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Antioxidant Properties and Electrochemical Behavior of some Acetyl Salicylic Acid Derivatives

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ABSTRACT

Antioxidants are frequently used in several dietary supplements and have been probed for their significant role in the prevention of diseases like cancer or coronary heart disease. Acetyl salicylic acid has grabbed substantial attention due to their ability in impeding oxidative stress. In the present study, nine new salicylic acid derivatives: 5-(2-hydroxyphenyl-5-substituted)-1,3,4-oxadiazole-2(3H)-thiones, N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hydroxy-5-substituted benzamides, 2-hydroxy-5-substituted-N'-[1-(2-oxo-2substituted-chromen-3-yl)ethylidene] benzohydrazides and Hg (II) complex of N'-[(1Z)-1-(6-bromo-2-oxo-2H-chromen-3-yl) ethylidene]-2-hydroxybenzohydrazide have been synthesized and characterized in the light of advanced spectral techniques. The antioxidant properties of these compounds were investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrometric assay and compared with the results of cyclic voltammetry studies. The redox properties shown by the active molecule using cyclic voltammetry correlated well with the biochemical study.

INTRODUCTION

Reactive oxygen species (ROS) are extremely reactive molecules that contain oxygen like superoxides, hydroxyl radicals, peroxides etc. which are normally generated as a byproduct of the natural oxygen metabolism that occurs within cells (Muller, 2000; Schumacker, 2006). Oxidative stress produced due to increased levels of either endogenous or exogenous ROS on exposure to ionic radiations or alterations in metabolic pathways can lead to severe consequences like ageing, DNA damage, lipid peroxidation, oxidation of amino acids in proteins, deactivation of certain enzymes eventually resulting in conditions like inflammation, cardiovascular disorders, neurodegenerative diseases or cancer (Renschler 2004; Li *et al.*, 2013). An antioxidant is any chemical substance that can either react with or quench ROS and thereby protects the biological systems from several lethal diseases (Trachootham *et al.*, 2009; Tochhawng *et al.*, 2013). In the last few decades, several antioxidant supplements have been extensively used in different fields like cosmetic formulations, pharmaceutical products etc. to inhibit/delay oxidative stress. Phenolic compounds like acetyl salicylic acid and its metabolite salicylic acid exhibit significant non-enzymatic antioxidant potential via donation of hydrogen atom from the hydroxyl moiety attached to the phenyl ring and hence prevent oxidative stress (Ghasemzadeh *et al.*, 2012; Bal-Demirci *et al.*, 2015).

Transition metal coordination compounds are particularly suitable as biologically active ligands as they can adopt a wide variety of oxidation states, coordination numbers and geometries (Kostova and Balkansky, 2013). Metal complexes, based on their structure and on the source of the oxidative stress, might act as antioxidants or pro-oxidants. The potential prominence of antioxidants has encouraged us to investigate the cooperative effects of metal complexes and salicylic acid derivatives for improving antioxidant activity. The current study provides the synthesis of few salicylic acid derivatives and their mercury based complexes followed by the investigation of their antioxidant potential.

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Antioxidant compounds are highly capable in acting as reducing agents and their solutions get easily oxidized on the surface of inert electrodes. Centered on this fact, the relationship between electrochemical behaviors and the data obtained from the spectrometric assay of the potent antioxidant compound was also explored.

MATERIALS AND METHODS

Chemistry

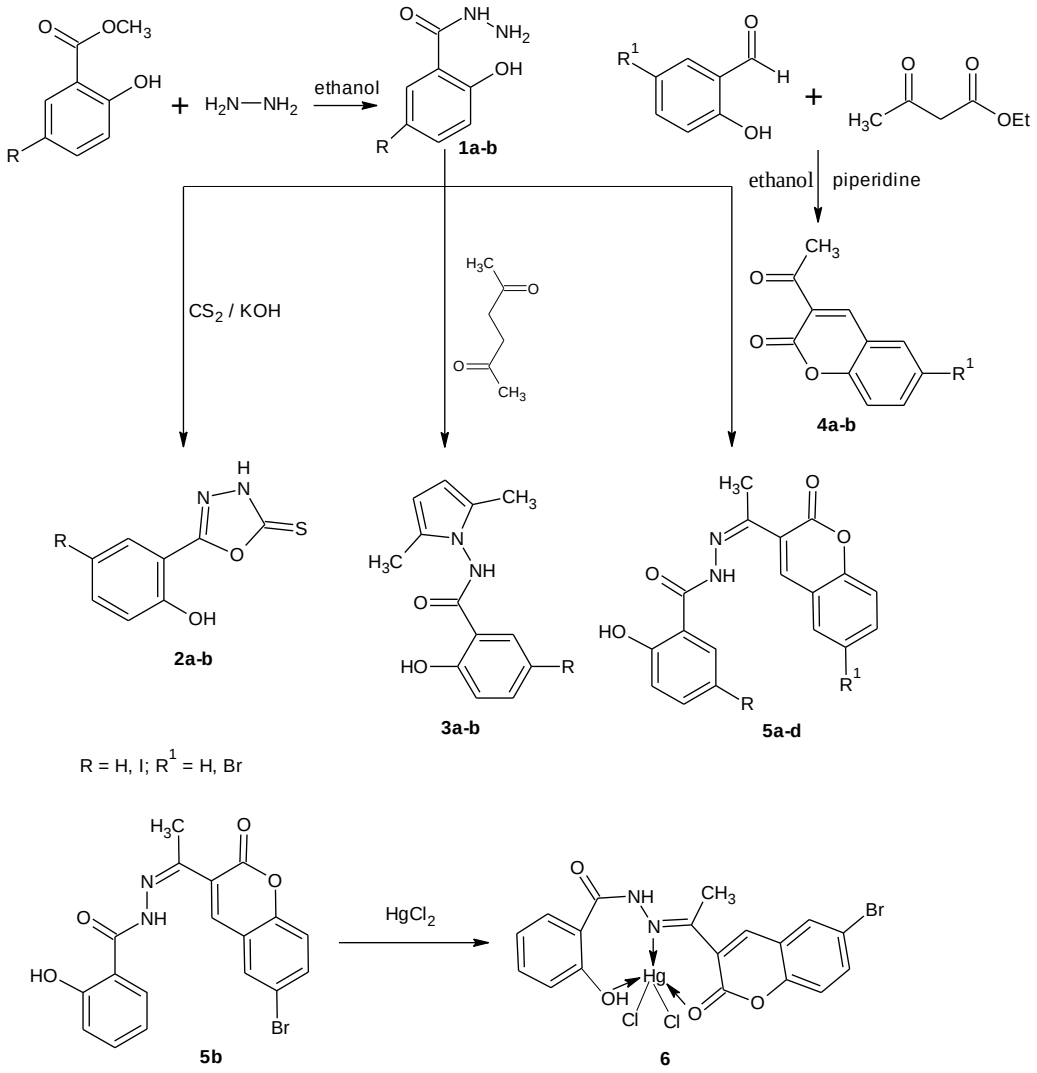
The chemicals and reagents used for synthesis and biochemical assays were procured commercially from Sigma-Aldrich, E. Merck India Ltd and Himedia. 2-Hydroxybenzohydrazides (**1a-b**) were prepared from substituted methyl salicylates using hydrazine hydrate as per literature methods (Sahoo *et al.,* 2010). The reaction between compounds (**1a-b**) and carbon disulphide in presence of a base yielded 2-(5-sulfanyl-1,3,4-oxadiazol-2-yl)-4-substitutedphenol (**2a-b**).

Compounds (**3a-b**) were obtained when (**1a-b**) was treated with an acidic solution of acetonyl acetone.

Substituted 3-acetyl-2*H*-chromen-2-ones (**4a-b**) were prepared by the cyclization reaction between salicylaldehydes and ethyl acetoacetate in presence of pyridine under sonication as given in the literature (Kamath *et al.,* 2015). The condensation of compounds (**1a-b**) and (**2a-b**) gave 2-hydroxy-5-substituted-N'-[1-(2-oxo-2-substituted-chromen-3-yl)ethylidene] benzohydrazide (**5a-d**). Schiff base **5b** was treated with mercury (II) chloride to give the mercury (II) complex **6**.

Thin layer chromatography was carried out using pre-coated aluminum sheets with Aluchrosep silica Gel 60/ UV_{254}. A mixture of ethyl acetate and hexane (1:1) was used as the eluent and the spots were observed using a UV chamber. The melting points of the new derivatives were determined via the open capillary method and are uncorrected.

The IR and mass spectra of salicylic acid derivatives were taken using Schimadzu FTIR 8400S spectrophotometer and Agilent 6510 series mass spectrometer. The ^{1}H and ^{13}C NMR were recorded on a Bruker 400 MHz instrument. The elemental analyses were performed using a Flash thermo 1112 series CHN analyser.



Scheme-1: Synthetic pathway for the preparation of salicylic acid derivatives.

EXPERIMENTAL

General procedure for synthesis of 5-(2-hydroxyphenyl-5-substituted)-1,3,4-oxadiazole-2(3H)-thiones (2a-b)

About 6 mmol of KOH dissolved in 2 mL of water and 2 mL of CS_2 was added with stirring to 3.8 mmol of 2-hydroxybenzohydrazide in ethanol and refluxed for 8 h. The solvent was evaporated, the residue treated with water, filtered, dried and recrystallized from methanol.

5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2(3H)-thione (2a)

White solid (77%); 254-256°C; R_f: 0.92; IR (KBr) [cm^{-1}]: 3440 (O-H *str*.), 3155 (N-H *str*.), 3055 (Ar. C-H *str*.), 1600 (Ar.C-O-C *str*.), 1539 (C=C *str*.), 1272 (C=S *str*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.12 (s, 1H, O-H), δ 10.73 (s, 1H, oxadiazole NH), δ 7.56 (d, 1H, phenyl 3H), δ 7.33 (d, 1H, phenyl 6-H), δ 7.00 (m, 2H, phenyl 4-H & 5-H); ^{13}C NMR (100 MHz, DMSO-d6) δ = 110.12, 116.86, 120.11, 125.68, 131.57, 156.20, 161.49, 179.66; MS (m/z): 195 (M+1); Anal. Calcd. for $C_8H_6N_2O_2S$: C, 49.48; H, 3.09; N, 14.43; found: C, 49.61; H, 3.10; N, 14.46.

5-(2-hydroxyphenyl-5-iodo)-1,3,4-oxadiazole-2(3H)-thione (2b)

White solid (77%); 222-224°C; R_f: 0.89; IR (KBr) [cm^{-1}]: 3410 (O-H *str*.), 3140 (N-H *str*.), 3068 (Ar. C-H *str*.), 1608 (Ar.C-O-C *str*.), 1545 (C=C *str*.), 1264 (C=S *str*), 510 (C-I str.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.14 (s, 1H, O-H), δ 10.73 (s, 1H, oxadiazole NH), δ 7.91 (s, 1H, phenyl 6-H), δ 7.40 (d, 1H, phenyl 4-H), δ 6.99 (d, 2H, phenyl 3-H); ^{13}C NMR (100 MHz, DMSO-d6) δ = 101.44, 117.64, 119.8, 137.91, 139.15, 156.88, 158.10, 178.35; MS (m/z): 321 (M+1); Anal. Calcd. for $C_8H_5N_2O_2SI$: C, 30.00; H, 1.56; N, 8.75; found: C, 30.12; H, 1.57; N, 8.77.

General procedure for synthesis of N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hydroxy-5-substitutedbenzamides (3a-b)

About 6 mmol of acetonyl acetone and 1mL of acetic acid in 10mL of ethanol was added to 3.8 mmol of 2-hydroxybenzohydrazide dissolved in ethanol, refluxed for 4-5 h, concentrated to half and then poured into 50 g crushed ice, filtered and recrystallized.

***N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hydroxybenzamide* (3a)**

Pink solid (81.2%); 178-180°C; R_f: 0.91; IR (KBr) [cm^{-1}]: 3220 (O-H *str*.), 3150 (N-H *str*.), 3100 (Ar. C-H *str*.), 2939 (CH_3 *asym. str*.), 2896 (CH_3 *sym. str*.), 1673 (C=O *str*.), 1608 (Ar. C=C *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.44 (s, 1H, OH), δ 11.20 (s, 1H, NH), δ 7.85 (d, 1H, phenyl 6-H), δ 7.5 (d, 1H, phenyl 3-H), δ 6.9-7.0 (m, 2H, phenyl 4-H & 5-H), δ 5.71 (d, 2H, pyrrol 3-H & 4-H), δ 2.04 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ = 15.52, 103.66, 115.89, 117.84, 119.40, 127.45, 129.06, 134.53, 159.43, 168.18; MS (m/z): 231 (M+1); Anal. Calcd. for $C_{13}H_{14}N_2O_2$: C, 67.83; H, 6.09; N, 12.17; found: C, 68.02; H, 6.10; N, 12.20.

***N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hydroxy-5-iodo-benzamide* (3b)**

Pink solid (83.6%); 188-190 °C; R_f: 0.98; IR (KBr) [cm^{-1}]: 3215 (O-H *str*.), 3150 (N-H *str*.), 3090 (Ar. C-H *str*.), 2985 (CH_3 *asym. str*.), 2876 (CH_3 *sym. str*.), 1679 (C=O *str*.), 1610 (Ar. C=C *str*.), 514 (C-Br *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.44 (s, 1H, OH), δ 11.20 (s, 1H, NH), δ 8.00 (s, 1H, phenyl 6-H), δ 7.36 (d, 1H, phenyl 4-H), δ 6.94 (d, 1H, phenyl 3-H), δ 5.85 (d, 2H, pyrrol 3-H & 4-H), δ 2.08 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ = 15.51, 103.4, 111.87, 118.92, 119.8, 137.48, 139.15, 140.5, 158.74, 163.01; MS (m/z): 357 (M+1); Anal. Calcd. for $C_{13}H_{13}N_2O_2I$: C, 43.82; H, 3.65; N, 7.86; found: C, 43.99; H, 3.66; N, 7.88.

General procedure for synthesis of 2-hydroxy-5-substituted-N'-[1-(2-oxo-2substituted- chromen-3-yl) ethylidene] benzohydrazide (5a-d)

About 0.002 mol of 2-hydroxybenzohydrazide in a minimum amount of ethanol was added to 0.002 mol of 3-acetyl coumarin and refluxed for 5 h. The product obtained was filtered, recrystallized from methanol.

2-hydroxy-N'-[(1Z)-1- (2-oxo-2H-chromen-3-yl) ethylidene] benzohydrazide.(5a)

Yellow solid (73%); 210-212 °C; R_f. 0.23; IR (KBr) [cm^{-1}]: 3250 (O-H *str*.), 3085 (N-H *str*.), 3055 (C-H *str*.), 2923 (C-H *asym. str*.), 2875 (C-H *sym. str*.), 1693 (coumarin C=O *str*.), 1604 (C=N *str*.), 1555 (Ar. C=C *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.42 (s, 1H, NH), δ 8.29 (s, 1H, coumarin 4-H),δ 8.00 (d, 1H, coumarin 5-H), δ 7.91 (d, 1H, coumarin 8-H), δ 7.45 (m, 3H, phenyl), 7.68 (m, 1H, phenyl), δ 7.03 (t, 2H, coumarin 6-H & 7-H), δ 5.41 (s, 1H, OH) δ 2.29 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ = 16.14, 23.79, 116.51, 116.86, 117.36, 118.31, 119.29, 120.23, 125.32, 127.02, 129.76, 131.24, 132.97, 133.99, 142.15, 153.95, 156.88, 159.78; MS (m/z): 323 (M+1); Anal. Calcd. for $C_{18}H_{14}N_2O_4$: C, 67.08; H, 4.35; N, 8.70; found: C, 67.28; H, 4.37; N, 8.73.

N'-[(1Z)-1-(6-bromo-2-oxo-2 H-chromen-3-yl)ethylidene]-2-hydroxybenzohydrazide (5b)

Yellow solid (74%); 248-250°C; R_f: 0.5; IR (KBr) [cm^{-1}]: 3298 (O-H *str*.), 3100 (N-H *str*.), 3074 (Ar. C-H *str*.), 2974 (C-H *asym. str*.), 2850 (C-H *sym. str*.), 1745 (coumarin C=O *str*.), 1610 (C=N *str*.), 1554 (Ar. C=C *str*.), 752 (C-Br *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.42 (s, 1H, NH), δ 7.82 (s, 1H, coumarin 4-H), δ 7.74 (d, 1H, phenyl 6-H), δ 7.73 (s, 1H, coumarin 5-H), δ 7.47 (t, 1H, phenyl 4-H), δ 7.43 (d, 1H, coumarin 8-H), δ 7.35 (d, 1H, coumarin 7-H), δ 7.29 (t, 1H, phenyl 5-H), δ 7.03 (d, 1H, phenyl 3-H), δ 5.43 (s, 1H, OH), δ 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ = 13.45, 111.97, 112.30, 117.26, 118.92, 120.86, 121.98, 128.15, 130.40, 131.85, 134.00, 135.22, 140.23, 151.55, 153.11, 158.73, 163.01, 165.13; MS (m/z): 402 (M+1), 404 (M+3); Anal. Calcd. for $C_{18}H_{13}N_2O_4Br$: C, 53.73; H, 3.23; N, 6.96; found: C, 53.97; H, 3.25; N, 7.00.

2-hydroxy-5-iodo-N'-[(1Z)-1- (2-oxo-2H- chromen-3-yl)ethylidene]benzohydrazide (5c)

Yellow solid (60%); 160-162°C; R_f: 0.92; IR (KBr) [cm^{-1}]: 3300 (OH str.), 3155 (N-H *str*.), 3031 (Ar. C-H *str*.), 1739 (Coumarin C=O *str*.), 1608 (C=N *str*.), 1568 (Ar. C=C *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.42 (s, 1H, NH), δ 8.02 (s, 1H, phenyl 6-H), δ 7.88 (d, 1H, coumarin 5-H), δ 7.76 (s, 1H, coumarin 4-H), δ 7.65 (t, 1H, coumarin 7-H), δ 7.47 (t, 1H, coumarin 6-H), 7.41 (d, 1H, coumarin 8-H), δ 7.37 (d, 1H, phenyl 4-H), δ 6.94 (d, 1H, phenyl 3-H), δ 5.41 (s, 1H, OH) δ 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ = 13.45, 116.40, 118.92, 118.37, 119.8, 121.87, 123.40, 127.67, 131.34, 134.00, 139.15, 140.23, 140.50, 151.55, 155.20, 158.73, 163.01, 165.14; MS (m/z): 449 (M+1); Anal. Calcd. for $C_{18}H_{13}N_2O_4I$: C, 48.21; H, 2.90; N, 6.25; found: C, 48.37; H, 2.91; N, 6.27.

N'-[(1Z)-1- (6-bromo-2-oxo-2H-chromen-3-yl) ethylidene]-2-hydroxy-5-iodobenzohydrazide(5d)

Yellow solid (67%); 252-254°C; R_f: 0.47; IR (KBr) [cm^{-1}]: 3200 (O-H *str*.), 3150 (N-H *str*.), 3055 (Ar. C-H *str*.), 1701 (coumarin C=O *str*.), 1600 (C=N *str*.), 1546 (Ar. C=C *str*.), 813 (C-Br *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 11.42 (s, 1H, NH), δ 8.02 (s, 1H, phenyl 6-H), δ 7.82 (s, 1H, coumarin 4-H), δ 7.73 (s, 1H, coumarin 5-H), δ 7.43 (d, 1H, coumarin 8-H), δ 7.37 (d, 1H, phenyl 4-H), δ 7.35 (d, 1H, coumarin 7-H), δ 6.94 (d, 1H, phenyl 3-H), δ 5.41 (s, 1H, OH) δ 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ = 13.45, 111.97, 112.3, 118.92, 119.8, 120.86, 124.31, 130.40, 134, 135.22, 139.15, 140.23, 140.5, 151.55, 153.11, 158.73, 163.01, 165.13; MS (m/z): 528 (M+1), 530 (M+3); Anal. Calcd. for $C_{18}H_{12}N_2O_4IBr$: C, 40.99; H, 2.28; N, 5.31; found: C, 41.12; H, 2.29; N, 5.33.

General procedure for Hg(II) complex of N'-[(1Z)-1-(6-bromo-2-oxo-2*H*-chromen-3-yl) ethylidene]-2-hydroxybenzohydrazide (6)

About 0.002 mol of mercury(II) chloride in 2-3 mL of water was added to 0.002 mole of N'-[(1Z)-1-(6-bromo-2-oxo-2**H**-chromen-3-yl)ethylidene]-2-hydroxybenzohydrazide (**5b**) dissolved in 1 mL of DMSO, stirred vigorously, kept aside for 15-20 min and filtered.

N'-[(1Z)-1- (6-bromo-2-oxo-2H-chromen-3-yl) ethylidene]-2-hydroxybenzohydrazidecomplex (6)

Yellow solid (74%); 248-250°C; R_f: 0.5; IR (KBr) [cm^{-1}]: 3286 (O-H *str*.), 3205 (N-H *str*.), 3132 (Ar. C-H *str*.), 2908 (CH_3 *asym. str*.), 2850 (CH_3 *sym. str*.), 1737 (Coumarin C=O *str*.), 1604 (C=N *str*.),1552 (Ar. C=C *str*.), 752 (C-Br *str*.), 530 (Hg-O *str*.), 510 (Hg-N *str*.), 411 (Hg-Cl *str*.); MS (m/z): 672 (M+1).

DPPH radical scavenging assay

The DPPH radical scavenging technique is based on the ability of the stable free radical DPPH, to become decolorized when they are exposed to antioxidants. About 100 µL each of various concentrations of test compounds and DPPH were added to respective wells of a micro plate, in order to make up a final volume of 200 µL. An equal amount of methanol and DPPH were used as the control. Ascorbic acid was employed as the standard reference. The potential of the test agents to quench DPPH was colorimetrically assessed after 20 min incubation in the dark. The absorbance was recorded using an ELISA plate recorder at 517 nm. The experiment was performed in triplicates and the mean values were considered (Shekhar and Anju, 2014; Sharma and Bhat, 2009; Manjula *et al.*, 2010).

$$\% \text{ scavenging of DPPH} = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{\text{Absorbance of control}} \times 100$$

Electrochemical Measurements

Electrochemical measurements hold many advantages over DPPH, they are fast, cheap and the oxidation potentials can be determined with high accuracy. Cyclic voltammetry studies were carried out using a three-electrode system comprising of glassy carbon working electrode, saturated calomel reference electrode and a platinum counter electrode which is used as a reference (Sochor *et al.*, 2013). The electrodes were cleaned and polished using 0.3 µm alumina powders and rinsed before the commencement of the experiment (Brcanovic *et al.*, 2013). Subsequent to mechanical treatment, electrochemical pretreatment of glassy carbon was carried out by potentio-dynamic cycling through11.0 - 0.8V in the supporting electrolyte at a slow sweep rate 10 mV/s for approximately 15-30 min. The electrolyte was de-aerated during the potential cycling process.

About 20 mL of the supporting electrode solution was taken in the electro-chemical cell. The molecule under study was dissolved in DMSO and added to it so that the total volume was maintained at 30 mL. The solution was mixed well using a magnetic stirrer for a minute and cyclic voltammetry measurements were performed from +2 V to -2 V at the glassy carbon electrode at a scan rate of 50 mV/s.

RESULTS AND DISCUSSION

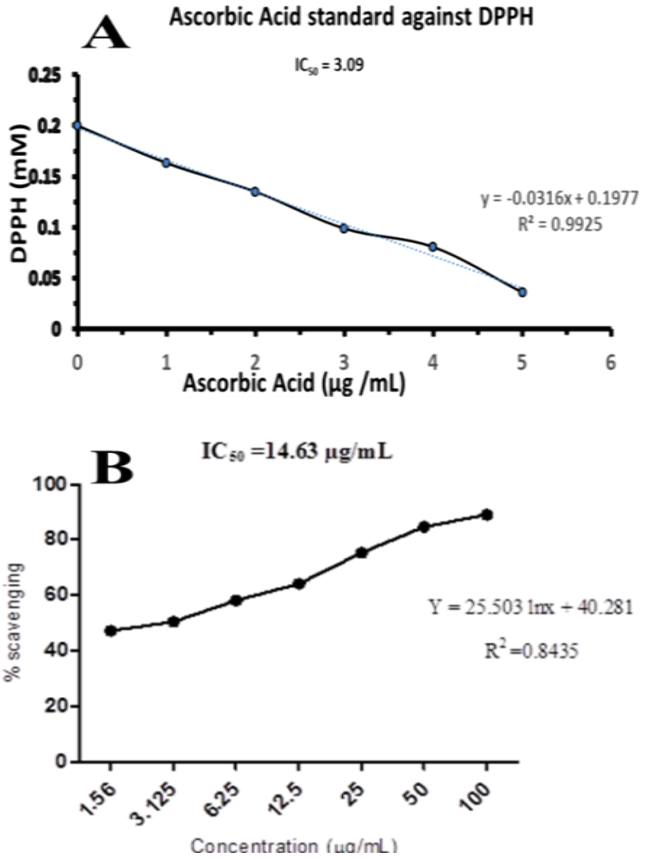
Chemistry

The IR spectra of oxadiazole thiones (**2a-b**) showed the characteristic C=S stretching band at 1260-1270 cm^{-1} and their ^{1}HNMR spectra displayed the NH protons at 10.73 ppm. The NH and C=O stretching vibrations were observed at 3150 and 1670-1680 cm^{-1} in the IR spectra and the NH proton resonated at δ 11.2-11.4 in the ^{1}HNMR spectra of pyrrole derivatives (**3a-b**). The schiff bases (**5a-d**) exhibited the characteristic IR stretching absorption at 1600-1610 cm^{-1} for the C=N group. The Schiff base ligand prepared under mild conditions reacted with $HgCl_2$ in DMSO at 298 K to afford the corresponding mercury (II) complex **6** in 74% yield. The IR analysis showed that in this mononuclear mercury (II) complex, the Hg^{2+} ions are surrounded by one nitrogen and two oxygen atoms from the schiff base ligand and the two Cl atoms. In comparison with the spectra of the schiff base **5b**, the Hg(II) complex displayed the band of (HC=N) at 1604 cm^{-}

[1]; showing a shift to lower wave numbers from 1610 cm^{-1} suggesting that, the azomethine nitrogen is coordinated to Hg(II) ion (Ngai *et al.*, 2013; Abdel-Hamid and Newair, 2011). The OH stretching vibration at 3298 cm^{-1} in **5b** shifted to a lower frequency to 3286 cm^{-1} in the complex. The band of lactone (C=O) at 1737 cm^{-1} in the metal complex showed an absorption shift to lower wave number from 1745 cm^{-1} confirmed that, the oxygen of the carbonyl group is coordinated to the metal ion. The unchanged position of NH band in the metal complex indicated that this group is not involved in coordination with Hg^{2+} ion. The new bands that appeared in the region of 510 and 530 cm^{-1} in the spectra of the complexes are attributed to (M-N) and (M-O) bond stretchings respectively. Yet another band in the region 410 cm^{-1} is assigned to (M-Cl) bond vibration. Thus the IR spectrum provides strong evidence for the complex formation of **5b** with Hg. The molecular weight of the synthesized molecules obtained from the mass spectra was in accordance with their respective molar masses.

Antioxidant studies

All the salicylic acid derivatives were screened for their antioxidant potential using DPPH assay. The ascorbic acid which was used as the standard exhibited an IC50 value of 3.09 µg/mL. All the molecules studied exhibited moderate to good antioxidant capabilities. Especially, Schiff base **5b** exhibited a good IC50 value of 14.63 µg/mL, whereas its Hg(II) complex did not show any antioxidant capacity. The graph depicting the antioxidant nature of ascorbic acid, **5b** and **6** is presented in **Fig.1**.



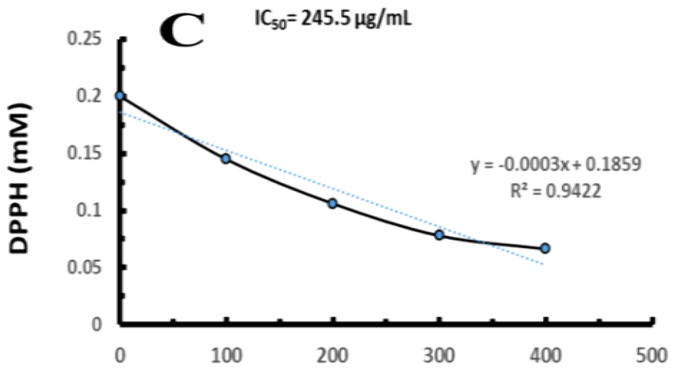


Fig. 1: Graphs depicting antioxidant potentials of A) Standard Ascorbic acid, B) Schiff base 5b and C) 5b-Hg(II) complex, 6.

Cyclic voltammetry

Cyclic voltammetry technique was used to assess the antioxidant activity of the most active molecule 5b Cyclic voltammetry is a frequently employed technique in electrochemistry to characterize the antioxidant potential of electro-active molecules as well as electrode surfaces, mainly attributed to its simplicity, speed, and amenability to be used directly on biological samples. In this method, the redox potential of probable antioxidants is scanned at a controlled rate, which is oxidized and the current produced during redox reactions that occur on the surface of the working electrode is recorded continuously (Hynek *et al.*, 2012). Thus in cyclic voltammetry, the antioxidants are characterized by their current–potential relationships offered at an inert glassy carbon electrode. An antioxidant can be easily oxidized at an electrode. The more powerful the reducing agent, the lower is its positive oxidation potential (Sun-Waterhouse *et al.*, 2008).

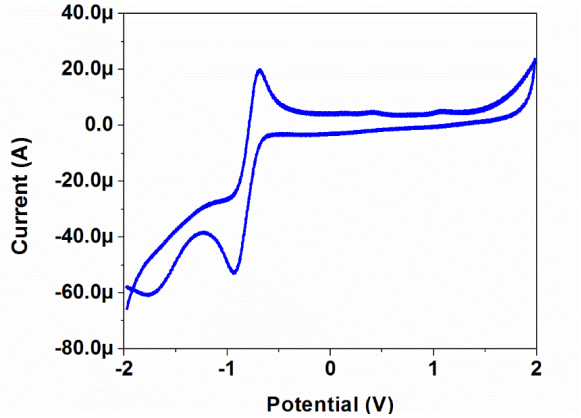


Fig. 2: Cyclic voltammogram of **5b** taken at a glassy carbon electrode at 50 mV/s.

Schiff base **5b** was subjected to cyclic voltammetry in order to assess its antioxidant behavior. The cyclic voltammogram of **5b** is presented in **Fig.2**. The cyclic voltammogram of 5b shows an increase in current that starts at 0.9 V in the forward scan, after which the potential climbs exponentially (**Fig. 2**). However, the swift upsurge in current does not persist for long. A peak is observed (Ep,a) at 0.65 V followed by decrease in current.

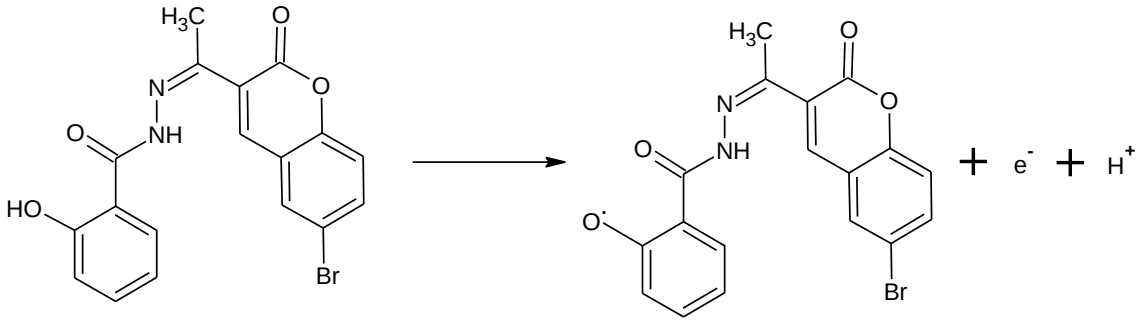


Fig. 3: Reaction of Schiff base 5b in the glassy carbon electrode surface.

This fall in the current is attributed to the depletion of **5b** that is expected to occur near the electrode surface as a result of the diffusion of **5b** from the bulk solution. The anodic peak potential was determined from the forward cyclic voltammogram. On the reverse scan as the electrode potential is lowered, the oxidation product of 5b (O) can be reduced. This is because 5b (O) is yet to diffuse away from the electrode surface and is practically absent from the bulk solution.

A cathodic peak is seen as the (O) levels near the electrode become depleted and the reaction is once again diffusion controlled. The cathodic peak current is lower than the anodic peak current because of continued oxidation of the reduction product (R) and also due to inadequate availability of O from the bulk solution.

The results reveal good antioxidant potential and electrochemical behavior of **5b** at glassy carbon electrode. Cyclic voltammogram presents single anodic and cathodic peaks with corresponding Epa and Epc values of -0.65 V and -0.95 V, the ratio of anodic and cathodic peak current value indicates a reversible electrode process. Electrolysis occurs generally at the electrode surface due in response to a potential change so as to sustain the surface concentrations of the oxidized and reduced species as demanded by the Nernst equation. The schiff base **5b** displays a single electron quasi reversible transfer process with a corresponding reduction and oxidation peak at a scan rate of 50 mV/s (**Fig.1**).

The low oxidation potential of **5b** indicates its ability to donate the electron with high ease to the system to generate free radicals and this data correlates well with its higher antioxidant activity in the biological assay (Arteaga *et al.*, 2012).

Compound **5b** acts as antioxidant by radical scavenging through oxidation leading to the formation of a substituted phenoxy radical through hydrogen atom donation leading to a lowering of the electrochemical potential (**Fig.3**) (Blomhoff, 2005)

CONCLUSIONS

Nine new salicylic acid derivatives were synthesized, characterized, and their resulting antioxidant capacity was explored. The redox potential of the most active compound **5b** obtained using cyclic voltammetry was compared with the radical-scavenging data obtained from DPPH spectrometric assay. The "low oxidation potential" of **5b** corresponds to its strong scavenging capabilities and high antioxidant power substantiating the results from the DPPH biological assay.

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