Journal of Saudi Chemical Society (2016) **20**, 45–48

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Biological evaluation of potent antioxidant, lipoxygenase inhibitor and antibacterial: A comparative study

Muhammad Aslam [a,b,*], Itrat Anis [b], Nighat Afza [a], Lubna Iqbal [a], Samina Iqbal [a], Ajaz Hussain [c], Rashad Mehmood [d], Muhammad Tahir Hussain [e], Muhammad Khalid [f], Haq Nawaz [f]

[a] *Pakistan Council of Scientific and Industrial Research, Karachi 75280, Pakistan*
[b] *Department of Chemistry, University of Karachi, Karachi 75270, Pakistan*
[c] *Department of Chemistry, Government College University, Faisalabad 38040, Pakistan*
[d] *Department of Conservation Studies, Hazara University, Mansehra 21120, Pakistan*
[e] *Department of Applied Sciences, National Textile University, Faisalabad 37610, Pakistan*
[f] *Institute of Chemistry, University of São Paulo, São Paulo 05513-970, Brazil*

Received 5 June 2012; accepted 8 September 2012
Available online 13 October 2012

KEYWORDS

Schiff bases;
Synthesis;
Antioxidant activity;
Lipoxygenase inhibition activity;
Antibacterial activity;
Urease activity

Abstract Three biologically active new Schiff bases, 2-[(3-hydroxybenzylidene)amino]phenol **5**, 2-[(4-hydroxybenzylidene)amino]phenol **6** and 4-[(2-hydroxyphenylimino)methyl]benzene-1,3-diol **7**, were synthesized by the reaction of 2-aminophenol **1** with three different hydroxyl-benzaldehydes **2**–**4**. They were characterized by spectroscopic analysis (IR, ^{1}H NMR, EI-MS) along with elemental analyses. The products were biological screened out for antioxidant, lipoxygenase inhibition, antibacterial and urease inhibition activities. The compounds **5** and **6** showed potent while **7** showed moderate antioxidant activity. Compound **6** showed potent whereas **5** and **7** showed significant lipoxygenase inhibition activity. All the target compounds showed excellent activities against *Staphylococcus intermedius*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* bacteria. All the compounds showed non-significant activity against urease enzyme.

1. Introduction

Schiff bases are azomethine compounds, after the name of Hugo Schiff (Da Silva et al., 2011), and are prepared by the condensation of primary amines with aldehydes or ketones in acid or base catalyst (Dueke-Eze et al., 2011). In organic, Schiff bases are important intermediates in the synthesis (Rana et al., 2012). Due to their azomethine moiety, they possess a remarkable anticancer (Shkawat et al., 1973), antibacterial

* Corresponding author. Tel.: +92 333 9955488.
E-mail address: maslamchemist@hotmail.com (M. Aslam).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1319-6103
http://dx.doi.org/10.1016/j.jscs.2012.09.009

(More et al., 2001), antifungal (Chaitanya et al., 2010), anti-tumor (Hodnett and Dunn, 1970), herbicides (Samadhiya and Halve, 2001), anti-inflammatory (Sathe et al., 2011) and analgesic (Chinnasamy et al., 2010) activities. They are also used as pigments, dyes, catalysts, and as polymer stabilizers (Taggi et al., 2002). Due to the attractive biological activities of Schiff bases, we have reported the synthesis and biological activities of various Schiff bases (Aslam et al., 2012).

We herein report the syntheses and characterizations of three Schiff bases, named 2-[(3-hydroxybenzylidene)amino]-phenol **5**, 2-[(4-hydroxybenzylidene)amino]phenol **6** and 4-[(2-hydroxyphenylimino)methyl]benzene-1,3-diol **7**, derived from 2-aminophenol **1** and hydroxyl-benzaldehydes **2**–**4**, along with their antioxidant, lipoxygenase inhibition, antibacterial and urease inhibition activities.

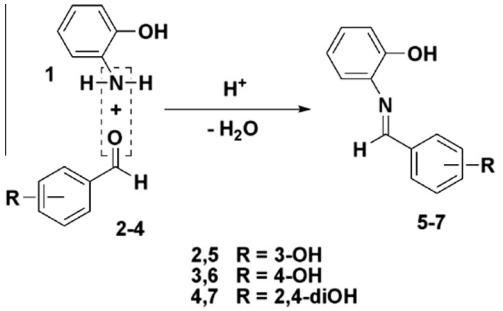
2. Experimental

2.1. Material and methods

All the solvents and chemicals were purchased from Merck. The melting points were determined by Gallenkamp apparatus and are uncorrected. Elemental analyses were carried out by Perkin–Elmer 2400 Series II elemental analyzer. IR spectra were measured on Thermo Nicolet Avatar 320 FT-IR spectrometer by using KBr pellets. Mass spectra on electron impact mode were measured on Finnigan MAT-112 spectrometer and ions are given in m/z. TLC was performed on pre-coated silica gel G-25-UV_{254} plates to check the purity. The ^{1}H NMR spectra were performed on Bruker AMX-400 spectrometer in DMSO-d_6 solvent and TMS used as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) are reported in Hertz.

2.2. General procedure for the synthesis of Schiff bases 5–7

The reaction mixture of 2-aminophenol **1** (0.01 mol in 50 mL EtOH) and hydroxybenzaldehydes **2**–**4** (0.01 mol in 50 mL EtOH) followed by 3–4 drops of conc. H_2SO_4, was refluxed for 3 h at 70 °C with constant stirring. The mixture was concentrated to one-third of its volume by using rotary evaporator. The conc. mixture was placed at ambient temperature to obtain the solid products. The products were filtered, washed with cold methanol and recrystallized with absolute methanol. The final products were dried on anhydrous calcium hydroxide at reduced pressure. The completion of reaction was monitored by TLC from time to time (Scheme 1).



Scheme 1 Synthetic scheme of the Schiff bases **5**–**7**.

2.3. 2-[(3-Hydroxybenzylidene)amino]phenol 5

Chocolate solid; yield 75.02%; m.p. 119 °C; Anal. Calcd. for $C_{13}H_{11}NO_2$: C, 73.23, H, 5.20, N, 6.57; found C, 73.41, H, 5.31, N, 6.51; IR (KBr) ν_{max} cm^{-1}: 1680 (C═N); ^{1}H NMR (DMSO-$_6d$, 400 MHz, ppm) δ: 8.56 (1H, s, ─N═CH, azomethine); EI-MS: m/z [M]$^+$ 213.3.

2.4. 2-[(4-Hydroxybenzylidene)amino]phenol 6

Fire brick solid; yield 81.07%; m.p. 123 °C; Anal. Calcd. for $C_{13}H_{11}NO_2$: C, 73.23, H, 5.20, N, 6.57; found C, 73.33, H, 5.39, N, 6.63; IR (KBr) ν_{max} cm^{-1}: 1671 (C═N); ^{1}H NMR (DMSO-$_6d$, 400 MHz, ppm) δ: 8.53 (1H, s, ─N═CH, azomethine); EI-MS: m/z [M]$^+$ 213.7.

2.5. 4-[(2-Hydroxyphenylimino)methyl]benzene-1,3-diol 7

Chocolate solid; yield 73.21%; m.p. 132 °C; Anal. Calcd. for $C_{13}H_{11}NO_3$: C, 68.11, H, 4.84, N, 6.11; found C, 68.29, H, 4.97, N, 6.26; IR (KBr) ν_{max} cm^{-1}: 1625 (C═N); ^{1}H NMR (DMSO-$_6d$, 400 MHz, ppm) δ: 8.76 (1H, s, ─N═CH, azomethine); EI-MS: m/z [M]$^+$ 229.1.

2.6. Biological assays

2.6.1. Antioxidant: DPPH radical scavenging assay

The free radical scavenging activity was carried out by 1,1-diphenyl-2-picryl-hydrazil (DPPH) (Ferheen et al., 2009). The solution of DPPH (0.3 μM) was prepared in ethanol. The solution of each sample was prepared in methanol. Five microliters of solution of each sample (with concentration range 5–500 μg) was added to 95 μL of DPPH solution, the mixture was then dispersed in 96 well plates and placed for 30 min into the incubator at 37 °C. Then absorbance was recorded at 515 nm by elisa plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was assessed in contrast to methanol treated control (DMSO). BHA (butylated hydroxylanisole) used as standard.

$$\text{DPPH scavenging effect } (\%) = \text{Ac} - \text{As}/\text{Ac} \times 100$$

where Ac, absorbance of control (DMSO treated); As, absorbance of sample.

IC_{50} values were checked by observing the effect of different concentrations (1–1000 μM) and were calculated using EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, USA).

2.6.2. Urease inhibition assay

The urease enzyme solution was prepared by taking 0.125 units in each well in phosphate buffer ($K_2HPO_4{\cdot}3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Each well was filled with 80 μL of 0.05 M potassium phosphate buffer (pH 8.2), 10 μL of the sample (concentration range 5–500 μM), contents were mixed and incubated for 15 min at 30 °C. Forty microliters of substrate solution (urea, 50 mM) was poured in each well for initiating reaction. Then, 70 μL alkaline reagent (0.5% NaOH and 0.1% active NaOCl) and 40 μL of phenol reagent (1% phenol and 0.005% w/v sodium nitroprusside) were introduced to each well. The well plate, containing reaction mixture, was

Table 1 IC_{50} values of antioxidant, lipoxygenase inhibition and urease inhibition activities of the compounds **5**–**7**.

Compound	DPPH scavenging activity IC_{50} (μM)	Lipoxygenase inhibition activity IC_{50} (μM)	Urease inhibition activity IC_{50} (μM)
5	15.3	51.6	+
6	34.0	17.1	+
7	118	67.4	+
BHA	44.2	–	–
Baicalein	–	22.6	–
Thiourea	–	–	21.6

+, Non-significant.

incubated for 50 min and absorbance was recorded at 630 nm. IC_{50} values were determined by monitoring the effect of increasing concentrations of Schiff bases **5**–**7** on extent of inhibition (Ferheen et al., 2009).

2.6.3. Lipoxygenase inhibition assay

Hundred and sixty microliters of 100 mM sodium phosphate buffer (pH 8.0) and 10 μL of sample in methanol (concentration's range 5–500 μM) was added to each labeled well. Twenty microliters of lipoxygenase (LOX) solution (enzyme 130 units per well) was added, mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 μL substrate solution (linoleic acid, 0.5 mM, 0.12% w/v tween 20 in the ratio of 1:2) to each well and the absorbance was measured after 15 min at 234 nm (Ali et al., 2009). Baicalein was used as standard

2.6.4. Antibacterial assay

Antibacterial activities of **5**–**7** were carried out against Gram-positive: *Staphylococcus intermedius*, *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative: *Escherichia coli* and *Salmonella typhi* bacteria by agar well diffusion method using Mueller Hinton agar medium. Test sample (200 mg) was dissolved in 10 ml DMSO (99.9%) and final concentration was made up to 20 mg/mL. The microorganism was grownup overnight individually in tryptic Soya broth and finally it mixed with physiological saline until colony formation was achieved in accordance with turbidity standard. The agar medium used for individual organisms was Molton Mueller Hinton agar medium, and used with 10 mL of prepared inoculum (inoculum size was 10^8 cells/ml as per McFarland standard). It was transferred to 20×100 mm petri dishes after proper homogenization. The required numbers of wells were adjusted in the seeded plates with the help of a sterile crock-borer (8.0 mm) after solidification. Hundred microliters of test sample was poured into respective wells. All the plates were incubated at 37 °C for 24 h after making positive (gentamicin 0.3%) and negative control (DMSO) plates ready. The diameter of the zone of inhibition and percentage of growth inhibition was calculated to determine the antibacterial activity (Bibi et al., 2011).

3. Results and discussion

3.1. Chemistry and characterization

The Schiff bases, 2-[(3-hydroxybenzylidene)amino]phenol **5**, 2-[(4-hydroxybenzylidene)-amino]phenol **6** and 4-[(2-hydroxyphenylimino)methyl]benzene-1,3-diol **7**, were synthesized by the condensation of 2-aminophenol **1** with 3-hydroxybenzaldehyde **2**, 4-hydroxybenzaldehyde **3** and 2,4-dihydroxybenzaldehyde **4**, respectively, in ethanol followed by 3–4 drops of conc. sulfuric acid as catalyst (Scheme 1). The purity of the products was checked by TLC and was very pure. Products have sharp melting points and are stable in air. In the IR spectra of products **5**–**7**, the presence of characteristic bands of azomethine moiety at 1680, 1671 and 1625 cm^{-1}, respectively and the absence of bands of the carbonyl and amine moieties, reveal the formation Schiff bases. In the ^1H NMR spectra of the compounds **5**–**7**, the singlet of the protons at δ 8.56, 8.53 and 8.76, respectively, evident the formation of azomethine linkage (Bhattacharjee et al., 2012). The EI mass spectra of the products **5**–**7**, showed that the molecular ion peaks at m/z 213.3, 213.7 and 229.1, respectively, were in complete agreement with the expected structure. Elemental analyses further supported the masses and the structures of the products (Scheme 1).

3.2. Biological evaluation

3.2.1. Antioxidant: DPPH radical scavenging study

The antioxidant activity of compounds **5**–**7** was carried out with DPPH by a well diffusion method in comparison with the BHA. The results show that compounds **5** and **6** are potent antioxidant active while **7** is moderate antioxidant active (Table 1). Generally, it is well known that compounds having functional groups such as –OH, –SH, –COOH, –N, –S–, and

Table 2 Results of antibacterial activities of the compounds **5**–**7**.

Bacteria	Gentamicin (0.3%)	**5**		**6**		**7**	
	Zone inhibition (mm)	Zone inhibition (mm)	Inhibition (%)	Zone inhibition (mm)	Inhibition (%)	Zone inhibition (mm)	Inhibition (%)
S. intermedius	30	19	63.3	26	86.6	23	76.6
B. subtilis	27	21	77.7	23	85.1	18	66.6
S. aureus	30	20	66.6	24	85.7	22	73.3
E. coli	30	25	83.3	23	76.6	26	86.6
S. typhi	24	16	66.6	19	79.1	14	58.3

–O– can show antioxidant activity. The present compounds having OH group in their structure, might be for this reason, the compounds that are antioxidant active.

3.2.2. Urease inhibition study

Compounds **5**–**7** under investigation were evaluated against urease enzyme and they showed non-significant activity (Table 1).

3.2.3. Lipoxygenase (LOX) inhibition study

All synthesized compounds **5**–**7** were screened out for lipoxygenase inhibition activity in comparison with baicalein. The results show that compound **6** is potent active while compounds **5** and **7** are significant active against it (Table 1). May be the reason is that, *para*-hydroxyl product is more compatible with LOX than the *ortho*- and *meta*-hydroxyl positions.

3.2.4. Antibacterial study

Antibacterial activities of compounds **5**–**7** under investigation were carried out against Gram-positive and Gram-negative: *S. intermedius*, *B. subtilis, S. aureus*, *E. coli* and *S. typhi* bacteria by the agar well diffusion method in comparison with gentamicin. All Schiff bases are excellently active against all bacteria (Table 2). It is reported that Schiff bases having hydroxyl moiety in their structures have a greater activity (Rehman et al., 2005). Compounds under investigation contain one and two hydroxyl groups, and due to this reason, these compounds have excellent antibacterial activities.

4. Conclusion

Schiff bases **5**–**7**, were synthesized by the reaction of 2-aminophenol **1** and hydroxylbenzaldehydes **2**–**4** in ethanol. The Schiff bases **5** and **6** are potent antioxidant agents and 6 is also a potent lipoxygenase inhibition active. All target compounds **5**–**7** have excellent antibacterial activities but not urease inhibition activity at all.

Acknowledgments

Dr. Nighat Afza and Muhammad Aslam express their compliments to Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi for providing financial support. Dr. Itrat Anis expresses her thanks to the Department of Chemistry, University of Karachi for providing research facilities.

References

Ali, S., Iqbal, L., Lateef, M., Riaz, N., Afza, N., Malik, A., 2009. Lipoxygenase inhibitory tetraketones: potential remedial source for inflammation and asthma. West Indian Med. J. 58, 92–98.

Aslam, M., Anis, I., Afza, N., Hussain, M.T., Yousuf, S., 2012. 2-[(2-Meth-oxy-benzyl-idene)amino]-phenol. Acta Crystallogr. Sect. E, Struct. Rep. Online 68, o1447.

Bhattacharjee, S., Saravanan, J., Mohan, S., Arrora, M., 2012. Synthesis, characterization and CNS depressant activity of some Schiff bases of 2-amino-N-(o-fluorophenylcarboxamido)-4-(p-methoxyphenyl) thiophenes. Int. J. Pharm. Pharm. Sci. 4, 528–532.

Bibi, Y., Nisa, S., Chaudhary, F.M., Zia, M., 2011. Antibacterial activity of some selected medicinal plants of Pakistan. BMC Complement. Altern. Med. 11, 52.

Chaitanya, M.S., Nagendrappa, G., Vaidya, V.P., 2010. Synthesis, biological and pharmacological activities of 2-methyl-4*H*pyrimido[2,1-b][1,3]benzothiazoles. J. Chem. Pharm. Res. 2 (3), 206–213.

Chinnasamy, R.P., Sundararajan, R., Govindraj, S., 2010. Synthesis, characterization and analgesic activity of novel Schiff base of isatin derivatives. J. Adv. Pharm. Technol. Res. 3, 342–347.

Da Silva, C.M., Da Silva, D.L., Modolo, L.V., 2011. Schiff bases: a short review of their antimicrobial activities. J. Adv. Res. 2, 1–8.

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

Ferheen, S., Rehamn, A., Afza, N., Malik, A., Iqbal, L., Rasool, M.A., Ali, M.I., Tareen, R.B., 2009. Galinsosides A and B, bioactive flavanone glucosides from *Galinsoga parviflora*. J. Enzyme Inhib. Med. Chem. 24 (5), 1128–1132.

Hodnett, E.M., Dunn, W.J., 1970. Structure–antitumour activity correlation of some Schiff bases. J. Med. Chem. 13, 768–770.

More, P.G., Bhalvankar, R.B., Pattar, S.C., 2001. Synthesis and biological activities of Schiff bases of aminothiazoles. J. Indian Chem. Soc. 78, 474–475.

Rana, K., Pandurangan, A., Singh, N., Tiwari, A.K., 2012. A systemic review of Schiff bases as an analgesic, anti-inflammatory. Int. J. Curr. Pharm. Res. 4, 5–11.

Rehman, W., Baloch, M.K., Badshah, A., 2005. Comparative study of structure–activity relationship of Di and Triorganotin (IV) complexes of monomethyl glutarate. J. Braz. Chem. Soc. 16, 827–834.

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

Sathe, B.S., Jagtap, V.A., Sreenivasa, G.M., 2011. Synthesis, characterization and in-vitro anti-inflammatory evaluation of new fluorobenzothiazole Schiff bases. Int. J. Pharm. Technol. Res. 3, 164–169.

Shkawat, D.R., Sabnis, S.S., Deliwala, C.V., 1973. Potential anticancer agents, Schiff bases from p-(3-azaspiro[5,5]undec-3-yl)benzaldehydes. Bull. Haffkine. 1, 35–39.

Taggi, A.E., Hafez, A.M., Wack, H., Young, B., Ferraris, D., Lectka, T., 2002. The development of the first catalysed reaction of ketenes and imines: catalytic asymmetric synthesis of β-lactams. J. Am. Chem. Soc. 124, 6626–6635.