International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 3, Issue 3, 2011

Research Article

SYNTHESIS AND ANTIOXIDANT EVALUATION OF NOVEL 5-METHOXY INDOLE ANALOGUES

NAGARAJA NAIK*, H.VIJAY KUMAR, T. SHUBHAVATHI

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India
Email: drnaik_chem@yahoo.co.in

Received: 04 June 2011, Revised and Accepted: 28 June 2011

ABSTRACT

An efficient synthesis of 5-Methoxyindole conjugated with aniline and substituted anilines by base catalyzed condensation reaction was described. *N*-acylation of 5-Methoxyindole with 3-chloro acetylchloride afforded 2-Chloro-1-(5-Methoxy-1H-indole-yl)ethanone (**2**), a key intermediate. Antioxidant activities of synthesized compounds (**2a–g**) were determined by *in vitro* assays such as 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay and microsomal lipid peroxidation (LPO) assay. Moreover, these activities were compared to butylated hydroxylanisole (BHA), an internal standard antioxidant. Among the synthesized anlogues compound **2a, 2f** and **2g** exhibits effective antioxidant properties.

Keywords: 5-Methoxyindole, 2-Chloro-1-(5-Methoxy-1H-indole-yl)ethanone, DPPH, Antioxidants, LPO

INTRODUCTION

Antioxidants or free-radical scavengers extend the shelf life of food and pharmaceutical products during processing and storage by retarding the process of lipid peroxidation.[1] Antioxidants are often used to prevent the radical chain oxidation reactions. They act by inhibiting the initiation and propagation steps, leading to the termination of the reaction, and delay the oxidation process.[2,3] At the present time; BHA, BHT, propyl gallate (PG), and tertiary butyl hydroquinone (TBHQ) are the most commonly used antioxidants. Besides, the use of BHA and BHT is restricted by legislative rules due to doubts about toxic and carcinogenic effects.[4] Therefore, a growing interest in natural antioxidants in food applications and consumer preferences has given impetus to exploring naturally derived antioxidants.[5,6]

Indole moieties occur widely in synthetic and natural products containing an important class of therapeutic agents.[7,8] In the last decade, antioxidant activity of synthetic indole derivatives and their possible activity mechanisms have been widely studied.[8] Phenols are reported to quench oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical. Many of these type of compounds have been reported to possess potent antioxidant activity, anticarcinogenic, antimutagenic, antibacterial, antiviral, and anti-in.ammatory activities to a greater or lesser extent.[9]

5-Methoxyindole and its derivatives are reported to exhibit broad spectrum chemotherapeutic properties such as anti bacterial, antifungal [10], anti HIV-1 [11], anticancer [12], antitumour [13], antiheptatis [14], antiinflammetry [15] and antiviral [16] etc. Owing to the wide spread application, synthetic and biological activity evaluation of 5-Methoxyindole and their derivatives has been subject of intense investigation. In the course of the development of new antioxidants, we are interested in 5-Methoxyindole derivatives based on the preliminary findings that 5-Methoxyindole has an antioxidant property. Even though many biological studies has been carried out on 5-Methoxyindole analogues, the antioxidant activities on the same 5-Methoxyindole analogues bearing substituted aniline moieties were not been done.

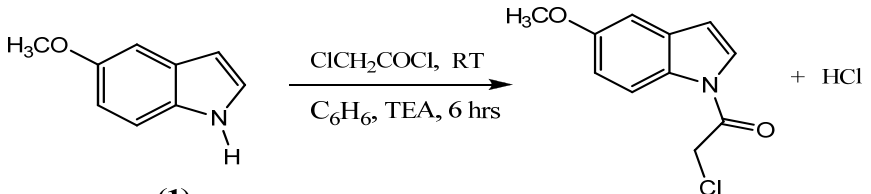
Recently, we have reported the antioxidant properties of 5H-dibenz[b,f]azepine, a tricyclic amine and some of its analogues, and their structure–activity relationships was established based on the different substituent's and positions [17-19]. In this paper, we have reported on the synthesis of 5-Methoxyindole analogues bearing substituted aniline moieties and their antioxidant properties were assessed by various *in vitro* assays and compared to the standard antioxidant.

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, anhydrous sodium sulphate and anilines and substituted anilines like 4-hydroxy-3-nitro aniline and 4-hydroxy-3-methoxy aniline, 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.

Synthesis of 2-chloro-1-(5 methoxy-1H-indole-1-yl) ethanone (2)

To the well stirred solution of 5-Methoxyindole (2 mM) and triethylamine (2.2 mM) in 10 ml benzene, 3-Chloro acetylchloride (2.2 mM) in 10 ml benzene was added drop by drop for about 30 min. Then the reaction mixture is stirred at room temperature for about 6 hrs. 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 extracted in diethyl ether. The ether layer was washed with 5% $NaHCO_3$ followed by distilled water. Finally the ether layer is dried over anhydrous Na_2SO_4. The brownish semi solid product was obtained by desolventation through rotary evaporator at 35°C.

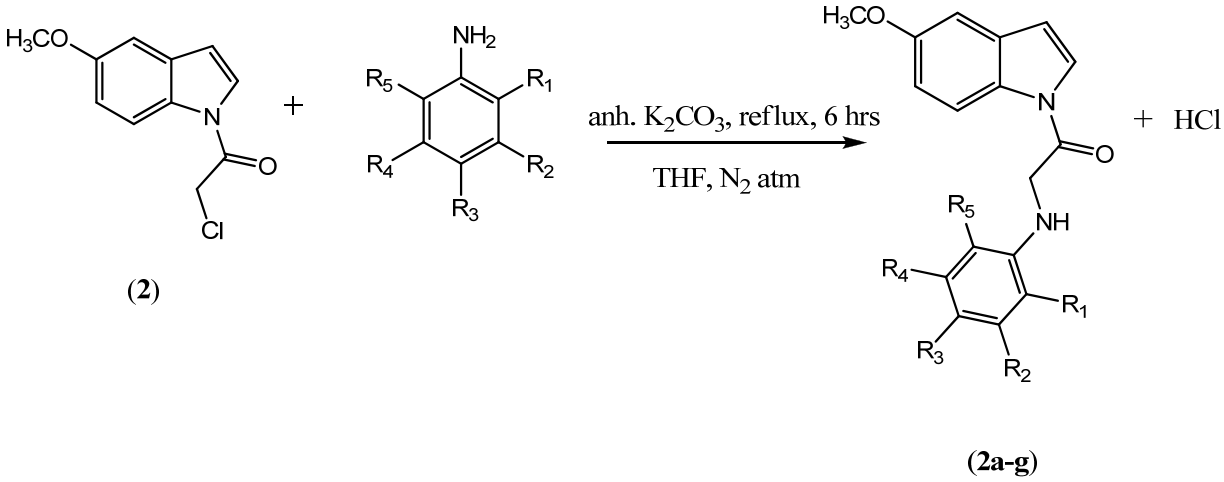


(1) (2)

Scheme 1: **Reaction protocol for the synthesis of 2-chloro-1-(5-Methoxy-1H-indole-1-yl)ethanone (key intermediate)**

2-Chloro-1-(5-methoxy-1H-indole-1-yl) ethanone (2)

Brown semi solid, yield 70% , IR(KBr) ν_{max} (cm^{-1}): 1698.20 (C=O), 3124.06-2917.77 (Ar-H) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 4,47 (d, CH_2-C=O, 2H), 6.69-7.89 (m, Ar -H, 3H), 3.8 (m, OCH_3, 3H), Anal.calcd. for $C_{11}H_{10}ClNO_2$: C, 59.07 ; H, 4. 51 ; Cl,15.85 ; N,6.26; O,14.31; Found: C,59.04; H, 4.49; Cl,15.83; N,6.24, O,14.33.



Scheme 2: **Synthetic pathway for 5-Methoxyindole conjugated products (2a-g)**

Compound	R_1	R_2	R_3	R_4	R_5
2a	OH	H	H	H	H
2b	H	OH	H	H	H
2c	H	H	Br	H	H
2d	H	H	H	H	H
2e	H	H	OH	NO_2	H
2f	H	H	OH	OCH_3	H
2g	H	H	OH	H	H

General procedure for the synthesis of 2-Chloro-l-(5-Methoxy-1H-indole-1-yl)ethanone conjugated with aniline and substituted anilines.

Aniline (1.2 mM) in THF (10 mL) was treated with anhydrous K_2CO_3 (600 mg) in N_2 atmosphere. Later the solution of 2-Chloro-1-(5 methoxy-1H-indole-1-yl)ethanone (1 mM) in THF (10 mL) was added drop by drop for 15 min. The reaction mixture was refluxed for 6-8 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 and dried over anhydrous Na_2SO_4. The brown semisolid was obtained by further desolventation in rotary evaporator at 50°C.

Synthesis of 2-chloro-1-(5 methoxy-1H-indole-1-yl) ethanone conjugated with substituted anilines like 2-hydroxy aniline, 3-hydroxy aniline, 4-hydroxy aniline, 4-hydroxy-3-nitro aniline, 4-hydroxy-3-methoxy aniline and bromo aniline were obtained by the same procedure. The analogues were separated and purified by column chromatography by using mixture of chloroform / methanol / acetic acid = 85: 12: 3. The products were characterized by IR, ^1H NMR and elemental Analysis.

2-(2-hydroxy phenyl amino)-1-(5-methoxy-1H-indol-1-yl)ethanone (2a)

Light brown semi solid; yield 76% ; IR(KBr) ν_{max} (cm^{-1}): 3401.21 (N-H), 1601.8 (C=O), 2360-2921.59 (Ar-H) cm^{-1}, 3378.2-3446.6 (Ph-OH), 2.3 (s, Indole CH, 2H); ^1H NMR ($CDCl_3$) δ: 4.1 (d, CH_2-C=O,2H), 6.5-7.8 (m, Ar-H, 3H), 4.0 (s, N-H, 1H), 6.7-6.9 (m, Ar-H, 4H), 5.3 (s, Ph-OH, 1H), 3.8 (s, OCH_3, 3H); Anal.calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45; O, 16.20 Found: C, 68.95; H ,5.41; N, 9.42; O, 16.23.

2-(3-hydroxy phenyl amino)-1-(5-methoxy-1H-indol-1-yl)ethanone (2b)

Yellow semisolid; yield 83%; IR(KBr) ν_{max} (cm^{-1}): 3412.21 (N-H); 1600.6 (C=O), 2363-2920.59 (Ar-H) cm^{-1}, 3377.3-3456.4 (Ph-OH), 2.3 (s, Indole CH, 2H); ^1H NMR ($CDCl_3$) δ: 4.18 (d, CH_2-C=O, 2H), 6.6 -7.8 (m, Ar-H, 3H), 4.0 (s, N-H, 1H), 6.1-7.0 (m, Ar-H, 4H), 5.4 (s, Ph-OH,1H), 3.8 (s, OCH_3, 3H); Anal.calcd.for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44 ; N, 9.45; O,16.20; Found: C, 68.92; H, 5.41; N, 9.43; O,16.23.

2-(4-bromophenylamino)-1-(5-methoxy-1H-indol-1-yl)ethanone (2c)

Dark yellow semisolid; yield 80%; IR(KBr) ν_{max} (cm^{-1}): 3412.22 (N-H), 1600.6(C=O), 2362-2920.56 (Ar-H) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 4.16 (d, CH_2-C=O, 2H), 6.58-7.13 (m, Ar-H, 3H), 4.1 (s, N-H, 1H), 6.58-7.13 (m, Ar-H, 4H), 3.8 (s, OCH_3, 3H), 2.3 (s, Indole CH, 2H); Anal.calcd. for $C_{17}H_{15}BrN_2O_2$: C, 56.84; H, 4.21; N, 7.80; O, 8.91; Br, 22.24; Found: C, 56.62; H,4.19; N,7.82; O, 8.93; Br, 22.22.

1-(5-methoxy -1H-indol-1-yl)-2-(phenylamino)ethanone (2d)

Brown semisolid; yield 86 %; IR(KBr) ν_{max}(cm^{-1}): 3409.33 (N-H), 1601 (C=O), 2360-2924.57 (Ar-H) cm^{-1}; ^1H NMR ($CDCl_3$) δ :4.19 (d, CH_2-C=O, 2H), 6.58 -7.23 (m, Ar-H, 3H), 3.9 (s, N-H,1H), 6.77 -7.23 (m, Ar-H, 5H), 3.8 (s, OCH_3, 3H), 2.3 (s, Indole CH, 2H); Anal.calcd. for $C_{17}H_{16}N_2O_2$: C,72.84; H, 5.75; N, 9.99; O,11.42; Found : C, 72.82 ;H, 5.72; N ,9.97; O,11.40.

2-(4-hydroxy-1-nitro phenyl amino)-1-(5-methoxy-1H-indol-1-yl)ethanone (2e)

Yellow semisolid; yield 78 %; IR(KBr) ν_{max} (cm^{-1}): 3411.18 (N-H); 1611.3 (C=O), 2943.59 (Ar-H) cm^{-1}; 3312.2-3445.5 (Ph-OH),^{1} H NMR ($CDCl_3$) δ: 4.15 (d, CH_2-C=O, 2H), 6.53 -7.15 (m, Ar-H, 3H), 3.9 (s, N-H,1H), 5.35-7.56 (m, Ar-H ,3H), 3.8 (s, OCH_3, 3H), 5.34 (s ,Ph-OH,

1H), 2.3 (s, Indole CH, 2H); Anal.calcd. for $C_{17}H_{15}N_3O_5$: C, 59.82; H, 4.43; N, 12.31; O, 23.44, Found: C, 59.80; H, 4.40; N, 12.33; O, 23.42.

2-(4-hydroxy-3-methoxy phenyl amino)-1-(5-methoxy-1H-indol-1-yl)ethanone (2f)

Dark brown semisolid; yield 77 % ; IR (KBr)ν_{max} (cm^{-1}): 3412.21 (N-H); 1613.9 (C=O), 2932.59 (Ar-H) cm^{-1} ; ^1H NMR ($CDCl_3$) δ: 4.18 (d, CH_2-C=O, 2H), 6.64 -7.89 (m, Ar-H, 3H), 4.0 (s, N-H. 1H), 598-6.62 (m, Ar-H, 3H), 3.82 (s, OCH_3, 3H), 3.82 (s, OCH_3 of methoxy indole, 3H), 5.36 (s, Ph-OH, 1H), 2.3 (s, Indole CH, 2H); Anal.calcd. for $C_{18}H_{18}N_2O_4$: C, 66.23; H, 5.53; N, 8.60; O, 19.61, Found: C, 66.20; H, 5.51; N , 8.59 ; O,19.63.

2-(4-hydroxy phenyl amino)-1-(5-methoxy-1H-indol-1-yl)ethanone (2g)

Brown semisolid; yield 80%; IR(KBr) ν_{max} (cm^{-1}): 3402.21 (N-H); 1603.9 (C=O), 2361-2929.59 (Ar-H) cm^{-1}, ^1H NMR ($CDCl_3$) δ : 4.19 (d, CH_2-C=O, 2H), 6.63 -7.89 (m, Ar-H, 3H), 3.83 (s ,N-H, 1H), 6.71-6.73 (m, Ar-H, 4H), 3.82 (s, OCH_3,3H), 5.36 (s ,Ph-OH, 1H), 2.3 (s, Indole CH, 2H); Anal. Calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44 ; N ,9.45 ; O,16.20 , Found : C, 68.90 ; H, 5.41; N , 9.42;O ,16.22.

BIOLOGICAL EVALUATION – ANTIOXIDANT ACTIVITY STUDIES

The newly synthesized compounds were screened for their antioxidant activities using a stable free radical, 2,2–diphenyl-1-picrylhydrazyl (DPPH) assay and Inhibition of microsomal (LPO) assay. The compounds under studies were dissolved in distilled ethanol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10 µM, 25 µM, 50 µM, 100 µM, 200 µM, and 500 µM) were prepared by serial dilution and the antioxidant activity was studied.

DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois. [20] Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 µM, 25 µM 50 µM, 100 µM, 200 µM and 500 µM) were taken in different test tubes; 4 mL of a 0.1 mM 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:

Radical scavenging activity (%) = $[(A_o - A_1) / A_o X100]$

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 BHA was also measured and compared with that of the newly synthesized compound.

Inhibition of microsomal lipid peroxidation assay

Liver excised from adult male Wister rats, was homogenized (20 g/100 ml Tris buffer) in 0.02 mol/L, tris buffer (pH 7.4). Microsomes were isolated by the calcium aggregation method. [21] 100 µl of liver microsomal suspension (0.5 mg protein) was incubated with 1 mmol/L each of $FeSO_4$ and ascorbic acid with or without compounds in a total volume of 1 ml in 0.1 mol/L phosphate buffer (pH 7.4). After incubation at 37 ^{0}C for 60 min, the reaction mixture was boiled with TBA (0.67 g/ 100 ml water) for 15 min. Formation of TBA reactive substances (TBARS) was calculated from the absorbance at 535 nm. [22] BHA was used as the positive control.

RESULTS AND DISCUSSIONS

5-Methoxyindole was synthesized by applying known method.[23] The active site for the coupling of aniline and substituted anilines to the basic moiety was very less, thus we carried out the *N*-acylation reaction in order to obtain a key intermediate in which, the coupling of aniline and substituted anilines can be execute very easily with simple experiment protocol with good yield. The synthesis of 5-Methoxyindole analogues conjugated with aniline and substituted anilines were realized in two steps. First step, the key intermediate, 2-chloro-1-(5 methoxy-1H-indole-1-yl) ethanone (**2**) was prepared in good yield by *N*-acylation reaction using 3-chloro acetylchloride (**Scheme 1**). In the second step, coupling of aniline and respective substituted anilines to the key intermediate were done to obtain the novel 5-Methoxyindole analogues **2a-g** (**Scheme 2)**. Our preliminary finding reveals that 5-Methoxyindole (**2**) exhibits some antioxidant properties (**Figure 1** and **2**). Thus, in order to establish some structure activity relationship based on the position and presence of different substituent and to understand how the different functionalities affect the antioxidant properties, we have synthesized a series of 5–Methoxy indole analogues. The synthesized compound was characterized by various physico-chemical and spectroscopic techniques. The IR spectra reveal the absence of secondary amine at 3301 cm^{-1} and also the presence of carbonyl stretching at 1698.28 cm^{-1}. ^1H NMR spectra of *N*-acylated analogues showed the absence of N-H band in the region at 4 ppm conforms the *N*-acylation of 5-Methoxyindole. In the course of our research aimed at the development of new antioxidants, we have been interested to synthesized 2-choloro-1-(5-methoxy-1H-indole-1-yl) ehanone **(2)** conjugated with aniline and substituted anilines. The procedure for the synthesis of the novel compounds is outline in **Scheme 2**. Here, we have carried out base condensation reaction at inert atmosphere (N_2 atmosphere) with good yield (80-90%). The newly synthesized compounds were purified by column chromatography using silica gel (60-120 mesh) as adsorbent and chloroform – methanol (70:30) as eluent. IR spectra of all conjugated analogues indicates the presence of N-H stretching in the region of 3401.22-3412.22 cm^{-1} and the presence of carbonyl (C=O) stretching was observed in the region 1600.6-1613.9 cm^{-1} conforms the expected products. All the aromatic peaks (Ar-H) of all conjugated analogues showed at respective regions (3400-3450cm^{-1}). ^1H NMR spectra of all conjugated analogues **(2a-g)** showed N-H protons as singlet at 3.8-4.0 ppm. The signal due to phenolic – OH in compound **2a, 2b, 2e, 2f** and **2g** appeared as singlet at about 5.3-5.4 ppm. A sharp singlet at 3.5 ppm corresponding to methoxy group in compound (**2f**) was observed. Other aromatic protons were observed at expected region (5.3-7.8 ppm). In the present investigation the evaluation study was carried out at various concentration and comparative studies were done with standard antioxidant BHA.

The evaluation of the antioxidant effects of the newly synthesized compounds having different concentrations were examined by two well documented *in vitro* assays like DPPH free radical scavenging assay and Inhibition of microsomal lipid peroxidation (LPO) assay. 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 5-Methoxyindole **(1)**, 2-chloro-1-(5-methoxy-1H-indole-1yl) ethanone **(2)** and 5-Methoxyindole conjugated with aniline and substituted aniline **(2a-g)** were examined and the compared with the standard **(Figure 1).**

Initially, our model compound **(1)** showed considerable activity, where as, 2-chloro-1- (5-methoxy-1H-indole-1-yl) ethanone **(2)** exhibits negligible activity. Further, coupling of aniline and substituted anilines gives the significant enhancement of activity which is depicted in the **Figure 1**. From the Figure we can conclude that, introduction of aniline showed very less activity. Whereas, coupling of substituted anilines to 2-choloro-1-(5-methoxy-1H-indole-1-yl) ethanone **(2)** increases the DPPH activity.

The presence of electron donating methoxy substituent in the phenolic compounds is known to increase the stability of the radical and hence, the antioxidant activity [24]. Thus, the Compound **2f**

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 **2e** instead of methoxy group in the same position exhibit slightly less to that of a compound **2f.** Compounds **2a, 2b, 2e** and **2g** showed good activity but slightly less activity to the standard BHA. On the other hand, compound **2c** and **2d** exhibits negligible activity towards DPPH. 50% inhibitions (IC_{50}) of DPPH for the entire synthesized compound were also calculated and are depicted in the **Table (1).**

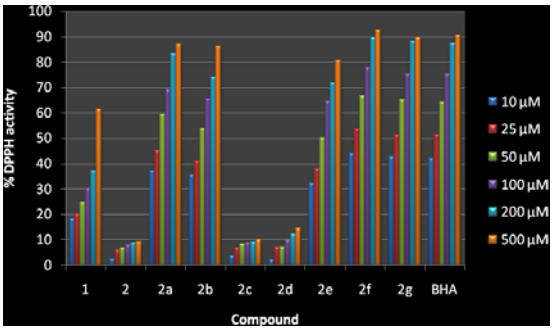


Fig. 1: DPPH activity of 5-Methoxyindole and its analogues at different concentrations

Table 1: 50% Inhibition of DPPH radical and LPO inhibition by 5-Methoxyindole and its analogues each value represents mean ± SD (n=3)

Compounds	DPPH activity IC_{50}(µM/ml)[a]	LPO inhibition IC_{50}(µM/ml)[b]
1	107±0.7	100±0.7
2	162±0.2	120±0.4
2a	19±0.5	15±0.3
2b	22±0.8	20±0.8
2c	166±0.2	135±0.1
2d	160±0.1	125±0.3
2e	27±0.3	22±0.5
2f	9±09	7±0.9
2g	12±0.6	11±0.1
BHA	11±0.5	10±0.2

[a]IC_{50} = the concentration (µM) exhibiting 50% inhibition of DPPH radical.

[b]IC_{50} = the concentration (µM) exhibiting 50% inhibition of LPO oxidation.

Each experiment was performed at least three times in duplicate.

The increasing orders of DPPH activity of newly synthesized analogues are as follows: **2f > BHA > 2g > 2e > 2a >2b > 2c> 2d >1 > 2**

From LPO studies, (**Figure 2)** among the synthesized compounds (**2a**), (**2b**), (**2g**) and (**2e**) showed good activity. Whereas (**2**), (**2c**) and (**2d**) showed negligible activity. Compound (**2g**) 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.[25] 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.[26] 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.[27]

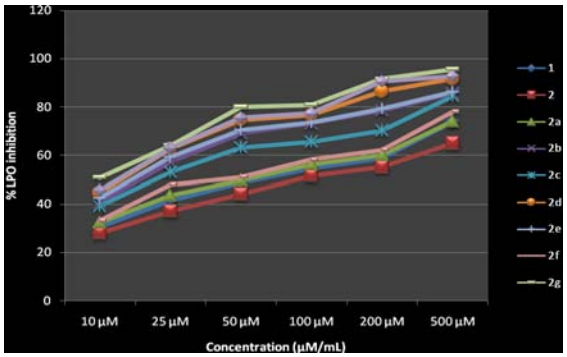


Fig. 2: % Inhibition of microsomal LPO of 5-Methoxyindole and newly synthesized analogues (2a-g). Each value represents the mean ±SD (n=3) derivatives

All the synthesized compounds exhibit same order of activity in both the assay performed. As a result, from the observed results we can conclude that our study may provide an evidence that the coupling of aniline and substituted aniline to 5-Methoxyindole had significant influence for antioxidant activity. Our investigations may be useful in academy and as well as in the treatment of pathologies in which free radical oxidation place of fundamental role.

ABBRIVATIONS

°C = degree centigrade
min = minute
hr = hour
mL = milli Liter
µM = micro molar
mg/mL = milli gram per milli Leter
g/mL= gram per milli Liter
% = percentage
IC_{50} = 50 percent Inhibition concentration
nm = nano meter
mM = milli molar
RT = room temperature
DPPH = 2, 2-diphenyl-1-picrylhydrazyl
TBA = thiobarbituric acid
TBARS= thiobarbituric acid reactive species
BHA = butylated hydroxy anisole
RSA =radical scavenging activity
< = less than
> = greater than
LPO= lipid peroxidation
$FeSO_4$= ferrous sulphate

REFERENCES

1. Halliwell B. Antioxidants in Human Health and Disease. Annu. Rev. Nut. 1996; 16: 33-50.
2. Gülcin I, Sat I. G, Beydemir S, Elmastas M, Küfreviog¢lu, Ö I. Comparison of antioxidant activity of clove (*Eugenia caryophylata Thunb*) buds and lavender (*Lavandula stoechas L.*). Food Chem. 2004; 87: 393-400.
3. Gülçin I. Seeds. Int. J. Food Sci. Nutr. 2005; 56: 491-499.
4. Moure A, Cruz J M, Franco D, Dominguez J M, Sineiro J, Dominguez H, Nunez M J, Parajo J C. Natural antioxidants from residual sources. Food Chem. 2001; 72:145-171.
5. Oktay M, Gülçin I, Küfreviogclu ÖI. Determination of *in vitro* antioxidant activity of fennel (Foeniculum vulgare)seed extracts. Lebensm. Wiss. Technol. 2003; 36: 263-271.
6. Gülçin I, Mshvildadze V, Gepdiremen A, Elias R. The antioxidant activity of a triterpenoid glycoside isolated from the berries of *Hedera colchica*: 3-*O*-(*β*-D-glucopyranosyl)-hederagenin. Phytother. Res. 2006; 20: 130-132.

7. Saracoglu N. Functionalization of Indole and Pyrrole Cores via Michael-Type Additions. Top. Heterocycl. Chem. 2007; 11: 1.
8. Suzen S. Antioxidant Activities of Synthetic Indole Derivatives and Possible Activity Mechanisms. Top. Heterocycl. Chem. 2007; 11: 145.
9. Gülçin I. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids. 2007; 32: 431-438.
10. Leboho T C, Michael J P, Van Otterlo W A L, Van Vuurer S F, De koning C B, The synthesis of 2- and 3-aryl indoles and 1,3,4,5-tetrahydropyrano[4,3-b]indoles and their antibacterial and antifungal activity. Bioorg. Med. Chem. Lett, 2009; 19: 494-4951.
11. Isidro M, Antonio M, Maria F, JUAN Jose Martinez de Irujo, Elena, Synthesis and anti-HIV-1 activities of new pyrimido[5,4-b]indoles. II Formaco, 1999; 54: 255-261.
12. Gerard A. P, Maria A P, Mario S, Giuseppe P. Synthesis and in vitro anticancer activity evaluation of biscarbamic esters of 2,3-bis (hydroxymethyl)-1-methyl-7- and 7,8 -substituted-benzo[g]indoles. II Farmaco, 1998; 53: 161-168.
13. Adel S. Girgis. Regioselective synthesis and stereochemical structure of anti-tumor active dispiro[3H-indole-3,2′-pyrrolidine-3′,3″-piperidine]-2(1H),4″-diones, Europ. J. Med. Chem. 2009; 44: 1257-1264.
14. Chai H, Zhao Y, Zhao C, Gong P, Synthesis and in vitro anti-hepatitis B virus activities of some ethyl 6-bromo-5-hydroxy-1H-indole-3-carboxylates. Bioorg. Med. Chem. 2009; 14: 911-917.
15. Sudhir Kumar Bhati, Ashok Kumar, Synthesis of new substituted azetidinoyl and thiazolidinoyl-1,3,4-thiadiazino (6,5-b) indoles as promising anti-inflammatory agents Eur. J. Med. Chem. 2008; 43: 2323-2330.
16. Michele Giampieri, Alessandro Balbi, Mauro Mazzei, Paolo La Calla, Cristina Ibba, Robert Lodda, Antiviral activity of indole derivatives. Anti viral research, 2009; 83: 179-185.
17. Vijay Kumar H, Gnanendra C R, Channe Gowda. D, Nagaraja Naik. In vitro antioxidant activity of 5H-dibenz[b,f]azepine and its analogues. E-J Chem. 2008, 5(S2), 1123–1132.
18. Vijay Kumar H, Kishor Kumar C, Nagaraja Naik. Synthesis of novel 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one derivatives with antioxidant activity. Med. Chem. Res. , 2011, 20(1),101-108.
19. Vijay Kumar H, Nagaraja Naik. Synthesis and antioxidant properties of some novel 5H-dibenz[b,f]azepine derivatives in different in vitro model systems. Eur. J. Med. Chem. 2010; 45(1): 2- 10
20. Blois M. S. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958; 26: 1199.
21. Kamath SA, Ryubin, E. Interaction of calcium with microsomes: A modified method for the rapid isolation of rat liver microsomes. Biochem Biophys Res Comm. 1972; 49: 52-59
22. Buege JA, Aust ST. Microsomal lipid peroxidation. Method in Enzymology. 1978; 52: 302-310
23. Sakamoto T, Hosoda I, Kikugawa Y. A simple synthesis of 5-methoxyindole and 5-methoxy-2-oxindole. J. Hetero. Chem, 1998; 25: 1279-1281.
24. Ajan P R, Vedrnikova I, Cos P, Berghe V D, Augustunsa K, Haemersa A, Synthesis and evaluation of caffeic acid amides as antioxidants. Bioorg. Med. Chem. Lett, 2001; 11: 215.
25. Kappus H. Lipid peroxidation- Mechanism and biological relevance. In: O.I. Aruoma, & B. Halliwell (Eds.). Free radicals and Food additives. London: Taylor and Francis. 1991.
26. Jadhav S J, Nimbalkar S S, Kulkarani A D, Madhavi D L. Lipid oxidation in biological and food systems. In: Madhavi D L, Deshpande S S, and Salunke D K., (Eds), Food antioxidants. New York: Marcel dekker.1996.
27. Janero, D., Malondialdehyde and thiobarbituric acid-reactivityas diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Rad. Bio. Med. 1990; 9: 515-540.