Int. J. Chem. Sci.: 13(2), 2015, 837-848
ISSN 0972-768X
www.sadgurupublications.com

SYNTHESIS, CHARACTERIZATION AND BIOCHEMICAL EVALUATION OF N-[3-(SUBSTITUTED PHENYL)-1-OXO-2-PROPHENYL] ALANINE

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ABSTRACT

Cinnamic acids **1** was prepared by the condensation of various substituted aldehydes with malonic acid in pyridine and piperidine. Condensation of **1** with alanine in, CH_2Cl_2, PPAA and triethylamine results in the formation of cinnamoyl alanine **2a-h**. The synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral data. The newly synthesized compounds were screened for their *in vitro* antioxidant activity against nitric oxide (NO) scavenging, DPPH radical and lipid peroxidation inhibition effect.

Key words: Cinnamic acids, Alanine, Amino acids, Antioxidant activity.

INTRODUCTION

Cinnamic acid, a substance that was first reported in the 1800s is a naturally occurring aromatic fatty acid of low toxicity has a long history of human exposure. Cinnamic acid (3-phenyl-2-propenoic acid) has been known over many years, having been isolated in 1872 by Beilstein and Kahlbery[1]. Esters of cinnamic acid are used as a fixing agent in perfumes[2]. Various derivatives of substituted cinnamic acid possess a wide range of activities such as anti-allergic agents[3], anti-inflammatory[4], Food preservative[5], antibacterial and antifungal[6] activities, veterinary preparations[7], topical formulations[8], and in the synthesis of substituted styrenes[9]. Based on these findings and in continuation of our work on the synthesis of biologically active compounds, in the present communication, we report the synthesis and antioxidant activity of title compounds.

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EXPERIMENTAL

Materials and methods

Melting points of all synthesized compounds were determined in open capillary tube and are uncorrected. The purity of compounds were checked by TLC using glass plates coated with silica gel G. UV spectra were recorded on systronic UV VIS spectrophotometer-117, FTIR were recorded using thermo nicolet nexus 670 spectrometer (cm^{-1}) in KBr disc, ^1H NMR (Avance-300 MHz) using DMSO as solvent and Mass spectra from IICT, Hyderabad.

Synthesis of 3-phenyl-2-propenoic acids (1a-h)

A mixture of substituted aldehydes (5 g, 0.33 mol) malonic acid (7.5 g, 0.72 mol) dissolved in pyridine (15 mL) and piperidine (0.25 mL) was heated under reflex for 2 hrs on water bath. The reaction mixture was cool and poured into excess of water containing enough HCl. The solid mass which separated out was filtered and washed with little water, dried and recrystallized from glacial acetic acid.

Similarly, all other cinnamic acids (**1a-h**) were synthesized. The physical data of compounds (**1a-h**) are given in Table 1.

Table 1: Physical data and yields of cinnamic acids (1a-1h)

Compound	R	M.P. (^oC)	Yield (%)	Molecular formula
1a	H	134-138	63	$C_9H_8O_2$
1b	4-Cl	240-245	73	$C_9H_7O_2Cl$
1c	4-OCH_3	175-177	77	$C_{10}H_{10}O_3$
1d	4-OH, 4-OCH_3	162-164	49	$C_{10}H_{10}O_4$
1e	4-$CH(CH_3)_2$	125-126	55	$C_{12}H_{14}O_2$
1f	3,4-di OCH_3	128-130	85	$C_{11}H_{13}O_4$
1g	4-$NHCOCH_3$	202-205	38	$C_{11}H_{11}NO_3$
1h	4-CH_3	196	71	$C_{10}H_{10}O_2$

Synthesis of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine (2a-2h)

To a cooled 0ºC solution of cinnamic acid (250 mg, 1.67 mol) in CH_2Cl_2 (5 mL) were added alanine (200 mg, 1.67 mmol, 1 molar equiv), triethylamine (950 µM, 690 mg,

3.3 mmol, 2 molar equiv), and propane phosphoric acid anhydride (PPAA) (50% ethyl acetate solution, 1 mL, 3.3 mol, 2 molar equiv). After the mixture was stirred for 15 min at 0ºC, the ice bath was removed, and the reaction mixture was allowed to warm to room temp in Ca for 1 hr, concentrated under reduced pressure. The residue was treated with cold water to afford a solid, which was filtered and recrystallized from petroleum ether or ethyl acetate.

Similarly, all other compounds **(2a-h)** were synthesized. The physical data of compounds are given in Table 2.

Table 2: Physical data and yields of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine (2a-2h)

Compound	R	M.P. (^oC)	Yield (%)	Molecular formula
2a	H	185-188	25	$C_{12}H_{13}NO_3$
2b	4-Cl	190-193	46	$C_{12}H_{12}NO_3Cl$
2c	4-OCH_3	142-145	54	$C_{13}H_{15}NO_4$
2d	4-OH, 4-OCH_3	200-203	20	$C_{13}H_{15}NO_5$
2e	4-$CH(CH_3)_2$	128	38	$C_{15}H_{19}NO_3$
2f	3,4-di OCH_3	142-146	42	$C_{14}H_{17}NO_5$
2g	4-$NHCOCH_3$	220-224	48	$C_{14}H_{16}N_2O_4$
2h	4-CH_3	154-157	51	$C_{13}H_{15}NO_3$

Spectral data of compounds

(2a). N-[3-(phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 302 nm; **IR** (KBr) cm^{-1}: 3341 (N-H), 2931 (Ar C-H str), 1694 and 1631 (C=O), 1585 (C=C); **^{1}H NMR** (300 MHz, DMSO-d6): δ 1.3 (d, 3H,-CH_3), 4.25-4.35 (m, 1H, -CH), 6.6 (d, 2H, CH=CH), 7.3-7.8 (m, 5H, Ar-H, -NH), 12.2 (s, br, 1H, COOH); **Mass** (m/z): 218 (M-1) peak.

(2b). N-[3-(4-chloro phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 272 nm; **IR** (KBr) cm^{-1}: 3339 (N-H), 2925 (Ar C-H str), 1696 & 1626 (C=O), 1593 (C=C); **^{1}H NMR** (300 MHz, DMSO-d6): δ 1.2 (d, 3H,-CH_3), 4.25-4.35 (m, 1H, -CH), 6.5-6.6 (d, 2H, CH=CH), 7.4-7.8 (m, 5H, Ar-H, -NH), 12.1 (s, br, 1H, COOH); **Mass** (m/z): 256 (M+3) peak.

(2c). N-[3-(4-methoxy phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 314 nm; **IR** (KBr) cm^{-1}: 3345 (N-H), 2940 (Ar C-H str), 1656 (C=O), 1572 (C=C); **^{1}H NMR** (300 MHz, DMSO-d6): δ 1.4 (d, 3H,-CH_3), 4.3-4.4 (m, 1H, -CH), 6.5-6.6 (d, 2H, CH=CH), 6.9-7.6 (m, 5H, Ar-H, -NH), 3.8 (s, 3H, OCH_3), 11.6 (s, br, 1H, COOH); **Mass** (m/z): 250 (M+1) peak.

(2d). N-[3-(4-hydroxy, 3-methoxy phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 234 nm; **IR** (KBr) cm^{-1}: 3321 (N-H), 2952 (Ar C-H str), 1671 (C=O), 1569 (C=C); **^{1}H NMR** (300 MHz, DMSO-d6): δ 1.32 (d, 3H,-CH_3), 4.2-4.4 (m, 1H, -CH), 6.4-6.6 (d, 2H, CH=CH), 7.2-7.9 (m, 4H, Ar-H, -NH), 4.9 (s, 1H, OH), 3.2 (s, 3H, OCH_3), 12.1 (s, br, 1H, COOH).

(2e). N-[3-(4-isopropyl phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 253 nm; **IR** (KBr) cm^{-1}: 3342 (N-H), 2935 (Ar C-H str), 1642 (C=O), 1587 (C=C); **^{1}H NMR** (300 MHz, DMSO-d6): δ 1.42 (d, 3H,-CH_3), 4.36-4.39 (m, 1H, -CH), 6.46-6.51 (d, 2H, CH=CH), 7.25-7.81 (m, 5H, Ar-H, -NH), 1.24-1.26 (d, 6H, $(CH_3)_2$), 2.67 (m, 1H, CH of isopropyl),11.9 (s, br, 1H, COOH).

(2f). N-[3-(3, 4-di methoxy phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 231 nm; **IR** (KBr) cm^{-1}: 3329 (N-H), 2921 (Ar C-H str), 1637 (C=O), 1594 (C=C).

(2g). N-[3-(4-acetamido phenyl)-1-oxo-2-propenyl] alanine

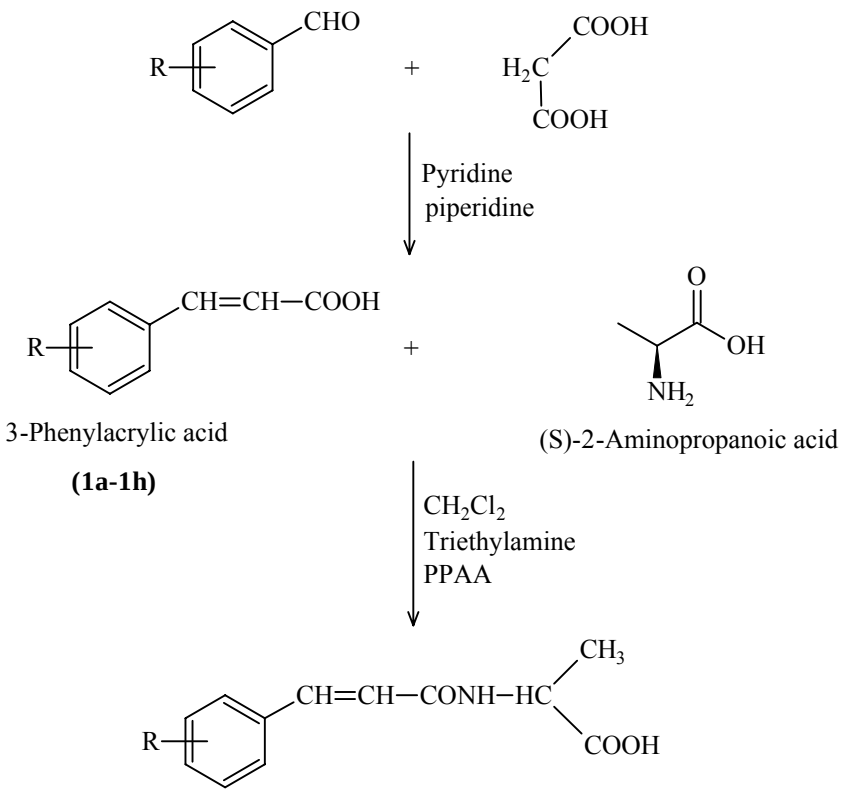
UV (Methanol): λ_{max} 287 nm; **IR** (KBr) cm^{-1}: 3346 (N-H), 2920 (Ar C-H str), 1686 (C=O), 1598 (C=C).

(2h). N-[3-(4-methyl phenyl)-1-oxo-2-propenyl] alanine

UV (Methanol): λ_{max} 314 nm; **IR** (KBr) cm^{-1}: 3320 (N-H), 2928 (Ar C-H str), 1643 (C=O), 1584 (C=C); **^{1}H NMR** (300 MHz, DMSO-d6): δ 1.36 (d, 3H,-CH_3), 4.39-4.4 (m, 1H, -CH), 6.4-6.6 (d, 2H, CH=CH), 7.1-8.2 (m, 5H, Ar-H, -NH), 2.5 (s, 3H, CH_3), 11.9 (s, br, 1H, COOH).

Antioxidant activity

Free radical scavenging activity of the test compounds (**2a-2h**) were performed by nitric oxide (NO) scavenging, DPPH radical and lipid peroxidation inhibition methods.



N-[3-(Substituted phenyl)-1-oxo-2-propenyl] alanine

(2a-2h)

R = 2a: H, 2b: 4-Cl, 2c: 4-OCH_3, 2d: 4-OH, 3-OCH_3, 2e: 4-$CH(CH_3)_2$, 2f: 3,4-diOCH_3, 2g: 4-$NHCOCH_3$, 2h: 4-CH_3

Scheme

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution of physiological pH spontaneously generates nitric oxide[10]. This nitric oxide reacts with oxygen to produce nitrite ions, which can be estimated using griess reagent[11]. Scavenger of nitric oxide (NO), complete with oxygen leading to a reduced production of nitric oxide[10].

Sodium nitroprusside in phosphate buffer was incubated with 100 μM of drug dissolved in methanol and tubes were incubated at 25°C for 30 mins. Control experiment was kept with test compound but equal amount of solvent was added in identical manner. At intervals 0.5 mL incubation solution was removed and diluted with 0.5 mL of Griess reagent.

The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with napthyl ethylene diamine was read at 546 nm and converted into percentage radical scavenging activity as follows. Methanol was used as a solvent for above studies and α-tocopherol was used as a standard. The trials were done in triplicate.

The data on the reactivities of compounds (**2a-2h**) at 100 µM concentration is given in Table 3 and their % of inhibition graph is shown in the Fig. 1.

% Scavenging activity was calculated using the formula

% Scavenging activity = [Absorbance of control-Absorbance of test sample/ Absorbance of control] x 100

Table 3: Nitric oxide scavenging of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine (2a-2h) at 100 µM concentration

Compound	R	% Inhibition (100 µM)
2a	H	37.7
2b	4-Cl	30
2c	4-OCH_3	36.2
2d	4-OH, 3-OCH_3	40
2e	4-$CH(CH_3)_2$	30.5
2f	3,4-di OCH_3	30.1
2g	4-$NHCOCH_3$	29.4
2h	4-CH_3	26.8
α-Tocopherol		50

DPPH free radical scavenging activity

The nitrogen centered stable free radical 1,1-di phenyl-2-picryl hydrazyl (DPPH) has been used to characterize phenolic antioxidants[12]. Reduction of DPPH free radical by test compounds **2a-2h** at 100 µM was estimated in alcoholic solution and the absorbance was measured at 517 nm. Methanol was used as a solvent for above studies and α-tocopherol was used as a standard. The trials were done in triplicate.

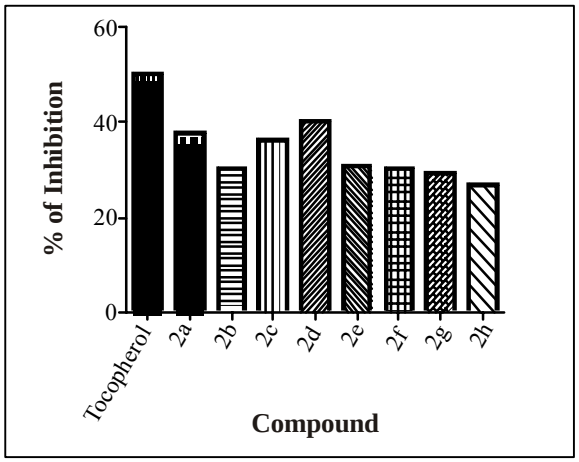


Fig. 1: % Inhibition of compounds 2a-2h in nitric oxide method

The data on the reactivities of compounds at 100 µM with DPPH at 100 µM concentration is given in Table 4 and their % of inhibition graph is shown in the Fig. 2.

% Scavenging activity was calculated using the formula

% Scavenging activity = [Absorbance of control - Absorbance of test sample/ Absorbance of control] x 100

Table 4: Reduction by DPPH stable free radical of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine (2a-2h) at 100 µM concentration

Compound	R	% Inhibition (100 µM)
2a	H	46.8
2b	4-Cl	47.3
2c	4-OCH_3	49
2d	4-OH, 3-OCH_3	43.7
2e	4-$CH(CH_3)_2$	50.7
2f	3,4-di OCH_3	46.6
2g	4-$NHCOCH_3$	45.9
2h	4-CH_3	47.8
α-Tocopherol		52

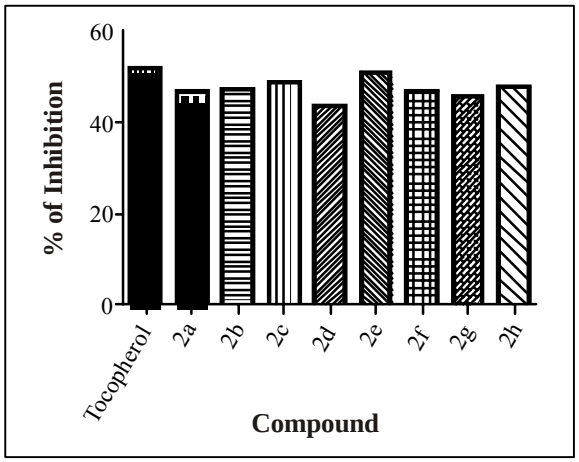


Fig. 2: % Inhibition of compounds 2a-2h in DPPH method

Ferrous (Fe^{2+}) induced lipid peroxidation in rat brain homogenate

Lipid peroxidation is an important pathophysiological event in illness and drug toxicities. Compounds that inhibit lipid peroxidation by interfering with the chain reaction of peroxidation and by scavenging reactive free radical mediated tissue damage could be of great therapeutic importance.

Ferrous (Fe^{2+}) stimulates lipid peroxidation through various mechanisms[13], as the generation of hydroxyl radical, decomposition of lipid peroxides[14], or forming perferryl or ferryl species.

Lipid peroxidation was initiated by adding ferrous chloride (100 μM) to a mixture containing rat brain homogenate (10%) in a total volume of 2 mL. The reaction was stopped after 20 min by adding ice cold TCA-TBA-BHT-HCl reagent (2 mL). Following heating in boiling water bath for 30 min, samples were cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm. Ethanol was used as a solvent for above studies and α-tocopherol was used as a standard. The trials were done in triplicate. The inhibition of lipid peroxidation in percent (%) was calculated according to the following equation:

$$\% \text{ Scavenged} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{sample} is the absorbance of the sample solution and A_{blank} is the absorbance of the blank without sample.

where A_{sample} is the absorbance of the sample solution and A_{blank} is the absorbance of the blank without sample.

The lipid peroxidation in the ferrous induced model in rat brain homogenate was tested by test compound **(2a-2h)** at 100 μM concentration is given Table 5 and their % of inhibition graph is shown in the Fig. 3.

Table 5: Effect of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine (2a-2h) at 100 μM concentration and ferric induced lipid peroxidation in rat brain homogenate

Compound	R	% Inhibition (100 μM)
2a	H	46
2b	4-Cl	47.5
2c	4-OCH_3	43
2d	4-OH, 3-OCH_3	50.1
2e	4-$CH(CH_3)_2$	47.1
2f	3,4-di OCH_3	39.9
2g	4-$NHCOCH_3$	38
2h	4-CH_3	48
α-Tocopherol		62

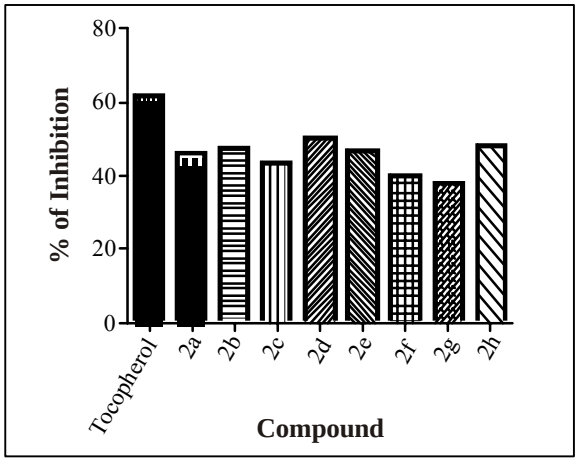


Fig. 3: % Inhibition of compounds 2a-2h in liquid peroxidation method

RESULTS AND DISCUSSION

The reaction of various substituted cinnamic acids **(1a-1h)** with CH_2Cl_2, triethyl amine and PPAA gave the target compounds N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine **(2a-2h)**.

In present study, the intermediate substituted cinnamic acids **(1a-1h)** formed from the reaction of substituted aldehydes with malonic acid in presence of pyridine and piperidine. It is further taken for next step with alanine in CH_2Cl_2, PPAA and triethyl amine to produce N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine **(2a-2h)**. The structures of newly synthesized compounds **(2a-2h)** were investigated according to their analytical and spectral data.

The IR spectra of compounds **2a-2h** displayed bands at 3339-3346 cm^{-1} due to N-H str, 2925-2952 cm^{-1} due to aromatic C-H str, 1631-1696 cm^{-1} due to the C=O str of –CONH group, a band at 1585-1593 cm^{-1} due to C=C (styryl) str.

The ^1H NMR spectra were taken for compounds **2a-2h**, which also supported the structures assigned. The compounds showed a doublet at δ 1.3 due to methyl protons, and multiplets at δ 4.25-4.35 due to –CH protons, doublet at δ 6.4-6.6 due to olefinic protons, multiplets in the region of δ 6.9-8.0 due to Ar, N-H protons, singlet at δ 11.6-12.2 due to –COOH protons. All of the possible fragmentations peaks are observed from the mass spectrum analysis, as expected.

All the above synthesized compounds were screened for nitric oxide, DPPH and ferric induced lipid peroxidation activities. The data in Table 3, 4 and 5, reveal that the synthesized compounds showed significant antioxidant activity compared to the standard.

Thus a series of N-[3-(substituted phenyl)-1-oxo-2-propenyl] alanine **(2a-2h)**, were synthesized and screened for nitric oxide scavenging activity. Among all these, the vanillinyl derivative **(2d)** showed 40% activity. The non-phenolic compounds, isopropyl **(2e)**, 4-methoxy **(2c)**, 4-chloro **(2b)** showed good scavenging (50.7%, 49%, and 47.3%, respectively) of DPPH stable free radical. The data of inhibition of ferric induced lipid peroxidation showed that the vanillinyl derivative **(2d)** showed highest activity (50.1%).

CONCLUSION

In the entire three models vanillinyl derivative showed good activity. An interesting observation is that the non-phenolic substitutions bearing the electron donating group

(methoxy, methyl and isopropyl) showed good inhibition of ferric induced lipid peroxidation, also though the unsubstituted compounds showed moderate activity, the substitution on the 4-phenyl group was found to enhance the potentiality of the pharmacophoric group.

ACKNOWLEDGEMENT

The author express sincere thanks to Dr. K. Bharathi, Dean, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, (Women's University), Tirupati. It's my privilege to express my sincere thanks to IICT, Hyderabad for the spectral analysis of compounds.

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Revised : 02.03.2015 *Accepted : 05.03.2015*