# Synthesis of Benzophenone Hydrazone Analogs and their DPPH Radical Scavenging and Urease Inhibitory Activities

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**Summary:** Benzophenone hydrazone analogs **1**-**25** were synthesized and evaluated for antioxidant (DPPH radical scavenging), and urease inhibitory activities. Out of twenty-five analogs, compounds **8**, **23**, and **1** showed potent free radical scavenging activities with $IC_{50}$ values 19.45 ± 1.25, 21.72 ± 1.49, and 26.0 ± 0.52 $\mu$M, respectively, while compound **8** ($IC_{50}$ = 36.36 ± 0.94 $\mu$M), and **15** ($IC_{50}$ = 55.5 ± 0.69 $\mu$M), showed good to moderate urease inhibitory potential.

Keywords: Benzophenone hydrazone, Antioxidant, Urease inhibition.

## Introduction

Benzophenones (also called diphenyl ketones) are a class of compounds having a range of biological and chemical significances. Benzophenones display substantial antitumor activity both *in vivo* and *in vitro* [1]. Synthetic benzophenones, for instance, dihydroxy-4-methoxy benzophenone [2], and 2-aminobenzophenone [3] have turned out to be anticancer and antimitotic agents, respectively. Benzophenones bearing an amino or a methoxy substituents are found to be potent cytotoxic agents against a panel of multi-drug-resistant cell lines [4]. Some derivatives of benzophenones showed a selective toxicity for the proliferation of endothelial cells by apoptosis induction [16]. Polyprenylated benzophenones were found to have the ability to cause induction of caspasemediated apoptosis [5]. Few years ago, benzophenones with *para*-methoxy substitutions were assessed as p38$\alpha$ inhibitors and were found to have high selectivity and efficacy [6].

Benzophenone hydrazone analogs are important scaffolds for a variety of biological activities. A nitro-substituted analog has completed a phase-I clinical trials, and objective responses were seen in advanced breast cancer, non-Hodgkin's lymphoma, and melanoma [7]. An important requirement of an iron-chelating drug, such as an antimalarial is a high attraction for iron. Arylhydrazones are biologically important Fe chelators and have been found to possess excellent antimalarial activity [8]. The affinity constant of acylhydrazones for iron (III) is about 1 x $10^{28}$ [9]. Some hydrazone derivatives are proteinase inhibitors with antiparasitic activity against *Trypanosoma brucei* [10].

Due to the biological importance of hydrazone molecules, here we are reporting the potential activity of benzophenone hydrazones against urease**,** and reactive oxygen species (ROS). Ureases are good target for the gastric and peptic ulcers [11]. Reactive oxygen species (ROS) damage the DNA of most of biological systems, which lead to carcinogenesis, heart disease, and many other health problems related to advancing age [12]. Synthetic antioxidants are used by many industries at low concentrations for suppression of radical generation for the prevention of premature polymerization during the course of processing, storage and transportation of the unsaturated monomers *etc*. Antioxidants scavenge or prevent the generation of ROS [13], thus preventing free radicals formation that would otherwise lead to cancer, cardiovascular diseases, inflammation and neurodegenerative *problems* [14].

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## Experimental

*Urease Inhibition Assay*

Reaction mixtures having 1 unit of urease solution and 55 $\mu$L of buffer containing 100 *m*M urea were subjected to incubation with 5 $\mu$L of test compounds (1 *m*M concentration) at 30 °C in 96-well plates for 15 min. Urease activity was assessed by measuring the generation of ammonia evolution by applying the indophenol method. Phenol reagent (45 $\mu$L) and alkali reagent (70 $\mu$L) were added to each well. The increasing absorbance was measured after 50 min at 630 nm, with the help of a microplate reader (Molecular Device, USA). All reactions were carried out in triplicate. The results (change in absorbance per min) were processed with the help of Soft-Max Pro software (Molecular Device, USA) [15].

*Determination of % Inhibition*

% Inhibition=100-(OD test well / OD control) ×100

*Antioxidant assay (DPPH Scavenging assay)*

Modified 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods [16, 17] were used for the measurement of potential of free radical scavenging of the compounds. Test compounds were countenanced to react with the stable free radical, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) for the span of 30 min at temperature 37 °C. The molarity of DPPH was held as 300 *m*M. The test samples were dissolved in DMSO while DPPH was ethanol. After the incubation, decline in absorption was determined at 515 nm with the help of multiplate reader (Spectra MAX-384). Percentage (%) radical scavenging activity (RSA) of the samples was measured by comparison with control, however, DMSO was kept as a positive control [16] using the following formula. All analyses were done three times.

% RSA = 100 - {(OD test compound/OD control) x 100}

*Determination of $IC_{50}$ Values*

The concentrations of samples, those which were inhibited the hydrolysis of substrates by 50% ($IC_{50}$), were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The $IC_{50}$ values were then calculated with the help of EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, U.S.A.).

## Results and Discussion

*Chemistry*

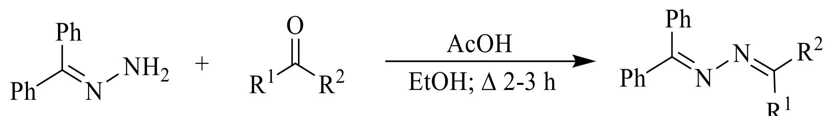
Benzophenone hydrazone derivatives **1**-**25** were synthesized by reacting commercially available benzophenone hydrazone with various aromatic aldehydes and ketones in anhydrous ethanol [18].

In a typical reaction, few drops of acetic acid were added to a stirred mixture of benzophenone hydrazone (3.0 mmol) and a substituted aromatic aldehyde or ketone (3.0 mmol) in anhydrous ethanol (10 ml), and heated at refluxed for 2-3 h. The reaction progress was monitored by TLC. When the reaction was complete, mixture was allowed to cool at room temperature and precipitates of benzophenone hydrazone derivatives were collected. The precipitates were washed with hexane and dried to afford compounds **1**-**25** in high yields. Recrystallization from methanol afforded pure crystals of synthetic compounds **1**-**25** (Scheme 1, Table-1).

*Bioactivities*

All the synthetic derivatives **1**-**25** were submitted to *in vitro* DPPH radical scavenging activity as per literature protocol [19, 20]. The derivatives showed reasonable free radical scavenging activities. Out of twenty-five derivatives, three compounds, **8**, **23**, and **1** displayed potent free radical scavenging activities bearing $IC_{50}$ values 19.45 ± 1.25, 21.72 ± 1.49, and 26.0 ± 0.52 $\mu$M, respectively, upon comparison with the standard, *n*-propyl gallate ($IC_{50}$ = 30.27 ± 1.6 $\mu$M) as depicted in Table-2.

All the compounds **1**-**25** were also randomly screened for their urease inhibitory activities. Compounds **7** ($IC_{50}$ = 65.77 ± 0.89 $\mu$M), **6** ($IC_{50}$ = 98.21 ± 1.67 $\mu$M), **13** ($IC_{50}$ = 101.08 ± 1.07 $\mu$M), **21** ($IC_{50}$ = 122.45 ± 3.17 $\mu$M), **25** ($IC_{50}$ = 154.13±3.52 $\mu$M), and **2** ($IC_{50}$ = 286.59 ± 1.56 $\mu$M) were found to be moderately to weakly active. All the compounds **1**-**25** were also randomly screened for their urease inhibitory activities. For urease inhibition, compounds, **8** ($IC_{50}$ = 36.36 ± 0.94 $\mu$M), **15** ($IC_{50}$ = 55.5 ± 0.69 $\mu$M), **1** ($IC_{50}$ = 102.66 ± 1.5), **6** ($IC_{50}$ = 128.76 ± 0.61), **7** ($IC_{50}$ = 139.5 ± 1.12), **23** ($IC_{50}$ = 192.83 ± 3.65), **13** ($IC_{50}$ = 202.46 ± 2.8), **25** ($IC_{50}$ = 223.12 ± 3.52), and **21** ($IC_{50}$ = 425.62 ± 2.36) were found to be good to weakly active.




Scheme-1: Syntheses of benzophenone hydrazone derivatives **1**-**25**.

Table-1: Synthesis of benzophenone hydrazones analogs **1-25.**

| S. No. | $R^1$ | $R^2$ | S. No. | $R^1$ | $R^2$ | S. No. | $R^1$ | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| **1** | **H** | OH, HO, OH | **10** | **H** | Me | **18** | **H** | Cl, Cl |
| **2** | **H** | OMe, HO | **11** | **Me** | $NO_2$ | **19** | **H** | Cl, Cl |
| **3** | **H** | $NO_2$ | **12** | **Me** | HO | **20** | **H** | OH |
| **4** | **H** | SMe | **13** | **Me** | HO, OH | **21** | **H** | OH, HO |
| **5** | **H** | OH | **14** | **Me** | HO, OH, OH | **22** | **H** | Cl, HO, Cl |
| **6** | **H** | OH, OH | **15** | **Me** | HO, OH | **23** | **H** | HO, OH, OH |
| **7** | **H** | HO, OH | **16** | **H** | OEt, HO | **24** | **Me** | OH |
| **8** | **H** | HO, OH, OH | **17** | **H** | HO, Cl | **25** | **Et** | HO, OH |
| **9** | **H** | HO | - | - | - | - | - | - |

*DPPH Radical Scavenging Studies*

Compounds **8**, **23** and **1** were explored to be the most active antioxidants among the library of synthesized compounds, with $IC_{50}$ values of 19.45 ± 1.25, 21.72 ± 1.49, and 26.0 ± 0.52 $\mu$M, respectively, in comparison with standard, *n*-propyl gallate ($IC_{50}$ = 30.27 ± 1.6 $\mu$M). The free radical scavenging potential of a compound depends upon the ability to stabilize the free radicals. All these compounds **8**, **23**

and **1** have three hydroxyl groups installed at the ring. The abstraction of hydrogen by DPPH, generates the stable phenoxide radical. Its stability is due to the extended conjugation. Consequently, good free radical scavenging potential is observed.

Compounds **7**, 2,5-dihydroxy, **6**, 3,4-dihydroxy and **23**, 2,3-dihydroxy analogs of benzophenone hydrazones also showed good DPPH scavenging effects with $IC_{50}$ values 65.77 ± 0.89, 98.21 ± 1.67, and 122.45 ± 3.17 $\mu$M, respectively. The difference in activities seems to be due to the orientation of hydroxyl group on the phenyl part.

Compound **13** ($IC_{50}$ = 101.08 ± 1.07 $\mu$M) was found to be more active than compound **25** ($IC_{50}$ = 154.13 ± 3.52 $\mu$M). The difference in activity may be due to difference in $R^1$ group. However, decline in activity was observed in compound **2** ($IC_{50}$ = 286.59 ± 1.56 $\mu$M), which might be due to presence of one hydroxyl and one methoxy group.

*Urease Inhibition Studies*

Benzophenone hydrazone derivatives **1**-**25** were screened against urease enzyme according to the literature protocol [18]. These residues showed a diversified degree of urease inhibitory potential having $IC_{50}$ values in the range of 36.36 ± 0.94 - 425.62 ± 2.36 $\mu$M comparing with the standard (thiourea $IC_{50}$ = 21 ± 0.11 $\mu$M) (Table-2). Compounds, **8** ($IC_{50}$ = 36.36 ± 0.94 $\mu$M), and **15** ($IC_{50}$ = 55.5 ± 0.69 $\mu$M), displayed good urease inhibitory potential, while compounds, **1** ($IC_{50}$ = 102.66 ± 1.5 $\mu$M), **6** ($IC_{50}$ = 128.76 ± 0.61 $\mu$M), **7** ($IC_{50}$ = 139.5 ± 1.12 $\mu$M), **7** ($IC_{50}$ = 192.83 ± 3.65 $\mu$M), **13** ($IC_{50}$ = 202.46 ± 2.8 $\mu$M), and **25** ($IC_{50}$ = 223.12 ± 3.52 $\mu$M) showed moderate inhibition potential against urease. However, compound **21** ($IC_{50}$ = 425.62 ± 2.36 $\mu$M) exhibited a weak inhibitory potential.

2,4,6-Trihydroxy substituted analog **8** and 2,6-dihydroxy **15** compound showed inhibitory activities among the series with $IC_{50}$ values 36.36 ± 0.94, and 55.5 ± 0.69 $\mu$M, respectively. 2,3,4-Trihydroxy analog **1** was found to be third most active among the series with $IC_{50}$ value of 102.66 ± 1.5 $\mu$M. Interestingly, compound **1** having 2,3,4-trihydroxyl group showed a weak activity than compound **8** (2,4,6-trihydroxy analog). The difference in activity between compound **8** and **1** may be due to the position of hydroxyl groups suggesting that position of hydroxyl group at phenyl ring also plays an important role in urease inhibitory activity. Similarly, compound **6**, 3,4-dihydroxy residue and compound **7**, 2,5-dihydroxy analog have $IC_{50}$ values 128.76 ± 0.61 and 139.5 ± 1.12 $\mu$M, respectivel,y by comparing with **15** ($IC_{50}$ = 55.5 ± 0.69 $\mu$M) analog which is also a dihydroxy analog. Weak inhibitions of these compounds may be because of variation in the position of hydroxyl group. Compounds **13**, 2,5-dihydroxy with $R^2$, as a methyl group and **25**, a 2,5-dihydroxy analog with $R^2$, as an ethyl group have $IC_{50}$ values 202.46 ± 2.8 and 223.12 ± 3.52 $\mu$M, respectively. Variation in $R^2$ group may be the reason in slight difference in $IC_{50}$ values.

Among the trihydroxy analogs, compound **23**, 2,4,5-trihydroxy ($IC_{50}$ = 192.83 ± 3.65 $\mu$M), exhibited weak inhibition. The reason may be the change in the position of hydroxyl group. Among the dihydroxy analogs, compound **21**, 2,3-dihydroxy has an $IC_{50}$ value of 425.62 ± 2.36 $\mu$M, showed weak inhibition against urease, not only among the dihydroxy analog but also in whole series. The reason seems to be due to the variation in the position of hydroxyl group on benzene ring. All the remaining compounds showed less than 50% inhibition and hence are considered to be inactive.

Table-2: Activities results of benzophenone hydrazone analogs 1-25.

| Compound No. | Urease Inhibition $IC_{50}$ ± SEM[a] [$\mu$M] | Antioxidant $IC_{50}$ ± SEM[a] [$\mu$M] |
|---|---|---|
| 1 | 102.66 ± 1.5 | 26.0 ± 0.52 |
| 2 | NA[b] | 286.59 ± 1.56 |
| 3 | NA[b] | NA[b] |
| 4 | NA[b] | NA[b] |
| 5 | NA[b] | NA[b] |
| 6 | 128.76 ± 0.61 | 98.21 ± 1.67 |
| 7 | 139.5 ± 1.12 | 65.77 ± 0.89 |
| 8 | 36.36 ± 0.94 | 19.45 ± 1.25 |
| 9 | NA[b] | NA[b] |
| 10 | NA[b] | NA[b] |
| 11 | NA[b] | NA[b] |
| 12 | NA[b] | NA[b] |
| 13 | 202.46 ± 2.8 | 101.08 ± 1.07 |
| 14 | NA[b] | NA[b] |
| 15 | 55.5 ± 0.69 | NA[b] |
| 16 | NA[b] | NA[b] |
| 17 | NA[b] | NA[b] |
| 18 | NA[b] | NA[b] |
| 19 | NA[b] | NA[b] |
| 20 | NA[b] | NA[b] |
| 21 | 425.62 ± 2.36 | 122.45 ± 3.17 |
| 22 | NA[b] | NA[b] |
| 23 | 192.83 ± 3.65 | 21.72 ± 1.49 |
| 24 | NA[b] | NA[b] |
| 25 | 223.12 ± 3.52 | 154.13 ± 3.52 |
| Standard | Thiourea[c] = 21 ±.0.11 | *n*-Propyl gallate[c] = 30. 27 ± 1.6 |

SEM[a] is the standard error of the mean, NA[b] Not active, Thiourea[c] standard inhibitor for anti-urease activity, *n*-propyl gallate[c] standard for DPPH radical scavenging assay.

## Conclusion

Out of twenty-five benzophenone hydrazone screened analogs, compounds **8** ($IC_{50}$ = 19.45 ± 1.25), **23** ($IC_{50}$ = 21.72 ± 1.49) and **1** ($IC_{50}$ = 26.0 ± 0.52

$\mu$M) showed good radical scavenging activities. However, compound **8** ($IC_{50}$ = 36.36 ± 0.94 $\mu$M), and **15** ($IC_{50}$ = 55.5 ± 0.69 $\mu$M), showed good to moderate urease inhibitory potential. Compounds **1**, **8**, **15**, and **23** may serve as lead compounds.

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