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Research Article

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Synthesis and evaluation of some novel 5-(1-benzoylamino-2-(substituted phenyl) vinyl)-2-amino-1,3,4-oxadiazoles for antimicrobial and antioxidant activities

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ABSTRACT

*A series of 5-(1-benzoylamino-2-(substitutedphenyl)vinyl)-2-amino-1,3,4-oxadiazoles derivatives **3(a-n)** were synthesized by warming α-benzamido-cinnamhydrazides **2(a-n)** and cyanogen bromide in ethanol. α-Benzamido-cinnamhydrazides **2(a-n)** were obtained by stirring 4-benzylidine-2-phenyloxazole-5-ones **1(a-n)** with a 90 % hydrazine hydrate in ethanol. The chemical structures of title compounds were established by IR, ^{1}H NMR. All the compounds were screened for antimicrobial and antioxidant activities .Among the series of the compounds i.e., 4-methoxy derivative **(3g)** showed significant antibacterial activity. Bromo vanillinyl derivative **(3k)** showed good nitric oxide scavenging activity. 4-dimethylamino derivative **(3f)** showed significant activity in reduction of DPPH.*

Key words: Benzamides, 1,3,4-oxadiazoles, antimicrobial activity, antioxidant activity

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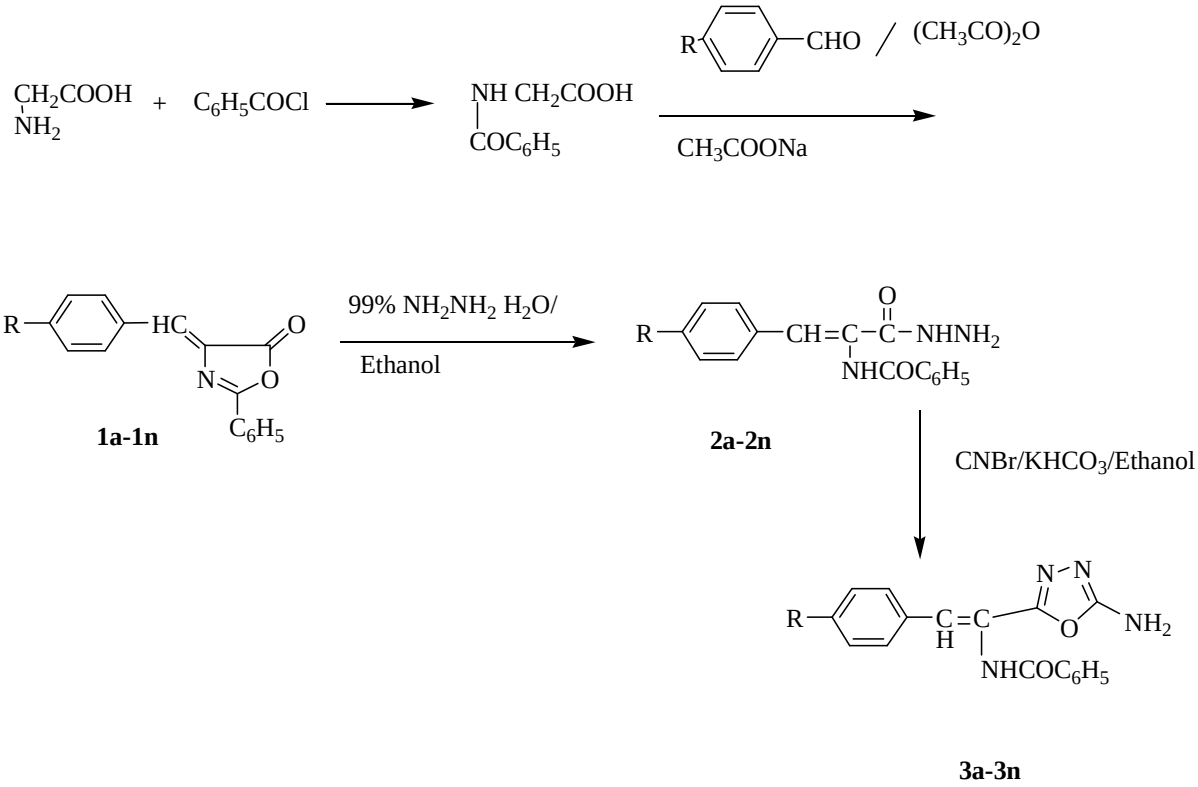
INTRODUCTION

The substituted oxadiazoles are five membered hetero cyclic compounds which serve both as biomimetic and reactive pharmacophores with potential biological activities [1] such as antiinflammatory [2], antimicrobial [3], anticonvulsant [4], antimalarial [5], antidiarrhoeal [6], etc.

EXPERIMENTAL SECTION

All the melting points reported in this series were determined in open capillaries using Thermonik Precision Melting Point cum Boiling Point Apparatus Model C-PMB-2 and are 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 (cm^{-1}). ^{1}H NMR spectra were recorded on GE Omega 400 MHz spectrometer or Bruker AVANCE 300 MHz spectrometer, using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL-JMS-D-300 spectrometer. All solvents are procured from Aldrich and Sigma and are used without further purification



SCHEME

Synthesis of 4-benzylidine-2-phenyloxazole-5-ones 1(a-n)

The various 4-benzylidine-2-phenyloxazole-5-ones were prepared by reported procedure [7], after drying, they were used for subsequent reaction.

Synthesis of α-benzamido-cinnamhydrazides 2(a-n)

4-benzylidine-2-phenyloxazole-5-ones 1 (0.03 mole) was stirred with a solution of hydrazine hydrate (0.06 mole) in ethanol (25 mL) for 30 min. The deep yellow colour of the oxazolone immediately changed to the light yellow coloured solid, which was filtered, washed and recrystallized from methanol.

Table 1 Physicochemical data of the compounds 3(a-n)

Compound	R	Yield(%)	m.p °C
3a	H	84	142-144
3b	4-CH_3	76	150-152
3c	4-CH-$(CH_3)_2$	71	128-129
3d	4-NO_2	76	152-154
3e	4-Cl	74	184-186
3f	4-$N(CH_3)_2$	82	152-154
3g	4-OCH_3	73	170-172
3h	4-OH	65	166-168
3i	4-OH,3-OCH_3	62	176-178
3j	4-OH,3,5-$(OCH_3)_2$	72	186-188
3k	5-Br,4-OH,3-OCH_3	76	175-176
3l	3,4-$(OCH_3)_2$	86	203-206
3m	3,4,5-$(OCH_3)_2$	72	135-136
3n	4-$NHCOCH_3$	70	194-196

Synthesis of 5-(1-benzoylamino-2-(substituted phenyl) vinyl)-2-amino-1,3,4-oxadiazoles 3(a-n)
To an ethanolic solution (60ml) of different substituted hydrazides (12.1gm)(0.05M), cyanogen bromide (5.8g,0.055M) was added. The reaction mixture was warmed at 45ºC for 30 minutes. The reaction mixture was cooled and neutralized with potassium bicarbonate solution; the solid thus separated was filtered, washed with water and recrystallised from methanol.

Antibacterial activity
The title compounds were screened for antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (gram-positive), *Escherichia coli* and *Pseudomonas aureginosa* (gram-negative) strains as bacteria, for antifungal activity against *Aspergillus niger* and *Aspergillus flavus* as fungi. Antimicrobial activity of these compounds was tested by using filter paper disc method [8] in the nutrient agar media by measuring zone of inhibition in millimeter.DMF was run as the control and the compounds were tested at 100µg/ml concentration. Streptomycin was used as a standard drug at 50µg/ml concentration.

Filter Paper Disc Method:
The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1ºC for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of stock culture to nutrient broth. The flasks were incubated at 37±1ºC for 48 hours before the experimentation.

Solutions of the test compounds were prepared by dissolving 5 mg each in dimethylformamide (DMF, 5ml). A reference standard for Gram-positive and Gram-negative bacteria was made by dissolving accurately weighed quantities of streptomycin (50µg/ml).

The nutrient agar medium was sterilized by autoclaving at 121ºC (15 lb/sq.inch) for 15min. Petri-plates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160ºC for an hour. Into each sterilized Petri-plate (10 cm diameter), about 27ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50µl of inoculum to each plate) was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, three discs of 5 mm diameter were used. The test solution at the concentration of 100µg/ml was added to the respective disc aseptically and labeled accordingly. The plates were kept undisturbed for 2 hours at room temperature to allow diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37±1ºC for 24 hours, the diameter of zone of inhibition surrounding each of the discs was measured. All the experiments were carried out in Triplicate. Simultaneously, controls were maintained employing 0.1 ml of DMF to observe the solvent effects.

Table 2 Antimicrobial activity of selected compounds of 3(a-n)

Comp. code	R	Zone of inhibition in mm					
		Antibacterial activity				Antifungal activity	
		E.C	P.A	S.A	B.S	A.N	A.F
3a	H	6	6	NA	NA	8	7
3b	4-CH_3	8	5	NA	NA	8.5	6
3d	4-NO_2	7	7	NA	NA	13	12
3e	4-Cl	7	8	NA	NA	10	11
3g	4-OCH_3	7	-	NA	NA	12	12.5
3h	4-OH	-	6	NA	NA	8.5	12.5
3k	5-Br,4-OH,3-OCH_3	-	-	-	-	14	12
3l	3,4-$(OCH_3)_2$	-	-	-	-	12	9

NA: No activity

Zone of inhibition of test compounds at a concentration of 100µg/mL was measured
E.C *: Esherichia coli* **P.A**: *Pseudomonas aureginosa*
S.A : *Staphylococcus aureus* **B.S**: *Bacillus subtili*
A.N : *Aspergillus niger* **A.F** : *Aspergillus flavus*

Lipid Peroxidation
All the compounds were tested for inhibition of lipid peroxidation induced by ferric ions in rat brain homogenate according to the method of M. Sreejayan and N. A Rao [9]

Nitric Oxide Scavenging Activity
All the compounds were evaluated for antioxidant activity against sodium nitroprusside induced nitric oxide (NO) production, measured by Griess reagent [10]

Reduction of Stable free radical DPPH
All these compounds were screened for stable free radical DPPH scavenging activity by the reported procedure [11].

Table 3. Antioxidant activities of selected compounds of 3(a-n) at 100µM

Comp.code	R	%inhibition of lipid peroxidase	%scavenging of nitric oxide	% reduction of DPPH
3a	H	44.91	35.96	44.16
3b	4-CH_3	28.80	44.40	36.14
3c	4-CH-$(CH_3)_2$	49.50	56.60	56.14
3d	4-NO_2	47.39	42.78	27.65
3e	4-Cl	41.31	28.80	14.51
3f	4-$N(CH_3)_2$	56.59	48.88	70.45
3g	4-OCH_3	41.80	24.20	29.94
3h	4-OH	40.18	48.86	15.53
3i	4-OH,3-OCH_3	48.18	49.19	49.60
3j	4-OH,3,5-$(OCH_3)_2$	57.90	56.18	58.18
3k	5-Br,4-OH,3-OCH_3	57.91	68.28	52.82
3l	3,4-$(OCH_3)_2$	51.98	38.78	46.60
3m	3,4,5-$(OCH_3)_2$	59.35	24.46	42.46
3n	4-$NHCOCH_3$	67.91	36.06	46.60
	α-tocopherol	51.6	-	-

RESULTS AND DISCUSSION

Synthesis of title compounds by the earlier described method resulted in products with good yield. The final products were purified by the recrystallisation techniques with methanol. The synthesised compounds were established on the basis of IR and ^{1}H NMR spectroscopy.

5-(1-benzoylamino-2-(methyl phenyl) vinyl)-2-amino-1,3,4-oxadiazoles (3b) :
3388(NH_2),2926(aliphatic C-H),1641(C=N of 1,3,4-oxadiazole nucleus),1361(penta atomic ring)831(C-N),1676(C=O of $NHCOC_6H_5$) ^{1}H NMR (δ),2.8(s,3H,CH_3),7.0-8.2(m,2H,NH_2)9.8(br,1H,CO-NH)

5-(1-benzoylamino-2-(isopropyl phenyl) vinyl)-2-amino-1,3,4-oxadiazoles (3c) :
3240(NH_2),2961(aliphatic C-H),1036(C-O-C of 1,3,4-oxadiazole nucleus),1603(C=N of 1,3,4-oxadiazole nucleus),1652(C=O of $NHCOC_6H_5$),1365(penta atomic ring),825(C-N) ^{1}H NMR (δ),1.2(d,6H,$CH(CH_3)_2$,2.8(m ,1H ,$CH(CH_3)_2$),6.8(s,2H,NH_2),7-8.2(s,10H,Ar-H& Olefinic protons),10.0(s,1H,CO-NH)

5-(1-benzoylamino-2-(nitro phenyl) vinyl)-2-amino-1,3,4-oxadiazoles (3d) :
3053(Ar-H),1552 and 1330(NO_2),3398(NH_2),2924(aliphatic C-H),1013(C-O-C of 1,3,4-oxadiazole nucleus),1624(C=N of 1,3,4-oxadiazole nucleus),1692(C=O of $NHCOC_6H_5$),1330(penta atomic ring),831(C-N) ^{1}H NMR (δ),6.2(s,2H,NH_2),7-8.4(m,10H,Ar-H & Olefinic protons)10.0(s,1H,CO-NH)

5-(1-benzoylamino-2-(3,4-dimethoxy phenyl) vinyl)-2-amino-1,3,4-oxadiazoles (3l) :
3378(N-H),3086(Aromatic C-H),1596(aromatic C-C),1097(C-O of 1,3,4-oxadiazole nucleus),1667(C=O of $NHCOC_6H_5$),1270(C-O of OCH_3),1021(N-N of 1,3,4-oxadiazole nucleus),1330(penta atomic ring) ^{1}H NMR (δ), 3.84(s,3H,OCH_3),3.86(s,3H,OCH_3),6.2(s,2H,NH_2),6.9-8.6(m,10H,Ar-H & Olefinic protons)10.0(s,1H,CO-NH)

5-(1-benzoylamino-2-(3,4,5-trimethoxy phenyl) vinyl)-2-amino-1,3,4-oxadiazoles (3m) :
^{1}HNMR(δ),3.6-3.8(t,9H,OCH_3),6.8(s,1H,NH_2),6.9-8.2(m,8H,Ar-H&Olefinic protons)10.0(br,1H,CO-NH)

Antimicrobial activity
Among the series of the compounds, substitution by electron withdrawing groups showed significant antibacterial activity against *E.coli and P.aeruginosa*.

4-methoxy **(3g)**, 4-nitro (**3d)** and 5-bromovanillyl **(3k)** derivatives showed good antifungal activity against both *Aspergillus niger* and *A.flavus*. 3,4-dimethoxy **(3l)** derivative showed moderate activity against both *A.niger* and *A.flavus*. (Table 2)

Lipid peroxidation

Among the series of compounds, 4-acetamido derivative **(3n)** showed highest activity. 4-dimethylamino **(3f)** and 3,4-dimethoxy **(3l)** derivatives showed activity comparable to α-tocopherol. 3,4,5-trimethoxy derivative **(3m)** and phenolic derivatives showed significant activity. Other compounds were found to be less active. (Table 3)

Nitric Oxide Scavenging Activity

Among the series of compounds, 5-bromo vanillin derivative **(3k)** showed highest activity. Substitution by 4-methyl group **(3b)** showed significant activity. 4-isopropyl **(3c)** and 4-hydroxy-3,5-dimethoxy **(3j)** derivatives showed moderate activity. (Table 3)

Reduction of Stable free radical DPPH

Among the series of compounds, 4-dimethylamino derivative **(3f)** showed good activity. 4-isopropyl derivative **(3c)** showed moderate activity and the phenolic derivatives showed significant activity [12]. (Table 3)

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