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RESEARCH ARTICLE

Synthesis and Biological Evaluation of Novel N-(2-benzamido-3-phenylacryloyl) Nicotinamide Derivatives.

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ABSTRACT

A series of novel N-(2-benzamido-3-phenylacryloyl) nicotinamide derivatives has been synthesized successfully under mild reaction conditions. All synthesized molecules were characterized on the basis of IR, ^{1}H NMR, Mass spectrometry and Elemental analysis data. The compounds were tested in vitro for their antioxidant ability and for their antimicrobial activity. Compounds 3b, 3c and 3e possess significant biological and biochemical activity.

Keywords: Oxazolones, Nicotinamide, Perkin condensation, Antioxidant activity, Antimicrobial activity.

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INTRODUCTION

The Erlenmeyer reaction was first described in 1893 by Friedrich Gustav Carl Emil Erlenmeyer [1] who condensed benzaldehyde with N-acetyl glycine in the presence of acetic anhydride and sodium acetate. The reaction goes via a Perkin condensation following the initial cyclization of then acetylglycine yielding the so-called Erlenmeyer azlactones. Erlenmeyer azlactones have been used in a wide variety of reactions as precursors for biologically active peptides [2], herbicides, fungicides [3], and as drugs, pesticides and agrochemical intermediates [4].

Oxazolones are heterocyclic compounds which perform an important role in the synthesis of several organic molecules including amino acids [5], amino alcohols, thiamine [6], amides [7], peptides [8] and polyfunctional compounds [9]. Certain natural and synthetic oxazolone also including benzoxazolone [10, 11] derivatives possess important biological activities; such as antimicrobial [12], anti-inflammatory [13], anticancer [14], anti-HIV [15], antiangiogenic [16], anticonvulsant [17], antitumor, antagonistic, sedative [18] activity. These are used as synthons for the construction of various alkaloid skeletons, immunomodulators and biosensors [19] or photosensitive composition devices for proteins.

Nicotinamide (Niacinamide) is a water-soluble vitamin of the B complex, which together with nicotinic acid belongs to vitamin B3 or vitamin PP. Nicotinamide is the active form that acts as constituent of the enzyme cofactors NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) (pyridine nucleotides). These function as electron carriers in cell metabolism of carbohydrates, fatty acids and amino acids [20].

A 2015 trial found nicotinamide to reduce the rate of new nonmelanoma skin cancers and actinic keratoses in a group of people at high risk for the conditions [21]. Nicotinamide has been investigated for many additional disorders, including treatment of bullous pemphigoid nonmelanoma skin cancers [22]. Nicotinamide may be beneficial in treating psoriasis [23]. There is tentative evidence for a potential role of nicotinamide in treating acne, rosacea, autoimmune blistering disorders, ageing skin, and atopic dermatitis [22]. Niacinamide also inhibits poly (ADP-ribose) polymerases (PARP-1), enzymes involved in the rejoining of DNA strand breaks induced by radiation or chemotherapy [24]. ARCON (accelerated radiotherapy plus carbogen inhalation and nicotinamide) has been studied in cancer [25]. HIV Research has suggested nicotinamide may play a role in the treatment of HIV [26]. Based on these findings and in continuation of our work on the synthesis of biologically active compounds in the present work, we report the synthesis, antioxidant and antimicrobial activity of targeted compounds.

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 using BRUKER AV I, 500 MHz in DMSO-d_6 using TMS as an internal standard, IR spectra were recorded on Bruker Alpha FTIR Spectrometer with universal sampling model using KBr pellets and Mass spectra were recorded in apex mass spectrophotometer. TLC was carried out using pre coated Silica gel-G plates. All the chemicals and solvents used were of LR grade and obtained from Sd fine Chem Limited.

Chemistry

Various 4-substituted benzylidene-2-phenyl oxazole-5-one 2a-2e 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 nicotinamide in aqueous acetone containing sodium hydroxide to obtain the title compounds 3a-3e (Scheme).

Experimental Section

Protocol for the synthesis of N-(2-benzamido-3-phenylacryloyl) nicotinamide derivatives:

The starting compound benzoyl glycine (**1**) for the preparation of oxazolones was obtained as described [27].

Synthesis of 4-Benzylidine-2-phenyl oxazolone-5-one (2a-e):

A mixture of substituted aldehydes (0.0476 mmol) benzoyl glycine I (0.0476 mmol) dissolved in acetic anhydride (0.146 mmol) and anhydrous sodium acetate (0.0476 mmol) was heated on electric hot plate with constant stirring. As soon as the mixture liquefies completely transfer the flask to water both and heat for 2 hrs. Then add (10ml) ethanol slowly to contents of flask and mixture was allowed to stand for overnight. Filter the crystalline precipitate with suction and wash the precipitate with 2 portion of ice-cold alcohol (6ml) and finally with 2 portions of boiling water. Dry the product and recrystallized from benzene.
Yield = 3.6g (72%), M.P = 156-158^{0}c.

General protocol for synthesis of N-(2-benzamido-3-phenylacryloyl)nicotinamide (3a-3e):

A solution of Nicotinamide (0.01 mmol) in 1N NaOH and acetone was stirred with 2-oxazoline-5-one (2a-2e) (0.01 mmol) for about 12-14 hr. a clear solution obtained was filtered and acidified with 1N HCl. The solid was separated was filtered and washed thoroughly with cold water and recrystallized from water. The various N-(2-benzamido-3-phenylacryloyl) nicotinamide were prepared in a similar manner.

IIIa: N-(2-benzamido-3-phenylacryloyl) nicotinamide:

IR (KBr) (cm-1): 2952.16(Ar C-H str), 3293.26(N-H str amide),2848.22(C-H str ethylene), 1485.83(Ar C=C str), 1603.94(C=N str), 1698.25(C=O str amide),1114.61(C-N str); **^{1}H NMR (DMSO-d_6), δ ppm:** 7.34 – 7.35 (3H, m, Ar-H), 7.35 – 7.37(1H, S, ethylene CH), 7.37 – 7.39 (2H, m, Ar-H), 7.40 – 7.46 (2H, m, Ar-H),7.51 – 7.60 (2H, m, Ar-H), 7.62 – 7.67 (2H, m, pyridine H), 7.98 – 7.99 (2H, m, pyridine H),9.93 (1H, S, NH), 12.74 (1H, S, NH); **GC-MS (m/z,%)** : 372 (M+1); **Anal. Calcd for $C_{22}H_{17}N_3O_3$:** C, 71.15; H, 4.61; N, 11.31; O, 12.92. Found: C, 71.24; H, 4.12; N, 11.39; O, 13.07.

IIIb: N-(2-benzamido-3-(4-chlorophenyl)acryloyl) nicotinamide:

IR (KBr) (cm-1): 2925.33(ArC-H str),3244.09(N-H str amide), 2853.53(C-H str ethylene), 1479.58(Ar C=C str), 1509.98(C=N str),1644.30(C=O str amide), 709.78(C-Cl str), 1091.00(C- N str); **^{1}H NMR (DMSO-d_6), δ ppm:** 7.43 (1H, S, ethylene CH), 7.45 (2H, m, Ar-H), 7.46 – 7.47 (3H, m, Ar-H), 7.51 – 7.54 (4H, m, Ar-H), 7.59 – 7.68 (2H, m, pyridine H),7.96 -7.98 (2H, m, pyridine H), 9.94 (1H, S, NH), 12.85 (1H, S, NH); **GC-MS (m/z,%)** : 405 (M+); **Anal. Calcd for $C_{22}H_{16}ClN_3O_3$:** C, 65.11; H, 3.97; Cl, 8.74; N, 10.35; O, 11.83. Found: C, 65.42; H, 3.76; Cl, 8.22; N, 10.31; O, 11.92.

IIIc: N-(2-benzamido-3-(4-nitrophenyl)acryloyl) nicotinamide:

IR (KBr) (cm-1): 3065.82(Ar C-H str), 3296.13(N-H str),2850.89 (C-H str ethylene), 1478.75 (Ar C=C str), 1578.27(C=N str), 1689.59 (C=O str amide),1027.45(C-N str), 1443.63(N-O str); **^{1}H NMR (DMSO-d_6), δ ppm:** 7.45 - 7.48 (1H, s, ethylene –CH), 7.50–7.55 (5H, m, Ar-H),7.60- 7.63 (2H, m, Ar-H),7.80 -7.89 (2H, m, Ar-H),7.97-7.98 (2H, m, pyridine H),8.23 – 8.25 (2H, m, pyridine H),10.11 (1H, S, NH),13.06 (1H, S, NH); **GC-MS (m/z,%)** : 417 (M+1); **Anal. Calcd for $C_{22}H_{16}N_4O_5$:** C, 63.46; H, 3.87; N, 13.46; O, 19.21Found: C, 63.26; H, 3.89; N, 13.22; O, 19.47.

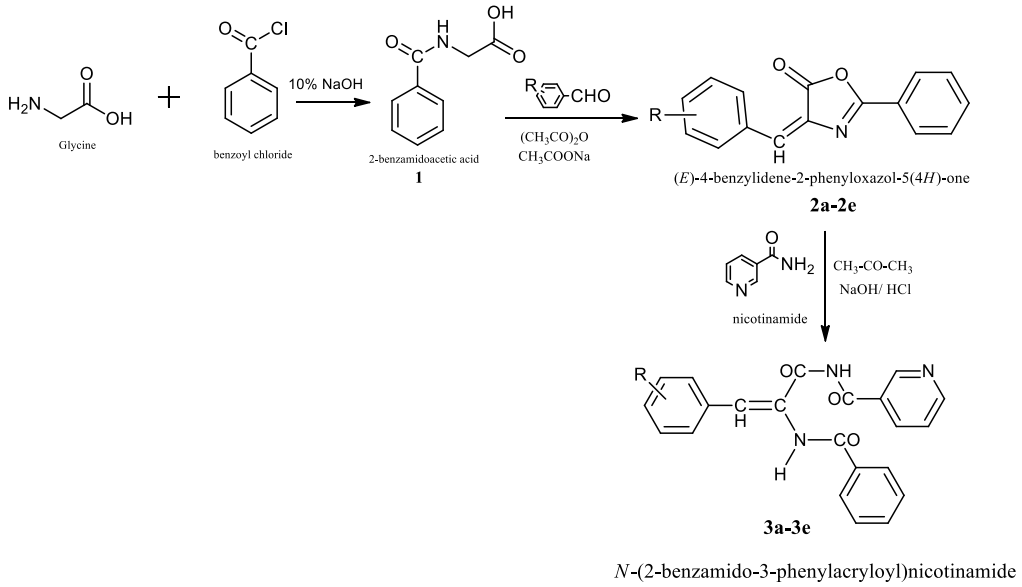
IIId: N-(2-benzamido-3-(4-methoxyphenyl)acryloyl) nicotinamide:

IR (KBr) (cm-1): 2947.15(Ar C-H str), 3216.93(N-H str amide), 2852.13(C-H str ethylene), 1476.87(Ar C=C str), 1597.09(C=N str), 1706.73(C=O str amide),1110.13(C=N str), 2633.96(C-H str OCH_3); **^{1}H NMR (DMSO-d_6), δ ppm:** 6.95 – 6.97 (1H, S, ethylene),7.47 (2H, m, Ar-H), 7.52 – 7.52 (3H, m, Ar-H), 7.59 (4H, m, Ar-H), 7.60 – 7.66 (2H, m, pyridine),7.99 – 8.01 (2H, m, pyridine), 9.83(1H, s, NH), 12.58 (1H, s, NH),

3.76 (3H, S, methoxy); **Anal. Calcd for $C_{23}H_{19}N_3O_4$:** C, 68.82; H, 4.77; N, 10.47; O, 15.94. Found: C, 68.45; H, 4.94; N, 10.42; O, 15.21.

IIIe: N-(2-benzamido-3-(4-hydroxyphenyl)acryloyl) nicotinamide:

IR (KBr) (cm-1): 2950.14(Ar C-H str), 3240.57(N-H str amide), 2872.10(C-H str ethylene), 1462.50 (Ar C=C str), 1621.32(C=N str), 1698.32(C=O str amide), 1115.18(C-N str); **^{1}H NMR (DMSO-d_6), δ ppm:** 6.71-6.77 (1H, S, ethylene), 6.51-6.63(2H, m, Ar-H), 7.92-8.02 (3H, m, Ar-H), 7.65-7.81 (4H, m, Ar-H), 8.12-8.24 (2H, m, pyridine), 9.01 (1H, m, pyridine), 9.54 (1H, s, NH), 10.18 (1H, s, NH), 5.26 (1H, S, hydroxy); **Anal. Calcd for $C_{22}H_{17}N_3O_3$:** C, 68.03; H, 4.67; N, 10.82; O, 16.48. Found: C, 68.20; H, 4.62; N, 10.89; O, 16.43.



Scheme: Synthesis of N-(2-benzamido-3-phenylacryloyl)nicotinamide derivatives (3a-3e).

Antioxidant activity

DPPH free radical scavenging activity

The DPPH free radical scavenging capability was performed as the method described by Koto, K et al [28]. 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. Ascorbic acid was used as a standard. The inhibition of DPPH radical scavengining activity in percent (I %) was calculated according to the following equation (1).

$$I\% = [(A_{blank} - A_{sample}) / A_{sample}] \times 100 \longrightarrow 1$$

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

Assay for Nitric Oxide (NO) scavenging activity:

Nitric oxide radical scavenging activity was determined according to the method reported by Marcocci et.,al [29]. 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 (0.5 ml) 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. The experiment was performed in triplicate, ascorbic acid was used as positive control and percentage scavenging activity was calculated using the equation 1.

Antimicrobial activity

The synthesized compounds were screened for their *in vitro* antimicrobial activity against *E. coli, P. aerugenosa, B. subtilis, S. aureus* by using disc diffusion method [30]. Streptomycin was used as reference standard. The test compounds were tested at a concentration of 50 µg/ml and 100 µg/ml. The average zone diameter of the plates was measured and compared with standard. The Results of antibacterial screening studies are reported in Table 3.

RESULTS AND DISCUSSION

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. Various 4-substituted benzylidene-2-phenyl oxazole-5-ones which are prepared by condensing aromatic aldehydes with benzyl glycine in the presence of acetic anhydride and anhydrous sodium acetate. Finally oxazolones (2a-e) were condensed with nicotinamide is aqueous acetone containing sodium hydroxide resulted in the formation of N-(2-benzamido-3-phenylacryloyl) nicotinamide derivatives (3a-e) with the yields in the range of 30-70%. The structures of prepared compounds have been confirmed by FTIR, ^{1}H NMR, Mass and elemental analysis.

Free radical scavenging activity of synthesized compounds (3a-3e) was performed by DPPH radical and nitric oxide (NO) scavenging methods. The data in table 1 and table 2 revealed that the nicotinamide derivatives showed significant antioxidant activity compared to standard. In DPPH method the compounds show the reactivities at 100µM with DPPH of 100 µM concentration (Fig 1). Among all these 4-hydroxy and 4-methoxy derivatives exhibited the maximum activity was found to 81.33% & 75.73% respectively. The substitution with electron withdrawing groups like 4-nitro (49.01%) & 4-chloro (43.54%) exhibited significant activity at 100 µM (Table 1). All the compounds were tested for their ability to scavenging nitric oxide at 100 µM concentration. Fig 2 showed the scavenging of nitric oxide free radical activity of nicotinamide derivatives. Among the substituted compounds, phenolic derivative i.e 4-hydroxy group showed highest activity (76.13%). The 4-methoxy (68.49%) & unsubstituted (55.93%) derivatives showed good to moderate inhibition at100 µM (Table 2).

The synthesized compounds were screened for their antimicrobial activity against *B.Subtilis, S.aureus, E.coli & P. aerugenosa* bacterial strains by disk diffusion method. All tested compounds have shown significant antimicrobial activity. Compound 3b, 3c and 3e showed good activity at 100 µg/ml concentration where as compound 3d showed good activity against gram negative bacteria. The results revealed that presence oxazolone moiety and hybridization of nicotinamide ring is a satisfactory backbone for antimicrobial activity. The presence of electron donating groups in the compounds may be responsible for enhancing biological and biochemical activity.

Table 1: % inhibition of DPPH radical by compounds (3a-3e) and ascorbic acid:

Compound	R	% Inhibition
Ascorbic acid		88.16
3a	H	32.1
3b	4-Cl	43.54
3c	4-NO_2	49.01
3d	4-OCH_3	75.73
3e	4-OH	81.33

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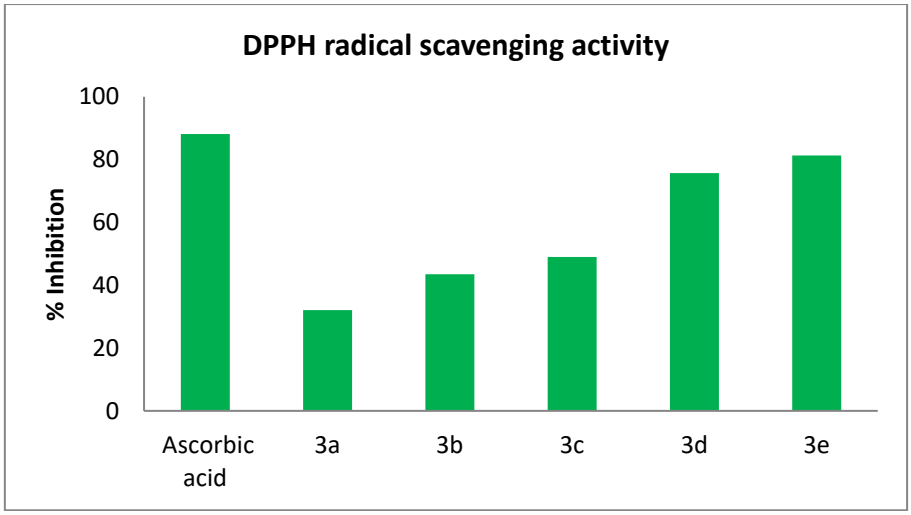

Figure 1: Reduction of DPPH stable free radical of N-(2-benzamido-3-phenylacryloyl) nicotinamide (3a-3e).

Table 2: %inhibition of Nitric Oxide radical by compounds (3a-3e) and ascorbic acid:

Compound	R	% Inhibition
Ascorbic acid		80.13
3a	H	55.93
3b	4-Cl	42.88
3c	4-NO_2	32.23
3d	4-OCH_3	68.49
3e	4-OH	76.13

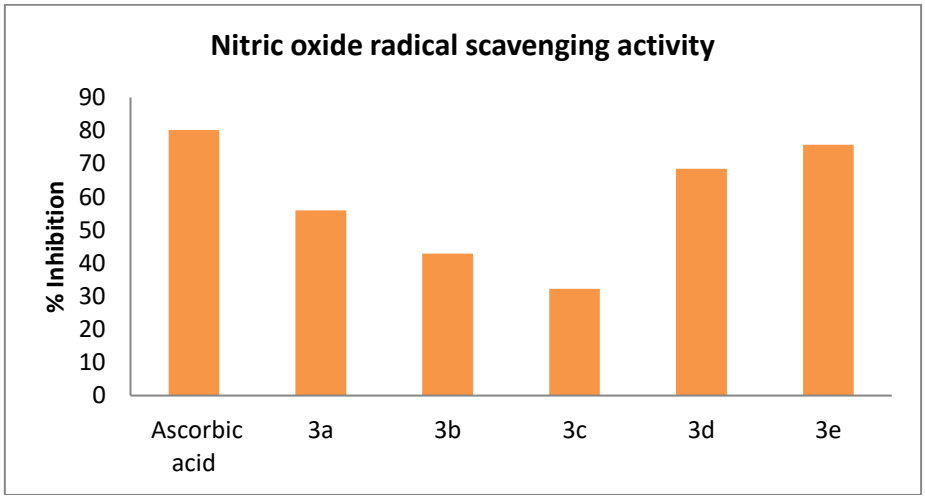

Figure 2: Nitric oxide free radical scavenging activity of N-(2-benzamido-3-phenylacryloyl) nicotinamide (3a-3e).

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Table 3: Antimicrobial activity of N-(2-benzamido-3-phenylacryloyl) nicotinamide (3a-3e):

Compound code	Conc. (µg/ml)	Zone of inhibition (mm)			
		Gram-Positive Organism		Gram-Negative Organism	
		B.Subtilis	*S.aureus*	*E.coli*	*P. aerugenosa*
3a	50	10	11	13	12
	100	16	15	16	15
3b	50	14	14	18	16
	100	20	19	22	23
3c	50	12	16	19	9
	100	17	20	25	12
3d	50	N	N	12	11
	100	8	11	16	19
3e	50	16	14	13	13
	100	21	19	17	18
Streptomycin	50	21	24	26	20
Control	-	-	-	-	-
N = No inhibition.					

CONCLUSION

In present investigation we have synthesized successfully a series of N-(2-benzamido-3-phenylacryloyl) nicotinamide derivatives with good antioxidant and antimicrobial activity. Obtained results revealed that most of the synthesized compounds showed promising activity as antioxidant and antimicrobial agents therefore might serve as lead molecules for further biological evaluation.

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