Antimicrobial Activity of Schiff Bases Substituted by Mannich Side Chain

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Abstract
Two Schiff bases (3-[(1E)-N-phenylethanimidoyl]phenol(I) and 2,6-bis(dimethylamino)3[(1E)phenylethanimidoyl]phenol(II) were synthesized via a general synthesis protocol. The target molecules were identified by spectroscopic tools: UV, IR, NMR and mass spectrometry. The synthesized bases were evaluated for their antimicrobial activity against six standard human pathogens. Both compounds showed significant activity against all tested organisms at a concentrations of 50, 100 and 200mg/ml.

Keywords: Schiff bases, Synthesis, Antimicrobial activity.

1-Introduction
Studies on the chemistry of Mannich bases are of interest in various areas of applications. A large number of Mannich bases have been synthesized in order to correlate their structure and reactivity with their pharmacological potential. Some Mannich bases possess antibacterial activity (Tamas and Bela, 2002; Gabriela et.al., 2009; Prashant and Kapandnis, 2004; Pernak et.al., 1999; Surendra et.al., 2000; Pandeya et al., 1999; Pandeya and Nath, 1999). Some were evaluated as novel potential antimalarial agents (Saudrine et.al., 2008; Kaylene, 1999; Alex and Jirigut, 2007; Ying et.al., 2003; Mannich bases with putative cytotoxic activity were reported (Jonathan et.al., 2002; Dimmock et.al., 1995; Jonathan et.al., 2000; Inci et.al., 2000). Some Mannich bases possess anticonvulsant activity, others exhibit promising antiamoebic potency (Mohammad and Amir, 2009; Kiran and Ashok, 2004).

Base exchange reactions involving Mannich bases with primary aromatic amines have been reported by a number of workers (Ardashev and Malik, 1967; Abdalla and Ramli, 1990; Greenhill and Ramli, 1997; Craig et.al., 1964). Some of these reactions are of particular practical interest, since the resultant secondary amines are not, in general, available by other synthetic routes.

Industrially, Mannich bases are utilized as dyes for synthetic fabrics (Makra et.al., 2001), as polymers in treatment of wastewater (Bakes et.al., 1991) as antioxidants and corrosion inhibitors (Farng and Horodysky, 1990) as active ingredients in lubricant composition and also as surface active components.

2- Materials and Methods
2.1- Materials
2.1.1- Test organisms
The synthesized compounds were screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

<table>
<thead>
<tr>
<th>Ser. No</th>
<th>Micro organism</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus subtilis</td>
<td>G+ve</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>G+ve</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas aeruginosa</td>
<td>G-ve</td>
</tr>
</tbody>
</table>
2.1.2- Chemicals and Solvents
Analytical grade reagents (Table .4) were used. They were purchased from Sigma – Aldrich company (UK)

2.2- Methods
2.2.1-Synthesis protocols
2.2.1.1- Synthesis of Schiff bases (I) : 3-[(1E)-N-phenylethanimidoyl]phenol
3-hydroxyacetophenone (2.72g ,20mmol) was added to aniline (1.86g,20mmol) in methanol (40ml). Then 3drops of concentrated H₂SO₄ were added. The mixture was then stirred for 1 hour at room temperature and left overnight. Removal of the solvent under reduced pressure gave the product.

2.2.1.2- Synthesis of Compound (II) : 2,6-bis(dimethylamino)-3-[(1E)-N-phenylethanimidoyl]phenol
Formalin(0.4g,5mmol) was added dropwise with stirring to a mixture of compound (I) (1.055gm,5mmol) and dimethylamine (0.22gm,5mmol) in absolute ethanol (10ml) at 0°C. The mixture was then stirred for 1 hour and left overnight. Removal of the solvent under reduced pressure gave the product.

2.2.2-Antimicrobial Assay
2.2.2.1-Preparation of Bacterial Suspensions
One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10⁸-10⁹ colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspensions were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

2.2.2.2-Preparation of fungal suspensions
Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

2.2.2.3-Testing for antimicrobial activity
The cup-plate agar diffusion assay was adopted with some minor modifications, to assess the antibacterial activity of synthesized compounds. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divid-
ed into two halves, two cups in each half (10 mm in
diameter) were cut using sterile cork borer (No 4),
each one of the halves was designed for one of the
compounds. Separate Petri dishes were designed for
standard antibacterial and antifungal chemothera-
peutic agents.
The agar discs were removed, alternate cup were
filled with 0.1 ml samples of each compound using
adjustable volume microtiter pipette and allowed to
diffuse at room temperature for two hours. The
plates were then incubated in the upright position at
37°C for 24 hours.
The above procedure was repeated for different
concentrations of the test compounds and the
standard antimicrobial chemotherapeutics. After
incubation, the diameters of the resultant growth
inhibition zones were measured in triplicates and
averaged.

3-Results and Discussion
A Schiff base (I) was synthesized via the reaction of
3-hydroxyacetophenone with aniline. Schiff base
(I) was then aminomethylated by dimethylamine to
obtain (II). Products were identified by spectro-
scopic tools (IR, UV, NMR, and MS) and then
screened for their antimicrobial activity.

3.1-Synthesis of the Schiff base (I) : 3-[(1E)-N-
phenylethanimidoyl]phenol

\[
(I)
\]

The Schiff base (I) was synthesized by adding 3-
hydroxyacetophenone to aniline in methanol (40ml).
Then 3 drops of concentrated H_2SO_4 were added.
The mixture was stirred for 1 hour at room tempera-
ture and left overnight. Removal of the solvent un-
der reduced pressure gave the product.

The IR spectrum of (I) (Fig.1) gave \( \nu \) (KBr)
609,688,794,877 (C-H, Ar. bending) .
1363,1429,1490,1577 (C= C, Ar). 1577,1664( C=N )
2831 (C-H,aliph.). and 3178 (OH stretching).

Fig.1: IR spectrum of the Schiff bases (I)
The UV spectrum showed \( \lambda_{max}(\text{MeOH}) \) 249 ,309
nm which is due to a benzonitrile chromophore
extended by additional phenyl function. The
\(^1\)H NMR spectrum (Fig. 2) revealed the following
signals:

<table>
<thead>
<tr>
<th>( \delta )</th>
<th>multiplet</th>
<th>( \delta )</th>
<th>double</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.41</td>
<td>singlet</td>
<td>7.01</td>
<td>4H</td>
</tr>
<tr>
<td>7.25-7.41</td>
<td>multiplet</td>
<td>5H</td>
<td></td>
</tr>
</tbody>
</table>
The signal at δ 3.41 (s, 3H) was assigned for the methyl group in \( \text{N} \equiv \text{C} - \text{CH}_3 \). The resonances at δ 7.01 (d, 4H) and δ 7.25-7.41 (m, 5H) account for the aromatic protons. The mass spectrum (Fig. 3) gave m/z 211 corresponding to M⁺.

The Mannich base (II) was synthesized by adding formalin dropwise to a mixture of compound (I) and dimethylamine in absolute ethanol (10ml) at room temperature. The IR spectrum (Fig. 4) gave \( \nu \) (KBr) 684, 790, 871 (C-H, Ar. bending), 1299 (C=N), 1363, 1436, 1492, 1575 (C=C, Ar.), 1575, 1666 (C=N). 2827 (C-H, aliph.) and 3178 cm⁻¹ (OH stretching).

The UV spectrum showed \( \lambda_{\text{max}} \) (MeOH) 249, 308 nm which corresponds to benzonitrile chromophore extended by a phenyl function. The \(^1\)HNMR spectrum of (II) (Fig. 5) revealed the following signals:

<table>
<thead>
<tr>
<th>Signal</th>
<th>Multiplicity</th>
<th>Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ 3.15</td>
<td>singlet</td>
<td>6H</td>
</tr>
<tr>
<td>δ 3.27</td>
<td>singlet</td>
<td>12H</td>
</tr>
<tr>
<td>δ 3.52</td>
<td>doublet</td>
<td>3H</td>
</tr>
<tr>
<td>δ 7.40</td>
<td>doublet</td>
<td>5H</td>
</tr>
<tr>
<td>δ 7.85</td>
<td>doublet</td>
<td>2H</td>
</tr>
</tbody>
</table>

Fig. 2: \(^1\)HNMR spectrum of the Schiff bases (I)

Fig. 3: Mass spectrum of the Schiff bases (I)

Fig. 4: IR spectrum of the Mannich base (II)

3.2 Synthesis of the Mannich base (II) : 2,6-bis(dimethylamino)-3-[(1E)-N-phenylethanimidoyl]phenol

![Mannich base (II)](image)
The signal at $\delta$ 3.15(6H) was assigned for two methyl functions. The resonance at $\delta$3.27 (12H) accounts for the four methyl functions of dimethylamino moiety, while the signal at $\delta$ 3.52(3H) was attributed to one methyl group. The resonances at $\delta$7.40(d,5H) and $\delta$7.85(m,2H) account for the aromatic protons. The mass spectrum (Fig.6) gave the ion m/z382 corresponding to M$^+$+1.

3.3- Antimicrobial activity
The synthesized compounds were screened for their antimicrobial activity against six standard organisms. The average of the diameters of the growth inhibition zones are displayed in Table (2). The results were interpreted in terms of the commonly used terms. Compounds resulting in 13mm or more growth inhibition zones were considered to be active and those resulting in less than 9mm were considered inactive; 9-12mm being partially active.

Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively. Both compounds showed significant activity against all tested organisms at a concentrations of 50, 100 and 200mg/ml.
Table (4) : Antifungal activity of standard chemotherapeutic agent

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. mg/ml</th>
<th>A.n</th>
<th>C.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotrimazole</td>
<td>30</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>17</td>
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</tr>
<tr>
<td></td>
<td>7.5</td>
<td>16</td>
<td>29</td>
</tr>
</tbody>
</table>

Acknowledgement
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References
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Ying Li-Zhong Shun, Yang Hong Zhang, Ben Jun Cao, Bioorganic And Medicinal Chemistry, 11, 4363 (2003).