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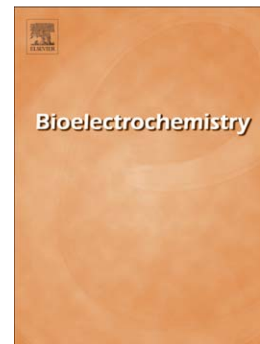

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Synthesis, biological and electrochemical evaluation of novel nitroaromatics as potential anticancerous drugs

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Abstract
Nitroaromatics i.e. 1-nitro-4-phenoxybenzene **(1)**, 4-(4-nitrophenyloxy) biphenyl **(2)**, 1-(4-nitrophenoxy) naphthalene **(3)** and 2-(4-nitrophenoxy) naphthalene **(4)** were synthesized by Williamson etherification and characterized by elemental analysis, FTIR, NMR (^{1}H, ^{13}C), UV-visible spectroscopy, mass spectrometry and single crystal X-ray diffraction analysis. Their brine shrimp cytotoxicity resulted LD_{50} values < 1 μg/mL indicating significant antitumor activity with IC_{50} values ranging from 29.0-8.4 μg/mL. They are highly active in protecting DNA against hydroxyl free radicals in concentration dependent manner. Voltammetric studies showed one electron reversible reduction at platinum electrode with diffusion coefficient (D_o) values of the order ~10^{-6}-10^{-7} cm^2s^{-1}. Strong interaction with the human blood DNA through intercalative mode was contemplated through electrochemical and UV-visible spectroscopic studies which are in agreement with the conclusions drawn from biological analysis, unravelling the potential anticancerous nature of the synthesized compounds.

Key Words: Nitroaromatics; Reversible electron transfer; Drug-DNA Intercalation

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1. Introduction

Nitroaromatic compounds have gained a huge prospective in the synthetic chemistry due to their great contribution in diverse applied fields. They have been used as synthons for a number of materials like polyamides, pesticides, agrochemicals, azodyes, and explosives etc., including drugs. Many pharmaceuticals like phenothiazines, paracetamol and antibiotics are synthesized from nitroaromatics [1, 2]. These are also used as anticancer drugs owing to their inhibiting ability of DNA replication [3-5]. The interaction of drugs with DNA is the central aspect of biological studies, in pharmaceutical development and drug discovery processes. Structural properties of DNA, origin of diseases and mechanism of antitumor activity are helpful to design new and more efficient DNA targeted drugs [6, 7]. Nitroaromatics have great affinity for DNA binding due to their structural features and show various modes of interactions like electrostatic, intercalative and groove binding which can be monitored by cyclic voltammetry, UV–vis., fluorescence, Raman and NMR spectroscopy but cyclic voltammetry is the reported reliable method for DNA interaction studies [8-13].

Herein we report the synthesis, characterization, electrochemical and biological evaluation of 1-nitro-4-phenoxybenzene **(1)**, 4-(4-nitrophenyloxy) biphenyl **(2)**, 1-(4-nitrophenoxy) naphthalene **(3)** and 2-(4-nitrophenoxy) naphthalene **(4)**. The biological activities of the compounds were evaluated by brine shrimp cytotoxicity, potato disc antitumor and hydroxyl free radical ($^{\bullet}$OH) scavenging assays. The cytotoxic ability was further complemented by voltammetric and UV-vis spectroscopic studies while quantitating and determining the mode of interaction with the human blood ds-DNA.

2. Material and methods

2.1. Chemicals

Anhydrous K_2CO_3, 4-chloronitrobenzene, phenol, biphenyl-4-ol, 1-naphthol, 2-naphthol, tetrabutylammonium perchlorate (TBAP), N,N□-dimethylformamide(DMF) and ethanol, were purchased from Sigma Aldrich. Solvents were purified and dried by standard techniques [14].

2.2. Instrumentation

Elemental analysis was carried out on CHNS 932 (Leco-USA) elemental analyzer. Melting points were determined, using a MPD Mitamura Riken Kogyo (Japan) electro thermal melting point apparatus. FTIR spectra were recorded on a Bio-Rad Excalibur FTIR, model FTS 4800 MX spectrophotometer (USA) in the frequency range of 4000-400 cm^{-1}.^{1}H and ^{13}C NMR spectra were obtained on a Bruker 300 MHz NMR Spectrophotometer in deuterated chloroform using teteramethylsilane as internal reference. GC-MS spectra were recorded in methanol on a micromass platform II instrument. Single crystal X-ray data for **1** and **4** were collected on a Bruker Apex II CCD diffractometer at 150(2) K while for **3** was collected on a STOE IPDS-II two-circle diffractometer at 173(2) K. All data sets were measured using graphite monochromated MoKα, radiation (λ= 0.71073Å).UV-visible spectra were recorded with a systronic double beam UV-visible Spectrophotometer 2202 in the range 200-800 nm. Electrochemical studies (cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV)) were carried out using Eco Chemie Autolab PGSTAT 302 potentiostat/galvanostat operated through GPES 4.9 software (Utrecht, The Netherlands). Conventional three-electrode cell with Ag/AgCl as a reference, platinum wire as counter and platinum disc (0.025 cm^2) as working electrode were used. DNA interaction studies by CV were performed by employing glassy carbon (GC) disc (0.09 cm^2) working electrode.

2.3. Procedure for analysis

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The toxicity of the compounds was determined by brine shrimp cytotoxicity assay [15]. Brine shrimp (*Artemia salina*) eggs (Ocean Star Inc., USA) were hatched in seawater (34 g/L) under constant aeration at room temperature. After 24 hours of hatching, phototropic nauplii (brine shrimp larvae) were collected by pipette from the lightened side separated by the divider from their shells. Ten shrimps were transferred to each vial using Pasteur pipette and the volume was raised up to 5 mL with artificial seawater having concentrations 10.0, 1.0, 0.5, 0.25, 0.125 and 0.0625 µg/mL of each tested compound. Three replicates were prepared for each concentration. The vials were maintained under illumination at room temperature. After 24 hours of incubation survivors were counted with the aid of 3× magnifying glasses. LD_{50} (lethal dose that killed 50% of shrimps) values were calculated by using Finny computer program [16]. The antitumor activity of compounds was examined by potato disc antitumor method [17]. In this assay inoculum was prepared with 24-48 hours old AT-10 strain of *Agrobacterium tumefaciens* having concentrations of 10, 100, 1000 µg/mL. Red skinned potatoes were surface sterilized in 0.1% mercuric chloride ($HgCl_2$) solution in distilled water for 7-10 minutes and were washed with autoclaved distilled water. Ten potato discs (5 mm × 8 mm) per plate were placed on solidified agar. Then 50µL of inoculums was applied to the top of each disc and petriplates were wrapped with parafilm strips and were placed in incubator at 28^oC.After 21 days, number of tumours was counted by staining with the Lugol's solution (10% KI and 5% I_2) and percentage inhibition was determined by using formula (Percentage inhibition = 100 – average number of tumors of sample/average number of tumors of negative control × 100)
DNA protection activity of the compounds was conducted in vitro by inhibition of hydroxyl free radical induced DNA damage assay [18].Reaction mixtures of 15 µL containing 0.5 µg pBR322, 3 µL of 50 mM phosphate buffer (pH = 7.4), 3 µL of 2 mM $FeSO_4$, 4 µL of 30% H_2O_2 and 5 µL of compounds having concentrations 10, 100 and 1000 µg/L were prepared. The mixture was incubated at 37 °C for 1 h. pBR322 treated with 2 mM $FeSO_4$ + 30% H_2O_2 (positive control), untreated pBR322 (negative control) and damaging effect of compound on DNA(compound control) were used.. The mixtures were subjected to 1% agarose gel electrophoresis in 1× Tris/Borate/EDTA, buffer. DNA bands (linear, open circular and super coiled) were compared with ladder (1Kb) after staining with ethidium bromide. Gels were analysed qualitatively by scanning with Gel-Doc (Bio Rad) computer program and intensity of the bands was determined.
DNA binding study by UV-visible spectroscopy was carried out by dissolving samples in the solvent mixture consisting of methanol and water (9:1, *v: v*). DNA was added in different concentrations starting from lowest concentration to higher concentrations (0.5×10^{-6} M, 1.0×10^{-6} M, 0.5×10^{-5} M and 1.0×10^{-5} M) keeping the compound concentration in the reaction mixture constant. The mixture was equilibrated for 5 minutes before recording the spectrum. The concentration of DNA stock solution was determined spectrophotometrically by calculating the absorption at 260 nm (characteristic λ_{max} value) and using molar absorption coefficient value 6600 M^{-1} cm^{-1} [19]. The purity of DNA solution was checked by ratio of absorbance at 260 nm and 280 nm (A260/A280) > 1.8 [20].
Electrochemical analysis was performed by making stock solutions of compounds in DMSO. Analyte solution of required concentration, containing tetrabutylammonium perchlorate (TBAP) of 0.1 M as supporting electrolyte was prepared by diluting the stock solution and resultant 10 mL solution was taken in electrochemical cell. Nitrogen gas was purged for 10 minutes prior to each voltammetric experiment. The GC surface was polished by alumina powder followed by extensive rinsing with acetone, water and respective solvent for reproducible readings. For studying DNA interactions by cyclic voltammetry, solutions of the compounds were prepared in

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water and DMSO (1:9, *v: v*) and DNA binding was studied at scan rate of 100 mV/s by stepwise increasing concentration of DNA.

3. Results and discussion

3.1. Chemistry

Nitroaromatics (1-4) were successfully synthesized by refluxing corresponding aromatic hydroxy compound, anhydrous K_2CO_3 and 4-chloronitrobenzene following the literature procedures [21] and were purified by recrystallization in ethanol at room temperature (scheme S1).

The structures of the synthesized 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 spectra exhibited characteristic absorption bands of nitro functionality at 1506-1508 cm^{-1} and 1339-1342 cm^{-1} corresponding to asymmetric and symmetric stretches respectively. A characteristic broad absorption band at 1244-1260 cm^{-1} was due to C-O-C whereas the band at 1585-1610 cm^{-1} was assigned to C=C vibrations (aromatic) [22,23](fig.S1).

The NMR (^{1}H and ^{13}C) spectra for the compounds in $CDCl_3$ were recorded. The chemical shifts values were assigned to protons by their intensity, multiplicity patterns and integration values which matched well with the expected molecular structures of the compounds. The synthesized compounds have phenyl (aromatic) protons only which showed their characteristic signals between 8.00-6.00 ppm, however the protons at ortho positions of nitro moiety were strongly deshielded due to its electron withdrawing effect displaying downfield signals at 8.26-8.19 ppm.

^{13}C NMR spectra also exhibited the characteristics signals. The carbon atoms of the aromatic rings attached directly to the oxygen atoms of ether linkage were strongly deshielded showing downfield signals at 163.96-154.21 ppm, carbons directly bonded with nitrogen of nitro moiety were comparatively less deshielded appearing at 145.54-141.83 ppm while rest of the carbons showed peaks ranging from 137.47-118.93 ppm.

The mass spectral data of the nitroaromatics justified their formation as molecular ion peaks were obtained at (m/z) 215, 291, 265 and 265 for compounds **1, 2, 3** and **4** respectively.

The UV-vis spectra of nitroaromatics in ethanol were measured in 200-800 nm range. The bands in the region of 205-256 nm corresponds to π-π^* transitions (benzene rings) [24].

3.2. X- ray crystallographic analysis

Single crystals suitable for X-ray diffraction analysis of compounds **1, 3** and **4** were grown by slow evaporation of ethanolic solution at ambient temperature. The structures were solved by direct methods using the program SHELXS and refined against F^2 with full-matrix least-squares techniques using the program SHELXL-97 [25]. H atoms were geometrically positioned and refined using a riding model with C-H = 0.95 Å. and U (H) = 1.2 U_{eq}C.

The general features of the molecular structures of these compounds are very similar (tables S1 & S2) and their perspective views are shown in schemes 1-3. The aromatic moieties are essentially planar and the dihedral angles between the two ring systems in each molecule are broadly comparable 63.58(5), 58.87(3) and 79.63(3)º for compounds **1, 3** and **4** respectively.

(Here Schemes 1-3)

In compound **3** the nitro group is essentially coplanar with the ring to which it is attached (torsion angle O3-N1- C4-C3 - 0.38(17)^o but there is a notable twist in this angle for **1** (-76.34(16)º) and **4** (-175.21(11)º). It is possible that this rotation is a consequence of intermolecular interactions involving the nitrophenol groups. In compound **1** pairs of nitrophenol

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groups show π-π interactions with centroid-centroid distance 3.699Å. There is also weak C-H···O hydrogen bonding to O3 from C6 (3.405(2) Å under 1-x, ½+y, ½-z) and from C12 (3.534(2) Å under 1-x, -y, -z); additionally, there is a long C-H···O interaction linking C5 to O1 (3.713(2) Å under 1-x, -½+y, ½-z). These interactions are shown in figure 1. Similar interactions occur in **4** (fig. 2), again the molecules are linked in pairs (under symmetry operation 2-x, -y, 1-z). The centroid-centroid distance for the nitrophenol groups is 3.742 Å and there is a C-H···O hydrogen bond linking C16 to O3 of a symmetry related molecule (3.538(2) Å under 2-x, -y, and 1-z). Surprisingly, there are no significant interactions involving the naphthyl group (although there is some further long C-H···O interactions).

(Here Fig.1 & Fig.2)

3.3. Biological studies

3.3.1. Cytotoxic activity

Brine shrimp lethality assay is a prescreen test to check the bioactive nature of compounds as anticancerous. The LD_{50} data (table 1) showed that all the compounds are cytotoxic with LD_{50} values in the range of 0.2-0.9 µg/mL. Toxicity of compound **3** was highest (LD_{50} value 0.2 µg/mL) while it was least for compound **2** (LD_{50} value 0.9 µg/mL)

(Here Table 1)

3.3.2. Antitumor activity

Antitumor potato disc assay resulted IC_{50} values in the range of 8.4-29.0 µg/mL (table 1). These results indicated significant antitumor activity, proposing these compounds as potential antitumor agents.

3.3.3. Inhibition of hydroxyl (•OH) free radical induced DNA damage assay

Inhibition of hydroxyl (•OH) free radical induced DNA damage assay was performed in vitro in order to check the antioxidant and pro-oxidant behaviour of compounds at three different concentrations (10 µg/mL, 100 µg/mL and 1000 µg/mL). The assay is based upon Fenton reaction. In a Fenton reaction Fe^{2+} reacts with H_2O_2, resulting in the production of •OH, which is thought to be the most harmful radical to bio-molecules [26]. With the attack of •OH produced from the Fenton reaction, super coiled plasmid DNA (SC) is broken into two forms, including open circular (OC) and linear form (linear). The degree of pro-oxidant or antioxidant effect of the test samples is computed by observing topology and intensity of bands produced after each treatment [27]. By analysing the intensity of bands formed on 1% agarose gel, results are noted and tabulated (table 1, figs. 3 & 4). All the compounds showed good protection against •OH radicals with no significant difference at 1000 µg/mL and 100 µg/mL concentrations, while slight protection was observed at 10 µg/mL concentration. So the DNA protection was observed in concentration dependent manner.

(Here Fig.3 & Fig.4)

3.4. Electrochemical Studies

Electrochemical behaviour of compounds **1**, **2, 3** and **4** was investigated on platinum electrode employing cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) techniques. The response of each compound (1 mM) was recorded in their

argon saturated 0.1 M solution of DMSO at potential scan rate of 50 mVs^{-1} at 25 ^oC. The CV signals and the variation of peak current with potential scan rate are presented in figure 5. The later was used to calculate the diffusion coefficient (D_o) for the four nitroaromatics. The D_o values and other useful parameters are gathered in table 2.

(Here Table 2)

The voltammograms reveal a pair of well-defined and stable redox peaks with peak separation values (ΔEp) of ≈ 63 mV in the potential range of -1.2 to -0.7 V. The observed peaks are attributed to the one electron reduction of the nitro group into free radical nitro anion and gives the neutral compound on electrooxidation as given below in equation 1 [28].

$$R-NO_2 + e^- \rightleftharpoons R-NO_2^{-\cdot} \quad (1)$$

Both the ΔEp values and the cathodic to anodic peak current ratio ($i_{pc}/i_{pa} \approx 1$) establish the response as a single electron transfer (ET) reversible redox process. Further, the electrochemical data (table 2) reveal that the substituents have no effect on the redox potential but slightly affected the kinetics of the ET process as apparent from varied peak currents (fig. 5).

(Here Fig.5)

The reversibility of the redox process was also confirmed by SWV (fig. S2).
The DPV was used to estimate the number of electrons involved in the electrochemical process from the peak width value at half of the maximum height employing equation 2 [29]

$$W_{1/2} = 3.52RT/nF = 90.4/n \text{ mV} \qquad (2)$$

Where F is Faraday constant, n is the number of electrons and $W_{1/2}$ is the peak width at half height.
In the DPV graphical data (fig. 6) the peak currents are in the same order i.e. **1** > **2** > **3** and **4** as observed in CV studies (table 2). The facile ET process in **1** is supportive from its simple and planar structure. On the other hand **4** with lowest value of peak current informs about its structural hindrance to the charge transfer process. The observed $W_{1/2}$ values (≈ 107 mV) of all the four molecules are close to the theoretical value of 90 mV for one-electron transfer reversible process.

(Here Fig.6)

3.5. Drug-DNA interaction studies
3.5.1. DNA binding study by UV-Vis spectroscopy
The potential of an anticancerous drug depends on its ability to interact with DNA molecules in such a way that the further replication of the latter stops. To assess this ability the binding of such potent compounds to the DNA can be evaluated through absorption spectrum analysis study [30]. UV-vis spectra of the pure compounds in water methanol (1:9, *v:v*) mixture were recorded for their λ_{max} values in the UV-vis region, *i.e.* **1** and **2** featured close to 205 nm while **3** and **4** responded near 225nm. Hyperchromism effect was observed on incremental addition of DNA to

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the solutions in each case. This behaviour was recorded for the step wise addition of DNA at 0.5 $\times 10^{-6}$ M, 1 $\times 10^{-6}$ M, 0.5 $\times 10^{-5}$ M and 1 $\times 10^{-5}$ M concentrations. The spectral response for compound **1** is shown in figure 7, for compounds **2**, **3** and **4** in figure S4 while absorption spectrum of the DNA without drug in figure S5.Additionally, a slight red shift in the λ_{max} values was also noted, however, due to the substantial broadening in peak shape it was not quantifiable. The observed hyperchromism in this case is ascribed to the unstacking of the DNA base pairs as reported previously [31]. The observed red shift favours the classical mode of intercalation [32]. A weak bathochromic effect is suggestive of drug interacting with the grooves of DNA molecule. However, later in CV studies the intercalation mode of interaction is evidently proved.

(Here Fig.7)

3.5.2. DNA binding study by cyclic voltammetry

The interaction of nitroaromatics with the DNA was also studied electrochemically. The amount of 2.90, 5.60 and 8.10 μM DNA was added into the 2 mM solution each of compounds **1, 2, 3** & **4** and the CV response was recorded. It was observed that the anodic and cathodic peak currents decreased considerably accompanied by a positive shift of the oxidation and reduction peak potentials (fig.8).

(Here Fig.8)

The formal potential (E^o) was shifted towards less negative potential (table 3) in each compound.

(Here Table 3)

The systematic positive shift along with decrease in peak current is an indicative of intercalative mode of interaction [33] while clearly depicting the decrease in the amount of unbound drug molecule (the nitroaromatic used).

To quantify the interaction of the compounds with DNA, CV studies were performed at different scan rates. The variation of anodic peak current with square root of scan rate ($v^{1/2}$) for all the compounds was found linear both in the absence and presence of DNA (but with decreased slope, fig. S6) and indicated that the redox process of compounds remained diffusion controlled even in the presence of DNA. Expectedly, the diffusion coefficient values obtained for compound-DNA complexes are much smaller than that of free compounds, demonstrating that compound-DNA complexes diffused slowly as compared to free compounds (table. 3). This result could be attributed to the diffusion of an equilibrium mixture of the free and DNA-bound compound to the electrode surface [34]. To further support the argument, the values of complex stability constant (K) were calculated by using equation 3 [35] (fig. S7).

$$1/[DNA] = K(1-A)/(1-I/I^0) - K \quad (3)$$

Where A is an empirical constant. The K values, given in table 3, determine the strength of binding of a molecule to the DNA. Significantly, large K compared to the reported for different compounds [36, 37] suggest the potential ability of these nitroaromatics to interact with DNA. Further, the values are also higher than that of the typical intercalatorlumazine-DNA complex

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(1.74×10^4 M^{-1}) [38]. The number of binding sites (s) in terms of concentration of base pairs were calculated from equation 4 [39] and given in table 3 (fig. S7).

$$C_b/C_f = K \{[DNA]/2s\} \qquad (4)$$

Where C_f and C_b are the concentrations of free and DNA-bound species respectively. The C_b/C_f can be defined by equation $C_b/C_f = (I_o - I)/I$ as reported elsewhere 4 [40]. The values of binding site size depicts that the compounds **2** and **4** incorporate more than one base pairs of the DNA and thus give more strength to the interaction.

4. Conclusion

Biological, electrochemical and drug-DNA interaction studies of nitroaromatics **1, 2, 3** and **4** respectively were explored. Biological studies (cytotoxic, antitumor and inhibition of hydroxyl ($^{\bullet}$OH) free radical induced DNA damage assays) indicated significant anticancerous nature. Voltammetric investigations showed reversible one electron transfer for all the four nitroaromatics.The binding ability of the compounds with human blood DNA was established through high drug-DNA adducts formation constants of the order of 10^4 M^{-1}. Both UV-visible spectroscopy and voltammetry techniques deduced the intercalative mode of interaction of the compounds with DNA. The electrochemical findings contemplated the results of biological assays depicting the substantial anticancerous potential in the compounds.

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Table 1. 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} (μg/mL)	10 (μg/mL)	100 (μg/mL)	1000 (μg/mL)
		10 (μg/mL)	100 (μg/mL)	1000 (μg/mL)				
1	0.4	27.5±1.9	32.5±1.7	42.5±1.6	29.0	+	+++	+++
2	0.9	15.0±1.6	65.0±0.8	70.0±0.9	27.0	+	+++	+++
3	0.2	52.5±1.6	72.5±1.0	77.5±0.9	8.4	+	+++	+++
4	0.3	10.0±1.8	77.5±1.1	80.0±1.0	17.6	+	+++	+++

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

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Table 2. Electrochemical parameters of the nitroaromatics on Pt disc electrode vs. Ag/AgCl in DMSO at 50 m Vs^{-1} scan rate at 25°C.

Compound	E_{pa} (V)	E_{pc} (V)	ΔE_p (V)	E^o (V)	i_{pc}/i_{pa}	$D_o \times 10^7$ (cm^2s^{-1}) ± SD
1	-0.922	-0.985	0.063	-0.954	0.95	13.20±0.016
2	-0.916	-0.980	0.064	-0.948	0.94	7.58±0.015
3	-0.922	-0.985	0.063	-0.954	0.99	6.09±0.016
4	-0.922	-0.985	0.063	-0.954	0.97	4.59±0.073

E_{pa}: anodic peak potential; E_{pc}: cathodic peak potential; ΔE_p: peak separation; E^o: Standard electrode potential; i_{pc}/i_{pa}: cathodic to anodic peak current ratio; D_o: diffusion coefficient

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Table 3. The drug-DNA interaction electrochemical parameters of compounds on glassy carbon electrode vs. Ag/AgCl in aqueous DMSO (1:9, *v: v*) solution at 50 m Vs^{-1} scan rate at 25^oC.

	In the absence of DNA		In the presence of DNA			
Compound	E^o (V)	$D_o \times 10^7(cm^2s^{-1})$ ±SD	E^o (V)	$D_o \times 10^7(cm^2s^{-1})$ ± SD	$K \times 10^{-4}(M^{-1})$	s(bp)
1	-0.937	7.72±0.020	-0.895	4.3±0.02	2.1	0.7
2	-0.921	4.19±0.037	-0.901	2.5±0.02	8.0	2.6
3	-0.924	8.21±0.040	-0.912	4.5±0.05	4.1	0.4
4	-0.936	5.70±0.075	-0.892	2.7±0.09	3.4	2.8

DNA: Deoxyribonucleic acid; D_o: diffusion coefficient; E^o: standard electrode potential; K: complex stability constant; s: number of binding sites

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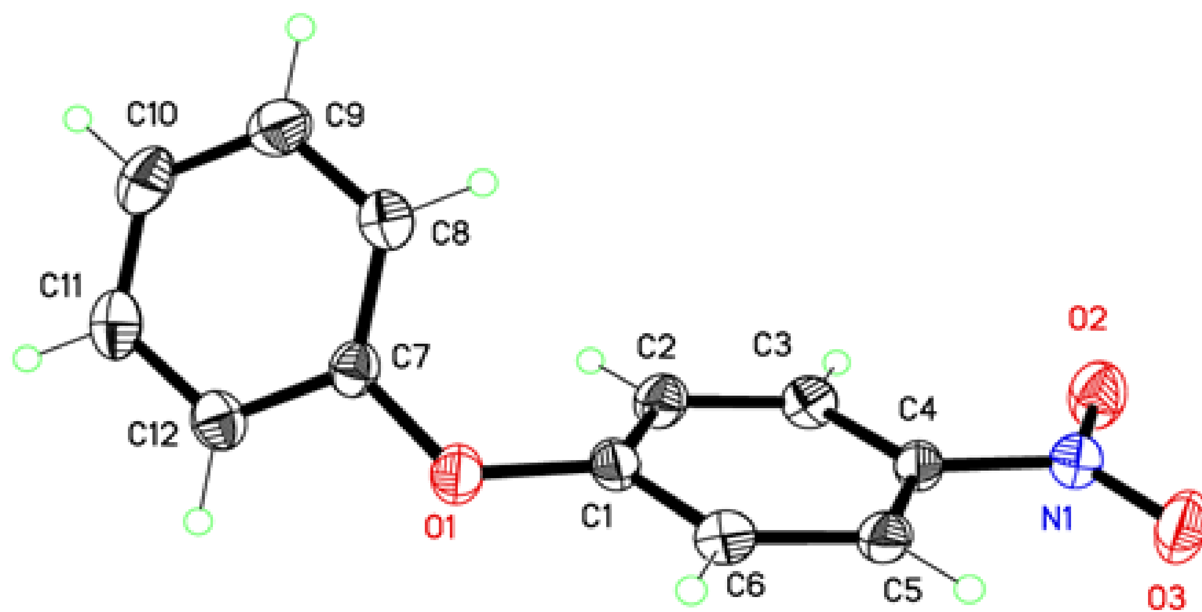
Scheme 1.



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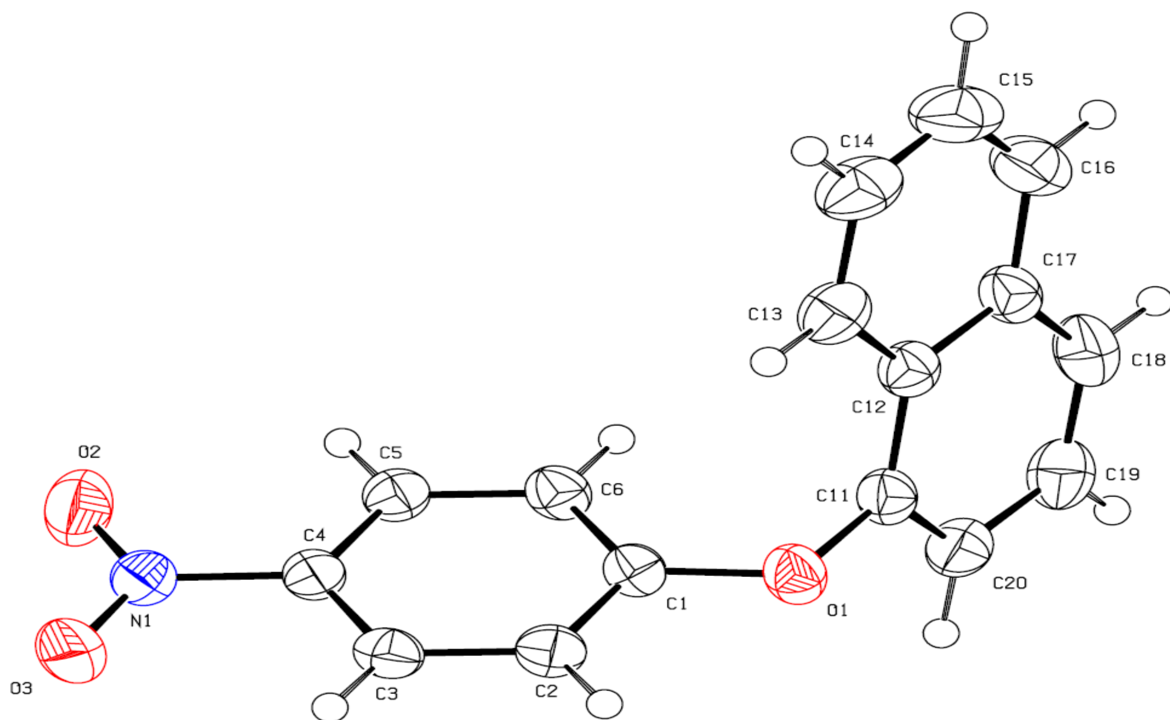
Scheme 2.



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Scheme 3.

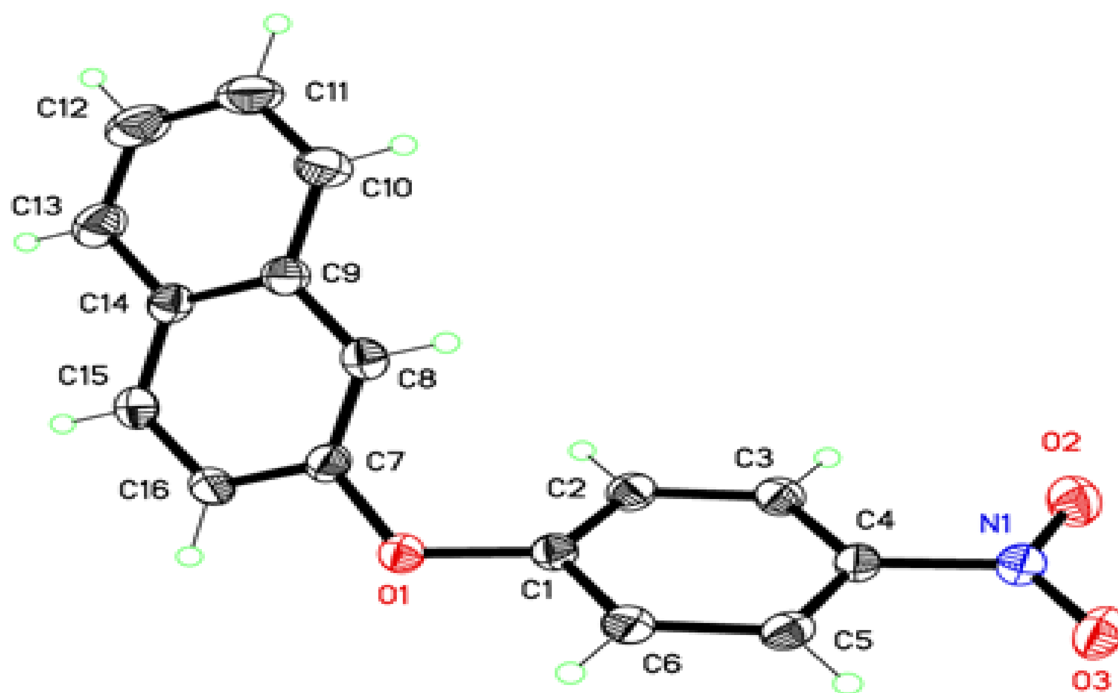

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Fig.1

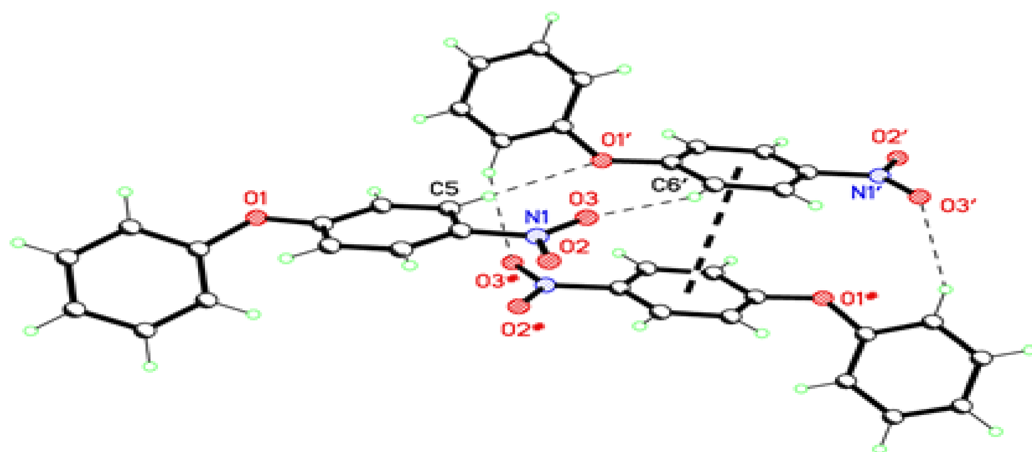

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Fig.2

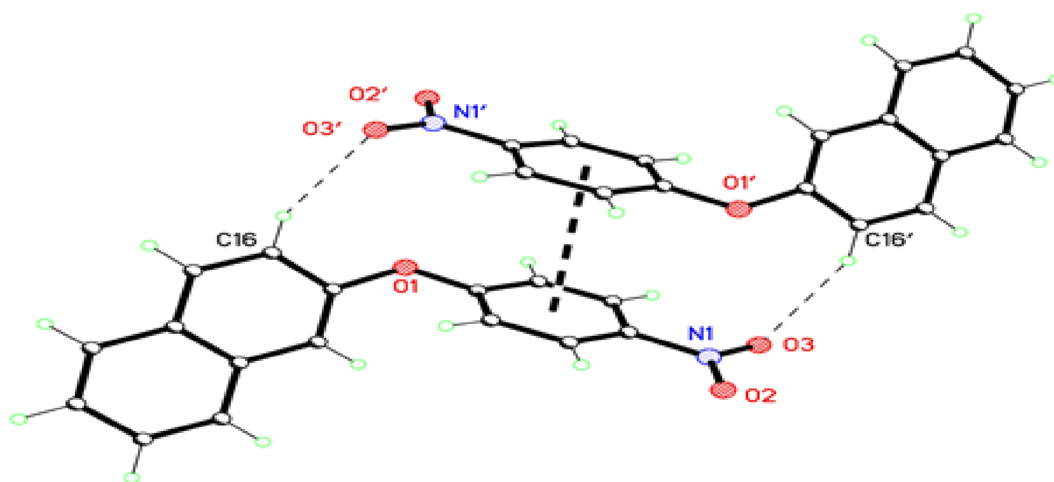

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Fig.3

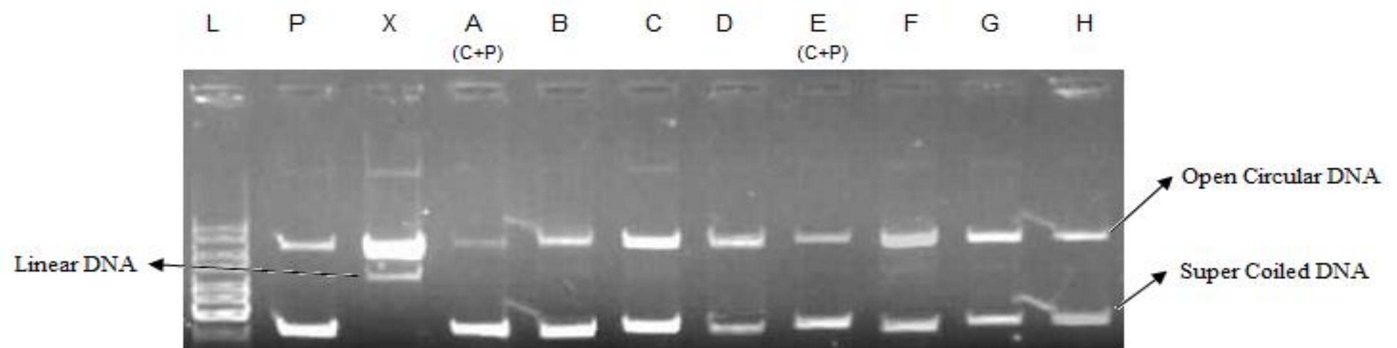

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Fig.4

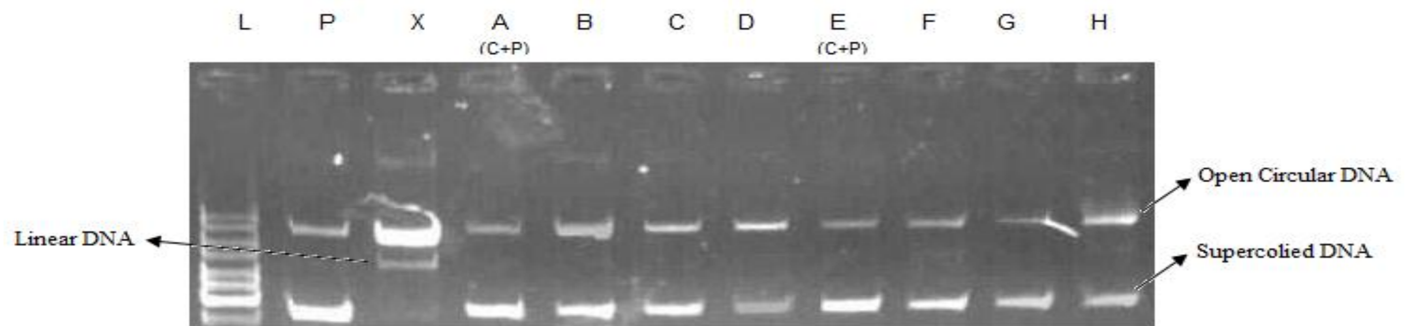

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Fig.5

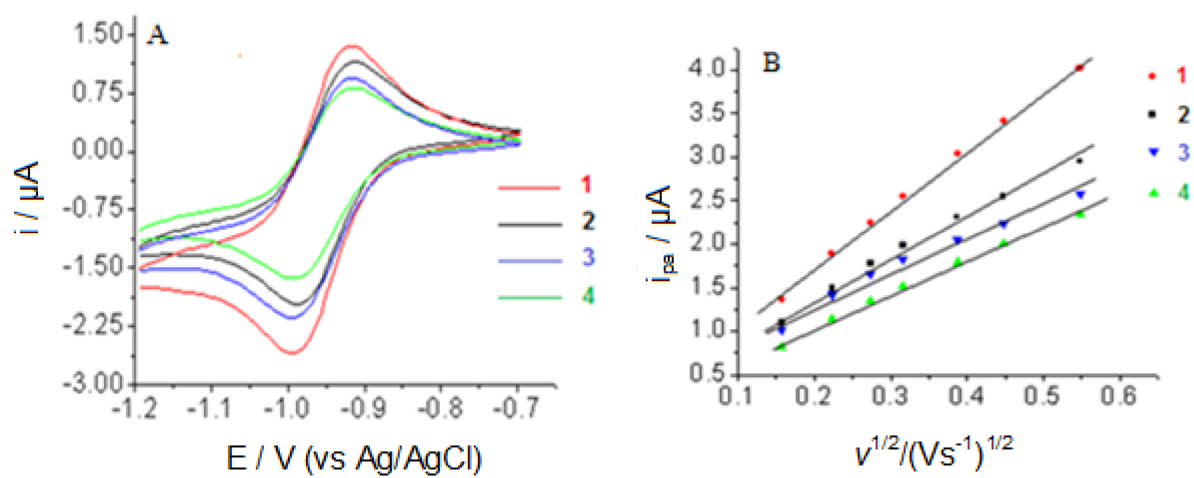

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Fig.6

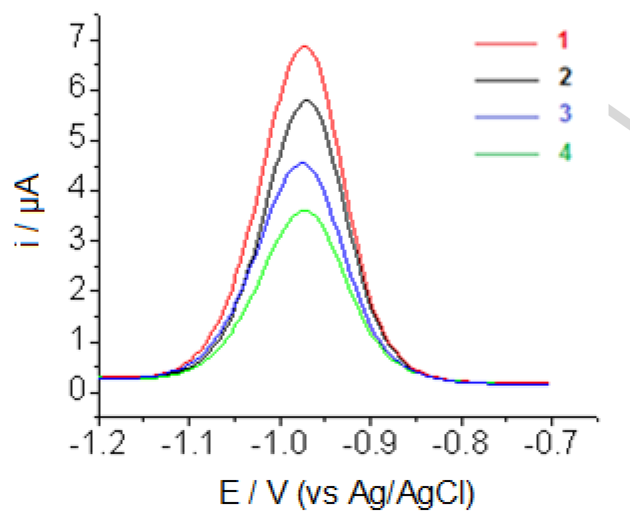

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Fig.7

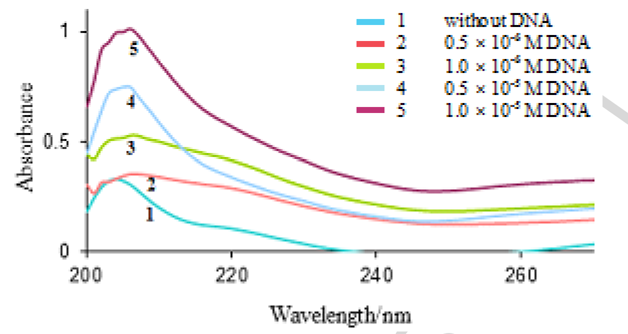

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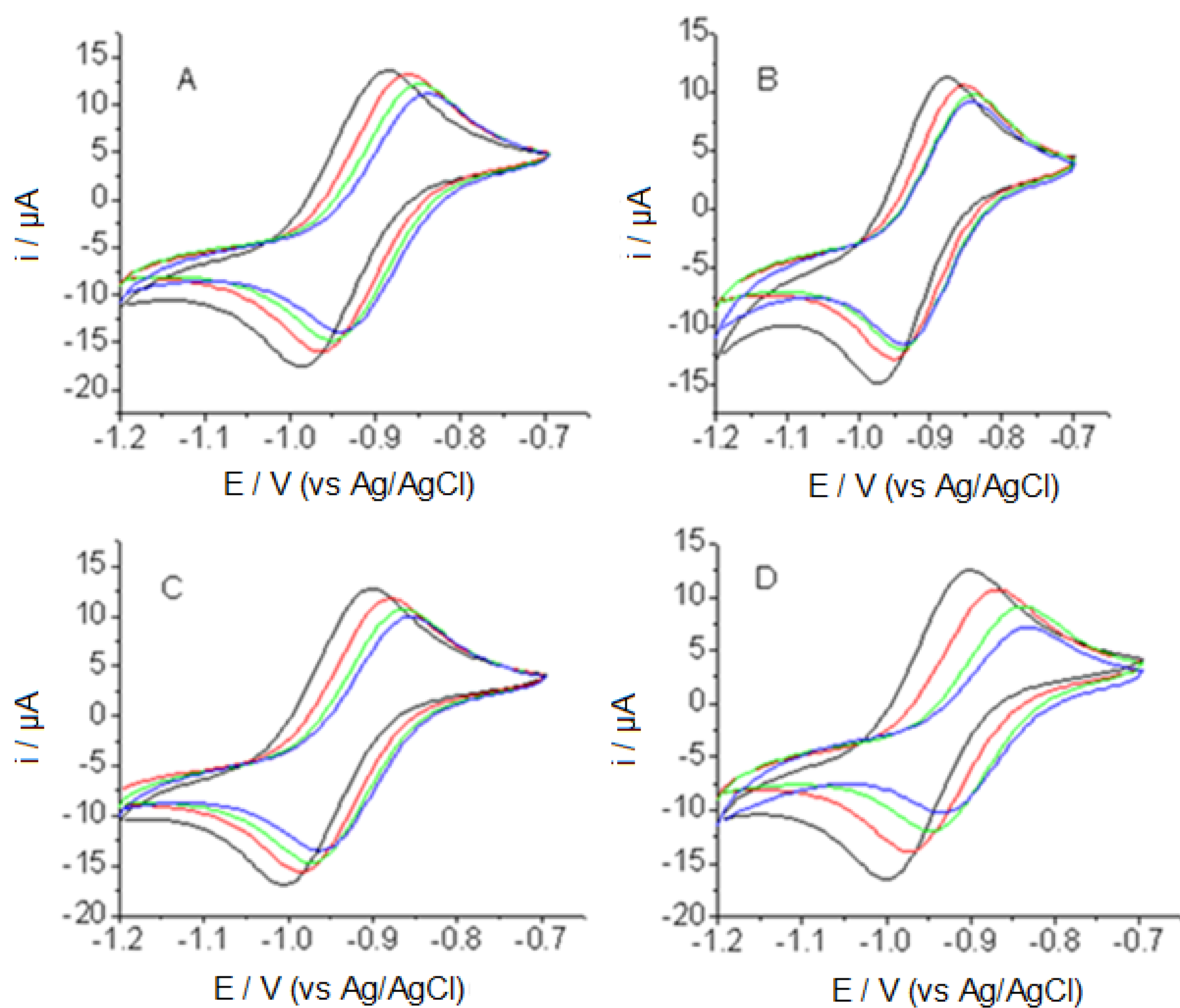
Fig.8



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Captions

Scheme 1. Crystal structure of compound **1**; thermal ellipsoids shown at the 50% probability level.

Scheme 2. Crystal structure of compound **3**; thermal ellipsoids shown at the 50% probability level.

Scheme 3. Crystal structure of compound **4**; thermal ellipsoids shown at the 50% probability level.

Fig.1. Intermolecular interactions in compound **1**; light dashed lines CH···O H-bonds, heavy dashed line π-π interaction (symmetry equivalents '1-x, ½+y, ½-z, # x, ½-y, ½+z).

Fig.2. Intermolecular interactions in compound **4**; light dashed lines CH···O H-bonds, heavy dashed line π-π (symmetry equivalents 2-x, -y, 1-z).

Fig.3. Effect of compounds **1** and **2** on pBR322 plasmid DNA [L = 1Kb ladder, P = pBR322 plasmid, X = pBR322 plasmid treated with $FeSO_4$ and H_2O_2 (positive control), A (C+P) = pBR322 plasmid + 1000 µg/mL of **1** control for the pro-oxidant effect of the compound on DNA, B = plasmid + 1000 µg/mL of **1**+ $FeSO_4$ + H_2O_2, C = plasmid + 100 µg/mL of **1** + $FeSO_4$ + H_2O_2, D = plasmid + 10 µg/mL of **1** + $FeSO_4$ + H_2O_2, E (C+P) = pBR322 plasmid + 1000 µg/mL of **2**; control for the pro-oxidant effect of the compound on DNA, F = plasmid + 1000 µg/mL of **2** + $FeSO_4$ + H_2O_2, G = plasmid + 100 µg/mL of **2** + $FeSO_4$ + H_2O_2, H = plasmid + 10 µg/mL of **2** + $FeSO_4$ + H_2O_2].

Fig.4. Effect of compounds **3** and **4** on pBR322 plasmid DNA [L = 1Kb ladder = pBR322 plasmid, X = pBR322 plasmid treated with $FeSO_4$ and H_2O_2 (positive control), A (C+P) = pBR322 plasmid + 1000 µg/mL of **3**; control for the pro-oxidant effect of the compound on DNA, B = plasmid + 1000 µg/mL of **3** + $FeSO_4$ + H_2O_2, C = plasmid + 100 µg/mL of **3**+ $FeSO_4$ + H_2O_2, D = plasmid + 10 µg/mL of **3** + $FeSO_4$ + H_2O_2, E (C+P) = pBR322 plasmid + 1000 µg/mL of **4** ; control for the pro-oxidant effect of the compound on DNA, F = plasmid + 1000 µg/mL of **4** + $FeSO_4$ + H_2O_2, G = plasmid + 100 µg/mL of **4** + $FeSO_4$ + H_2O_2, H = plasmid + 10 µg/mL of **4** + $FeSO_4$ + H_2O_2].

Fig.5. (A) Cyclic voltammograms of compounds **1, 2, 3** and **4** (1mM each) recorded in their argon saturated 0.1 M solution of DMSO at 50 m Vs^{-1} scan rate at 25°C. (B) Variation of anodic peak current (i_{pa}) with square root of scan rate $v^{1/2}/(Vs^{-1})^{1/2}$.

Fig.6. Differential pulse voltammograms of the nitroaromatics **1**(**—**), **2** (**—**), **3** (**—**) and **4** (**—**) with 1mM each recorded in their argon saturated 0.1 M solution of DMSO at 25°C.

Fig.7. Absorption spectra of 25µM aqueous-methanol mixture (1: 9; *v*: *v*) of compound **1** with varying concentrations of DNA at 25°C.

Fig.8. Cyclic voltammograms of 2mM aqueous-DMSO (1: 9; *v: v*) of **1** (A)**, 2** (B)**, 3** (C) and **4** (D) without DNA (**—**), in the presence of 2.90 µM DNA (**—**), 5.60 µM DNA (**—**) and 8.10 µM DNA (**—**) on glassy carbon electrode at scan rate of 100 mV/s.

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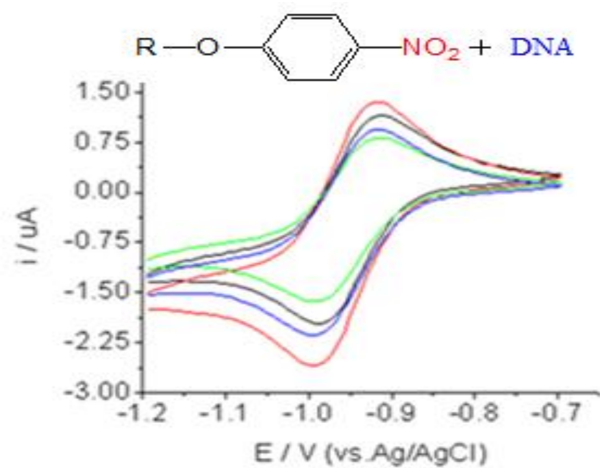
Graphical abstract



Four novel nitroaromatics were investigated for their cytotoxic, antitumor, •OH scavenges and DNA interaction studies through biological and electrochemical methods. The compounds showed significant anticancerous response.

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Highlights

- Four new nitroaromatics were synthesized with yields around 75%.
- Voltammetric studies revealed reversible one electron transfer with $D_o \sim 10^{-6}$-10^{-7} cm^2s^{-1}.
- Biological studies have indicated significant anticancerous nature.
- Drug-DNA interaction was shown by voltammetry and UV-visible spectroscopy.
- The compound-DNA adducts formation constants were in the range of $2\text{-}8 \times 10^4$ M^{-1}.

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