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Evaluation of *in vitro* antioxidant activity of benzophenone-pyridine analogues

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ABSTRACT

A series of synthesized benzophenones possessing pyridine nucleus ***8a–l*** *were evaluated for antioxidant activity by in vitro studies through different in vitro models such as DPPH, nitric oxide and hydrogen peroxide free radical-scavenging activity. The antioxidant activity result shows that compound* ***8k*** *with hydroxy and methyl groups exhibited highest activity. Compounds* ***8l*** *with amino and methyl groups has shown second highest activity****.*** *Further, compounds* ***8b*** *with fluoro and hydroxy groups****,*** ***8e*** *with chloro and hydroxy groups and* ***8h*** *with iodo and hydroxy groups also exhibited activity next to compounds* ***8k*** *and* ***8l..***

Keywords: Antioxidant activity, DPPH, nitric oxide, hydrogen peroxide.

INTRODUCTION

Oxidation, caused by reactive oxygen species (ROS), is a pervasive biological process in physiology and metabolism of many organisms [1]. ROS are normally generated in the human body and scavenged by antioxidant defenses system when ROS remains at physiological concentrations [2]. It is essential to preserve the endogenous antioxidant defense systems and normal cell functions when ROS remains at physiological concentrations. Therefore, the body can have the capacity to avoid many harmful damages [3]. However, these systems are insufficient to prevent the harm entirely [4]. It is reported that free radicals, including superoxide anion, hydroxyl radical, and hydrogen peroxide can cause pathological damages like cancer disease, diabetes, atherosclerosis, coronary heart disease, and many other diseases associated with aging to the organism, and lead to harmful alterations in foods and pharmaceutical industries [5-7]. Therefore, it is urgent to develop antioxidant supplements to help the human body reduce oxidative scratch.

Pyridine is one of the most prevalent heterocyclic compounds in nature. For example, it is present in the coenzyme vitamin B_6 family and in numerous alkaloids, further it plays a central role as versatile building block in the synthesis of natural products as well as biologically active compounds. Further, pyridine bases are widely used in pharmaceutics as nicotinamides and nicotinic acid derivatives. The various therapeutic potential of pyridine derivatives have been reported in the treatment of cancers of diverse cells, by targeting angiogenesis [8-11], apoptosis

[12,13] and by inhibiting wide range of tumour promoting factors like, FAK [14], CDK [13,16] and topoisomerase II [17]. Nevertheless, benzophenone derivatives are extensively used in medicine research for their recognized potencies against various pathological conditions including cancer [18–22]. In recent years, our group has reported a

number of novel benzophenone conjugated analogues as potent inhibitors targeting angiogenesis [21–26] and apoptosis. [27, 28]

On the basis of aforementioned encouraged results, we investigate the antioxidant activities of the synthesized compounds **8a-l.**

MATERIALS AND METHODS

***In vitro* antioxidant activity**

DPPH radical scavenging assay

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1, 1-diphenyl-1-picrylhydrazyl (DPPH) [29-33]. The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (25, 50, 75, 100 and 250 mg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation (1)

% of scavenging = [(A control - A sample)/A blank] ×100 --------- (1)

Where A is the absorbance of the control reaction (containing all reagents except the test compound) and a sample is the absorbance of the test compound. Tests were carried at in triplicate.

Nitric oxide radical scavenging assay

Nitric oxide scavenging activity was measured by slightly modified methods of Marcocci et al [34]. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 mg/mL) of the test compounds and incubated for 150 minutes at 25^{O} C and 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm and nitric oxide scavenging activity was calculated using Eq. (1).

H_2O_2 radical scavenging assay

The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch et al [35]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75 and 100 mg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated using Eq. (1).

RESULTS AND DISCUSSION

The reaction sequence for various title compounds **8a-l** was outlined in Scheme 1. Substituted phenyl benzoates **3a-d** were synthesized by stirring 2-chloro-6-fluoro phenol **1** with substituted acid chlorides **2a-d** in alkaline medium using triethylamine. The phenyl benzoates **3a-d** were subjected to Fries rearrangement to afford hydroxy benzophenones **4a-d.** Condensation of **4a-d** with ethyl chloroacetate in the presence of anhydrous potassium carbonate in dry acetone gave ethyl (2-aroyl-4-methylphenoxy) acetates **5a-d**, which on treatment with 99% hydrazine hydrate in ethanol gave 4-aryloylaryloxyacethydrazides **6a-d**. Finally, the title compounds **8a-l** were achieved in excellence yield by coupling **6a-d** with substituted nicotinic acids **7a-c** in the presence of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as coupling agent and 2,6-dimethyl pyridine (lutidine). The structures of the compounds were elucidated by IR, ^{1}H NMR and mass spectral studies and also by microanalyses. [36]

All the synthesized compounds **(8a-l)** were screened for *in vitro* antioxidant activity through different *in vitro* models such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) free radical-scavenging activity and the results were tabulated in table **1, 2** and **3** respectively. Observing these results it indicates that, some of the tested compounds are significant in their antioxidant properties. Mainly compounds **8k, 8l, 8b, 8e** and **8h** were most efficient among the series with its IC_{50} value almost comparable with the standard drug ascorbic acid in all the above three methods. In DPPH method compounds **8k, 8l, 8b, 8e** and **8h** shown IC_{50} values of 15.67, 15.78, 16.85, 16.86 and 18.78 µg/mL respectively, compared to the standard ascorbic acid 14.97µg/mL.

The same compounds **8k, 8l, 8b, 8e** and **8h** in nitric oxide scavenging assay shows IC_{50} values of 15.98, 25.97, 16.11, 14.84 and 18.77 µg/mL respectively, compared to the standard ascorbic acid 13.97 µg/mL. Further, in hydrogen peroxide radical scavenging assay compounds **8k, 8l, 8b, 8e** and **8h** shown IC_{50} values of 17.54, 16.35, 19.66, 17.70 and 18.93 µg/mL respectively, compared to the standard ascorbic acid 15.83 µg/mL.

Table 1: *In vitro* antioxidant activity of compounds 8a-l in DPPH method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
8a	46.36±0.57	51.21±1.20	54.98±0.53	60.31±0.79	23.78±1.01
8b	69.84 ± 0.24	73.85 ± 0.41	76.69 ± 0.63	80.56 ± 0.68	16.85 ± 0.59
8c	64.33 ± 0.31	66.74 ± 0.47	69.51±0.66	73.49 ± 0.79	19.98 ± 1.27
8d	54.26 ± 0.90	57.13 ± 1.40	60.25± 0.79	65.64 ± 1.06	24.79 ± 0.43
8e	64.19±1.08	67.83± 1.27	69.75± 0.58	72.36±0.69	16.86±0.53
8f	59.74 ± 1.17	64.47 ± 1.24	68.91 ± 0.88	72.17 ± 0.95	20.94 ± 0.78
8g	48.62 ± 0.60	52.85± 1.24	56.61 ±0.55	61.92 ± 0.82	25.67 ± 1.10
8h	62.56 ±0.69	66.74 ±1.30	70.69±1.27	75.77 ±1.32	18.78 ±1.21
8i	60.41±1.18	65.76 ± 1.25	69.52±0.89	73.64 ± 0.96	21.36 ± 0.77
8j	51.87 ± 1.70	57.95± 1.00	60.68± 0.90	66.99 ± 1.58	22.83 ± 1.45
8k	72. 94 ± 0.17	76.78 ± 0.43	78.95 ± 0.44	82.79± 0.68	15.67 ± 0.58
8l	78.85± 0.27	79.85 ± 0.48	82. 92± 0.59	84.89 ± 0.76	15.78 ± 0.26
Ascorbic acid	81.97±0.11	82.89±0.36	84.84±0.42	86.78±0.51	14.97±0.42
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ±SD.

Table 2: The *in vitro* antioxidant activity of compounds 8a-l in nitric oxide (NO) method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
8a	71.48 ±0.83	73.78 ± 0.92	77.82 ± 1.04	81.96 ± 1.39	24.88 ± 0.79
8b	75.41 ± 0.17	77.60 ± 0.32	81.39 ± 0.60	85.59 ± 0.70	16.11 ±0.25
8c	62.83 ± 1.30	70.84 ± 1.22	75.79 ±1.02	77.41 ±0.77	19.89±1.16
8d	74.75 ± 0.24	78.92 ± 0.35	81.71 ± 0.55	84.82±0.70	26.86±0.91
8e	70.53 ±0.14	75.72 ± 0.32	78.91 ±0.51	82.89 ± 0.66	14.84 ± 0.79
8f	54.38± 1.19	58.07± 0.88	62.75 ± 1.49	66.19 ± 1.00	22.98 ± 0.69
8g	72.86 ± 0.24	77.83 ± 0.43	81.79±0.60	83.68 ± 0.77	16.67 ±0.89
8h	63.85 ± 1.16	68.95 ± 1.55	73.82 ± 1.39	78.92 ± 0.69	18.77 ± 1.21
8i	66.86 ± 0.84	69.89 ± 1.37	73.95 ± 0.93	78.96 ± 1.19	23.89 ± 1.37
8j	65.67 ±1.65	68.78 ± 1.30	72.83 ± 1.40	77.56 ± 0.74	25.97 ± 1.19
8k	84.19 ± 0.38	89.28 ± 0.57	92.62± 0.71	98.18± 0.89	15.98 ± 0.77
8l	77.58 ± 0.26	82.84 ± 0.45	83.73 ± 0.59	85.79±0.78	14.62 ± 0.75
Ascorbic acid	83.63±0.17	84.65±0.35	87.54±0.51	89. 97±0.68	13.97±0.54
Blank	-	-	-	-	-

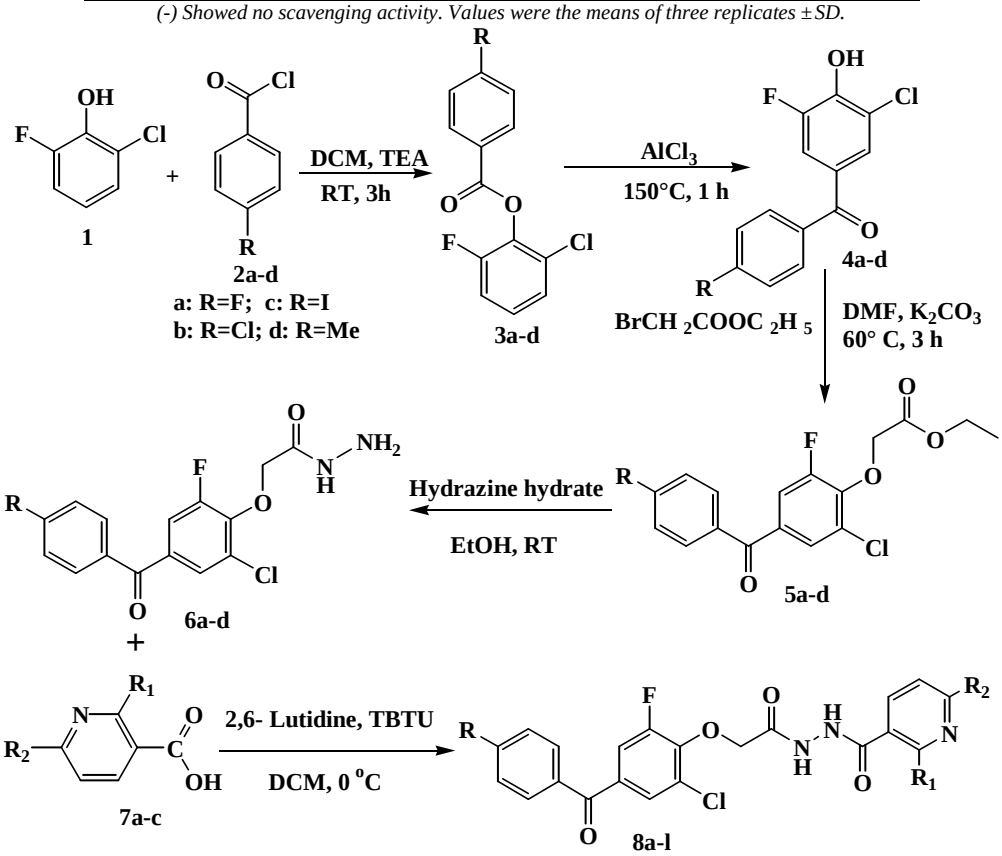
(-) Showed no scavenging activity. Values were the means of three replicates ±SD.

The antioxidant activity result shows that compound **8k** with hydroxy and methyl groups exhibited highest activity. Compounds **8l** with amino and methyl groups has shown second highest activity**.** Further, compounds **8b** with fluoro and hydroxy groups**, 8e** with chloro and hydroxy groups and **8h** with iodo and hydroxy groups also exhibited activity next to compounds **8k** and **8l.**

Table 3: The in vitro antioxidant activity of compounds 8a-l in hydrogen peroxide (H_2O_2) method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
8a	47.65 ± 0.59	51.86 ± 1.22	55.63 ± 0.54	60.93 ± 0.81	25.69 ± 1.10
8b	60.76 ±1.30	63.83 ± 1.16	67.84±1.05	70.66 ± 1.55	19.66±0.75
8c	59.63 ± 1.16	63.44 ± 1.21	67.82 ±0.86	71.11±0.93	20.91±0.77
8d	53.66±1.17	57.73 ± 0.86	61.81 ± 1.47	65.73 ± 0.80	21.55±0.66
8e	70.59 ±0.26	74.21 ± 0.43	77.85 ±0.65	81.92 ±0.70	17.70 ± 0.61
8f	50.84 ± 1.14	53.78 ± 0.84	57.83 ± 0.95	62.91 ± 0.61	24.59 ± 1.05
8g	65.84 ± 1.10	68.68 ± 1.29	70.88 ±0.58	73.85 ± 0.69	17.64±0.58
8h	61.63 ± 0.27	64.84 ± 0.44	67.52 ± 0.63	71.74 ± 0.76	18.93±1.24
8i	52.88 ± 0.87	55.76 ± 1.37	58.74 ± 0.76	63.69 ± 1.03	22.77 ± 0.39
8j	45.02 ± 0.88	47.38 ± 1.17	50.44 ± 1.27	53.61 ± 1.39	27.76 ± 0.64
8k	67.84 ± 0.26	70.58 ± 0.43	73.76 ± 0.59	78.74 ± 0.80	17.54 ± 0.91
8l	76.41±0.18	78.60 ± 0.33	82.39±0.61	86.59 ± 0.71	16.35 ± 0.27
Ascorbic acid	75.99±0.18	77.76±0.33	81.49±0.61	85.72±0.71	15.83±0.26
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ±SD.



Scheme 1. Synthesis of nicotinic acid N′-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazides 8a–l

CONCLUSION

In summary, antioxidant activity of a series of benzophenone analogues bearing pyridine ring **8a-l**, achieved successfully, among the series **8a-l** compounds **8k, 8l, 8b, 8e** and **8h** shown were recognized as convincing compounds towards activity.

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