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Synthesis, characterization, biological and electrochemical evaluation of novel ether based ON donor bidentate Schiff bases

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ABSTRACT

Four novel ON donor Schiff bases (E)-2-((4-phenoxyphenylimino)methyl)phenol (HL1), (E)-2-((4-(4-biphenyloxy)phenylimino)methyl)phenol(HL2), (E)-2-((4-(naphthalen-1-yloxy) phenylimino)methyl) phenol(HL_3)and(E)-2-((4-(2-naphthoxy)phenylimino)methyl)phenol (HL_4)have been synthesized and characterized by various spectroscopic, analytical and electro-analytical techniques. Single crystal X-ray diffraction analysis of Schiff base (HL_3) revealed that phenol and anthracene rings are inclined at 30.25(9)° and 89.64(4)° to the central phenyl ring, respectively. Intra and inter molecular interactions are observed in single crystal analysis of HL_3 Intramolecular interactions are hydrogen bonding but most of the intermolecular interactions are of the C–H … π type. There is a bit of π … π stacking between the anthracene groups. Only compounds (HL_1) and (HL_3) have been investigated for the biological activities due to slight solubility of (HL_2) and (HL_4) in DMSO. The results of brine shrimp cytotoxicity assay indicated LD_{50} values <1 μg/ml showing significant antitumor activity with IC_{50} values 14.20 and 4.54 μg/ml respectively. The compounds were highly active in protecting DNA against hydroxyl free radicals in concentration dependent manner. Voltammetric results indicated that one electron irreversible oxidation product is formed due to hydroxyl moiety and the process is diffusion controlled. On exposing to DNA environment the electrooxidised product developed electrostatic linkage and groove binding intercalation while consuming the DNA concentration substantially. The binding strength was quantitative in terms of drug-DNA binding of the order of 10^4 M^{-1}.

1. Introduction

Schiff bases are most widely used organic compounds that coordinate to metal ions via azomethine nitrogen and have a wide variety of applications in many fields including analytical, biological, and inorganic chemistry. In azomethine derivatives, the C=N linkage is present in various natural (ancistrocladidine have antimalarial activity), natural-derived (chitosin-derived Schiff bases have antifungal activity), and non-natural compounds which is essential for biological activity [1]. Several azomethines possess remarkable antibacterial, antifungal, anticancer and diuretic activities. The nitrogen atom of azomethine may be involved in the formation of a hydrogen bond with the active centres of cell constituents and interferes in normal cell processes [2]. Apart from biological activities, they have found applications in many other fields such as intermediates in organic synthesis, dyes, pigments, polymer stabilizers, corrosion inhibitors, fungicidal, agrochemical, analytical chemistry, electrical conductivity, magnetism, host guest chemistry, ion exchange, nonlinear optics and catalysis [3–10]. Schiff bases have played an important role in the development of coordination chemistry and inorganic biochemistry as well. They have been used for the synthesis of a number of biologically and industrially active compounds like formazans, 4-thiazolidinines, benzoxazines, and so forth, via ring closure, cycloaddition and replacement reactions [11].

Particularly salicyaldehyde-Schiff bases derived from salicyaldehyde and primary amines have recently acquired a considerable importance due to their promising biological properties.

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Such Schiff bases are found to be a versatile pharmacophore for design and development of various bioactive lead compounds. These Schiff bases may act as bidentate-O, N and a tridentate −O, O, N donor ligand etc [12]. which can be employed for the synthesis of various coordination complexes. Cambridge structural database have shown that about 42% of Schiff bases synthesized worldwide are derived from salicylaldehyde based aldehydes and almost 68% of Schiff base complexes are synthesized from such ligands [13]. A very useful application of such new compounds is that they specially target DNA molecule with significant potential and thus can be proposed as drugs. Among other techniques the voltammetric methods have established a prominent role to study drug-DNA interaction due to high sensitivity, selectivity, versatility and fast detection ability in addition to cost effectiveness. Monitoring of the reaction of interest at the electrode surface helps to elucidate the mechanism of drug- DNA interactions [14–16].

In the present work (E) -2- ((4-phenoxyphenylimino) methyl) phenol (HL_1), (E)-2-((4-(4-biphenyloxy)phenylimino)methyl)phenol(HL_2), (E)-2-((4-(naphthalen-1-yloxy)phenylimino)methyl) phenol(HL_3)and(E)-2-((4-(2-naphthoxy)phenylimino)methyl)phenol(HL_4)were synthesised and characterized successfully by various spectroscopic, analytical, advanced electro-analytical techniques and single crystal analysis. As Schiff bases are biological active in nature therefore synthesised compounds (HL_1-HL_4) were studied to acquire valuable information about their role in cellular vicinities. Biological studies (cytotoxic, antitumor and inhibition of hydroxyl (OH) free radical induced DNA damage assay) were proceeded to screen the pharmacological importance of the compounds. Voltammetric studies were carried to investigate the redox behaviour and to identify the electrophoric centres in the compounds. Further, the interactions with the DNA and the binding mode of the synthesised compounds were also investigated.

2. Experimental

2.1. Materials and methods

Solvents used were purified by standard distillation procedure and drying methods [17]. The Schiff bases (HL_1–HL_4) were prepared by condensation reactions of salicylaldehyde and corresponding aromatic amines [18] already reported by our research group in ethanol following the reported method [19,20]. The progress as well as purity of products was checked by thin layer chromatography on pre-coated Kieselgel 60HF TLC plates. Elemental analysis was carried out on a CHNS 932 (Leco-USA) elemental analyzer. Melting points were determined, using a MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus. FTIR spectra were recorded on a Thermoscientific (USA) Nicolet 6700 spectrometer in the frequency range of 4000–400 cm^{-1}. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker NMR Spectrometer.

Single crystal of Schiff base (HL_3) was obtained by the slow evaporation of ethanol from the mother liquor at room temperature. X-ray data were collected at 150(2) K on a Bruker Apex II CCD diffractometer using MoK_α radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods [21] and refined on F^2 using all the reflections [22]. All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters and hydrogen atoms bonded to carbon were inserted at calculated positions using a riding model. The phenolic proton was located and its coordinates refined. Parameters for data collection and refinement are summarised in Table 1.

The bioactive nature of the synthesized compounds was evaluated by brine shrimp lethality assay [23]. Brine shrimp (*Artemia-salina*) eggs (Ocean Star Inc., USA) were hatched in shallow rectangular dish (22 × 32 cm) filled with prepared seawater (34 g/l) under constant aeration for 48 h at room temperature. After 24 h, phototropicnauplii (brine shrimp larvae) were shifted to glass vial by Pasteur pipette and 25 µl of the each stock solution (0.1 µ g/ml, 1 µ g/ml, and 10 µ g/ml) of the test compound was added. The volume of test compounds from their stock solutions was raised up to 5 ml of artificial seawater with 10, 1, 0.5, 0.25, 0.125 and 0.0625 µg ml^{-1} final concentration. Three replicates were prepared for each concentration. The vials were maintained under illumination at room temperature. After 24 h of incubation survivors were observed and LD_{50} (Lethal Dose that killed 50% of shrimps) was calculated by using Finny (1971) software [24].

Antitumor activity of the synthesized compounds was checked by executing modified potato disc antitumor assay [25]. Inoculum with three different concentrations (1000, 100 and10 µg/ml) was prepared containing 48 h bacterial culture of *Agrobacterium tumefaciens*(At 10). Red-skinned potatoes were surface sterilized in 0.1% $HgCl_2$ solution and potato discs of size 8 mm × 4 mm were prepared with the help of sterilized cork borer. Ten discs were placed on the agar plates along with 50 µl of inoculum on the surface of each disc. After 21 days of incubation at 28 °C, discs were stained with Lugol solution (10% KI & 5% I_2) and tumors were counted on each disc. The tumour inhibition was calculated by formula, (Percentage inhibition = 100 − average number of tumors of sample/average number of tumors of negative control × 100).

Antioxidant or prooxidant activity of the synthesized compounds was assessed by DNA damage assay [26]. Plasmid DNA (pBR322 Fermentas) with a concentration of 0.5 µg/3 µl was treated with three different concentrations of test samples (1000, 100, and 10 ppm). Fenton reaction was induced by addition of 30% H_2O_2 (4 µl) and 2 mM $FeSO_4$ (3 µl) into the reaction mixture. Three controls, untreated pBR322 DNA as negative, DNA treated with compound (C + P), DNA treated with 2 mM $FeSO_4$ and 30% H_2O_2 as positive control were run simultaneously. Each reaction mixture

Table 1
Crystal data and structure refinement for HL_3.

Empirical formula	$C_{23}H_{17}NO_2$	
Formula weight	339.38	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a = 13.3952(11) Å	α = 90°.
	b = 8.8205(7) Å	β = 100.9600(10)°
	c = 14.7042(12) Å	γ = 90°.
Volume	1705.6(2) $Å^3$	
Z	4	
Density (calculated)	1.322 Mg/m^3	
Absorption coefficient	0.084 mm^{-1}	
F(000)	712	
Crystal size	0.44 × 0.30 × 0.04 mm^3	
Crystal description	Yellow plate	
Theta range for data collection	1.55–26.40°	
Index ranges	$-16 \le h \le 16$, $-11 \le k \le 11$, $-18 \le l \le 18$	
Reflections collected	14712	
Independent reflections	3497 [R(int) = 0.0439]	
Completeness to theta = 28.32°	100.0%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9966 and 0.9638	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	3497/0/235	
Goodness-of-fit on F^2	0.993	
Final R indices [I > 2sigma(I)]	$R_1 = 0.0423$, $wR_2 = 0.0975$	
R indices (all data)	$R_1 = 0.0752$, $wR_2 = 0.1127$	
Largest diff. peak and hole	0.264 and −0.173 $e.Å^{-3}$	

K: kelvin temperature; Å: angstrom; $Å^3$: volume; Z: number of chemical formula units per unit cell; D: density; F: structure factor; R: reliability factor.