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Synthesis and evaluation of N-substituted indole-3-carboxamide derivatives as inhibitors of lipid peroxidation and superoxide anion formation

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Abstract

The *in vitro* antioxidant effects of novel N-substituted indole-3-carboxamides **(I3CDs) 1-10** on rat liver microsomal NADPH-dependent lipid peroxidation (LP) levels and their free radicals scavenging properties were determined by the inhibition of superoxide anion formation (SOD). Among the synthesized compounds, **4**, **5**, **8** and **9** significantly inhibited SOD with an inhibition range at 84–100% at 10^{-3} M concentration. The presence of halo substituents both ortho- and para- positions of these compounds resulted 100% inhibition of SOD. Comparison the activity results of halogenated and non-halogenated derivatives suggested that the halogenated compounds are more active than the non-halogenated compounds. On the other hand, the introduction of a para fluoro benzyl in the 1-position of indole (compounds **7**, **8**) has more impact on the SOD inhibition when the benzamide ring was mono halogenated. However, none of other compounds had a significant inhibitory effects on the level of lipid peroxidation.

Keywords: *Antioxidant, N-substituted indole-3-carboxamides, lipid peroxidation, superoxide anion formation, reactive oxygen species*

Introduction

Oxygen is essential for life but also can harm cells. The active forms of oxygen includes superoxide anion radical ($O_2^{\cdot -}$), hydroxide radical ($HO^{\cdot}$), hydroperoxide $HO_2^{\cdot}$, alkoxide ($LO^{\cdot}$), peroxide ($LOO^{\cdot}$) and non-radical derivatives of oxygen, such as singlet oxygen ($^1O_2$), lipid peroxides or H_2O_2 arise during oxidative stress [1]. Directly or indirectly, these chemical species of oxygen, as well as reactive species of nitrogen, can transiently or permanently damage nucleic acids, lipids, and proteins. Oxidative damage to these cellular macromolecules is implicated in the genesis of several diseases such as; inflammatory, cardiovascular diseases, atherosclerosis, cancer and age-related molecular degeneration [2,3]. Reactive oxygen species (ROS) within cells act as second messengers in intracellular signaling cascades, which induce and maintain the oncogenic phenotype of cancer cells. ROS are tumorigenic by virtue of their ability to increase cell proliferation, survival, cellular migration, and also by inducing DNA damage leading to genetic lesions that initiate tumorigenicity and sustain subsequent tumor progression. Antioxidant compounds prevents the development of certain cancer by elimination of ROS [4].

Current evidence from our laboratory demonstrated that a series of 3-(substituted-benzylidene)-1, 3-dihydro indolin-2 one and thione derivatives (Figure 1) had effective activities as radical scavengers and may be considered as an effective source for combating oxidative damage [5]. These compounds have been previously reported in the literature as inhibitors of protein kinase [6].

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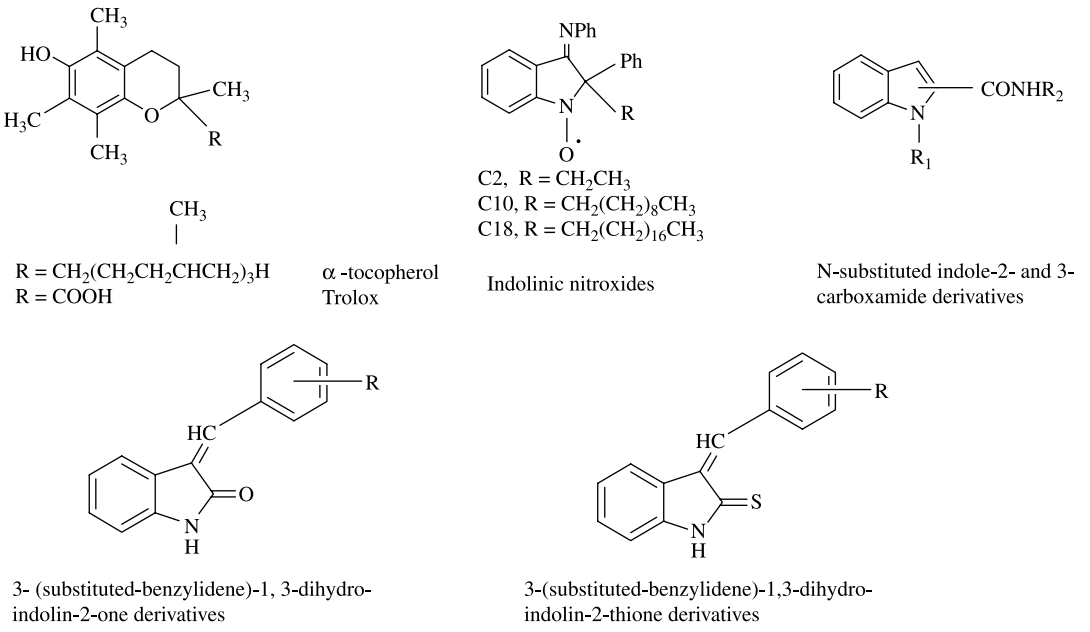


Figure 1. The structure of some anti-oxidant compounds.

There is a close relationship with inflammation and cellular oxidation. Inflammatory cells are capable of inducing genetoxic effects such as DNA strand break and mutation. Therefore, it is considered that anti-inflammatory and antioxidant agents may play an imported role in cancer prevention [7]. Several studies indicated that COX-2 (cyclooxygenase 2) inhibition can be beneficial for the prevention of cancer such as colon and prostate [8]. It was found that small-molecule antioxidants inhibit cytokine production [9]. Among them, several coumarin derivatives have been reported as significant anti-inflammatory/antioxidants [10]. Some studies from our laboratory also supported similiar findings that compounds have been shown both anti-inflammatory and antioxidant activities. Several indole amide derivatives (IADs) (Figure 1) which were previously found as selective cyclooxygenase-2 (COX-2) inhibitors [11], can scavenge oxygen free radicals, and some of them also inhibited the $^1O_2$, which were produced by cyclooxygenases. The importance of this antioxidant property may also be significant in treating human disease that involves ROS and antioxidant damage [12]. Further studies from our laboratory determined that several indole esters and amide derivatives are very efficient to inhibit superoxide anion and lipid peroxidation [13,14].

A hydrophilic analogue of α-tocopherol (Trolox) and indolinic nitroxides (Figure 1) were also found very efficient antioxidants, protecting both lipids and proteins from peroxidation [15]. Recently, several benzimidazole containing indole and tetrahydro-naphthalene-indole derivatives were explored as significant inhibitor of lipid peroxidation and superoxide anion formation [16,17]. In our previous publication, we reported that some novel N-substituted indole-2-carboxamides (CDs) were active inhibitors of SOD and LP [18]. Due to promising antioxidant activities of **I2CDs**, we planned to synthesize their congeners at position 3. We aimed to explore the activity pattern of congeners at different positions. In this study, we examined *in vitro* free radical scavenging properties of **I3CDs** by determining their capacity to scavenge superoxide anion formation and inhibition of lipid peroxidation. **I3CDs** are currently under investigation on the role of oxygen free radical and $^1O_2$-generating systems, in order better understanding the mechanism of action. Therefore, the hydroxyl radical ($HO^{\cdot}$) - and superoxide anion radical ($O_2^{\cdot -}$)-scavenging activity, as well as the singlet oxygen ($^1O_2$)-quenching property of compounds is going to be determined by deoxyribose degredation assay.

Experimental

Materials

Indole-3-carboxylic acid, *p*-chlorobenzyl amine, *p*-fluorobenzyl amine, 2,4-dichlorobenzyl amine were from Aldrich; deutoro chloroform, potassium carbonate, *p*-fluorobenzyl chloride, dichloromethane, pyridine, hexane, ethylacetate from Merck; deuterated dimethyl-sulfoxide, benzyl bromide, 2,4-difluorobenzyl amine from Acros; methanol, hydrochloric acid, acetic acid, sodium hydroxide, toluene, ethanol from Riedel-de Häen; sulphiric acid, sodium hydride, dimethyl formamide, thionyl chloride from Fluka. Cytochrome *c*, α-tocopherol (Vit E) and thiobarbituric acid (TBA) were from Sigma Chemicals Co.

Chemistry

Melting points were measured with a capillary melting point apparatus (Electrothermal 9100) and uncorrected. ^{1}H-NMR spectra were recorded on Varian Mercury 400 NMR spectrometer for 400 MHz, with Me_4Si as internal standard. Chemicals shifts (δ) were reported in parts per million (ppm), and signals were expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on a Waters ZQ Micromass LC-MS Spectrometer Electrosprey Ionization (ESI). Infrared (IR) spectra of compounds were measured on Jasco FT/IR-420. Elemental analyses were done on a Leco-932 CHNS-O analyzer. The column chromatography was accomplished on silica gel 60 (Merck).

General Procedure for Synthesis of N-substituted Indole-3-carboxamide Derivatives ***1-10***. 1-Benzyl and *p*-fluorobenzyl indole-3 carboxylic acids (0.002 mol) were refluxed in 5 ml toluene with 2.5 ml $SOCl_2$ for 2 h at 80°C. The solvent and $SOCl_2$ were removed by co-evaporation with toluene (3 × 10 ml). The residue was dissolved in 10 ml chloroform and an equivalent amount of pyridine, the corresponding amine derivatives were added and the mixture was stirred at room temperature overnight. The solvent was evaporated to give crude compounds, which were purified by silicagel column chromatography (hexanes: EtOAc = 7:3) and then recrystallized from ethanol.

*N, 1-Dibenzyl-1H-indole-3-carboxamide (**1**)*. Recrystallization from ethanol gave pure **1** (0.575 g, yield 84.6%). M.P. 175–178°C. $Rf_1 = 0.25$ (n-hexanes: ethyl acetate; 7:3), $Rf_2 = 0.16$ (dichloromethane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.70 (d, 2H, J = 5.2, NH—C*H*$_2$-Ph), 5.31 (s, 2H, C*H*$_2$-Ph), 6.23 (**t**, 1H, N*H*-CH_2-Ph), 7.09 (d, 2H, J = 6.6, H-2, 6), 7.22–7.37 (m, 10H, H-b, c, 3, 4, 5, 2′, 3′, 4′, 5′, 6′), 7.40 (d, 1H, J = 6.8, H-d), 7.73 (s, 1H, H-a), 7.97 (d, 1H, J = 9.6, H-e). IR (KBr) cm^{-1}: 1616 (CO), 1537, 3343 (N—H), 1465 (C—N). ES-MS *m/z*: 341.17 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{20}N_2O$: C, 80.72; H, 5.94; N, 8.18; (0.1 H_2O). Found: C, 80.52; H, 5.77; N, 8.15%.

*1-Benzyl-N-(4-chlorobenzyl)-1H-indole-3-carboxamide (**2**)*. Recrystallization from ethanol gave pure **2** (0.623 g, yield 83.2%). M.P. 230–233°C. $Rf_1 = 0.24$ (n-hexanes: ethyl acetate; 7:3), $Rf_2 = 0.20$ (dichloromethane). ^{1}H-NMR (400 MHz, DMSO- d_6) δ: 4.45 (d, 2H, J = 5.6, NH—C*H*$_2$-Ph), 5.47 (s, 2H, C*H*$_2$-Ph), 8.53 (**t**, 1H, N*H*—CH_2-Ph), 7.12–7.42 (m, 11H, H-b, c, 2, 3, 4, 5, 6, 2′, 3′, 5′, 6′), 7.53 (d, 1H, J = 7.2, H-d), 8.17 (s, 1H, H-a), 8.17 (d, 1H, J = 7.6, H-e). IR (KBr) cm^{-1}: 1617 (CO), 1538, 3332 (N—H), 1466 (C—N). ES-MS *m/z*: 375.23 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{19}N_2ClO$: C, 73.69; H, 5.11; N, 7.47. Found: C, 73.44; H, 4.99; N, 7.52%.

*1-Benzyl-N-(4-Fluorobenzyl)-1H-indole-3-Carboxamide (**3**)*. Recrystallization from ethanol gave pure **3** (0.593 g, yield 82.9%). M.P. 201–204°C. $Rf_1 = 0.22$ (n-hexanes: ethyl acetate; 7: 3), $Rf_2 = 0.18$ (dichloromethane). ^{1}H-NMR (400 MHz, DMSO- d_6) δ: 4.45 (d, 2H, J = 6.0, NH—C*H*$_2$-Ph), 5.46 (s, 2H, C*H*$_2$-Ph), 8.51 (**t**, 1H, N*H*—CH_2-Ph), 7.12–7.35 (m, 9H, H-b, c, 2, 3, 4, 5, 6, 3′, 5′), 7.38 (dd, 2H, $J_0 = 9.0$, $J_{m1} = 5.4$, $J_{m2} = 5.6$, H-2′, 6′), 7.53 (d, 1H, J = 7.2, H-d), 8.17 (s, 1H, H-a), 8.18 (d, 1H, J = 7.6, H-e). IR (KBr) cm^{-1}: 1619 (CO), 1539, 3343 (N—H), 1465 (C—N). ES-MS *m/z*: 359.15 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{19}N_2FO$: C, 77.08; H, 5.43; N, 7.82. Found: C, 76.82; H, 5.25; N, 7.78%.

*1-Benzyl-N-(2,4-dichlorobenzyl)-1H-indole-3-carboxamide (**4**)*. Recrystallization from ethanol gave pure **4** (0.670 g, yield 82.0%). M.P. 162–164°C. $Rf_1 = 0.38$ (n-hexanes: ethyl acetate; 7: 3), $Rf_2 = 0.28$ (dichloromethane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.72 (d, 2H, J = 6.4, NH—C*H*$_2$-Ph), 5.31 (s, 2H, C*H*$_2$-Ph), 6.46 (**t**, 1H, N*H*—CH_2-Ph), 7.13 (dd, 2H, $J_0 = 7.2$, $J_{m1} = 1.6$, $J_{m2} = 2.4$, H-2, 6), 7.20–7.34 (m, 7H, H-b, c, 3, 4, 5, 5′, 6′), 7.39 (d, 1H, $J_m = 1.6$, H-3′), 7.46 (d, 1H, J = 8.8, H-d), 7.73 (s, 1H, H-a), 7.96 (d, 1H, J = 9.2, H-e). IR cm^{-1}: 1628 (CO), 1537, 3335 (N—H), 1467 (C—N). ES-MS *m/z*: 409.19 (M^+). Anal. Calcd for $C_{23}H_{18}N_2Cl_2O$: C, 67.49; H, 4.43; N, 6.84. Found: C, 67.11; H, 4.34; N, 6.89%.

*1-Benzyl-N-(2,4-Difluorobenzyl)-1H-indole-3-carboxamide (**5**)*. Recrystallization from ethanol gave pure **5** (0.560 g, yield 74.5%). M.P. 188–189°C. $Rf_1 = 0.32$ (n-hexanes: ethyl acetate; 7:3), $Rf_2 = 0.25$ (dichloromethane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.68 (d, 2H, J = 6.4, NH—C*H*$_2$-Ph), 5.31 (s, 2H, C*H*$_2$-Ph), 6.32 (**t**, 1H, N*H*—CH_2-Ph), 6.78–6.87 (m, 2H, H-3′, 5′), 7.13 (dd, 2H, $J_0 = 7.6$, $J_{m1} = 1.6$, $J_{m2} = 2.4$, H-2, 6), 7.22–7.34 (m, 6H, H-b, c, 3, 4, 5, 6′), 7.48 (d, 1H, $J_o = 8.4$, H-d), 7.72 (s, 1H, H-a), 7.96 (d, 1H, J = 8.8, H-e). IR cm^{-1}: 1625 (CO), 1539, 3335 (N—H), 1466 (C—N). ES-MS *m/z*: 377.18 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{18}N_2F_2O$: C, 73.39; H, 4.82; N, 7.44. Found: C, 73.52; H, 4.71; N, 7.50%.

*N-Benzyl-1-(4-fluorobenzyl)-1H-indole-3-carboxamide (**6**)*. Recrystallization from ethanol gave pure **6** (0.536 g, yield 74.8%). M.P. 205–207°C. $Rf_1 = 0.18$ (n-hexanes: ethyl acetate; 7: 3), $Rf_2 = 0.19$ (dichloromethane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.71 (d, 2H, J = 5.6, NH—C*H*$_2$-Ph), 5.29 (s, 2H, C*H*$_2$-Ph), 6.23 (**t**, 1H, N*H*—CH_2-Ph), 6.99 (t, 2H, J = 17.6, 3, 5), 7.12 (dd, 2H, $J_0 = 9.0$, $J_{m1} = 4.8$, $J_{m2} = 5.6$, H-2, 6), 7.23-7.38 (m, 7H, H-b, c, 2′, 3′, 4′, 5′, 6′), 7.41 (d, 1H, J = 8.4, H-d), 7.72 (s, 1H, H-a), 7.96 (d, 1H, J = 8.8, H-e). IR cm^{-1}: 1613 (CO), 1540, 3343 (N—H), 1465 (C—N). ES-MS *m/z*: 359.14 ($M^+ + 1$).

Anal. Calcd for $C_{23}H_{19}N_2FO$: C, 77.08; H, 5.34; N, 7.82. Found: C, 76.68; H, 5.24; N, 7.77%.

N-(4-Chlorobenzyl)-1-(4-fluorobenzyl)-1H-indole-3-carboxamide (7). Recrystallization from ethanol gave pure **7** (0.665 g, yield 84.7%). M.P. 200–202°C. $Rf_1 = 0.19$ (n-hexanes: ethyl acetate; 7: 3), $Rf_2 = 0.22$ (dichloromethane). ^{1}H-NMR (400 MHz, DMSO- d_6) δ: 4.45 (d, 2H, J = 6.0, NH—CH_2-Ph), 5.46 (s, 2H, CH_2-Ph), 8.53 (**t**, 1H, NH—CH_2-Ph), 7.12–7.21 (m, 4H, H-2, 6, 3′, 5′), 7.33–7.40 (m, 4H, H-b, c, 3, 5), 7.32 (dd, 2H, $J_o = 8.4$, $J_{m1} = 5.6$, $J_{m2} = 5.6$, H-2′, 6′), 7.55 (d, 1H, J = 8.4, H-d), 8.15 (s, 1H, H-a), 8.16 (d, 1H, J = 8.8, H-e). IR cm^{-1}: 1620 (CO), 1536, 3372 (N—H), 1464 (C—N). ES-MS *m/z*: 393.12 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{18}N_2FClO$: C, 70.32; H, 4.62; N, 7.13. Found: C, 69.97; H, 4.58; N, 7.11%.

N, 1-Bis (4-fluorobenzyl)-1H-indole-3-carboxamide (8). Recrystallization from ethanol gave pure **8** (0.625 g, yield 83.0%). M.P. 191–192°C. $Rf_1 = 0.16$ (n-hexanes: ethyl acetate; 7: 3), $Rf_2 = 0.16$ (dichloromethane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.59 (d, 2H, J = 5.6, NH—CH_2-Ph), 5.21 (s, 2H, CH_2-Ph), 6.18 (**t**, 1H, NH—CH_2-Ph), 6.90–6.97 (m, 4H, H-3, 5, 3′, 5′), 7.04 (dd, 2H, $J_0 = 8.8$, $J_{m1} = 4.8$, $J_{m2} = 5.6$, H-2, 6), 7.16–7.25 (m, 3H, H-b, c, d), 7.29 (dd, 2H, $J_0 = 8.6$, $J_{m1} = 5.2$, $J_{m2} = 5.6$, H-2′, 6′), 7.64 (s, 1H, H-a), 7.87 (d, 1H, J = 9.6, H-e). IR cm^{-1}: 1617 (CO), 1540, 3351 (N—H), 1464 (C—N). ES-MS *m/z*: 377.14 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{18}N_2F_2O$: C, 72.69; H, 4.88; N, 7.37 (0.2 H_2O). Found: C, 72.57; H, 4.71; N, 7.35%.

*N-(2,4-Dichlorobenzyl)-1-(4-fluorobenzyl)-1H-indole-3-carboxamide (**9**)*. Recrystallization from ethanol gave pure **9** (0.626 g, yield 73.3%). M.P. 139–142°C. $Rf_1 = 0.29$ (n-hexanes: ethyl acetate; 7: 3), $Rf_2 = 0.32$ (dichloromethane) ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.66 (d, 2H, J = 5.6, NH—CH_2-Ph), 5.23 (s, 2H, CH_2-Ph), 6.35 (**t**, 1H, NH-CH_2-Ph), 6.93 (t, 2H, J = 17.2, H-3, 5), 7.05 (dd, 2H, $J_0 = 9.0$, $J_{m1} = 5.2$, $J_{m2} = 5.6$, H-2, 6), 7.15–7.24 (m, 4H, H-b, c, 5′, 6′), 7.34 (d, 1H, $J_m = 2.0$, H-3′), 7.40 (d, 1H, J = 8.0, H-d), 7.65 (s, 1H, H-a), 7.87 (d, 1H, J = 9.2, H-e). IR cm^{-1}: 1616 (CO), 1540, 3305 (N—H), 1463 (C—N). ES-MS *m/z*: 427.11 (M^+). Anal. Calcd for $C_{23}H_{17}N_2FCl_2O$: C, 64.65; H, 4.01; N, 6.56. Found: C, 64.96; H, 4.40; N, 6.32%.

*N-(2,4-Difluorobenzyl)-1-(4-fluorobenzyl)-1H-indole-3-carboxamide (**10**)*. Recrystallization from ethanol gave pure **10** (0.621 g, yield 78.7%). M.P. 177–180°C. $Rf_1 = 0.26$ (n-hexanes: ethyl acetate; 7:3), $Rf_2 = 0.28$ (dichloromethane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.69 (d, 2H, J = 5.6, NH—CH_2-Ph), 5.29 (s, 2H, CH_2-Ph), 6.32 (**t**, 1H, NH—CH_2-Ph), 6.80–6.88 (m, 2H, H-3′, 5′), 6.99 (t, 2H, J = 17.6, H-3, 5), 7.11 (dd, 2H, $J_0 = 8.8$, $J_{m1} = 4.8$, $J_{m2} = 5.6$, H-2, 6), 7.24-7.32 (m, 3H, H-b, c, 6′), 7.46 (d, 1H, J = 8.4, H-d), 7.71 (s, 1H, H-a), 7.95 (d, 1H, J = 8.8, H-e). IR cm^{-1}: 1624 (CO), 1540, 3347 (N—H), 1466 (C—N). ES-MS *m/z*: 395.16 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{17}N_2F_3O$: C, 70.04; H, 4.34; N, 7.10. Found: C, 69.87; H, 4.44; N, 7.08%.

Antioxidant activity of compounds

Superoxide radical scavenging activity. The capacity of the compounds to scavenge superoxide anion formation was determined spectrophotometrically on the basis of inhibition of cyctochrome *c* (from horse heart, Sigma Co. St. Louis, MO) reduction as per the modified method of McCord and Fridovich [19]. Superoxide anion was generated in the xanthine/xanthine oxidase (from milk, Sigma Co. St. Louis, MO) system. The reaction mixture contained in a final volume of 1.0 ml, 0.05 M phosphate buffer pH 7.8, 0.32 Units/ml xanthine oxidase, 50 μM xanthine, 60 mM ctytochrome *c* and different concentration of synthesized compounds at 100 μl solution in DMSO/MeOH (5:95). Xanthine oxidase was finally added to this mixture to start the reaction. The absorbance was measured spectrophotometrically at 550 nm for cytochrome *c* reduction. Each experiment was performed in triplicate, and the results were expressed as a percent of the control.

Assay of lipid peroxidation. The effect of synthesized compounds on rat liver homogenate lipid peroxidation level in the presence of $FeCl_2$-ascorbic acid was determined by the modified method of Mihara et al. [20]. Male albino Wistar rats (200–225 g) were fed a standard laboratory rat chow and allow to drink tap water *ad libitum*. Procedures involving the animals and their care conformed to Institutional guidelines, in compliance with National and International laws and guidelines for the use of animals in biomedical research. The animals were starved for 24 h prior to execution by decapitation under anaesthesia. The livers were immediately removed and washed with ice-cold distilled water, and immediately homogenized with an ice chilled Teflon homogenizer. LP was measured spectrophotometrically by estimation of thiobarbituric acid reactant substances (TBARS) [20]. The amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA)/g tissue. This optimized assay mixture contained 0.5 ml of liver homogenate, 0.1 ml of Tris–HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid, 0.05 ml of 4 mM $FeCl_2$ and 0.05 ml of various concentrations of the synthesized compounds in DMSO/MeOH (5:95), or α-tocopherol (Vit E). The mixture was incubated for 1 h at 37°C. After incubation, 3.0 ml of H_3PO_4 and 1.0 ml of 0.6% TBA were added and the mixture shaken vigorously then boiled for 30 min. After

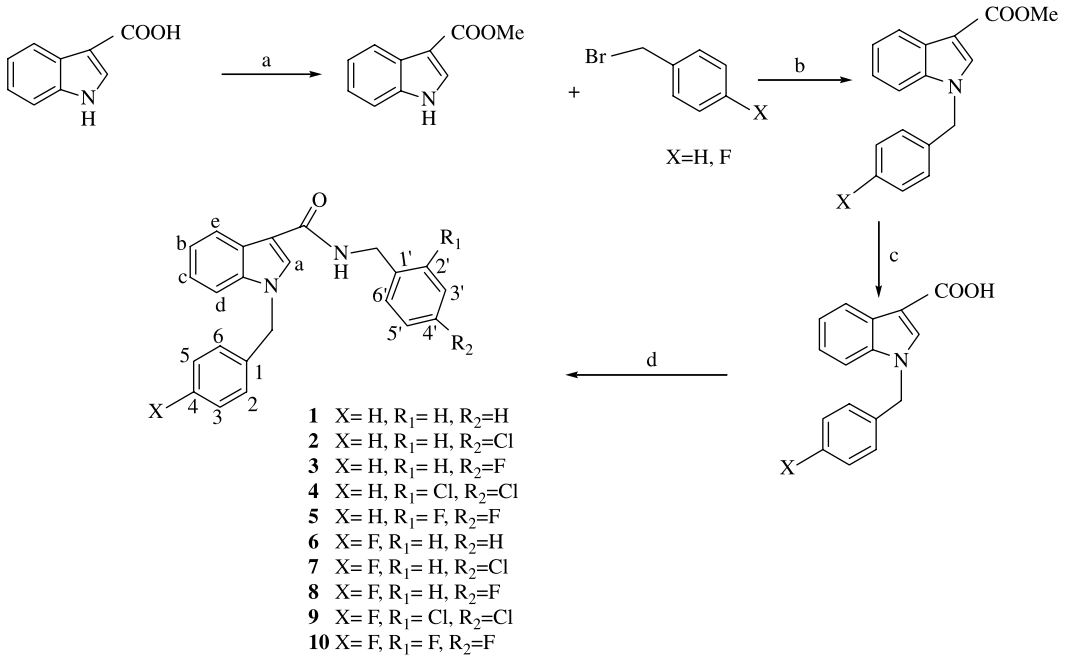
cooling, n-butanol was added and whole mixture was again shaken vigorously. The n-butanol phase was separated by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm against a blank, which contained all reagents except the liver homogenate.

Results and Discussion

The synthesis of the compounds was carried out as shown in Scheme 1. Initially, methyl indole-3-carboxylate was synthesized to protect the carboxylic acid. The substitution of indole N—H with unsubstituted benzyl or *p*-fluorobenzyl was accomplished by reaction with NaH in DMF [21]. N-substituted indole-3-carboxylic acid was obtained by the hydrolysis of N-substituted methyl indole carboxylate. The carboxyl group was converted to acyl chloride and displaced with the appropriate amines in the presence of pyridine [11].

The structural elucidation of compounds were established by NMR, IR and MS. Elemental analyses of compounds was satisfactory and within the range of ± 0.40%. In the IR spectra, absorption bands for all compounds were detected in the range 1613–1628 cm^{-1} corresponding to a CO stretching vibration. NH stretching of amide values were found as 3305–3372 cm^{-1} and NH bending values were found as 1536–1540 cm^{-1}. The C—N stretching vibration of the compounds was detected as 1463–1467 cm^{-1}. Numbering for NMR interpretation is shown in Scheme 1. The characteristic NH triplets of CON*H*—CH_2-Ph were found at 6.18–6.32 ppm and CH_2 protons were detected as doublets at 4.45–4.72 ppm with a coupling constant 5.2–6.4 Hz. N-substituted benzyl CH_2 protons were observed as a sharp singlet at ppm 5.21–5.47 ppm. The chemical shifts of all aromatics protons were found at 6.78–8.18 ppm.

The inhibiting properties of compounds on SOD and LP were determined *in vitro* and the results are shown in Table I. The scavenging extent of compounds, **4**, **5**, **8** and **9** were in the range of 84–100% at 10^{-3}M concentration. Dichloro-(compound **4**) and difluoro-(compound **5**) substituted indole-3-carboxamide derivatives showed 100% inhibition for SOD. The substitution at the indole nitrogen needs to be benzyl to obtain the maximum ativity for these compounds. Mono halogen substituted derivatives (compounds **2** and **3**) did not show any activity, when they had benzyl substitution at the indole nitrogen. As far as *p*-fluoro benzyl substitution at indole nitrogen is concerned, the dichlorinated compound **9** showed activity by about 90% and the difluorinated compound **10** had 22% inhibition. On the other hand, monohalogenated compounds **7** and **8** showed an activity of 58% and 84%, respectively. The activity was still found higher for



Reagents: (a) 10% HCl gas in MeOH, reflux; (b) NaH, DMF, RT; (c) 10%NaOH, MeOH, 65 ºC, AcOH; (d) 1. $SOCl_2$, toluene, reflux, 2. corresponding amines, $CHCl_3$, pyridine, RT.

Scheme 1. The synthesis of N-substituted indole-3-carboxamide derivatives **1–10**.

Table I. The activity results for compounds **1–10**.

Comp.	Concentration in incubation medium (M)	% Inhibition of SOD	% Inhibition* of LP
1	10^{-3}	NA	NA
	10^{-4}	NA	NA
2	10^{-3}	NA	NA
	10^{-4}	NA	NA
3	10^{-3}	NA	NA
	10^{-4}	NA	10 ± 1.4
4	10^{-3}	100 ± 1.0	4 ± 2.8
	10^{-4}	33 ± 4.0	NA
5	10^{-3}	100 ± 4.0	51 ± 2.1
	10^{-4}	12 ± 2.0	NA
6	10^{-3}	NA	NA
	10^{-4}	NA	NA
7	10^{-3}	58 ± 5.0	NA
	10^{-4}	NA	NA
8	10^{-3}	84 ± 4.0	5 ± 1.4
	10^{-4}	NA	NA
9	10^{-3}	90 ± 6.0	13 ± 2.8
	10^{-4}	NA	NA
10	10^{-3}	22 ± 4.0	NA
	10^{-4}	NA	NA
Vit E	10^{-3}	83 ± 6.0	95 ± 3.2
	10^{-4}	10 ± 2.0	93 ± 2.0

NA; not active.

*Each value represents the mean ± SD of three independent experiments.

the monofluorinated compound **8**. This result indicated that fluoro substitution on the benzyl ring at the indole nitrogen of compound **8** has a positive effect for inhibition of SOD. It was also found that only compound **5** decreased the LP level by 51% at 10^{-3} M concentration. However, but none of the other **I3CDs** showed significant inhibitory effects on lipid peroxidation. Vit E caused 83% inhibition on superoxide anion production and 95% inhibition for lipid peroxidase at 10^{-3} M concentration. Comparison of the activity results for the compounds and Vit E revealed that the compounds are equally active or slightly more active than Vit E on SOD inhibition.

In this study, the relationship between the activity, type of substituents and their position in the **I3CDs** were evaluated. Activity was not observed when N-benzyl substituted compounds had a chloro- or a fluoro atom at the para position of the benzamide ring. Both ortho, para positions of the benzamide ring needs to be dichlorinated or difluorinated in these compounds in order to get maximum inhibitory effects on superoxide anion. On the other hand, the para fluoro substituted compound **8** showed more a positive effects on activity than a para chloro substituted compound **7**. This suggested that para fluoro benzyl substitutions at the indole nitrogen showed positive effects on the activity for the fluorinated compound **8** compared with the chlorinated compound **7**. On the contrary, para fluoro benzyl substitution at the indole nitrogen had a positive impact for the dichloro benzamide compound **9** compared with the difluorobenzamide compound **10**. Comparing the activity results with halogenated and non-halogenated derivatives, it was found that the halogenated compounds are generally more active then the non-halogenated compounds. Since 1-benzyl-*N*-(2, 4-difluorobenzyl)-1*H*-indole-3-carboxamide **5**, had 100% inhibition for SOD and 51% for LP, it can be considered that compound **5** is the most active one among all the compounds.

Same congeners of compounds at position-2 were reported in our previous publication [18]. The activity results of congeners at both positions-2 and -3 were found slightly different. Dichloro and difluoro substituted benzamide compounds at position-3 showed 100% activity. Difluoro substituted benzamide compound **5** at position-3 is 50 fold higher than its congener at position-2. While the monofluoro compound at position-2 showed 97% inhibition, the same compound at position-3 did not show any activity. Same substitutions at position-1 also affected the activity results of congeners at positions-2 and -3. None substituted compound at position-2 was found more active than compound at position-3, when position-1 was substituted with *p*-fluorobenzyl. In conclusion, the same substitutions on different position of indole carboxamide may lead to different role of scavenging effects on the superoxide anion radicals. It was found that congeners at position-2 are more effective for the inhibition of lipid peroxidation. For this reason, it can be considered that N-substituted indole 2-carboxamide derivatives are more active than N-substituted indole-3-carboxamide derivatives. An extension of these design concepts by alteration of the length of carbon chain between benzamide and the indole ring and the attachment of more hydrophilic groups on benzamide is underway test these modifications on biological activity.

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