



Novel indole-2-carboxylic acid analogues: Synthesis and a new light in to their antioxidant potentials

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ABSTRACT

Two series of novel indole-2-carboxylic acid derivatives is reported. In the first series, *N*-substituted derivatives (**3a-h**) were synthesized via acylation of indole-2-carboxylic acid followed by aldol condensation reaction. Whereas, in the second series, indole-2-carboxamides (**5a-g**) were synthesized through conversion of acid to its acid chloride followed by coupling of substituted anilines. Structures of the newly synthesized compounds were confirmed by elemental analysis and spectral IR, ¹H NMR and mass data and were screened for antioxidant activity. Among the first series, compound **3g** showed higher antioxidant activity and whereas, in the second series compounds **5b** and **5c** exhibited potential antioxidant activity. Compounds **3g**, **5b** and **5c** exhibited for its enhanced antioxidant activity.

1. Introduction

Evidences suggests that free radicals, which are generated in many bioorganic redox processes, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids and may also be involved in the processes leading to the formations of mutations [1]. The deleterious effects of an imbalance between reactive oxygen species (ROS) production and the available antioxidant defense capacity, termed oxidative stress, as well as its role in the aggravation of a plethora of pathological conditions, are widely documented in the literature [2]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants [3,4]. Antioxidants play a significant role in several important biological processes such as immunity, protection against tissue damage, reproduction and growth or development and can prevent cardiovascular disease, cancer, cataracts and various other ailments associated with ageing [5,6].

Indole and its derivatives are found abundantly in nature and are known to exhibit potent physiological properties [7-10]. Substituted indoles are capable of binding to many receptors with high affinity. Therefore, the synthesis and selective functionalization of indoles have been the focus of active research over the years [11-17]. Promoted from the above findings and as a continuation of our research interest in synthesis and biological activities of novel derivatives of some heterocyclic compounds [18-20], the present study aimed to synthesis and to evaluate antioxidant potentials of novel indole-2-carboxylic acid analogues.

2. Experimental

2.1. Instrumentation

All chemicals used were of laboratory grade (Qualigen, Merck). The melting points were determined by open capillary method on a Campbel electronic apparatus and are uncorrected. The IR spectra of synthesized compounds were recorded on a Shimadzu 8400S FT-IR in potassium bromide disks. The ¹H NMR was recorded in DMSO-*d*₆ using a NMR Varian-Mercury 400 MHz spectrometer and chemical shifts are given in units as δ ppm, downfield from tetramethylsilane (TMS) as an internal standard. 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. The progress of reactions was monitored by thin layer chromatography using chloroform-methanol and hexane-ethyl acetate as the solvent systems and spots were visualized after exposure to iodine vapours or under ultraviolet (UV) light.

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

To a well stirred solution of 1H-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.

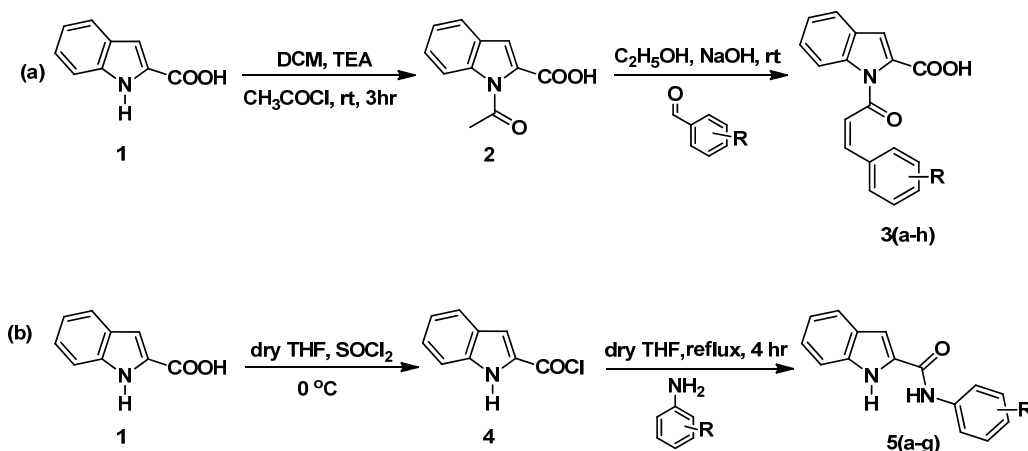


Figure 1. Protocol for the synthesis of indole-2-carboxylic acid derivatives, (a) *N*-substituted indole-2-carboxylic acid analogues, (b) indole-2-carboxamides.

The organic layer was washed with 5% NaHCO₃ followed by distilled water. Finally the organic layer was dried over anhydrous Na₂SO₄. The brown solid product was obtained by desolventation through rotary evaporator.

1-acetyl-1H-indole-2-carboxylic acid (2): Brown solid. Yield: 87%. M.p.: 175-178 °C. FT-IR (KBr, cm⁻¹): 1664 (C=O), 3347 (OH), 2853-2943 (Ar-H). ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 11.1 (s, 1H, COOH), 6.92-7.50 (m, 5H, Ar-H), 2.36 (s, 3H, COCH₃). MS (EI, m/z): 204.10 (M+1)⁺. Anal. calcd. for C₁₁H₉NO₃: C, 65.02; H, 4.46; N, 6.89; O, 23.62%. Found; C, 65.05; H, 4.43; N, 6.85; O, 23.65%.

2.3. Synthesis of 1H-indole-2-carbonyl 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 3mL dry THF was added drop wise at 0 °C, then the reaction mixture is stirred at room temperature for about 3 h. 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 **3a-h** and **5a-g** in moderate good yield, which were identified by spectroscopic techniques: ¹H NMR, FT-IR and MS-EL. The synthetic strategies of the synthesized compounds are depicted in Figure 1 (Table 1).

1H-indole-2-carbonyl chloride (4): Light brown solid. Yield: 78%. M.p.: 157-160 °C. FT-IR (KBr, cm⁻¹): 1695 (C=O), 3338 (N-H), 2873-2927 (Ar-H). ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 11.91 (s, 1H, N-H), 6.91-7.67 (m, 5H, Ar-H). MS (EI, m/z): 181.08 (M+1)⁺. Anal. calcd. for C₉H₆ClNO: C, 60.19; H, 3.37; Cl, 19.74; N, 7.80; O, 8.91%. Found; C, 60.17; H, 3.39; Cl, 19.78; N, 7.84; O, 8.93.

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

Table 1. Chemical structures of the synthesized compounds.

Entry		Entry	
3a-h		5a-g	
3a		5a	
3b		5b	
3c		5c	
3d		5d	
3e		5e	
3f		5f	
3g		5g	
3h			

(Z)-1-(3-phenylacryloyl)-1H-indole-2-carboxylic acid (3a): Brown semi solid. Yield: 88%. FT-IR (KBr, cm⁻¹): 1664 (C=O), 3505 (OH), 2863-2918 (Ar-H). ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 11.3 (s, 1H, COOH), 7.45-7.50 (m, 5H, Ar-H), 6.91-7.10

(m, 5H, Ar-H of R-CHO), 6.63 (d, 1H, CH=CH), 6.59 (d, 1H, CH=CH). MS (EI, m/z): 292.10 (M+1)⁺. Anal. calcd. for C₁₈H₁₃NO₃: C, 74.22; H, 4.50; N, 4.81; O, 16.48%. Found; C, 74.25; H, 4.52; N, 4.84; O, 16.45%.

(Z)-1-(3-(4-chlorophenyl)acryloyl)-1H-indole-2-carboxylic acid (**3b**): White semi solid. Yield: 81%. FT-IR (KBr, cm⁻¹): 1674 (C=O), 3445 (OH), 2853-2948 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.1 (s, 1H, COOH), 7.21-8.10 (m, 5H, Ar-H), 7.22 (d, 4H, Ar-H of R-CHO), 6.73 (d, 1H, CH=CH), 6.68 (d, 1H, CH=CH). MS (EI, m/z): 326.12 (M+1)⁺. Anal. calcd. for C₁₈H₁₂ClNO₃: C, 66.37; H, 3.71; Cl, 10.88; N, 4.30; O, 14.73%. Found; C, 66.39; H, 3.71; Cl, 10.85; N, 4.27 O, 14.76%.

(Z)-1-(3-(4-hydroxyphenyl)acryloyl)-1H-indole-2-carboxylic acid (**3c**): Light brown solid. Yield: 87%. M.p.: 105-108 °C. FT-IR (KBr, cm⁻¹): 1624 (C=O), 3338 (OH), 2849-2923 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.24 (s, 1H, COOH), 7.21-7.86 (m, 5H, Ar-H), 6.83-7.10 (d, 4H, Ar-H of R-CHO), 7.22 (d, 1H, CH=CH), 6.28 (d, 1H, CH=CH), 5.34 (s, 1H, Phenolic -OH). MS (EI, m/z): 308.12 (M+1)⁺. Anal. calcd. for C₁₈H₁₃NO₄: C, 70.35; H, 4.26; N, 4.56; O, 20.83%. Found; C, 70.37; H, 4.24; N, 4.58; O, 20.87%.

(Z)-1-(3-(4-nitrophenyl)acryloyl)-1H-indole-2-carboxylic acid (**3d**): Brown solid. Yield: 77%. M.p.: 115-118 °C. FT-IR (KBr, cm⁻¹): 1664 (C=O), 3445 (OH), 2838-2988 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.1 (s, 1H, COOH), 7.45-7.50 (m, 5H, Ar-H), 6.86-7.48 (d, 4H, Ar-H of R-CHO), 6.73 (d, 1H, CH=CH), 6.27 (d, 1H, CH=CH). MS (EI, m/z): 337.20 (M+1)⁺. Anal. calcd. for C₁₈H₁₂N₂O₅: C, 64.29; H, 3.60; N, 8.33; O, 23.79%. Found; C, 64.25; H, 3.63; N, 8.35; O, 23.75%.

(Z)-1-(3-(4-methoxyphenyl)acryloyl)-1H-indole-2-carboxylic acid (**3e**): Yellow solid. Yield: 85%. M.p.: 124-127 °C. FT-IR (KBr, cm⁻¹): 1683 (C=O), 3447 (OH), 2847-2943 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.0 (s, 1H, COOH), 7.45-7.50 (m, 5H, Ar-H), 6.91-7.32 (d, 4H, Ar-H of R-CHO), 7.11 (d, 1H, CH=CH), 6.93 (d, 1H, CH=CH), 3.84 (s, 3H, OCH₃). MS (EI, m/z): 322.20 (M+1)⁺. Anal. calcd. for C₁₉H₁₅NO₄: C, 71.02; H, 4.71; N, 4.36; O, 19.92%. Found; C, 71.04; H, 4.75; N, 4.33; O, 19.90%.

(Z)-1-(3-(p-tolylacryloyl)-1H-indole-2-carboxylic acid (**3f**): Brown semi solid. Yield: 85%. FT-IR (KBr, cm⁻¹): 1663 (C=O), 3505 (OH), 2853-2918 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.0 (s, 1H, COOH), 7.45-7.50 (m, 5H, Ar-H), 6.83-7.10 (d, 4H, Ar-H R-CHO), 6.79 (d, 1H, CH=CH), 6.63 (d, 1H, CH=CH), 2.33 (s, 3H, CH₃). MS (EI, m/z): 306.30 (M+1)⁺. Anal. calcd. for C₁₉H₁₅NO₃: C, 74.74; H, 4.95; N, 4.59; O, 15.72%. Found; C, 74.72; H, 4.96; N, 4.59; O, 15.71%.

(Z)-1-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)-1H-indole-2-carboxylic acid (**3g**): Yellow solid. Yield: 88%. M.p.: 162-165 °C. FT-IR (KBr, cm⁻¹): 1675 (C=O), 3388 (OH), 2853-2918 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.2 (s, 1H, COOH), 7.45-7.68 (m, 5H, Ar-H), 7.10-7.22 (d, 3H, Ar-H of R-CHO), 7.10 (d, 1H, CH=CH), 6.64 (d, 1H, CH=CH), 5.35 (s, 1H, phenolic OH), 3.83 (s, 3H, -OCH₃). MS (EI, m/z): 338.20 (M+1)⁺. Anal. calcd. for C₁₉H₁₅NO₅: 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%.

(Z)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-1H-indole-2-carboxylic acid (**3h**): White solid. Yield: 82%. M.p.: 126-129 °C. FT-IR (KBr, cm⁻¹): 1653 (C=O), 3433 (OH), 2854-2938 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.0 (s, 1H, COOH), 6.26-7.11 (m, 5H, Ar-H), 7.67 (d, 2H, Ar-H of R-CHO), 7.42 (d, 1H, CH=CH), 7.46 (d, 1H, CH=CH), 3.83 (m, 9H, OCH₃). MS (EI, m/z): 382.23 (M+1)⁺. Anal. calcd. for C₂₁H₁₉NO₆: C, 66.13; H, 5.02; N, 3.67; O, 25.17%. Found; C, 66.15; H, 5.06; N, 3.63; O, 25.14%.

2.5. General procedure for the synthesis of 1H-indole-2-carbonyl chloride analogues (5a-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 (Figure 1). 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.

N-phenyl-1H-indole-2-carboxamide (**5a**): Yellow solid. Yield: 82%. M.p.: 115-118 °C. FT-IR (KBr, cm⁻¹): 1656 (C=O), 3265 (N-H), 2853-2927 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.9 (s, 1H, NH of indole), 8.35 (s, 1H, NH of amine), 7.1-7.45 (m, 5H, Ar-H), 7.5-7.75 (d, 4H, Ar-H R-NH₂). MS (EI, m/z): 237.28 (M+1)⁺. Anal. calcd. for C₁₅H₁₂N₂O: C, 76.25; H, 5.12; N, 11.86; O, 6.77%. Found; C, 76.26; H, 5.15; N, 11.88; O, 6.75%.

N-(4-hydroxyphenyl)-1H-indole-2-carboxamide (**5b**): White solid. Yield: 76%. M.p.: 125-128 °C. FT-IR (KBr, cm⁻¹): 1695 (C=O), 3338 (N-H), 2873-2956 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.7 (s, 1H, NH of indole), 9.15 (s, 1H, NH of amine), 6.91-7.54 (m, 5H, Ar-H), 7.45-7.70 (d, 4H, Ar-H R-NH₂), 5.35 (s, 1H, phenolic OH). MS (EI, m/z): 253.21 (M+1)⁺. Anal. calcd. for C₁₅H₁₂N₂O₂: C, 71.42; H, 4.79; N, 11.10; O, 12.68%. Found; C, 71.40; H, 4.77; N, 11.13; O, 12.65%.

N-(2-hydroxyphenyl)-1H-indole-2-carboxamide (**5c**): White solid. Yield: 85%. M.p.: 176-179 °C. FT-IR (KBr, cm⁻¹): 1695 (C=O), 3348 (N-H), 2863-2956 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.9 (s, 1H, NH of indole), 9.13 (s, 1H, NH of amine), 6.81-7.59 (m, 5H, Ar-H), 7.44-7.99 (d, 4H, Ar-H R-NH₂), 5.3 (s, 1H, phenolic OH). MS (EI, m/z): 253.18 (M+1)⁺. Anal. calcd. for C₁₅H₁₂N₂O₂: C, 71.42; H, 4.79; N, 11.10; O, 12.68%. Found; C, 71.41; H, 4.77; N, 11.14; O, 12.66%.

N-(4-methoxyphenyl)-1H-indole-2-carboxamide (**5d**): Bright yellow solid. Yield: 82%. M.p.: 111-114 °C. FT-IR (KBr, cm⁻¹): 1689 (C=O), 3317 (N-H), 2873-2933 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.62 (s, 1H, NH of indole), 9.41 (s, 1H, NH of amine), 7.11-7.57 (m, 5H, Ar-H), 7.51-7.88 (d, 4H, Ar-H R-NH₂), 3.83 (s, 3H, OCH₃). MS (EI, m/z): 267.32 (M+1)⁺. Anal. calcd. for C₁₆H₁₄N₂O₂: C, 72.16; H, 5.30; N, 10.52; O, 12.02%. Found; C, 72.17; H, 5.27; N, 10.55; O, 12.06%.

N-(2-methoxyphenyl)-1H-indole-2-carboxamide (**5e**): White solid. Yield: 72%. M.p.: 185-188 °C. FT-IR (KBr, cm⁻¹): 1695 (C=O), 3378 (N-H), 2823-2986 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.6 (s, 1H, NH of indole), 9.43 (s, 1H, NH of amine), 6.86-7.86 (m, 5H, Ar-H), 7.51-7.90 (d, 4H, Ar-H R-NH₂), 3.83 (s, 3H, OCH₃). MS (EI, m/z): 267.30 (M+1)⁺. Anal. calcd. for C₁₆H₁₄N₂O₂: 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%.

N-(4-bromophenyl)-1H-indole-2-carboxamide (**5f**): White semi solid. Yield: 77%. FT-IR (KBr, cm⁻¹): 1775 (C=O), 3407 (N-H), 2833-2936 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.91 (s, 1H, NH of indole), 9.5 (s, 1H, NH of amine), 7.1-7.67 (m, 5H, Ar-H), 7.61-8.11 (d, 4H, Ar-H R-NH₂), MS (EI, m/z): 316.36 (M+1)⁺. Anal. calcd. for C₁₅H₁₁BrN₂O: C, 57.16; H, 3.52; Br, 25.35; N, 8.89; O, 5.08%. Found; C, 57.16; H, 3.54; Br, 25.37; N, 8.85; O, 5.05%.

N-(4-nitrophenyl)-1H-indole-2-carboxamide (**5g**): White solid. Yield: 70%. M.p.: 106-109 °C. FT-IR (KBr, cm⁻¹): 1677 (C=O), 3438 (N-H), 2873-2956 (Ar-H). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 11.76 (s, 1H, NH of indole), 9.1 (s, 1H, NH of amine), 7.33-7.71 (m, 5H, Ar-H), 7.64-7.97 (d, 4H, Ar-H R-NH₂). MS (EI, m/z): 282.10 (M+1)⁺. Anal. calcd. for C₁₅H₁₁N₃O₃: C, 64.05; H, 3.94; N, 14.94; O, 17.07%. Found; C, 64.09; H, 3.92; N, 14.97; O, 17.03%.

2.6. Antioxidant activity studies

The novel synthesized molecules were further subjected for the antioxidant evaluation by various *in vitro* assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2-azino bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radical cation decolorization assays, Ferric ion (Fe³⁺) reducing antioxidant power assay (FRAP) and Cupric ion (Cu²⁺) reducing ability (CUPRAC method).

2.6.1. Free radical scavenging activity

The newly synthesized compounds were screened for free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [21]. Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions (**3a-h**) and (**5a-g**) having different concentrations (10, 25, 50, 100, 200 and 500 μM) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The test tubes were then incubated in the dark room at room temperature for 20 min. A DPPH blank was prepared without the compound and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrometer (Shimadzu 160 A). The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula (Equation 1):

$$\text{Radical scavenging activity (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (1)$$

where A_c is absorbance of the control (without compound) and A_s is absorbance of the compounds **3a-h** and **5a-g**. The radical scavenging activity of BHA and ascorbic acid was also measured and compared with that of the different synthesized compounds.

2.6.2. ABTS^{•+} radical scavenging activity

The synthesized indole-2-carboxylic acid analogues were subjected to 2,2-azino bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radical scavenging activity [22]. The ABTS^{•+} cation was produced by the reaction between 7 mM ABTS in H₂O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 hr. Before the usage, the ABTS^{•+} solution was diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with phosphate buffer (0.1 M, pH = 7.4). Then, 1 mL of ABTS^{•+} solution was added to the compounds **3a-h** and **5a-g** solution in ethanol at different concentrations (1.5 mL, 10, 25, 50, 100, 200, 500 $\mu\text{M}/\text{mL}$). After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance (ethanol).

The scavenging capability of ABTS^{•+} radical was calculated using the equation 2.

$$\text{ABTS}^{\bullet+} \text{ scavenging effect (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (2)$$

where, A_{control} is the initial concentration of the ABTS^{•+} and A_{sample} is the absorbance of the remaining concentration of ABTS^{•+} in the presence of the compounds **3a-h** and **5a-g**.

2.6.3. Ferric ion (Fe³⁺) reducing antioxidant power assay (FRAP)

All the novel indole-2-carboxylic acid analogues were screened for ferric reducing antioxidant power [23]. The compounds **3a-h** and **5a-g** having concentration (10 $\mu\text{M}/\text{mL}$) 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 (2.5 mL, 10%). After FeCl₃ (0.5 mL, 0.1%) was added to this solution, the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicates an increased reducing power.

2.6.4. Cupric ion (Cu²⁺) reducing ability (CUPRAC method)

All the synthesized compounds were performed cupric ion reducing ability assay [24]. Briefly, a mixture of CuCl₂ (1 mL, 0.01 M) solution, ethanolic neocuproine (Nc) (1 mL, 7.5×10^{-3} M) solution and ammonium acetate (1 mL, 1.0 M) in a test tube were added to a solution of compounds **3a-h** and **5a-g** (1 mL,

10 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.

2.6.5. Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of compound needed to inhibit DPPH free radicals and ABTS radicals concentration by 50%, (IC₅₀) was graphically estimated using a linear regression algorithm.

3. Results and discussion

3.1. Chemistry

To keep the electron-withdrawing and electron-donating character of the substituents in the molecule, we decided to study several indole-2-carboxylic acid analogues bearing electron rich and deficient substituents. In the first series, the key intermediate (**2**) was obtained by *N*-acylation using acetyl chloride in the presence of triethylamine as base. Further, aldol condensation of the key intermediate (**2**) with various substituted aldehydes in the presence of sodium hydroxide as base, afforded the corresponding *N*-substituted indole-2-carboxylic acid analogues (**3a-h**) (Figure 1 (a)). Further, in the second series indole-2-carboxamides (**5a-g**) was obtained by conversion of acid to acid chlorides in the presence of thionyl chloride followed by coupling of substituted anilines through base condensation reaction in good yield (Figure 1 (b)). The newly synthesized compounds were purified by column chromatography using silica gel 60-120 mesh and chloroform:methanol (60:40) as eluent. The synthesized compounds were characterized by various physico-chemical and spectroscopic techniques like IR, ¹H NMR, mass and elemental analysis. The absence of a broad absorption band at 3300 cm⁻¹ corresponding to -NH absorption and a sharp band at 3347 cm⁻¹ corresponding to acid group in IR spectrum of key intermediate (**2**) and the absence of signal at 11.91 ppm in ¹H NMR confirms the acylation to -NH proton. Similarly, in the IR spectra of key intermediate (**4**) exhibited the absence of a broad absorption band at 3300 cm⁻¹ which corresponds to acid group and the presence of a sharp absorption band at 3338 cm⁻¹ for the NH absorptions confirms the acid to acid chloride reaction successfully.

The IR spectra of all the substituted aldehyde analogues (**3a-h**) showed the absence of sharp N-H band i.e., indole (N-H) band at around 3300-3500 cm⁻¹ and also reveals the presence of aromatic peaks (Ar-H) at the respective region 2853-2943 cm⁻¹. Similarly, the IR spectra of all the substituted aniline analogues (**5a-g**) showed a sharp N-H band i.e., indole (N-H) band at around 3304-3438 cm⁻¹ and also reveals the presence of aromatic peaks (Ar-H) at the respective region 2823-2986 cm⁻¹. ¹H NMR spectra of all conjugated analogues (**3a-h**) showed the absence of N-H protons as singlet at 11.91 ppm. The signal due to acid -OH in all the analogues appeared as singlet at about 11.0-11.4 ppm. In addition to acid -OH, the phenolic -OH resonated at 5.34 ppm in compound **3c** and -OCH₃ protons present in the compound **5e** resonated as singlet at 3.83 ppm, other aromatic protons were observed at expected regions 6.26-8.10 ppm for (**3a-h**) and 6.81-8.11 ppm for (**5a-g**).

3.2. Antioxidant activities

3.2.1. DPPH radical scavenging activity

The DPPH radical scavenging activity assay is a simple method for measuring the antioxidant ability to trap free radicals. The scavenging effects of compounds (**3a-h**) and (**5a-g**) and two controls, BHA and ascorbic acid are evaluated (Table 2). Compound **3g** containing hydroxy and methoxy group and **5b** and **5c** containing a hydroxy moiety on phenyl

ring showed the highest activity ($IC_{50} = 23 \mu M, 35 \mu M$ and $45 \mu M$) among the synthesized analogues. Compound **5e** possessing methoxy group and **3c** having hydroxy group on phenyl moiety displayed promising activity ($IC_{50} = 50 \mu M$ and $65 \mu M$).

Table 2. IC_{50} (Concentration required for 50% inhibition) values of DPPH• and ABTS•+ radical scavenging activities of the compounds (**3a-h**) and (**5a-g**) and the standard antioxidant compounds such as BHA and Ascorbic acid.

Tested compounds	DPPH•	Scavenging activity (IC_{50}) ^a
		ABTS•+
BHA	12	15
Ascorbic acid	10	12
3a	190	140
3b	290	265
3c	65	52
3d	400	200
3e	74	86
3f	145	123
3g	23	20
3h	200	315
5a	190	175
5b	35	28
5c	45	40
5d	85	68
5e	50	62
5f	285	375
5g	195	164

* The values are expressed as μM concentration. Lower IC_{50} values indicate higher radical scavenging activity.

3.2.2. ABTS radical cation scavenging activity

The ABTS assay is a widely used method for measuring the antioxidant ability to trap free radicals [22]. The ABTS radical cation scavenging capacity of the synthesized compounds (**3a-h**) and (**5a-g**) are screened Table 2. Scaffold **3g** containing hydroxy and methoxy group and **5b** and **5c** containing a hydroxy moiety on phenyl ring showed good ABTS scavenging capacity ($IC_{50} = 20 \mu M, 28 \mu M$ and $40 \mu M$). Whereas, compounds **3c**, **5e** and **5d** displayed moderate activity ($IC_{50} = 52 \mu M, 62 \mu M$ and $68 \mu M$).

3.2.3. Ferric reducing antioxidant power (FRAP)

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 FRAP assay provides a reliable method to study the antioxidant activity of various compounds [23]. Ferric reducing ability of compounds (**3a-h**) and (**5a-g**) are studied Table 3. Compounds **3g**, **5b** and **5c** showed the best reducing power (absorbance value = 0.4144, 0.3989 and 0.3791) 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 -Cl, -NO₂ and Br showed least activity. The reducing power of all compounds and standards showed an increase by rising concentrations.

3.3.4. Cupric ion (Cu^{2+}) 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 Table 3. 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 (absorbance value= 0.2980, 0.2132 and 0.2104). Whereas, compounds **3c** and **5d** showed average activity.

Table 3. Comparison of ferric ions (Fe^{3+}) reducing ability by Fe^{3+} - Fe^{2+} transformation methods and Cu^{2+} - Cu^{+} reducing ability of the compounds **3a-h** and **5a-g** and the standard antioxidant compounds such as BHA and Ascorbic acid at the concentration of $10 \mu M$.

Tested compounds	Fe^{3+} - Fe^{2+} reducing ability*	Cu^{2+} - Cu^{+} reducing ability*
BHA	0.5462	0.3534
Ascorbic acid	0.6362	0.4391
3a	0.1577	0.0562
3b	0.0971	0.0486
3c	0.3556	0.2022
3d	0.1137	0.0862
3e	0.3410	0.1598
3f	0.1520	0.1462
3g	0.4144	0.2980
3h	0.2362	0.1639
5a	0.2163	0.1430
5b	0.3989	0.2132
5c	0.3791	0.2104
5d	0.3218	0.1980
5e	0.3164	0.1532
5f	0.1116	0.1429
5g	0.1034	0.1110

* The values are expressed as absorbance. High absorbance indicates high reducing power.

4. Conclusion

In the present investigation, two series of novel indole-2-carboxylic acid analogues (**3a-h**) and (**5a-g**) have been synthesized by a simple and convenient method. All the synthesized analogues were screened for their antioxidant activity by various *in vitro* assays. Although most of the above compounds exhibited more or less antioxidant activity compared to standards. Compounds **3g**, **5b** and **5c** exhibited for its enhanced antioxidant activity. The antioxidant activities of these compounds are attributed to the presence of electron donating group such as -OH and -OCH₃ on the phenyl moiety. Along with, we observed the presence of electron withdrawing groups such as -Cl, -NO₂ and -Br on phenyl ring exhibited lowest antioxidant activity.

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