Substituent effect on spectral and antimicrobial activity of Schiff bases derived from aminobenzoic acids

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ABSTRACT

The dependence of electronic absorption spectra antimicrobial property on the substituent position was investigated using three Schiff bases derived from salicylaldehyde and isomeric aminobenzoic acids in three solvents of different polarities. The absorption maxima in all three solvents exhibited dependence on the position of the substituent with the absorption maxima undergoing a red shift as solvent polarity increased. The in vitro antibacterial activity of the compounds against some clinically important bacteria namely Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923), Enterococcus feacalis (ATCC 29212) was determined in N,N”-dimethylformamide and 1,4-dioxane using the agar dilution method. The results revealed that the ortho and meta substituted Schiff bases exhibited better antimicrobial activity in the non-polar solvent.

Keywords: Schiff Base; Aminobenzoic Acid; Antibacterial Activity; Salicyaldehyde; Electronic Spectra

1. INTRODUCTION

Schiff base compounds have been shown to be promising leads for the design of efficient antimicrobial agents as a result of the broad range of biological activities exhibited by these compounds. These compounds and their metal complexes are reported to exhibit antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties [1-3].

The mode of interaction and inhibition effectiveness of Schiff bases with bacteria and fungi is expected to depend on the molecular structure of the compounds. Thus, advances in this field will require analyses of structure activity relationships of Schiff bases along with investigation of the mechanism of action of these compounds [4].

In particular, Schiff bases composed of salicylaldehydes are very promising in the search of new functional materials. They exhibit a variety of biological activities [5,6] as well as show important photochromism where light absorption causes interconversion between enol-imine and keto-amine tautomers through intramolecular hydrogen transfer.

As part of our efforts to study structure activity relationship of Schiff bases, we report the effect of substituent position on the electronic spectra and antimicrobial activity of Schiff bases of isomeric aminobenzoic acids with salicylaldehyde.

2. EXPERIMENTAL

2.1. Material and Methods

All chemicals were obtained commercially from Sigma-Aldrich Chemicals. The solvents: ethanol, 1,4-dioxane, N,N”-dimethylformamide (DMF) and acetonitrile were of spectroscopic grade and used without further purification.

Infra-Red spectra were recorded as KBr pellets on a Shimadzu FT-IR 157 Spectrophotometer. ¹H NMR Spectra were obtained using a Bruker 400 MHz spectrometer in d⁶-dimethylsulfoxide (DMSO) solution with tetramethylsilane (TMS) as internal standard. Microanalytical data were determined using a CE-440 Elemental analyser, EAI Exeter Analytical Inc. Melting points were determined with Gallenkamp melting point apparatus.

The electronic spectra of the solution were investigated in various solvents of different polarities: 1,4-dioxane, N,N”-dimethylformamide (DMF) and acetonitrile. The electronic spectra were recorded on a T80/T80+ UV-VIS spectrophotometer using 1 cm quartz cell at room temperature immediately after preparing the so-
lutions in order to obtain the spectra of mainly the enol-imine tautomer.

2.2. Typical Synthesis of a Schiff Base

Equimolar quantities (10 mmol) of salicylaldehyde and primary amine were dissolved in ethanol (25 ml). Two drops of ethanoic acid was added and the mixture allowed to reflux at 70°C for 6 h. The reaction mixture was cooled to room temperature, the precipitate collected by filtration, re-crystallized from ethanol, and dried in a desiccator.

2-(2-hydroxybenzylideneamino) benzoic acid (1). Orange crystalline product, yield 52%, mp: 198°C - 199°C, IR (KBr) \( \nu/cm^{-1} \): 3440, 1682, 1618, 1567, 1459, 919, 677. \(^1\)H NMR (\(p\) DMSO-\(d_6\)): 13.06 (s, 1H, OHC=O), 8.95 (s, 1H, HC=N) 7.97 (d, 1H); 7.78 (d, 1H); 7.74 (t, 1H); 7.09 (t, 1H); 7.07 (d, 1H); 7.05 (d, 1H).

Anal. Calcd. for C\(_{14}\)H\(_{11}\)NO\(_3\): C, 69.70; H, 4.56; N, 5.80. Found: C, 69.23; H, 4.53; N, 5.77.

3-(2-hydroxybenzylideneamino) benzoic acid (2). Yellow powdery solid, yield 86%, mp: 183°C - 184°C, IR (KBr) \( \nu/cm^{-1} \): 3408, 3057, 1679, 1599, 1568, 1493, 941 - 753. \(^1\)H NMR (\(p\) DMSO-\(d_6\)): 12.84 (s, 1H, OHC=O), 9.00 (s, 1H, HC=N), 7.92 (s, 1H); 7.91 (d, 1H); 7.89 (t, 1H); 7.87 (d, 1H); 7.61 (d, 1H); 7.59 (d, 1H); 6.69 (t, 1H); 6.67 (t, 1H).

Anal. Calcd. for C\(_{14}\)H\(_{11}\)NO\(_3\): C, 69.70; H, 4.56; N, 5.80. Found: C, 69.24; H 4.66; N, 5.78.

4-(2-hydroxybenzylideneamino) benzoic acid (3). Yellow solid, yield 72%, mp: >220°C decomp. IR (KBr) \( \nu/cm^{-1} \): 3410, 3055, 1622, 1679, 1573, 1451, 930-678. \(^1\)H NMR (\(p\) DMSO-\(d_6\)): 12.70 (s, 1H, OHC=O), 8.99 (s, 1H, HC=N), 8.01 (dd, 2H); 7.70 (dd, 2H); 7.49 (d, 1H); 7.44 (t, 1H); 6.99 (t, 1H); 6.97 (d, 1H).

Anal. Calcd. for C\(_{14}\)H\(_{11}\)NO\(_3\): C, 69.70; H, 4.56; N, 5.80. Found: C, 69.28; H 4.64; N, 5.80.

3. RESULTS AND DISCUSSION

3.1. Synthesis

The Schiff bases 1 - 3 were prepared by the condensation reaction of salicylaldehyde with the corresponding aminobenzoic acid as illustrated in Scheme 1 below. The salicylidimineaminobenzoic acids 1 - 3 differed only in the position of the carboxylic functional group on the amine ring. The compounds are soluble in organic solvents such as ethanol, chloroform, DMF, DMSO and dioxane but insoluble in hexane and toluene.

The physical and analytical data are presented in Table 1. The low yield reported for ortho substituted compound, 1, is attributed to steric hindrance resulting from close proximity of the aldehyde and carboxylic acid functional groups. Important IR and \(^1\)HNMR peaks for the compounds are listed in Table 2.

The Infrared spectra of the all the compounds is composed of a band in the region 1599 - 1622 cm\(^{-1}\) attributed to \(\nu(C=N)\) of the azomethine group at 1618, 1599 cm\(^{-1}\) and 1622 cm\(^{-1}\) respectively. The lower frequency of this band compared to that of normal C=N (1680 - 1650 cm\(^{-1}\)) may be due to intramolecular interaction between the imine nitrogen and the hydrogen atom which results in a lengthening of the C=N bond [8-10]. Furthermore,
Table 1. Physical properties and analytical data of Schiff bases.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Empirical formula</th>
<th>Formula weight</th>
<th>Yield %</th>
<th>Color</th>
<th>M.pt</th>
<th>Microanalysis (caled)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%C %H %N</td>
</tr>
<tr>
<td>1</td>
<td>C_{14}H_{11}NO_{3}</td>
<td>241.24</td>
<td>52</td>
<td>Orange</td>
<td>198 - 199</td>
<td>69.23 4.53 5.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(69.70) (4.56) (5.80)</td>
</tr>
<tr>
<td>2</td>
<td>C_{14}H_{11}NO_{3}</td>
<td>241.24</td>
<td>86</td>
<td>Yellow</td>
<td>183 - 184</td>
<td>69.24 4.66 5.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(69.70) (4.56) (5.80)</td>
</tr>
<tr>
<td>3</td>
<td>C_{14}H_{11}NO_{3}</td>
<td>241.24</td>
<td>72</td>
<td>Yellow</td>
<td>&gt;220</td>
<td>69.28 4.64 5.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(69.70) (4.56) (5.80)</td>
</tr>
</tbody>
</table>

Table 2. Important IR and $^1$H NMR data of Schiff bases.

| Compd | $\delta$ (ppm) | $v$(O-H)$_{ar}$ | $v$(O-H)$_{acid}$ | $v$(C=O) | $v$(C=N) | $v$(COO)$_{asy}$ | $v$(COO)$_{sym}$ | C=N | OHC=O
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>3440</td>
<td>3050</td>
<td>1682</td>
<td>1618</td>
<td>1567</td>
<td>1459</td>
<td>8.95</td>
<td>13.06</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3408</td>
<td>3057</td>
<td>1679</td>
<td>1599</td>
<td>1568</td>
<td>1493</td>
<td>9.00</td>
<td>12.84</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3410</td>
<td>3070</td>
<td>1679</td>
<td>1622</td>
<td>1573</td>
<td>1451</td>
<td>8.99</td>
<td>12.70</td>
</tr>
</tbody>
</table>

The absorption spectra of Schiff bases 1 - 3 was investigated in three solvents of different polarities namely 1,4-dioxane, N,N'-dimethylformamide (DMF) and acetonitrile and the results summarized in Table 3. The use of non H-bonding solvents allows a study of solvent effect on the shift in absorption maxima for the enol-imine tautomer with minimum interference from the keto-anime tautomer.

The spectra comprise of absorption bands in the 200 - 500 nm region. The first one or two bands located in wavelength range 210 - 280 nm in the spectra of all Schiff bases can be assigned to the excitation of the $\pi$ electrons of the aromatic system [11]. The band in the 330 - 360 nm region is due to transition intramolecular charge transfer involving the whole molecule. This band observed in salicylaldimine compounds is facilitated by the presence of intramolecular hydrogen bonding between the hydroxyl group and the azomethine nitrogen [12]. In addition 1 showed band in the region 438 - 520 nm. This is probably due to conjugation between the ortho-COOH group and the imine functional group which results in cyclization.

Spectra data are good evidence for the presence of solute-solvent interactions between the active solvent and Schiff bases. Difference in position of the carboxylic group in these compounds is reflected in the interaction within the molecules. Substitution of the carboxylic group in the compounds results in an inductive effect of the order $para < meta < ortho$ [13]. Based on this trend, the absorption maxima ($\lambda_{max}$) of the compounds is expected to be in the order $o < m < p$. This is observed in the non-polar solvent dioxane. The absorption spectra are influenced by the physical properties of the solvent. It is observed that acetonitrile gives better resolution of the spectrum for all compounds and both the low and medium energy $\pi - \pi^*$ bands are clearly observed. This may be due to the lower hydrogen bonding ability of this dipolar aprotic solvent.

The low energy $\pi - \pi^*$ band of 2 containing the carboxylic acid group in the meta position shows maximum deviation suggesting that the COOH group in these compounds behaves as a meta director. The proton NMR of all the Schiff bases showed a singlet at 8.95 - 9.00 ppm thus confirming the presence of the azomethine proton.

3.2. Electronic Absorption Spectra

The electronic absorption spectra of Schiff bases 1 - 3 was investigated in three solvents of different polarities namely 1,4-dioxane, N,N'-dimethylformamide (DMF) and acetonitrile and the results summarized in Table 3. The use of non H-bonding solvents allows a study of solvent effect on the shift in absorption maxima for the enol-imine tautomer with minimum interference from the keto-anime tautomer.

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The low energy $\pi - \pi^*$ band of 2 containing the meta-substituent appears to be insensitive to solvent polarity. This is consistent with earlier reports that indicate that this highly intense $\pi - \pi^*$ band is sensitive to substitution in the aromatic part of the molecule and is little influenced by changing solvent polarity [14]. However, for 1 and 3 the band is solvent dependent with the more polar solvent, DMF having highest value of $\lambda_{max}$. This suggests that the mesomeric effect of the ortho and para groups stabilizes the excited state more than the ground state thereby giving rise to a red shift in absorption maxima in the polar solvent. Red shift in $\lambda_{max}$ value is observed in the following order: dioxane < acetonitrile > DMF.

The charge transfer band is more sensitive to solvent
changes. The CT band exhibits a shift of the $\lambda_{\text{max}}$ of CT band to longer wavelength in more polar DMF. Thus, indicating better stabilization of the polar excited state of the compounds in the more polar solvent. The change parallels the refractive indices of the solvents with acetonitrile < dioxane < DMF.

### 3.3. Antibacterial Activity

Schiff bases 1 - 3 were screened for their *in vitro* antimicrobial activity against bacteria pathogens *S. aureus*, *E. coli*, *E. feacalis* and *P. aeruginosa* using agar dilution technique in DMF and dioxane. The results and MIC values are reported in Table 4. The minimum inhibitory concentration gives approximation to the least concentration of an antimicrobial needed to prevent microbial growth.

Antimicrobial activity depends on the nature of bacterial strain, the solvent and chelating ability of the Schiff base. It is believed that Schiff bases act by forming a chelate with the bacterial strain. This may involve hydrogen bonding through the azomethine group with the active centres of cell constituents thus resulting in an interference with normal cell process [15]. Hence, the better the hydrogen bonding ability, the more active the compound.

The morphology of the cell wall is a key factor that influences the activity of antibacterial agents. *E. feacalis* and *S. aureus* has the highest MIC values of 25 mg/ml and 12.5 mg/ml respectively in all the compounds tested. For these strains, the activity is independent on position of substituent, interference of compounds with bacterial cell wall and solvent used.

In this study, Gram positive bacteria gave higher MIC values compared to gram negative bacteria tested. This may be due to the nature of the bacteria cell wall composed of peptidoglycan which is thicker in gram positive bacteria and this usually poses a barrier to the degree of diffusion of antibacterial agents into the enzyme. The thinner cell wall of the gram negative bacteria leads to easier penetration/diffusion of the compound across the cell wall thereby giving better screening effect.

Gram negative strains were more sensitive to solvent variation. The lowest MIC values were reported in dioxane against *P. aeruginosa* for 1 and 2 and *E. coli* for 3. The effect of solvent on inhibitory activity is pronounced in 3 with MIC values of 12.5 mg/ml in DMF and 3.125 mg/ml in dioxane when screened against *E. coli*. Thus, use of non-polar dioxane medium showed better inhibition with widely varying effects.

It can be deduced that the different response of the studied Schiff bases arises because of their structural difference and are also solvent dependent.

### 4. CONCLUSION

We have synthesized and characterized a series of Schiff bases of salicyaldehyde and isomeric aminobenzoic acids. The activity data suggest that the antibacterial activity is dependent on the molecular structure of the compound and solvent used. The non-polar solvent exhibits a better screening activity. The compounds possessing broad spectrum of *in vitro* antimicrobial activity hence can be used for treatment of some common diseases caused by these pathogens such as septicemia. Furthermore, the higher activity reported for gram negative bacteria suggests that

### Table 3. Electronic absorption bands of Schiff bases 1 - 3.

<table>
<thead>
<tr>
<th>Schiff base</th>
<th>Solvent</th>
<th>Band A</th>
<th>Band B</th>
<th>Band C</th>
<th>Band D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dioxane</td>
<td>2.2</td>
<td>252</td>
<td>302</td>
<td>334</td>
</tr>
<tr>
<td></td>
<td>Acetonitrile</td>
<td>37.5</td>
<td>293</td>
<td>343</td>
<td>497</td>
</tr>
<tr>
<td></td>
<td>DMF</td>
<td>36.7</td>
<td>303</td>
<td>346</td>
<td>520</td>
</tr>
<tr>
<td>2</td>
<td>Dioxane</td>
<td>244</td>
<td>319</td>
<td>338</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetonitrile</td>
<td>220</td>
<td>268</td>
<td>339</td>
<td></td>
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<td></td>
<td>DMF</td>
<td>360</td>
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</tr>
<tr>
<td>3</td>
<td>Dioxane</td>
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<td>323</td>
<td>343</td>
<td></td>
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<tr>
<td></td>
<td>Acetonitrile</td>
<td>274</td>
<td>320</td>
<td>344</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMF</td>
<td>324</td>
<td>342</td>
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</tbody>
</table>

*D* = dielectric constant.

### Table 4. Minimum inhibitory concentration values of compounds 1 - 3 by dilution method.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>E. coli</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>E. feacalis</em></th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMF</td>
<td>DIOXANE</td>
<td>DMF</td>
<td>DIOXANE</td>
</tr>
<tr>
<td>1</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>1.56</td>
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<td>2</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>1.56</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>3.125</td>
<td>6.25</td>
<td>6.25</td>
</tr>
</tbody>
</table>
these compounds may also have possible antitumor effects since gram negative bacteria are considered a quantitative microbial method for testing beneficial and important drug candidates.

5. ACKNOWLEDGEMENTS

The authors are grateful to Department of Medical Microbiology, University of Lagos where the standard organisms were obtained.

REFERENCES


