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Synthesis, Anti-Oxidant and Analgesic Activity of N-(1-(4-Hydroxy-3-Methoxybenzyl Carbamoyl)-2-Phenylvinyl) Benzamide Derivatives

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Abstract : series of novel N-acylvanillamide derivatives were synthesized by the reaction of substituted 4-benzylidene-2-phenyl oxazol-5-one (2a-2j) with vanillamine HCl in the presence of acetone and alkali. The compounds were characterized using IR, ^{1}H NMR & Mass analysis. All the synthesized N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-phenylvinyl) benzamide derivatives (3a-3j) were screened for their antioxidant and analgesic activity by known experimental models.
Key words : Vanilloid receptors, N- acylvanillamides, Azlactones, Antioxidant activity, Analgesic activity.

Introduction :

N-acylvanillamines (N-AVAM), a class of compounds unique to the genus *capsicum*, are exemplified by capsaicin (CPS) [1], the major pungent principle of hot pepper and the archetypal vanilloid[2]. N-AVAM has been at the center of intense research activity aimed at elucidating the basis of their antinociceptive properties and exploiting their therapeutic potential. A simple and general synthesis of vanillamides was developed and employed to screen acids from the fatty and isoprenoid pools for new acyl templates of biological relevance as capsaicin analogues. Potent activation of the human vanilloid receptor 1 (VR1) was observed for the vanillamides of certain polyfunctional acids from both pools.

Vanilloid receptors 1 (VR1) is essential for normal thermal nociception and for thermal hyperalgesia induced by inflammation[3]. Consequently, VR1 receptor antagonists may be useful in the study of inflammatiory hyperalgesia and pain[2].

Oxazolone is a versatile lead molecule for designing potential bioactive agent. They are derived from natural amino acid derivatives, have received much attention during recent years on account of their prominent potential as antimicrobial[4], analgesic[5], anti-fungal [6], anti-cancer [7,8],anti-inflammatory[9], anti-HIV[10], anti-

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angiogenic[11], anti-convulsant[12,13], ant-viral[14], neuroleptic[15], sedative[16],anti-diabetic[17],antiobesity[18], and cardiotonic activity[19]. Therefore, it was planned to synthesize hybrid compounds that comprise both the oxazolone and vanillamine ring system.N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-phenylvinyl) benzamide (3a-3j) were prepared according to the procedure outlined in scheme 1. The structure of synthesized compounds was confirmed byelemental analysis and spectral (IR.^{1}H NMR & Mass) data and evaluated them for their potential as antioxidant and analgesic agents.

Materials and Methods:

General

Melting points were taken in open capillary tubes using Arson digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on BRUKER AV I, 300 MHz NMR spectrometer, IR spectra were recorded on Thermo Nicolet Nexus 670 Spectrometer with universal sampling model using KBr pellets.TLC was carried out using pre coated Silica gel plates. All the chemicals and solvents used were of LR grade and obtained from Sd-Fine and Merck.

Chemistry

Various 4-substituted benzylidene-2-phenyl oxazole-5-one 2a-2j which are required as starting materials were prepared according to reported method[20], the reaction of an aldehyde with hippuric acid usually is referred to as the Erlenmeyer azalactone synthesis. The action of acetic anhydride on an α-acylamino acid in aqueous solution yields an azalactone, provided that a basic catalyst, such as sodium acetate, is present. The oxazolones were condensed with vanillamine HCl is aqueous acetone containing sodium hydroxide to obtain the title compounds 3a-3j (Fig 5).

Synthesis

The starting compound (**1**) for the preparation of oxazolones was obtained as described[21]. The 2-oxazoline-5-one (**2a-2j**) was prepared by according to reported method[22].

General procedure for synthesis of N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-phenylvinyl) benzamide (3a-3j)

A solution of vanillamine HCl (0.01 mmol) in 1N NaOH and acetone was stirred with 2-oxazoline-5-one (2a-2h) (0.01 mmol) for about 3-4 hr. a clear solution obtained was filtered and acidified with 1N HCl. The product obtained was filtered, washed thoroughly with cold water and crystallized from water to give compounds (3a-3j).

3a:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-phenylvinyl) benzamide

IR (KBr) (cm-1): 3248 (N-H str),2928 (C-H Ar Str) 1690 (C=O str), 1655 (C=C str), 3320 (O-H Str), 2662 (C-H str, OCH_3), 3010 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.3 (s, 3H, OCH_3), 4.2 (s, 2H, N-CH_2), 7.1-7.9 (m, 15H, Ar-H, -NH & Olefinic), 9.8 (s, 1H, Ar-OH).**GC-MS (m/z,%)** : 403 (M+H).Anal. Calcd for$C_{24}H_{22}N_2O_4$:C, 71.63; H, 5.51; N, 6.96; O, 15.90. Found: C, 71.60; H, 5.42; N, 6.84; O, 15.79.

3b:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-chlorophenyl)vinyl] benzamide

IR (KBr) (cm-1): 3258 (N-H str),2935 (C-H Ar Str) 1695 (C=O str), 1645 (C=C str), 3319 (O-H Str), 2639 (C-H str, OCH_3), 3009 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.7 (s, 3H, OCH_3), 4.3 (s, 2H, N-CH_2), 7.4-7.9 (m, 15H, Ar-H, -NH & Olefinic), 9.9 (s, 1H, Ar-OH). Anal. Calcd for$C_{24}H_{21}N_2O_4Cl$: C, 65.98; H, 4.84; N, 6.41; O, 14.65;Cl, 8.11. Found: C, 65.06; H, 4.20; N, 6.35; O, 14.24; Cl, 8.10.

3c:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-methoxyphenyl)vinyl]benzamide

IR (KBr) (cm^{-1}): 3264 (N-H str),2926 (C-H Ar Str) 1684 (C=O str), 1592 (C=C str), 3320 (O-H Str), 2627 (C-H str, OCH_3), 3011 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.4 (s, 6H, OCH_3), 4.2 (s, 2H, N-CH2), 6.9-8.1

(m, 15H, Ar-H, -NH & Olefinic), 9.6 (s, 1H, Ar-OH). Anal. Calcd for$C_{25}H_{24}N_2O_5$:C, 69.43; H, 5.59; N, 6.48; O, 18.50. Found: C, 68.24; H, 5.52; N, 6.32; O, 18.47.

3d:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-hydroxyphenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3250 (N-H str),2960 (C-H Ar Str) 1692 (C=O str), 1548 (C=C str), 3328 (O-H Str), 2729 (C-H str, OCH_3), 3022 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.5 (s, 3H, OCH_3), 4.0 (s, 2H, N-CH2), 7.1-7.9 (m, 15H, Ar-H, -NH & Olefinic), 9.8 (s, 1H, Ar-OH), 5.2 (s,1H,Ar-OH). Anal. Calcd for$C_{24}H_{22}N_2O_5$:C, 68.89; H, 5.30; N, 6.69; O, 19.12. Found: C, 68.57; H, 5.39; N, 6.51; O, 19.06.

3e:N-(1-(4-hydroxy-3-methoxybenzylcarbamoyl)-2-[(4-hydroxy-3-methoxyphenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3258 (N-H str),2928 (C-H Ar Str) 1689 (C=O str), 1546 (C=C str), 3320 (O-H Str), 2626 (C-H str, OCH_3), 3006 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.2 (s, 6H, OCH_3), 4.3 (s, 2H, N-CH2), 7.2-8.0 (m, 15H, Ar-H, -NH & Olefinic), 9.9 (s, 1H, Ar-OH), 4.9 (s,1H, Ar-OH). Anal. Calcd for$C_{25}H_{24}N_2O_6$:C, 66.95; H, 5.39; N, 6.25; O, 21.41. Found: C, 66.89; H, 5.37; N, 6.11; O, 21.32.

3f:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-dimethylaminophenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3247 (N-H str),2926 (C-H Ar Str) 1674 (C=O str), 1542 (C=C str), 3322 (O-H Str), 2630 (C-H str, OCH_3), 2820 (C-H str, CH_3) 3012 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.1 (s, 3H, OCH_3), 4.2 (s, 2H, N-CH2), 7.1-8.0 (m, 15H, Ar-H, -NH & Olefinic), 9.8 (s, 1H, Ar-OH), 2.0 (d, 6H, $N(CH_3)_2$). Anal. Calcd for$C_{26}H_{27}N_3O_4$:C, 70.09; H, 6.11; N, 9.43; O, 14.37. Found: C, 70.01; H, 6.06; N, 9.32; O, 14.32.

3g:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-isopropylphenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3260 (N-H str),2958 (C-H Ar Str) 1697 (C=O str), 1641 (C=C str), 3314 (O-H Str), 2725 (C-H str, OCH_3), 2832 (C-H str, CH_3) 3010 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 1.3 (d, 6H, $(CH_3)_2$), 2.8-3.0 (m, 1H, CH),3.9 (s, 3H, OCH_3), 4.5 (s, 2H, N-CH2), 7.2-7.9 (m, 15H, Ar-H, -NH & Olefinic), 9.9 (s, 1H, Ar-OH). Anal. Calcd for$C_{27}H_{28}N_2O_4$:C, 72.95; H, 6.35; N, 6.30; O, 14.40. Found: C, 72.90; H, 6.28; N, 6.22; O, 14.38.

3h:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-nitrophenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3240 (N-H str),2932 (C-H Ar Str) 1672 (C=O str), 1594 (C=C str), 3320 (O-H Str), 2635 (C-H str, OCH_3), 3019 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.26 (s, 3H, OCH_3), 4.3 (s, 2H, N-CH2), 7.0-7.9 (m, 15H, Ar-H, -NH & Olefinic), 9.9 (s, 1H, Ar-OH). Anal. Calcd for$C_{24}H_{21}N_3O_6$:C, 64.42; H, 4.73; N, 9.39; O, 21.45. Found: C, 64.29; H, 4.65; N, 9.31; O, 21.40.

3i:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(3-nitrophenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3245 (N-H str),2929 (C-H Ar Str) 1670 (C=O str), 1594 (C=C str), 3321 (O-H Str), 2638 (C-H str, OCH_3), 3021 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 3.22 (s, 3H, OCH_3), 4.2 (s, 2H, N-CH2), 7.0-8.0 (m, 15H, Ar-H, -NH & Olefinic), 9.9 (s, 1H, Ar-OH). Anal. Calcd for$C_{24}H_{21}N_3O_6$:C, 64.42; H, 4.73; N, 9.39; O, 21.45. Found: C, 64.36; H, 4.62; N, 9.36; O, 21.37.

3j:N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-[(4-methylphenyl)vinyl] benzamide

IR (KBr) (cm^{-1}): 3263 (N-H str),2921 (C-H Ar Str) 1699 (C=O str), 1505 (C=C str), 3317 (O-H Str), 2634 (C-H str, OCH_3), 3010 (=CH str) ; **^{1}H NMR (DMSO-d_6), δ ppm:** 2.3 (s, 3H, CH3),3.75 (s, 3H, OCH_3), 4.42 (s, 2H, N-CH2), 7.1-8.0 (m, 15H, Ar-H, -NH & Olefinic), 9.9 (s, 1H, Ar-OH). Anal. Calcd for$C_{25}H_{24}N_2O_4$:C, 72.10; H, 5.81; N, 6.73; O, 15.37. Found: C, 72.02; H, 5.75; N, 6.59; O, 15.31.

Antioxidant Studies:

Assay for Nitric Oxide (NO) scavenging activity:

The scavenging effect of N-Acylvanillamides (3a-3j) on nitric oxide was measured according to the method of marcocci et al[23]. 100µM concentration of drug dissolved in a suitable solvent, were then added in

the test tubes to 10 µM of sodium nitroprusside solution, and the tubes incubated at 25ºC for 120 min. an aliquot (0.5ml) of incubation solution was removed and diluted with 0.5ml of Griess reagent.The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent. α- tocopherol was used as a standard.

DPPH free radical scavenging activity:

The DPPH free radical scavenging capability was performed as the method described by Koto, K et al[24]. Solutions of test samples at 100 µM concentration were added to 100 µM DPPH in 95% ethanol. Incubation was carried out at room temperature for 30 min. for each concentration; the assay was run in triplicate. At end of the incubation period, the optical density of each sample was determined at 517 nm against a blank. Results are expressed as means of triplicate.α- tocopherol was used as a standard. The inhibition of DPPH radical scavengining activity in percent (I %) was calculated according to the following equation:

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

Where $A_{sample}$is the absorbance of a sample solution and A_{blank} is the absorbance of the blank solution.

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

Inhibition of lipid peroxidation was assayed by the method described by Braughler JM et al; Ciuffi M et al[25, 26]. Wistar rats (180-200 g) were fasted for 16 h and sacrificed by decapitation. The homogenate (10% w/v) of brain tissue was prepared. 1.0ml of homogenate was mixed with 100 µM of sample solution. The mixture was incubated for 2 h at 37ºC. then, reaction were stopped by adding 2 ml of ice-cold 0.25 N HCl containing trichloroacetic (15 %), thibarbituric acid (0.38%) & BHT (0.05%). This mixture was heated in a boiled water bath for 15 min and then cooled to room temperature and centrifuged at 1000 rpm for 10 min. the absorbance of the supernatant solution was recorded at 532 nm.α- tocopherol was used as a standard. The inhibition of lipid peroxidation in percent (I %) was calculated according to the following equation.

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

Where $A_{sample}$is the absorbance of a sample solution and A_{blank} is the absorbance of the blank solution without sample.

Pharmacology

Acute toxicity studies:

It is found that, all the compounds have shown good safety profile till the highest dose. No mortality of animals observed even after 24 h.

Analgesic activity:

In this method Wistar rats (150 to 200 gm) of either both sex were obtained from the Sreenivasa enterprise, Bangalore. The animals were maintained under environmental condition and had free access to standard diet and fresh water ad libitum. They were housed in animal cages at room temperature (30±2ºC) and 60-65% relative humidity. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animal was devoid of water and food 12 hours before the administration of treatment. The animals were divided into twelve groups, each consisting of six animals was fasted overnight prior to the experiments. Ten groups were for single dose strength (20 mg/kg) of the test drug, while one each for standard (diclofenac sodium 10 mg/1Kg) drug and control (Tween 80, 0.1ml/10Kg) respectively.

Acetic acid induced writhing response method:

The compounds were selected for investigating their analgesic activity in acetic acid induced writhing response in Wistar rats, following the method of Collier et al[27]. Seventy two rats were divided into 12 groups

(six in each group) starved for 16 h pretreated as follows, the 1st group which served as control positive orally received distilled water in appropriate volumes. The 3rd to 12th groups received the aqueous suspension of synthesized compounds orally at a dose of 20 mg/kg. The 2nd group orally received diclofenac sodium in a dose of 10 mg/kg. After 30 min, each rat was administrated 0.6% of an aqueous solution of acetic acid (0.1 ml) and the rats were then placed in transparent boxes for observation. The number of writhes was counted for 20 min after acetic acid injection. The number of writhing was recorded and the percentage protection was calculated. The observations are tabulated as Table 4.

Statistical analysis:

All the results are expressed as mean ± standard error of mean (SEM). The data were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph Pad Prism, version 6.Values of $p< 0.01$ and $p<0.001$ were considered statistically significant.

Results and Discussion

Chemistry

The usefulness of 2-oxazolin-5-one as synthons has generated much interest in their chemistry in recent years consequently; the reaction of an aldehyde with hippuric acid usually is referred to as the Erlenmeyer azalactone synthesis. The action of acetic anhydride on an α-acylamino acid in aqueous solution yields an azalactone, provided that a basic catalyst, such as sodium acetate, is present. Various 4-substituted benzylidene-2-phenyl oxazole-5-one which are required as starting materials were prepared by condensing aromatic aldehydes with benzyl glycine in the presence of acetic anhydride and anhydrous sodium acetate. The oxazolones were condensed with vanillamine HCl is aqueous acetone containing sodium hydroxide to obtain the title compound (3a-3j). The presence of alkali is essential to open the oxazolone ring, in the case of compounds (4-OH & 4-OH, 3-OCH_3) the phenolic hydroxyl group was acetylated during the preparation of oxazolone in the presence of acetic anhydride. Synthesized compounds were characterized by spectral data.

Antioxidant studies

Scavenging of Nitric oxide free radical

Sodium nitroprusside in aqueous solution of physiological pH spontaneously generates nitric oxide. This nitric oxide reacts with oxygen to produce nitrite ions which can be estimated using griess reagent. All the compounds were tested for their ability to scavenging nitric oxide at 100 μM concentration. Fig 1 showed thescavenging of Nitric oxide free radical activity of N-acylvanillamide and its derivatives. Among the substituted compounds, 4-dimethyl amino group showed highest activity (64.9%). The vanillinyl derivative showed good activity (62.5%) greater than the simple phenolic 4-hydroxyl derivative (43.5%) which may be due to the presence of –OCH_3 group ortho to –OH group (Table 1).

Table 1: Nitric Oxide Scavenging of N-(1-(4-Hydroxy-3-Methoxybenzylcarbamoyl)-2-Phenylvinyl) Benzamide of 100 μM Concentration.

Compound	R	% inhibition
3a	H	53.2
3b	4-Cl	43.08
3c	4-OCH3	32.3
3d	4-OH	43.5
3e	4-OH, 3-OCH_3	62.5
3f	4-$N(CH_3)_2$	64.9
3g	4-$CH(CH_3)_2$	33.37
3h	4-NO_2	41.8
3i	3-NO_2	40.01
3j	4-CH_3	41.59
α-tocopherol		55

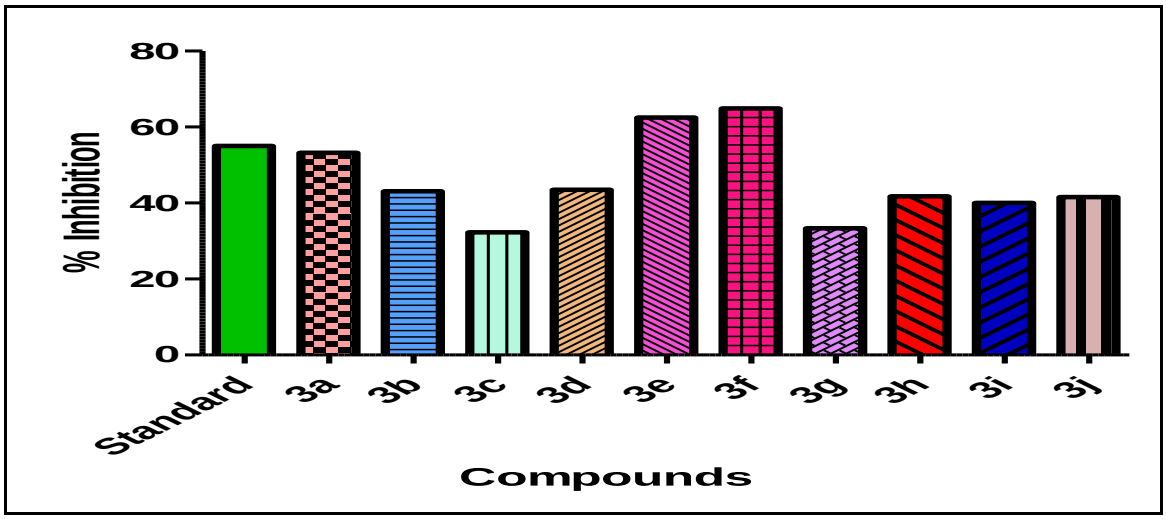


Fig 1: Nitric oxide scavenging activity of N-acylvanillamide derivatives. Each value is expressed as mean ± SD (n = 3).

DPPH radical scavenging activity

The nitrogen centered stable free radical 1,1-diphenyl-1,2-picryl hydrazyl (DPPH) has often been used to characterize phenolic antioxidants. It is reversibly reduced, and due to its unpaired electrons, densely colored. This property makes it suitable for spectrophotometric studies. A scavenging antioxidant reacts with DPPH stable free radical and converts it to 1,1-diphenyl-2-picryl hydrazine. The change in absorbance produced in this reaction has been used to measure antioxidant properties. The compounds show the reactivities at 100 μM with DPPH of 100 μM concentration (Fig 2). Among the compounds tested for antioxidant activity, phenolic compounds: 4-hydroxy & vanillinyl derivatives exhibited the highest antioxidant activity was found to be 96% & 95.1% respectively, and also dimethyl amino derivative shows grater activity (95.1%). The non-phenolic compounds also exhibited good reduction. The electron-donating groups like 4-methyl, 4-methoxy and isopropyl showed good activity (89.6%, 87.9%, and 82.8% respectively). The substitution with electron withdrawing group resulted in slightly reduction in activity (78.2%) at 100 μM (Table 2).

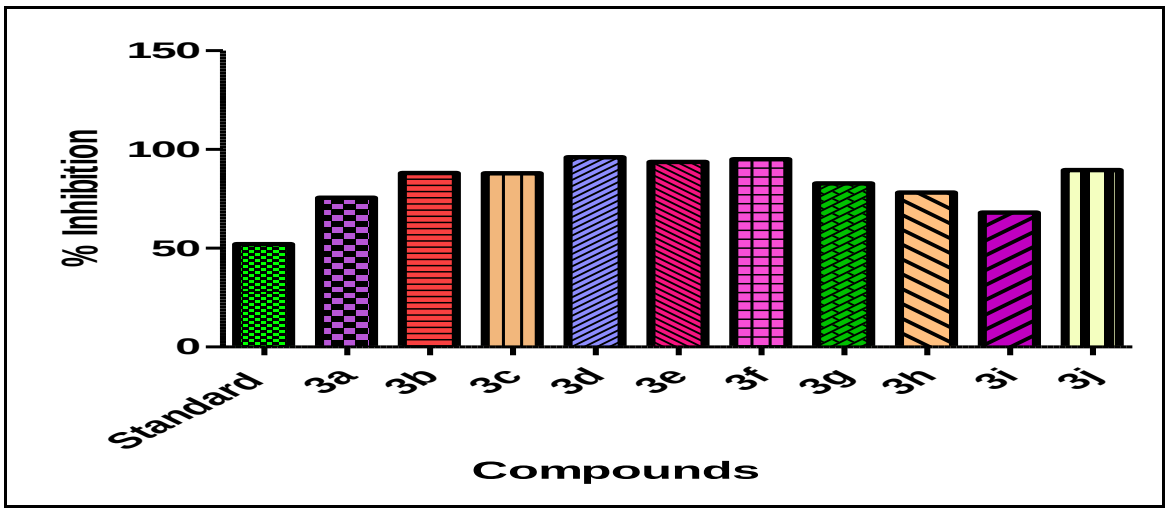


Fig 2: DPPH radical scavenging activity of N-acylvanillamide derivatives. Each value is expressed as mean ± SD (n = 3).

Table 2: Reduction of Dpph Stable Free Radical of N-(1-(4-Hydroxy-3-Methoxybenzylcarbamoyl)-2-Phenylvinyl)Benzamide of 100 μM Concentration.

Compound	R	% inhibition
3a	H	75.6
3b	4-Cl	88
3c	4-OCH3	87.9
3d	4-OH	96
3e	4-OH, 3-OCH_3	93.7
3f	4-$N(CH_3)_2$	95.1
3g	4-$CH(CH_3)_2$	82.8
3h	4-NO_2	78.2
3i	3-NO_2	68
3j	4-CH_3	89.6
α-tocopherol		52

Table 3: Effect of Interaction of Compounds at Of 100 μM Concentration and Ferrous Sulphate Induced Lipid Peroxidation In Rat Brain Homogenate.

Compound	R	% inhibition
3a	H	69.3
3b	4-Cl	77.7
3c	4-OCH3	42.8
3d	4-OH	80
3e	4-OH, 3-OCH_3	83
3f	4-$N(CH_3)_2$	83.2
3g	4-$CH(CH_3)_2$	82.3
3h	4-NO_2	81.7
3i	3-NO_2	75.4
3j	4-CH_3	82
α-tocopherol		79.7

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. Fig 3 showed the inhibition of Ferrous (Fe^{2+}) induced lipid peroxidation in rat brain homogenate. Among the substituted compounds, the vanillinyl derivatives showed the highest activity (83%). The substitution with electron-donating groups like 4-methyl, and 4- isopropyl showed good activity (82.3% & 82% respectively). The substitution with electron withdrawing groups like 4-chloro (77.7%) and 4-NO_2 (81.7%) exhibited good activity at 100 μM (Table 3).

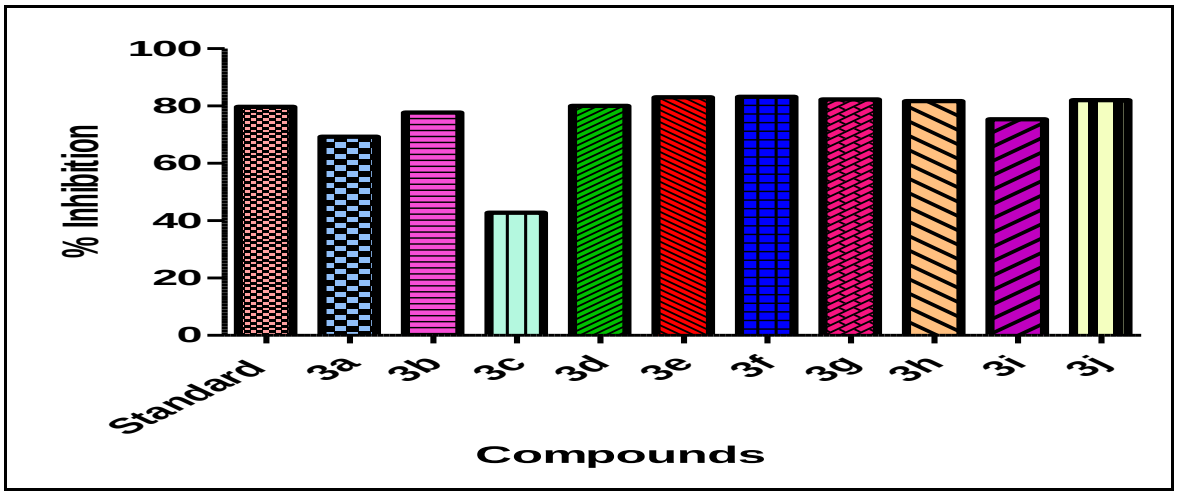


Fig 3: Inhibition of lipid peroxidation in brain tissue of rats by N-acylvanillamide derivatives. Each value is expressed as mean ± SD (n = 3).

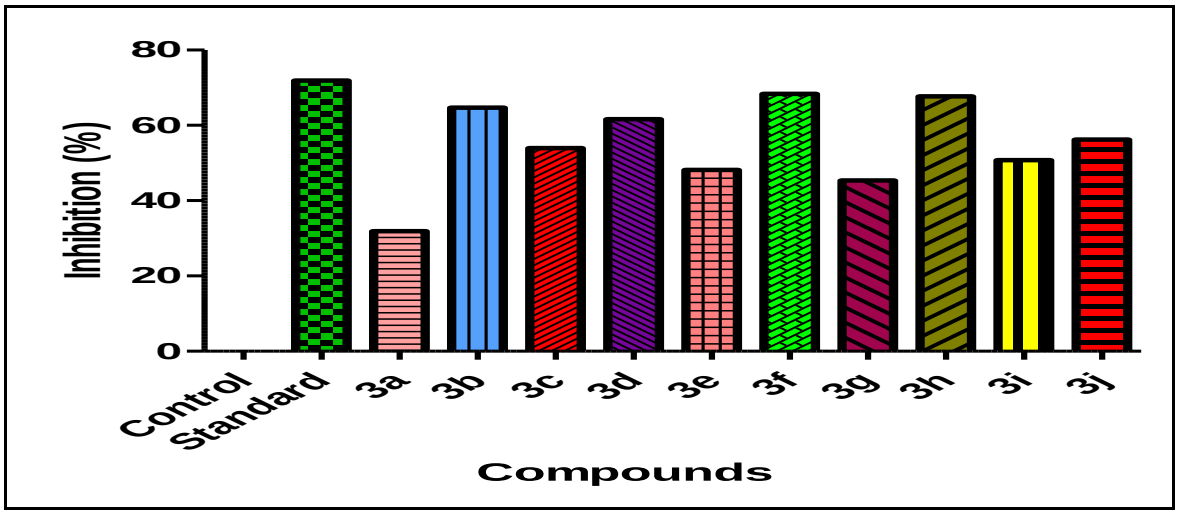


Fig4: inhibition of analgesic activity in rats by N-acylvanillamide derivatives.

Biological Studies

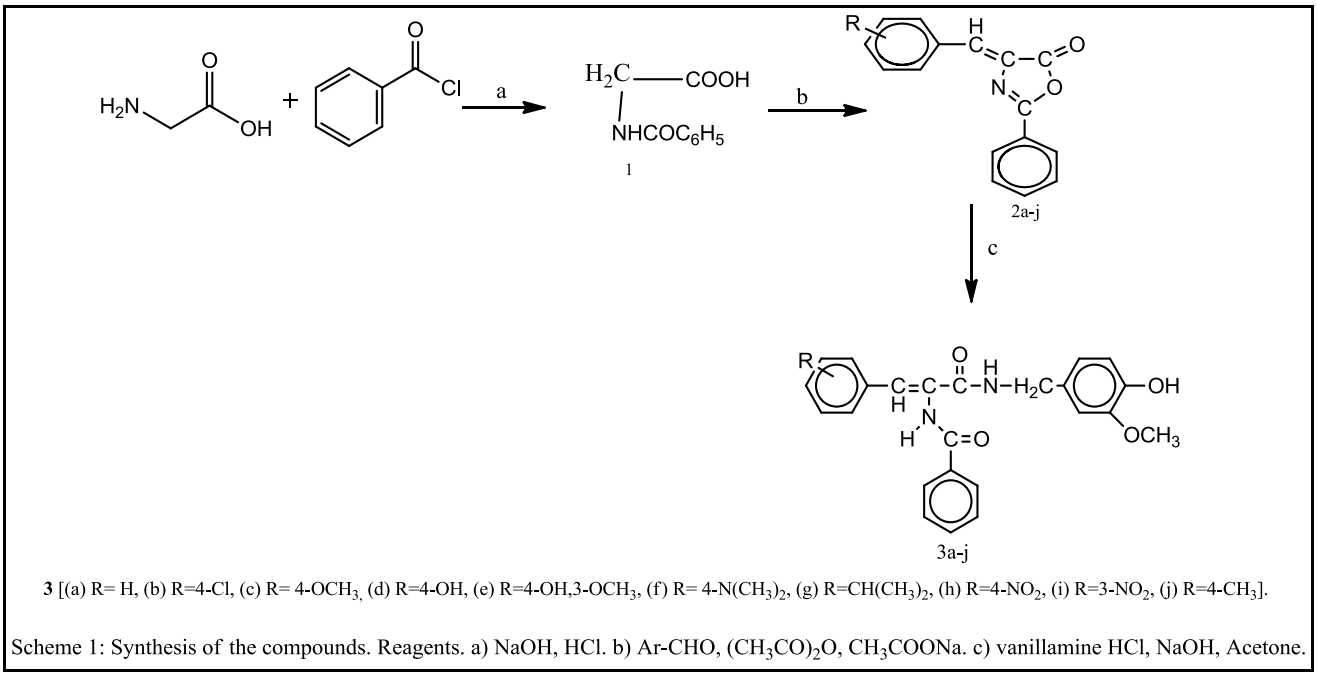
Analgesic activity by Acetic acid induced Writhing in rats

Abdominal constriction response induced by acetic acid is sensitive procedure to establish efficacy of peripherally acting analgesics. Intraperitonial administration of acetic acid causes increase in the level of PGE2 and PGF 2a. Fig 4 showed the percentage inhibition of analgesic activity by Acetic acid induced Writhing in mice. Amongst the tested compounds, **3f**, **3h** and **3b** significantly inhibited the acetic acid induced writhing (up to 68%, 67% and 64%, respectively). Compound **3d** possess good analgesic activity (61%) and other compounds (3j, 3c, 3i, 3e and 3g) in the range of 56-45% as compare to the standard. Thus compounds **3f** and **3h** appear to possess significant peripheral anti-nociceptive activity. Percentage analgesic activity shown by the tested compounds is recorded in Table 4.

Table 4: Analgesic Activity by Acetic Acid Induced Writhing in Rats

Group	Treatment	No. of writhes in 30 min mean±SEM	Inhibition (%)
I	Control	31.3±5.29	0
II	Standard	8.8±2.13***	71.88
III	3a	21.3±5.25	31.90
IV	3b	11.04±2.34***	64.70
V	3c	14.4±3.27*	53.99
VI	3d	12.2±3.27**	61.61
VII	3e	9.90±4.47	48.18
VII	3f	15.3±3.65***	68.37
IX	3g	17.10±5.23	45.36
X	3h	10.10±2.17***	67.73
XI	3i	15.40±4.56*	50.79
XII	3j	13.70±5.87**	56.23

Results are expressed as Mean ± SEM, relative to their respective standard and data were analyzed by Oneway ANOVA followed by Dunnett's test for (n=6); *p < 0.05, **p < 0.01, ***p<0.001.



3 [(a) R= H, (b) R=4-Cl, (c) R= 4-OCH_3, (d) R=4-OH, (e) R=4-OH,3-OCH_3, (f) R= 4-$N(CH_3)_2$, (g) R=$CH(CH_3)_2$, (h) R=4-NO_2, (i) R=3-NO_2, (j) R=4-CH_3].

Scheme 1: Synthesis of the compounds. Reagents. a) NaOH, HCl. b) Ar-CHO, $(CH_3CO)_2O$, CH_3COONa. c) vanillamine HCl, NaOH, Acetone.

Fig 5: Conventional route for synthesis of N-(1-(4-hydroxy-3-methoxybenzyl carbamoyl)-2-phenylvinyl) benzamide derivatives.

Conclusion

It was interesting to note that the all compound showed good reduction of DPPH, the substituted phenolic compounds exhibited good activity in both DPPH and NO scavenging activities. An interesting observation which is derivates from general conclusion for antioxidant activity of phenolic compounds is that the non-phenolic compounds bearing the electron-donating groups (methoxy and isopropyl) showed good inhibition of ferrous induced lipid peroxidation indicating that the phenolic group itself is not responsible for antioxidant activity, but the pharmacophoric group also plays a good role. Compounds of 3a-3j series were tested for the analgesic activity using diclofenac sodium as a standard drug. The parameter percentage of protection was calculated through Acetic acid induced Writhing test in albino rats. Compounds 3f, 3h, 3b & 3d have good inhibition by 68.37, 67.73, 64.7 & 61.61% compared to the standard drug, which showed 71.88% of

inhibition. Since antioxidant therapy seems to offer protection against a wide range of free radical induced diseases, it could be concluded that compounds 3f & 3e can be selected as the lead moiety in the analogue designing process of developing an ideal analgesic agent as Vanilloid receptors 1 antagonist.

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Reference

1. Suzuki, T.,Jwai, K.K.,Constituents of red-pepper species: chemistry, biochemistry, pharmacology, and food science of the pungent principle of capsicum species. Academicpress: Orlando FL, 1984, 227-229.
2. Szallasi, A., vanilloid receptor ligands: Hopes and realities for the future, Drugs aging,2001, 18 (8), 561-573.
3. Catherina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I., Julius, D.,Impaired nociception and pain sensation in micelacking the capsaicin receptor,Science, 2000, 288 (5464), 306-313.
4. Desai, N.C., Bhavasar, A.M., Baldaniya, B.B.,Synthesis and antimicrobial activity of 6-imidazolione derivatives,Indian J Pharm Sci, 2009,71, 90-94.
5. Jakeman, D.L.,Farrell, S.,Yong N.,Doucet, R.J.,Timmons, S.C.,Revel jadomycins incorporation of non-natural and natural amino acids, BioorgMed Chem Lett, 2005, 15(5), 1447-1449.
6. Sah, P.,Nair, S., Garg, S.P., Synthesis and antimicrobial activity of some new oxazolone derivatives of 4,5-disubstituted-2-aminothiazole, J Indian Chem Soc,2006, 83(2),205-207.
7. Violeta Benedetti-Doctorovich., Edward M Burgess., John, L., Daniel, L., Donald Van Derveer.,Leon, H.,Synthesis of 2-Methyl-(Z)-4-(phenylimino)naphth[2,3-d]oxazol-9-one, Monoimine Quinone with Selective Cytotoxicity toward Cancer Cells, J. Med.Chem, 1994, 37(5),710-712.
8. Jat, L.R., Mishra, R., Pathak, D., Synthesis and anticancer activity of 4-benzylidene-2- phenyloxazol-5(4h)-one derivatives, Int J Pharm Pharm Sci, 2012, 4 (1), 378-380.
9. Crespo, M.I.,Synthesis and biological evaluation of 3,4-diaryloxazolones.A new class of orally active cyclooxygenase-2 inhibitors, J Med Chem, 2000, 43 (2),214-223.
10. Witvrouw, M., Pannecouque, C., Clercq, D.E., Fernandez-Alvarez, E., Marco, J.L., Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by some diversely functionalized spirocyclopropyl derivatives,Arch. Pharm. Med. Chem, 1999, 352, 163-166.
11. Perron-Sierra, F.M., Pierre, A., Burbridge, M., Guilband, N., Novel bicyclic oxazolone derivatives as anti-angiogenic agents, Bioorg Med Chem Lett,2002,12,1463–1466.
12. Madkour, H.M.F.,Simple One-Step Syntheses of Heterocyclic Systems from (4Z)-2-Phenyl-4-(thien-2-ylmethylene)-1,3(4H)-oxazol-5-one, Chem. Pap, 2002, 56, 313-319.
13. Fareed, G., Afza, N., Versiani, A.M., Fareed, N., Mughal, R.U., Kalhoro, A.M., Iqbal, L., Lateef, M., Synthesis, spectroscopic characterization and pharmacological evaluation ofoxazolone derivatives, J. Serb. Chem. Soc, 2013, 78(8), 1127–1134.
14. Pinto, I.L., West, A., Debouckm, C.M., DiLella, A.G., Gorniak, J.G., O'Donnell K.C.,Shannessy, D.J., Patel, A., Jarvest, R.L.,Novel, selective mechanism-based inhibitors of the herpes proteases, Bioorganic Med. Chem. Lett, 1996,6, 2467-2472.
15. Cascio, G., Manghisi, E., Fregnan, G.,5-piperazinylalkyl-2-3(H)-oxazolones with neuroleptic activity, J Med Chem, 1989, 32(10),2241-2247.
16. Mesaik, A., Rahat, S., Khan, M., Ullah, Z., Choudary,M.I., Murad, S., Ismail, Z., Rahman, A., Ahmad, A., Synthesis and immunodilatory properties of selected oxazolone derivatives, Bioorg Med Chem, 2004, 12, 2049-2057.
17. Pereira,E.R., Sancelme, M., Voldoire, A., Prudhomme, M., Synthesis and antimicrobial activities of 3-N-substituted-4,5-bis(3-indolyl)oxazol-2-ones,Bioorg Med. Chem.Lit, 1997, 7(190), 2503-2506.
18. Viti, G., Namnicine, R., Ricci, R., Pestelline, V., Abeli, L., Funo, M., Newantagonists of platelet-activating factor containing 2-oxazolidinone or 2- morpholinone, Euro J. Med.Chem,1994, 29, 401-406.

19. Dobhal, Y., Rawat, R., Parcha, V., Sharma, R., Chaudhary, R., Design, synthesis and evaluation of phenyl oxazolone derivatives as Cardioprotective Drugs, World JPharma Sci, 2014, 2(12), 1712-1722.
20. Crane, B.R., Aravai, A.S., Gachhul, R., The structure of nitric oxide synthase oxygenase domain and inhibitor complexes, Science, 1997,278 (5337), 425-431.
21. Vogel, A.I., In: A Text Book of Practical Organic Chemistry, Longman, London., 1971, 909.
22. Bhatt, P.V., Wadia, D.N., Patel, P.M., Patel, R.M.,Synthesis of Some Novel Imidazolinone Derivatives with Dibenzo(b,f)azepine Nucleus,Asian J Chem,2006, 18(1),1248-1254.
23. Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T., Packer, L., The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Bioche. Biophys, Res. Commu, 1994,201(2), 748–755.
24. Kato, K., Terao, S., Shimamoto, N., Hirato, M., Studies of active oxygen species, synthesis and biological activity of 2-O-alkyl ascorbic acids, J.Med.Chem, 1998,31(4), 793-798.
25. Braughler**,** J**.**M., Duncan, L.A., Chase, R.L.,The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation, J Biol Chem, 1986, 261(22), 10282-10289.
26. Ciuffi, M., Gentilini, G., Franchi-Micheli, S., Zilletti, L., Lipid peroxidation induced "*invivo*" by iron-carbohydrate compels in the rat brain cortex, Neurochem Res, 1991, 16, 43-49.
27. Collier, H.O., Dinneen, L.C., Johnson, C.A., Schneider, C., The Abdominal Constriction Response and its Suppression by Analgesic Drugs in the Mouse, Br J Pharmacol Chemother, 1968, 32(2), 295–310.

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