

## Original Research Article

# Evaluating the antidiabetic and antioxidant properties of 5-benzyl-1,3,4-oxadiazole-2-thiol

Shaista Qamar<sup>1,2</sup>, Khalid Hussain<sup>1\*</sup>, Nadeem I Bukhari<sup>1</sup>, Sabahat Z Siddique<sup>3</sup>, Aziz-ur-Rehman<sup>3</sup>, Muhammad A Abbasi<sup>3</sup>, Sajida Parveen<sup>1</sup>, Abida Latif<sup>1</sup>, Abida Qamar<sup>1</sup>, Ejaz Ali<sup>1</sup>, Naureen Shehzadi<sup>1</sup>, Muhammad Islam<sup>1</sup>, Surriya Naheed<sup>1</sup>

<sup>1</sup>Punjab University College of Pharmacy, University of the Punjab, Allama Iqbal Campus, <sup>2</sup>Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, <sup>3</sup>Department of Chemistry, Government College University, Lahore-54000, Pakistan

\*For correspondence: **Email:** [khussain.pharmacy@pu.edu.pk](mailto:khussain.pharmacy@pu.edu.pk), [hussain\\_761@yahoo.com](mailto:hussain_761@yahoo.com); **Tel:** +92-4299211617

Sent for review: 17 December 2018

Revised accepted: 21 April 2019

### Abstract

**Purpose:** To evaluate 5-Benzyl-1,3,4-oxadiazole-2-thiol (OXPA) for antidiabetic and antioxidant properties.

**Methods:** Antidiabetic activity was evaluated using three *in vitro* models, glucose uptake by yeast cells, alpha amylase inhibition assay and hemoglobin glycosylation inhibition assays. Antioxidant potential was determined by DPPH radical scavenging, reducing power and lipid peroxidation assays.

**Results:** OXPA showed antidiabetic activity in all the three models. The activity of the compound was comparable with that of metronidazole in glucose uptake by yeast cells, but the alpha amylase inhibition activity of the compound was slightly lower than that of acarbose, whereas the hemoglobin glycosylation inhibition activity of the compound was higher than that of vitamin E. DPPH free radical and hydrogen peroxide scavenging activity of the compound was comparable with that of vitamin C. In reducing power assay, the activity of the compound was lower than that of vitamin C ( $p > 0.05$ ).

**Conclusion:** The results of antidiabetic and antioxidant activity indicate that OXPA may be a drug-candidate for treating both diabetes and its associated oxidative stress.

**Keywords:** Antidiabetic, Glucose uptake, Antioxidant, Reactive oxygen species, Hemoglobin glycosylation, Alpha amylase

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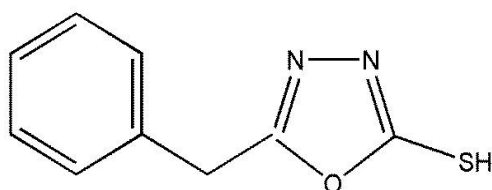
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## INTRODUCTION

The compound under investigation, 5-Benzyl-1,3,4-oxadiazole-2-thiol (OXPA), is a derivative of 1,3,4-oxadiazole having benzyl and thiol at positions 5 and 2, respectively (Figure 1). It was synthesized to prepare a number of s-substituted derivatives possessing antibacterial and

hemolytic activities [1]. The compound was also investigated for its behavior toward different stressors using UV spectrophotometry [2]. The compound was selected for antioxidant activity due to proton donating property of free thiol group.

Thiol group and oxadiazole ring are the two major functional entities which can make it an antidiabetic and antioxidant drug-candidate. It is reported that thiol containing compounds act as antioxidants due to proton donating potential [3-5], while oxadiazole ring is a pharmacophore possessing a number of pharmacological activities including antidiabetic activity [3-5]. Therefore, OXPA is expected to have antidiabetic and antioxidant potential. An association of diabetes with oxidative stress has warranted the need to find compounds which can manage diabetes and control diabetes-associated oxidative stress, simultaneously.



**Figure 1:** Chemical structure of 5-Benzyl-1,3,4-oxadiazole-2-thiol (OXPA)

Diabetes is a metabolic disorder which has several etiologies including insulin deficiency, insulin resistance and genetic defects of  $\beta$ -cells of the pancreas. Obesity, age and lack of physical activity are also the contributors of the disorder. This disorder may be congenital – Type-I diabetes – or acquired called Type-II diabetes. The prevalence of both types of the disease in all age groups is alarmingly escalating. Type-I diabetes in young-adults is reported to be 5-10 %, while 90–95 % population of the diabetics is suffering from Type-II diabetes [6,7]. The complications of the disease are more fatal than the disease itself, and the same is witnessed by 1.5 million deaths due to diabetes and 2.2 million due to its co-morbidities [6,7]. These studies also indicated higher death toll in the low and middle income countries [6,7].

Uncontrolled diabetes leads to hyperglycemia which results in glycosylation of blood-proteins, which causes co-morbidities such as neuropathy, retinopathy, cardiomyopathy and stroke. This situation disturbs the equilibrium between the generation of reactive oxygen species and antioxidant defense capacity [8]. The advanced glycation end products being the source of free radicals further aggravate oxidative stress [9]. Such complications can be reduced using antioxidants or compounds having both antidiabetic and antioxidant activities [10]. Therefore, the present study aimed to investigate OXPA for antidiabetic and antioxidant activities using various models.

## EXPERIMENTAL

### Chemicals

Glucose anhydrous (Riedel-deHaen), commercial grade baker's yeast, 2,2-diphenyl-picrylhydrazyl and disodium hydrogen phosphate (Sigma Aldrich), alpha amylase (UniChem), hydrogen peroxide ( $H_2O_2$ ), hemoglobin and potato starch (China), potassium ferricyanide, trichloroacetic acid, potassium dihydrogen phosphate, sodium dihydrogen phosphate and NaOH (Merck), dimethyl sulfoxide (Panreac Quimica, SAU), ascorbic acid, ferric chloride, potassium sodium tartrate and 3,5-dinitrosalicylic acid (BDH, England), acarbose (Bayer), and vitamin E (Fluka) were procured from the local market. Metronidazole (MTZ) was gifted by M/S Siza International Pvt. Ltd. Lahore, Pakistan. 5-Benzyl-1,3,4-oxadiazole-2-thiol was obtained from the Department of Chemistry, Government College University, Lahore, Pakistan.

### Determination of anti-diabetic activity

#### Glucose uptake by yeast cells

Yeast powder was washed by 0.9 % ice-cold sodium chloride solution and centrifuged ( $3500 \times g$ ; 5 min). The supernatant was removed and yeast cells were again washed using the same procedure until the supernatant became clear [11]. Then, yeast cell pellet was suspended in distilled water to prepare 10 % (V/V) suspension. One milliliter of sample prepared in DMSO was added in test tubes containing 1 mL of 5, 10 and 25 mM aqueous glucose solution, and test tubes were incubated at  $37^\circ C$  for 10 min. Then, 100  $\mu L$  of the yeast suspension was added in test tubes and contents were further incubated at  $37^\circ C$  for 60 min. Afterwards, each tube was centrifuged for 10 min at  $3000 \times g$ , and the supernatant was analyzed at 620 nm against a blank containing the vehicle. A control was prepared like the sample using all the components, except the test solution. Metronidazole solution, treated like the sample, served as a standard. The activity was determined using Eq. 1.

$$\text{Glucose uptake (\%)} = \frac{Ac - As}{Ac} \times 100 \dots \dots (1)$$

where Ac is absorbance of control and As is absorbance of sample.

#### Hemoglobin glycosylation inhibition activity

The sample and reagents were prepared in 0.2 M phosphate buffer. One milliliter of 5, 10 and 25

mM glucose solution was added in test tubes, separately. Then, 1 mL of 0.06 % hemoglobin solution, phosphate buffer and sample/vitamin E were added in all the tubes. Finally, 5  $\mu$ L of 0.02 % gentamicin solution was added and the mixture was kept in the dark at room temperature. The absorbance was measured at 443 nm at different time intervals for 72 h [12].

#### **Alpha amylase inhibition activity**

The activity was determined using the method described earlier [13]. The sample, standard and the enzyme dissolved in sodium phosphate buffer of pH 6.9. Potato-starch was suspended in phosphate buffer (1 %, w/v) and boiled for 30 min. One milliliter solution of sample, potato starch, alpha amylase (0.025 %, W/V) were mixed with 1 mL of sodium phosphate buffer (pH 6.9) and incubated at 37 °C for 30 min. Then, 0.3 mL of 3,5-dinitrosalicylic acid solution was added and the reaction was ceased by adding 0.2 mL of 2 N NaOH and heating for 20 min in a water bath at 85 °C. Finally, the absorbance of the mixture was measured at 540 nm to calculate the alpha amylase inhibition activity.

#### **Evaluation of antioxidant activity**

##### **DPPH radical scavenging activity**

Three milliliters of 0.1 mM methanolic solution of DPPH was mixed with 1 mL of sample/standard (ascorbic acid) and incubated in the dark at 37 °C for 30 min. A blank control was prepared by replacing the sample solution with 1 mL of methanol. Vitamin C (10  $\mu$ g/mL) was used as a standard. The absorbance of the sample/standard and control was measured at 517 nm against methanol as a blank [14].

##### **Reducing power activity**

The reducing power activity of the compound was determined using the method described earlier [15]. Briefly, a reaction mixture was prepared by mixing 1 mL of sample/standard solution (vitamin C), 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1 %, W/V potassium ferric cyanide.

The reaction mixture was kept at 50 °C in water bath for 20 min. Then, 2.5 mL of trichloroacetic acid (10 %, W/V) was added and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL), distilled water (2.5 mL) and 0.1 %, W/V ferric chloride solution (0.5 mL) were mixed and the absorbance was measured at 700 nm.

#### **Hydrogen peroxide scavenging activity**

The sample/standard solution (3.4 mL) and 43 mM H<sub>2</sub>O<sub>2</sub> (0.6 mL), prepared in phosphate buffer (pH 7.4), were mixed and kept at room temperature at dark for 50 min. The absorbance of the reaction mixture was measured at 230 nm against phosphate buffer as a blank [16].

#### **Statistical analysis**

The samples and the standards were analyzed in triplicate and the results are presented as mean  $\pm$  standard deviation. Half maximal effective concentration (EC<sub>50</sub>) or half maximal inhibitory concentration (IC<sub>50</sub>) was determined applying linear regression on dose-response curves. The activity of OXPA, compared to that of standard drug, was analyzed by independent samples *t*-test using IBM, SPSS (version 20). *P* < 0.05 was considered statistically significant.

## **RESULTS**

#### **Antidiabetic activity**

The results of antidiabetic activity of OXPA using glucose uptake in yeast cells model are given in Table 1. These results showed that the compound facilitated the transport of glucose in the yeast cells in all the three glucose solutions (5, 10 and 25 mM). At equivalent concentration (250  $\mu$ g/mL) activity of the compound was found to be higher than metronidazole (*p* < 0.05). The half-maximal effective concentration (EC<sub>50</sub>) of the compound determined in a concentration range 50-250  $\mu$ g/mL in 5 mM glucose solution was found to be 52.77  $\mu$ g/mL ( $y = 0.1121x + 44.856$ ,  $R^2 = 0.9959$ ). This indicated that activity of the compound at a concentration of 150  $\mu$ g/mL was comparable to metronidazole (250  $\mu$ g/mL) in 5 mM glucose solution. However, in 10 and 25 mM glucose solutions, the compound at a concentration of 100  $\mu$ g/mL showed comparable activity to that of the metronidazole (250  $\mu$ g/mL).

The findings of hemoglobin glycosylation inhibition activity of the compound are given in Table 2. Free hemoglobin concentration decreased with the passage of time in all the three glucose, which indicated that glycosylation took place. In this assay, the activity of the compound was found to be higher as compared to vitamin E (*p* < 0.05). IC<sub>50</sub> of the compound determined in a concentration range (40-250  $\mu$ g/mL) in 5, 10 and 25 mM glucose solutions was found to be 210.75  $\mu$ g/mL ( $y = 0.1055x + 27.788$ ,  $R^2 = 0.9724$ ), 125.93  $\mu$ g/mL ( $y = 0.0932x + 38.263$ ,  $R^2 = 0.9122$ ) and 19.23  $\mu$ g/mL ( $y = 0.0726x + 48.604$ ,  $R^2 = 0.9828$ ), respectively.

These results showed that the compound inhibited the reaction of glucose and hemoglobin. Furthermore, the inhibition of glycosylation was higher in concentrated glucose solutions.

Alpha amylase inhibition activity of the compound on at a concentration of 1.4 mg/mL was 51.80 %, whereas acarbose at the same concentration showed 65.64 % activity (Table 3). In this model,  $IC_{50}$  of the compound was found to be 1.33 mg/mL ( $y = 36.645x - 1.6795$ ,  $R^2 = 0.979$ ). These results indicated that OXPA has a promising antidiabetic activity.

**Table 3:** Alpha amylase inhibition activity of 5-benzyl-1,3,4-oxadiazole-2-thiol (OXPA)

Drug	Inhibition(%)	SD
0.8 mg/mL	30.01	1.85
1.0 mg/mL	33.15	1.07
1.2 mg/mL	39.16	0.95
1.4 mg/mL	51.80	0.21
1.6 mg/mL	57.33	0.25
Acarbose (1.2 mg/mL)	65.63	0.36

SD = standard deviation

**Table 1:** The effect of 5-Benzyl-1,3,4-oxadiazole-2-thiol (OXPA) on glucose uptake by yeast cells in glucose solutions of different concentrations

Drug	Activity at 5 mM (%)	Activity at 10 mM (%)	Activity at 25 mM (%)
50 µg/mL	49.83±2.06	61.17±3.04	48.76±1.93
100 µg/mL	56.47±2.23	63.77±1.55	54.81±2.51
150 µg/mL	62.18±4.05	71.09±0.48	60.27±0.58
200 µg/mL	67.61±3.20	72.70±0.59	62.65±0.54
250 µg/mL	72.29±0.83	76.80±2.64	70.09±1.99
MTZ 250 µg/mL	64.03±1.23	61.97±2.74	60.71±2.31

MTZ = metronidazole

**Table 2:** Hemoglobin glycosylation inhibition activity of 5-Benzyl-1,3,4-oxadiazole-2-thiol (OXPA)

Drug	Activity at 5 mM (%)	Activity at 10 mM (%)	Activity at 25 mM (%)
50 µg/mL	33.83±1.78	44.83±2.53	51.63±1.44
100 µg/mL	38.73±2.06	47.29±2.30	56.84±0.99
150 µg/mL	42.06±2.76	49.84±2.53	59.67±0.88
200 µg/mL	47.48±3.12	54.93±2.15	62.20±0.14
250 µg/mL	55.83±1.19	64.31±1.10	67.09±0.71
Vitamin-E	40.31±1.39	49.03±2.00	58.30±0.38

**Table 4:** Antioxidant activity of 5-benzyl-1,3,4-oxadiazole-2-thiol (OXPA) based on three models (n = 3)

Drug (µg/ml)	DPPH assay (mean±SD)	Drug µg/ml	RPA (mean±SD)	Drug µg/ml	HPO (mean±SD)
5	27.7±1.20	50	29.0±0.40	20	22.0±0.50
10	37.5±1.60	100	33.8±1.40	40	32.1±0.50
25	76.2±0.10	150	38.8±0.90	80	43.3±0.20
50	92.3±0.90	200	49.3±0.20	100	50.9±0.90
100	92.9±0.70	250	63.6±0.30	150	59.3±0.60
S 10	33.2±0.40	S 250	84.9±0.10	S 80	28.4±0.60

DPPH = DPPH assay; RPA = reducing power assay; HPO = hydrogen peroxide assay

## Antioxidant activity

The results of dose-dependent antioxidant activity of OXPA, determined using three *in vitro* models, are shown in Table 4. The activity of the compound was found to be comparable to the vitamin C in the DPPH assay ( $p > 0.05$ ), whereas reducing power activity of the compound was less than that of vitamin C. In hydrogen peroxide assay, activity of the compound was significantly higher than vitamin C ( $p < 0.05$ ). The plots of concentration versus antioxidant-activity using different antioxidant-activity models were used to determine half-maximal effective concentration ( $EC_{50}$ ). The  $EC_{50}$  of the compound was found to be 14.50, 206.00 and 97.00 µg/mL in the DPPH assay, reducing power assay and hydrogen peroxide assay, respectively. These findings showed that OXPA is a promising antioxidant drug-candidate.

## DISCUSSION

The transport of glucose in yeast cells is a complex phenomenon involving some stereo-specific transporters, glycolytic enzymes and concentration gradient [17-20].

Glucose transport was found to be higher in concentrated glucose solutions, which indicated that higher glucose concentration and glucose utilization in fermentation had facilitated glucose influx in yeast cells. Moreover, the influx was faster in 5 and 10 mM glucose solutions as compared to 25 mM glucose solution. The reason of slow glucose uptake in 25 mM glucose solution could be due to decreased activity of the transporters as a result of establishing equilibrium between intracellular and extracellular glucose concentration. These results are consistent with that reported earlier indicating that intracellular glucose concentration during its uptake inhibits the glucose-influx and in some cases, promotes efflux [17,18].

Glycosylated hemoglobin (HbA1c) is produced by an increased blood-glucose concentration. Glycosylation of hemoglobin results in the formation of reactive oxygen species which cause diabetes-associated complication [8]. The literature review indicated that terminal nitrogen of valine of hemoglobin is glycosylated with glucose [21]. In the present study, OXPA inhibited the glycosylation of hemoglobin that might be due to the interaction of nitrogen atoms of the oxadiazole ring with glucose. Digestive enzymes such as alpha-amylase and alpha-glucosidase convert starch into glucose and maltose in the intestine [22]. Therefore, the inhibitors of such enzymes (acarbose, voglibose and miglitol) are used to manage Type-II diabetes [23]. In the present study, the compound inhibited the alpha-amylase activity significantly, which indicated that OXPA may be used to decrease glucose availability from the intestine from digestible carbohydrates, hence may be used as an oral anti-hyperglycemic agent.

Antioxidants act through various mechanisms, therefore, in the present study, antioxidant potential of the compound was evaluated using three antioxidant models. The DPPH free radical is reduced by proton donors. Thiol group of the compound can donate proton, therefore, DPPH free radical scavenging may be due to proton of the thiol group. DPPH scavenging activity of the compound was comparable with vitamin C (standard drug). Likewise, the compound showed promising antioxidant activity in reducing power assay. In this assay, the compound reduced the ferric to ferrous that was indicated by change of color. Thiol group may chelate ferric ions as reported earlier that such group containing compound chelate metals [24]. Therefore, hydrogen donating capacity and metal chelation are expected to be the mechanism of antioxidant activity of the compound. Hydrogen peroxide

(H<sub>2</sub>O<sub>2</sub>) scavenging activity of the compound indicated its usefulness in the living system. In the living system, hydrogen peroxide formed by biological processes produces highly toxic hydroxyl radical that can attack many cellular energy-producing glycolytic enzyme. Therefore, the tendency of thiol containing compounds in scavenging of H<sub>2</sub>O<sub>2</sub> is very important for protecting living organisms from the oxidative stress. The unsaturation of the compound may also be involved in scavenging H<sub>2</sub>O<sub>2</sub> as reported earlier [24,25]. Antioxidants help in preventing diabetes-associated complications. As stated earlier that thiol group makes OXPA a strong reducing agent. Hence, the compound may be an effective antioxidant and antidiabetic agent.

## CONCLUSION

The results of the present study indicate that 5-benzyl-1,3,4-oxadiazole-2-thiol (OXPA) has antidiabetic activity by acting via the various mechanisms. Moreover, the compound has antioxidant potentials which is comparable to that of vitamin C. Therefore, OXPA may be beneficial in managing diabetes and reducing oxidative-induced co-morbidities.

## DECLARATIONS

### *Acknowledgement*

SQ is thankful to Dr. Quaid Zaman, Assistant Professor, University of Veterinary and Animal Sciences, Lahore, Pakistan, for providing laboratory facilities to perform hemoglobin glycosylation studies.

### *Conflict of interest*

No conflict of interest is associated with this work.

### *Contribution of authors*

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. KH and NIB conceived and designed the study. SZS, AR and MAA synthesized the compound and NS checked its purity. SP, AL, MI, SN and EA helped in performing the experiments. The manuscript was drafted by SQ and reviewed by KH and NIB.

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