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Synthesis and Evaluation of Substituted Cinnamoyl Alanines for Antiinflammatory, Analgesic and Antioxidant Activities

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A series of substituted cinnamoyl alanines were synthesized by condensation of substituted 4-benzylidene-2-phenyloxazol-5-ones with alanine. The chemical structures of synthesized compounds were confirmed by means of IR, ^{1}H NMR, mass spectral and elemental analysis. All the compounds were screened for their antiinflammatory, analgesic and antioxidant activities. Out of these compounds, N-[2-(benzoylamino)-3-(3,5-dimethoxy-4-hydroxyphenyl)-1-oxo-2-propenyl]alanine (**24**) exhibited good anti-inflammatory activity (70 %) comparable to the standard drug phenylbutazone (73 %). This compound also showed good analgesic activity. Several phenolic compounds in this series showed good antioxidant activity.

Key Words: Cinnamoyl alanines, Antiinflammatory, Analgesic, Antioxidant.

INTRODUCTION

Amino acids such as tryptophan[1], phenyl alanine[2], creatinine[3], glutamine[4] and sulphur containing amino acids like methionine[5] were reported to possess good antiinflammatory activity. These amino acids were also reported to act as scavengers of oxygen free radicals[5,6]. Curcumin, a natural constituent of *Curcuma longa* is known to possess antiinflammatory activity[7]. It has a styryl carbonyl moiety in its structure, display dual inhibition of 5-LOX and COX *in vitro*. Curcumin and dehydrozingerone were reported to be potent scavengers of oxygen free radicals and also possess good antiinflammatory activity. Both are styryl ketones with similar substituents on the phenyl ring. Like curcumin, cinnamic acid and its derivatives exhibited antiinflammatory and analgesic activities similar to those of aspirin[8]. These findings prompted us to synthesize compounds containing both styryl carbonyl pharmacophore[9,10] and constrained amino acid such as alanine as these interesting structural features possesses antiinflammatory activity. These compounds also present the structural features of dehydropeptides which are an important class of bioactive peptides. The starting compounds 4-substituted benzylidine-2-phenyl-oxazol-5-ones were prepared according to known method[11]. The oxazolones when stirred with alanine in the presence of 1 N NaOH and acetone yielded substituted cinnamoyl alanines.The chemical structures of the synthesized compounds were

confirmed by means of IR, ^{1}H NMR, mass spectral and elemental analysis. The compounds were screened for their antiinflammatory, analgesic and antioxidant activities.

EXPERIMENTAL

The melting points were determined in open capillaries and were uncorrected. Purity of the compounds was checked by using precoated TLC plates (E. Merck Kieselgel 60 F_{254}). The IR spectra were recorded using KBr Pellets on a Perkin-Elmer 1760 spectrophotometer (ν_{max}, cm^{-1}). ^{1}H NMR spectra were recorded on GE Omega 400 MHz spectrometer or Bruker Avance (300 MHz) spectrometer using TMS as an internal standard (chemical shift in δ ppm). Mass spectra were recorded on a Jeol-JMS-D-300 spectrometer. Elemental analysis (C, H and N) were undertaken with Perkin-Elmer model 240C analyzer: the found values were within ± 0.4 % of the theoretical values, unless otherwise indicated.

General method of synthesis of 2-phenyl-4-(substituted benzylidene) oxazol-5-ones: (1-12): Various 2-phenyl-4-(substituted benzylidene)-oxazol-5-ones **1-12** were prepared by reported procedure[11].

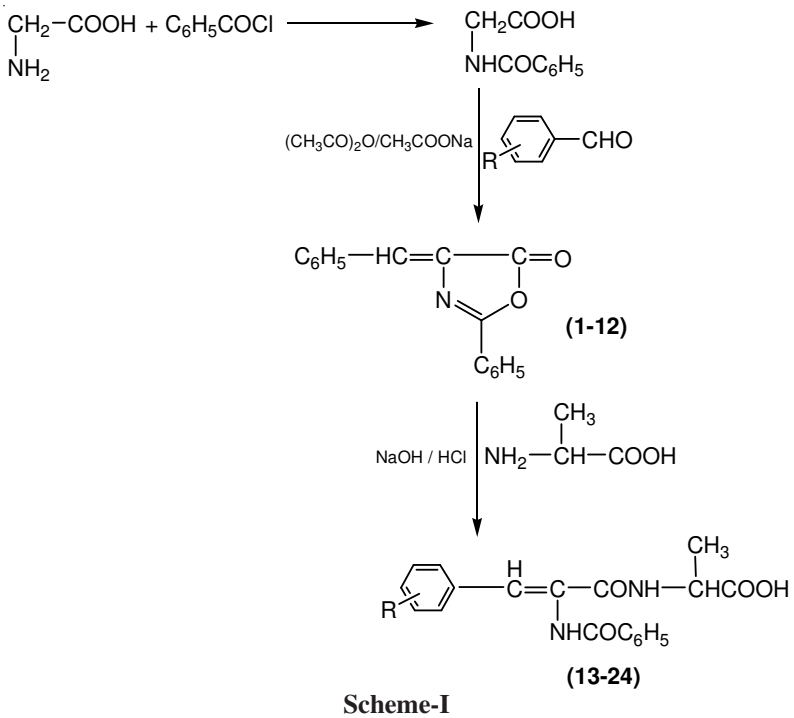
General method of synthesis of cinnamoyl alanines: (13-24): Synthesis of N-[2-(benzyolamino)-1-oxo-3-phenyl-2-propenyl] alanine (13): A solution of alanine (0.016 mol) in 1 N NaOH (16 mL) and acetone (10 mL) was stirred with 4-benzylidene-2-phenyloxazol-5-one (0.014 mol). After 2-3 h, the resulting clear solution was acidified by the addition of excess of 1N HCl. The completion of the reaction was monitored by TLC. The solid that separated was filtered and washed thoroughly with cold water. The product was dissolved in acetone and crystallized on addition of water.

Compounds **14-24** were prepared in a similar manner **(Scheme-I)**. Physical data of these compounds are given in Table-1.

N-[2-(Benzoylamino)-1-oxo-3-phenyl-propenyl]alanine(13): m.f. $C_{19}H_{18}N_2O_4$, IR (KBr, ν_{max}, cm^{-1}): 3417 (N–H), 3238 (O–H), 1652 and 1792 (C=O), 1593 (C=C). ^{1}H NMR (DMSO-d_6) δ: 12.5 (br s, –COOH, 1H), 9.9 (s, 1H, =C–NHCO), 7.2-8.3 (m, 11H, ArH and =CH–Ar), 4.3-4.4 (m, 1H, CH–CH_3), 1.3 (d, 3H, CH–CH_3). EI-MS m/z: 339.

N-[2-(Benzoylamino)-3-(4-chlorophenyl)-1-oxo-2-propenyl]alanine(14): m.f. $C_{19}H_{17}N_2O_4Cl$, IR (KBr, ν_{max}, cm^{-1}): 3419 (N–H), 3234 (O–H), 1740 and 1651 (C=O), 1624 (C=C).^{1}H NMR ($CDCl_3$) δ: 12.6 (br s, –COOH, 1H), 9.8 (s, 1H, = C–NHCO), 7.3-8.0 (m, 10H, ArH and =CH–Ar), 4.5-4.6 (m, 1H, CH–CH_3), 1.4 (d, 3H, CH–CH_3).

N-[2-(Benzoylamino)-3-(4-methylphenyl)-1-oxo-2-propenyl]alanine(15): m.f. $C_{20}H_{20}N_2O_4$, IR (KBr, ν_{max}, cm^{-1}): 3410 (N–H), 3220 (O–H), 1780 and 1650 (C=O), 1600 (C=C). ^{1}H NMR (DMSO-d_6) δ: 12.4 (br s, –COOH, 1H), 9.2 (s, 1H, =C–NHCO), 7.3-8.1 (m, 10H, ArH and =CH–Ar), 4.4-4.5 (m, 1H, CH–CH_3), 2.5 (s, 3H, CH_3), 1.4 (d, 3H, CH–CH_3).



Scheme-I

N-[2-(Benzoylamino)-3-(4-isopropylphenyl)-1-oxo-2-propenyl]alanine (16): m.f. $C_{22}H_{23}N_2O_4$, IR (KBr, ν_{max}, cm^{-1}): 3406 (N–H), 3226 (O–H), 1765 and 1656 (C=O), 1602 (C=C).

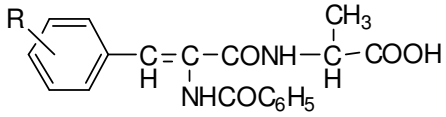
N-[2-(Benzoylamino)-3-(4-dimethylaminophenyl)-1-oxo-2-propenyl]-alanine (17): m.f. $C_{21}H_{23}N_3O_4$, IR (KBr, ν_{max}, cm^{-1}): 3500 (N–H), 3140 (O–H), 1761 and 1645 (C=O), 1600 (C=C). ^{1}H NMR (DMSO-d_6) δ: 12.5 (br s,1H, –COOH), 9.8 (s, 1H, =C–NHCO), 6.9-7.9 (m, 10H, ArH and =CH–Ar), 4.3-4.4 (m, 1H, CH–CH_3), 3.0 (s, 6H, $N(CH_3)_2$), 1.3 (d, 3H, CH–CH_3).

N-[2-(Benzoylamino)-3-(4-methoxyphenyl)-1-oxo-2-propenyl]alanine (18): m.f. $C_{20}H_{20}N_2O_5$, IR (KBr, ν_{max}, cm^{-1}): 3438(N–H), 3224 (O–H) 1733 and 1648 (C=O), 1626 (C=C). ^{1}H NMR (DMSO-d_6) δ: 12.5 (br s, 1H, COOH), 9.8 (s, 1H, =C–NHCO), 6.9-7.9 (m, 10H, ArH and =CH–Ar), 4.3-4.4 (m, 1H, CH–CH_3), 3.7 (s, 3H, OCH_3), 1.3 (d, 3H, CH–CH_3).

N-[2-(Benzoylamino)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]alanine (19): m.f. $C_{20}H_{20}N_2O_5$,IR (KBr, ν_{max}, cm^{-1}): 3440 (N–H), 3120 (O–H), 1733 and 1648 (C=O), 1626 cm^{-1} (C=C).

N-[2-(Benzoylamino)-3-(3,4,5-trimethoxyphenyl)-1-oxo-2-propenyl]alanine (20): m.f. $C_{22}H_{24}N_2O_7$, IR (KBr, ν_{max}, cm^{-1}): 3234 (N–H), 3061 (O–H), 1745 and 1642 (C=O), 1582 (C=C). ^{1}H NMR ($CDCl_3$) δ:12.4 (br s, 1H, COOH), 8.5 (s, 1H, =C–NHCO), 6.6-7.9 (m, 8H, ArH and =CH-Ar), 4.5-4.6 (m, 1H, CH–CH_3), 3.9 (s, 3H, OCH_3), 3.8 (s, 6H, OCH_3) 1.4 (d, 3H, CH–CH_3). EI-MS m/z: 400.

TABLE-1
PHYSICAL AND PHARMACOLOGICAL PROPERTIES OF COMPOUNDS **13-24**



Compound	R	Recrystallization solvent	m.p. ºC	Yield (%)	Edema inhibition (%)	Writhing inhibition (%)[b]
13	H	A	196-198	76	18	–
14	4-Cl	A	283-285	66	32	–
15	4-CH_3	B	165-167	70	19	–
16	4- CH $(CH_3)_2$	B	170-172	75	38*	10
17	4-$N(CH_3)_2$	A	150-152	70	60*	–
18	4-OCH_{31}	A	168-170	75	44*	–
19	3,4-$(OCH_3)_2$	B	160-162	70	25	–
20	3,4,5-$(OCH_3)_3$	B	164-167	72	19	–
21	4-OH	A	141-142	74	45*	–
22	4-OH, 3-OCH_3	A	142-144	70	52*	7
23	5-Br, 4-OH, 3-OCH_3	A	140-142	68	66*	49.7*
24	4-OH, 3,5-$(OCH_3)_2$	A	140-145	70	70*	51.1*
	Pheylbutazone	–	–	–	73*	–
	Aspirin	–	–	–	–	63.8*

A: Acetone – water B: Methanol – water.
(a) At 100 mg/kg (P.O.) per cent edema inhibition calculated by comparing edema volume with that of respective vehicle treated control animals. (b) Per cent writhing inhibition was calculated comparing number of writhes with that for the respective vehicle treated control animals *Statistically significant ($p < 0.05$ Mann – Whitney). ($p < 0.05$, Student's 't' test).

N-[2-(Benzoylamino)-3-(4-hydroxyphenyl)-1-oxo-2-propenyl]alanine (21): m.f. $C_{19}H_{18}N_2O_5$, IR (KBr, ν_{max}, cm^{-1}): 3550 (Ar–OH), 3445 (N–H), 3110 (O–H), 1740 and 1690 (C=O), 1625 (C=C).

N-[2-(Benzoylamino)-3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propenyl]alanine (22): m.f. $C_{20}H_{20}N_2O_6$, IR (KBr, ν_{max}, cm^{-1}): 3400 (Ar–OH), 3340 (N–H), 3098 (O–H), 1754 and 1648 (C=O), 1598 (C=C). ^1H NMR ($CDCl_3$) δ:12.3 (br s, 1H, COOH), 8.8 (s, 1H, =C–NHCO), 6.6-7.9 (m, 9H, ArH and =CH–Ar), 4.5-4.6 (m, 1H, CH–CH_3), 3.9 (s, 3H, OCH_3), 1.46 (d, 3H, CH–CH_3).

N-[2-(Benzoylamino)-3-(5-bromo-4-hydroxy-3-methoxyphenyl)-1-oxo-2-propenyl]alanine (23): m.f. $C_{20}H_{19}N_2O_6Br$, IR(KBr, ν_{max}, cm^{-1}): 3400 (N–H), 3102.3 (O–H), 1754 and 1675 (C=O), 1585 (C=C).

N-[2-(Benzoylamino)-3-(3,5-dimethoxy-4-hydroxyphenyl)-1-oxo-2-propenyl]alanine (24): m.f. $C_{21}H_{22}N_2O_7$, IR(KBr, ν_{max}, cm^{-1}): 3397 (N–H), 3104 (O–H), 1787and 1649 (C=O), 1594 (C=C). ^1H NMR ($CDCl_3$) δ:12.3(br s, 1H, COOH), 8.9 (s, 1H, =C–NHCO), 6.6-7.9 (m, 8H, ArH and =CH–Ar), 4.5-4.6 (m, 1H, CH–CH_3), 3.9 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 1.46 (d, 3H, CH–CH_3).

Antiinflammatory activity: The method of Winter *et al.*[12] was followed. The compounds were given orally to groups of male albino rats (150-180 g, wistar strain) 1 h before injection of 0.05 mL of 1 % suspension of carrageenan into the subplantar region of the rat hind paw. Additional groups were similarly treated with 100 mg/kg of phenylbutazone (positive controls) or 10 mL/kg of 0.5 % sodium carboxy methylcellulose (vehicle controls). The volume of the injected paw was measured by water displacement in a plethysmograph immediately after carrageenan injection.The paw volume was again measured after 3 h. The results were expressed as the per cent edema inhibition which was calculated using the following formula.

$$\text{Edema inhibition (\%)} = 100(1 - V_t/V_c)$$

where, V_c = Volume of the edema in the control group, V_t = Volume of the edema in the treated group.

Analgesic activity: This method was based on acetic acid induced writhings in mice[13]. Groups of 6 mice (albino mice of either sex, 18-25 g) each were dosed with the test compounds or with the aspirin, at a dose of 100 mg/kg p.o, 1 h before intraperitoneal injection of 0.6 % acetic acid (10 mL/kg). Mice were observed for the total number of writhes for 15 min beginning 5 min after the acetic acid injection and the total number of writhes were recorded. The mean value of writhes for each group was calculated and compared statistically with the vehicle treated control group. Results were expressed in terms of per cent inhibition of the number of writhes which was calculated by using the formula.

$$\text{Inhibition (\%)} = 100\ (1\text{-}W_t/W_c)$$

where, W_c = No. of writhes for the control group, W_t = No. of writhes for the treated group.

Gastric ulcerogenicity: The ulcerogenic liability was determined in albino rats of either sex (60-100 g) following the method of Saxena *et al.*[14].

Statistical analysis: All values are expressed as mean ± standard deviation. Tests of significance were analyzed by the Mann – Whitney method or Student's 't' – test and $p < 0.05$ was accepted as statistically significant.

Inhibition of lipid peroxidation in rat brain homogenate: Albino wistar rats (180-200 g) of either sex were used for study. Decapitated and removed the brain and perfused transcardially with ice-cold normal saline to prevent contamination of brain tissue with blood. Tissue was weighed and homogenate (10 % w/v) was prepared in 0.15 M KCl and centrifuged at 800 g for 10 min. The supernatant was used immediately for the study of *in vitro* lipid peroxidation. The incubation mixture contained in a final volume of 1.5 mL, brain homogenate (0.5 mL), KCl (0.15 M) and ethanol (10 µL) or test compound dissolved in ethanol. Peroxidation was initiated by adding, ferric chloride (100 µM) to give the final concentration stated. After incubating for 20 min at 37 °C, reactions were stopped by adding 2 mL of ice-cold 0.25 M HCl containing 15 % trichloroacetic acid, 0.38 % thiobarbituric acid and 0.05 % butylated hydroxyl toluene. Following heating at 80 ºC for 15 min, samples

were cooled and centrifuged at 1000 g for 10 min. The absorbance of the supernatant was measured at 532 nm [15]. Percentage inhibition of TBARS formed by test compound was calculated by comparing with vehicle only experiments. Control experiments without test compound were conducted in an identical manner.

Assay of nitric oxide (NO) scavenging activity: Sodium nitroprusside (10 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentrations of drug dissolved in a suitable solvent (dioxane/methanol) and tubes were incubated at 25 ºC for 2 h. 2 mL of incubation solution was removed and diluted with 2 mL of Griess reagent (sulphanilamide: 4 g, N-naphthylethylene diamine dihydrochloride: 0.2 g, 10 % orthophosphoric acid: 10 mL; distilled water: 100 mL). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and on subsequent coupling with N-naphthylethylene diamine was read at 546 nm[16].

Interaction with stable free radical DPPH: DPPH assay was performed as described[17]. Solutions of various drugs at 100 μM concentration were added to 100 μM DPPH in 95 % ethanol and tubes were kept at an ambient temperature for 20 min and absorbance was measured at 517 nm. Ethanol was used as blank and DPPH solution in ethanol served as the control.

RESULTS AND DISCUSSION

Antiinflammatory activity: Antiinflammatory activity of all the 12 compounds were screened by carrageenan induced hind paw edema model in rats (dose 100 mg/kg). The data is given in Table-1. Phenolic compounds in this series exhibited good activity. 4-Hydroxy derivative showed significant activity (45 %). Introduction of methoxy group at *ortho* position to the phenolic hydroxyl group as in compounds **22** and **23** resulted in a significant increase in activity. 3,5-dimethoxy 4-hydroxy derivative (70 %) (compound **24**) exhibited activity comparable to phenylbutazone (73 %). Similar results were reported with compounds such as curcumin, dehydrozingerone and substituted phenyl styryl ketones containing sterically hindered phenolic groups[18]. Substitution by electron releasing groups such as 4-isopropyl (38 %), 4-methoxy (44 %) and 4-dimethylamino (60 %) derivatives at *para* position increased the activity.

Analgesic activity: Compounds **16**, **22**, **23** and **24** exhibited good antiinflammatory activity were screened for analgesic activity (Table-1). 5-Bromo-vanillinyl and 4-hydroxy-3,5-dimethoxy derivatives showed significant activity.

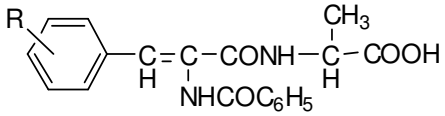
Gastric ulcerogenicity: The active compound **24** was tested for gastric ulcerogenicity. The ulcerogenic potential of this compound (Lesion index, 8.17) was found to be less than phenylbutazone (Lesion index, 45.17).

Antioxidant activities of N-[2-(benzoylamino)-1-oxo-3-phenyl-2-propenyl]alanines

Inhibition of lipid peroxidation: All the compounds were tested for inhibition of lipid peroxidation induced by ferric ions in rat brain homogenate. Data is shown in Table-2. Among the phenolic compounds, vanillinyl (70 %), 5-bromo-vanillinyl

(60 %) and 3,5-dimethoxy-4-hydroxy (50 %) analogues were found to be more active than the standard drug α-tocopherol (55 %). The good activity of these might be due to inductive effect of the methoxy group *ortho* to be phenolic group. Many studies have shown that *ortho* substitution with groups like alkyl or alkoxy increases the antioxidant properties of phenols. Among the non-phenolic compounds containing electron donating groups at the *para* position such as 4-methoxy, 4-methyl and 4-isopropyl showed good inhibition of lipid peroxidation.

TABLE-2
ANTIOXIDANT ACTIVITIES OF COMPOUNDS **13-24** At 100 μM



Compound	R	% inhibition of lipid peroxidation	% scavenging of nitric oxide	% reduction of DPPH
13	H	30.0	NA	20.0
14	4-Cl	38.0	26	30.0
15	4-CH_3	49.0	22	26.0
16	4-CH-$(CH_3)_2$	50.0	40	45.0
17	4-N$(CH_3)_2$	46.0	15	20.0
18	4-OCH_3	43.0	36	28.0
19	3,4-$(OCH_3)_2$	30.0	29	27.0
20	3,4,5-$(OCH_3)_3$	18.0	20	26.0
21	4-OH	36.0	33	48.0
22	4-OH, 3-OCH_3	70.0	40	60.0
23	5-Br, 4-OH, 3-OCH_3	60.0	28	75.0
24	3,5-$(OCH_3)_2$, 4-OH	50.0	26	70.0
	α-tocopherol	55.6	–	58.6

Nitric oxide scavenging activity: Table-2 shows the scavenging activity of nitric oxide. Among the phenolic compounds, vanillinyl analogue which exhibited good inhibition of lipid peroxidation also showed significant nitric acid scavenging activity. Among the other non-phenolic analogues 4-isopropyl and 4-methoxy showed significant activity.

Reduction of DPPH free radical: Table-2 shows the reactivities of title compounds with DPPH at 100 μM concentration. 4-hydroxy derivative showed only 38 % reduction. Introduction of methoxy group adjacent to phenolic hydroxy led to enhanced activity. Vanillinyl (60 %), 5-bromovanillinyl (75 %) and 3,5-dimethoxy-4-hydroxy (70 %)) analogues showed good activity. These results agree with earlier work indicating that the antioxidant efficiency of monophenols is strongly enhanced by the introduction of one or two methoxy substitutions in position *ortho* to the phenolic group[19].

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