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In vitro antiglycation and antioxidant properties of benzophenone thiosemicarbazones

Arshia[1], Sanaullah Abbasi[2], Saima Rasheed[1], Jalaluddin AJ Khan[2], Syed Muhammad Saad[1,3], Khalid Mohammed Khan[1,4*] and M Iqbal Choudhary[1,2,5]

[1]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

[2]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

[3]Department of Chemistry, University of Karachi, Karachi, Pakistan

[4]Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

[5]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract: Fifteen benzophenone thiosemicarbazones were synthesized and their *in vitro* antiglycation activity was evaluated. The most active compound 2 (IC_{50} = 118.15±2.41μM) showed two folds potent activity than the standard, rutin (IC_{50} = 294.5±1.5μM). Compounds 1 and 3-7 showed good to moderate antiglycation activity in the range of 204.14 - 488.54μM. These compounds were also evaluated for antioxidant activity. Their structure-activity relationships have been developed. The results reveal the potential of these compounds as leads for further studies towards the development of antidiabetic drugs.

Keywords: Benzophenone, thiosemicarbazone, advanced glycation end products (AGEs), antioxidant, diabetes, structure-activity relationship (SAR).

INTRODUCTION

Diabetes mellitus (DM) is mainly characterized by defects in insulin secretion, or action of insulin, or both. Chronic hyperglycemia is linked with multi organ failure (Zimmet 2003; Finlayson and Zimmerman 2009; Su *et al*., 2008).The process of glycation occur slowly in normal body, but in diabetic patients due to higher sugar levels (Monnier 2003; Gugliucci 2000), protein alteration take places with much factor rate and the changes in protein structures cause degenerative disorders, *e.g.* Alzheimer's disease (AD), cataract (Stern, 1995). (Gomes *et al*., 2005; Kapurniotu *et al*., 1998) *etc*. Diabetic impediments are multifactorial but glycoxidative stress is the unifying linkage between the various disorders in DM. Due to the chronic hyperglycemia-induced oxidative stress, formation of AGEs is also accelerated (Farah and Bukhari, 2018). The consequences of glycoxidative stress include, damage to proteins, DNA and aminolipids and buildup of damaged molecules in the cells (Gomes *et al*., 2005; Kapurniotu *et al*., 1998).

Benzophenone, also called as benzoyl benzene, is an essential organic molecule that is naturally found in *Moraceae*, *Clusiaceae* and few other plant families (Baggett *et al*., 2005). The pharmacological significances of this nucleus include anti-microtubule (Yamazaki, *et al*., 2012), Alzheimer's disease (Belluti, and Simone, 2014), antileishmanial (Maciel-Rezende and de Almeida 2013), anti-inflammatory (Bandgar and Chavan 2013), antifungal and antibacterial (Sun and Wu 2011) properties. Benzophenone nuclei also have properties such as adhesives, coatings, and photoinitiator materials (Wang, and Yang 2014; Karahan, Balta; 2014, Wang and Jiang 2009; Wang and Ma, 2010). Thiosemicarbazone moiety is the product of condensation of aldehyde or ketone with thiosemicarbazide. Thiosemicarbazones may exist in thioamido-thioiminol tautomeric forms (fig. 1). Benzophenone thiosemicarbazones are molecules of great interest due to their potential pharmacological properties, such as anti-asthmatic (Hsieh and Liou 2003), antimalarial (Pingaew and Prachayasittikul, 2010), cathespin L. inhibition (Kumar and Chavarria 2010), and antiprotozal properties (Glinma and Kpoviessi 2011).

As described earlier, diabetes is a major health problem and to combat this chronic health problem, our research group is involved in search of, safe and effective antidiabetic agent (Khan and Rahim 2014; Khan and Mughal 2009). Recently, our research group has reported antiglycation activities of benzophenone hydrazone Schiff bases (Khan and Rahim 2013). In this context, we have synthesized new benzophenone thiosemicarbazone analogs (1-15) and evaluated their protein antiglycation activity. The compounds which were found to be active in *in vitro* antiglycation assay (*i.e.* compounds 1-7) were subjected to antioxidant studies. To the best of our knowledge, the antiglycating activity of these compounds is reported for the first time.

**Corresponding author:* e-mail: khalid.khan@iccs.edu

MATERIAL AND METHODS

Quercetin 3-rutinoside, pyruvaldehyde (40% aqueous solution) and bovine serum albumin were purchase from Merck (Germany). Benzophenone, 4-hydroxybenzophenone, hexane, ethanol, (NaH_2PO_4), (DMSO), (Na_2HPO_4), and (NaN_3) were purchased from Sigma-Aldrich (Japan). All chemicals were of analytical grade.

In vitro antiglycation assay

The *in vitro* antiglycation assay contains 14mM pyruvaldehyde, 10mg/mL BSA, and 1mM test compound. BSA and pyruvaldehyde solution were prepared in 0.1M phosphate buffer (pH 7.4). NaN_3 (3mM) was used as an antibacterial agent. However, 1mM solution of test analog composed in DMSO. Every reaction consists of 20μL of test compound, 50μL of BSA, 50μL of pyruvaldehyde, and 80μL phosphate buffer (pH 7.4), 50μL pyruvaldehyde and 20μL of test compound. Volume of reaction mixture was 200μL at per well of 96-well plate. Incubation of reaction mixture was carried out at 37°C for 9 days under sterile conditions. Examination of sample were carried out by fluorescence at 330 nm and emission at 420 nm, on a microtiter plate reader (Spectra Max M5, Molecular Devices, CA, USA). The assay was perfomed in three times (Choudhary and Adhikari 2011; Choudhary and Ali 2011).

Following formula was used for calculating percentage inhibition:

$$\% \text{ Inhibition} = \left(\frac{1 - \text{Fluorescence of test sample}}{\text{Fluorescence of the control}} \right) \times 100$$

Diphenyl picryl hydrazine (DPPH) radical-scavenging activity

The antioxidant property of the synthetic compounds against radical scavenging potential was evaluated by measuring the DPPH scavenging activity. For DPPH radical scavenging assay, test compounds were dissolved in DMSO, while 3mM soloution of DPPH was prepared in ethanol. The reaction mixture was prepared by mixing 5μL test compound with 95μL of DPPH. These reaction mixtures were distributed in 96-well plate. Incubation of reaction mixture was performed at 37°C for 0.5 an hour and the variation in absorbance was calculated at 515 nm on SpectraMax M5 (Molecular Devices, CA, USA) (Orhan and Kartal 2007). The percent radical scavenging activity (% RSA) was determined by using following formula.

$$\% \text{ RSA} = \left[1 - \left(\frac{\text{Absorbance of test}}{\text{Absorbance of control}} \right) \right] \times 100$$

STATISTICAL ANALYSIS

All of biological assays were performed in 96-well plate by using SpectraMax M5 (Molecular Devices, CA, USA). Results were obtained as mean standard deviation in triplicate manner using SoftMaxPro 4.8, GraphPad Prism 5.0, and Microsoft Office Excel 2007. 50% inhibitory concentration values were determined by using EZ-FIT software by Perrella Scientific, Inc., USA.

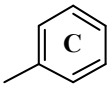
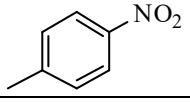
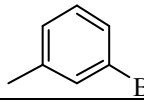
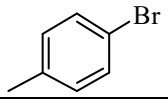
General procedure for the synthesis of compounds 1-15

Synthesis and structure elucidation of compounds 1-15 have been previously reported by our scientific group (Scheme 1) (Arshia and Khan 2016).

RESULTS

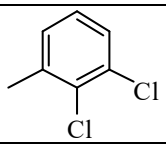
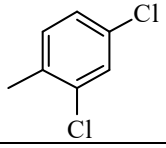
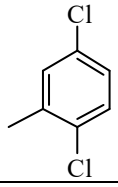
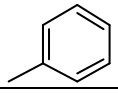
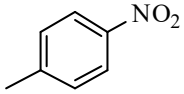
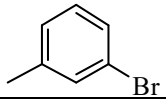
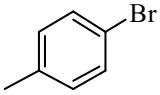
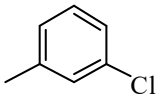
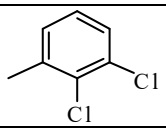
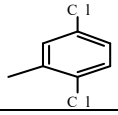
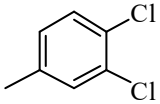
All compounds were synthesized as per Scheme 1. Their tautomeric structures are shown in Fig 1 and general structures in fig 2. *In vitro* bovine serum albumin methylglyoxal (BSA-MG) protein glycation model, employed in this study, has provided a useful tool for evaluating the effects of fifteen benzophenone

Table 1: Antiglycation activity of benzophenone thiosemicarbazones 1-15

Compound No.	R	$IC_{50} \pm SEM^a$
1		293.02 ± 2.36
2		118.15 ± 0.41
3		222.11 ± 1.53
4		204.14 ± 2.01

Continue…

Table 1: Continued…

Compound No.	R	$IC_{50} \pm SEM^a$
5		247.75 ± 1.18
6		488.54 ± 6.93
7		282.64 ± 0.37
Series B		
8		N. A.[b]
9		N. A.[b]
10		N. A.[b]
11		N. A.[b]
12		N. A.[b]
13		N. A.[b]
14		N. A.[b]
15		N. A.[b]
Rutin (Standard)		294 ± 1.5

thiosemicarbazones 1-15 against the non-enzymatic glycation processes (Table 1) (Fig. 3). Out of fifteen synthetic compounds, seven were found to be active against antiglycation assay (Fig. 4). The active compounds were then screened for antioxidant assay, however weak antioxidant results were obtained as shown in table 2.

DISCUSSION

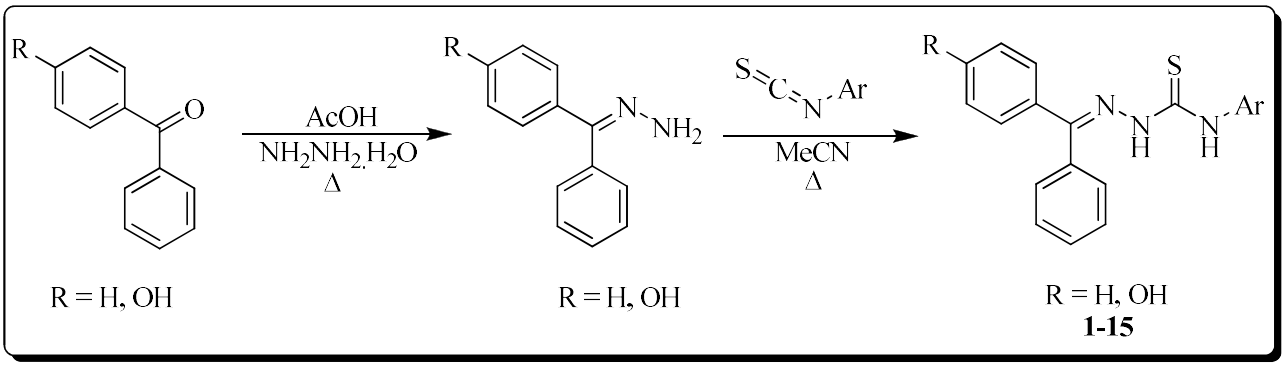
In vitro antiglycation activity

The difference in antiglycation activity of benzophenone thiosemicarbazone derivatives are mainly due to *para*-hydroxy substituent on ring A and varied substituents on ring C (fig. 2). In this scenario, compound 2 (IC_{50} = 118.15±0.41μM) with hydroxy at *para* position of ring A

Table-2: *In vitro* antioxidant activity of benzophenone thiosemicarbazones 1-7

Compound No.	$IC_{50} \pm SEM^a(\mu M)$
1	71.77 ± 3.25
2	85.05 ± 0.06
3	84.09 ± 8.03
4	75.89 ± 1.54
5	86.99 ± 6.78
6	79.45 ± 1.04
7	76.73 ± 0.45
Gallic acid[b]	23.34 ± 0.43

[a]IC_{50} Values are presented in μM and as mean ± standard error of mean; [b]Standard for antioxidant studies.



Scheme 1: Synthesis of benzophenone thiosemicarbazones 1-15.

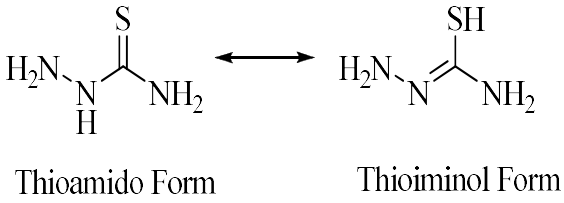


Fig. 1: Tautomeric forms of thiosemicarbazone

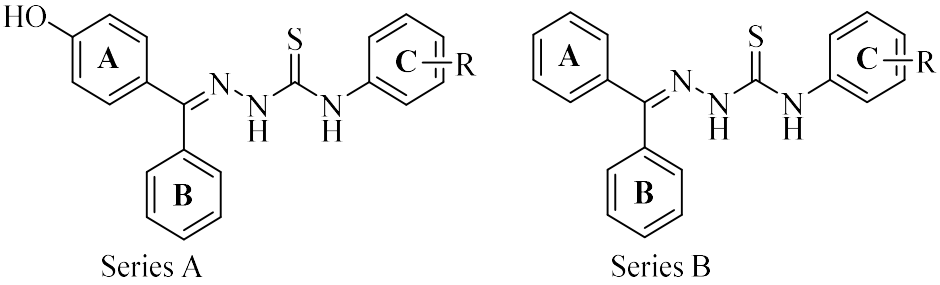


Fig. 2: General structure of synthetic compounds

and nitro group at *para* position of ring C, was found to be several folds (significant; p values < 0.05) more active than the standard. *i.e.* rutin (IC_{50} = 294.11±1.5μM) (fig. 4). Replacement of nitro substituent with bromo substituent in ring C, as in compound 4 (IC_{50} = 204.14± 2.01μM), resulted in decreased activity. When a bromo group was shifted to *meta* position, as in compound 3 (IC_{50} = 222.11±1.53μM), a further slight decline in activity was observed (fig. 4).

Among the dichlorinated analogs, *ortho* or *para* linked isomers showed a better activity than the standard, as may be seen in compounds 5 (IC_{50} = 247.75±1.18μM) and 7 (IC_{50} = 282.64±0.37μM). However, *meta* linked

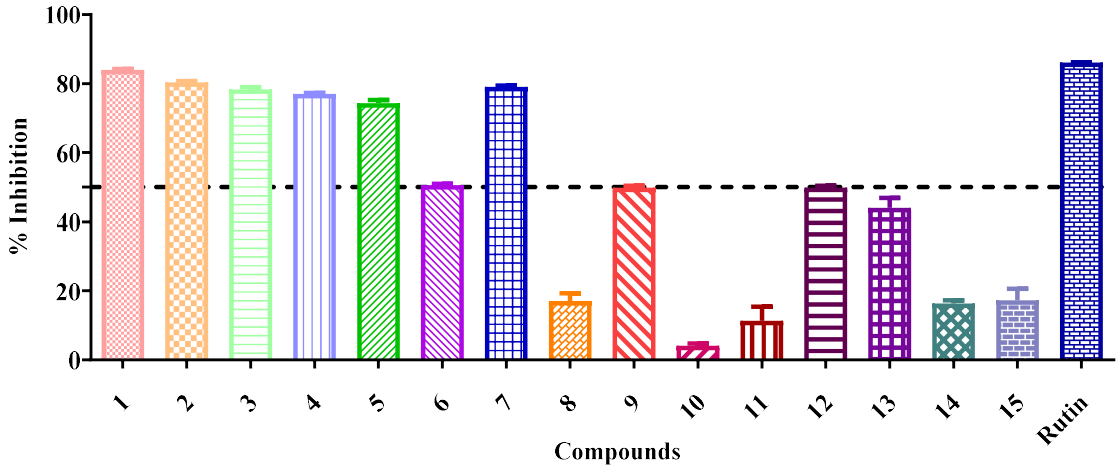


Fig. 3: Anti-glycation activity of benzophenone thiosemicarbazones (1-15).

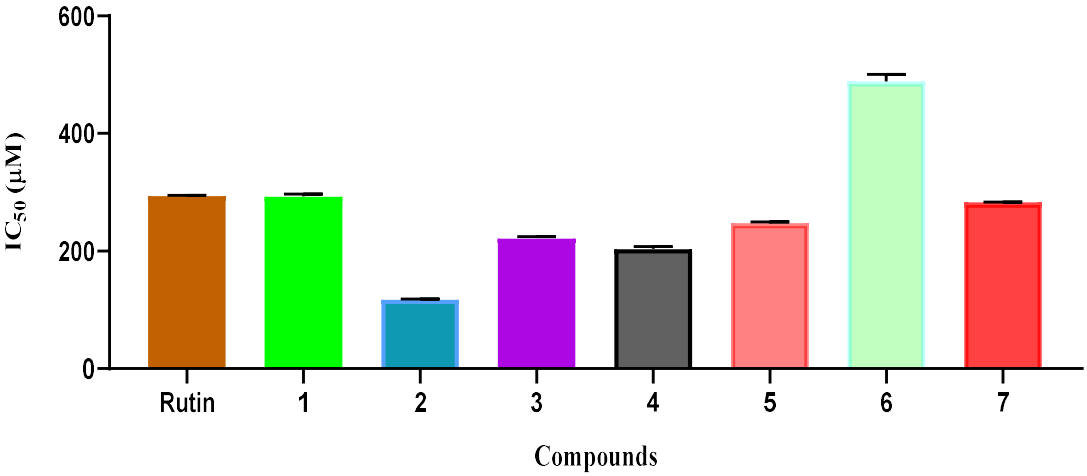


Fig. 4: Comparison of the anti-glycation activity of active compounds 1-7 with standard inhibitor Rutin.

dichlorinated analog 6 (IC_{50} = 488.54±6.93μM) displayed a weak antiglycating activity (fig. 4).

Surprisingly, compound 1 (IC_{50} = 293.02±2.36μM) with no substitution at ring C but hydroxyl at ring A, showed compareable IC_{50} value with the standard, rutin (IC_{50} = 294.11±1.5μM) (fig. 4, table 1). This structural configuration suggests that variation of subtituents on ring C ends up with a range of varied antiglycation activity.

The antiglycating activity was found to be entirely diminished when the hydroxyl group installed at ring A was absent (compounds 8-15). This clearly depicts the obvious presence of hydroxyl group in ring A for the compounds to be active.

In vitro antioxidant activity

Out off fifteen benzophenone thiosemicarbazones, all hydroxyl containing compounds 1-7 were found to be active against antiglycation assay. These active compounds were further subjected for the assessment of antioxidant potential. Results reveal weak antioxidant activities in the range of 71.77-86.99μM, when compared with the standard, gallic acid (IC_{50} = 23.24± 0.43μM).

CONCLUSION

Fifteen different substituted benzophenone thiosemicarbazones were evaluated for their antiglycation activity. Among all the screened compounds, only hydroxylated analogs were found to be active. Compound 2 was found to be the most potent *in vitro* protein antiglycating agent in BSA-MG glycation model. Five compounds 1, 3-5, and 7 showed good to moderate antiglycation activity, however, compound 6 displayed a weak activity. These active compounds were then subjected to antioxidant activity and were found to be

active. The scavenging of free radicals plays an important role in the inhibition of protein glycation and thus provides protection against hyperglycemia-mediated protein damage. The *in vivo* studies of the active compounds followed by refining in SAR may result in the development of a novel class of antiglycating agents.

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