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CYCLIC VOLTAMETRIC AND DNA CLEAVAGE STUDIES OF INDOLYL CARBOHYDRAZIDES

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ABSTRACT
The indole analogues were investigated for the electrochemical behavior using the glassy carbon electrode as the working electrode. In the present study electrochemical reduction or oxidation of indole analogues in sulphuric acid media was reported. We can see that the C=N functional groups in the molecule is easily reduced at the glassy carbon electrode. These compounds were also subjected to DNA cleavage study. In this study Compounds 1a-c have shown highest activity and remaining compounds shown moderate activity. Compounds with halogen, methyl substitution at five positions and phenyl ring at third position of indole nucleus have exhibited very good activity when compared to standard.

Key words: Indole, Carbohydrazide, DNA Cleavage activity, Cyclicvoltametry

INTRODUCTION

Synthesis of indole analogues have found great significance due to their wide spectrum of biological and pharmacological activities [1].Indole derivatives were reported to have biological effects [2], anti-infammatory [3], antitubercular [4] and antimicrobial [5] activities. Tio-indole derivatives possess antifungal [6], antimicrobial [7], antibacterial, analgesic [8, 9], anticonvulsant [10], antioxidant [11], antidepressant [12], antihypertensive [13], and antiviral agents [14]. For the synthesis of heterocyclic compounds carbohydrazides are well known as useful building A large number of heterocyclic carbohydrazides derivatives are reported to exhibit significant biological activites [15,16]. Cyclic voltammetry (CV) is an widely used electroanalytical technique that allows studying redox processes of molecules particularly in organic and metal-organic systems, electrochemical behaviour of complex chemical and biochemical systems [17]. Indole analogues are well-known electro active compounds that are readily oxidized or reduced at carbon based electrodes, i.e. glassy carbon electrode [18-20]. Some new indole analogues with highly potent antioxidant, DNA cleavage, antimicrobial, anti-inflammatory, analgesic, CNS depressant, activities and cyclic voltametric studies [21-25]. were described previously. As the indole derivatives exhibit broad spectrum of biological activities we thought of making molecules which are bioactive compounds.

EXPERIMENTAL

CYCLIC VOLTAMETRY STUDIES

INSTRUMENTATION

The electrochemical experiments were carried out using a model-660 Electrochemical Workstation (CHI660c). All experiments were carried out in a conventional three-electrode system. The electrode system contains a working Glassy Carbon electrode, a platinum wire as counter electrode and saturated calomel electrode as reference electrode (a reference electrode, typically Ag/AgCl). Experimentally, the potential of a working electrode is linearly scanned Vs a reference electrode from an initial value to a final value and back. Thus, forward and backwards electrochemical reactions can be studied. This in a typical cyclic voltammetric experiment of substrate (indole derivative), reaction mixture consisted of indole derivative solution, ethyl alcohol and sulphuric acid media was used for the electrolytic reduction. The three electrodes were connected to a computer controlled potentiostat and required potential scan rate, current sensitivity, initial potential and final potential were fixed and the resulting current measured as a function of applied potential

Pre-treatment of glassy carbon electrode

Before each measurement, Glassy-carbon electrode is generally pre-treated either electrochemically or mechanically to obtain a reproducible electrode surface. Electrochemical pre-treatment is usually performed by cyclic scanning over a wide potential range. Mechanical polishing is generally performed by polishing the glassy-carbon electrode with alfa alumina powder (0.3μ) on rubbing pad and then rinsed with purified water until there were no visible markings or scratches. This procedure was repeated after each set of experiment.

DNA CLEAVAGE ACTIVITY

Preparation of culture media

DNA cleavage experiments were done according to the literature [26]. Nutrient broth [peptone, 10; yeast extract, 5; NaCl, 10; in (g/l)] was used for culturing of Escherichia coli. Fifty-milliliter media was prepared, autoclaved for 15min at 121°C under 15lb pressure. The autoclaved media were inoculated for 24h at 37°C.

Isolation of DNA

The fresh bacterial culture (1.5mL) was centrifuged to obtain the pellet which was then dissolved in 0.5mL of lysis buffer (100mM tris pH 8.0, 50mM EDTA, 10% SDS). To this 0.5mL of saturated phenol was added and incubated at 55°C for 10min, then centrifuged at 10,000 rpm for 10min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3M sodium acetate (pH 4.8) was added. Centrifuging at 10,000 rpm for 10min and to the supernatant, 3 volumes of chilled absolute alcohol were added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10mM tris pH 8.0, 1mM EDTA) and stored in cold condition.

Agarose gel electrophoresis

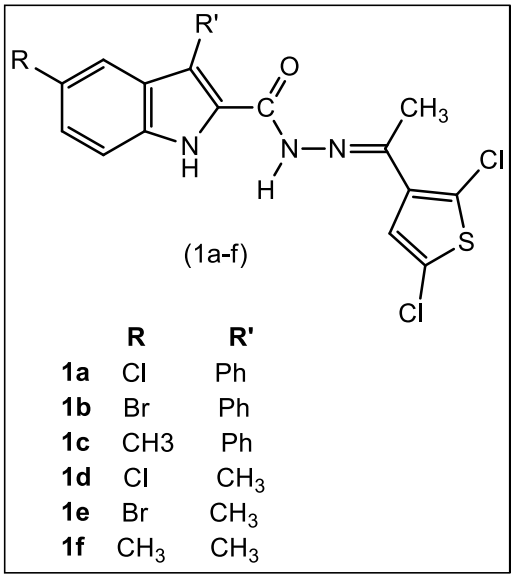
Cleavage products were analyzed by agarose gel electrophoresis method27. Test samples (1 μg/mL) were prepared in DMF. The samples (25μg) were added to the isolated DNA of E. coli. The samples were incubated for 2hr at 37°C and then 20mL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, 0.5M EDTA/1L) and finally loaded on agarose gel and passed the constant 50V of electricity for 30min. Removing the gel and stained with 10.0 mg/mL ethidium bromide for 10-15min, the bands were observed under Vilber Lourmat Gel documentation system and then photographed to determine the extent of DNA cleavage. The results are compared with standard DNA marker.

RESULT AND DISCUSSION

Cyclic Voltametry Studies

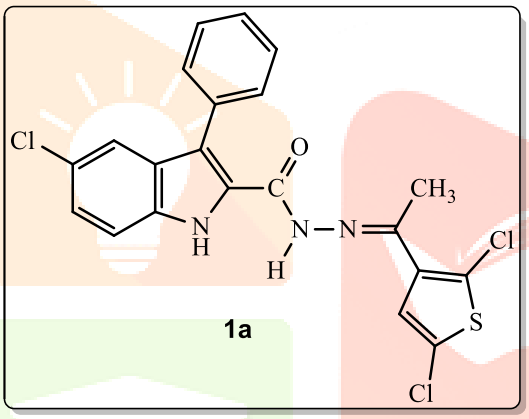
Cyclic voltametric studies of 3,5-Disubstituted-N'-(1-(2,5- dichlorothiophen-3-yl)ethylidene)-1H-indole-2-carbohydrazide (1a-f). 3,5-Disubstituted-N'-(1-(2,5-dichlorothiophen-3-yl)ethylidene)-1H-indole-2-carbohydrazide derivatives were synthesized by reported methods [23] scheme-1. Cyclicvoltammogram and reduced structure of 5-chloro-N'-(1-(2,5-dichlorothiophen-3-yl)ethylidene)-3-phenyl-1H-indole-2-carbohydrazide (1a) as shown in Scheme-2 (Fig. 01) at a glassy carbon electrode in 25mM sulphuric acid media at scan rates 100 mVs-1. The compound showed a cathodic peak potential at -0.636 V for the reduction of the C=N moiety. The quasirreversibility was confirmed by the presence of an anodic peak between the potential of +1.0 mV to –1.0 mV. The effect of scan rate was studied and cathodic peak current was proportional to the scan rate and the process is diffusion-controlled. From the Fig. 01, we can see that the C=N functional groups in the molecule is easily reduced at the glassy carbon electrode. The reduction potential value is -0.636V which has chloro substitution at five position of indole. Variation of scan rate from 50 mVs−1 to 250 mVs−1 is shown in Fig. 02. With the increase of scan rate, the quasireversibility becomes completely irreversible in nature and the absence of anodic peak in the reverse direction.

Cyclic voltammogram of (1-(2,5-dichlorothiophen-3-yl)ethylidene)-5-methyl- 3-phenyl-1H-indole-2-carbohydrazide (80c) (Fig. 03) studied at a glassy carbon electrode in 25mM of sulphuric acid media at a scan rates of 100 mVs-1. The compound showed a cathodic peak potential at -0.633 V for the reduction of the C=N function. The irreversibility was confirmed by the absence of an anodic peak between the potential of +0.8 mV to –1.0 mV. The effect of the scan rate was studied and the cathodic peak current was proportional to the scan rate and the process is diffusion-controlled. From Fig. 03 We can see that the C=N functional groups is easily reduced at the glassy carbon electrode. The reduction potential value is -0.633 V for methyl substituted at five position of indole. Variation of scan rate from 50 mVs−1 to 300 mVs−1 there is no change in the nature and remains irreversible as shown in Fig. 04.



	R	R'
1a	Cl	Ph
1b	Br	Ph
1c	CH3	Ph
1d	Cl	CH_3
1e	Br	CH_3
1f	CH_3	CH_3

Scheme1. Schematic representation of indole analogues (1a-f).



Scheme 2. Probable structure of reduced compound 1a

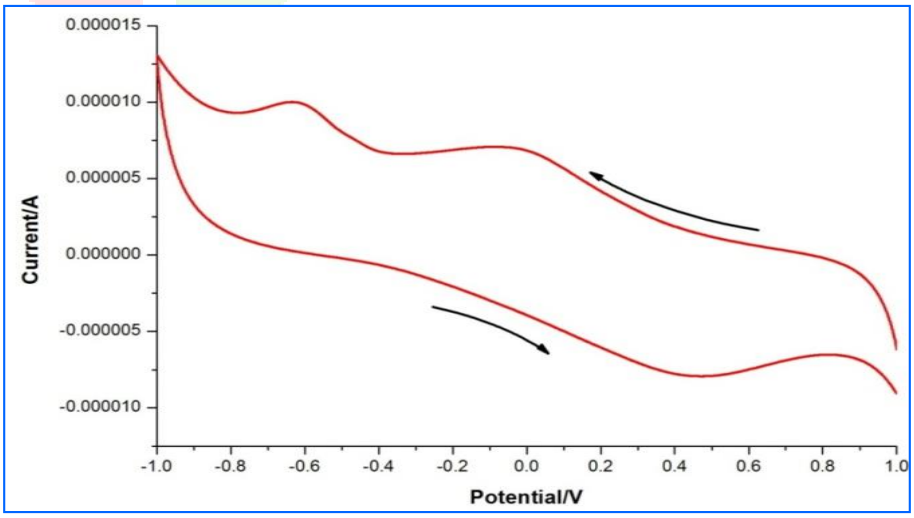


Fig.1. Cycli Votammogram of 80a at glassy carbon electrode in 25mM Sulfuric acid at scan rate $0.1Vs^{-1}$ (1a)

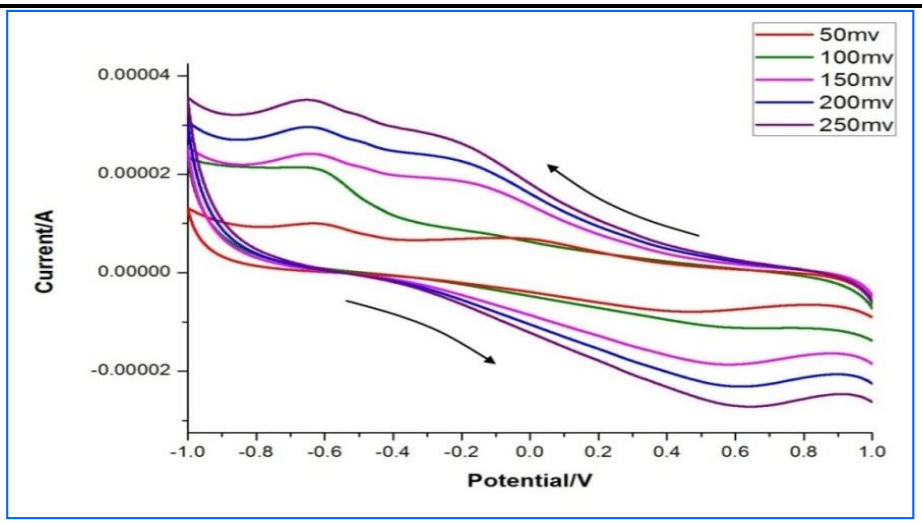
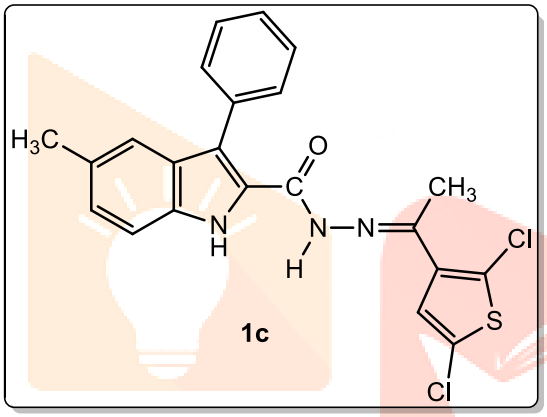


Fig.2. Variation of scan rate from 50mVs^{-1} to 250mVs^{-1} (1a)



Scheme 3. Probable structure for reduced compound (1c).

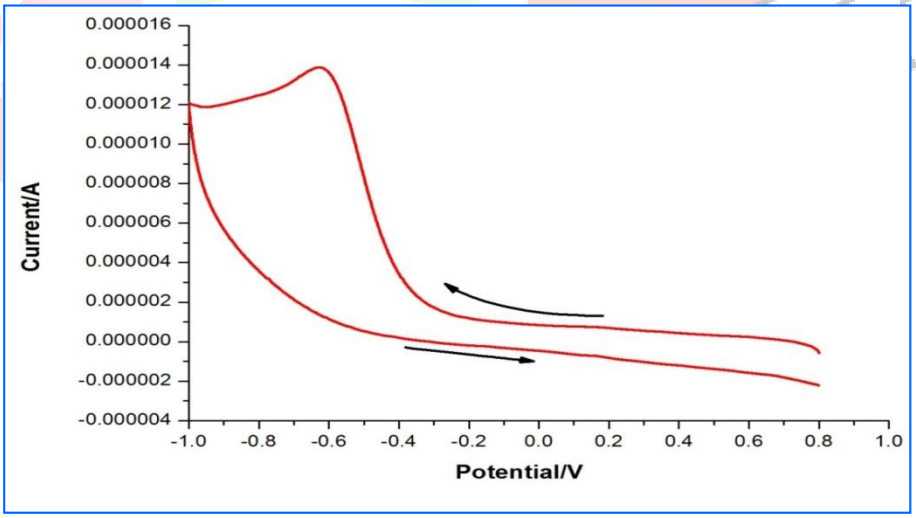


Fig.3. Cyclic Votammogram of 80c at glassy carbon electrode in 25mM Sulfuric acid at scan rate 0.1Vs^{-1} (1c).

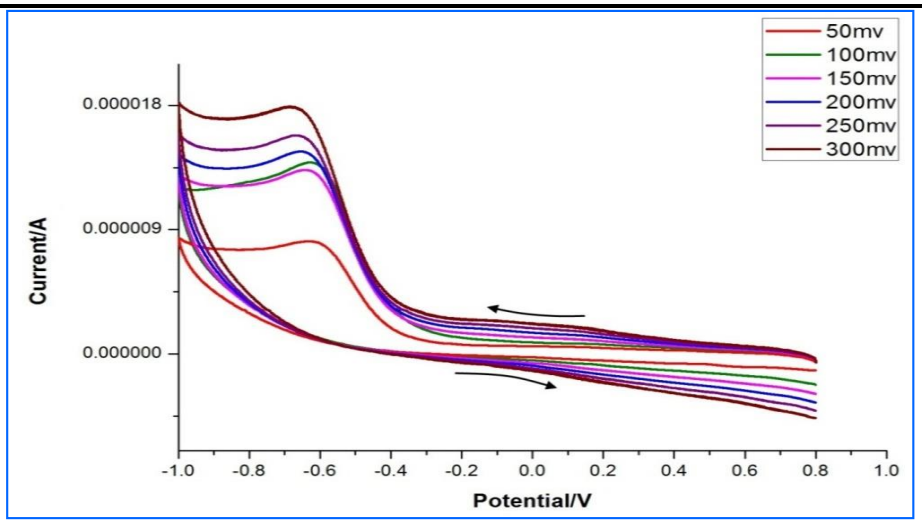


Fig. 4. Variation of scan rate from 50mVs^{-1} to 300mVs^{-1} (1c)

DNA CLEAVAGE ACTIVITY

Among the synthesized compounds have been subjected to DNA Cleavage studies. The DNA cleavage activity was determined using gel electrophoresis by Sambrook et.al [26]. The pictures of the gels are presented in Fig. 5. When the compound is made to interact with DNA could induce the breakage of DNA strands. The gel electrophoresis clearly reveal that, all the tested compounds did act on the DNA as little tailing in the bands can be observed in treated samples. The difference was observed in bands of all the compounds compared to the control DNA (C). This shows that the control DNA alone does not show any apparent cleavage as the compounds did. With this, it can be concluded that the compounds inhibits the growth of the pathogenic organism by cleaving the genome. Compounds 1a-c, have shown highest activity and remaining compounds shown moderate activity. Compounds with halogen and methyl substitution at five positions and phenyl ring at third position of indole nucleus have exhibited very good activity when compared to standard.

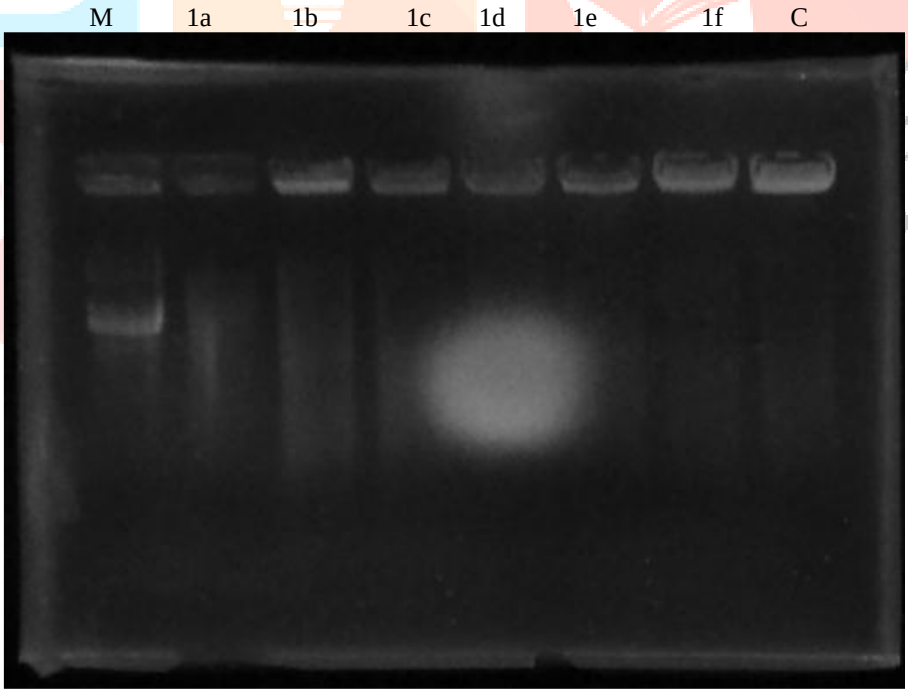


Fig. 5. DNA Cleavage Activity of (1a-f).

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