



Electrochemical Study of 6-Bromo-3-[N-(3-Chloro-Benzylidene)-Hydrazino]-Chromene-2-One by Cyclic Voltammetry, Its DNA Interaction Study using UV-Visible Spectroscopy

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ARTICLE DETAILS

Article history:

Received 22 September 2015

Accepted 03 October 2015

Available online 05 October 2015

Keywords:

6-Bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one

Cyclic Voltammetry

UV-Visible Spectroscopy

ABSTRACT

The present paper investigates the electrochemical behavior of 6-Bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one synthesized earlier and its interaction with salmon sperm fish DNA. Electrochemical study of these compounds done in 0.1 molL⁻¹ BR buffer (Britton Robinson) and 0.1M LiCl used as a supporting electrolyte by cyclic voltammetry. CV study reveal that synthesized compound show 1e⁻ diffusion control, irreversible reduction peak. The diffusion coefficients of these derivatives were also calculated, after that DNA-interaction study done by UV-Visible spectrophotometer that show coumarin interacts with DNA via intercalation mode involving outside edge stacking interactions with the oxygen atom of the phosphate backbone of DNA and the result of these type of interaction are denaturation of DNA strand which seem by hypochromism in the emission spectra of these complex. Binding/association constants of these complexes were also calculated in this paper.

1. Introduction

There has been considerable interest in the binding studies of small molecules with DNA owing to their diverse applications [1, 2]. DNA is the pharmacological target of many drugs that are currently in clinical use or are in advanced clinical trials [3, 4]. DNA has been the recognition and characterization, site for the interaction of small molecules as they yield effective information for the development of therapeutic agents for controlling gene expressions [5, 6]. Studying the interaction of pharmaceutical agents with DNA is also essential for understanding their mode of action and structural specificity of their binding reactions [7]. Interaction between small molecules and DNA provides a structural guideline in rational drug designing. It helps in the synthesis of new and improved drug entities with more selective activity, greater clinical efficacy and lower toxicity. Small molecules may bind to DNA double helical structures through three different modes (i) Electrostatic binding: occurs due to interaction between negatively charged DNA phosphate backbone and positively charged end of small molecules (ii) Intercalative binding: occurs when small molecules intercalate within stacked base pairs thereby distorting the DNA backbone conformation [8] (iii) Groove binding: occurs due to hydrogen bonding or Van der Waals interaction with nucleic acid bases and small molecules in the deep major groove or the shallow minor groove. Groove binders cause no or little distortion of the DNA backbone [9]. However, many small molecules can directly interact with DNA and the factors for these interactions are quite complex. Studying DNA as a drug target is attractive due to the availability of the genome sequence, well-studied three-dimensional DNA structure and the predictability of their accessible chemical functional groups. However, the number of known DNA-based drug targets is still very limited as compared to the protein-based drug targets [10].

Coumarin(1,2-benzopyrone), the parent molecule of coumarin derivatives, is the simplest compound of a large class of naturally occurring polyphenolic substances made of fused benzene and apyrone rings [11]. Coumarin is present in a wide variety of plants including cassia, lavender, yellow sweet clover, tonka beans, green tea, woodruff and in fruits such as bilberry and cloudberry. Coumarins have recently attracted much attention because of their broad pharmacological properties. Coumarin has been reported to exhibit antioxidant, anti inflammatory, anti-

mutagenic and anti-cancer properties [12-14]. In spite of vast pharmacological properties of coumarin, its mode of binding with DNA has not been elucidated. It is thus pertinent to study the interaction of coumarin with DNA to reveal how this compound may be further modified to enhance its biological activities. The source being natural dietary constituents, an understanding of the interactions of coumarin and other related derivatives has the potential to provide guidelines for the development of more potent compounds. Present study is concerned to give the information of redox behavior of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one by CV at carbon paste electrode and its interaction with deoxyribonucleic acid (DNA) by UV-Visible spectrophotometer.

2. Experimental Methods

2.1 Chemicals and Apparatus

Chemicals purchased from Sigma-Aldrich, Himedia and used without purification. Melting point was determined by using open capillary tube melting point apparatus. The IR spectra were recorded on a FTIR Shimadzu-8400S spectrometer using KBr pellets. The ¹H NMR and ¹³C NMR spectra were recorded on Varian 300 spectrometer taking TMS as standard and DMSO as a solvent. Sonication was done with the help of frontline sonicator (with a frequency of 22 KHz with a normal power of 225 W). The pH measurement was carried out by μ pH system 361 digital pH meter.

2.2 Electrochemical Cells and Voltammetric Parameters

Voltammetric experiments were carried out using a Metrohm 797 V.A. Computrace (Swiss made). Cyclic voltammetry were carried out using a carbon paste electrode ($d = 0.2$ cm) as working electrode, a Pt wire as a counter electrode, and a Ag/AgCl (3 M KCl) as a reference electrode, in one-compartment electrochemical cell. Carbon paste electrode ($d = 0.2$ mm) were prepared by mixing graphite powder with paraffin wax in 3:7 ratio, then it was sonicated for 1 min in an ultrasound bath and again rinsed with water. After this mechanical treatment, the carbon paste electrode was placed in three cell system. This procedure ensured very reproducible experimental systronics 2201 double beam UV-Visible spectrophotometer were used for performing drug-DNA interaction.

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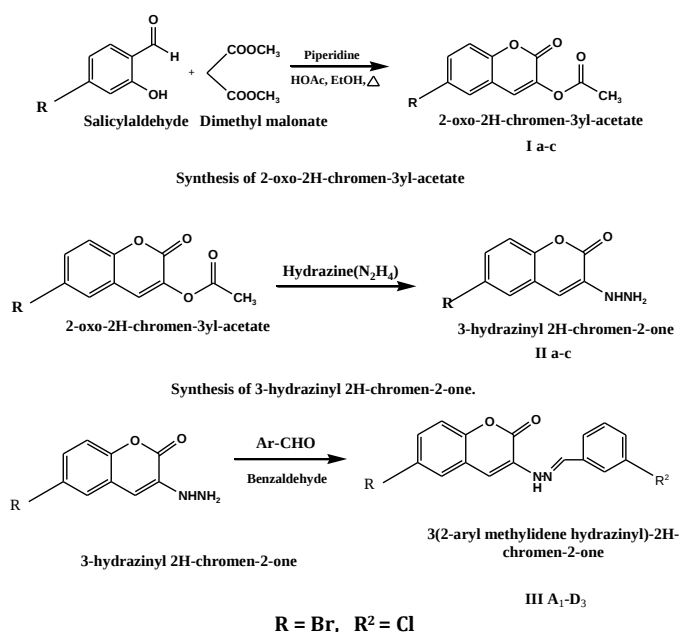
2.3 Sample Preparation

For cyclic voltammetry (CV) solutions were prepared by mixing 7.0 mL of 0.01 M stock solution and 1.0 mL of 0.1 M LiCl (as supporting electrolyte) and 2.0 mL of 0.1 mol L⁻¹ BR (Britton Robinson) buffer. Nitrogen gas was passed in the solution for ~15 minutes and, thereafter, a blanket of nitrogen gas was maintained throughout the experiment. During the experiment solution was subjected to controlled potential electrolysis. For UV-Visible study Salmon fish sperm DNA was used without further purification, its concentration was identified spectrophotometrically using the molar absorption coefficient $\epsilon_{277} = 2480$ cm⁻¹mol⁻¹. But here known concentration are used that are 0.0016, 0.00232, 0.00476 M and its 1.1, 1.6, 2.32, 4.76 mL volume will taking for analysis.

2.4 Synthesis of 6-Bromo-3-[N-(3-Chloro-Benzylidene)-Hydrazino]-Chromene-2-One

For 2–6 h, 0.01 mol of compound 3-hydrazinyl-2H-chromene-2-one and 0.011 mol of appropriate aromatic aldehydes and 25 mL of ethanol (96%) were refluxed. The solid that separate was filtered and recrystallized from ethanol.

FTIR (KBr) ($\nu_{\max}$ cm⁻¹): 1590(NH₂), 1550(N-N), 1100(C-N), 1625(C=N), 1675(C=C), 690–515 (C-Br), 910–630 (C-Cl), 1575 (C-C). ¹H NMR data - (ppm, 300MHz, TMS) - 7.7(m, 8H, Ar-H), 2.44 (s, 1H, NH), 6.55 (s, C-H). ¹³C NMR data - (ppm, 100MHz, DMSO) δ ppm: 162 (C=O), 120–128 (C=C), 20.9 (CH₃), 131.1(C=N).



Scheme 1 General scheme for the synthesis of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one.

3. Results and Discussion

3.1 Cyclic Voltammetric Studies

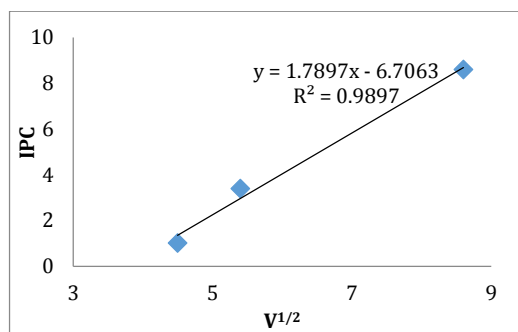


Fig. 1 Plot of peak current (I_{pc}) vs square root of the sweep rate ($v^{1/2}$) for 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one

The electrochemical behaviour of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one at a carbon paste electrode and this experiment were carried out in 0.1 mol L⁻¹ BR (Britton Robinson) buffer and 0.1 M LiCl as supporting electrolyte. Compound 6-bromo-3-[N-

(3-chloro-benzylidene)-hydrazino]-chromene-2-one show voltammograms at pH 10. During the voltammetric measurement a constant flux of N₂ was kept over the solution surface in order to check the diffusion of atmospheric oxygen into the solution. In this study purging time is 10 sec, deposition time is 60 sec. and deposition potential is -1.100 V. Several peaks were observed (Fig. 2). A study of effect of scan rate is made in order to find out the feasibility of electrochemical reactions and linear plots of I_{pc} vs $v^{1/2}$ are obtained, that show the reduction of derivatives in this medium is diffusion controlled with increasing scan rate (Fig. 1).

3.2 Electrochemical Study of 6-Bromo-3-[N-(3-Chloro-Benzylidene)-Hydrazino]-Chromene-2-One

The cyclic voltammogram of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one shows one electron reduction peak on different scan rate. The anodic half cycle and cathodic half cycle show one peak as mentioned in Table 1 and shown in Fig. 2. Good linear plots of I_{pc} vs $v^{1/2}$ are obtained that show the reduction of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one in this medium is diffusion controlled with employed scan rate (30, 50 and 75 mVs⁻¹) in Fig. 1. The shift of cathodic peak potential towards more positive values with the increase in scan rate indicates irreversible nature of the system [15].

Peak current for irreversible system is given by Randle Sevcik equation

$$I_{pc} = (2.99 \times 10^5) n (\alpha n_1)^{1/2} A C D_0^{1/2} v^{1/2}$$

Where A is the area of electrode in cm², D₀ the diffusion coefficient in cm² s⁻¹, C the concentration in mol l⁻¹ and v is in mVs⁻¹.

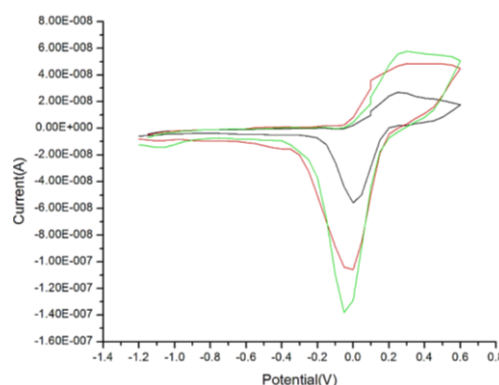


Fig. 2 Cyclic voltammogram at different scan rate (30, 50 and 75 mVs⁻¹) of 6-Bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one.

Table 1 Electrochemical parameters of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one.

E_{pc} (mV)	I_{pc} (pA)	Scan rate (mVs ⁻¹)	E_{pa} (mV)	I_{pa} (pA)	$D_0^{1/2} \times 10^{-3}$ (cm ² s ⁻¹)
0.29140	3.39475	30	0.00119	-7.60114	5.985

3.3 Drug –DNA Interaction Study by UV-Visible Spectroscopy

UV –visible absorption spectroscopy is very useful technique for study the drug alone or interaction with others molecules they may be small or large. It is very simplest, reliable and most commonly employed technique for study the drug–polynucleotide interaction. Drug–polynucleotide interaction are based on the absorption phenomena in the UV spectroscopy means each molecule show maximum absorption at particular wavelength, when these molecule interact with other molecule which may be small or large show maximum absorption at different wave length so, by analyzing the changes in the absorption properties (including $\lambda_{\max}$ or intensity of band) of drug and the drug–DNA complex. Generally drug works as a ligand exhibit an absorption maxima that can easily recognize in the visible region. An easy route for the determining the interaction between drug and polynucleotide (or protein or enzyme) is to scan the shifting the position of the absorption maximum when drug is free in the solution or when the drug is attached with polynucleotide. The magnitude of this displacing of the absorption band could explain as a signal for the analyzing the strength of the interaction between the DNA and drug molecule [16–18].

Drug–polynucleotide (DNA) interaction are different type it could covalent or non-covalent depends upon the force that present between them, which are identify by comparing the UV spectra of drug molecule and drug–DNA complex. If after interaction spectra show hypochromism and bathochromic shift (red shift) then its mean compound attached with

DNA through intercalation. Degree of hypochromism is depends on the strength of intercalation [19], and strength of intercalation depends on the distance between DNA and drug molecule, when distance decrease then intensity of absorption band decrease result an hypochromic shift and in this case difference between π bonding and π^* energy level also decrease, so electron transition from π bonding orbital of drug to π^* orbital of polynucleotide easily takes place then result a red or bathochromic shift [20–21].

On other hand hyperchromic effect is observed when drug molecule attach with DNA by electrostatic attraction (presence of cation), hyperchromism also reflect the structural or conformational changes in DNA molecule after binding with drug molecule, this phenomena occur due to presence of charged cation in the drug molecule, charge cation bind with more electro negative oxygen atom of phosphate group present on DNA backbone by electrostatic attraction [22] then hydrogen bonding between purine and pyrimidine base disrupted (A and T, G and C) and DNA denaturation takes place, by DNA denaturation purine and pyrimidine base are free as a result surface area (active site) increase so, the absorption intensity of band extremely increase about 40% more than free double strand DNA at the same concentration.

On the bases of maximum absorption in free drug and drug combine with DNA we can find out the binding/association constant of the drug with DNA according to Benesi-Hildebrand equation [23]

$$A_0/A-A_0 = \epsilon_D/\epsilon_{D-D}-\epsilon_D + \epsilon_D/\epsilon_{D-D}-\epsilon_D \times 1/K_b [\text{DNA}]$$

Where K_b is the association/binding constant, A_0 , and A are the absorbance of drug and its complex with DNA, respectively, and ϵ_D and ϵ_{D-D} are the absorption coefficient of the drug and the DNA- drug complex respectively. The association constant can be determined from the intercept-to-slope ratios of $A_0/A-A_0$ vs. $1/[\text{DNA}]$ plot.

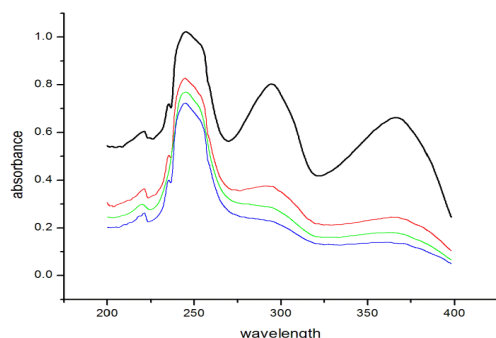


Fig. 3 UV visible absorption spectra of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one and arrow represent the increasing concentration of DNA in the solution.

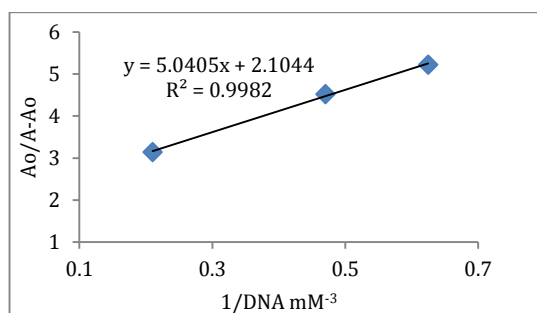


Fig. 4 Graph between $A_0/A-A_0$ vs. $1/[\text{DNA}]$ plot

Fig. 3 shows the interaction of 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one with DNA, this show with increasing concentration of DNA in the drug solution the intensity of absorption band decreases, so the consequence of DNA addition is hypochromism. Fig. 4 represents the plot between $A_0/A-A_0$ vs. $1/[\text{DNA}]$, with the help of this plot we can determine the binding constant of Drug-DNA complex i.e. $K_b = 4.174 \times 10^4 \text{ M}^{-1}$.

4. Conclusion

Electrochemical studies revealed that 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one are electro active as it undergoes oxidation and reduction processes at a carbon paste electrode. Compound shows one electron irreversible reduction. Diffusion coefficient $D_0^{1/2}$ for compound 6-Bromo-3-[N-(3-chloro-benzylidene)-

hydrazino]-chromene-2-one is $5.985 \times 10^{-3} \text{ cm}^2\text{s}^{-1}$. Drug-DNA complex interaction has been investigated by UV-Visible spectroscopy. All spectral data and figure indicate the well binding interaction between DNA and drug complexes. In the drug 6-bromo-3-[N-(3-chloro-benzylidene)-hydrazino]-chromene-2-one bind to DNA via intercalation mode involving outside edge stacking interactions with the oxygen atom of the phosphate backbone of DNA with binding constant $k_b = 4.174 \times 10^4 \text{ M}^{-1}$ respectively show hypochromism.

Acknowledgement

I express my thanks to Department of Chemistry, Dr. Harisingh Gour central University, Sagar (M.P) and the MPCST Bhopal, for the financial support in the form of research fellowship.

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