

SYNTHESIS AND EVALUATION OF NOVEL *p*-COUMARIC ACID AMIDES AS ANTIOXIDANTS

NAGARAJA NAIK*, H. VIJAY KUMAR, ANITHA

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India.

Email: drnaik_chem@yahoo.co.in

Received: 8 May 2011, Revised and Accepted: 6 July 2011

ABSTRACT

An efficient, two steps synthesis of *p*-coumaric acid amides was described. The key intermediate, 3-(4-hydroxyphenyl) acryloyl chloride (**2**) was obtained by conversion of *p*-coumaric acid (**1**) to its acid chloride in presence of thionyl chloride. Further, coupling of different aminophenols and substituted aminophenols by base condensation reaction affords *p*-coumaric acid amides (**2a-e**). The newly synthesized compounds were characterized by spectral and elemental analysis data and studied for their antioxidant properties using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay and inhibition on human Low-density lipoprotein (LDL) assay. All the compounds exhibited good potent activity dose dependently. Butylated hydroxy anisole (BHA) was used as a reference antioxidant and also the comparative study with newly synthesized compounds was done.

Keywords: *p*-Coumaric acid, 3-(4-Hydroxyphenyl) acryloyl chloride, DPPH, Aminophenols.

INTRODUCTION

Numerous lines of evidence have indicated that free radicals play a critical role in a variety of pathological conditions including the processes of aging, cancer, multiple sclerosis, inflammation, coronary heart and cardiovascular diseases, senile dementia, arthritis and atherosclerosis¹⁻³. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk⁴⁻⁵. Recently, much attention has been focused on dietary natural antioxidants capable of inhibiting reactive oxygen radical mediated oxidative stress, which is involved in several pathological diseases, such as cancer, atherosclerosis, diabetes, inflammation, and aging⁶⁻⁷. Particularly, superoxide and hydroxyl radicals of reactive oxygen species are known to cause severe oxidative damage to susceptible biomolecules, thereby eventually contributing to deleterious biological effects, including carcinogenesis, inflammation, mutagenesis, and cytotoxicity⁸⁻⁹. For these reasons, an extensive search for novel natural antioxidants acting as radical scavenger was undertaken. Naturally occurring hydroxycinnamic acid derivatives, such as *p*-coumaric acid (CA), ferulic acid (FA), and caffeic acids, as anticancer¹⁰, antiinflammation¹⁰, antihepatotoxicity¹¹, antibacterial¹², antimutation¹³, and antioxidation¹⁴⁻¹⁵. Particularly, several hydroxycinnamic acids and their derivatives have been found to possess strong antioxidant activities as radical scavengers against reactive oxygen species¹⁶⁻¹⁹ and to have considerable tyrosinase inhibitory activities²⁰⁻²¹. Oxidation of low density lipoprotein (LDL) has been suggested to play a key role in the development of atherosclerosis and as a consequence an important role in the prevention of LDL oxidation has been attributed to the antioxidants contained within LDL and in plasma²². It has been suggested that oxidative modification of LDL may play a role in the development of atherosclerosis²³. Such modification of LDL can be inhibited by antioxidants²⁴⁻²⁶. *p*-Coumaric acid has antioxidant properties and is believed to reduce the risk of stomach cancer²⁶ by reducing the formation of carcinogenic nitrosamines²⁷ and also the radical scavenging activity of coumaric acid and thioamide was recently reported²⁸. In light of the above observations, it was thought worthwhile to synthesize a system that brings *p*-coumaric acid and aminophenols together to furnish the corresponding amides (**2a-e**) with the hope of developing some promising antioxidant agents. However, their molecular characteristics indicate that these compounds may serve as effective radical scavenging agents. Owing to the widespread applications, synthetic and biological activity evaluation of coumaric acid and their derivatives has been a subject of intense investigations. In

this report, some conjugated amides of *p*-coumaric acid was synthesized and reported to behave as efficient DPPH radical scavenging activity and inhibition on human Low-Density Lipoprotein oxidation.

MATERIALS AND METHODS

DPPH was purchased from Sigma Aldrich, thionyl chloride, triethylamine, ethyl acetate, *n*-hexane, tetrahydrofuran, anhydrous potassium carbonate, methanol, chloroform, anhydrous sodium sulphate and aminophenols like 2-aminophenol, 3-aminophenol, 4-aminophenol, substituted aminophenols like 4-nitro-2-aminophenol and 4-methoxy-2-aminophenol were all of analytical grade and procured from Merck. TLC aluminum sheets -Silica gel 60 F₂₅₄ was also purchased from Merck. All the reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed with aluminium sheets-Silica gel 60 F₂₅₄ purchased from Merck. The compounds were purified by using column chromatography with silica gel (60-120 mesh), using chloroform:methanol, 65:35 as eluent. IR: Nicolet 5700 FT-IR spectrophotometer; ¹H NMR: Bruker 250 MHz spectrometer; mass spectra were obtained by Waters-Q-TOF ultima spectrometer. Micro analytical data were obtained by Elemental-Vario EL-III.

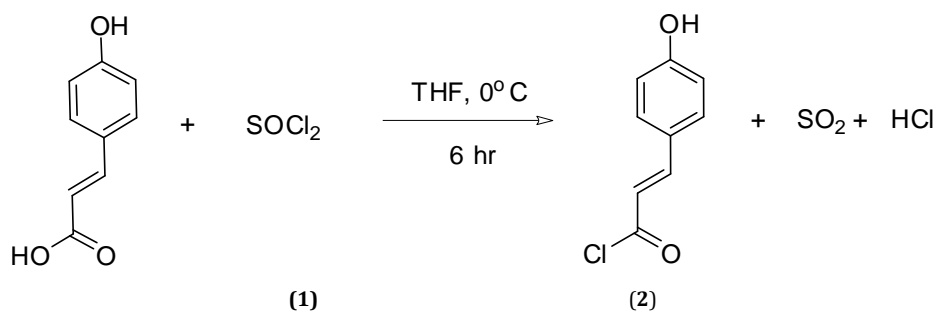
Chemistry

p-coumaric acid was synthesized by applying known method²⁷. The synthesis of *p*-coumaric acid amides obtained by conjugated with aniline and substituted anilines were realized in two steps. First step, the key intermediate 3-(4-hydroxyphenyl) acryloyl chloride (**2**) was prepared in good yield by conversion of *p*-coumaric acid to its acid chloride in the presence of thionyl chloride (Scheme1).

Synthesis of 3-(4-hydroxyphenyl) acryloyl chloride (**2**)

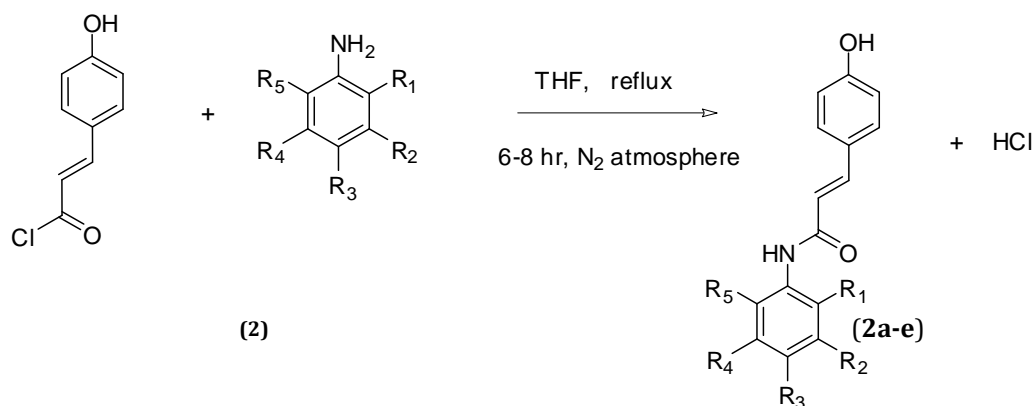
To the well stirred solution of *p*-coumaric acid (2 mM) in 25 ml dry THF, freshly distilled thionyl chloride (2.2 mM) in 10 ml dry THF was added drop by drop for about 15 min. Then the reaction mixture is stirred at 0 °C for about 6 hr. Progress of the reaction is monitored by TLC using 5:5 *n*-hexane: ethyl acetate mixture as mobile phase. Finally the reaction mixture was concentrated in rotary evaporator at 80 °C, a light yellow solid product was obtained upon recrystallization using acetonitrile.

Light yellow solid, yield 82%, m.p 190-192 °C. ¹H NMR (250 MHz) (CDCl₃) δ (ppm): δ 6.2-7.4 (m, Ar-H, 4H), 9.4 (s, Ar-OH, 1H), 8.2 (m, 1H, alkyl CH proton), 6.7 (m, 1H, alkyl proton). IR (KBr) ν_{max} cm⁻¹: 3049-2967 (Ar-H), 3278-3545 (phenolic-OH), 1683 (C=O). M⁺ 182.01. Anal.Calcd for C₉H₇ClO₂: C, 59.20; H, 3.86. Found C, 59.18; H, 3.84.



Scheme 1: Synthetic pathway for the preparation of 3-(4-hydroxyphenyl)acryloyl chloride (2)

In the second step, coupling of respective amino phenols and substituted amino phenols to the key intermediate by based condensation reaction to afforded novel *p*-coumaric acid amides (2a-e) (Scheme2).



Where,

Compound	R ₁	R ₂	R ₃	R ₄	R ₅
2a	H	H	OH	H	H
2b	OH	H	H	H	H
2c	H	OH	H	H	H
2d	H	H	OH	NO ₂	H
2e	H	H	OH	OCH ₃	H

Scheme 2: General reaction protocol for the synthesis of *p*-coumaric acid amides(2a-e)

General procedure for the synthesis of *p*-coumaric acid amides (2a-e)

2-aminophenol (1.2 mM) in dry THF (10 mL) was treated with K₂CO₃ (600mg). Later the solution of 3-(4-hydroxyphenyl) acryloyl chloride (1 mM) in dry THF (10 mL) was added drop by drop for 30 min, under N₂ atmosphere. The reaction mixture was refluxed for 6-8 hr. 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 Na₂SO₄. The brown semisolid product (2a) was obtained by further desolventation in rotary evaporator at 50 °C. Other respective *p*-coumaric acid amides were obtained by conjugated with 3-aminophenol, 4-aminophenol, substituted aminophenols like 4-nitro-2-aminophenol and 4-methoxy-2-aminophenol by following the same procedure. The analogues were separated and purified by column chromatography by using mixture of chloroform / methanol = 85: 15. The products were characterized by IR, ¹H NMR, mass and elemental analysis.

3-(4-hydroxyphenyl)acryloyl chloride (2)

Light yellow solid , yield 82%, m.p 190-192 °C . ¹H NMR (250 MHz) (CDCl₃) δ (ppm): δ 6.6-7.4 (m, Ar-H, 4H), 9.4 (s, Ar-OH, 1H), 7.2 (m, 1H, alkyl CH proton), 6.7 (m, 1H, alkyl proton). IR (KBr) ν_{max} cm⁻¹: 3049-2967 (Ar-H), 3278-3545 (phenolic-OH), 1683(C=O). M⁺ 182.01. Anal.Calcd for C₉H₇ClO₂: C, 59.20; H, 3.86. Found C, 59.18; H, 3.84.

4-(4-hydroxyphenyl)-1-(4-hydroxyphenylamino)but-3-en-2-one (2a)

Brown semi solid, yield 71%, ¹H NMR (250 MHz) (CDCl₃) δ (ppm): δ 6.1- 7.1 (m, Ar-H, 4H), 9.3 (s, Ar-OH, 1H), 8.2 (m, 1H, alkyl CH proton), 6.7 (m, 1H, alkyl proton), 9.0 (s, N-H, 1H), 6.7-7.3 (m, Ar-H of aminophenol, 4H), 9.6 (s, phenolic -OH of aminophenol, 1H). IR (KBr) ν_{max} cm⁻¹: 3066-2898 (Ar-H), 3287- 3554 (phenolic-OH), 3316 (N-H); M⁺ 269.11; Anal.Calcd for C₁₆H₁₅NO₃: C, 71.36; H, 5.61; N, 5.20. Found C, 71.34; H, 5.60; N, 5.22.

4-(4-hydroxyphenyl)-1-(2-hydroxyphenylamino)but-3-en-2-one (2b)

Brown semi solid, yield 73%, ¹H NMR (250 MHz) (CDCl₃) δ (ppm): δ 6.0-7.2 (m, Ar-H, 4H), 9.3 (s, Ar-OH, 1H), 8.2 (m, 1H, alkyl CH proton), 6.7 (m, 1H, alkyl proton), 10.5 (s, N-H, 1H), 6.6-7.3 (m, Ar-H, 4H), 10.1 (s, phenolic -OH of aminophenol, 1H). IR (KBr) ν_{max} cm⁻¹: 3039-2877 (Ar-H), 3283-3544 (phenolic-OH), 3341 (N-H). M⁺ 269.11. Anal.Calcd for C₁₆H₁₅NO₃: C, 71.36; H, 5.61; N, 5.20. Found C, 71.34; H, 5.59; N, 5.18.

4-(4-hydroxyphenyl)-1-(3-hydroxyphenylamino)but-3-en-2-one (2c)

Brown semi solid, yield 70%, ¹H NMR (250 MHz) (CDCl₃) δ (ppm): δ 6.4-7.6 (m, Ar-H, 4H), 9.2 (s, Ar-OH,1H), 8.2 (m, 1H, alkyl CH proton), 6.6-7.3 (m, Ar-H, 4H),6.7 (m, 1H, alkyl proton), 9.2 (s, N-H,1H), 9.6 (s,

phenolic-OH of aminophenol, 1H). IR (KBr) ν_{\max} cm^{-1} : 3086-2857 (Ar-H), 3213-3505 (phenolic-OH), 3331 (N-H). M^+ 269.11. Anal.Calcd for $\text{C}_{16}\text{H}_{15}\text{NO}_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.34; H, 5.62; N, 5.22.

1-(4-hydroxy-3-nitrophenylamino)-4-(4-hydroxyphenyl)but-3-en-2-one (2d)

Brown semi solid, yield 68%, ^1H NMR (250 MHz) (CDCl_3) δ (ppm): δ 6.5-7.3 (m, Ar-H, 4H), 9.4 (s, Ar-OH, 1H), 8.2 (m, 1H, alkyl CH proton), 6.7 (m, 1H, alkyl proton), 9.2 (s, N-H, 1H), 6.8-7.1 (m, Ar-H of aminophenol, 3H), 9.4 (s, phenolic -OH of aminophenol, 1H). IR (KBr) ν_{\max} cm^{-1} : 3049-2827 (Ar-H), 3213-3504 (phenolic-OH), 3334 (N-H). M^+ 314.09. Anal.Calcd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_5$: C, 61.14; H, 4.49; N, 8.91. Found C, 61.16; H, 4.47; N, 8.89.

1-(4-hydroxy-3-methoxyphenylamino)-4-(4-hydroxyphenyl)but-3-en-2-one (2e)

Brown semi solid, yield 76%, ^1H NMR (250 MHz) (CDCl_3) δ (ppm): δ 6.3-7.5 (m, Ar-H, 4H), 9.4 (s, Ar-OH, 1H), 8.3 (m, 1H, alkyl CH proton), 6.8 (m, 1H, alkyl proton), 9.4 (s, N-H, 1H), 7.1-7.8 (m, Ar-H of aminophenol, 3H), 3.8 (s, $-\text{OCH}_3$, 3H), 9.4 (s, phenolic -OH of aminophenol, 1H). IR (KBr) ν_{\max} cm^{-1} : 3049-2827 (Ar-H), 3214-3544 (phenolic-OH), 3371 (N-H). M^+ 299.12. Anal.Calcd for $\text{C}_{17}\text{H}_{17}\text{NO}_4$: C, 68.21; H, 5.72; N, 4.68. Found C, 68.19; H, 5.70; N, 4.70.

Antioxidant activity

The newly synthesized *p*-coumaric acid amides were screened for their potential over antioxidant activity by using two well established invitro methods like DPPH radical scavenging activity and inhibition of LDL oxidation activity. The compounds under studies were dissolved in distilled ethanol (50 mL) to prepare 1000 μM solution. Solutions of different concentrations (10 μM , 50 μM , 100 μM , 200 μM and 500 μM) were prepared by serial dilution and the free radical scavenging 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 Blois³⁰. Compounds at different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 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 decrease in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1) / A_0 \times 100]$$

Where A_0 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.

Human LDL oxidation assay

Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4 °C. LDL (0.1 mg LDL protein/mL) was isolated from freshly separated plasma by preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge. The LDL was prepared from the plasma according to the method of ³¹. The isolated LDL was extensively dialyzed against phosphate buffered saline (PBS) pH 7.4 and sterilized by filtration (0.2 μm Millipore membrane system, USA) and stored at 4 °C under nitrogen. 1 mL of various concentrations (10, and 25 μM) of compounds were taken in test tubes, 40 μL of copper sulphate (2 mM) was added and the volume

was made up to 1.5 mL with phosphate buffer (50 mM, pH 7.4). A tube without compound and with copper sulphate served as a negative control, and another tube without copper sulphate with compound served as a positive control. All of the tubes were incubated at 37 °C for 45 min. To the aliquots of 0.5 mL drawn at 2, 4 and 6 hr intervals from each tube, 0.25 mL of thiobarbituric acid (TBA, 1% in 50 mM NaOH) and 0.25 mL of trichloro acetic acid (TCA, 2.8%) were added. The tubes were incubated again at 95 °C for 45 min and cooled to room temperature and centrifuged at 2500 rpm for 15 min. A pink chromogen was extracted after the mixture was cooled to room temperature by further centrifugation at 2000 rpm for 10 min. Thiobarbituric acid reactive species in the pink chromogen were detected at 532 nm by a spectrophotometer against an appropriate blank. Data were expressed in terms of malondialdehyde (MDA) equivalent, estimated by comparison with standard graph drawn for 1,1,3,3-tetramethoxy-propane (Which was used as standard) which give the amount of oxidation and the results were expressed as protection per unit of protein concentration (0.1 mg LDL protein/mL). Using the amount of MDA, the percentage protection was calculated using the formula:

$$\% \text{ inhibition of LDL oxidation} = (\text{Oxidation in control} - \text{Oxidation in experimental}) / \text{Oxidation in control} \times 100$$

RESULTS AND DISCUSSION

p-coumaric acid was synthesized by applying known method²⁹ by converting coumarin to *p*-hydroxy-trans-cinnamic acid (*p*-coumaric acid) by shaking with dilute alkali (NaOH) and freshly precipitated mercuric oxide. *p*-coumaric acid amides was realized in two steps. First step, the key intermediate 3-(4-hydroxyphenyl)acryloyl chloride (2) was prepared by reaction of *p*-coumaric acid with thionyl chloride, in good yield (Scheme 1). In the second step, further coupling of respective aminophenols and substituted aminophenols to the intermediate afforded novel *p*-coumaric acid amides (2a-e). (Scheme 2). Structural conformation was done using IR, ^1H NMR, mass spectra and elemental analysis. The IR spectrum of compound (2) showed characteristic absorption at 1683 cm^{-1} for carbonyl group. Aromatic stretching was observed at 3049-2967 cm^{-1} . From the ^1H NMR spectra, the absent of carboxylic -OH at 12 ppm conformed the conversion of *p*-coumaric acid to its amides. The IR spectrum of all *p*-coumaric acid amides (2a-e) reveals the presence of N-H stretching band at 3213-3354 cm^{-1} in all the analogues and showed broad phenolic stretching at 3210-3561 cm^{-1} . All the aromatic (Ar-H) absorption band was appeared at the expected regions. ^1H NMR spectra of all *p*-coumaric acid amides showed N-H proton as singlet at 9.8-10.5 ppm. The signal due to phenolic -OH in all the amides appeared as singlet at about 9.4-10.1 ppm. In addition to phenolic -OH, $-\text{OCH}_3$ protons present in the compounds 2e resonated as a singlet at 3.8 ppm. Other aromatic protons were observed at expected regions. Mass spectra of all newly synthesized compounds showed M^+ peak, in agreement with their molecular formula. The newly synthesized *p*-coumaric acid amides were evaluated for their potential over antioxidant activity by using two well documented assays namely DPPH radical scavenging assay and inhibition of LDL oxidation assay. The evaluation study was carried out at various concentrations and also comparative studies was done with the standard antioxidant (BHA). 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 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 atoms or by electron donating, conceivable) and convert them to a colorless/bleached product (i.e., 2,2-diphenyl-1-picrylhydrazine, or a substituted analogues hydrazine), resulting in a decrease in absorbance. Hence, more rapidly the absorbance decrease, the more potent the antioxidant activity of the compound.

Percentage activity (%) of ethanolic solutions of 3-(4-hydroxyphenyl)acryloyl chloride (2) and *p*-coumaric acid amides (2a-e) were examined and compared with the standard (Figure 1).

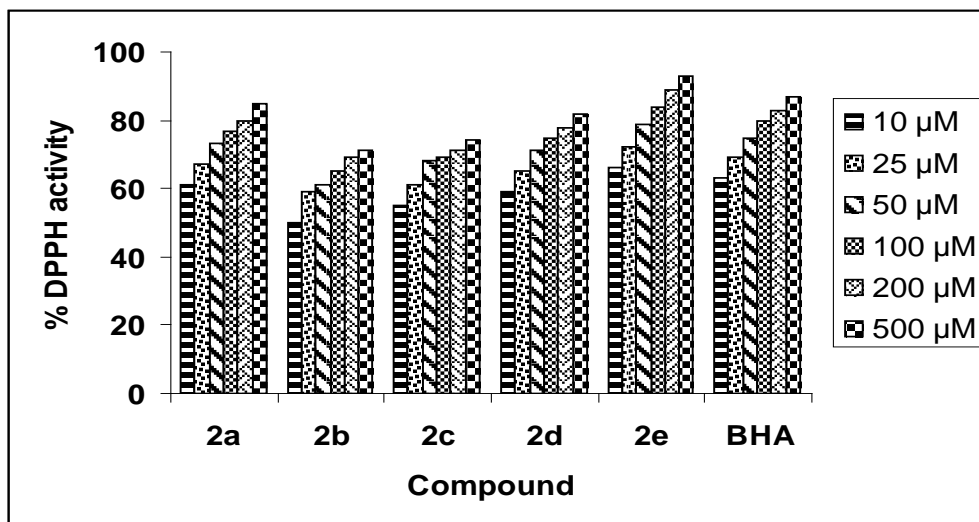


Fig. 1: % DPPH activity of *p*-coumaric acid amides. Each values represents mean± SD (n=3)

Initially, compound (2) showed considerable DPPH activity, Whereas coupling of amino phenols and substituted amino phenols to key intermediate (2) exhibits increase in the antioxidant activity (Fig.1).Among the analogues compound 2e bearing methoxy group

(electron donating group) addition to the phenolic moiety showed dominant DPPH activity, where as compound 2d having nitro group (electron withdrawing group) exhibit slightly less activity to that of compound 2e. IC₅₀ for all the compounds were calculated (Table.1)

Table 1: 50% Inhibition of DPPH radical and human LDL oxidation by *p*-coumaric amides

Compound	DPPH activity IC ₅₀ (µM/ml)	LDL oxidation inhibition IC ₅₀ (µM/ml)
2a	14±0.90	10±0.33
2b	17±1.45	15±0.23
2c	21±1.43	19±0.21
2d	15±0.67	12±0.39
2e	9±0.76	6±0.21
BHA	12±0.55	8±0.76

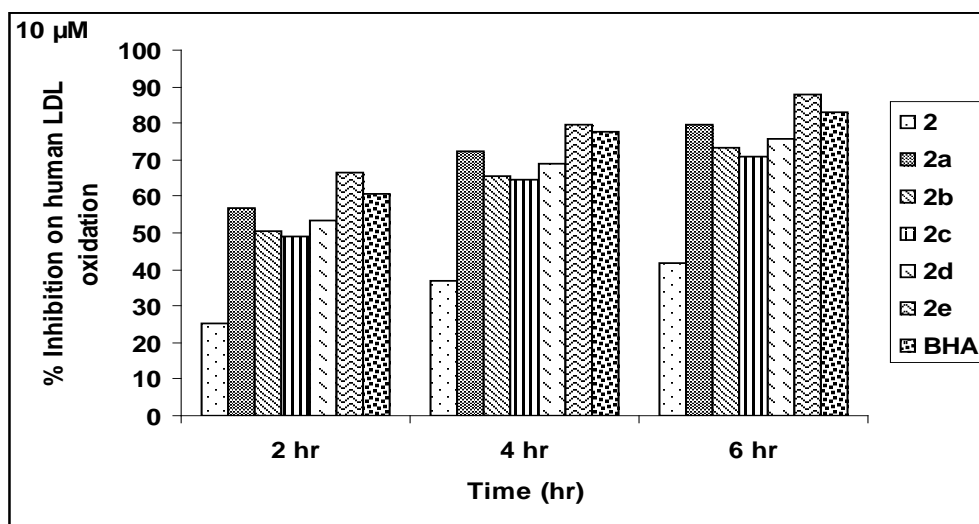
Each value represents means ± SD (n=3)

Table 1 reveals the 50% inhibitory concentration towards DPPH activity of newly synthesized compounds. The increasing order of DPPH activity of newly synthesized analogues is as follows

2e> BHA> 2a> 2d> 2b> 2c> 2

Oxidation modification is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases³² and the dietary antioxidants that protect LDL from oxidation

may therefore reduce atherogenesis and coronary heart diseases³³. In general, oxidation of LDL follows a radical chain reaction that generates conjugated diene hydroperoxides as its initial products. It has been reported that inhibition of human LDL oxidation may arise due to free-radical scavenging³⁴. The antioxidant activity of *p*-coumaric acid amides (2a-e) against human LDL oxidation with different concentrations (10 µM and 25 µM) is shown in the Figure 2.



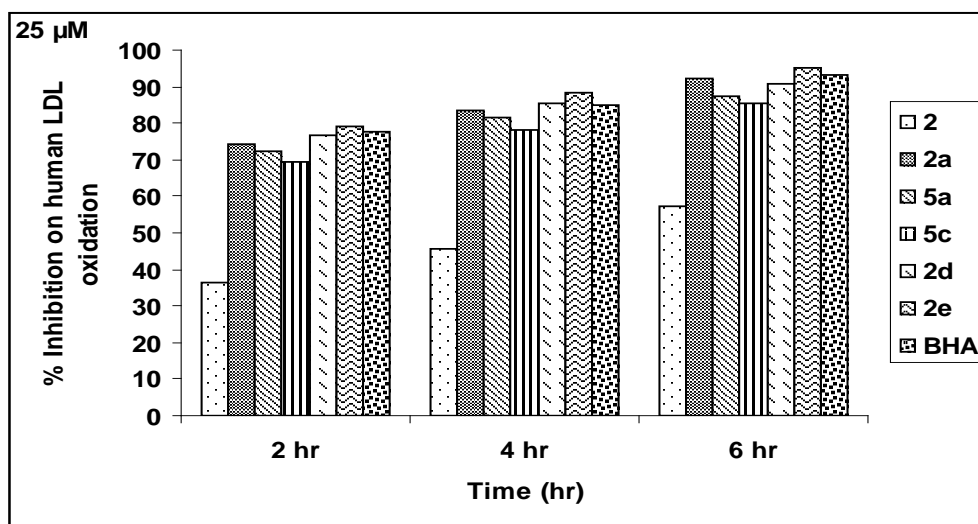


Fig. 2: Percentage of inhibition of human LDL oxidation by *p*-coumaric acid amides and standards at different concentration at different time interval. Each value represents means \pm SD (n=3)

The poly unsaturated fatty acid (PUFA) of human LDL were oxidized, and the malonaldehyde (MDA) formed have been estimated by using thiobarbituric acid (TBA) method. Initially the formation of conjugated dienes due to copper-induced LDL oxidation was unaffected by compound (2) showing less activity but coupling of aminophenols and substituted aminophenols i.e., compound (2a-e) effectively inhibited LDL oxidation showed good activity. The average induction time for copper mediated LDL oxidation was around 4 min without the addition of compounds. The compounds protected LDL from oxidation as measured by the prolongation of the induction time of the formation of conjugated dienes. Among the synthesized compounds, 2e exhibited 66.74 and 76.25% protection at the 10 and 25 μ M levels at the end of 2 hr after the induction of oxidation. Whereas, it was 87.65 and 95.25% protection at the end of 6 hr showing dominant inhibition over LDL oxidation and also comparable or slightly less protection with respect to the standards. Compound (2) exhibited 5.34 and 7.43% protection at the 10 and 25 μ M levels at the end of 2 hr after the induction of oxidation. Whereas, it showed 8.87 and 12.43% protection at the end of 6 hr showing less activity. IC_{50} values are calculated and depicted in the table 1. The results indicate a dose-dependent inhibition effect of compounds against LDL oxidation.

CONCLUSION

In conclusion, a ray of *p*-coumaric acid amides were synthesized in two steps in moderate to high yield. The antioxidant properties of the new analogues were evaluated by DPPH free radical scavenging activity and inhibition on human LDL oxidation. In both the assays, initially 3-(4-hydroxyphenyl) acryloyl chloride (2) showed considerable activity. Coupling of aminophenol and substituted aminophenols to key intermediate enhance the antioxidant activities. In contrast coupling of substituted aminophenols containing methoxy group i.e., compound 2e revealed high antioxidant activity and even more than the standard. The coupling of different aminophenol and substituted aminophenols is the most important feature for the significant antioxidant activity of the *p*-coumaric acid amides studied. These effects may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role.

Abbreviations

$^{\circ}$ C = centigrade
min = minute
hr = hour
mL = milli Liter

μ m = micro molar

% = percentage

IC_{50} = 50 percent Inhibition concentration

nm = nano meter

mM = milli molar

M= molar

RT = room temperature

BHA = Butyrate hydroxyl anisole

DPPH = 2, 2-diphenyl-1-picrylhydrazyl

TLC = Thin layer chromatography

IR= Infrared

1 H NMR= proton nuclear magnetic resonance

MP= melting point

KBr= potassium bromide

RSA= radical scavenging activity

LDL = Low density lipoprotein

TBA= Thiobarbituric acid

REFERENCE

- Blake D, Winyard PG. Immunopharmacology of Free Radical Species. Academic Press: San Diego, CA; 1995.
- Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol; 1990; 186B; 1-85.
- Halliwell B, Gutteridge J MC. Free Radicals in Biology and Medicine. 3rd ed. Oxford University Press: Oxford, UK; 1999.
- Rumit S, Heena K, Rajal S, Naveen S. In vitro antioxidant activity of roots of *tephrosia purpurea linn*. Int. J. Pharmacy. Pharm. Sci; 2010;30-33
- Arief N, Arief RH, Supardjan AM, Sardjiman, Nunung Y. Evaluation of hepatoprotective and antioxidant activity of hexagamavunon-1 against carbon tetrachloride-induced hepatic injury in rats. Int. J. Pharmacy. Pharm. Sci; 2010; 45-48.
- Free B. Nonenzymatic antioxidant defense systems. In Natural Antioxidants in Human Health and Disease; Briviba, K., Sies, H., Eds.; Academic Press: London, 1994; 107-116.

7. Rice-Evans C. The role of antioxidants in biological systems and human studies of disease prevention. In *Advances in Applied Lipid Research*; Padley, F. B., Ed.; JAI Press Inc.: London. 1996; 2, 183-211.
8. Halliwell B, Gutteridge JMC. Free radicals, ageing, and disease. *Free Radicals in Biology and Medicine*; Clarendon Press: London, 1996; 416-427.
9. Cross EE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord, JM. Oxygen radicals and human disease (clinical conference). *Ann. Intern. Med*; 1987; 107: 526-545.
10. Zhang LP and Ji ZZ. Synthesis, anti inflammatory and anticancer activity of cinnamic acids, their derivatives and analogues. *Yao Xue Xue Bao*; 1992; 27: 817-823.
11. Perez-Alvarez V, Bobadilla RA, Muriel P. Structure hepato-protective activity relationship of 3,4-dihydroxycinnamic acid (caffeic acid) derivatives. *J. Appl. Toxicol*; 2001; 21: 527-531.
12. Ramos-Nino ME, Clifford MN, Adams MR. Quantitative structure activity relationships for the effects of benzoic acids, cinnamic acids and benzaldehydes on *Listeria monocytogenes*. *J. Appl. Bact*; 1996; 8: 303-310.
13. Yamada J, Tomita Y. Antimutagenic activity of caffeic acid and related compounds. *Biosci., Biotechnol., Biochem*; 1996; 60: 328-329.
14. Graf E. Antioxidant potential of ferulic acid. *Free Rad. Biol. Med*; 1992; 13: 435-448.
15. Natella F, Nardini M, Di Felice M, Scaccini C. Benzoic and cinnamic acid derivatives as antioxidants: Structure-activity relation. *J. Agric. Food Chem*; 1999; 47: 1453-1459.
16. Toda S, Kumura M, Ohnishi M. Effect of phenolcarboxylic acids on superoxide anion and lipid peroxidation induced by superoxide anion. *Planta Med*; 1991; 57: 8-10.
17. Chan WS, Wen PC, Chiang HC. Structure-activity relationship of caffeic acid analogues on xanthine oxidase inhibition. *Anticancer Res*; 1995; 15: 703-707.
18. Kanski J, Aksenova M, Stoyanova A, Butterfield A. Ferulic acid antioxidant protection against hydroxyl and peroxy radical oxidation in synaptosomal and neuronal cell culture systems in vitro: Structure-activity studies. *J. Nutr. Biochem*; 2002; 13: 273-281.
19. Mansouri A, Makris DP, Kefalas P. Determination of hydrogen peroxide scavenging activity of cinnamic and benzoic acids employing a highly sensitive peroxyoxalate chemiluminescence-based assay: Structure-activity relationship. *J. Pharm. Biomed. Anal*; 2005; 39: 22-26.
20. Billaud C, Lecornu D, Nicolas J. Substrates and carboxylic acid inhibitors of a partially purified polyphenol oxidase from gum Arabic. *J. Agric. Food Chem*; 1996; 44: 1668-1675.
21. Lee HS. Tyrosinase inhibitors of *Pulsatilla cernua* root-derived materials. *J. Agric. Food Chem*; 2002; 50: 1400-1403.
22. Esterbauer H, Schmidt R, Hayn M. *Adv. Pharmacol*; 1997; 38: P-425-456.
23. Jialal I, Devaraj S. *Clin. Chem*; 1996; 42: 498.
24. Frankel EN, Kanner JL. *Cancer*; 1993; 341: 454.
25. Mangiapane H, Thomson J, Salter A, Brown S, Bell GD, White DA. *Biochem. Pharmacol*; 1992; 43: 445.
26. Ferguson LR, Shuo-tun Z, Harris P. *J. Mol. Nut & Food. Res*; 2005; 49: 585-693.
27. Kikugawa K, Hakamada T, Hasunuma M, Kurechi T. *J. Agric. Food Chem*; 1983; 1: 780-785.
28. Zhivko Velkov, Elena Balabanova, Alia Tadjer. *J. Mol. Structure: TheoChem*; 2007; 821: 133-138.
29. McIntyre BG, Martínez Bermejo F, Srivastava SK, Husbands SM, Lewis JW, Crosby J, Simpson TJ. Solid phase synthesis of 4-hydroxycinnamic acid and its derivatives for potential. *Comb.Chem*; 2001; 4: 111-114.
30. Blois MS. *Nature*; 1958; 1199.
31. Princen HM, Poppel GV, Vogelesang C, Buytenhek R, Kok FJ. Supplementation with vitamin E but not beta-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. Effect of cigarette smoking. *Arter. Thromb. Vasc. Biol*; 1992; 12: 554-562.
32. Teinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *The New Eng. J. Med*; 1989; 320: 915-924.
33. Kinsella JE, Frankel E, German B, Kanner J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol*; 1993; 47: 85-89.
34. Decker EA, Ivanov V, Zhu BZ, Frei B. Inhibition of Low-Density Lipoprotein Oxidation by Carnosine, Histidine. *J. Agric. Food chem*; 2001; 49: 511-516.