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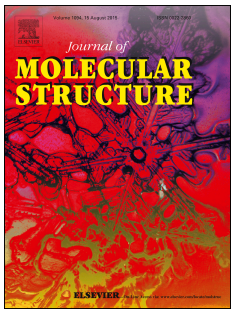

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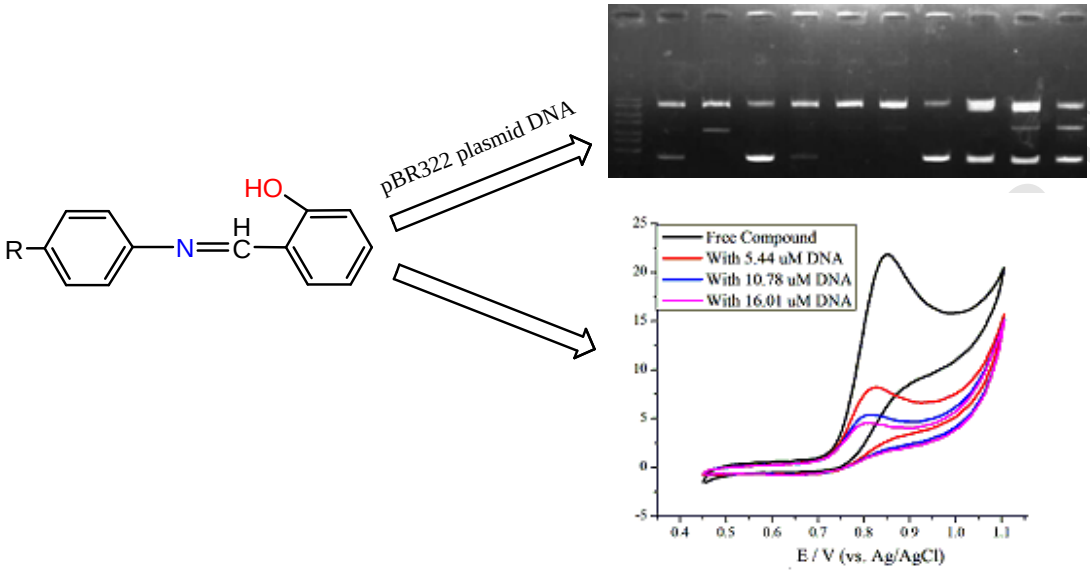


Synthesis, characterization, biological and electrochemical evaluation of novel ether based ON donor bidentate Schiff bases

Muhammad Shabbir[a], Zareen Akhter[a], Iqbal Ahmad[a], Safeer Ahmed[a], Hammad Ismail[b], Bushra Mirza[b], Vickie McKee[c], Michael Bolte[d]

[a]Department of Chemistry, Quaid-i-Azam University, Islamabad-45320. Pakistan.
[b]Department of Biochemistry, Quaid-i-Azam University, Islamabad-45320. Pakistan.
[c]School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland.
[d]Institutfür Anorganische Chemie, J.W. Goethe-Universität Frankfurt, Max-Von-Laue-Strasse 7, Frankfurt/Main 60438, Germany.

Corresponding author
Telephone +92 051 90642111; fax: +92 051 9064224
E-mail address: zareenakhter@yahoo.com, zareen_a@qau.edu.pk

Abstract

Four novel ON donor Schiff bases (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)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 <1µg/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 the 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}.

Keywords: Schiff bases, electrochemical studies, pharmacological studies, DNA-interaction

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 centers 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. 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}.^{1}H NMR (300MHz) and^{13}C NMR (75MHz) 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 (λ = 0.71073Å). 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 (*Artemiasalina*) 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 µg/ml, 1 µg/ml, and 10 µg/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 µgml^{-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 8mm×4mm 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 tumor 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/3µl 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 was incubated at 37ºC for an hour. After incubation, the sample was loaded on a 0.9%

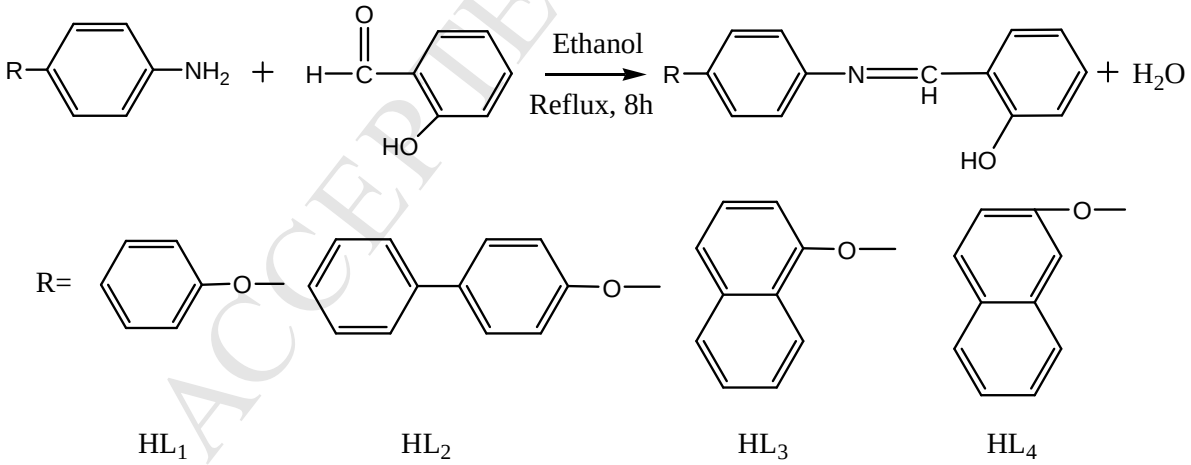
agarose gel and was visualized with Doc-IT (VWR). Estimation of antioxidant or pro-oxidant effects on DNA was estimated on the basis of percentage increase or loss of a super-coiled monomer, compared with the control.

Voltammetric studies (cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV)) were conducted by using Eco Chemie Autolab PGSTAT 302 potentiostat/galvanostat (Utrecht, the Netherlands) with software version GPES 4.9.The conventional three-electrode cell with Ag/AgCl(aq) as a reference, home-made platinum wire as a counter electrode and a glassy carbon electrode (GCE) with surface area of 0.09 as a working electrode was employed. The active surface area of GCE was polished with 0.25 m alumina paste on a nylon buffing pad followed by rinsing with distilled water and DMSO. The electrochemical behaviour of each compound (2mM) was investigated in argon saturated H_2O/DMF (1:9,v:v) solution containing 0.1M tetrabutylammoniumtetrafluroborate (TBATFB).Drug-DNA interaction studies by CV were carried out in aqueous-DMF mixture (1/9,v/v) at a scan rate of 100 mV/s by the sequential addition of DNA.

2.2 General procedure for the synthesis of ON donor Schiff bases (HL_1-HL_4)

ON donor Schiff bases (HL_1-HL_4) were synthesized by adopting the following general procedure:

In a pre-backed two necked round bottom flask (250 ml) equipped with magnetic stirrer and reflux condenser, the solution of salicyaldehyde was taken in dried ethanol. To this, solution of corresponding amine in dried ethanol was added drop wise with constant stirring. The reaction mixture was refluxed for 8 h under inert conditions using nitrogen. The progress of the reaction was monitored by TLC. After the completion of the reaction the ethanol was removed by rotary evaporator [27].The solid product was recrystallized from ethanol (scheme 1).



Scheme1: Synthesis of Schiff bases (HL_1-HL_4)

2.2.1. (E)-2-((4-phenoxyphenylimino)methyl)phenol (HL_1)

(E)-2-((4-phenoxyphenylimino)methyl)phenol (HL_1) was manufactured by refluxing equimolar quantities of 1-amino-4-phenoxybenzene 1.85 g (10 mmol) and 1.05 ml (10 mmol) 2-hydroxy benzaldehyde.
Color: yellow, Yield 90%, melting point 91^0C. FTIR: (υ /cm^{-1}) 3405 (–OH), 1603, (-CH=N),^{1}H NMR (300 MHz, $CDCl_3$, δ ppm):13.30 (s, 1 H, -OH), 8.65 (s, 1 H, -CH=N), 7.43-6.94 (13 H, m, Ar- H)., ^{13}C NMR (75 MHz ,$CDCl_3$, δ ppm): 162.13 (-C=N), 160.24-117.15 (18 C, Ar-C), MS (m/z): 289(M^+) CHN found (calcd.) for $C_{19}H_{15}NO_2$: C: 78.79 (78.89), H: 5.73 (5.19), N: 4.72 (4.84).

2.2.2. (E)-2-((4-(4-biphenyloxy)phenylimino)methyl)phenol (HL_2)

(E)-2-((4-(4-biphenyloxy)phenylimino)methyl)phenol (HL_2) was synthesized by refluxing equimolar quantities of 4-4(aminophenyloxy) biphenyl 2.61 g (10 mmol) and 1.05 ml (10 mmol) 2-hydroxy benzaldehyde.
Color: light yellow, Yield 92%, melting point 173^0C. FTIR: (υ /cm^{-1}) 3400 (–OH), 1601 (-CH=N), ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm):13.30 (s, 1 H, -OH),8.66 (s, 1 H, -CH=N), 7.63-6.95 (17 H, m, Ar- H), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 161.73 (-C=N), 160.15-117.28 (24 C, Ar-C), MS (m/z): 365 (M^+) CHN found (calcd.) for $C_{25}H_{19}NO_2$: C: 82.15 (82.19), H: 5.58 (5.20), N: 3.94 (3.84).

2.2.3. (E) - 2-((4-(naphthalen-1-yloxy)phenylimino)methyl)phenol (HL_3)

(E) - 2-((4-(naphthale,n-1-yloxy)phenylimino)methyl)phenol (HL_3) was prepared by taking equimolar quantities of 1-(4-aminophenoxy) naphthalene 2.35 g (10 mmol) and 1.05 ml (10 mmol) 2-hydroxy benzaldehyde.
Color: yellow, Yield 87%, melting point 105^0C. FTIR: (υ /cm^{-1}) 3412 (–OH), 1613 (-CH=N), ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 13.32, (s, 1 H, -OH), 8.65 (s, 1 H, -CH=N), 8.23-6.94 (15 H, m, Ar- H), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 162.27 (-C=N), 159.01-114.72 (22 C, Ar-C), MS (m/z): 339(M^+) CHN found (calcd.) for $C_{23}H_{17}NO_2$: C: 81.69 (81.41), H: 5.06 (5.01), N: 4.06 (4.13).

2.2.4. (E)-2-((4-(2-naphthoxy)phenylimino)methyl)phenol (HL_4)

(E)-2-((4-(2-naphthoxy)phenylimino)methyl)phenol (HL_4) was synthesized by using equimolar quantities of 2-(4-aminophenoxy) naphthalene 2.35g (10 mmol) and 1.05 ml (10mmol) 2-hydroxy benzaldehyde.
Color: yellow, Yield 93%, melting point 120 ^{0}C. FTIR :(υ /cm^{-1}) 3408 (-OH), 1610 (-CH=N), ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm):13.30 (s, 1H, -OH),8.68 (s, 1 H ,-CH=N), 7.90-6.96 (15H, m, Ar- H), ^{13}C NMR (75 MHz , $CDCl_3$, δ ppm): 162.25 (-C=N), 161.08-114.27 (22 C, Ar-C), MS (m/z): 339(M^+) CHN found (calcd.) for $C_{23}H_{17}NO_2$: C: 81.39 (81.41), H: 5.75 (5.01), N: 4.06 (4.13).

3 Results and Discussion

Schiff bases (HL_1-HL_4) have been successfully synthesised in a single step as described in experimental section and were characterised by spectroscopic, analytical, voltammetric and X-ray diffraction analysis. They are air stable under ambient conditions and soluble in common organic solvents such as C_2H_5OH, CH_2Cl_2, $CHCl_3$, DMSO, and DMF. The synthesized Schiff bases (HL_1-HL_4) were tested for biological, voltammetric and DNA interaction studies by cyclic voltammetrically.

3.1 Spectral characterization

The structures of the synthesised compounds were established by means of spectral studies (FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry), elemental analysis, and single crystal X-ray diffraction studies.

The FTIR spectral analysis of Schiff bases indicated the presence of all expected functionalities.Schiff bases having O-hydroxy group on an aldehyde can form intramolecular hydrogen bonding which affects ν(OH) vibration by shifting towards lower frequency with broadening. The extent of shift depends on the strength of hydrogen bonding.The FTIR spectra of these compounds exhibited the strong absorptions bands in the region of 1601-1613 cm^{-1} due to azomethine bond (CH=N).The hydroxyl groups (-OH) of the Schiff bases showed broad absorption bands in the region of 3400-3412 cm^{-1}.The disappearance of peaks in the region 3391-3468 (asymmetric) and 3315-3372 (symmetric) cm^{-1} for $–NH_2$ moiety confirmed the conversion of amino group into azomethine bond. They also exhibited absorption bands of C-O-C vibrations in the region of 1244-1260 cm^{-1} [28]. The FTIR spectra of Schiff bases as presented in Fig. S1(a-d).

The ^{1}H NMR and^{13}C NMR spectra of all the Schiff bases are consistent with the proposed molecular structures. The ^{1}H NMR spectra showed the characteristic azomethine singlet in the region of 8.68-8.65 ppm. Phenyl protons were present in all the compounds which were verified by the appearance of multiplets in the range of 7.43-6.94 (HL_1), 7.63-6.95 (HL_2), 8.23-6.94 (HL_3) and 7.90-6.96 (HL_4) ppm, according to the substituents attached. The resonance signals observed around 13.30-12.14 were assigned to phenolic proton as shown in Fig. S2(a-d).

^{13}C NMR spectra of Schiff bases show all the characteristic signals of azomethine and aromatic carbon atoms in their respective ranges of chemical shift values. Azomethine carbon resonated around 162 ppm. The resonance signals observed around 160, 155-150 and 141 ppm were assigned to C–OH, C-O-C and C-N carbons respectively. The rest of the peaks from 146-110 ppm were due to aromatic carbon atoms of Schiff bases as displayed in Fig. S3(a-d).

The mass spectral data of the Schiff bases justified their formation as their molecular ion peaks were obtained at (m/z) 289, 365, 339 and 339 respectively as revealed in Fig. S4(a-d).

3.2 X- ray structure analysis of HL_3

The single crystals of Schiff base (HL_3) for X-ray crystallographic analysis were grown by the slow evaporation of its ethanolic solution at room temperature. A single suitable crystal was selected for X-ray diffraction analysis and was mounted on Bruker Apex II CCD diffractometer. The crystal was kept at 150(2)K during analysis. A perspective diagram of HL_3 is shown in Fig. 1. There are no unusual bond lengths; the phenol and anthracene rings are inclined at 30.23(8)° and 89.64(4)° to the central phenyl ring, respectively and the phenol group makes an intramolecular hydrogen bond to the imine nitrogen ()1 – N1, 2.5591(18)Å) .There is some π···π

stacking between anthracene groups, but most of the intermolecular interactions are of the C-H···π type as shown in Fig. 2.

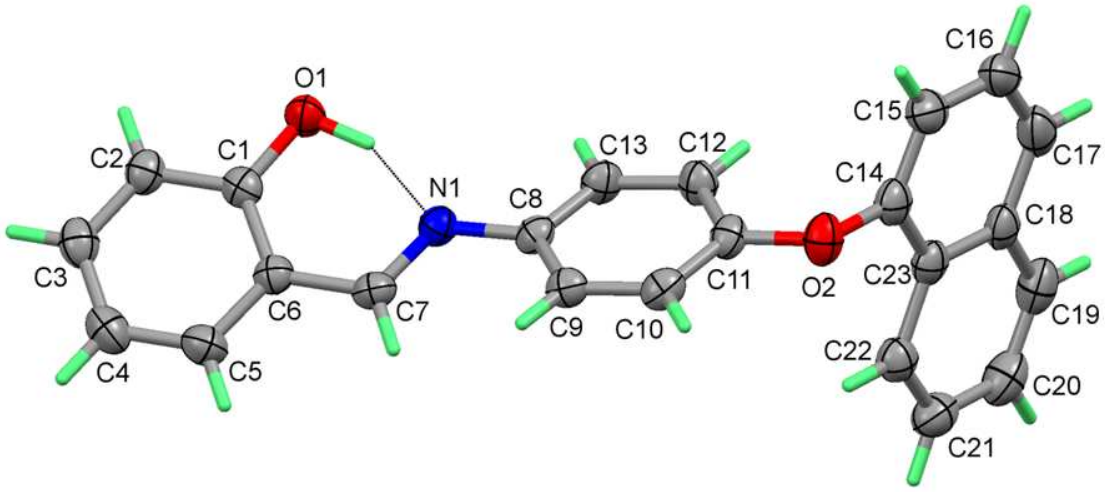


Fig.1 Perspective diagram of HL_3 showing 50% probability ellipsoids.

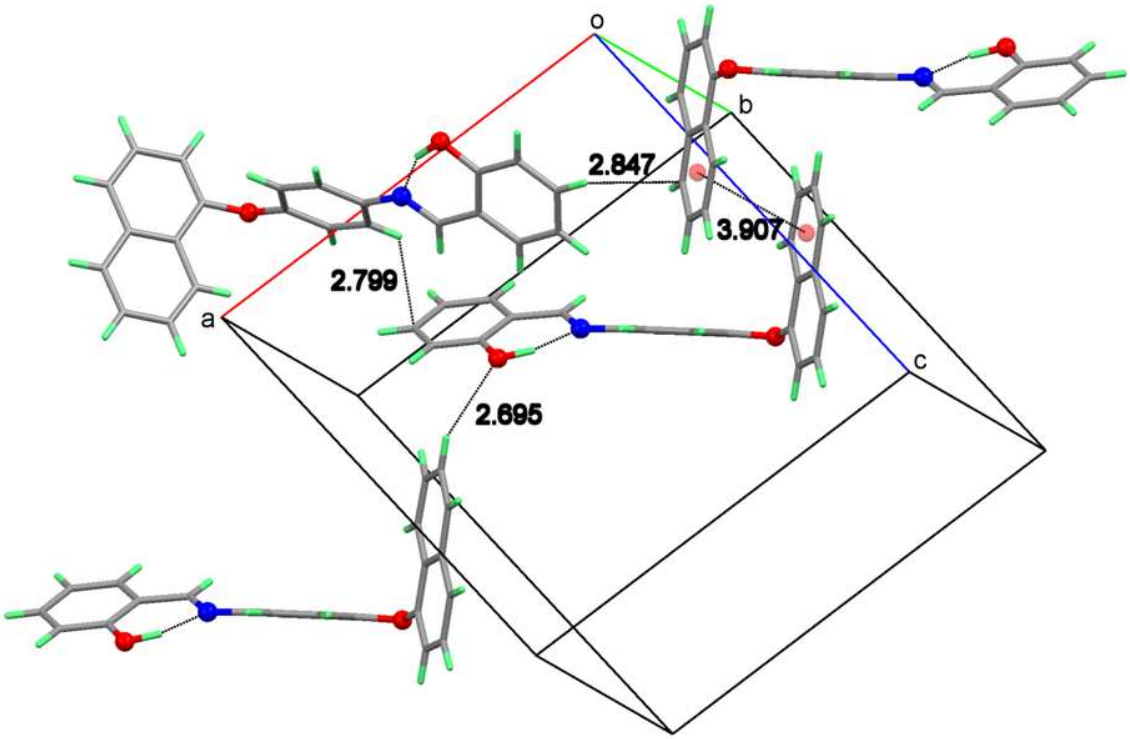


Fig. 2 Intermolecular interactions in HL_3

Crystal data and refinement details are listed in Table 1. Selected bond lengths and angles for HL_3 are summarized in Table 2 and hydrogen bonding parameters in Table 3.

Table 1.Crystal data and structure refinement for HL_3

Empirical formula	$C_{23}\ H_{17}\ N\ O_2$	
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 × 0.04mm^3	
Crystal description	Yellow plate	
Theta range for data collection	1.55 to 26.40°	
Index ranges	-16<=h<=16, -11<=k<=11, -18<=l<=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

Table 2. Selected bond lengths [Å] and angles [°] for HL_3

Bond	Length Measurement(Å)	Bond angle	Angle measurement(°)
O(1)-C(1)	1.356(2)	O(1)-C(1)-C(2)	118.46(16)
C(7)-N(1)	1.286(2)	O(1)-C(1)-C(6)	121.05(15)
N(1)-C(8)	1.415(2)	N(1)-C(7)-C(6)	120.79(16)
C(11)-O(2)	1.387(2)	C(11)-O(2)-C(14)	117.31(13)
O(2)-C(14)	1.399(2)	C(15)-C(14)-O(2)	120.58(18)

Table 3.Hydrogen bonds for HL_3 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...N(1)	0.84	1.81	2.5613(18)	147.6

3.3. Pharmacological studies

3.3.1. Brine shrimp cytotoxic assay

Brine shrimp cytotoxicity assay is a pre-screen test to observe the bioactive nature of compounds. The compounds HL_1 and HL_3 showed significant activity against brine shrimp nauplii to be highly active with LD_{50} values 0.18 and 0.06 µg/ml respectively. This cytotoxic action of a drug is simply provided by disturbing the basic mechanisms concerned with mitotic activity, cell growth, function and differentiation (Table 4).

Table 4. Results of cytotoxicity, potato disc antitumor and inhibition of hydroxyl free radical induced DNA damage assays.

Compound	Cytotoxic activity	Antitumor activity			DNA protection activity			
	LD_{50}value (µg/ml)	percentage inhibition± SD			IC_{50}			
		10(µg/ml)	100(µg/ml)	1000(µg/ml)	(µg/ml)	10(µg/ml)	100(µg/ml)	1000(µg/ml)
HL_1	0.18	40±1.8	82.5±0.8	95±0.4	14.20	+	+++	+++
HL_3	0.06	55±1.4	75±1.3	100±0	4.54	+	+++	+++

DNA: Deoxyribonucleic acid; LD_{50}: lethal dose 50 or median lethal dose; SD: standard deviation; IC_{50}: half maximal inhibitory concentration

3.3.2. Potato disc antitumor assay

The compounds were screened for possible antitumor activity by using potato disc antitumor assay and results are summarized in Table 4. The results of compounds HL_1 and HL_3 showed significant antitumor activity with IC_{50} values 14.20 and 4.51 µg/ml respectively. It is thought that these compounds at different doses may be used against different cancer-chemopreventive models and may appear to contrive safer drugs for future.

3.3.3. DNA protection assay

To check the antioxidant and pro-oxidant behavior of compounds free radical induced DNA damage assay was performed. With the attack of $^{\bullet}OH$ produced from the Fenton reaction, supercoiled plasmid DNA is broken into two forms, including open circular (OC) and linear form (linear). By analyzing the intensity of bands formed on 1% agarose gel, results are noted and tabulated (Fig.3). The compounds $HL_1$and HL_3 showed moderate protection at 1000 µg/ml and 100 µg/ml concentrations; while slight protection at 10 µg/ml concentration. So, it was concluded that these compounds showed DNA protection in concentration dependent manner i.e. DNA protection increased with the increase in concentration of the test compound.

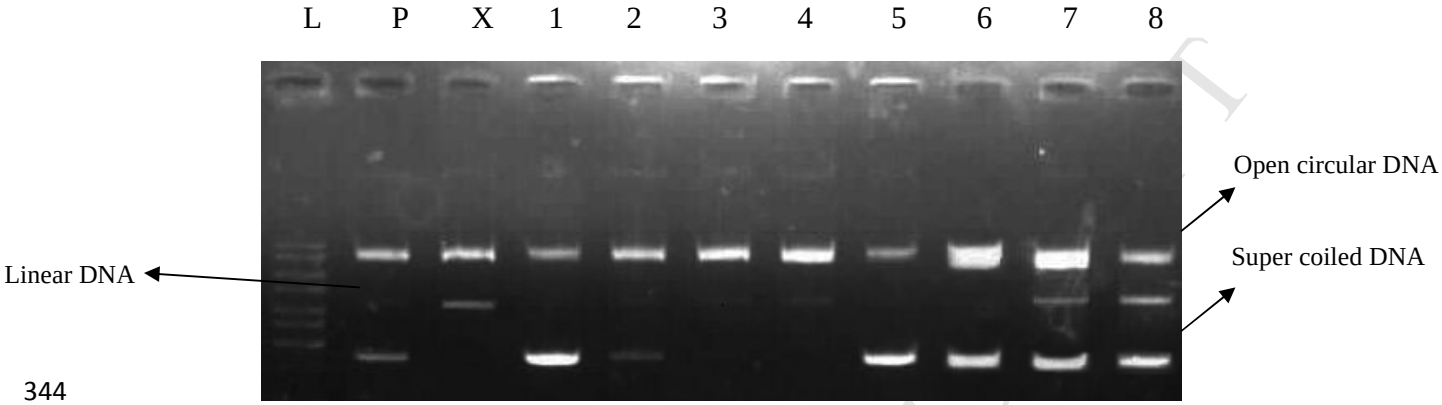


Fig.3 Effect of compounds $HL_1$and HL_2 on pBR322 plasmid DNA [L = 1Kb ladder, P = pBR322 plasmid, X = pBR322 plasmid treated with $FeSO_4$ and H_2O_2 (positive control), 1 (C+P) = pBR322 plasmid + 1000 µg/ml of HL_1 control for the pro-oxidant effect of the compound on DNA, 2 = plasmid + 1000 µg/ml of HL_1+ $FeSO_4$ + H_2O_2, 3 =plasmid + 100 µg/ml of HL_1+ $FeSO_4$ + H_2O_2, 4 = plasmid + 10 µg/ml of HL_1 + $FeSO_4$ + H_2O_2, 5 = pBR322 plasmid + 1000 µg/ml of HL_2; control for the pro-oxidant effect of the compound on DNA, 6 = plasmid + 1000 µg/ml of HL_2+ $FeSO_4$ + H_2O_2, 7 = plasmid + 100 µg/ml of HL_2+ $FeSO_4$ + H_2O_2, 8= plasmid + 10 µg/ml of HL_2+ $FeSO_4$ + H_2O_2

3.2 Voltammetric studies.

Electrochemical behaviour of the Schiff bases (HL_1-HL_4, 2mM) was investigated by employing three voltammetry techniques (CV, DPV and SWV) in oxygen free atmosphere in DMF solution. The cyclic voltammograms are displayed in Fig. 4. During anodic scan only one oxidation peak was observed for all the four Schiff bases, which appears in the range of 0.815 to 0.844 V, without corresponding reduction peak on the backward scan. This describes the irreversible oxidation of OH moiety present on the Schiff base molecule. To inspect the adsorption of the oxidation product, which is likely in such type of compounds, the successive scans were recorded at constant potential scan rate (100 mVs^{-1}). A systematic decrease in peak current was observed in following cycles for all the compounds revealing adsorption of oxidation product while hindering the further electron transfer process on the surface of working electrode. The GCE was very carefully renewed each time for a new compound. While increasing the scan rate for all the Schiff bases, it was found that oxidation peak current increases linearly with square root of scan rates as shown in Fig. 5 and follows the Randles-Sevcik equation of an irreversible process. The details about the equation and parameters are given in our earlier papers[29-32].The diffusion coefficients evaluated from the slope of anodic peak current vs. square root of scan rate were found to be 4.65×10^{-6}, 3.18×10^{-7}, 5.15×10^{-10} and 1.55×10^{-7} for HL_1, HL_2, HL_3 and HL_4 respectively. The values depict that the ET process is diffusion controlled.

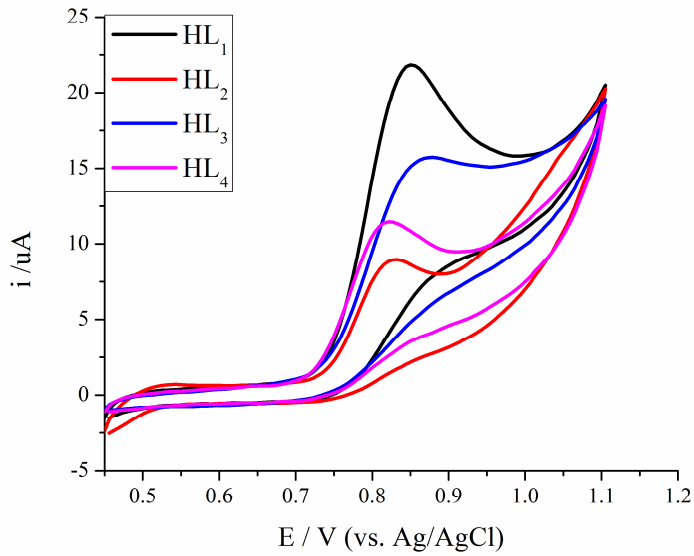


Fig.4 Cyclic voltammograms of Schiff bases HL_1, HL_2, $HL_3$and HL_4 (2mM each) recorded in their argon saturated H_2O/DMF (1/ 9; *v/v*) + 0.1M TBATFB solution at 100 m $Vs^{-1}(Vs^{-1})^{1/2}$scan rate at 25°C.

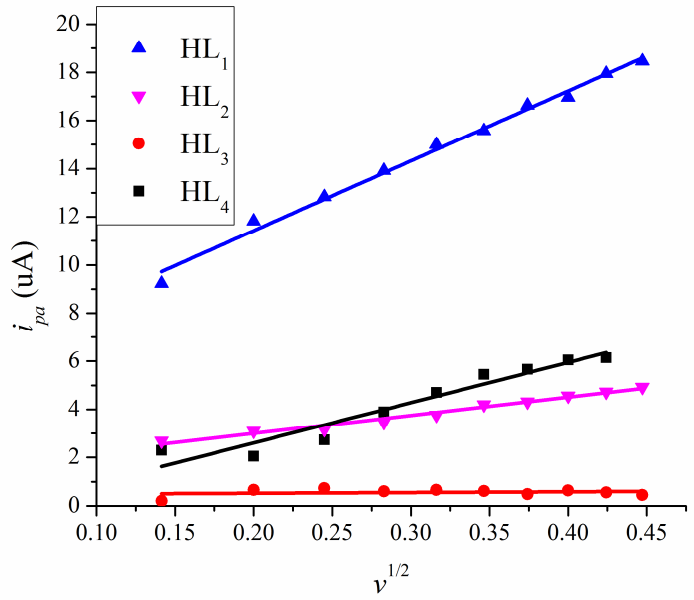


Fig. 5 Plots of anodic peak current (i_{pa}) with square root of scan rate $v^{1/2}/ (Vs^{-1})^{1/2}$for HL_1, HL_2, HL_3 and HL_4.

To further confirm the irreversibility of the ET process SWV was run. The resultant voltammograms of HL_1, HL_2, HL_3 and HL_4 are shown in Fig. 6 (A-D). The absence of reduction current in all the four cases evidently indicates the complete irreversible nature of ET process as shown in CV results.

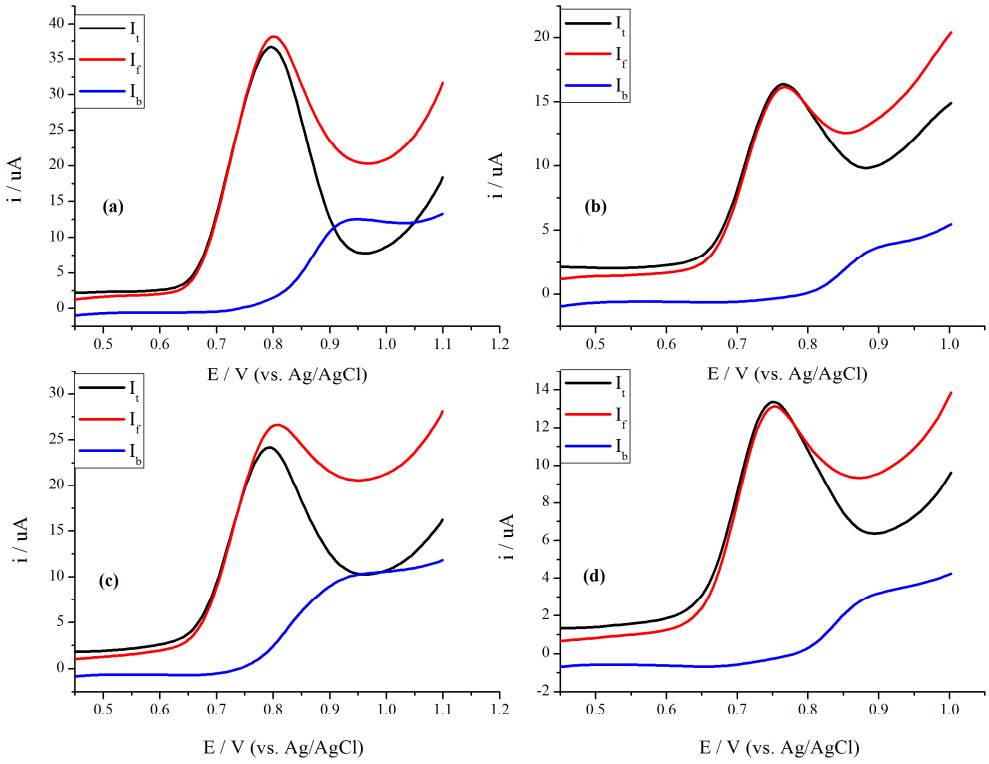


Fig.6 Square wave voltammograms of compound, HL_1(a), HL_2(b), HL_3(c) and HL_4(d) (2 mM each) recorded at GCE in argon saturated H_2O/DMF (1/ 9; *v* / *v*) + 0.1 M TBATFB solution at 25^oC, E_{sw} =5 mV frequency = 10 Hz . Pulse amplitude =25mV. The symbols are; I_t– total current, I_f – forward current, I_b –backward current.

DP voltammograms of the Schiff bases, displayed in Fig. 7, were recorded to estimate the number of electrons involved in the electrooxidation. The recorded peak currents are in the order of HL_1> HL_3> HL_2>HL_4 same as observed in CV studies indicating the enough sensitivity of CV for these compounds in the given time scale. The full peak width at half peak current($W_{1/2}$) values are 122, 88, 117 and 88 mV for HL_1, HL_2, $HL_3$and HL_4,respectively and are close to the theoretical value of 90 mV for ET process involving one electron only. Hence, it is concluded on the basis of voltammetry results that there is single electrophore in the studied Schiff bases offering one ET irreversible diffusion controlled oxidation process. Corollary of this result is that the electro-oxidised species will act as a good nucleophile for the DNA molecules.

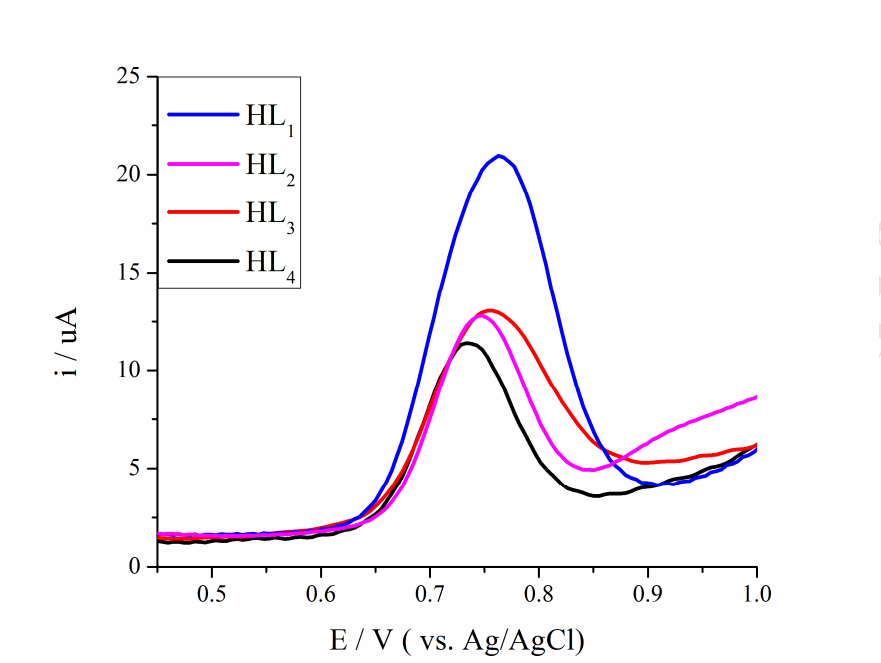


Fig.7 Differential pulse voltammograms of the Schiff bases with 2 mM each recorded in argon saturated H_2O /DMF(1/9; *v* /*v*) + 0.1M TBATFB solution at 25^oC.

4 DNA-drug interaction studies

The CV was employed to envisage the role of Schiff bases as drug molecules against DNA degradation. The characteristic parameters of the peak potential and peak current of a compound are prone to change on addition of DNA if the former is 'active'. The variation in peak current with varying concentration of DNA at fixed concentration of a Schiff base was utilized to determine the quantitative binding parameters (Fig. 8, Table 4) whereas the shift in peak potential was used to get an idea about the modes of interaction.

The values of binding constant (K) were calculated from equation 1 which determines the strength of binding of a molecule to the DNA.

$1/ [DNA] = K (1-A) / (1-I/I^0) – K$ (1) [33]

Where A is an empirical constant, I^0 and I are peak currents in the absence and presence of DNA, respectively.

From Fig. 8 and the tabulated data (Table 4) it is evident that on addition of first increment of DNA (5.44 µM) to the electro-oxidised product of the Schiff base there is a marked decrease in the peak current which is about two third of the original peak current. To ensure that this decrease is not due to adsorption of the molecule on the electrode surface, the GCE surface was renewed each time before running the next increment of DNA. On the other hand the addition of DNA caused a very small negative shift in the peak potential even up to the 16.01µM DNA. The negative shift in peak potential is revealing the electrostatic interaction between the electrooxidised electrophile of Schiff base and the negative phosphate component of DNA molecules. The result is in agreement to that conferred above in DPV analysis of the compounds. The sequential drop in peak current has attributed the decrease in free drug concentration due to formation of slow diffusing Schiff base-DNA adducts. Formation of this slow diffusing complex

is also evident from lowering in diffusion coefficient values upon the addition of DNA as compared to free compounds as shown in Table 4. The binding constant values given in Table 4 are in the range of the order of ≈ 10^4 M^{-1}. These moderately high values (K = 3.6 x 10^4 $M^{-1}$for cisplatin[34]) suggest that ON donor Schiff bases are efficient DNA binders.
As a whole it can be safely interpreted from the results that the investigated Schiff bases possess sufficient potential to be pursed as drug molecules whereas they were found interacting with DNA while being consumed chemically and partially due to electrostatic linkage.

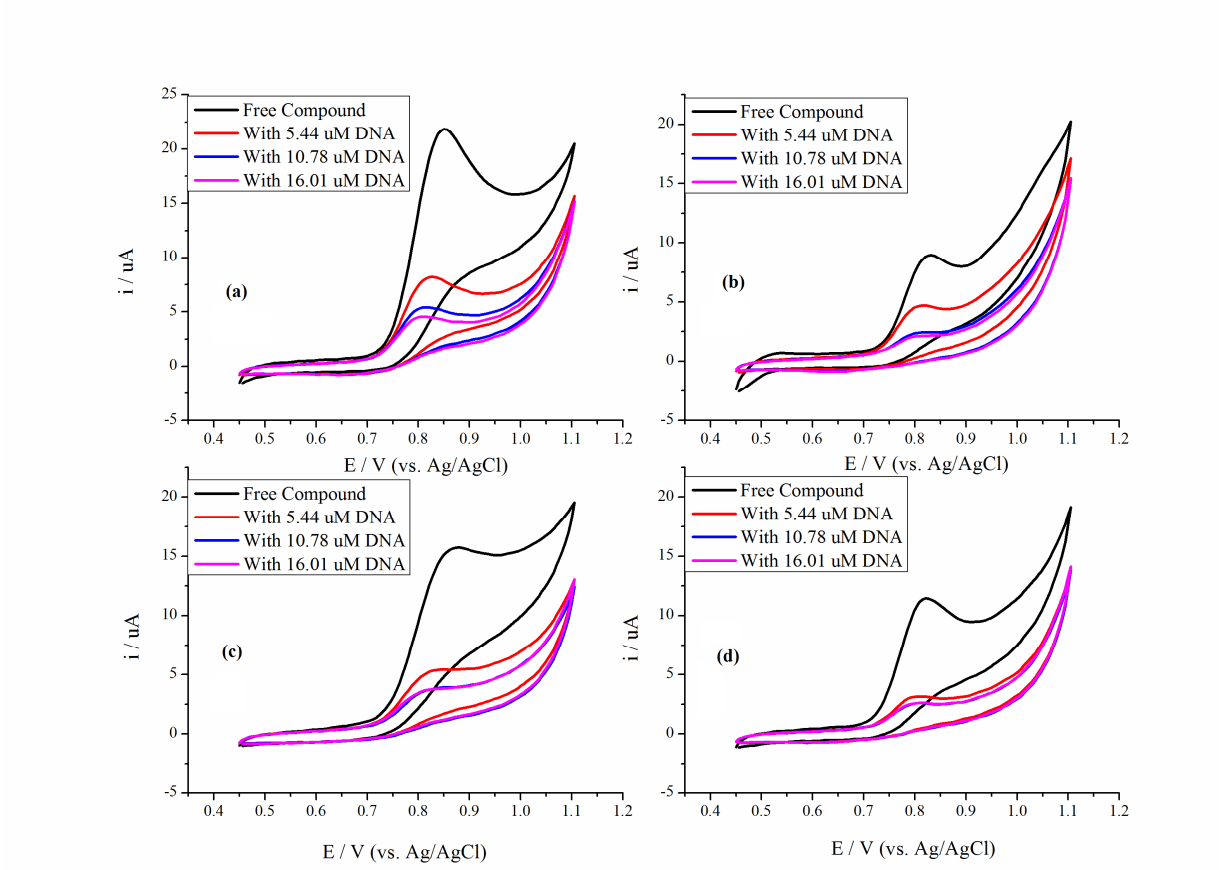


Fig.8 Cyclic voltammograms of 2mM H_2O/DMF (1/ 9; *v/v*) of HL_1 (a), HL_2 (b), HL_3 (c) and HL_4 (d) (—) without DNA, (—) in the presence of 5.44 µM DNA, (—) 10.78 µM DNA and (—) 16.01 µM DNA on glassy carbon electrode at scan rate of 100 mV/s.

Table 5. The drug-DNA interaction electrochemical parameters of compounds on glassy carbon electrode vs. Ag/AgCl in H_2O/DMF (1/ 9; *v/v*) solution at 50 mVs^{-1} scan rate at 25°C.

Compound	In the absence of DNA		In the Presence of DNA		
	E_p (V)	$D_o(cm^2 s^{-1})$	E_p (V)	$D_o(cm^2 s^{-1})$	K (M^{-1})
HL_1	0.844	4.65×10^{-6}	0.833	2.25×10^{-6}	2.04×10^4
HL_2	0.815	3.18×10^{-7}	0.809	2.27×10^{-7}	1.77×10^4
HL_3	0.838	5.15×10^{-10}	0.833	1.62×10^{-10}	4.16×10^4
HL_4	0.821	1.55×10^{-7}	0.809	1.12×10^{-7}	3.06×10^4

DNA: Deoxyribonucleic acid; D_o: diffusion coefficient; E_p: peak potential; K: complex stability constant.

4 Conclusions

Four novel ON donor Schiff bases (HL_1-HL_4) have been successfully synthesized and characterized by various spectroscopic, analytical and electro-analytical techniques. Single crystal X-ray diffraction analysis of Schiff base (HL_3) reveals intra (hydrogen bonding) and inter molecular interactions(C-H…π type, π…π stacking between anthracene groups).The biological findings showed significant activity in inhibiting the tumour formation by *A. tumefaciens* on potato discs. These compounds also have potential to prevent H_2O_2 induced oxidative damage to pBR322 DNA. Overall these compounds displayed a variant degree of activity in all the assays, further investigations are required to explore the exact mechanism of biological activities which may be helpful to explore new and alternative chemotherapeutic agent(s) in clinical implications. The compounds were found to respond irreversible one electron diffusion controlled electrooxidation on glassy carbon electrode. Further, they were found to interact to DNA in electrooxidised form through combined mode of groove binding and electrostatically with sufficiently good strength to be pursued further as drug molecules.

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Highlights

- Four novel ON donor Schiff bases were synthesized with yields around 90%.
- Exhibited strong •OH scavenging activity in concentration dependent manner.
- Voltammetry revealed irreversible single electron oxidation with D_o ~10^{-10}-10^{-6} cm^2s^{-1}.
- Anticancerous nature, intercalating with DNA having binding constants of 10^4 M^{-s1}.