CHAPTER-2

Synthesis, Characterisation and Antioxidant Studies
Indole-2-carboxylic Acid Analogues
2.1. INTRODUCTION

Indole derivatives have generated considerable interest and has been reported as having diverse biological activity which includes fused indole derivatives which forms the basis of a range of pharmaceuticals.\(^1\)\(^,\)\(^2\) Indole moiety occurs widely in synthetic and natural products containing an important class of therapeutic agents.\(^3\)\(^,\)\(^4\) In the last decade, antioxidant activity of synthetic indole derivatives and their possible activity mechanisms have been widely studied.\(^4\)

The efficient synthesis of novel substituted indole derivative compounds still represents the highly pursued target. Many compounds containing the indole skeleton have indeed shown biological properties as HIV-1 integrase inhibitor,\(^5\) inhibitors of cytosolic phospholipase A\(_2\).\(^6\) Several 3-acylindole-2-carboxylic acid derivatives are inhibitors of the cPLA\(_2\)-mediated arachidonic acid release induced by calcium ionophore.\(^7\) Indole acetic acid has been shown to be active against E. coli.\(^8\) Also certain indole related derivatives such as \(\{5\text{-}\[2\text{-}(\text{benzoxazol-2-ylmethylamino)}\text{ethoxy}\}\text{-}1\text{-methyl-1H-indol-3-yl}\}\text{acetic acids (a) and 6-alkoxy-1-alkyl-1H-indole-2-carboxylic acids (b) serves as Aβ aggregation inhibitors.}\(^9\)

![Chemical structures of Aβ aggregation inhibitors.](image)

**Figure- 2.1:** Chemical structures of Aβ aggregation inhibitors.

Studies on the effect of amino acid receptor antagonist indole-2-carboxylic acid on memory and motor dysfunction and changes in regional total tissue brain following brain injury in the rat has been done. It was found that indole-2-carboxylic
acids produced beneficial effects on both behavioral and neurochemical sequelae of traumatic brain injury. Compounds demonstrated potential clinical utility with respect to the improvement of behavioral and neurochemical outcome of experimental brain injury.¹⁰

Novel Indole-2-carboxamide and cycloalkeno[1,2-b]indole derivatives. Structure activity relationships for high inhibition of human LDL peroxidation has been studied.¹¹

**Figure-2.2a**

**Figure-2.2b**

**Figure-2.2a:** Structure of indole-2-carboxamide derivatives.

**Figure-2.2b:** Structure of cycloalkeno[1,2-b]indole derivatives.

Synthesis of series of indole-2-carboxylic acid benzylidene hydrazides as a new series of potent apoptosis inducers has been reported.¹² A structure activity relationship (SAR) study was carried out by modification of the substitutions on the indole and benzene rings. Substitution at the 3-position of the indole ring was found to be important for apoptotic activity.
Figure-2.3: Structure of substituted indole-2-carboxylic acid benzylidene hydrazides where $R_1=\text{Cl, CH}_3, \text{NO}_2$, $R_2=\text{CH}_3, \text{H, C}_6\text{H}_5$, $R_3=\text{H, p-Cl, p-CH}_3, \text{p-OCH}_3$………

Vibrational spectroscopic studies of indole carboxylic acids and their metal complexes especially 5-Methoxyindole-2-carboxylic acid and its Zn(II) complex were reported.\textsuperscript{13}

These findings prompted the synthesis of a number of indole derived systems of potential biological activity.

2.2. MATERIALS AND METHODS

A brief description of purification of solvents, the analytical procedure followed and the different physico-chemical techniques utilized for the characterization of the synthesized compounds and experiments are presented here.

2.2.1. Reagents

2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich), BHA and ascorbic acid (s.d.fine-chem), silica gel, TLC silica gel 60F\textsubscript{254} Plates (MERCK), BHA, ascorbic acid, ammonium chloride (s.d.fine-chem. Pvt. Ltd. India), sodium sulphate anhydrous (s.d.fine-chem), neocuproine, (Avra synthesis Pvt. Ltd. India) potassium ferricyanide, potassium per sulphate, ferric chloride, ammonium acetate, potassium carbonate (s.d.fine-chem), sodium bicarbonate (Rankem. Pvt. Ltd. India), sodium chloride (Rankem), potassium bromide (KBr) (Sisco Research Laboratories Pvt. Ltd. India), Silica gel (Qualigens 60-120 and 230-400mesh).

2.2.2. Solvents

The solvents thionyl chloride, acetyl chloride, tetrahydrofuran, trichloro acetic acid, ethyl acetate, dichloro methane, hexane, ethanol, methanol were of analytical
grade and purchased from Merck. Anilines, substituted anilines, aldehydes and substituted aldehydes were brought from S.D fine chemicals. And distilled water–double distilled by quartz distillation unit.

2.3. ANALYTICAL TECHNIQUES

2.3.1. Thin layer chromatography (TLC)

For TLC, Merck silica gel F$_{254}$. Different hexane:ethyl acetate and chloroform:methanol solvents were used as mobile phases depending on the polarities of the compounds for TLC systems and spots were visualized after exposure to iodine vapours or under ultraviolet (UV) light.

2.3.2. Column chromatography

For column chromatography silica gel (Qualigens 60-120 mesh and 230-400 mesh) was used and hexane:ethyl acetate and chloroform:methanol mobile phases with different ratio depending on the polarity of the compounds.

2.3.3. Determination of melting point

The melting points were determined by open capillary method on a campbel electronic apparatus and are uncorrected.

2.3.4. Instrumentation

The instrumental techniques employed for the characterization of the newly synthesized compounds includes FT-IR, $^1$H-NMR, LC-MS and elemental analysis. The details of instrumentation are briefly given below.

2.3.4.1. Infrared (FT-IR)

The IR spectra of synthesized compounds were recorded on a shimadzu 8400S FT-IR in potassium bromide disks.
2.3.4.2. $^1$H NMR

The $^1$H-NMR was recorded in DMSO-$d_6$ and CDCl$_3$ using NMR Varian-Mercury 400 MHz spectrometer and chemical shifts are given in units as $\delta$ ppm, downfield from tetramethylsilane (TMS) as an internal standard. Chemical shift values were expressed in ppm relative to internal tetra methyl silane (TMS) as the standard.

2.3.4.3. LC-MS

Mass spectra were obtained on an electron impact mass spectrometer using micromass Q-Tof- 2 mass spectrometers at 70 eV ionizing beam and using a direct insertion probe.

2.4. EXPERIMENTAL METHODS

2.4.1. Synthesis of 1-acetyl-$1H$-indole-2-carboxylic acid (2)

To a well stirred solution of indole-2-carboxylic acid (1 mM) and triethylamine (1.2 mM) in 15 mL dichloromethane, acetyl chloride (1.3 mM) in 5 mL was added drop by drop for 10 min, then the reaction mixture is stirred at room temperature for about 3 hr. Progress of the reaction was monitored by thin layer chromatography (TLC) using hexane:ethylacetate (6:4) mixture as mobile phase. After the completion of reaction, the reaction mass was quenched in ice cold water and the product was extracted with ethyl acetate. The organic layer was washed with 5% NaHCO$_3$ followed by distilled water. Finally the organic layer was dried over anhydrous Na$_2$SO$_4$. The brown solid product was obtained by desolventation through rotary evaporator.
2.4.2. Synthesis of 1H-indole-2-carboxyl chloride (4)

To a well stirred solution of 1H-indole-2-carboxylic acid (1 mM) in 15 mL dry tetrahydrofuran (THF), thionyl chloride (1.2 mM) in 3 mL dry THF was added drop wise at 0 °C, then the reaction mixture is stirred at room temperature for about 3 hr. Progress of the reaction was monitored by TLC using hexane:ethylacetate(6:4) mixture as mobile phase. After the completion of reaction, the product was extracted with ethyl acetate. The organic layer was washed with 5% NaHCO₃ followed by distilled water. Finally the organic layer was dried over anhydrous Na₂SO₄. The light brown solid product was obtained by desolventation through rotary evaporator.

Further, coupling of substituted aldehydes and substituted anilines to obtain a series analogues of 1H-indole-2-carboxylic acid 3(a-h) and 5(a-g) in moderate to good yield, which were identified by spectroscopic techniques using ¹H NMR, FT-IR and LC-MS.

2.4.3. General procedure for the synthesis of 1-acetyl-1H-indole-2-carboxylic acid analogues 3(a-h).

To a solution of 1-acetyl-1H-indole-2-carboxylic acid (1 mM) in ethanol (10 mL) substituted benzaldehydes were added in the presence of 10% NaOH at room temperature (scheme-2.I) Progress of the reaction was monitored by TLC using chloroform:methanol (6:4) mixture as mobile phase. After the completion of reaction, the product was extracted with ethyl acetate. The organic layer was acidified with dilute HCl and washed with brine solution followed by distilled water. Finally the organic layer was dried over anhydrous Na₂SO₄. Further the product was obtained by desolventation through rotary evaporator.
2.4.4. General procedure for the synthesis of 1-acetyl-1H-indole-2-carboxylic acid analogues 5(a-g).

To a solution of 1H-indole-2-carbonyl chloride (1 mM) in dry THF (10 mL) substituted anilines were added in the presence of TEA (3 mL) under inert (N₂) atmosphere. The reaction mixture was refluxed for 4 hr (Scheme-2.II). Progress of the reaction was monitored by TLC using chloroform:methanol (6:4) mixture as mobile phase. After the completion of reaction, the product was extracted with ethyl acetate. The organic layer was washed with 5% NaHCO₃ solution followed by distilled water. Finally the organic layer was dried over anhydrous Na₂SO₄. Further the product was obtained by desolventation through rotary evaporator.

Reaction Scheme-2.I and 2.II: Reaction scheme for the synthesis of indole-2-carboxylic acid analogues
**Table-2.1:** Chemical structures and yields of indole-2-carboxylic acid analogues.

<table>
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<tr>
<th>Compound</th>
<th>R</th>
<th>Yield (%)</th>
<th>compound</th>
<th>R</th>
<th>Yield (%)</th>
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<td><img src="3a.png" alt="structure" /></td>
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<td>5a</td>
<td><img src="5a.png" alt="structure" /></td>
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<tr>
<td>3b</td>
<td><img src="3b.png" alt="structure" /></td>
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<td><img src="5b.png" alt="structure" /></td>
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<tr>
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<td><img src="5c.png" alt="structure" /></td>
<td>85</td>
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<tr>
<td>3d</td>
<td><img src="3d.png" alt="structure" /></td>
<td>77</td>
<td>5d</td>
<td><img src="5d.png" alt="structure" /></td>
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<tr>
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<td><img src="5e.png" alt="structure" /></td>
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<tr>
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<td>5f</td>
<td><img src="5f.png" alt="structure" /></td>
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<tr>
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<td>5g</td>
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<tr>
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### 2.5. CHARACTERISATION TABLES

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<td><strong>Physical State</strong></td>
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<td><strong>Melting Point</strong></td>
</tr>
<tr>
<td><strong>IR</strong></td>
</tr>
<tr>
<td><strong>Mass</strong></td>
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<tr>
<td><strong>$^1$H NMR</strong></td>
</tr>
<tr>
<td><strong>$^{13}$C NMR</strong></td>
</tr>
<tr>
<td><strong>Elemental Analysis</strong></td>
</tr>
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## Compound 3b

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<td><strong>Chemical Formula</strong></td>
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<td><strong>$^1$H NMR</strong></td>
<td>(400 MHz, DMSO-d$_6$, $\delta$, ppm): 11.1 (s, 1H, COOH), 7.21-8.10 (m, 9H, Ar-H), 6.73 (d, 1H, CH=CH), 6.68 (d, 1H, CH=CH).</td>
</tr>
<tr>
<td><strong>$^{13}$C NMR</strong></td>
<td>168.6, 160.4, 151.7, 146.3, 145.4, 134.2, 133.1, 130.3, 128.7, 128.5, 127.6, 127.1, 125.2, 124.8, 117.3, 112.2, 110.1, 107.5</td>
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<td>C, 66.39; H, 3.71; Cl, 10.85; N, 4.27</td>
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![Compound 3b structure](image-url)
# Compound 3c

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<tr>
<td><strong>Chemical Formula</strong></td>
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<td>IR(KBr) (\lambda_{\text{max}}(\text{cm}^{-1})): 3338 (OH), 2849-2923 (Ar-H), 1624 (C=O).</td>
</tr>
</tbody>
</table>
| **Mass**               | MS (EI, m/z) 308.12 (M+1)
| \(^1\text{H} \text{NMR}\) | (400 MHz, DMSO-\text{d}_6, \delta, ppm): 11.24 (s, 1H, COOH), 6.21-7.86 (m, 9H, Ar-H), 7.22 (d, 1H, CH=CH), 6.28 (d, 1H, CH=CH), 5.34 (s, 1H, Phenolic -OH). |
| \(^{13}\text{C} \text{NMR}\) | 169.1, 160.6, 158.2, 151.5, 146.5, 145.4, 137.2, 136.4, 134.1, 129.9, 127.6, 127.4, 123.4, 123.0, 119.1, 116.2, 108.3 |
| **Elemental Analysis** | **Calculated** | **Found** |
|                        | C, 70.35; H, 4.26; N, 4.56; O, 20.83 |
                        | C, 70.37; H, 4.24; N, 4.58; O, 20.87 |
### Compound 3d

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<td>(Z)-1-(3-(4-nitrophenyl)acryloyl)-1H-indole-2-carboxylic acid</td>
</tr>
<tr>
<td><strong>Chemical Formula</strong></td>
<td>(\text{C}<em>{18}\text{H}</em>{12}\text{N}_2\text{O}_5)</td>
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<td><strong>Molecular Weight</strong></td>
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<tr>
<td><strong>Physical State</strong></td>
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<td><strong>Mass</strong></td>
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<tr>
<td><strong>(^1\text{H} \text{NMR</strong>}**</td>
<td>(400 MHz, DMSO-(d_6), (\delta) ppm): 11.1 (s, 1H, COOH), 6.86-7.50 (m, 9H, Ar-H), 6.73 (d, 1H, CH=CH), 6.27 (d, 1H, CH=CH).</td>
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<tr>
<td><strong>(^{13}\text{C} \text{NMR}</strong></td>
<td>170.4, 160.1, 148.2, 146.1, 151.2, 138.1, 136.6, 131.2, 128.6, 127.1, 122.1, 121.6, 118.6, 112.1, 111.1, 110.2, 108.1</td>
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<td><strong>Elemental analysis</strong></td>
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<tr>
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<td><strong>IUPAC Name</strong></td>
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<td><strong>Chemical Formula</strong></td>
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<td><strong>Molecular weight</strong></td>
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<td><strong>Mass</strong></td>
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<td>(400 MHz, DMSO-$d_6$, $\delta$, ppm): 11.0 (s, 1H, COOH), 6.91-7.68 (m, 9H, Ar-H), 7.11 (d, 1H, CH=CH), 6.93 (d, 1H, CH=CH), 3.84 (s, 3H, OCH$_3$).</td>
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<td>170.6, 160.1, 150.1, 145.9, 142.4, 137.6, 136.6, 130.6, 127.6, 122.2, 120.8, 119.4, 112.6, 110.1, 109.9, 104.1, 66.5</td>
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<td><strong>Chemical Formula</strong></td>
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<td><strong>Molecular Weight</strong></td>
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<td><strong>Physical State</strong></td>
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<td><strong>IR</strong></td>
<td>IR(KBr) $\lambda_{\text{max}}$(cm$^{-1}$): 3505 (OH), 2853-2918 (Ar-H), 1663 (C=O).</td>
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<tr>
<td><strong>Mass</strong></td>
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<td><strong>$^1$H NMR</strong></td>
<td>(400 MHz, DMSO-$d_6$, $\delta$, ppm): 11.0 (s, 1H, COOH), 6.83-7.50 (m, 9H, Ar-H), 6.79 (d, 1H, CH=CH), 6.63 (d, 1H, CH=CH), 2.33 (s, 3H, CH$_3$).</td>
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<td><strong>$^{13}$C NMR</strong></td>
<td>170.8, 160.3, 151.2, 146.2, 141.3, 137.1, 136.5, 130.8, 127.2, 121.9, 120.9, 119.5, 112.7, 111.4, 109.8, 103.4, 21.5</td>
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<td><strong>Elemental Analysis</strong></td>
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<td>C, 74.74; H, 4.95; N, 4.59; O, 15.72</td>
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<th>Property</th>
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| IUPAC Name                     | (Z)-1-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)-1H-indole-2-carboxylic acid |<br>![IUPAC structure](image)
| Chemical Formula               | C_{19}H_{15}NO_{5}                                                      |
| Molecular Weight               | 337.33                                                                 |
| Physical State                 | yellow solid                                                           |
| Melting Point                  | 162-165 °C                                                             |
| IR                             | IR(KBr)γ_{max}(cm^{-1}): 3388 (OH), 2853-2918 (Ar-H), 1675 (C=O).       |
| Mass                           | MS (EI, m/z) 338.30 (M+1)^+                                             |
| \(^1\)H NMR                     | (400 MHz, DMSO-\(d_6\), δ, ppm): 11.2 (s, 1H, COOH), 7.10-7.68 (m, 8H, Ar-H), 7.10 (d, 1H, CH=CH), 6.64 (d, 1H, CH=CH), 5.35 (s, 1H, phenolic OH), 3.83 (s, 3H, \(-\text{OCH}_3\)). |
| \(^{13}\)C NMR                   | 170.6, 160.8, 150.9, 147.3, 145.3, 141.2, 137.4, 135.3, 130.1, 127.5, 120.8, 120.1, 119.2, 114.3, 110.3, 102.6, 65.3 |
| Elemental Analysis             | Calculated: C, 67.65; H, 4.48; N, 4.15; O, 23.72                      | Found: C, 67.63; H, 4.44; N, 4.17; O, 23.76 |
# Compound 3h

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<td><strong>IR</strong></td>
<td>IR(KBr) $\lambda_{\text{max}}$ cm$^{-1}$: 3433 (OH), 2854-2938 (Ar-H), 1653 (C=O).</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>MS (EI, m/z) 382.23 (M+1)$^+$</td>
</tr>
<tr>
<td><strong>$^1$H NMR</strong></td>
<td>(400 MHz, DMSO-$_d_6$, $\delta$, ppm): 11.0 (s, 1H, COOH), 6.26-7.67 (m, 7H, Ar-H), 7.46 (d, 1H, CH=CH), 7.42 (d, 1H, CH=CH), 3.83 (m, 9H, OCH$_3$).</td>
</tr>
<tr>
<td><strong>$^{13}$C NMR</strong></td>
<td>168.9, 161.2, 156.1, 152.1, 150.1, 150.1, 140.1, 139.8, 134.1, 130.2, 126.4, 121.1, 120.8, 118.3, 114.2, 109.1, 104.3, 68.3, 64.2, 64.1</td>
</tr>
<tr>
<td><strong>Elemental Analysis</strong></td>
<td><strong>Calculated</strong></td>
</tr>
<tr>
<td></td>
<td>C, 66.13; H, 5.02; N, 3.67; O, 25.17</td>
</tr>
</tbody>
</table>
**Compound 5a**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC Name</td>
<td>N-phenyl-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>C_{15}H_{12}N_{2}O</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>236.27</td>
</tr>
<tr>
<td>Physical State</td>
<td>Yellow solid</td>
</tr>
<tr>
<td>Melting Point</td>
<td>115-118 °C</td>
</tr>
<tr>
<td>IR</td>
<td>IR(KBr) $\lambda_{max}$(cm$^{-1}$): 3265 (N-H), 2853-2927 (Ar-H), 1656 (C=O).</td>
</tr>
<tr>
<td>Mass</td>
<td>MS (EI, m/z) 237.28 (M+1)$^+$</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>(400 MHz, DMSO-$d_6$, $\delta$, ppm): 11.9 (s, 1H, NH of indole), 8.35 (s, 1H, NH of amine), 7.1-7.5 (m, 10H, Ar-H).</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>161.9, 152.6, 153.4, 138.1, 131.2, 130.8, 129.5, 128.6, 128.2, 112.6, 110.9, 110.1, 108.3, 100.1, 102.4</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td>Calculated Found</td>
</tr>
<tr>
<td></td>
<td>C, 76.25; H, 5.12; N, 11.86; O, 6.77 C, 76.26; H, 5.15; N, 11.88; O, 6.75</td>
</tr>
</tbody>
</table>
### Compound 5b

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IUPAC Name</strong></td>
<td>N-(4-hydroxyphenyl)-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td><strong>Chemical Formula</strong></td>
<td>C₁₅H₁₂N₂O₂</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>252.27 g/mol</td>
</tr>
<tr>
<td><strong>Physical State</strong></td>
<td>White solid</td>
</tr>
<tr>
<td><strong>Melting Point</strong></td>
<td>125-128 °C</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>IR(KBr) $\lambda_{\text{max}}$(cm$^{-1}$): 3338 (N-H), 2873-2956 (Ar-H), 1695 (C=O).</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>MS (EI, m/z) 253.21 (M+1)$^+$</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>(400 MHz, DMSO-$d_6$, $\delta$, ppm): 11.7 (s, 1H, NH of indole), 9.15 (s, 1H, NH of amine), 6.91-7.70 (m, 9H, Ar-H), 5.35 (s, 1H, phenolic OH).</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>162.3, 152.9, 153.1, 137.2, 131.3, 129.4, 128.9, 128.6, 112.4, 111.6, 110.9, 109.1, 101.2, 100.6.</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td><strong>Calculated</strong></td>
</tr>
<tr>
<td></td>
<td>C, 71.42; H, 4.79; N, 11.10; O, 12.68</td>
</tr>
<tr>
<td></td>
<td><strong>Found</strong></td>
</tr>
<tr>
<td></td>
<td>C, 71.40; H, 4.77; N, 11.13; O, 12.65</td>
</tr>
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**Compound 5c**

<table>
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<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC Name</td>
<td>N-(2-hydroxyphenyl)-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td>Molecular Weight</td>
<td>252.27</td>
</tr>
<tr>
<td>Physical State</td>
<td>White solid</td>
</tr>
<tr>
<td>Melting Point</td>
<td>176-179 °C</td>
</tr>
<tr>
<td>IR</td>
<td>IR(KBr) (\lambda_{max}(\text{cm}^{-1}):) 3348 (N-H), 2863-2956 (Ar-H), 1695 (C=O).</td>
</tr>
<tr>
<td>Mass</td>
<td>MS (EI, m/z) 253.18 (M+1)&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>(^1)H NMR</td>
<td>(400 MHz, DMSO-(d_6), (\delta), ppm): 11.9 (s, 1H, NH of indole), 9.13 (s, 1H, NH of amine), 6.81-7.99 (m, 9H, Ar-H), 5.3 (s, 1H, phenolic OH).</td>
</tr>
<tr>
<td>(^{13})C NMR</td>
<td>162.4, 153.3, 153.0, 138.3, 131.6, 131.0, 129.2, 128.6, 128.3, 112.1, 1111.0, 110.6, 109.3, 101.4, 101.3</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td>Calculated</td>
</tr>
<tr>
<td></td>
<td>Found</td>
</tr>
<tr>
<td></td>
<td>C, 71.42; H, 4.79; N, 11.10; O, 12.68</td>
</tr>
<tr>
<td></td>
<td>C, 71.41; H, 4.77; N, 11.14; O, 12.66</td>
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## Compound 5d

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<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IUPAC Name</strong></td>
<td>N-(4-methoxyphenyl)-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td><strong>Chemical Formula</strong></td>
<td>C_{16}H_{14}N_{2}O_{2}</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>266.29</td>
</tr>
<tr>
<td><strong>Physical State</strong></td>
<td>Bright yellow solid</td>
</tr>
<tr>
<td><strong>Melting Point</strong></td>
<td>111-114 °C</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>IR(KBr) ( \lambda_{\text{max}} ) (cm\textsuperscript{-1}): 3317 (N-H), 2873-2933 (Ar-H), 1689 (C=O).</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>MS (EI, m/z) 267.3 (M+1)\textsuperscript{+}</td>
</tr>
<tr>
<td>(^1\text{H NMR})</td>
<td>(400 MHz, DMSO-\textit{d}_6, ( \delta ), ppm): 11.62 (s, 1H, NH of indole), 9.41 (s, 1H, NH of amine), 7.11-7.59 (m, 9H, Ar-H), 3.83 (s, 3H, OCH\textsubscript{3}).</td>
</tr>
<tr>
<td>(^{13}\text{C NMR})</td>
<td>162.07, 154.4, 154.0, 131.2, 131.1, 129.4, 128.5, 128.2, 112.0, 111.8, 110.9, 109.2, 101.1, 66.7</td>
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<tr>
<td><strong>Elemental Analysis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Calculated</strong></td>
<td>C, 72.16; H, 5.30; N, 10.52; O, 12.02</td>
</tr>
<tr>
<td><strong>Found</strong></td>
<td>C, 72.17; H, 5.27; N, 10.55; O, 12.06</td>
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### Compound 5e

<table>
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<tr>
<td><strong>IUPAC Name</strong></td>
<td>N-(2-methoxyphenyl)-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td><strong>Chemical Formula</strong></td>
<td>C_{16}H_{14}N_{2}O_{2}</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>266.29</td>
</tr>
<tr>
<td><strong>Physical State</strong></td>
<td>White powder</td>
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<tr>
<td><strong>Melting Point</strong></td>
<td>185-188 °C</td>
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<tr>
<td><strong>IR</strong></td>
<td>IR(KBr) $\lambda_{\text{max}}$ (cm$^{-1}$): 3378 (N-H), 2823-2986 (Ar-H), 1695 (C=O).</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>MS (EI, m/z) 267.30 (M+1)$^+$</td>
</tr>
<tr>
<td><strong>$^1$H NMR</strong></td>
<td>(400 MHz, DMSO-$d_6$, $\delta$, ppm): 11.6 (s, 1H, NH of indole), 9.43 (s, 1H, NH of amine), 6.86-7.90 (m, 9H, Ar-H), 3.83 (s, 3H, OCH$_3$).</td>
</tr>
<tr>
<td><strong>$^{13}$C NMR</strong></td>
<td>162.0, 154.2, 154.1, 130.9, 129.2, 128.3, 128.0, 112.2, 111.9, 110.6, 108.1, 101.3, 101.2, 66.6</td>
</tr>
</tbody>
</table>
| **Elemental Analysis**    | **Calculated**

| C, 72.16; H, 5.30; N, 10.52; O, 12.02 |

**Found**

| C, 72.18; H, 5.29; N, 10.55; O, 12.05 |
### Compound 5f

<table>
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<tr>
<td><strong>IUPAC Name</strong></td>
<td>N-(4-bromophenyl)-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td><strong>Chemical Formula</strong></td>
<td>C$<em>{12}$H$</em>{11}$BrN$_{2}$O</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>315.16</td>
</tr>
<tr>
<td><strong>Physical State</strong></td>
<td>White solid</td>
</tr>
<tr>
<td><strong>Melting Point</strong></td>
<td>145-148 °C</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>IR(KBr) $\lambda_{\text{max}}$(cm$^{-1}$): 3407 (N-H), 2833-2936 (Ar-H), 1775 (C=O).</td>
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<tr>
<td><strong>Mass</strong></td>
<td>MS (EI, m/z) 316.26 (M+1)$^+$</td>
</tr>
<tr>
<td><strong>$^1$H NMR</strong></td>
<td>(400 MHz, DMSO-$d_6$, $\delta$, ppm): 11.91 (s, 1H, NH of indole), 9.5 (s, 1H, NH of amine), 7.1-7.67 (m, 9H, Ar-H).</td>
</tr>
<tr>
<td><strong>$^{13}$C NMR</strong></td>
<td>163.1, 153.2, 152.1, 130.6, 130.1, 126.2, 128.4, 128.0, 120.2, 114.1, 111.8, 110.2, 108.8, 101.4, 101.3.</td>
</tr>
<tr>
<td><strong>Elemental Analysis</strong></td>
<td><strong>Calculated</strong></td>
</tr>
<tr>
<td></td>
<td>C, 57.16; H, 3.52; N, 8.89; O, 5.08</td>
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</table>
### Compound 5g

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<tbody>
<tr>
<td><strong>IUPAC Name</strong></td>
<td>N-(4-nitrophenyl)-1H-indole-2-carboxamide</td>
</tr>
<tr>
<td><strong>Chemical Formula</strong></td>
<td>C₁₅H₁₁N₃O₃</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>281.27</td>
</tr>
<tr>
<td><strong>Physical State</strong></td>
<td>White solid</td>
</tr>
<tr>
<td><strong>Melting Point</strong></td>
<td>106-109 °C</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>IR(KBr) λ_{max}(cm⁻¹): 3438 (N-H), 2873-2951 (Ar-H), 1677 (C=O).</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>MS (EI, m/z) 282.10 (M⁺)</td>
</tr>
<tr>
<td><strong>¹H NMR</strong></td>
<td>(400 MHz, DMSO-d₆, δ, ppm): 11.76 (s, 1H, NH of indole), 9.1 (s, 1H, NH of amine), 7.33-7.97 (m, 9H, Ar-H).</td>
</tr>
<tr>
<td><strong>¹³C NMR</strong></td>
<td>162.2, 152.1, 151.0, 140.1, 132.6, 132.1, 128.4, 128.3, 126.1, 121.2, 116.1, 111.6, 106.1, 101.6, 101.2</td>
</tr>
<tr>
<td><strong>Elemental Analysis</strong></td>
<td><strong>Calculated</strong></td>
</tr>
<tr>
<td><strong>C, H, N, O</strong></td>
<td><strong>Found</strong></td>
</tr>
<tr>
<td>C, 64.05; H, 3.94; N, 14.94; O, 17.07</td>
<td>C, 64.09; H, 3.92; N, 14.97; O, 17.03</td>
</tr>
</tbody>
</table>
2.6. SPECTRAS:

**Figure-2.4:** IR spectrum of compound 3e
Figure-2.5: $^1$H NMR spectrum of compound 3a in DMSO-$d_6$
Figure-2.6: $^1$H NMR spectrum of compound 3e in DMSO-$d_6$
Figure-2.7: $^1$H NMR spectrum of compound 3h in DMSO-$d_6$
Figure-2.8: $^1$H NMR spectrum of compound 5a in DMSO-$d_6$
Figure-2.9: $^1$H NMR spectrum of compound 5d in DMSO-$d_6$
Figure-2.10: $^{13}$C NMR spectrum of compound 3e in DMSO-$d_6$
Figure-2.11: $^{13}$C NMR spectrum of compound 5d in DMSO-$d_6$. 
Figure 2.12: LC-MS spectrum of compound 3e
Figure-2.13: LC-MS spectrum of compound 5d
2.7. EVALUATION OF ANTIOXIDANT ACTIVITIES

**Background**

Oxidation of the organic compounds is one of the efficient methods of organic synthesis. On the other side the auto oxidation of the organic compounds, their mixtures and products promotes their rapid deterioration due to the action of the atmospheric oxygen. Antioxidant prevents the rapid development of these undesirable processes. The practical use of antioxidants began in the end of 19th century.

Different antioxidant compounds may act through different mechanisms; consequently, one method alone cannot be utilized to fully evaluate the antioxidant capacity of the compounds. For this reason, different antioxidant capacity tests with different approaches and mechanisms have been carried out. It is necessary to develop a rapid method for determining the potential antioxidants capacity. The antioxidants effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate.

**2.7.1. DPPH RADICAL SCAVENGING ACTIVITY**

The newly synthesized organic compounds were screened for their radical scavenging activities using stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

![Structure of DPPH](image)

*Figure-2.14: Structure of DPPH.*
Diphenyl picryl hydrazyl (DPPH) is a stable free radical, having molecular formula C_{18}H_{12}N_{5}O_{6} and molecular weight 394.3. It is soluble in dimethyl sulfoxide and ethyl alcohol. DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule i.e. 2,2-diphenyl-1-picrylhydrazine. Therefore DPPH is often used as a substrate to evaluate antioxidant activity of an antioxidant.

Stable free radical is used to trap other free radicals and thus reacting as an inhibitor in radical reactions. In this way one can study initiation reactions of radicals.

![Mechanism of DPPH radical.](image)

**Figure-2.15:** Mechanism of DPPH radical.

**DPPH Radical Scavenging Activity**

The compound under study was dissolved in distilled ethanol (50 mL) to prepare 1000 μM solution. Solutions of different concentrations (10 μM, 25 μM, 50 μM, 100 μM) were prepared by serial dilution and the free radical scavenging activity was studied. The DPPH scavenging effect was carried out according to the method first employed by Blois. Each compound solution having different concentrations were taken in different test tubes, 4mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at room temperature for 20 minutes.
A DPPH blank was prepared without compound and ethanol was used for the base line correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-Visible spectrophotometer and the remaining DPPH was calculated. The percentage decrease in the absorbance was recorded for each concentration, and percentage quenching of DPPH was calculated on the basis of the observed decrease in the absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the following equation:

**Radical Scavenging Activity (RSA) (%) = \[\frac{(A_0 - A_1)}{A_0} \times 100\]**

Where, \(A_0\) is the absorbance of control (blank, without compound).

\(A_1\) is the absorbance of the compound.

The radical scavenging activity of ascorbic acid and BHA was measured and compared with that of the newly synthesized compounds. \(IC_{50}\) (50% inhibitory concentration) was calculated by plotting graph (concentration v/s RSA).

### 2.7.2. FERRIC ION (Fe\(^{3+}\)) REDUCING ANTIOXIDANT POWER ASSAY

KFe[Fe(CN)\(_6\)]

Ferricyanide method: Prussian blue

Reducing power reflects the electron donating capacity of bioactive compounds, is associated with antioxidant activity. The reducing capacity of a compound can be measured by the direct reduction of Fe[(CN)\(_6\)]\(^{3-}\) to Fe[(CN)\(_6\)]\(^{2-}\). Addition of free Fe\(^{3+}\) to the reduced product leads to the formation of the intense Perl’s Prussian blue complex, which has a strong absorbance at 700 nm. An increase in absorbance of the reaction mixture would indicate an increase in the reducing capacity due to an increase in the formation of the complex. The ferric ion reducing antioxidant power assay takes advantage of an electron transfer reaction in which a
ferric salt is used as an oxidant. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this method, higher absorbance values indicate greater reducing capacity of ferric (Fe$^{3+}$) to ferrous (Fe$^{2+}$) ions.

All the novel indole-2-carboxylic acid analogues were screened for ferric reducing antioxidant power.$^{15}$ The compounds 3(a-h) and 5(a-g) having different concentrations (10 µM, 25 µM, 50 µM, 100 µM) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferric cyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. Later, the reaction mixture was acidified with trichloroacetic acid (TCA) (2.5 mL, 10%). After FeCl$_3$ (0.5 mL, 0.1%) was added to this solution, the absorbance was measured at 700 nm. Higher the absorbance value implies greater antioxidant power of the compounds.

**2.7.3. CUPRIC ION (Cu$^{2+}$) REDUCING ABILITY (CUPRAC METHOD)**

The method is based on reduction of Cu(II) to Cu(I) by reductants (antioxidants) present in a sample.

The plausible reaction of cuprac method is depicted below

\[
n \text{Cu(Nc)$_2^{2+}$ + Ar(OH)$_n$} \rightarrow n \text{Cu(Nc)$_2^{+}$ + Ar(=O)$_n$ + n H$^+$}
\]
Where, the polyphenol is oxidized to the corresponding quinone, and the reduction product, i.e., bis(neocuproine)copper(I) chelate, shows absorption maximum at 450 nm. Moreover, the results obtained from in vitro cupric ion (Cu$^{2+}$) reducing measurements might be more efficiently extended to the possible in vivo reactions of antioxidants. CUPRAC chromogenic redox reaction is carried out at a pH (7.0) close to the physiological pH, and the method is capable of measuring thiol-type antioxidants such as glutathione and non-protein thiols unlike the widely applied FRAP test, which is non-responsive to -SH group antioxidants. Of the eight criteria proposed by Prior et al. for defining an ideally standardized method of antioxidant capacity measurement, the CUPRAC method meets six, such as simplicity, clarity of end-point and mechanism, readily available instrumentation, good intra- and inter-assay reproducibility, adaptibility to simultaneously assay lipophilic and hydrophilic antioxidants and high throughput for routine analysis.

All the synthesized compounds were performed cupric ion reducing ability assay. Briefly, this method comprised of a mixture of CuCl$_2$ (1 mL, 0.01 M) solution, ethanolic neocuproine (Nc) (1 mL, 7.5 x 10$^{-3}$ M) solution and ammonium acetate (NH$_4$OAc) (1 mL, 1.0 M) were taken in a test tube and were added to a solution of compounds 3(a-h) and 5(a-g) with different concentrations (10 µM, 25 µM, 50 µM, 100 µM) along with 0.1 mL distilled water. The mixture was incubated for 30 min. Then the absorbance was measured at 450 nm against reagent blank.
2.7.4. PHOSPHOMOLYBDENUM ASSAY:

\[ \text{Mo(VI)} + e^- \rightarrow \text{Mo(V)} \quad \lambda_{\text{max}} = 695 \text{ nm} \]

\[ 3\text{H}_2\text{O}-\text{P}_2\text{O}_5-13\text{WO}_3-5\text{MoO}_3-10\text{H}_2\text{O} \]

**Figure-2.17:** Folin: molybdophosphotungstate heteropolyanion reagent, in which Mo(VI) is reduced to Mo(V) with an electron donated by an antioxidant.

The total antioxidant capacity was evaluated by using standard method. An aliquot of 0.1 mL of compound solutions with varying concentration was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank 0.1 mL of methanol was used in place of compound. The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to rt, the absorbance of the aqueous solution of each was measured at 695 nm against blank in spectrophotometer. For compounds of unknown composition, antioxidant capacity was expressed as equivalent of ascorbic acid (µM/mg of compound).

2.8. RESULTS AND DISCUSSION

2.8.1. Antioxidant evaluation

Evaluation of antioxidant activity for the newly synthesized analogues 3(a-h) and 5(a-g) was done by using *in vitro* bioanalytical methodologies such as 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity, ferric ion (Fe\(^{3+}\)) reducing antioxidant power assay, cupric ion (Cu\(^{2+}\)) reducing ability (CUPRAC method) and phosphomolybdenum assay. The antioxidant properties were expressed as 50% inhibitory concentration (IC\(_{50}\)) values for DPPH antioxidant assay.
2.8.1.1. DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical scavenging evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity (RSA) of specific compounds. The reaction of synthesized compounds with stable DPPH free radical indicates their free radical scavenging ability shown in table-2. In the present study, different electron withdrawing and electron donating groups attached to phenyl ring as substituent were studied for antioxidant efficacy. All the synthesized derivatives showed certain degree of antioxidant activity. The scaffold \( 3g \) containing hydroxy and methoxy group and \( 5b \) and \( 5c \) containing a hydroxy moiety on phenyl ring showed the effective activity among the synthesized analogues. This may be due to the presence of electron releasing substituents attached to the phenyl ring and in the compounds \( 5b \) and \( 5c \) in addition to the hydroxy group the presence of –NH group in indole moiety also adds for the achievement of lowest \( IC_{50} \) values there by showing good antioxidant activity when compared to the standards used. Compound \( 5e \) possessing methoxy group and \( 3c \) having hydroxy group on phenyl moiety displayed promising activity, but slightly less than that of \( 3g \), \( 5b \) and \( 5c \). The compounds \( 3e \), \( 5e \) and \( 5d \) having methoxy group on phenyl ring furnished moderate activity. Whereas compound \( 3a \) and \( 5a \) which does not have any substituents on the phenyl ring displayed comparably less activity when compared to standard. Also, compounds \( 3b \), \( 3d \), \( 5f \) and \( 5g \) having electron withdrawing groups displayed weak antioxidant activity. It can be concluded that the antioxidant nature of the synthesized scaffolds depends on the nature of the substituents present on phenyl ring and also the presence of –NH group in indole.
**Table-2.2:** Concentration required for 50% scavenging (IC$_{50}$) of DPPH• radical scavenging activity of the compounds 3(a-h) and 5(a-g) and the standard antioxidant compounds such as BHA and ascorbic acid. Each value represents mean ± SD (n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Scavenging activity (IC$_{50}$) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH•</td>
</tr>
<tr>
<td>3a</td>
<td>190±0.24</td>
</tr>
<tr>
<td>3b</td>
<td>290±0.62</td>
</tr>
<tr>
<td>3c</td>
<td>65±0.10</td>
</tr>
<tr>
<td>3d</td>
<td>400±0.57</td>
</tr>
<tr>
<td>3e</td>
<td>74±0.76</td>
</tr>
<tr>
<td>3f</td>
<td>145±65</td>
</tr>
<tr>
<td>3g</td>
<td>23±68</td>
</tr>
<tr>
<td>3h</td>
<td>200±34</td>
</tr>
<tr>
<td>5a</td>
<td>190±42</td>
</tr>
<tr>
<td>5b</td>
<td>35±0.55</td>
</tr>
<tr>
<td>5c</td>
<td>45±79</td>
</tr>
<tr>
<td>5d</td>
<td>85±0.12</td>
</tr>
<tr>
<td>5e</td>
<td>50±0.90</td>
</tr>
<tr>
<td>5f</td>
<td>285±0.48</td>
</tr>
<tr>
<td>5g</td>
<td>195±0.10</td>
</tr>
<tr>
<td>BHA</td>
<td>12±0.56</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>10±0.16</td>
</tr>
</tbody>
</table>

$^a$The values are expressed as $\mu$M concentration. Lower IC$_{50}$ values indicate higher radical scavenging activity.
Figure-2.18: Percentage (%) DPPH activity of substituted indole derivatives 3(a-h) and 5(a-g).

2.8.1.2. FERRIC ION (Fe³⁺) REDUCING ANTIOXIDANT POWER ASSAY:

The reducing power associated with antioxidant activity reflects the electron donating capacity of bioactive compounds. The reducing capacity for a compound can be measured by the direct reduction of Fe[(CN)₆]³⁻ to Fe[(CN)₆]²⁻. Ferric reducing power was determined using the iron(III) to iron(II) reduction assay. Since the antioxidant activity of a substance is usually correlated directly to its reducing capacity, the Ferric Ion reducing antioxidant power assay provides a reliable method to study the antioxidant activity of various compounds. Ferric reducing ability of compounds 3(a-h) and 5(a-g) are studied table-2.3 Compounds 3g, 5b and 5c showed the best reducing power among the synthesized analogues but slightly less compared to that of the standards BHA and ascorbic acid. This may be due to the presence of electron donating capacity of hydroxy and methoxy groups on phenyl ring. While the reducing power of the other synthesized compounds 3c, 5d and 3e showed moderate
activity and compounds 3b, 5g, 3d and 5f containing electron withdrawing groups like chloro, nitro and bromo showed least activity. Interestingly, the compound 3h which has three methoxy substituent groups on the phenyl ring showed very low antioxidant power, the reason for it may be due to the steric hindrance. The reducing power of all compounds and standards showed an increase by rising concentrations.

Table-2.3: Absorbance values of ferric ion (Fe\(^{3+}\)) reducing ability of the compounds 3(a-h) and 5(a-g) and the standard antioxidant compounds such as BHA and ascorbic acid.

<table>
<thead>
<tr>
<th>Compound</th>
<th>10µM</th>
<th>25µM</th>
<th>50µM</th>
<th>100µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>0.1577</td>
<td>0.3169</td>
<td>0.5361</td>
<td>0.6946</td>
</tr>
<tr>
<td>3b</td>
<td>0.0971</td>
<td>0.1798</td>
<td>0.2806</td>
<td>0.3089</td>
</tr>
<tr>
<td>3c</td>
<td>0.3556</td>
<td>0.7904</td>
<td>0.7904</td>
<td>1.0177</td>
</tr>
<tr>
<td>3d</td>
<td>0.1137</td>
<td>0.2588</td>
<td>0.3605</td>
<td>0.4647</td>
</tr>
<tr>
<td>3e</td>
<td>0.341</td>
<td>0.5718</td>
<td>0.767</td>
<td>0.9956</td>
</tr>
<tr>
<td>3f</td>
<td>0.152</td>
<td>0.2907</td>
<td>0.3861</td>
<td>0.4908</td>
</tr>
<tr>
<td>3g</td>
<td>0.4144</td>
<td>0.6507</td>
<td>0.8827</td>
<td>1.0861</td>
</tr>
<tr>
<td>3h</td>
<td>0.2362</td>
<td>0.419</td>
<td>0.6968</td>
<td>0.7746</td>
</tr>
<tr>
<td>5a</td>
<td>0.2163</td>
<td>0.3757</td>
<td>0.6307</td>
<td>0.7544</td>
</tr>
<tr>
<td>5b</td>
<td>0.3989</td>
<td>0.6033</td>
<td>0.8538</td>
<td>1.0506</td>
</tr>
<tr>
<td>5c</td>
<td>0.3791</td>
<td>0.5833</td>
<td>0.821</td>
<td>1.0409</td>
</tr>
<tr>
<td>5d</td>
<td>0.3218</td>
<td>0.5287</td>
<td>0.7471</td>
<td>0.9625</td>
</tr>
</tbody>
</table>
The values are expressed as absorbance. Higher absorbance indicates high reducing power.

<table>
<thead>
<tr>
<th></th>
<th>0.3164</th>
<th>0.5185</th>
<th>0.7399</th>
<th>0.9355</th>
</tr>
</thead>
<tbody>
<tr>
<td>5e</td>
<td>0.1116</td>
<td>0.2125</td>
<td>0.3001</td>
<td>0.3458</td>
</tr>
<tr>
<td>5f</td>
<td>0.1034</td>
<td>0.1831</td>
<td>0.2898</td>
<td>0.326</td>
</tr>
<tr>
<td>5g</td>
<td>0.5462</td>
<td>0.7876</td>
<td>1.045</td>
<td>1.2757</td>
</tr>
<tr>
<td>BHA</td>
<td>0.6362</td>
<td>0.8627</td>
<td>1.1487</td>
<td>1.3565</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>0.6362</td>
<td>0.8627</td>
<td>1.1487</td>
<td>1.3565</td>
</tr>
</tbody>
</table>

**Figure-2.19:** Antioxidant activity of the substituted indole derivatives 3(a-h) and 5(a-g) at different concentrations using ferric ion reducing antioxidant power assay.

### 2.8.1.3. CUPRIC ION (Cu²⁺) REDUCING ABILITY (CUPRAC METHOD)

The CUPRAC method is based on Cu(II)-Cu(I) reduction by antioxidants in the presence of neocuproine. The copper reducing ability of newly synthesized compounds are examined. Among the synthesized analogues, compound 3g,
containing hydroxy and methoxy group and 5b and 5c containing a hydroxy moiety on phenyl ring showed marked cupric ion reducing ability. Whereas, compounds 3c and 5d showed average activity. Also, the compounds 3a and 5a showed moderate antioxidant activity. The table shows the copper reducing ability of the newly synthesized analogues. Higher the absorbance greater is the antioxidant scavenging ability.

Unquestionably, the potent copper reducing ability found for the tested compounds relies mainly on their electron-rich substituents on indole moiety, which hold high resonance stability.

**Table-2.4:** Absorbance values of cupric ion reducing ability of the compounds 3(a-h) and 5(a-g) and the standard antioxidant compounds such as BHA and ascorbic acid.

<table>
<thead>
<tr>
<th>Compound</th>
<th>10µM</th>
<th>25µM</th>
<th>50µM</th>
<th>100µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>0.0562</td>
<td>0.0904</td>
<td>0.185</td>
<td>0.3071</td>
</tr>
<tr>
<td>3b</td>
<td>0.0486</td>
<td>0.084</td>
<td>0.1542</td>
<td>0.2749</td>
</tr>
<tr>
<td>3c</td>
<td>0.2022</td>
<td>0.2907</td>
<td>0.4534</td>
<td>0.6363</td>
</tr>
<tr>
<td>3d</td>
<td>0.0862</td>
<td>0.1301</td>
<td>0.1864</td>
<td>0.279</td>
</tr>
<tr>
<td>3e</td>
<td>0.1598</td>
<td>0.2448</td>
<td>0.4134</td>
<td>0.58</td>
</tr>
<tr>
<td>3f</td>
<td>0.1492</td>
<td>0.2196</td>
<td>0.2907</td>
<td>0.4023</td>
</tr>
<tr>
<td>3g</td>
<td>0.298</td>
<td>0.4485</td>
<td>0.6003</td>
<td>0.8664</td>
</tr>
<tr>
<td>3h</td>
<td>0.1639</td>
<td>0.2343</td>
<td>0.3851</td>
<td>0.5287</td>
</tr>
<tr>
<td>5a</td>
<td>0.143</td>
<td>0.185</td>
<td>0.2482</td>
<td>0.3736</td>
</tr>
<tr>
<td>5b</td>
<td>0.2132</td>
<td>0.3536</td>
<td>0.5346</td>
<td>0.7189</td>
</tr>
<tr>
<td>5c</td>
<td>0.2104</td>
<td>0.316</td>
<td>0.4801</td>
<td>0.6946</td>
</tr>
<tr>
<td>5d</td>
<td>0.198</td>
<td>0.2741</td>
<td>0.4329</td>
<td>0.6179</td>
</tr>
</tbody>
</table>


|    | 3a | 3b | 3c | 3d | 3e | 3f | 3g | 3h | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | 5a | 5b | 5c | 5d | 5e | 5f | 5g | 5h | BHA | AA |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 5e | 0.1532 | 0.2247 | 0.3089 | 0.4934 |
| 5f | 0.1429 | 0.1655 | 0.2213 | 0.308  |
| 5g | 0.3534 | 0.1605 | 0.2055 | 0.2898 |
| BHA| 0.3534 | 0.6615 | 0.9136 | 1.1674 |
| Ascorbic Acid (AA)| 0.4391 | 0.7304 | 0.9956 | 1.2676 |

The values are expressed as absorbance. Higher absorbance indicates high reducing power.

**Figure-2.15:** Antioxidant activity of the substituted indole derivatives 3(a-h) and 5(a-g) at different concentrations using cupric reducing antioxidant power assay.

2.8.1.4. PHOSPHOMOLYBDENUM ASSAY

The antioxidant activity for the synthesized compounds was evaluated by using phosphomolybdenum method. It determines the total antioxidant capacity. This assay is based on the reduction of Mo(VI) to Mo(V) in presence of the antioxidant compounds and the subsequent formation of a green phosphate/Mo(V) complex at
acidic pH, which is measured at 695 nm on spectrophotometer. The antioxidant capacity of the compounds was determined for different concentrations varying from 10 µM, 25 µM, 50 µM and 100 µM.

Among the synthesized derivatives 3(a-h), compounds 3g, 3c and 3e possessing electron releasing substituents like hydroxy and methoxy enhances the antioxidant scavenging potential whereas, compounds 3b, 3d and 3f having electron withdrawing groups such as chloro, nitro and methyl decreases the antioxidant activity. Whereas, the compound 3a which does not have either electron rich or deficient group displayed moderate antioxidant activity. The same results were observed in the series 5(a-g), compounds 5b, 5c and 5d showed good antioxidant activity whereas, compounds 5f and 5g decreases the antioxidant activity. The antioxidant capacities of the compounds are expressed as µM of ascorbic acid equivalent/mg and is showed in table 2.5 below. Higher value indicates more antioxidant activity of the compound.

Compound 5b reduces Mo(VI) to Mo(V) in better way due to the presence of –OH group. In addition to this, the presence of N-H group on indole moiety also helps to enhance the antioxidant activity. The antioxidant activity of the tested compounds were compared to standards (BHA and ascorbic acid) and found that compound 5b and 3g possess better phosphomolybdate activity than the standards. The antioxidant capacities of the compounds determined by phosphomolybdate method were expressed as µM of ascorbic acid equivalent/mg.
Table-2.5: Absorbance values are expressed as equivalent of ascorbic acid (µM/mg) for the compounds 3(a-h) and 5(a-g) and the standard antioxidant compounds such as BHA and ascorbic acid.

<table>
<thead>
<tr>
<th>Compound</th>
<th>10µM</th>
<th>25µM</th>
<th>50µM</th>
<th>100µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>39.98</td>
<td>59.12</td>
<td>87.03</td>
<td>131.14</td>
</tr>
<tr>
<td>3b</td>
<td>10.11</td>
<td>17.45</td>
<td>27.82</td>
<td>41.12</td>
</tr>
<tr>
<td>3c</td>
<td>122.11</td>
<td>233.22</td>
<td>367.89</td>
<td>622.08</td>
</tr>
<tr>
<td>3d</td>
<td>4.34</td>
<td>7.88</td>
<td>12.44</td>
<td>16.55</td>
</tr>
<tr>
<td>3e</td>
<td>82.22</td>
<td>186.11</td>
<td>300.77</td>
<td>553.8</td>
</tr>
<tr>
<td>3f</td>
<td>18.67</td>
<td>32.79</td>
<td>46.1</td>
<td>69.34</td>
</tr>
<tr>
<td>3g</td>
<td>181.02</td>
<td>346.15</td>
<td>588.04</td>
<td>816.1</td>
</tr>
<tr>
<td>3h</td>
<td>7.34</td>
<td>13.98</td>
<td>20.52</td>
<td>32.94</td>
</tr>
<tr>
<td>5a</td>
<td>47.56</td>
<td>78.67</td>
<td>120.98</td>
<td>170.02</td>
</tr>
<tr>
<td>5b</td>
<td>220.12</td>
<td>508.12</td>
<td>834.89</td>
<td>1110.19</td>
</tr>
<tr>
<td>5c</td>
<td>136.7</td>
<td>218.09</td>
<td>384.08</td>
<td>689.17</td>
</tr>
<tr>
<td>5d</td>
<td>106.78</td>
<td>211.12</td>
<td>319.09</td>
<td>598.06</td>
</tr>
<tr>
<td>5e</td>
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<td>174.12</td>
<td>297.67</td>
<td>502.18</td>
</tr>
<tr>
<td>5f</td>
<td>13.13</td>
<td>20.23</td>
<td>28.87</td>
<td>47.12</td>
</tr>
<tr>
<td>5g</td>
<td>23.34</td>
<td>39.54</td>
<td>57.73</td>
<td>96.56</td>
</tr>
<tr>
<td>BHA</td>
<td>378</td>
<td>724.76</td>
<td>1100.12</td>
<td>1533.08</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>400.34</td>
<td>789.34</td>
<td>1389.56</td>
<td>1665.1</td>
</tr>
</tbody>
</table>

The values are expressed as absorbance. Higher absorbance indicates high reducing power.
Antioxidant activity of the substituted indole derivatives 3(a-h) and 5(a-g) at different concentrations using phosphomolybdate antioxidant power assay.

2.9. CONCLUSION

Series of novel indole-2-carboxylic acid derivatives were synthesized and their antioxidant activities have been evaluated. The indole derivatives synthesized and tested in the present study were shown to be of reassuring importance for the development of new antioxidant drugs.

The efficacy of antioxidant activity for the synthesized analogues which were found exhibiting good antioxidant activity is in the order:

i) For DPPH activity:-  
   Ascorbic acid > BHA > 3g > 3c > 3e  
   3(a-h)
   Ascorbic acid > BHA > 5b > 5c > 5e  
   5(a-g)

ii) For FRAP assay :-  
   Ascorbic acid > BHA > 3g > 3c > 3e  
   3(a-h)
   Ascorbic acid > BHA > 5b > 5c > 5d  
   5(a-g)

iii) For CUPRAC assay :-  
   Ascorbic acid > BHA > 3g > 3c > 3e  
   3(a-h)
   Ascorbic acid > BHA > 5b > 5c > 5d  
   5(a-g)
iv) For phosphomolybdenum assay:-

Ascorbic acid > BHA > 3g > 3c > 3e \(3(a-h)\)

Ascorbic acid > BHA > 5b > 5c > 5d \(5(a-g)\)

Our study provides evidence that the synthesized novel compounds of indole-2-carboxylic acid has significant influence for the antioxidant activities in different in vitro model systems. These compounds \((3g, 3c, 3e, 5b, 5c, and 5d)\) may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role. Among the compounds \(5(a-g)\) the reason for the antioxidant activity shown by them will also be supported by the presence of hydrogen in the indole ring (N-H).

REFERENCES


