Ether based Schiff bases- potential antimicrobial agents

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Abstract

A series of Schiff bases 4-((4-phenoxyphenylimino)methyl)phenol(HL_1),4-((4-(biphenyl-4-yloxy)phenylimino)methyl)phenol(HL_2),4-((4-(naphthalen-1-yloxy)phenylimino)methyl)Phenol(HL_3),4-((4-(naphthalen-2-yloxy)phenylimino)methyl)phenol(HL_4) were synthesized from 4- hydroxybenzaldehyde and primary amines(1-amino-4-phenoxybenzene , 4-(4-aminophenyloxy) biphenyl , 1-(4-aminophenoxy) naphthalene and 2-(4-aminophenoxy) naphthalene). The manufactured compounds were characterized by elemental, mass spectrometric and spectroscopic (FTIR, NMR) techniques. These Schiff bases were then further evaluated for various biological studies (antifungal, cytotoxicity, antitumor and free radical hydroxyl (·OH) induced DNA damage assays) to examine their bioactive nature. Antifungal activity of the compounds was determined against Fusarium solani and Mucor species and the results exhibited that most of the compounds are active against Mucor species while only HL_4 showed antifungal activity against both strain. The results of brine shrimp cytotoxicity assay were found highly significant with LD_{50} in the range of 0.41-0.70µg/ml as compared with Doxorubicin. Similarly, in antitumor assay the prominent activity was recorded in IC_{50} range of 13.20-27.75µg/ml. Moreover, the biological investigations have shown their bioactive nature in protecting DNA against hydroxyl free radicals (·OH) in concentration dependent manner.

Keywords: *Schiff bases, antifungal, cytotoxic, potato disc antitumor, DNA damage assays.*

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Introduction

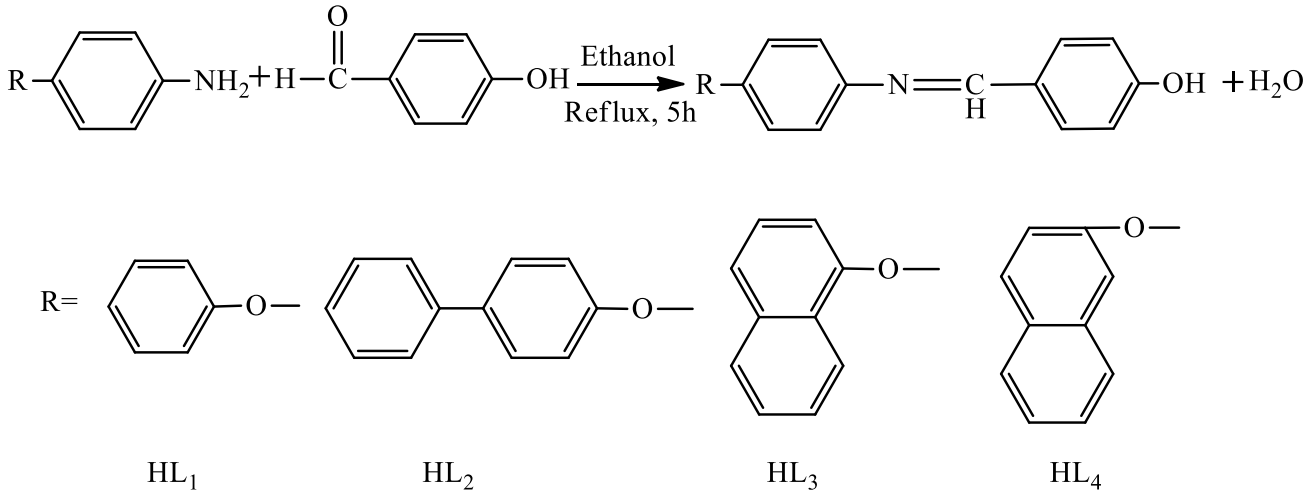
Schiff bases are an important group of organic molecules which make dative linkage to metal ions via azomethine nitrogen moiety (Vigato & Tamburini, 2004). The presence of imine linkage plays a vital role in biochemistry (antibacterial, antifungal, anticancer, diuretic activities etc) and applied chemistry (food, dye, analytical, catalysis & agrochemicals) (Supuran et al., 1996; Genin et al., 2000; Golcu, et al., 2005; Sundriyal et al., 2006].These molecules are precursors for many enzyme catalyzed reactions and they have acquired a prominent place in medicinal chemistry due to their broad range of bioactivities (Kumar & Parthiban, 2011; Boghaei, et al., 2008).
The pharmacologically useful bioactivities include anticonvulsant, anti-inflammatory, anti-hypertensive, antipyretic, antimicrobial, cytotoxicity, herbicidal and the destruction of various parasites (Shahabadi, et al., 2010; Pandeya, et al., 1999). In addition, such molecules are part of many biological systems and are useful synthetic tool in organic synthesis (Upadhyay, et al., 2008).
As our previous work on Schiff bases (Shabbir, et al.,2016; Shabbir, et al.,2017) reveal their pharmacological worth so continuing it we made an effort to synthesize a series of Schiff base ligands 4-((4-phenoxyphenylimino)methyl)phenol(HL_1), 4-((4-(biphenyl-4-yloxy)phenylimino)methyl)phenol(HL_2),4-((4-(naphthalen-1-yloxy)phenylimino)methyl)Phenol(HL_3),4-((4-(naphthalen-2-yloxy)phenylimino)methyl) phenol(HL_4). As literature studies illustrate their bioactive nature therefore synthesized molecules (HL_1-HL_4) were investigated for antifungal, cytotoxic, antitumor and inhibition of hydroxyl (OH) free radical induced DNA damage assays to assess their biological significance.

Experimental

Reported procedure was adopted for drying of solvents (Holmes, et al., 1987). 4-Hydroxy benzaldehyde was purchased from fluka and used as such. Melting points of the compounds were determined on MPD Mitamura Riken Kogyo (Japan). Their combustion analysis was performed on CHNS 932 (Leco-US**A)**. FTIR spectral studies (4000-400 cm^{-1}) were completed on Bruker Tensor II (Germany) spectrometer. NMR spectra of Schiff bases (^1H & ^{13}C) were recorded in deuterated chloroform on Bruker ARX 300 MHz. Mass spectral investigations were accomplished on micromass platform II instrument. The materials and methods for biological studies are same as reported in our earlier papers (Shabbir, et al., 2016; Shabbir, et al., 2017).

Synthetic procedure for ligands (HL_1- HL_4)

Compounds (HL_1-HL_4) were prepared from 4-hydroxy benzaldehyde and aromatic amines (Hammad, et al., 2015) by the same general synthetic procedure as reported earlier by our research group (Shabbir, et al., 2016; Shabbir, et al., 2017) (scheme 1).



Scheme1. Synthetic scheme for Schiff bases (HL_1-HL_4).

4-((4-phenoxyphenylimino)methyl)phenol(HL_1)

4-((4-phenoxyphenylimino)methyl)phenol was synthesized by taking 1.00g (5.40mmol) 1-amino-4-phenoxybenzene and 0.658g (5.40 mmol) of 4-hydroxybenzaldehyde.
Yield (78%), m.p 154 ºC. FTIR :(υ /cm^{-1}) 3427 (phenol), 1609 (imine), ^{1}H NMR (δ ppm): 5.06, (s, 1H, phenolic), 8.59 (s, 1H, imine), 6.87-7.77 (14H, m, aromatic), ^{13}C NMR (δ ppm): 162.59 (1C, imine), 160.29-117.54 (22C, aromatic), Anal. Calcd. for $C_{19}H_{15}NO_2$(289.0) : C, 78.89 ; H, 5.19 ; N, 4.84. Found: C, 78.83; H, 5.18; N, 4.81%.

4-((4-(biphenyl-4-yloxy)phenylimino)methyl)phenol(HL_2)

4-((4-(biphenyl-4-yloxy)phenylimino)methyl)phenol was prepared from 1.00g (3.89mmol) 4-(4-aminophenyloxy) biphenyl and 0.467 g (3.83 mmol) 4-hydroxybenzaldehyde.
Yield (81%), m.p 144 ºC. FTIR :(υ /cm^{-1}) 3426 (phenol), 1615 (imine), ^{1}H NMR (δ ppm): 5.08, (s, 1H, phenolic), 8.56 (s, 1H, imine), 6.61-7.77 (14H, m, aromatic), ^{13}C NMR (δ ppm): 161.87 (1C, imine), 160.35-117.81 (24C, aromatic), Anal. Calcd. for $C_{25}H_{19}NO_2$(365.0) : C,82.19; H, 5.20 ;N, 3.84 . Found: C, 82.20; H, 5.17; N, 3.86%.

4-((4-(naphthalen-1-yloxy)phenylimino)methyl)phenol(HL_3)

4-((4-(naphthalen-1-yloxy)phenylimino)methyl)phenol was synthesized by refluxing 1.00g (3.89mmol) 1-(4-aminophenoxy) naphthalene and 0.474g (3.89 mmol) 4-hydroxybenzaldehyde.
Yield (70%), m.p 81 ºC. FTIR :(υ /cm^{-1}) 3440 (phenol), 1606 (imine), ^{1}H NMR (δ ppm): 5.02, (s, 1H, phenolic), 8.56 (s, 1H, imine), 6.62-7.93 (14H, m, aromatic), ^{13}C NMR (δ ppm):162.89 (1C, imine), 159.57-116.45 (22C, aromatic), Anal. Calcd. for $C_{23}H_{17}NO_2$(339.0) : C, 81.41; H, 5.01; N, 4.13. Found: C, 81.40; H, 5.02; N, 4.15%.

4-((4-(naphthalen-2-yloxy)phenylimino)methyl)phenol(HL_4)

4-((4-(naphthalen-2-yloxy)phenylimino)methyl)phenol was manufactured from 1.00g (3.89 mmol) 2-(4-aminophenoxy) naphthalene and 0.474g (3.89mmol) of 4-hydroxybenzaldehyde.
Yield (72%), m.p 106 ºC. FTIR :(υ /cm^{-1}) 3442 (phenol), 1608 (imine), ^{1}H NMR (δ ppm): 5.04, (s, 1H, phenolic), 8.53 (s, 1H, imine), 6.66-7.87 (14H, m, aromatic), ^{13}C NMR (δ ppm): 162.76 (1C, imine), 161.21-115.79 (22C, aromatic), Anal. Calcd. for $C_{23}H_{17}NO_2$(339.0) : C,81.41; H, 5.01; N, 4.13. Found: C, 81.42; H, 5.02; N, 4.15%.

Results and discussion

The synthesized compounds (HL_1-HL_4) were characterized by spectroscopic (FTIR, NMR), mass spectrometric and elemental analysis. These molecules were then investigated for various biological assays (antifungal, cytotoxic, antitumor and hydroxyl (·OH) free radical induced DNA damage).

Spectral Characterization

The compounds were characterized by FTIR spectroscopy. The spectral bands in the region 3427, 3426, 3440 and 3442 cm^{-1} were due to phenolic protons whereas absorptions in the region 1609, 1615, 1606 and 1608 cm^{-1} in the ligands HL_1 , HL_2 , HL_3 and HL_4 were due to azomethine group respectively.
The structural identity of molecules has been further deduced from NMR (^1H &^{13}C) spectroscopic studies. The chemical shift values for these compounds (HL_1-HL_4) are summarized in Table 1. The azomethine singlet (^1H NMR) was shown in the region of 8.53-8.59 ppm. The presence of aromatic protons was confirmed by the emergence of multiplets in the range of 6.61-6.87 ppm. The resonance signals around 13.23- 5.02-5.08 were assigned to phenolic protons.
The ^{13}C NMR spectra of molecules (HL_1 – HL_4) displayed the characteristic signals of the carbon atoms. Azomethine carbon (-CH=N) resonated around 161.87-162.89 ppm whereas the signals for aromatic carbons were observed in the range of 159.57-115.79 ppm.

Table 1. NMR (^1H &^{13}C) spectral data of Schiff bases (HL_1-HL_4) δ (ppm)

Compound	^1H NMR			^{13}C NMR	
	δ (Ar-H)	δ (CH=N) s, 1H	δ (OH) s, 1H	δ (C=N)	δ (Ar-C)
HL_1	6.87-7.77 (m, 14H)	8.59	5.06	162.59	160.29-117.54(18C)
HL_2	6.61-7.77(m, 14H)	8.56	5.08	161.87	160.35-117.81(24C)
HL_3	6.62-7.93(m, 14H)	8.56	5.02	162.89	159.57-116.45(22C)
HL_4	6.66-7.87(m, 14H)	8.53	5.04	162.76	161.21-115.79(22C)

The molecular masses of the compounds were confirmed by mass spectrometry. The molecular ion peaks for compounds (HL_1-HL_4) obtained at (m/z) 289.0, 365.0, 339.0 and 339.0 respectively confirmed their formation.

Biological studies

The bioactive nature of the compounds was assessed by antifungal, brine shrimp cytotoxicity, potato disc antitumor and DNA damage assays.
The antifungal activity of the organic molecules was investigated by disc diffusion method. The activity was measured in terms of zone of inhibition and findings are tabulated in Table 2. The results exhibited that the compound HL_4 showed antifungal activity against both strain while the compounds HL_3 and HL_1 bioactive only against *Mucor species*. A literature survey showed that Schiff bases exhibit antimicrobial activity (Nair et al., 2006) and existence of metal ions attached to biologically active substance may increase the antibacterial activity.
Brine shrimp cytotoxic assay was performed to determine their bioactive character and the findings in the form of LD_{50} are given in Table 2. The results indicated highly significant activity. The synthesized compounds resulted higher activity than Doxorubicin (positive control). Highest activity was exhibited by HL_3 (LD_{50} 0.41µg/ml) followed by HL_4, HL_2 and HL_1 with LD_{50} values 0.61, 0.65 and 0.70µg/ml respectively. Cytotoxic action of a drug is simply provided by disturbing the basic mechanisms concerned with mitotic activity, cell growth, function and differentiation (Goodman *et al.*, 1980).
Potato disc antitumor assay was performed to investigate the antitumor behavior of the compounds and the results of IC_{50} are tabulated in Table 2. The results revealed that all compounds showed prominent tumor inhibition potential. The compounds showed IC_{50} values 27.75, 24.72, 13.20 and 22.74 µg/ml for HL_1, HL_2, HL_3 and HL_4 respectively. As it was previously observed that some Schiff bases and their metal complexes containing Cu, Ni, Zn and Co were synthesized from salicylaldehyde, 2, 4-dihydroxybenzaldehyde, glycine and L-alanine

possess antitumor activity (Kumar *et al.*, 2009). Interestingly, the results of antitumor assay are in accordance with the results of brine shrimp cytotoxicity assay. Overall, cytotoxic and antitumor nature of these compounds suggests their anticancer potential which can be revealed from further advanced studies.

The antioxidant and pro-oxidant behavior of the molecules was checked by H_2O_2 stimulated DNA damage assay. The extent of DNA damage/protection was determined from the intensity and thickness of bands formed on agarose gel. The assay was performed on concentrations 1000, 100, 10 and 1 µg/ml in triplicates. Prominent and significant DNA protection activity was shown by the examined molecules as shown in Table 2. The presence of CN linkage in Schiff base is very important for biological activity, such as antibacterial, antifungal, anticancer, antioxidant and diuretic activities. These Schiff bases can be used for further biological studies such as antimicrobial, antifungal and antitumor (Kumar *et al.*, 2017).

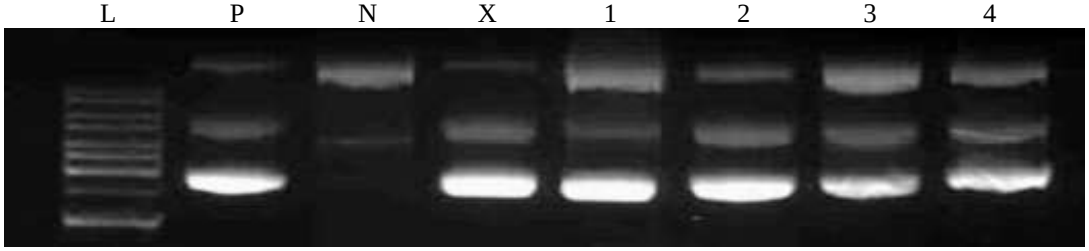


Fig. 1 Effect of compound HL_4 on pBR322 plasmid DNA [L= 1Kb ladder, P= pBR322 plasmid (nontreated), N= pBR322 plasmid treated with $FeSO_4$ and H_2O, X= pBR322 plasmid + 1000µg/ml of HL_4 (prooxidant control), 1= Plasmid + phosphate buffer + 1000µg/ml HL_4, 2= plasmid + phosphate buffer + 100µg/ml of HL_4 + $FeSO_4$ + H_2O_2, 3= plasmid+ phosphate buffer + 10µg/ml of HL_1 + $FeSO_4$ + H_2O_2, 4= plasmid+ phosphate buffer + 1µg/ml of HL_4 + $FeSO_4$ + H_2O_2].

Fig. 1 is the representative gel image of compound HL_4 showing DNA protection activity in concentration dependant manner. Additionally, no pro-oxidant effect was observed from any of the tested compound.

Table 2. Results of biological activities

Compound	Brine Shrimp cytotoxic assay (LD_{50} µg/ml)	Potato disc antitumor assay (IC_{50} µg/ml)	DNA protection assay	Antifungal activity (zone of inhibition at 200 µg/ml)	
				Fusarium solani	*Mucor species*
HL_1	0.70	27.75	+	-	7.6 mm
HL_2	0.65	24.72	+	-	-
HL_3	0.41	13.20	+	-	7.8 mm
HL_4	0.61	22.74	+	6.3	7.5 mm
Doxorubicin	0.71	0.45	NA	NA	NA
Terbinafine	NA	NA	NA	24.1 mm	26.6 mm

Where "+" represents good DNA protection activity, "-" represents no activity while "NA" represents not applicable.

Conclusions

Organic compounds (HL_1-HL_4) have been manufactured and characterized by FTIR, NMR (^1H &^{13}C), mass spectrometric and elemental analysis. The biological investigations described their vital bioactivity for brine shrimps along with inhibition potential for tumour formation on potato discs. These molecules have the ability to avert oxidative DNA damage induced by H_2O_2. Additionally, the molecules exhibit antifungal property. Overall all the synthesised compounds presented diverse bioactivities in observed investigations. Further biological studies may give an insight to reconnoitre their exact biological mechanism thus considering them useful drugs.

Author(s) Contribution Statement All the authors have contributed to prepare this manuscript. Further, manuscript has been read and approved by all the authors.

Declaration of interest The authors declare no conflict of interest.

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References

Vigato, P. A., & Tamburini, S. (2004). The challenge of cyclic and acyclic Schiff bases and relate derivatives. *Coordination Chemistry Reviews*, *248*(17-20), 1717-2128.

Supuran, C. T., Barboiu, M., Luca, C., Pop, E., Brewster, M. E., & Dinculescu, A. (1996). Carbonic anhydrase activators. Part 14. Syntheses of mono and bis pyridinium salt derivatives of 2-amino-5-(2-aminoethyl)-and 2-amino-5-(3-aminopropyl)-1, 3, 4-thiadiazole and their interaction with isozyme II. *European journal of medicinal chemistry*, *31*(7-8), 597-606.

Genin, M. J., Biles, C., Keiser, B. J., Poppe, S. M., Swaney, S. M., Tarpley, W. G., ... & Romero, D. L. (2000). Novel 1, 5-diphenylpyrazole nonnucleoside HIV-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: lead identification and SAR of 3-and 4-substituted derivatives. *Journal of medicinal chemistry*, *43*(5), 1034-1040.

Golcu, A., Tumer, M., Demirelli, H., & Wheatley, R. A. (2005). Cd (II) and Cu (II) complexes of polydentate Schiff base ligands: synthesis, characterization, properties and biological activity. *Inorganica Chimica Acta*, *358*(6), 1785-1797.

Sundriyal, S., Sharma, R. K., & Jain, R. (2006). Current advances in antifungal targets and drug development. *Current medicinal chemistry*, *13*(11), 1321-1335.

Kumar, B. S., & Parthiban, K. G. (2011). Synthesis of Schiff bases of some novel n-nitrosoisatin derivatives as potential antimicrobial agents. *Asian Journal of Pharmaceutical and Clinical Research*, *4*(4), 137-140.

Boghaei, D. M., Askarizadeh, E., & Bezaatpour, A. (2008). Synthesis, characterization, spectroscopic and thermodynamic studies of charge transfer interaction of a new water-soluble cobalt (II) Schiff base complex with imidazole derivatives. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *69*(2), 624-628.

Shahabadi, N., Kashanian, S., & Darabi, F. (2010). DNA binding and DNA cleavage studies of a water soluble cobalt (II) complex containing dinitrogen Schiff base ligand: The effect of metal on the mode of binding. *European journal of medicinal chemistry*, *45*(9), 4239-4245.

Pandeya, S. N., Sriram, D., Nath, G., & Clercq, E. D. (1999). Synthesis, Antibacterial, Antifungal And Anti-HIV Activity Of Schiff And Mannich Bases Of Istain With N-[6-Chlorobenzothiazol-2-yl] Thiosemicarbazide. *Indian journal of pharmaceutical sciences*, *61*(6), 358.

Upadhyay, K. K., Kumar, A., Upadhyay, S., & Mishra, P. C. (2008). Synthesis, characterization, structural optimization using density functional theory and superoxide ion scavenging activity of some Schiff bases. *Journal of Molecular Structure*, *873*(1-3), 5-16.

Shabbir, M., Akhter, Z., Ahmad, I., Ahmed, S., Ismail, H., Mirza, B., ... & Bolte, M. (2016). Synthesis, characterization, biological and electrochemical evaluation of novel ether based ON donor bidentate Schiff bases. *Journal of Molecular Structure*, *1116*, 84-92.

Shabbir, M., Akhter, Z., Ashraf, A. R., Bolte, M., Wahid, S., & Mirza, B. (2017). Pharmacological evaluation of ONNO donor quadridentate Schiff bases. *European Journal of Chemistry*, *8*(1), 46-51.

Nair, R., Shah, A., Baluja, S. & Chanda, S., (2006). Synthesis and antibacterial activity of some Schiff base complexes. *Journal of the Serbian Chemical Society*, 71(7), 733-744.

Goodman, L., S., Gilman, A., G., & Gilman, A. (1980). Antiproliferative agents and immunosuppressive drugs; The pharmacological basis of therapeutics. 6th Ed. Macmillan Publishing Co, Inc, USA, 1299-1313.

Kumar, P., Narasimhan, B., Sharma, D., Judge, V. & Narang, R., (2009). Hansch analysis of substituted benzoic acid benzylidene/furan-2-yl-methylene hydrazides as antimicrobial agents. European journal of medicinal chemistry, 44(5), 1853-1863.

Kumar, M., Padmini, T. & Ponnuvel, K., (2017). Synthesis, characterization and antioxidant activities of Schiff bases are of cholesterol. *Journal of Saudi Chemical Society*, 21, 322-328.

Holmes, R. R., Schmid, C. G., Chandrasekhar, V., Day, R. O., & Holmes, J. M. (1987). Oxo carboxylate tin ladder clusters. A new structural class of organotin compounds. *Journal of the American Chemical Society*, *109*(5), 1408-1414.

Ismail, H., Mirza, B., Haq, I. U., Shabbir, M., Akhter, Z., & Basharat, A. (2015). Synthesis, characterization, and pharmacological evaluation of selected aromatic amines. *Journal of Chemistry*, *2015*.