive: S. intermedius, B. subtilis and S. aureus, and Gram-negative: E. coli and S. typhi bacteria, as compounds 5 and 4 are excellent active against B. subtilis and S. typhi, respectively. These good to potent activities may be due to the presence of free hydroxyl and amino groups. They showed non-significant urease inhibition activity.

Key Words: Schiff bases, Synthesis, X-ray diffraction, Molecular docking, Biological activities.

INTRODUCTION

In the pharmaceutical field, study on antioxidants, antibacterial and enzyme inhibitions remain a vital area of research as these can depreciate human skin, body, can be the reason of different disease or even can cause death. This research has led to the discoveries of new drugs useful in different physiological conditions. Reactive oxygen species (ROS) oxidize the human cell or body and play an important role in the etiology [1] and pathophysiology such as coronary heart disease, cancer, Alzheimer’s disease [2], neurodegenerative disorders, atherosclerosis, cataracts and inflammation [3], and human aging [4]. Antioxidants fare the ROS and protect the human body. In the world, most people died due to bacterial infectious diseases [5]. The enzyme have various pathological effects such as lipoxigenase (LOX) causes inflammation and is also responsible in the growth of cancer cells, metastasis, invasiveness and tumor necrosis factor (TNF) induction, and urease enzyme causes gastric ulceration, urinary stone formation, pylonephritis, and other dysfunctions [7].

The Schiff bases engaged much attention as they demonstrated antimicrobial [8], anticancer [9], anticonvulsant [10], diuretic [11], herbicidal [12], anti-inflammatory [13], antitumor [14] and anti HIV [15] activities, can be prepared through condensation of an amine and a carbonyl compound with good yield. Generally, it is well known that the compounds having hydroxyl, thiol, carboxylic, amino, thio, etc functional groups are more active. Due to this reason and versatile biological uses of Schiff bases prompted us to synthesize the new Schiff bases by condensation of 2-aminophenol (1) with free hydroxyl and amino groups containing hydroxybenzaldehyde (2) and 4-dimethylaminocinnamaldehyde (3), respectively. It is expected that the products will show excellent results as antioxidant, lipoxigenase, antibacterial and urease activities. Herein, we report the synthesis, characterization, X-ray crystallography and biological screening of two new Schiff bases named 2-[(2-hydroxyphenyl)methylidene]amine)phenol (4) and 2-[(3-4-(dimethylamino)phenyl-2-propenylidene]amine)phenol (5). Biological screening of products exhibited that they have good lipoxigenase inhibition and antioxidant activities, as compound 4 showed potent antioxidant activity. Both target compounds also showed good activities against S. intermedius, B. subtilis, S. aureus, E. coli, S. typhi but insignificant against P. aeruginosa bacteria but showed non-significant activity against urease enzyme.

EXPERIMENTAL

All the chemicals and solvents purchased from E. Merck and used as obtained. Thin layer chromatography (TLC) was performed on pre-coated silica gel G-25-UV254, plates (E. Merck), and detection was carried out at 254 and 366 nm. The IR spectra were recorded on Thermo Nicolet Avatar 320 FTIR spectrometer using KBr pellets. Melting points were measured on a Gallenkamp apparatus and are uncorrected. Elemental analyses were performed on Perkin Elmer 2400 Series II elemental analyzer. X-ray diffraction was performed on Bruker Smart APEX II, CCD 4-K area detector diffractometer, SAINT program was used for data reduction, structure was solved by direct method and refined by full-matrix least squares on F2 using SHELEX LT-PC package. The figures were plotted with the help of ORTEP program. The FAB (Fast Atomic Bombardment) mass spectra were recorded on JEOL SX102/DA-6000 mass spectrometer using glycerol as matrix and ions are given in m/z. The ‘H NMR spectra were recorded on a Bruker AMX-400 spectrometer in DMSO-d6. The chemical shifts (δ) are given in ppm, relative to tetramethylsilane as an internal standard, and the scalar coupling constants (J) are reported in Hertz.

GENERAL PROCEDURE FOR THE SYNTHESIS OF SCHIFF BASES 4-5

The mixture of solutions of 2-aminophenol (0.01 mole in 50 mL EtOH, 1) and 2-hydroxybenzaldehyde (2) or 4-dimethylaminocinnamaldehyde (3) (0.01 mole in 50 mL EtOH) along with 3-4 drops of conc. H2SO4 was refluxed with stirring at 70 °C for 3 hours (Figure 1). After cooling, the mixture was concentrated to one third of its volume under reduced pressure. The concentrated mixture kept for overnight at ambient temperature and orange red crystalline solid, yield 96%. M.p. 186 °C. IR (KBr) ν cm−1: 3618, 3045, 1630, 1531. ‘H NMR (400 MHz, DMSO-d6) δ: 14.77 (OH), 8.95 (1H, s, H-1*), 7.60 (1H, dd, J = 7.2, 1.6 Hz, H-6), 7.37 (1H, dd, J = 8.4, 8.0, 1867

e-mail: maslamchemist@hotmail.com, rashadhej@gmail.com
2-aminophenol by modified agar well diffusion method

2-[3-(4-Dimethylamino)phenyl-2-propenylideneamino]phenol (5)
Orange red crystalline solid, Yield 89%. M.p. 174°C. IR (KBr) ν cm⁻¹: 3376, 3050, 1601, 1513. H¹ NMR (400 MHz, DMSO-d₆): δ 8.36 (1H, d, J = 16.0 Hz, H-1'), 7.46 (2H, d, J = 8.8 Hz, H-2', -6'), 7.19 (1H, d, J = 15.6 Hz, H-3'), 7.04 (1H, dd, J = 8.0, 1.6 Hz, H-6), 6.99 (1H, dd, J = 8.4, 8.0, 1.6 Hz, H-4), 6.89 (1H, d, J = 16.0, 15.6 Hz, H-2'), 6.82 (1H, dd, J = 8.0, 1.6 Hz, H-3), 6.78 (1H, d, J = 8.4, 8.0, 1.6 Hz, H-5), 6.73 (2H, d, J = 8.8 Hz, H-3', -5'), 2.97 (6H, s, N-CH₃) FAB-MS (+ve) m/z: 267.2 [M⁺]⁺ (calcd. for C₁₃H₁₁NO, Elemental analysis: found C 76.73, H 6.90, N 10.55; calcld. C 76.66, H 6.81, N 10.52).

Crystal Data of 4

C₁₀H₁₀N₂O₂, MS: 213.23, triclinic, space group P-1, a = 9.0456(12) Å, b = 10.1549(14) Å, c = 12.3667(17) Å, α = 69.680(3)°, β = 89.897(3)°, γ = 76.960(3)°, V = 1034.2(2) Å³, Z = 4, rₓᵧ = 1.370 mg/m², F(000) = 448, μ(Mo Kα) = 0.71073 Å, max/min transmission 0.8935/0.6105 crystal dimensions 0.28 x 0.08, 1.76 < q < 25.5, 11775 reflections were collected, of which 3830 (I > 2s(I), and R int = 0.1084 for I > 2s(I), and R int = 0.0436, wR int = 0.1362 for all data; max/min residual electron density: 0.225/ -0.180 e Å⁻³.

Crystal Data of 5

C₁₇H₁₇N₂O₂, MS: 266.33, monoclinic, space group P2₁/c, a = 6.3277(4) Å, b = 12.805(8) Å, c = 18.0358(12) Å, α = 90.000°, β = 97.926(2)°, γ = 90.000°, V = 1447.41(16) Å³, Z = 4, rₓᵧ = 1.222 mg/m², F(000) = 568, μ(Mo Kα) = 0.71073 Å, max/min transmission 0.9093/0.9633, crystal dimensions 0.9 x 0.14 x 0.14, 196 < q < 25.5, 8436 reflections were collected, of which 2698 reflections were observed (R exp = 0.0275). The R-values were: R exp = 0.0436, wR exp = 0.1084 for I > 2s(I), and R int = 0.0666, wR int = 0.1235 for all data; max/min residual electron density: 0.138/ -0.167 e Å⁻³.

Antioxidant: DPPH Radical Scavenging Activity Assay
The solution of DPPH (0.3 mM) was prepared in ethanol. 5 µL methanol solution of each sample of different concentrations (5-500 µg) was mixed with 95 µL of DPPH solution in ethanol. The mixture was then dispersed in 30 well plates and incubated at 37°C for 30 min, then absorbance was measured at 515 nm by microtitre plate reader (Spectramax plus 384 Molecular Device, Amherst, U.S.A). Butylated hydroxyanisole (BHA) was also used as standard. The percent inhibition was calculated by this formula:

\[ \text{Percentage inhibition} = \frac{(\text{Test sample} - \text{Solvent control})}{\text{Positive control} \times 100} \]

Lipoxygenase Inhibition Assay
The urease enzyme solution was prepared by taking 0.125 units in each well in phosphate buffer (K₂HPO₄, 1 mM EDTA and 0.01M LiCl). Each well was filled with 80 µL of 0.05 M potassium phosphate buffer pH 8.2), 10 µL of the test compound (concentration range 5 - 500 µM), contents were mixed and incubated for 15 min at 30 °C. 40 µL of substrate solution (urea) (50 mM) was added in each well except B enzyme for initiating reaction. Then, 70 µL alkaline reagent (0.5 % NaOH and 0.1 % active NaOCl) and 40 µL of phenol reagent (1% Phenol & 0.005 % w/v sodium nitroprusside) were introduced to each well. The reaction mixture containing well plates were incubated for 50 minutes and absorbance was recorded at 630 nm. IC₅₀ values were determined by monitoring the effect of increasing concentrations of test compounds on extent of inhibition [16].

Results and Discussion
Scaffold bases 4 and 5 were synthesized through condensation of 2-aminophenol (1) with 2-hydroxybenzaldehyde (2) or 4-N,N-dimethylinocinnamaldehyde (3), respectively, in ethanol at 70°C followed by few drops of conc. sulfuric acid as catalyst (Figure 1). Their structures were determined by spectroscopic data as shown in experimental part.

X-Ray Crystallography
The structure of the Schiff base 4 consists of two independent molecules in the asymmetric units (Figure 2). The planer phenyl rings of both molecules are twisted by 10.42° (C1–C6 and C8–C13) and 9.92° (11)° (C14–C19 and C21–C26) with respect to each other. The bond lengths and angles are similar to those in our previously published related compound [18]. In the crystal, intramolecular N1⋯-H1A ...O2 and N2⋯-H2A...O4 hydrogen bonds form S(6) and adjacent molecules are linked by intermolecular O1⋯H1B⋯O3 and O1⋯H1B⋯O2 hydrogen bonds, forming zigzag sheets parallel to the b axis (Figure 3). The crystal data is in Experimental Part (Table 4).

Single-crystal X-ray diffraction analysis was carried out to establish the structure of the Schiff base 5. The ORTEP diagrams of the Schiff base 5 showed that mono-substituted N,N-dimethyl phenyl and phenol rings are connected by aza-butadiene chain (Figure 4). The azomethine (C=NH, 1.278 (2) Å) and olefinic double bonds (C=C, 1.335 (2) Å) adopts E-configuration, further stabilized by O1-H1A...N1 intramolecular hydrogen bond forming a S(5) ring motif (Figure 4). In the crystal, molecules are linked by C-H⋯O, intermolecular hydrogen bonds to form chains arranged parallel to the b-axis (Figure 5) and crystal data is in Experimental Part (Table 5).
Table 1: Results of antibacterial activities of the Schiff bases 4-5.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gentamicin (0.3%)</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone inhibition (mm)</td>
<td>Zone inhibition (mm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. intermedius</td>
<td>30</td>
<td>17</td>
<td>57</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>27</td>
<td>20</td>
<td>74</td>
</tr>
<tr>
<td>S. aureus</td>
<td>30</td>
<td>19</td>
<td>63</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>30</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>S. typhi</td>
<td>24</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>25</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

S. Intermedius (Staphylococcus intermedius), B. subtilis (Bacillus subtilis), S. aureus (Staphylococcus aureus), E. coli (Escherichia coli), S. typhi (Salmonella typhi) and P. aeruginosa (Pseudomonas aeruginosa); + mean non-significant

**Figure 2:** The molecular structure of 4 with displacement ellipsoids drawn at 30% probability level. Dashed lines show the intramolecular hydrogen bonding.

**Figure 3:** The crystal packing of 4, only hydrogen atoms involve in the intramolecular hydrogen bonding, which are shown by dashed lines.

**Figure 4:** The molecular structure of 5 with displacement ellipsoids drawn at 30% probability level. Dashed lines indicate the intramolecular hydrogen bonding.

**Figure 5:** The crystal packing of 5, only hydrogen atoms involve in the intramolecular hydrogen bonding which are shown by dashed lines.

**ANTIOXIDANT: DPPH RADICAL SCAVENGING ACTIVITY**

The antioxidant activity of compounds 4-5 was carried out with DPPH by well diffusion method in comparison with the BHA. They showed good activity against DPPH, as 5 showed comparable activity with the standard and 4 showed more activity than the standard (BHA) (Table 2), which may lead to the potential antioxidant and it need to be further proceed.

**ANTI-BACTERIAL ACTIVITIES**

Activities of the products 4 and 5 were investigated against Gram-positive: Staphylococcus intermedius, Bacillus subtilis, Staphylococcus aureus and Gram-negative: Escherichia coli, Salmonella typhi and Pseudomonas...
*P. aeruginosa* bacteria in comparison with gentamicin as reference drug. Both
the compounds are good active against all bacteria except *P. aeruginosa*. The
results show that both compounds are more active against *B. subtilis* than
against the others bacteria (Table 1). The results also show that Gram-positive
bacteria are more active than the Gram-negative bacteria for the products.

### Lipoxygenase Inhibition Activity and Molecular Docking

Lipoxygenase inhibition activity of the synthetic compounds 4-5 were
examined with the comparison of baicalein. The results showed that both
compounds are good active against lipoxygenase (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urease Inhibition Activity IC_{50} (μM)</th>
<th>DPPH Scavenging Activity IC_{50} (μM)</th>
<th>Lipoxygenase Inhibition Activity IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>59.2</td>
<td>32.0</td>
<td>59.2</td>
</tr>
<tr>
<td>5</td>
<td>28.9</td>
<td>45.5</td>
<td>-</td>
</tr>
<tr>
<td>BHA</td>
<td>-</td>
<td>44.2</td>
<td>-</td>
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<tr>
<td>Baicalein</td>
<td>-</td>
<td>-</td>
<td>22.6</td>
</tr>
<tr>
<td>Thiourea</td>
<td>21.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Butylated hydroxyanisole (BHA); + mean non-significant.

<table>
<thead>
<tr>
<th>Compound</th>
<th>4 (C_{13}H_{13}NO); MS: 213.23</th>
<th>5 (C_{17}H_{18}N_{2}O); MS: 266.33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometry</td>
<td>Triclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P-1</td>
<td>P21/c</td>
</tr>
<tr>
<td>a</td>
<td>9.0456(12) Å</td>
<td>6.3277(4) Å</td>
</tr>
<tr>
<td>b</td>
<td>10.1549(14) Å</td>
<td>12.8050(8) Å</td>
</tr>
<tr>
<td>c</td>
<td>12.3667(17) Å</td>
<td>18.0358(12) Å</td>
</tr>
<tr>
<td>α</td>
<td>69.680(3)°</td>
<td>90.00°</td>
</tr>
<tr>
<td>β</td>
<td>89.897(3)°</td>
<td>97.926(2)°</td>
</tr>
<tr>
<td>γ</td>
<td>76.960(3)°</td>
<td>90.00°</td>
</tr>
<tr>
<td>V</td>
<td>1034.2(2) Å^3</td>
<td>1447.41(16) Å^3</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>( \rho_{calc} )</td>
<td>1.370 mg/m³</td>
<td>1.222 mg/m³</td>
</tr>
<tr>
<td>F(000)</td>
<td>448</td>
<td>568</td>
</tr>
<tr>
<td>Crystal dimension</td>
<td>0.38 x 0.28 x 0.08 mm</td>
<td>0.49 x 0.14 x 0.14 mm</td>
</tr>
</tbody>
</table>

Multi-conformer library FRED 2.1 used for the docking of OMEGA pre-
generated multi-conformer, exhaustive docking / scoring of all orientations and
position of ligand in the binding site. For exhaustive search, rigid rotations
and translations of each conformation within the binding site make the grounds.
Filtered by FRED and cause ensemble by rejecting those that have clashes
with the protein (LOX) or having no connection with protein. The smooth
shape-based Gaussian scoring function (shapegauss) was used for evaluating
the complementary shape of ligand and binding pocket to score or rescoring the
final poses. Default FRED protocol was used for defining the binding sites except the size of the box. To optimize the docking-scoring performance for
exhaustive docking, the optimization mode of shapegauss was used, which
involved the optimization of a solid body systematically of the top ranked
poses. For this, three different solid bodies or boxes LOX (PDB ID: 1JNQ)
were used. The method adopted, involved three different simulations around
the reference ligand with an additional value of 8 Å [19].

The inside active site of LOX consists of His 523, Ile 857, His 518 and iron which catalyze the polyunsaturated fatty acids to leukotrienes. The active
LOX inhibitors establish the strong molecular interactions at these residues
and consequently block this active site. In the present study, the compound 4
showed the LOX inhibition activity with IC_{50} 59.2 μM. A number of factors
are involve in the low LOX inhibition activity of 4 such as non-existence of
hydrogen bonding between phenolic moiety and the active site (His 518, His
523 and Ile 867), dipole-dipole interactions between nitrogen of imine group
and Ile 557, and charge-dipole interactions: non-hydrophilic interactions with
Phe 576 and Ile 770. The major factors are the defective electrostatic and steric
interactions.

The compound 5 showed LOX inhibition activity with IC_{50} 28.9 μM, which is near to the IC_{50} value of standard (Baicalein, IC_{50} 22.1 ± 0.03 μM).
The good LOX inhibition of 5 is due to the strong interactions with the active
site of LOX. The molecular docking simulations of 5 showed that it has strong
molecular contacts with the catalytic triad along with iron atom (Figure 6, 7).
The various factors involve in the good LOX inhibition activity of 5 include
hydrogen bonding, dipole-dipole interactions and charge-dipole interactions.
The most important part of the compound 5 is phenolic group, which is
involved in hydrogen bonding with all four components of the active site as
His 518 (3.58 Å), His 523 (2.57 Å) and Ile 867 (3.12 Å). These promising
interactions could be one of the major factors of its good LOX inhibition
activity. The other aspect of its activity is the dipole-dipole interactions of
nitrogen of imine with Ile 557. Alongside with the above factors, N-methyls
also provide the favorable hydrophobic interactions with Phe 576 and Ile
770. All the interactions form the compound 5 a good inhibitor of LOX. The
good LOX inhibition of 5 showing strong potential to be developed as anti-
inflammatory agent. Therefore, based on present studies, it can be proceeded to
develop a new anti-inflammatory drug.
UREASE INHIBITION ACTIVITY
Both the target compounds 4-5 were studied against urease enzyme and they showed non-significant activity (Table 2).

CONCLUSION
The Schiff bases 4 and 5 were derived from 2-aminophenol (1) and 2-hydroxybenzaldehyde (2) or 4-N,N-dimethylaminocinnamaldehyde (3), respectively. Both target compounds are good antioxidant agent whereas 4 is potent antioxidant as expecting due to presence of hydroxyl group. They are good lipoxygenase inhibitors and have good antibacterial activities against all mentioned bacteria except P. aeruginosa while compounds 5 and 4 are excellent active against B. subtilis and S. typhi, respectively.

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