International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 7 (2): 79-85, 2013
ISSN 1819-155X / DOI: 10.3923/ijbc.2013.79.85

Substituent Effect on the Antimicrobial Activity of Schiff Bases Derived from 2-aminophenol and 2-aminothiophenol

[1]T.M. Fasina, [1]F.N. Ejiah, [1]C.U. Dueke-Eze and [2]N. Idika
[1]Department of Chemistry, University of Lagos, Akoka, Nigeria
[2]Department of Microbiology, Nigeria Institute of Medical Research, Yaba, Nigeria

Corresponding Author: T.M. Fasina, Department of Chemistry, University of Lagos, Akoka, Nigeria

ABSTRACT

The biological activity of Schiff bases have been of interest in the design of new antimicrobial agents, however relationship between molecular structure and biological activity had been little studied. This study seeks to investigate the role of subtle electronic effect arising from substituent variation on the biological activity of salicyladimines. The effect of substituent variation on the antimicrobial activity of Schiff bases was therefore studied using aminophenol and aminothiophenol compounds. Four Schiff bases derived from condensation of 2-aminophenol or 2-aminothiophenol with 5-bromosalicylaldehyde and 5-nitrosalicyladehyde were synthesized and characterized by IR, NMR and elemental analysis. Electronic absorption spectra of the compounds were recorded in dioxane and methanol as solvents. The presence of absorption bands above 400 nm for the aminophenol compounds reveals the existence of keto-enol tautomerisim. *In vitro* antimicrobial screening against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Enterococcus feacalis* (ATCC 29212) in N,N'-dimethylformamide (DMF) and 1,4-dioxane as solvents show dependence of biological activity on the nature of substituent and solvent. The Schiff bases were active against gram positive bacteria and more potent in DMF with the bromine substituted imines exhibiting higher activity.

Key words: Aminophenol, aminothiophenol, Schiff base, electronic absorption spectra, biological activity

INTRODUCTION

Schiff bases are a class of important compounds owing to their wide range of properties and applications. These compounds have received much attention as a result of their use as models for biological systems (Hodnett and Dunn, 1970) and application as catalysts in various chemical and photochemical reactions (Gao and Zheng, 2002). Schiff bases are reported to show antibacterial (Da Silva *et al*., 2011; Shi *et al*., 2007), antifungal (Jarrahpour *et al*., 2007), anticancer (Tang *et al*., 1985) and herbicidal (Aggarwal *et al*., 2009; Samadhiya and Halve, 2001) activities.

Schiff bases derived from salicyaldehyde, salicylaldimines, in particular have been a subject of intense study probably as a result of the close proximity of the hydroxyl and imine groups. These compounds function as excellent chelating ligands with wide range of properties tunable by introduction of various substituents on either the carbonyl or amino phenyl rings. Furthermore, these compounds have been reported to exhibit keto-enol tautomerism on absorption of light. (Guha *et al*., 2000). It is known that the spectral behaviour is strongly related to the ground state and excited state structures of the compounds. Thus, spectra behaviour of Schiff bases has been investigated for use in structure elucidation (Schilf *et al*., 2002).

The effect of subtle electronic variations such as substituent effects on Chemistry and activity of Schiff bases and their metal complexes (Dueke-Eze *et al.*, 2011; Ejiah *et al.*, 2012) is of current interest. In this study, Schiff bases of 2-aminophenol and 2-aminothiophenol with 5-bromosalicyladehyde and 5-nitrosalicyladehyde have been synthesized and characterized to investigate the effect of substituent on the spectral behaviour and biological activity of the Schiff bases. It has been reported that the presence of sulphur atom in compounds improves biological activity possibly due to its specific interference with enzymes having sulfhydryl groups at their active sites (Ankri *et al.*, 1997). This and the lower electronegativity of sulphur compared to its lighter homologue, oxygen prompted the use of the amines in this study.

MATERIALS AND METHODS

All chemicals and solvents used were obtained from commercially from Sigma-Aldrich and used without further purification.

Infra-red spectra were recorded as nujol mulls on a Shimadzu FT-IR 157 Spectrophotometer. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded using dimethysulphoxide (DMSO-d_6) as solvent with TMS as internal standard on a Bruker 400 MHZ spectrometer. Elemental analyses were determined at the Durham University elemental analysis service. Melting points were determined with Gallenkamp melting point apparatus. The electronic absorption spectra of all the complexes were recorded in 1,4-dioxane and methanol on a PGT80/T80^{+} UV-VIS spectrophotometer in 1 cm quartz cell at room temperature immediately after preparing the solution.

TYPICAL SYNTHESIS OF SCHIFF BASE

Equimolar quantities (0.04 mol) of aldehyde and primary amine were dissolved in ethanol (45 mL) with the addition of 3 drops of formic acid. The mixture was heated under reflux at 70°C for 4 h. The precipitate formed was separated by filtration, re-crystallized from ethanol and dried in a desiccator.

Schiff base 1: (4-bromo-2-[(2-hydroxylphenyl)imino]methylphenol): Yield 69%; I. R. (cm^{-1}) 3280, 2363.2, 2158, 2042,1984,1807, 1695, 1622, 1589, 1501, 1454, 1401, 1316, 1267, 1189, 1127, 1023, 920, 819, 747. 688. ^{1}H NMR (DMSO): 8.97 (sEC), 6.91-6.98 (m) 7.49-7.86 (m). ^{13}C NMR: 160, 159.9, 151.3, 134, 134.4, 135.8, 126.5, 121.3, 119.6, 119.4, 119.1, 116.6, 109.9.

Anal calcd for $C_{13}H_{10}NBrO$: C 53.46; H 3.44; N4.43; found C 53.41; H 3.42; N 4.79.

Schiff base 2: (4-bromo-2-[(2-suphanylphenyl)imino]methylphenol): Yield 30%; I. R. (cm^{-1}) 2788, 2280, 1613, 1569, 1472, 1431, 1372, 1308, 1264, 1204, 1131, 1083, 1014, 976, 869, 815, 722. 622.. ^{1}H NMR(DMSO) 11.7 (s), 8.90 (s), 8.38 (s), 8.15(d), 8.08 (d), 7.56-7.45 (m), 7.07 (s). ^{13}C NMR 162, 155.2, 151.3, 135.4, 134.4, 130.1, 126.4, 125.1, 122.4, 121.9, 120.9, 119.1, 110.8,

Anal calcd for $C_{13}H_{10}NBrS$: C 50.65; H 3.25; N4.55; found C 50.66; H 3.27; N 4.54.

Schiff base 3: (4-nitro-2-[(2-hydroxylphenyl)imino]methylphenol): Yield 66%; IR (cm^{-1}) 3071; 2165, 1617,1577,1520,1483, 1329, 1278, 1210, 1101, 941, 815. ^{1}H NMR (DMSO): 10.5 (s) , 9.4 (s), 8.68, 8.55-8.22, 7.66 (d), 7.29-7.03, 6.98-6.95. ^{13}C NMR: 172.4, 159.2, 150.4, 136.8, 130.0, 129.8, 129.3, 128.7, 120.4, 119.8, 118.9, 116.6, 116.5.

Anal calcd for $C_{13}H_{10}N_2O$: C 60.47, H 3.88, N 10.85; found C 60.83, H 3.90, N 10.83.

Schiff base 4: (4-nitro-2-[(2-suphanylphenyl)imino]methylphenol): Yield 85%; IR (cm^{-1}); 3214.5, 2571.4, 1615, 1520. ^{1}H NMR (DMSO): 9.18 (s), 8.29-8.14(m), 7.58 -7.48 (m), 7.26 (d). ^{13}C NMR: 161.4, 161.2, 151.2, 139.9, 127.1, 126.5, 125.3, 124.3, 122.6, 122.0, 119.6, 117.6.

Anal calcd for $C_{13}H_{10}N_2OS$: C 56.93, H 3.65, N 10.21; found C 57.47, H 3.22, O 10.20.

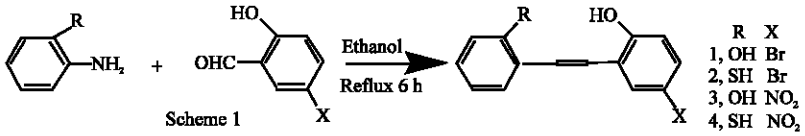
Biological activity: The *in-vitro* antimicrobial activity of compounds 1-4 against *Staphylococcus aureus*, *Entercoccus feacalis*, *Pseudomonas aeruginosa* and *Escherichia coli* were studied using the agar ditch method (Jigna *et al.*, 2005). The stock solution from which two-fold serial dilutions obtained was prepared by dissolving 40 mg of each compound in 1 mL of N,N-dimethylformamide (DMF) and 1,4-dioxane, respectively.

An aseptically prepared double layered Muller Hinton agar plate was flooded with standardized (0.5 McFarland) test microorganism and left to stand for two minutes. A sterilized cork borer was used to make five wells radially. The wells were filled with the test compounds using a micropipette and incubated at 37°C for 24 h. During this period, the test compounds diffused and the growth of the inoculated microorganism was affected. The diameter of the zone of inhibition surrounding each well was measured and recorded. In order to clarify any participating role of the solvent in the biological screening, control test was included using the solvent alone to fill the control well.

RESULTS AND DISCUSSION

The Schiff bases 1-4 were obtained in good yields from the reaction of 2-aminophenol and 2-aminothiophenol with 5-bromosalicylaldehyde and 5-nitrosalicyladehyde (Scheme 1) and purified by recrystallization from ethanol. The compounds were characterized by NMR, infrared spectroscopy and elemental analysis. Analytical and spectroscopic data for the synthesized compounds 1-4 are summarized in Table 1.

The IR spectra of all the compounds formation contains a band in the region 1622-1613 cm^{-1} attributed to the azomethine (HC = N) bond of the Schiff base which confirms the formation of the Schiff base. This formation was further reaffirmed by the singlet at 8.90-9.38 ppm in the ^{1}H NMR spectra. The elemental analysis data are in good agreement with the proposed formula of the compounds.



Scheme 1

Table 1: Analytical and spectroscopic data of schiff bases 1-4

		Microanalysis (calcd.)			IR($V\ cm^{-1}$)				^{1}H NMR
Compound	Empirical formula	C	H	N	OH	(C = N)	(C-Br)	(CO)	δ (HC = N)
1	$C_{13}H_{10}NBrO$	53.41	3.42	4.79	3280	1622	-	1316	8.97
		(53.46)	(3.44)	(4.43)					
2	$C_{13}H_{10}NBrS$	50.66	3.27	4.54	-	1613	2728	-	8.90
		(50.65)	(3.25)	(4.55)					
3	$C_{13}H_{10}N_2O$	60.83	3.90	10.83	3037	1617	-	1307	9.40
		(60.47)	(3.88)	(10.85)					
4	$C_{13}H_{10}N_2OS$	57.47	3.22	10.20	3214	1615	2571	-	9.18
		(56.93)	(3.65)	(10.21)					

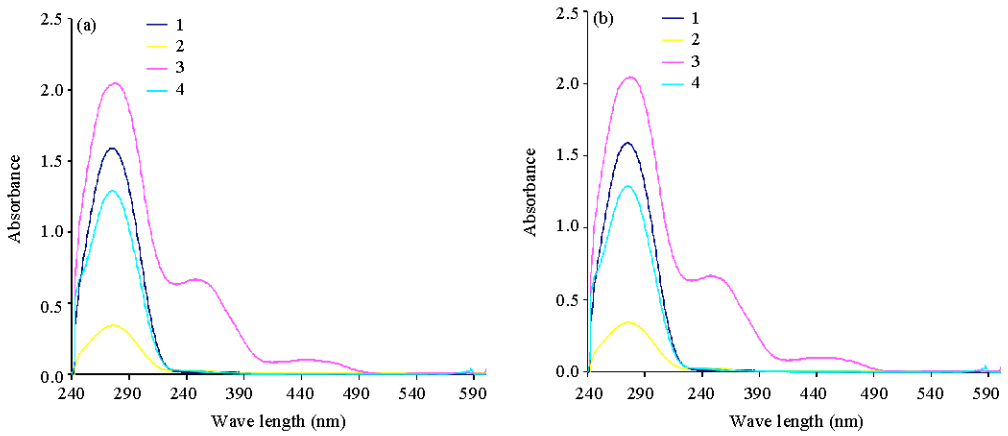


Fig. 1(a-b): Electronic absorption spectra for 1-4 from 200-600 nm in (a) Methanol (b) 1,4-dioxane

Table 2: Electronic absorption data of 1-4

	λ max (nm)			
Solvent	1	2	3	4
Methanol	294	290	268	287
	346	342	360	360
	430		446	396
1,4-dioxane	275	275	277	275
		342		
		443		

Electronic spectra: The electronic absorption spectra of the Schiff bases recorded in DMF and 1,4-dioxane (Fig. 1, Table 2) is composed of three bands in the 200 -500 nm region. The first band (Band A) appearing within the 250 -290 nm region can be assigned to the π-π* transition of the aromatic rings. The second band (Band B) observed within the wavelength range 340-360 nm is due to transition between the π-orbital localized on the central azomethine (-CH = N-) bond (Soliman, 1997) while the third band (Band C) located within the 390-450 nm region can be ascribed to charge transfer within the entire Schiff base molecule. This band is commonly observed in o-hydroxyl Schiff bases and is based on strong intramolecular hydrogen bonding between the hydroxyl group of the salicylidene and the azomethine nitrogen (Sovilja *et al.*, 1998). The charge transfer bands being more sensitive to solvent changes than bands resulting from local transitions.

In the studied compounds, it is observed that band A shifts to longer wavelengths (Δλ~12-20 nm) in the more polar solvent methanol, thereby confirming the π-π* nature of the electronic transition. This shift is attributed to better stabilization of the excited π* state in the polar solvent (Ahmed and Kassem, 2010). Expectedly, the charge transfer band, band C is much pronounced in methanol for the aminophenols 1 and 3. It also occurs in the thiophenol 4 containing the strongly electron withdrawing nitro (NO_2) substituent which also shows charge transfer in the nonpolar solvent dioxane. The high electron withdrawing power of the nitro group makes it behave as a good CT acceptor centre with the nonbonding orbital of the azomethine group as the main

Table 3: Antibacterial screening data of schiff bases 1-4 at concentration of 2.5 mg mL^{-1}

Compound	Solvent	*S. aureus*	*P. aueroginosa*	*E. feacalis*	*E. coli*
1	DMF	3+	-	3+	-
	Dioxane	2+	-	-	-
2	DMF	+	-	2+	-
	Dioxane	3+	-	-	-
3	DMF	3+	-	-	-
	Dioxane	-	-	-	-
4	DMF	+	-	-	-
	Dioxane	-	-	-	-

-: No activity, +: 0.1-0.5 cm beyond control (less active), 2+: 0.6 -1, 1 cm beyond control (moderately active), 3+: ≥1.2 cm beyond control (highly active)

participant of the electron donor group. The results show that although all compounds are salicylaldimines, the nature of substituent on the salicylaldehyde ring is important for charge transfer transitions.

Antimicrobial activity: The *in vitro* antimicrobial activity of the compounds against some clinically important gram positive and gram negative bacteria namely *S.aureus, P. aueroginosa, E. Feacalis* and *E. coli* was studied in DMF and dioxane. The compounds were tested at a concentration of 2.5 mg mL^{-1} using the agar ditch method (Jigna *et al.*, 2005). The diameter of growth inhibition zones were measured in mm and the results are summarized in Table 3. The results indicate that none of the studied compounds was active against the gram negative bacteria *P. aueroginosa* and *E. coli* but showed activity to different extents on the gram positive bacteria *S.aureus* and *E. feacalis*.

The activity of antibacterial agents is influenced largely by the morphology of the bacteria cell wall as diffusion of the antibacterial agent into the enzyme through the cell wall is a key step to facilitate interaction. The bacteria cell wall is composed of peptidoglycan which is thicker in the gram positive bacteria and this usually poses a barrier to the degree of diffusion of antibacterial agents into the enzyme (Mims *et al.*, 2004). According to the overtone concept of cell permeability, the lipid membrane surrounding the cell favours the passage of only lipid-soluble materials, which means that liposolubility is an important factor controlling antimicrobial activity (Dharamraj *et al.*, 2001). Hence, the higher the lipophilicity the greater the ease of diffusion in to the bacteria and this is expected to result in an increased biological activity of the compound.

Compounds 1 and 2 containing the bromine substituent showed high to moderate activity against *S. aureus* in both DMF and dioxane, while the nitro substituted compounds 3 and 4 were active only in DMF. Thus, suggesting that the activity against this bacterial strain is dependent more on the nature of substituent on the salicyaldehyde ring. However, even though the bromine substituted compounds were active in both solvents, 2 were more active in the non-polar solvent dioxane. This is probably due to the less polar nature of the thiophenol substituent which favours better interaction in the nonpolar solvent. The bromine substituted imines 1 and 2 also showed considerable activity against *E. feacalis* in DMF but no activity in dioxane. The decreased activity of the nitro substituted compounds is in line with an earlier observation that *p*-nitro substitution decreases antibacterial activity (Halve *et al.*, 2006).

CONCLUSION

Antibacterial activity of the synthesized compounds 1-4 studied in DMF and 1,4-dioxane was found to be dependent on both the substituent on the Schiff base and nature of solvent medium. Compounds 1 and 2 specialized in inhibiting Gram-positive bacteria; hence, these compounds can be used in formulation of narrow spectrum antibiotics for treatment of infections caused by *S. aureus*.

REFERENCES

Aggarwal, N., R. Kumar, P. Dureja and D.S. Rawat, 2009. Schiff base as potential fungicides and nitrification inhibitors. J. Agric. Food Chem., 57: 8520-8525.

Ahmed, I.S. and M.A. Kassem, 2010. Synthesis, solvatochromaticity and bioactivities of some transition metal complexes with 2-(R-benzylideneamino)-pyridin-3-ol Schiff base derivatives. Spectrochim. Acta A. Mol. Biomol. Spectros., 77: 359-366.

Ankri, S., T. Miron, A. Rabinkov, M. Wilckek and D. Mirelman, 1997. Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of *Entamoeba histolytica*. Antimicrob. Agents Chemother., 10: 2286-2288.

Da Silva, C.M., D.L. da Silva, L.V. Modolo, R.B. Alves, M.A. de Resende, C.V.B. Martins and A. de Fatima, 2011. Schiff bases: A short review of their antimicrobial activities. J. Adv. Res., 2: 1-8.

Dharamraj, N., P. Viswanathanmurthi and K. Natarajan, 2001. Ruthenium(II) complexes containig bidentate Schiff bases and their antifungal activity. Trans. Met. Chem., 26: 105-109.

Dueke-Eze, C.U., T.M. Fasina and N. Idika, 2011. Synthesis, electronic spectra and inhibitory study of some salicylaldehyde schiff bases of 2-aminopyridine. Afr. J. Pure Applied Chem., 5: 13-18.

Ejiah, F.N., T.M. Fasina, A.A. Nejo, N. Revaprasadu, A.R. Opoku and O.B. Familoni, 2012. Substituent effect on antibacterial activity of Schiff bases derived from aminophenol derivatives. Int. J. Chem. Sci., (In Press).

Gao, W.T. and Z. Zheng, 2002. Synthetic studies on optically active Schiff-base ligands derived from condensation of 2-hydroxyacetonephenone and chiral diamines. Molecules, 7: 511-516.

Guha, D., A. Mandal, A. Koll, A. Filarowski and S. Mukherjee, 2000. Proton transfer reaction of a new orthohydroxy Schiff base in protic solvents at room temperature. Spectrochimica Acta A Mol. Biomol. Spectroscopy, 56: 2669-2677.

Halve, A.K., R. Dubey, D. Bhadauria, B. Bhaskar and R. Bhadauria, 2006. Synthesis, ntimicrobial screening and structure-activity relationship of some novel 2-hydroxy-5-(nitro-substituted phenylazo) benzylidine anilines. Indian J. Pharm. Sci., 68: 510-514.

Hodnett, E.M. and W.J. Dunn, 1970. Structure-antitumor activity correlation of some Schiff bases. J. Med. Chem., 13: 768-770.

Jarrahpour, A., D. Khalili, E. De Clercq, C. Salmi and J.M. Brunel, 2007. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives. Molecules, 12: 1720-1730.

Jigna, P., R. Nairi, S. Baluja and S. Chandai, 2005. Synthesis and antibacterial activity of some Schiff bases derived from 4-amino benzoic acid. J. Serb. Chem. Soc., 70: 1115-1161.

Mims, C., H.M. Dockrell, R.V. Goering, I. Roitt, D. Wakelin and M. Zuckerman, 2004. Medical Microbiology. 3rd Edn., Elsevier Mosby, St. Louis.

Samadhiya, S. and A. Halve, 2001. Synthetic utility of Schiff bases as potential herbicidal agents. Orient. J. Chem., 17: 119-122.

Schilf, W., A. Szady-Chelmieniecka, E. Grech, P. Przybylski and B. Brzezinski, 2002. Spectroscopic studies of new schiff and Schiff-mannich bases of ortho-derivatives of 4-bromophenol. J. Mol. Struct., 643: 115-121.

Shi, L., H.M. Ge, S.H. Tan, H.Q. Li, Y.C. Song, H.L. Zhu and R.X. Tan, 2007. Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. Eur. J. Med. Chem., 42: 558-564.

Soliman, A.A., 1997. Effect of solvents on the electronic absorption spectra of some salicylidene thioschiff bases. Spect. Chimica Acta A, 53: 509-515.

Sovilja, S.P., V.M. Vasicb, D.L. Stojic and B. Stojceva-Radovanovic, 1998. Spectrophotometric studies of the influence of organic solvents and substituents on some schiff bases. Spect. Lett., 31: 1107-1122.

Tang, A., E.J. Lien and M.M.C. Lai, 1985. Optimization of the schiff bases of N-hydroxy-N-aminoguanidine as anticancer and antiviral agents. J. Med. Chem., 28: 1103-1106.