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SYNTHESIS, ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF NEWER 3-(1-BENZOFURAN-2-YL)-5-SUBSTITUTED ARYL-1, 2-OXAZOLE

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ABSTRACT:

The 3-(1-BENZOFURAN-2-YL)-5-SUBSTITUTED ARYL-1,2-OXAZOLE have been synthesized by the reaction of Benzofuran chalcone with hydroxylamine hydrochloride in presence of sodium acetate in ethanol. All the compounds were synthesized by conventional method and characterized by IR, ^{1}H NMR and mass spectral data. The synthesized compounds are screened for the antioxidant activity by Nitric oxide scavenging activity, H_2O_2 scavenging ability, Lipid peroxidation inhibition methods. Ascorbic acid was used as standard.

Keywords: Benzofuran, oxazole, chalcone, conventional, antioxidant activity

[I] INTRODUCTION:

Heterocyclic synthesis has emerged as powerful technique for generating new molecules useful for drug discovery [1]. Heterocyclic compounds provide scaffolds on which pharmacophores can arrange to yield potent and selective drugs [2].

Benzofuran nucleus may be combined with nitrogen heterocycles in different ways. Several benzofuran compounds are reported to posses, antibacterial [3], antifungal [4], Anti-inflammatory [5], antidepressant [6], analgesic [7] and hypoglycemic activities [8].

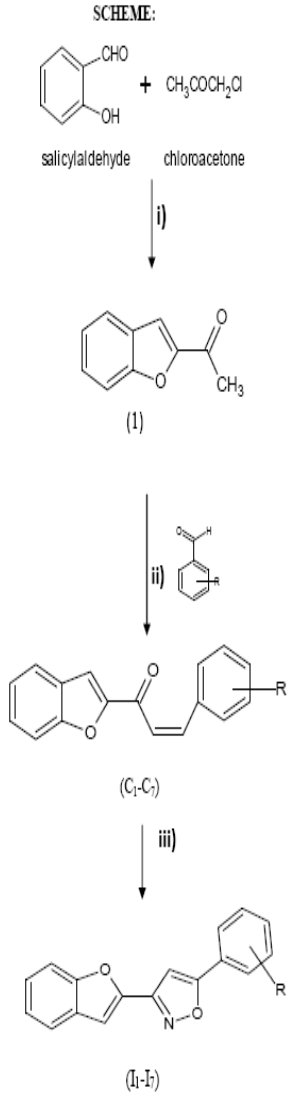
It has already been pointed out that; benzofuran nucleus is very rarely associated with a nitrogen heterocycle. Several isoxazole derivatives are found to possess antitubercular [9], antimicrobial [9]and anti-inflammatory [9] activities.

In our present thesis work we are synthesizing new compound i.e. benzofuran fused with isoxazole ring.

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors [10]. *In-vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intercellular signaling or synthesis of biologically important compounds [11]. However, ROS may also be very damaging; they can attack lipids in cell membranes and also attack DNA, inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity and also cause DNA mutation leading to cancer [12-13]. A potent scavenger of these species may serve as a possible preventive intervention for free

radical-mediated diseases [14]. In the present investigation, radical scavenging and antioxidative capacity for the newly synthesized compounds are evaluated using three anti-oxidant methodologies.

[II] MATERIALS AND METHODS:



i - K_2CO_3, dry acetone, ii - Ethanol (95%), KOH (10%), iii - NH_2OHHCl, CH3COONa, Ethanol.

2.1 Experimental section:

Reactions and purity of compounds were monitored by TLC (silica gel G60) using ethylacetate:benzene (1:1) solvent system and the spots were identified by iodine vapor chamber. Melting points were determined in open capillary using paraffin bath and are uncorrected. The IR spectra of the compounds were recorded on NICOLET380 FT-IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded in DMSO on a 300 MHz Shimadzu FT-NMR (δ in ppm) relative to TMS as internal standard. The mass spectra were recorded on Triple Quadupole LC-MS with ESI source. Mfg. SCIEX at 70eV.

Preparation of 2-acetyl benzofuran (Compound 1):

Salicylaldehyde (0.1 mole) was taken in 50 ml of absolute ethanol. To this, KOH (0.1 mole) crystals were added and the reaction mixture was stirred for 5 minutes in ice bath. To this reaction mixture chloroacetone (0.1 mole) was added drop by drop from dropping funnel about 10 minutes. Further whole reaction mixture was allowed to stir for another 20 minutes with catalytic amount of potassium iodide (KI). The resultant solution was poured into the crushed ice, the solid obtained was filtered and recrystallised from ethanol to produce 2- acetyl benzofuran and was used directly for the next step.

Compound 1: 90-95% yield, Melting Point (MP) 74-76^{0}C, R_f Value: 0.57

IR (KBr cm^{-1}): 1603 (C=C str Ar), 1163 (C-O-C), 2926 (-CH str in CH_3), 1651 (C=O).**^{1}H NMR (DMSO δ ppm):** 7.0-7.4 (m, 5H, Ar-H), 2.5 (s, 3H, CH_3).

Preparation of (2Z)-1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one (Chalcone, C1)

Equimolar quantities of benzaldehyde (0.01mol) and 2-acetyl benzofuran (0.01mol) were dissolved in minimum amount of ethanol. Potassium hydroxide solution (0.02mol) was added slowly and

the mixture stirred for 2 hrs until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol. Finally the compound synthesized namely, (2Z)-1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one (C1, Chalcone). The completion of the reaction was monitored by TLC. Similarly various chalcones C2-7 were prepared.

67% Yield, MP: 84-86^{o}C, Rf Value: 0.5

Compound C1: IR (KBr cm^{-1}): 2955 (-CH str Ar), 1648 (C=O), 750 (Ar-H), 1452 (C-O- C). **1H NMR (DMSO δ ppm):** 7.9-7.3 (m, 6H, Ar-H), 6.7-6.0 (d, 2H, chalcone).

Compound C2: IR (KBr cm^{-1}): 1344 (NO str), 2957 (Ar-CH str), 1519 (C=N), 1453 (CH=CH), 1107 (C-O-C).

Compound C3: IR (KBr cm^{-1}): 1357 (NO str), 2968 (Ar-CH str), 1527 (C=N), 1458 (CH=CH), 1116 (C-O-C).

Compound C4: IR (KBr cm^{-1}): 3415 (-OH str), 2929 (-CH str), 1646 (C=O), 756 (Ar-H), 1455 (CH=CH).

Compound C6: IR (KBr cm^{-1}): 1260 (C-O-C), 2926 (-CH str), 1455 (CH=CH), 753(Ar-H), 1652 (C=O).

Compound C7: IR (KBr cm^{-1}): 1268 (C-O-C), 2932 (-CH str), 1468 (CH=CH), 758(Ar-H), 1660 (C=O). **1H NMR (DMSO δ ppm):** 8.3 (S, 1H of Ar-H of benzofuran), 7.6 (s, 1H of Chalcones), 7.2-7.0 (m, 4H of Ar-H of benzofuran), 6.9 (s, 1H of chalcone).

Preparation of 3-(1-benzofuran-2-yl)-5-phenyl-1,2-oxazole (I1)

A mixture of chalcone, C1 (0.02 mol), hydroxylamine hydrochloride (0.02 mol) and catalytic amount of sodium acetate in ethanol (25 ml) was refluxed for 6 h. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized from ethanol. Finally the compound synthesized namely 3-(1-benzofuran-2-yl)-5-phenyl-1,2-oxazole (I1). The completion of the reaction was monitored by TLC. Similarly various isoxazole derivatives I2-7 were prepared.

Table 1: Physical data of synthesized compound

Product Code	R	Melting Point (^{0}C)	Yield (%)	R_f Value:
I1	-H	120-121	89	5.8
I2	-NO_2 (p)	172-174	54	5.4
I3	-NO_2 (m)	Semisolid	78	4.9
I4	-OH (p)	157-159	60	5.7
I5	-C_2H_5O (m),-OH (p)	117-119	58	6.1
I6	CH_3O (m)	125-126	90	5.8
I7	-CH_3O (o, m, p)	semisolid	74	5.3

Spectral studies of new synthesized compounds:

Compound I1: IR (KBr cm^{-1}): 2926 (-CH str), 1453 (CH=CH), 1378 (C-O-C), 753 (Ar-H).

Compound I2: IR (KBr cm^{-1}): 1454 (CH=CH), 1345 (NO str), 753 (Ar-H), 2957 (-CH str).

Compound C3: IR (KBr cm^{-1}): 1459 (CH=CH), 1357 (NO str), 753 (Ar-H), 2963 (-CH str).

Compound I4: IR (KBr cm^{-1}): 3356 (OH), 1456 (CH=CH), 2926 (-CH str), 1157 (C-O-C).

Compound I5: IR (KBr cm^{-1}): 3337 (OH), 2956 (-CH str), 1248 (C-O-C), 752 (Ar-H), 1454 (CH=CH).

Compound I6: IR (KBr cm^{-1}): 3372 (NO), 2955 (CH str), 1455 (CH=CH), 1601 (C=C). **1H NMR DMSO δ (ppm):** 3.8 (m, 3H of OCH_3), 8.0-6.9 (m, 8H, Ar-H), 5.4 (s, 1H of Isoxazole), 3.8 (m, 3H of OCH_3). **MS (m/z):** 291

Compound I7: IR (KBr cm^{-1}): 1125 (C-O-C), 1455 (CH=CH), 1590 (C=C), 2955 (-CH str). **1H NMR (DMSO δ ppm):** 7.4-7.1 (m, 7H, Aryl-H), 6.8 (s, 1H of isoxazole), 3.8-3.2 (m, 9H of OCH_3).

2.2 Evaluation of Antioxidant Activity:

2.2.1 Nitric oxide scavenging activity

The Nitric oxide scavenging activity of the synthesized compounds I1-**7** was determined according to the method of Green *et al* [15]. The different concentrations (12.5, 25, 50, 100 µg/ml) of test compound were taken in the volume of 200 µl. Nitric oxide production was initiated with the addition of 200 µl of phosphate buffer (0.1 M, pH 7.4) and 800 µl Sodium nitroprusside (10mM) and the reaction mixture were incubated at 25°C for 150 min. A control tube was also processed in the same way except for test extract ethanol was used. After the incubation time 1.2 ml of Griess reagent was added to the tubes and they were kept at room temperature for 30 min and the color developed, the optical density was read at 540 nm (Green et al., 1982).

% Inhibition of NO = $[A_0 - A_1]/A_0 \times 100$,

Where A_0 is the absorbance before reaction and A_1 is the absorbance after reaction has taken place.

Table 2: In vitro antioxidant activity of isoxazole derivatives against nitric oxide radical inhibition method

Compound Code	% inhibition ± S.D.*			
	100 µg/ml	**50 µg/ml**	**25 µg/ml**	**12.5 µg/ml**
Standard	93.14±0.56	68.13±1.46	32.34±1.36	8.89±0.37
I1	81.99±0.36	74.46±0.90	20.45±2.41	10.80±2.93
I2	56.23±0.35	75.15±0.73	21.75±1.37	13.07±0.07
I3	80.00±0.98	69.01±1.94	13.70±2.87	10.87±0.87

I4	92.14±0.65	65.87±3.08	40.76±2.14	21.45±1.07
I5	80.45±1.08	81.48±1.06	60.09±2.01	40.67±1.07
I6	20.15±1.10	15.50±1.20	13.23±2.01	10.56±1.10
I7	25.13±1.34	22.11±1.44	18.77±1.55	17.22±1.25

In nitric oxide radical scavenging method compound I5 has shown potent antioxidant activity at the concentration of 50, 25 and 12.5 µg/ml than the standard ascorbic acid. Whereas Compound I4 shown potent antioxidant activity then the standard ascorbic acid at the concentration of 100 µg/ml. compounds I1, I2 and I3 also shown moderate antioxidant activity at the concentration of 50 µg/ml. All other compounds found to be weak antioxidant activity.

2.2.2 H_2O_2 scavenging activity:

The H_2O_2 scavenging ability of the synthesized compounds **I1-7** was determined spectrophotometrically according to the method of Ruch *et al* [16]. Briefly, a solution of hydrogen peroxide (2 mM) was prepared in 0.17 M phosphate buffer (pH 7.4). Various concentrations of the samples (in methanol) were added to the reaction mixture containing 2 mM hydrogen peroxide. After 10 min incubation at room temperature, the absorbance was read against a blank at 230 nm.

H_2O_2 scavenging activity (%) = [(A control – A sample)/A blank] x 100.

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

Table 3: In vitro antioxidant activity of isoxazole derivatives against hydrogen peroxide radical inhibition method

Compound Code	**% inhibition ± S.D***			
	100 µg/ml	**50 µg/ml**	**25µg**	**12.5µg**
Standard	88.14±0.56	74.13±1.46	33.34±1.36	11.89±0.37
I1	27.14±0.07	25.77±0.76	13.23±0.43	7.06±1.26
I2	53.21±2.56	23.54±2.56	9.54±0.36	6.89±2.03
I3	71.21±1.53	43.67±1.58	28.55±1.26	12.45±0.34
I4	89.39±1.33	62.43±0.66	31.28±2.46	11.23±0.87
I5	91.23±0.33	59.67±2.54	28.67±1.56	10.68±0.59
I6	22.13±0.06	18.56±0.56	15.65±0.33	14.98±2.56

I7	58.76±0.96	27.54±0.11	8.56±0.58	4.23±0.44

*Average of three determinations

In the hydrogen peroxide method compounds I4 and I5 at concentration 100 µg/ml were showed potent antioxidant activity than the standard ascorbic acid. Compound I3 found moderate antioxidant activity. All the other compounds found low antioxidant activity against hydrogen peroxide.

2.2.3 Lipid peroxidation inhibitory activity

The Lipid peroxidation inhibitory activity of the synthesized compounds **I1-7** was determined according to the method of Okhawa *et al* [17]. The reaction mixture containing 0.5 ml of 10% rat liver homogenate in 0.1 M KCl (pH 7.4) and 0.5 ml of various concentrations (12.5, 25, 50, 100 µg/ml) of test compounds and 0.2 ml of $FeSO_4$ (15 mM) was incubated at 37°C for 30 min and it was again treated with 1.0 ml 10% TCA and 1 ml of 0.67% thiobarbituric acid. This mixture was heated in 90°C for 20 min. The pink colour developed was read at 532 nm. The control tube contains methanol instead of test sample.

Table 4: *In vitro* antioxidant activity of isoxazole derivatives and standard ascorbic acid against lipid peroxidation method.

Compound Code	**Percentage inhibition ± S.D*.**			
	100 µg/ml	**50 µg/ml**	**25 µg/ml**	**12.5 µg/ml**
Standard	80.33±1.54	81.12±2.54	60.41±0.70	40.32±0.64
I1	67.46±1.56	62.01±0.11	33.46±0.58	11.23±3.94
I2	64.21±2.56	55.54±2.56	11.54±1.56	1.89±2.03
I3	56.21±1.53	51.67±1.56	18.55±2.56	10.75±0.46
I4	94.39±2.52	69.43±0.56	30.28±0.51	12.43±2.76
I5	90.23±0.59	60.67±2.54	29.97±0.22	11.68±1.46
I6	68.53±0.74	53.76±1.54	13.97±0.22	6.68±0.56
I7	29.14±0.56	27.77±0.56	14.23±0.66	8.16±1.46

*Average of three determinations

Among the compounds tested for antioxidant activity in lipid peroxidation method, compounds I4 and I5 found to be good antioxidant activity. The compounds I1, I2, I3, I6 and I7 found to be weak antioxidant activity.

Table 5: List of IC50 value of synthesized compounds (I1-I7) and standard ascorbic acid.

Compound code	**IC50 ± S.D. (µg/ml)***		
	Nitric oxide	**H_2O_2**	**Lipid peroxidation**
I1	41±0.08	<100	65±0.10
I2	44±0.51	87±0.32	81±0.15

I3	39±0.41	65±0.18	97±0.16
I4	31±0.32	37±0.23	38±0.45
I5	34±0.01	40±0.02	41±0.10
I6	<100	<100	82±0.08
I7	<100	91±0.58	<100
Standard (Ascorbic acid)	36±0.14	38±0.11	34±0.12

*Average of three determinations

RESULTS AND DISCUSSION:

All the above-synthesized compounds were tested for their antioxidant activity, using *in vitro* Nitric oxide scavenging activity, Hydrogen peroxide scavenging activity and Lipid peroxidation inhibition methods. Among the tested compounds, compound I4 and I5 showing most potent activity by Nitric oxide scavenging activity method. The IC50 value of the compounds was found to be 31±0.32 µg/ml and 34±0.01 µg/ml. While I1, I2, I3 was found to be moderately active.

In the Hydrogen peroxide scavenging method, compound I4 and I5 showing most potent activity with IC50 value 37±0.23 and 40±0.02 respectively. Similarly I4 and I5 showed potent activity by Lipid peroxidation inhibition methods.

CONCLUSION:

Benzofuran with fused isoxazole compounds are reported to posses, antibacterial, antifungal, anti-inflammatory and antitubercular activities. Here when these moieties are fused and screened for antioxidant activitiy. They showed good antioxidant property. Among these derivatives 3-ethoxy,4-Hydroxybenzaldehyde substituted derivative (I5) showed good antioxidant activity. The free OH group on derivative is responsible for antioxidant activity as it proven by testing compound I4 and I5, which showed good activity. Above results establish the fact that benzofuran with fused isoxazole can be a rich source for exploitation.

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