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Full Paper

Synthesis, Antimicrobial and Electrochemical Studies of Four Substituted Isatin Derivatives at a Glassy Carbon Electrode

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Abstract- Isatins, derivatives of indole, represent important class of compounds belonging to nitrogen heterocycles. These compounds comprise synthetically vital substrates that are used as precursors for drug synthesis and raw materials for heterocycles etc. Research in this group of compounds has engrossed interest among scientific community in recent and past as Isatins are known to possess immense biological activities. Present work delineates synthesis, characterization, electrochemical and antimicrobial studies of four substituted derivatives of isatin derivatives. The cyclic voltammetric studies of all the analytes showed that four derivatives have better electro catalytic activity towards the analytes at glassy carbon electrode. These synthesized isatin derivatives were screened for their antimicrobial activity against Gram-negative bacteria (*Escherichia coli and Staphylococcus aureus*) and fungi such as *Candida albicans* and *Penicillin chrysogenum,* and found to possess considerable antimicrobial activity suggesting their effectiveness in developing antibiotics and novel drugs.

Keywords- Cyclic voltammetry; Glassy carbon electrode; Antimicrobial activity; Isatin derivatives

1. INTRODUCTION

Isatins which are naturally found in many plants are indole derivatives with keto moiety at 2nd and 3rd position of the pyrole ring fused with benzene ring. Synthesis of Istatin was first

reported in 1841 by Erdman and Laurent [1, 2]. Substituted forms of isatins are also found to occur in few plant species and also in humans as metabolic derivatives [3,4]. Various derivatives of istatins are known to possess potent antimicrobial and anti-human immune virus activities. They are also known to present anti-inflammatory, anti-mycobacterial, anticancer and anticonvulsant activities [5-9]. The wide spectrum applications of isatin derivatives and their various biological and chemical properties have led to their increasingly expanded use as precursors for the preparation of many biologically active compounds. They are extensively used as precursors for many of the synthetic drugs in medicinal chemistry [8,10,11]. The diverse applications of isatin derivatives are attributed to the occurrence of multiple reaction centers in isatin and their derivatives [12]. Isatin hydrazine derivatives were known to be active against Walker carcinosarcoma. Similarly, acetone and ketone-derivatives of isatin exhibited antioxidant activity [13,14]. They are also known to be effective against leprosy, tuberculosis, viral and protozoan infections [15-18].

Appropriate medical diagnosis of recurring diseases continues to be a major challenge task to doctors, patients and research community all over the world due to resistance of existing drugs and associated toxicities. These alarming issues have led to the unremitting exploration for novel compounds with antimicrobial activities [19, 20]. Since last three decades there has been significant progress in terms of investigation of synthetic and semi synthetic, substituted antimicrobial compounds for various disorders [21].

Various analytical techniques are deployed for numerous bio-molecules determination such as spectrometry, electrochemistry etc. in recent and past. Accuracy in sensitivity, cost effectiveness, simple procedures have made electrochemical sensors very attractive [22-24]. Electrochemical processes are highly efficient in assaying the concentration of electro active analyte at trace levels and beneficial in obtaining information regarding its physical and chemical characters like oxidation potential, diffusion coefficients, electron transfer rates and electron transfer number. Besides, these methods are of utmost interest in the investigation of pharmacologically active compounds and metabolites produced/synthesized by different metabolic pathways involving redox reactions [25].To evaluate the property of analytes, voltammetry studies are conducted. This technique is a function of unremitting change of the potential applied across the electrode-solution interface and resultant current is noted [26]. Cyclic Voltammetry studies of synthesized substituted isatin molecules were found worth exploring for their pharmaceutical applications. However, much studies have not been done on the electrochemical (CV) and biological activities of these derivatives so far. Also, the researches on cyclic voltammetry and antimicrobial activities of methyl isatin derivatives 4(a-d) have not been reported. The cyclic voltammetry studies on glassy carbon electrode (GCE) were carried out. These derivatives were also checked for their antimicrobial potential. In this study, a series of four different substituted isatin derivatives, were synthesized, characterized, investigated by cyclic voltammetry and tested for their antimicrobial activity.

2. Materials and method

2.1. Materials

Chemicals used in the synthesis of compounds were purchased from Spectrochem Pvt. Ltd. Bangalore, India. The solvents were of reagent grade, purified and dried. Melting points of the synthesized compounds were determined. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 and 100 MHz instruments using DMSO-d6/$CDCl_3$ as solvents and TMS as an internal standard; chemical shifts are expressed as δ values (ppm). The *J* values are expressed in Hertz (Hz). Mass spectra (MS) were recorded in GCMATE II LC–Mass spectrometer with electron impact ionization (EI) method.

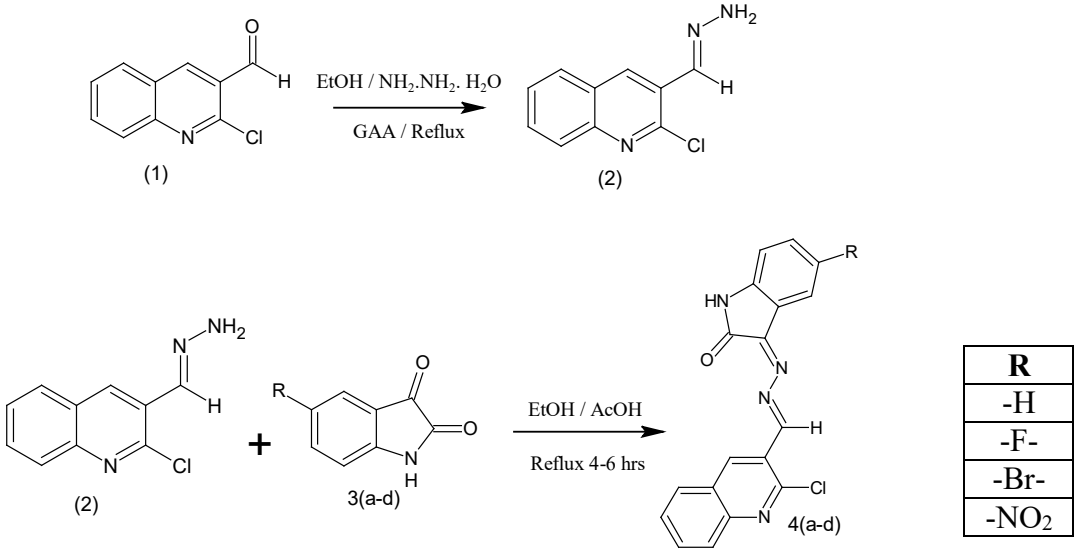
2.2. Methods

2.2.1. General procedure for synthesis of (2-chloroquinolin-3-yl)methylenehydrazine (2)

2-chloroquinoline-3-carbaldehyde**1**was taken sufficient quantity in a 100 ml round bottom flask, to this ethanol (50 ml) was added and refluxed around 4-6 hrs followed by the addition of hydrazine hydrate (excess) slowly. After completion of the reaction, the solid formed was filtered and recrystalized from ethyl alcohol.

2.2.2. General procedure for synthesis of 4- methyl phenol derivatives 4(a-d)

(2-chloroquinolin-3-yl) methylene hydrazine 2 was taken in ethanol (10 ml) and substituted isatins 3a-d (equivalent) was added, and continued with reflux for about 2-3 hrs. The obtained mass was filtered and dried. Purification of the synthesized compounds were carried by recrystalization with suitable solvents.



Scheme 1. Synthesis procedure of 4-methyl phenol derivatives 4(a-d)

2.2.3. Spectral characterization of (2-chloroquinolin-3-yl) methylidenehydrazinylidene 1, 3dihydro-2H-indol-2-one (4a)

Yield: 67 %. M. Pt. 148-150 °C;[1]H NMR (DMSO-d_6, 400 MHz, δ ppm):13.53 (s, 1H), 8.92 (s, 1H), 8.41 (s, 1H), 8.10-8.12 (d, 1H, J=8 Hz), 7.95-7.97 (d, 1H, J=8 Hz), 7.73 (m, 2H), 7.43-7.44 (d, 2H, J=4 Hz), 7.01-7.02 (d, 1H, J=4 Hz), 6.81-6.82 (d, 1H, J=4 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 163.9, 152.0, 148.1, 134.5, 130.8, 130.7, 130.0, 128.6, 124.3, 123.7, 122.9, 116.2, 111.5; Calcd. 334.7gm/ml. EI-MS (m/z): 332.9 (M-1).

2.2.4. Spectral characterization of (2-chloroquinolin-3-yl)methylidenehydrazinylidene-5-fluoro-1,3-dihydro-2H-indol-2-one(4b)

Yield: 61 %. M. Pt. 188-189 °C; 1H NMR (DMSO-d6,400 MHz, δ ppm): 13.52 (s, 1H), 8.91 (s, 1H), 8.41 (s, 1H), 7.99-8.10 (d, 1H, J=8 Hz), 7.95-7.96 (d, 2H, J=4 Hz), 7.45-7.46 (d, 2H, J=4 Hz), 6.89-6.90 (d, 2H, J=8 Hz); 13C NMR (DMSO-d6,100 MHz, δ ppm):163.9, 159.1, 156.8, 152.0, 148.1, 146.4, 142.3, 134.5, 130.8, 130.1, 128.1, 124.3, 123.7, 121.5, 116.6, 115.8, 115.5, 112.6; Calcd. 352.7gm/ml. EI-MS (m/z): 350.87 (M-1).

2.2.5. Spectral characterization of 5-bromo-3-(2-chloroquinolin-3-yl)methylidenehydrazinylidene1,3-dihydro-2H-indol-2-one(4c)

Yield: 63 %. M. Pt. 138-140 °C; 1H NMR (DMSO-d6, 400 MHz, δ ppm): 13.52 (s, 1H), 8.92 (s, 1H), 8.49-8.50 (d, 1H, J=4 Hz), 7.72-7.75 (m, 2H), 7.67-7.69 (d, 2H, J=8 Hz), 7.29-7.31 (d, 2H, J=8 Hz), 6.09 (s, 1H); Calcd. 413.6gm/ml. EI-MS (m/z): 413.87 (M+).

2.2.6. Spectral characterization of (2-chloroquinolin-3-yl)methylidenehydrazinylidene-5-nitro-1,3-dihydro-2H-indol-2-one (4d)

Yield: 66 %. M. Pt. 129-130 °C; [1]H NMR (DMSO-d_6, 400 MHz, δ ppm): 13.58 (s, 1H), 8.91 (s, 1H), 8.44-8.47 (t, 2H, *J*=12 Hz), 8.01-8.03 (d, 2H, *J*=8 Hz), 7.46-7.47 (d, 2H, *J*=4 Hz), 7.27 (s, 1H), 6.9-7.0 (d, 1H, *J*=4 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm):163.7, 159.3, 155.7, 151.2, 134.5, 133.4, 130.9, 130.8, 130.0, 128.3, 123.7, 123.5, 122.9, 122.4, 115.4, 112.5, 110.4, 110.0; Calcd. 379.7gm/ml. EI-MS (*m/z*): 379.8 (M^+).

2.3. Antimicrobial activity

The in vitro antibacterial and antifungal activities of 4(a-d) isatin derivatives were tested against gram negative bacterias *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (25923), and fungi *Penicilliumcrysogenium* and *Candida albicans* by agar disc diffusion

method. The microbial strains were cultured overnight at 37 ˚C in nutrient broth and potato dextrose agar medium. The broth cultures were compared to the turbidity with that of the standard 0.5 McFarland solution. All the Micro-organisms were stored at 4˚C for future use. Ciprofloxacin and Amphotericin were utilized as standard drugs for bacteria and fungi respectively. The agar plates of the media (Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water-bacteria and Czapek-Dox Agar: Composition (g/l) Sucrose-30.0; Sodium nitrate- 2.0; K_2HPO_4-1.0, $MgSO_4.7H_2O$-0.5; KCl-0.5; $FeSO_4$-0.01; Agar-20-fungi) were prepared and were made in the plate. Each plate was inoculated with minimum of 18 hrs old cultures (100 µl, 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells were filled with the compounds and antibiotic of different concentrations. All the plates were subjected to incubation at 37 ºC for 24 hrs for bacteria and at 27 ºC for 96 hrs for fungi. The zone of inhibition around the well in each plate was measured. The lowest concentration of the compounds fully hindering the growth of bacteria and fungi compared against standard antibiotic was recorded as minimum inhibitory concentration [25-27].

3. RESULTS AND DISCUSSION

Final synthesized compounds (derivatives) were subjected to purification by recrystallization. Purity of these derivatives was confirmed through studies like TLC, NMR, melting point. These title compounds were known to possess antimicrobial activity hence, antibacterial activities were investigated.

The electrochemical behavior of substituted isatin derivatives was studied at glassy carbon electrode using cyclic voltammetry studies. Present study delineates detailed information on structure and characterization of the title determination of their electrochemical and antimicrobial activity. Figure 1 to 4 shows the cyclic voltammograms obtained for 4a analyte at a scan rate of 10 and 50mV/s in the potential window -400 to 1800 mV. From the CV graphs we can note that, an irreversible peak for analyte 4a is present. Also for rest of the analytes that is from 4b-4d cyclic voltammetry studies were carried out and the obtained graphs are shown in Figure 2, 3 and 4. It is noticed that in these cases also an irreversible peak for analytes.

3.1. In vitro antimicrobial study

The synthesized compounds were screened for their antimicrobial activity. The results are tabulated in Tables-1, 2, 3 and 4. The MIC values of the synthesized compounds are shown. The antibacterial results revealed that compound 4d was the most effective against *Escherichia coli and Staphylococcus aureus*, with MIC values ranging from 250 to 1000 µg/ml. Compounds 4(a-d) showed less significant activity against fungi *Candida albicans*and *Penicillin chrysogenum* with MIC values of more than 1000 µg/ml, respectively. *C. albicans* were resistant to synthesized drug. Figure 2 and 3 are representative pictures of zone of

inhibition against *E. coli*and *S. aureus* as given by synthesized derivatives of (2-chloroquinolin-3-yl)methylidenehydrazinylidene-5-fluoro-1, 3-dihydro-2H-indol-2-one 4 (a-d).

Table 1. Zone of inhibition against E. coli as given by synthesized derivatives of (2-chloroquinolin-3-yl) methylidene hydrazinylidene-5-fluoro-1,3-dihydro-2H-indol-2-one 4(a-d)

Samples	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
4a	0	0	0	5	8	12	250
4b	0	0	3	5	7	8	100
4c	0	0	3	5	6	10	100
4d	0	0	0	0	6	12	500
Ciprofloxacin	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	26	29	32	34	38	*	25

Table 2. Zone of inhibition against *S. aureus* as given by synthesized derivatives of (2-chloroquinolin-3-yl) methylidene hydrazinylidene-5-fluoro-1, 3-dihydro-2*H*-indol-2-one 4(a-d)

Samples	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
4a	0	0	0	4	7	10	250
4b	0	0	0	4	5	6	250
4c	0	0	3	5	7	9	100
4d	0	0	0	0	3	6	500
Ciprofloxacin	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	25	28	31	34	36	*	25

Note: *zones could not be measured due to merging, Zones ≥3 mm considered for MIC

The MIC of the synthesized compounds were compared to standard antibacterial drug ciprofloxacin. Our study demonstrated significant antibacterial effect and mild antifungal activity suggesting wide range of antimicrobial potential against gram positive, gram negative bacteria and fungi.

3.2. Cyclic Voltammetric Studies

3.2.1. Electrochemical responses of (2-chloroquinolin-3-yl) methylidenehydrazinylidene-5-fluoro-1, 3-dihydro-2H-indol-2-one 4(a-d) at glassy carbon electrode

The analyte 4a showed a reduction peak with Glassy Carbon Electrode when compared to that of bare GCE. 400 µL of analyte 4a taken from the stock solution and 10 mL of phosphate buffer of pH 6.0 were added to the electrochemical cell. Then GCE (working electrode) with reference and auxiliary electrodes were dipped in the test solution and potential is applied in the range of 400 mV to +1800 mV. The cyclic voltammogram of analyte 4a in phosphate buffer

is shown in Fig. 1. The curve b is for the blank solution. Curve 'a' shows the reduction peak of analyte 4a at potential E_{pc} 865 mV and peak current I_{pc} 36.15µA indicate the sensitivity of GCE.

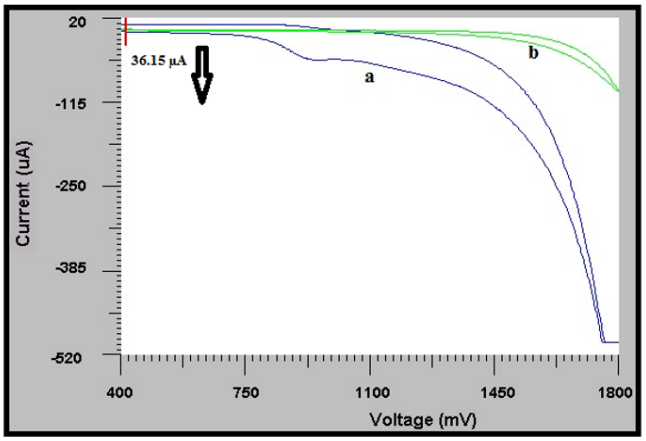


Fig. 1. Cyclic Voltammograms of 0.5 mM analyte (4a) at GCE curve a, curve b for blank solution in phosphate buffer of pH 6 at GCE, scan rate 50 mVs^{-1}

The analyte 4b showed a reduction peak with Glassy Carbon Electrode when compared to that of bare GCE. 500µL of analyte 4b taken from the stock solution and 10mL of phosphate buffer of pH 7.0 were added to the electrochemical cell. Then GCE (working electrode) with reference and auxiliary electrodes were dipped in the test solution and potential is applied in the range of 200 mV to 1200 mV. The cyclic voltammogram of analyte 4b in phosphate buffer is shown in Fig. 2. The curve b is for the blank solution. Curve 'a' shows the reduction peak of analyte 4b at potential E_{pc} 835 mV and peak current I_{pc} 6.15µA indicate the sensitivity of GCE.

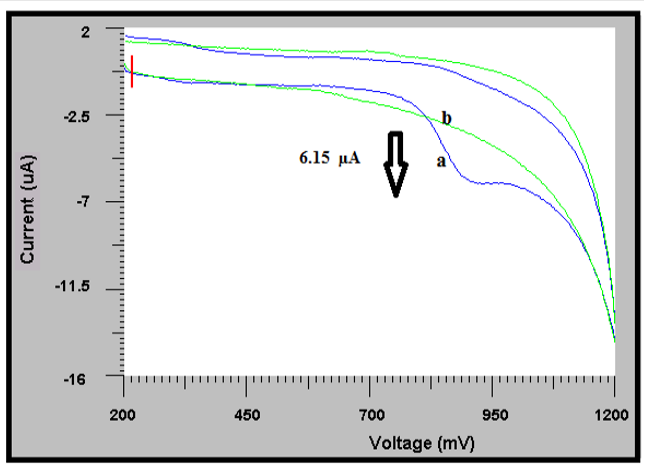


Fig. 2. Cyclic Voltammograms of 0.5 mM analyte (4b) at GCE curve a, curve b for blank solution in phosphate buffer of pH 7 at GCE, scan rate 100 mVs^{-1}

The analyte 4c showed a reduction peak with Glassy Carbon Electrode when compared to that of bare GCE. 500µL of analyte 4c taken from the stock solution and 10mL of phosphate buffer of pH 5.0 were added to the electrochemical cell. Then GCE (working electrode) with reference and auxiliary electrodes were dipped in the test solution and potential is applied in the range of 200 mV to 1200 mV. The cyclic voltammogram of analyte 4c in phosphate buffer is shown in Fig. 3. The curve b is for the blank solution. Curve a shows the reduction peak of analyte 4c at potential E_{pc} 911 mV and peak current I_{pc} 9.53 µA indicate the sensitivity of GCE.

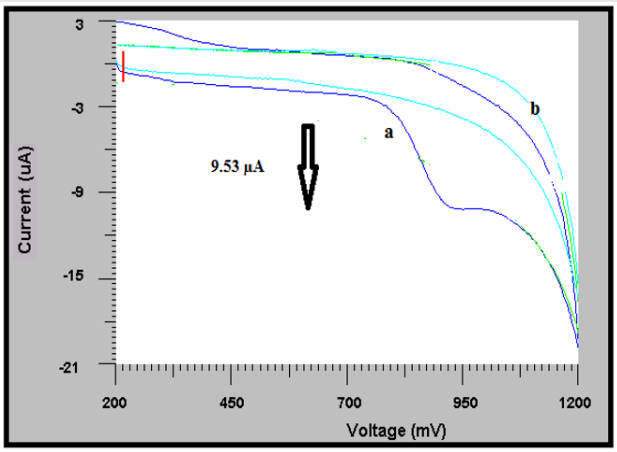


Fig. 3. Cyclic Voltammograms of 0.5 mM analyte (4c) at GCE curve a, curve b for blank solution in phosphate buffer of pH 5 at GCE, scan rate 100 mVs^{-1}

The analyte 4d showed a reduction peak with Glassy Carbon Electrode when compared to that of bare GCE. 500µL of analyte 4d taken from the stock solution and 10mL of phosphate buffer of pH 8.0 were added to the electrochemical cell.

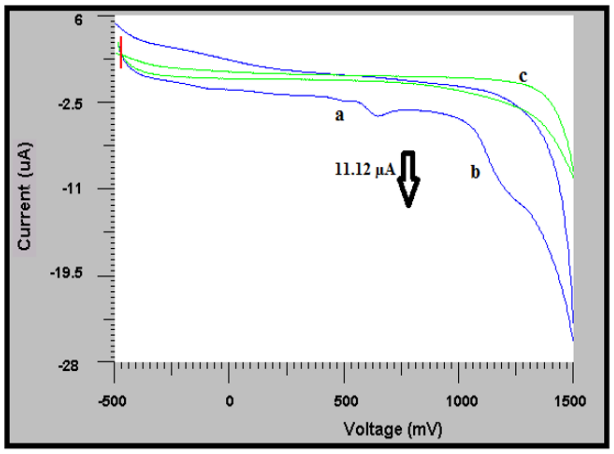


Fig. 4. Cyclic Voltammograms of 0.5 mM analyte (4d) at GCE curve a, curve b for blank solution in phosphate buffer of pH 8 at GCE, scan rate 50 mVs^{-1}

Then GCE (working electrode) with reference and auxiliary electrodes were dipped in the test solution and potential is applied in the range of 500 mV to 1500 mV. The cyclic voltammogram of analyte 4d in phosphate buffer is shown in Fig.4. The curve b is for the blank solution. Curve a shows the reduction peak of analyte 4d at potential E_{pc} 562 mV, E_{pc} 1201 mV and peak current I_{pc} 2.65 μA, I_{pc} 11.12 μA.

4. CONCLUSION

The present work reports the synthesis, spectral characterization, antimicrobial and electrochemical studies of synthesized series of Schiff base molecules. Molecules were screened for antimicrobial activity by agar well diffusion method. The work indicates that the titled compounds were electrochemically significant and possess good antimicrobial activity.

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Conflict of interest

There is no any conflict of interest.

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