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Novel indole-3-carboxaldehyde analogues: Synthesis and their antioxidant evaluation

Nagaraja Naik*[1], H. Vijay Kumar[2] and N. M. Roopashree[1]

[1]*Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore, Karnataka, India.*
[2]*Department of Organic Chemistry, Indian Institute of Science, Bangalore, Karnataka, India*

ABSTRACT

*A series of novel indole-3-carboxaldehyde conjugated with different aryl amines were synthesized and examined for their antioxidant potential to probe the most potent analogues using two in vitro models like 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and inhibition of microsomal lipid peroxidation (LPO) assay. Through the compounds showed various degree of activity whereas, compound (**5f**) displayed superior antioxidant activity relative to other examined analogues and also exhibit more activity than the standard, butylated hydroxy anisole (BHA).*

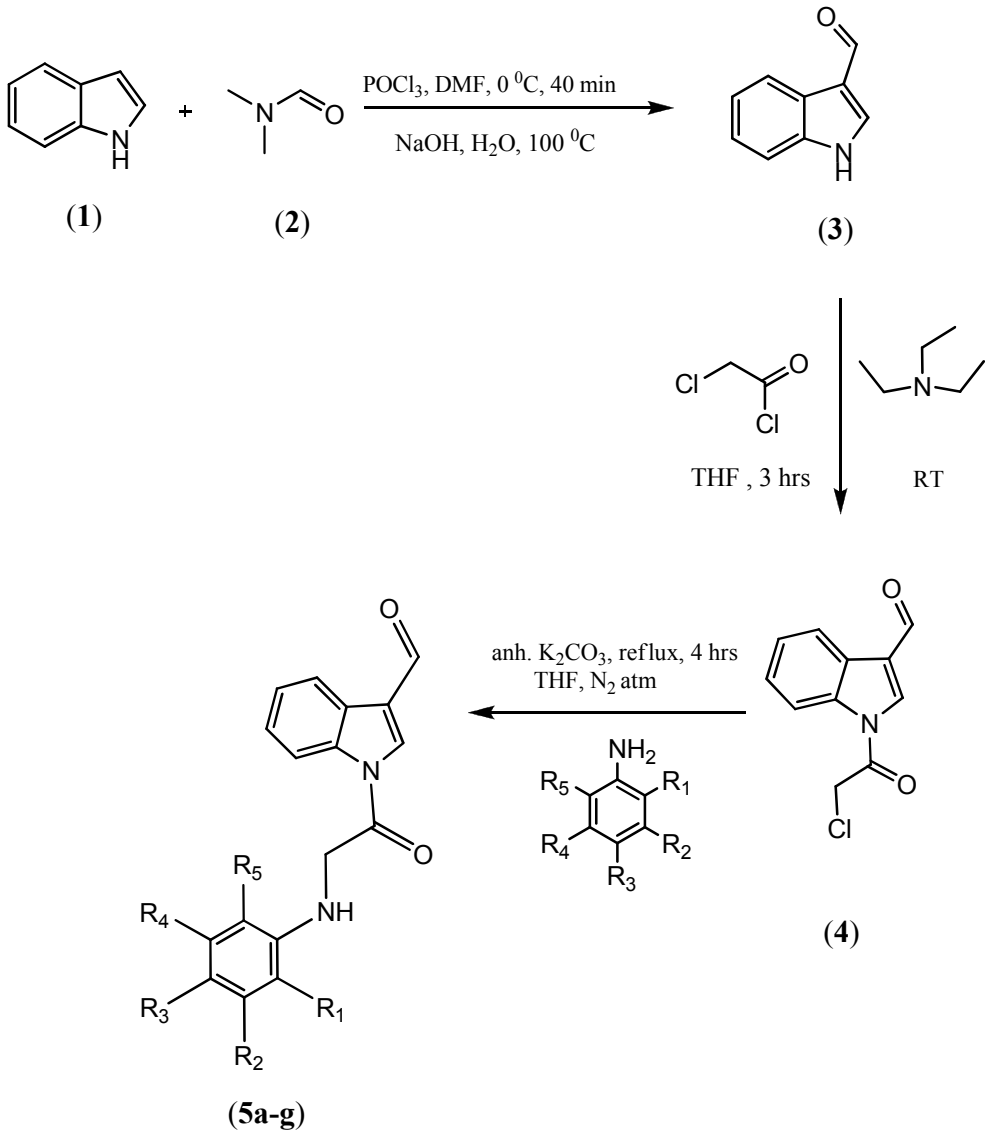
Keywords: Indole-3-carboxaldehyde, aryl amines, Free radical scavenging, Microsomal lipid peroxidation, Antioxidant activity.

INTRODUCTION

Free radical formation is associated with the normal natural metabolism of aerobic cells. The oxygen consumption inherent in cell growth leads to the generation of a series of oxygen free radicals. The interaction of these species with lipidic molecules produces new radicals: hydroperoxides and different peroxides [1, 2]. This group of radicals (superoxide, hydroxyl, and lipoid peroxides) may interact with biological systems in a cytotoxic manner. Free radicals and their uncontrolled production, in fact, are responsible for several pathological processes, such as certain tumours (prostate and colon cancers) and coronary heart disease [3]. Indole moieties occur widely in synthetic and natural products containing an important class of therapeutic agents. Many of these types of compounds have been reported to possess potent antiamoebic activity [4, 5], DNA cleavage [6], antifungal activity [7], analgesic and anti-inflammatory agents [8-9].

Owing to the wide spread application, synthetic and biological activity evaluation of indole-3-carboxaldehyde and their derivatives has been subject of intense investigation. In the course of the development of new antioxidants, we are interested in indole-3-carboxaldehyde derivatives based on the preliminary findings that indole-3-carboxaldehyde has an antioxidant property. Even though many biological studies has been carried out on indole-3-carboxaldehyde analogues, the antioxidant activities for the same indole-3-carboxaldehyde analogues bearing aryl amines moieties has not been done.

Recently, we have reported the antioxidant properties of 5H-dibenz[b,f]azepine, a tricyclic amine and some of its analogues, and their structure–activity relationships was established based on the different substituent's and positions [10-13]. In this paper, we have reported on the synthesis of indole-3-carboxaldehyde analogues bearing substituted aryl amines moieties. Their antioxidant were assessed by various *in vitro* assays and compared to the standard antioxidant.



Where,

Compound No	R_1	R_2	R_3	R_4	R_5
5a	OH	H	H	H	H
5b	H	OH	H	H	H
5c	H	H	Br	H	H
5d	H	H	H	H	H
5e	H	H	OH	NO_2	H
5f	H	H	OH	OCH_3	H
5g	H	H	OH	H	H

Scheme1: Protocol for the synthesis of indole-3-carboxaldehyde analogues.

MATERIALS AND METHODS

DPPH was purchased from Sigma Aldrich, 3-chloro acetylchloride, triethylamine, benzene, diethyl ether, ethyl acetate, n-hexane, tetrahydrofuran, anhydrous potassium carbonate, methanol, chloroform, sodium bicarbonate, ferrous sulphate, ascorbic acid, anhydrous sodium sulphate and aniline, 2-aminophenol, 3-aminophenol, 4-aminophenol, 4-hydroxy-3-nitro aniline, 4-hydroxy-3-methoxy aniline and bromo aniline were all of analytical grade and procured from S.d.fine chem. TLC aluminum sheets-Silica gel 60 F_{254} was purchased from Merck. The lR spectra were recorded on a FT-IR021 model in KBr disc. The ^{1}H NMR spectra were recorded on Joel GSX 400 MHz spectrophotometer using $CDCl_3$ as a solvent and the chemical shift (δ) are in ppm relative to internal standard.

Chemistry

The starting material indole-3-carboxaldehyde (**3**) was synthesized by known procedure [14]. N-acylation of indole-3-carboxaldehyde with 3-chloro acetylchloride in the presence of triethylamine as base afforded 1-(2-chloroacetyl)-1H-indole-3-carboxaldehyde (**4**), a key intermediate (**Scheme 1**). Further, coupling of aryl amines were done by base condensation reaction to obtain a series of novel indole-3-carboxaldehyde analogues (**5a-g**), as target compounds (**Scheme 1**).

Synthesis of 1-(2-Chloroacetyl)-1H-Indole-3-carbaxaldehyde (4)

To the well stirred solution of indole-3-carboxaldehyde (2 mM) and triethylamine (2.2 mM) in 10 ml tetrahydrofuran (THF), 3-chloro acetylchloride (2.2 mM) in 5 ml THF was added drop by drop. The reaction mixture was stirred at room temperature for about 3 hr. Progress of the reaction is monitored by TLC using 9:1 hexane:ethyl acetate mixture as mobile phase. After the completion of reaction, the reaction mass was quenched in ice cold water and the product was extracted in diethyl ether. The ether layer was washed with 5% $NaHCO_3$ followed by distilled water. Finally the ether layer was dried over anhydrous Na_2SO_4. The yellow solid product was obtained by desolventation through rotary evaporator at 35 ^{0}C.

Yellow solid, yield 95%, m.p: 79 ^{0}C, IR(KBr) (cm^{-1}): 3055-2568 (Ar-H), 1601.15 (C=O); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.11-8.95 (m, 4H, Ar-H), 4.27(s, 2H, CH_2, CH_2N-H), 10.1 (s, 1H, CHO), 7.1 (s, 1H, Indole ring), Anal.Calcd for $C_{11}H_8ClNO_2$, C, 59.61; H, 3.64; Cl, 16.00; N, 6.32; O, 14.44; Found C, 59.63; H, 3.62; Cl, 16.00; N, 6.32; O, 14.44.

General procedure for the synthesis of 1-(2-chloroacetyl)-1H-indole-3-carboxaldehyde conjugated with different aryl amines (5a-g)

Aryl amines (1.2 mM) in dry THF (10 ml) was treated with K_2CO_3 (600 mg) in N_2 atmosphere. Later the solution of 1-(2-chloroacetyl)-1H-indole-3-carboxaldehyde (1mM) in dry THF (5 ml) was added drop by drop. The reaction mixture was refluxed for 4 hrs. The progress of the reaction mixture was monitored by TLC. The reaction mixture was then desolventized in rotary evaporator and the compound is extracted in ethyl acetate. The ethyl acetate layer was washed with water 5% $NaHCO_3$ solution followed by water and dried over anhydrous Na_2SO_4. The respective products were obtained by further desolventation in rotary evaporator at 50 ^{o}C. All respective analogues were separated and purified by column chromatography by using mixture of chloroform/methanol= 85:15. The products were characterized by IR, ^{1}H NMR and elemental analysis.

1-(2-(2-hydroxyphenylamino)acetyl)-1H-indole-3-carboxaldehyde (5a)

Light brown semi solid, yield 84%, IR(KBr) (cm^{-1}): 3025-2541 (Ar-H), 1643.13 (C=O), 3209 (n-H); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.11-8.95 (m, 4H, Ar-H), 4.1 (s, 1H, N-H), 4.27(s, 2H, CH_2, CH_2N-H), 6.75-6.68 (m, 4H, Ar-H of aryl amine), 9.3 (s, 1H, OH), 10.1 (s, 1H, CHO), 7.1 (s, 1H, Indole ring),, Anal.Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; O, 16.31; Found C, 69.35; H, 4.77; N, 9.54; O, 16.34.

1-(2-(3-hydroxyphenylamino)acetyl)-1H-indole-3-carboxaldehyde (5b)

Brown semi solid, yield 82%, IR (KBr) (cm^{-1}): 3011-2989 (Ar C-H); 1621 (C=O); 3154-3426 (phenolic –OH), 3219 (N-H); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.02-8.6 (m, 4H, Ar-H), 4.2 (s, 1H, N-H), 4.4(s, 2H, CH_2, CH_2N-H), 6. 5-6.8 (m, 4H, Ar-H of aryl amine), 9.5 (s, 1H, OH), 10.2 (s, 1H, CHO), 7.2 (s, 1H, Indole ring),. Anal.Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; O, 16.31; Found C, 69.35; H, 4.81; N, 9.54; O, 16.31.

1-(2-(4-bromophenylamino)acetyl)-1H-indole-3-carboxaldehyde (5c)

Dark brown semi solid, yield 79%, IR (KBr) (cm^{-1}): 3123 (Ar C-H); 1682 C=O), 3244(N-H). ^{1}H NMR ($CDCl_3$) δ(ppm): 7.22-8.76 (m, 4H, Ar-H), 4.1 (s, 1H, N-H), 4.3(s, 2H, CH_2, CH_2N-H), 6.4-6.6 (m, 4H, Ar-H of aryl amine),

10.1 (s, 1H, CHO), 7.3 (s, 1H, Indole ring),. Anal.Calcd for $C_{17}H_{13}BrN_2O_3$: C, 57.16; H, 3.67; Br, 22.37; N, 7.84; O, 8.96. Found C, 57.17; H, 3.69; Br, 22.39; N, 7.82; O, 8.95.

1-(2-(phenylamino)acetyl)-1H-indole-3-carboxaldehyde (5d)
Light brown semi solid, yield 82%, IR (KBr) (cm^{-1}): 3396 (Ar C-H); 1663 C=O), 3232 (N-H). ^{1}H NMR ($CDCl_3$) δ(ppm): 6.9-8.5 (m, 4H, Ar-H), 4.1 (s, 1H, N-H), 4.1(s, 2H, CH_2, CH_2N-H), 6.75-6.68 (m, 5H, Ar-H of aryl amine), 10.1 (s, 1H, CHO), 7.1 (s, 1H, Indole ring), Anal.Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07; O, 11.50; Found C, 73.39; H, 5.09; N, 10.06; O, 11.52.

1-(2-(4-hydroxy-3-nitrophenylamino)acetyl)-1H-indole-3-carboxaldehyde (5e)
Yellow solid, yield 76%, m.p 87 ^{0}C, IR (KBr) (cm^{-1}): 3054-2832 (Ar C-H); 1644 (C=O), 3222(N-H); 3121-3521 (phenolic-OH); 2548-3012 (CH_2). ^{1}H NMR ($CDCl_3$) δ(ppm): 7.01-8.75 (m, 4H, Ar-H), 4.1 (s, 1H, N-H), 4.13 (s, 2H, CH_2, CH_2N-H), 6.55-6.88 (m, 4H, Ar-H of aryl amine), 10.0 (s, 1H, CHO), 7.1 (s, 1H, Indole ring),. Anal.Calcd for $C_{17}H_{13}N_3O_5$: C, 60.18; H, 3.86; N, 12.38; O, 23.58; Found C, 60.20; H, 3.88; N, 12.40; O, 23.59.

1(2-(4-hydroxy-3-methoxyphenylamino)acetyl)-1H-indole-3-carboxaldehyde (5f)
Brown solid, yield 88%, m.p 121 ^{0}C IR (KBr) (cm^{-1}): 3052-2830 (Ar C-H); 1600 (C=O), 3231 (N-H). ^{1}H NMR ($CDCl_3$) δ(ppm): 7.1-8.6 (m, 4H, Ar-H), 4.1 (s, 1H, N-H), 4.22 (s, 2H, CH_2, CH_2N-H), 6.25-6.98 (m, 3H, Ar-H of aryl amine), 9.4 (s, 1H, OH), 10.0 (s, 1H, CHO), 2.5 (s, 3H, OCH_3), 7.0 (s, 1H, Indole ring), Anal.Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; O, 19.73; Found C, 66.65; H, 4.95; N, 8.62; O, 19.75.

1-(2-(4-hydroxyphenylamino)acetyl)-1H-indole-3-carboxaldehyde (5g)
Brown solid, yield 92%, m.p 103 ^{0}C, IR (KBr) (cm^{-1}): 3042-2865 (Ar C-H); 1606 (C=O), 3256 (N-H). ^{1}H NMR ($CDCl_3$) δ(ppm): 7.2-8.9 (m, 4H, Ar-H), 4.0 (s, 1H, N-H), 4.2 (s, 2H, CH_2, CH_2N-H), 6.8-7. 1 (m, 4H, Ar-H of aryl amine), 9.5 (s, 1H, OH), 10.1 (s, 1H, CHO), 7.1 (s, 1H, Indole ring),. Anal.Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; O, 16.31; Found C, 69.40; H, 4.81; N, 9.53; O, 16.32.

Antioxidant activity
DPPH Radical scavenging activity
The newly synthesized compounds were screened for their antioxidant activity by following two well established *in vitro* models DPPH free radical scavenging activity and LPO assay. The compounds under study were dissolved in distilled ethanol (50 ml) to prepare 1000 µM stock solution. Solutions of different concentrations (10 µM, 50 µM, 100 µMM, 200 µM and 500 µM) were prepared by serialdilutions and the antioxidant activity was studied.

DPPH radical scavenging effect was carried out according to the method first employed by Blois [15]. Compound of different concentrations were prepared in distilled ethanol, 1ml of each compound solutions having different concentrations (10 µM, 50 µM, 100 µM, 200 µM and 500 µM) were taken in different test tubes; 4ml of a 0.1mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(A_o - A_1)/A_o \times 100]$$

Where A_o is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The radical scavenging activity of internal standard BHA was also measured and IC_{50} values was calculated and compared with that of the newly synthesized compounds.

Inhibition of microsomal lipid peroxidation (LPO) assay
Liver excised from adult male Wister rats, was homogenized (20g/100 ml tris buffer) in 0.02 mol/L, tris buffer (pH 7.4). Microsomes were isolated by the calcium aggregation method [16]. 100 µl of liver microsomal suspension (0.5 mg protein) was incubated with 1 mmol/L each of $FeSO_4$ and ascorbic acid with and without compound in a total volume of 1 ml in 0.1 mol/L phosphate buffer (pH 7.4). After incubation at 37 ^{o}C for 60 min, the reaction

mixture was boiled with thiobarbutyric acid (TBA) (0.67 g/100 ml water) for 15 min. Formation of TBA reactive substances (TBARS) was calculated from the absorbance at 535 nm [17].

RESULTS AND DISCUSSION

The starting material indole-3-carboxaldehyde was synthesized by the well known improved procedure. N-acylation of indole-3-carboxaldehyde with 3-chloro acetylchloride in the presence of triethylamine afforded to give 1-(2-chloroacetyl)-1H-indole-3-carboxaldehyde (**4**), a key intermediate. Further, coupling of aryl amines were done by base condensation reaction to obtain a series of novel indole-3-carboxaldehyde analogues (**5a-g**), as target compounds (scheme 1). The synthesized compounds were characterized by various physico-chemical and spectroscopic techniques. The IR spectrum of compound (**4**) reveals the absence of secondary amine at 3301cm^{-1} and also the presence of carbonyl stretching at 1601.15 cm^{-1}. ^{1}H NMR spectra of N-acylated analogues showed the absence of indole secondary N-H band at 4 ppm confirms the N-acylation of indole-3-carboxaldehyde. IR spectra of all conjugated analogues indicates the presence of aryl amines N-H stretching in the region of 3209-3256 cm^{-1} and the presence of carbonyl (C=O) stretching was observed in the region 1600-1682 cm^{-1} conforms the expected products. All the aromatic peaks (Ar-H) of all conjugated analogues displayed in the respective region (3396 -2865 cm^{-1}). ^{1}H NMR spectra of all conjugated analogues (**5a-g**) showed aryl amines N-H protons as singlet at 4.0-4.2 ppm. The signal due to phenolic – OH in compound **5a, 5b,** and **5g** appeared as singlet at about 9.3-9.5 ppm. Other aromatic protons were observed at expected region (6.2-8.8 ppm).

In order to explore the antioxidant properties and to establish the structure activity relationship of newly synthesized compounds, DPPH radical scavenging activity assay and LPO assay has been employed. DPPH radical scavenging activity evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds or extracts. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum (λmax) at 517 nm. This purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radical (i.e., by providing hydrogen atom or by electron donating, conceivable) and convert them to a colourless / bleached product (i.e., 2,2-diphenyl-1-picryl hydrazine, or a substituted analogues hydrazine), resulting in a decrease in absorbance. Hence, more rapidly the absorbance decreases, the more potent the antioxidant activity of the compounds. Percentage activity of ethanolic solution of Indole-3-carboxaldehyde **(3)**, and its derivatives (**4)** and **(5a-g)** were examined and the compared with the standard **(Figure 1).**

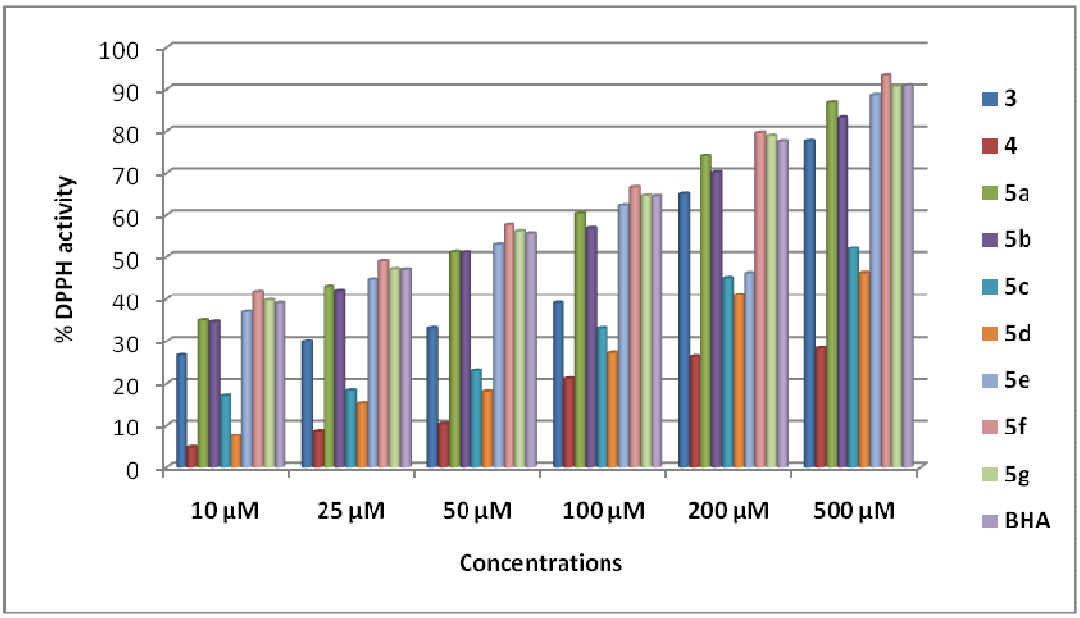


Figure 1. % DPPH activity of indole-3-carboxaldehyde and its analogues at different concentrations

Initially, model compound **(3)** possessing indole N-H showed considerable activity, where as, 1-(2-chloroacetyl)-1H-indole-3-carboxaldehyde **(4)** exhibits negligible activity. Further, coupling of aryl amines gives the significant enhancement in the activity which is depicted in the **Figure 1**. Compound (**5f)** bearing a methoxy group (electron donating group) addition to phenolic moiety showed dominant DPPH activity compare to BHA. The presence of nitro group (electron with drawing group) in (**5e)** instead of methoxy group in the same position exhibit slightly less to that of a compound (**5f)**. On the other hand substituted of bromine **(2c)** exhibits very less activity. Compounds (**5a), (5b), (5e)** and (**5g)** displayed good activity but slightly less to the standard BHA. On the other hand, compound (**5c)** and (**5d)** exhibits negligible activity towards DPPH. IC_{50} for the entire synthesized compound were also calculated and is depicted in the **table (1).**

Table 1: 50 % Inhibition of DPPH radical and LPO inhibition by indole-3-carboxaldehyde and its analogues

Each value represents mean ± S D (n=3)

Compound	DPPH activity IC_{50} (µM/ml)	LPO inhibition IC_{50} (µM/ml)
3	121±0.5	70±0.7
4	159±0.4	75±0.4
5a	18±0.1	24±0.3
5b	21±0.2	29±0.8
5c	109±0.5	118±0.1
5d	120±0.1	120±0.3
5e	16±0.8	21±0.5
5f	8±0.9	7±0.1
5g	13±0.2	16±0.9
BHA	11±0.5	9±0.1

Table (1) reveals the 50% inhibitory concentration towards DPPH activity of newly synthesized compounds. Initially, 1-(2-chloroacetyl)-1H-indole-3-carboxaldehyde **(4)** showed negligible activity towards DPPH but further coupling of aryl amines enhance the DPPH activity by showing significant activity. The increasing orders of DPPH activity of newly synthesized analogues are as follows: **5f > BHA > 5g > 5e > 5a >5b > 3 > 5c> 5d> 4**

From LPO studies, (**Figure 2)** among the synthesized compounds (**5a**), (**5b**), (**5g**) and (**5e**) showed good activity. Whereas (**4**), (**5c**) and (**5d**) showed negligible activity. Compound (**5g**) bearing methoxy group (electron donating group) additions to phenolic moiety demonstrate dominant on inhibiting LPO of liver microsomes. IC_{50} values of LPO inhibition for the newly synthesized analogues were depicted in **Table 1**.

LPO has been broadly defined as the oxidative deterioration of polyunsaturated lipids [18]. Initiation of a peroxidation sequence in a membrane or polyunsaturated fatty acid is due to abstraction of a hydrogen atom from the double bond in the fatty acid. The free radical tends to stabilize by a molecular rearrangement to produce a conjugated diene, which then readily reacts with oxygen molecule to give a peroxy radical [19]. Peroxy radicals can abstract a hydrogen atom from another molecule to give lipid hydroperoxide, R-OOH. A probable alternative fate of peroxy radicals is to form cyclic endoperoxides fragment to aldehydes such as malondialdehyde (MDA) and polymerization products. MDA and 4-hydroxy nonenal are the major break down products of LPO. MDA is usually taken as a marker of LPO and oxidative stress [20]. All the synthesized compounds exhibit same order of activity in both the assay performed. As a result, our study may provide evidence that the coupling of aryl amines to indole-3-carboxaldehyde had significant influence for antioxidant activity.

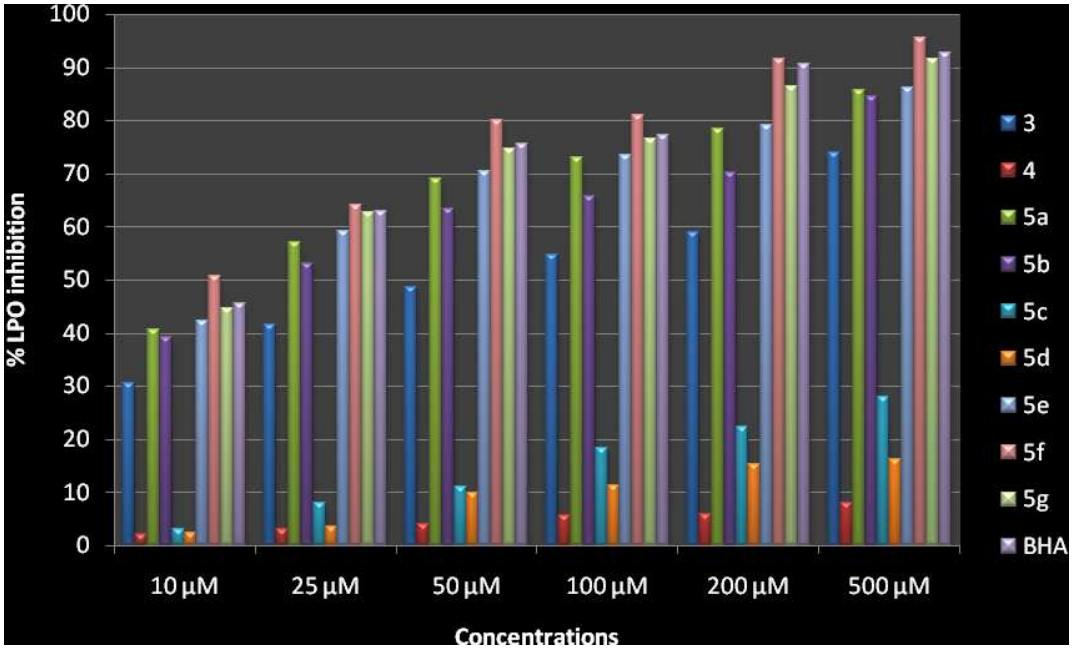


Figure. 2 % Inhibition of microsomal LPO of indole-3-carboxaldehyde and newly synthesized analogues (5a-g)
Each value represents the mean ±SD (n=3) derivatives.

CONCLUSION

A facile synthetic procedure has been developed for the synthesis of a new class of indole-3-carboxaldehyde analogues (**4**) and (**5a-g**). The synthesized analogues were subjected to screening of their activity as antioxidants which led to the identification that the antioxidant activity of compound (**4**) shows negligible activity. Further, coupling of aryl amines to compound (**4**) enhance the antioxidant property which shows comparable but slightly less activity than standard (BHA). In contrast, compound (**4g**) possessing methoxy substituent addition to the phenolic moiety exhibited antioxidant activity higher than BHA. Hence, it is clear that the coupling of aryl amines is the most important feature for the significant antioxidant activity of indole-3-carboxaldehyde analogues. These findings may be useful in the treatment of pathology in which free radicals oxidation plays a fundamental role and may warrant further in depth biological evaluations.

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