SYNTHESIS, CHARACTERIZATION AND STRUCTURE ACTIVITY RELATIONSHIP OF SCHIFF BASES DERIVED FROM 2-AMINOPHENOL AND SUBSTITUTED BENZALDEHYDES

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Abstract: The structure-antibacterial activity of six Schiff bases synthesized from 2-aminophenol and substituted benzaldehydes was studied by relating the effect of substituent groups on antibacterial activity. The study showed that compounds with electron donating methoxy substituent exhibited higher inhibitory activity against the tested microorganisms than compounds bearing an electron withdrawing chloroor nitro substituent. Quantum chemical studies carried out on the compounds using density functional theory calculations (DFT) revealed that the activity of the Schiff bases is related to the electrostatic potential, ionization potential and alignment of the dipole moment with respect to the imine bond.

Keywords: 2-aminophenol, antibacterial activity, schiff bases, quantum chemical studies

Introduction

Reports of resistance of micro-organisms particularly bacteria to current antimicrobial agents have led to resurgence in the search of potent antimicrobial agents. One of the useful class of compounds in the search for new drug candidates are Schiff bases which are nitrogen analogues of aldehydes in which the C=O group is replaced by a C=N bond (Dueke-Eze *et al*., 2011). These compounds exhibit a wide variety of biological activities including antibacterial, antifungal, antitumor, antioxidant and antitubercular activities (Jarrahpour *et al*., 2007; Sinha *et al*., 2008; Hearn *et al*., 2009; Abdel-Aal *et al*., 2010; Qiao *et al*., 2011; Da Silva *et al*., 2011; Anouar *et al*., 2013). The activity exhibited is attributed to the presence of the imine bond and the ease of electronic or structural modification of the Schiff base (Kajal *et al*., 2013).

A fundamental step in the development of new antimicrobial agents is the synthesis and screening of large number of potential candidates. The presence or absence of certain chemical groups can have profound effect on the biological activity of compounds; hence a study of structure-activity relationship (SAR) of potential drug candidates is important in the design of highly active chemical compounds. Such a study helps to determine the nature and position of chemical groups on the compound responsible for evoking a target biological effect in the organism of interest. Current efforts in Schiff base chemistry are directed to the understanding of structural effects on the observed properties/activities of designed compounds (Khan*et al*., 2012). Structure-antibacterial activity study of thiazolidine-4-ones, isatin and pyridine amide Schiff bases (Pandeya *et al*., 1999; Li *et al*., 2008; Devprakash and Udaykumar, 2011) and some related Mannich bases (Sivakumar *et al*., 2013), have indicated that substituent groups present on either the amine or aldehyde ring have significant effect on the biological activity observed for the compounds. The electronic property of substituents and conformation of the aromatic rings of enamines have pronounced effect on the structure-antibacterial activity of the compounds (Xiao *et al*., 2007). In addition to the nature of the group, the position of substituents on the aromatic ring is also of significance (Kuz'min *et al*., 2000; Singh *et al*., 2010; Aslam *et al*., 2016).

Aminophenols are powerful antibacterial agents and studies of the *in-vitro* activity of aminophenols and aminochlorophenols show that these compounds possess intrinsic biological activity (Valentovic *et al*., 1996).Schiff bases derived from aminophenols have also been reported to possess antibacterial, antifungal and anticancer activities (Mohamed, 2006; Baluja *et al*., 2009; Varghese and Nair, 2010; Prakash and Adhikari, 2011). In order to understand the role of substituent on the biological activity of aminophenol Schiff bases, the SAR in a series of Schiff bases of 2-aminophenol with substituted benzaldehydes bearing electron donating and electron withdrawing groups have been explored. A theoretical study was also undertaken to correlate the observed activity to some electronic parameters of the compounds. This study would provide a better understanding of the electronic effects on the bioactivity of these compounds and assist in design of new potent antimicrobial agents.

Materials and Methods

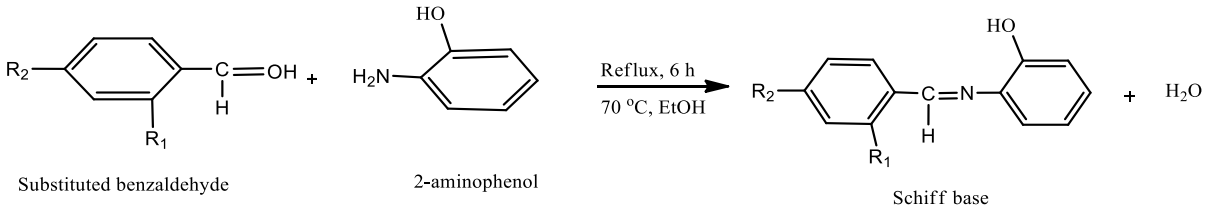
All reagents and solvents were of analar/spectroscopic grades were purchased from Aldrich-Sigma company Ltd and used without further purification. Infrared (IR) spectra of the compounds were recorded on a Bruker FT-IR (ATR) tensor 27 spectrophotometer directly on small samples of the compounds in the range 4000 to 400 cm^{-1}. ^{1}H-NMR spectra in chloroform and DMSO-d_6 solution of the ligands were recorded on a Bruker Avance III 400 MHz. Chemical shifts were reported in ppm relative to TMS as internal standard. Electronic absorption spectra of Schiff bases recorded from 200 to 600 nm using freshly prepared chloroform ($CHCl_3$) solution were measured on a Cary Model 50 spectrophotometer. Melting points were determined on a Reichert Thermovar melting-point apparatus and are uncorrected. Microanalytical data were obtained on a Perkin Elmer model 2400 series II CHNS/O elemental analyzer.

Antibacterial assay

The antibacterial sensitivity of Schiff bases was individually tested against a panel of standard microorganisms namely *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (10702), *Enterococcus faecalis* (ATCC 29212) *and Klebsiella pneumoniae* (ATCC 10031) using the paper disc diffusion method (Bauer *et al*., 1996). The compounds were prepared in DMSO to obtain a final concentration of 10 mg/ml. The minimum inhibitory concentration (MIC) was determined using the 96-well micro-plate dilution method (Eloff, 1998).

Results and Discussion

Schiff bases **1-6** were obtained in moderate to good yields from the reactions of 2-aminophenol with 2-substituted-benzaldehydes namely; 2-methoxybenzaldehyde (1), 2-chlorobenzaldehyde (2), 2-nitrobenzaldehyde (3) and 4-substituted-benzaldehydes namely; 4-methoxybenzaldehyde (4), 4-chlorobenzaldehyde (5), 4-nitrobenzaldehyde (6) in a 1:1 stoichiometric ratio under reflux conditions in ethanol (**Scheme 1)**. The purity of the compound was confirmed by the sharp melting points and elemental analysis data obtained as shown in Table 1.



Substituted benzaldehyde — 2-aminophenol — Schiff base

Compound	R_1	R_2
1	OMe	H
2	Cl	H
3	NO_2	H
4	H	OMe
5	H	Cl
6	H	NO_2

Scheme 1: Synthesis of Schiff bases

Table 1: Physical and analytical data of substituted 2-aminophenol Schiff bases

Compound	Empirical Formula (M.wt)	Yield (%)	Color	M.pt (°C)	% Found (cacld)		
					C	H	N
1	$C_{14}H_{13}NO_2$ (227)	72	Yellow	109-110	73.30(73.77)	5.77(5.77)	6.05(6.16)
2	$C_{13}H_{10}NOCl$ (231)	58	Yellow	98-99	67.38(67.39)	4.24(4.35)	6.03(6.05)
3	$C_{13}H_{10}N_2O_3$(242)	65	Yellow	106-107	64.62(64.46)	4.07(4.16)	11.60(11.56)
4	$C_{14}H_{13}NO_2$ (227)	69	Yellow	94-95	74.08(73.99)	5.67(5.77)	6.13(6.16)
5	$C_{13}H_{10}NOCl$ (231)	63	Yellow	120-121	67.26(67.39)	4.30(4.35)	6.05 (6.05)
6	$C_{13}H_{10}N_2O_3$ (242)	77	Yellow	163-165	64.82(64.46)	4.07(4.16)	11.64(11.56)

The IR spectra of the Schiff bases (Table 2) reveal a band in the region 1625-1601 cm^{-1} attributed to the imine bond (Curran and Siggia, 1970). The presence of OMe and NO_2 groups increased IR stretching frequency for imine while Cl group decreased the IR stretching frequency. These may be attributed to electronic effects (resonance effects in OMe, NO_2 and inductive effects in Cl). This effect is more pronounced in the *para* substituted compounds.The ^1H NMR of all Schiff bases exhibit a singlet in the region 7.89-8.37 attributed to the imine proton. Schiff bases with electron donating groups appear up-field due to increase in the electron density in the vicinity of the proton which causes shielding from the magnetic field while Schiff bases with electron withdrawing groups appear at a lower field as a result of reduced electron density in the vicinity of the proton.

Table 2: Spectroscopic data of substituted 2-aminophenol Schiff bases

Compound	ν cm^{-1}				δ, ppm		
	O-H	C=N	C-O	C-Cl	HC=N	OH	OCH_3
1	3438	1625	1284	-	8.17	9.17	3.93
2	3414	1616	1270	349	8.26	9.21	-
3	3360	1626	1269	-	8.25	9.15	-
4	3333	1618	1245	-	7.89	8.63	3.91
5	3291	1601	1238	350	7.98	8.65	-
6	3304	1623	1299	-	8.37	8.77	-

Table 3: Electronic absorption data of substituted 2-aminophenol Schiff bases

Compound	$CHCl_3$(ν/ cm^{-1})(logε)	Assignment
1	277 (4.00)	π→π*
	358 (4.40)	n→π*
2	276 (4.78)	π→π*
	355 (4.75)	n→π*
3	271 (4.90)	π→π*
	369 (4.80)	n→π*
4	287 (4.17)	π→π*
	346 (4.20)	n→π*
5	273 (4.15)	π→π*
	350 (4.05)	n→π*
6	273 (4.16)	π→π*
	384 (4.18)	n→π*

The electronic absorption data of the compounds in chloroform at room temperatures summarized in Table 3 comprises of two absorption bands; a high-energy band attributed to π→π* transitions of the aromatic rings and a lower energy band due to n→π* transition of the non-bonding electrons present on the nitrogen of the azomethine group (Issa *et al*., 2005). The absorption spectra of Schiff bases (Figs. 1 and 2) feature an intense band system with maxima in the region 271-277 nm and 355-369 nm attributed to π→π* and n→π* transitions for *o*-substituted Schiff bases and 273-287 nm and 346-384 nm for the *p*-substituted compounds. The n→π* band underwent a blue shift with change in

position of the methoxy group and a red shift for the electron-withdrawing nitro group.

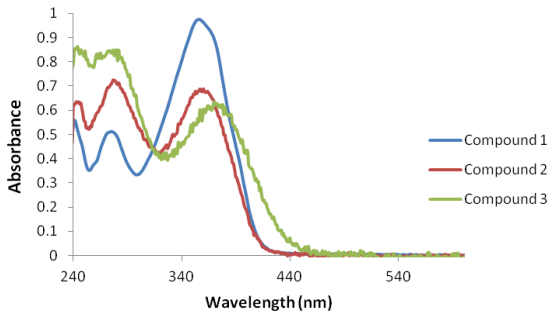


Fig. 1: Electronic absorption spectra of *o*-substituted Schiff bases 1-3

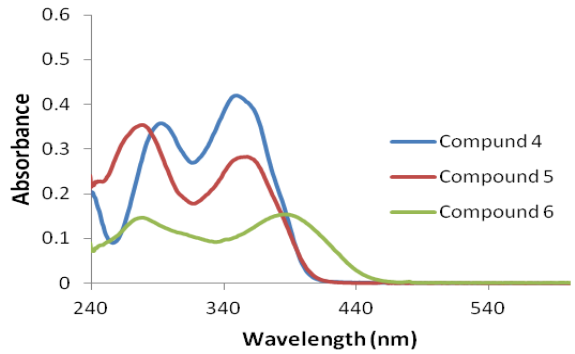


Fig. 2: Electronic absorption spectra of *p*-substituted Schiff bases 4-6

The in-vitro antibacterial activity of compounds **1-6** against six human pathogenic bacteria; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterococcus faecalis* and *Klebsiella pneumoniae* was carried out using the paper disc method with ampicillin as a reference compound. The minimum inhibitory concentration (MIC) values of the compounds against the bacterial strains are summarized Table 4.The results indicate the compounds exhibited strong to comparable activity when compared to ampicillin. A minimum inhibitory concentration value of 0.28-1.27 mg/ml has been attributed with extremely strong activity while MIC values of 1.81-8.85 mg/ml are attributed with weak activities (Aligiannis *et al*., 2001). The low MIC values of compounds **1** and **4** can be attributed to the presence of the methoxy group on the aldehyde ring. This is in line with previous observation by Xiao *et al*. (2007) that electron donating groups on amino acid Schiff bases result in high biological activity. The higher activity of compound 1 against all bacterial strains tested can be attributed to the better chelating properties of the *ortho*-substituted compound. The *para*-analogue, **4** however shows selectively higher activity against gram negative bacteria. In addition, compound **2**, shows comparable activity to ampicillin.

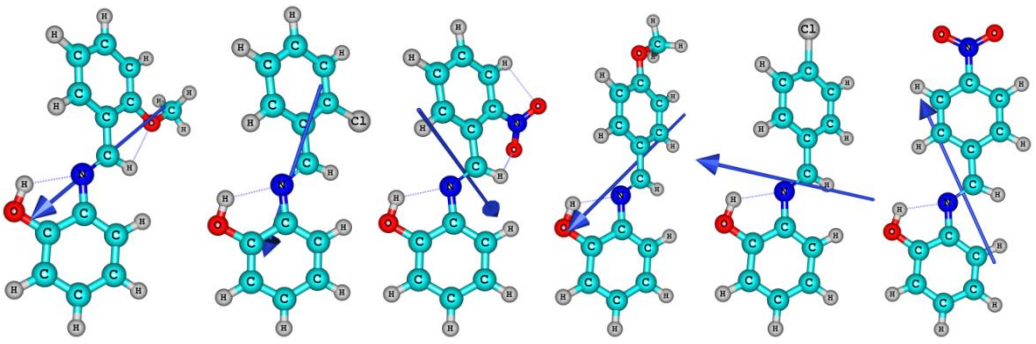
The results obtained validate the hypothesis that Schiff bases with methoxy and chloro groups at the phenyl ring are required for the antibacterial activity while nitro group at different positions in the aromatic ring may have varying antibacterial activity (Zhang *et al*., 2006).

Table 4: Minimum inhibitory concentration (MIC) mg/ml of substituted 2-aminophenol Schiff bases

Compound	*S.aureus* (ATCC 6538)	*E.feacalis* (ATCC 29212)	*B.cereus* (ATCC 10702)	*E.coli* (ATCC 8739)	*P.aeruginosa* (ATCC 19582)	*K.pneumonia* (ATCC 10031)
1	2.50	2.50	2.50	1.25	1.25	1.25
2	2.50	2.50	5.00	2.50	2.50	2.50
3	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
4	5.00	2.50	2.50	1.25	2.50	1.25
5	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
6	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
Ampicillin	2.50	5.00	5.00	1.25	5.00	2.50

Table 5: Minimum local ionization potential data of synthesized Schiff bases

Compound	*S.aureus (ATCC 6538)*	*E.feacalis (ATCC 29212)*	*B.cereus (ATCC 10702)*	*E.coli (ATCC 8739)*	*P.aeruginosa (ATCC 19582)*	*K.pneumonia (ATCC 10031)*	Min ElPot (kJ/mol)	Min LocIonPot (kJ/mol)	Max ElPot (kJ/mol)
1	*2.5*	*2.5*	*2.5*	*1.25*	*1.25*	*1.25*	-160	36.83	133.6
2	2.5	2.5	5	2.5	2.5	2.5	-143.3	37.76	159.9
4	*5*	*2.5*	*2.5*	*1.25*	*2.5*	*1.25*	-159.1	36.92	135.1



1 **2** **3** **4** **5** **6**

Fig. 3: The Optimized structures of 2-aminophenol Schiff bases

In order to further shed light on the SAR, quantum chemical studies were carried out on the Schiff base ligands. These calculations were performed using the Spartan 14 software at the B3LYP/6-31(g)d level of calculation (Hehre, 2003; Shao *et al.,* 2006). The B3LYP is a density functional model that contains the Becke 88 exchange functional and the correlation functional of Lee, Yang and Parr. A conformational search was performed on all the structures in order to identify the lowest energy structure. This lowest energy structure was further confirmed to be a minimum by frequency calculation at the same level of theory. A quantitative structure activity relationship (QSAR) calculation, as available within the Spartan software was then performed on this structure. The parameters that best describe the activities of this Schiff bases in relation to their MIC is the minimum local ionization potential and the maximum electrostatic potential. The maximum electrostatic potential is the most positive value of the electrostatic potential which is related to the hydrogen bond donating tendency of the molecule. The minimum local ionization potential value (Table 5) is related to the most reactive or least tightly bound electrons (Bulat *et al.,* 2010). These interactions are electrostatic in nature. Thus, the biological activity of these molecules is electrostatic in nature. We also observe that though the magnitude of the dipole moment does not correlate well to the structure activity, the net direction of the dipole moment correlates with the activity. The optimized structures showing the direction of the dipole moment of the six Schiff bases is displayed in Fig. 3.

The most active compounds are **1** and **4**, for these the direction of the dipole moment is parallel to the C=N bond. The more the direction of the dipole moment coincides with the C=N bond the more active the compound. **1** being the most active coincides with the C=N bond. We also observe that for **2** in which the dipole moment is slightly off the C=N bond is moderately active. Quantum chemical studies show that the properties of importance are the maximum electrostatic potential and minimum ionization potential

Conclusion

The structure-antibacterial activity of six substituted 2-aminophenol Schiff bases have been studied using six pathogenic bacterial strains. The compounds containing the methoxy groups **1 and 4** exhibited high activities with the *o*-substituted compound **1** being the most active against all bacterial strains tested. The results indicate that both the nature and position of the substituent affects the biological activity of the Schiff bases. Quantum chemical studies show that the most active compounds have the direction of dipole moment parallel to the imine bond.

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