IJPSR (2026), Volume 17, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 February 2026; received in revised form, 18 March 2026; accepted, 23 April 2026; published 01 July 2026

ANTIOXIDANT POTENTIAL OF SELECTED 1, 3, 4-OXADIAZOLE–BENZOTHIAZOLE DERIVATIVES: AN *IN-VITRO* STUDY

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Keywords:

Antioxidant activity, 1, 3, 4-oxadiazolederivatives, Benzothiazole derivatives, Oyaizu reducing power method, Oxidative stress, Free radicals

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ABSTRACT: Oxidative stress results from an imbalance between the generation of reactive oxygen species and the body's antioxidant defense mechanisms, leading to cellular damage. Antioxidants play a vital role in neutralizing free radicals and preventing oxidative injury. The present study was undertaken to evaluate the *in-vitro* antioxidant activity of synthesized 1, 3, 4-oxadiazole–benzothiazole derivatives using the Oyaizu reducing power method. Compound C3 and Compound V3 [2-(5′-substituted phenyl)-1′,3′,4′-oxadiazol-2′-yl amino)-6-fluoro-7-substituted (1,3)-benzothiazole derivatives], were assessed for their reducing ability by measuring the conversion of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) at 700 nm. Ascorbic acid was used as the standard antioxidant. Both compounds exhibited concentration-dependent antioxidant activity in the range of 20–120 µg/mL. Compound V3 showed a 34.65% increase in reducing power, while compound C3 showed a 32.33% increase at 120 µg/mL concentration. The results indicate that the synthesized 1, 3, 4-oxadiazole–benzothiazole derivatives may have antioxidant qualities based on their observed reducing potential. However, further research is needed to validate their biological relevance, including *in-vivo* studies, acute toxicity assessments, and other antioxidant assays such as DPPH, ABTS, or ORAC.

INTRODUCTION: A cellular reduction-oxidation imbalance in the body causes oxidative stress, a metabolic disorder marked by high levels of reactive oxygen species (ROS) [1]. The development and evolution of diabetes mellitus and its consequences are caused by excess ROS attacking intracellular proteins, lipids, DNA, and mitochondrial enzymes [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.17(7).2140-44

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(7).2140-44

Free radicals, which are dangerous to living things and present in biological cells, are counteracted by antioxidants. Superoxide dismutase (SOD) is an enzyme that plays a special role in mitigating the consequences of oxidative stress linked to the presence of free radicals.

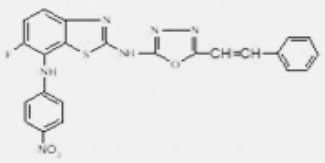
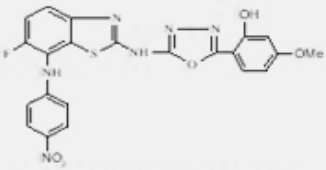
This enzyme catalyzes the recombination process of oxygen radicals. By reducing the synthesis of triplet oxygen and hydrogen peroxide, SOD-based antioxidant treatment effectively treats and prevents a range of degenerative disorders in the human body [3]. Antioxidants are substances that, when found in very small amounts in food or the human body, slow down, inhibit, or stop oxidative processes that lead to the deterioration of food

quality or the development and spread of degenerative diseases in the body. These antioxidant molecules use a range of strategies and actions to prevent oxidation [4]. Antioxidants can be categorized in a number of ways according to their physiological functions and surroundings [5, 6]. A chemical that may significantly slow down or completely stop substrate oxidation, even at low doses, is referred to as an antioxidant [7]. By providing electrons to free radicals, these substances reduce oxidative damage to biological systems [8, 9, 10]. Additionally, they prevent the production of free radicals and halt oxidative processes at any of the three crucial stages initiation, propagation, and termination [11, 12].

Compounds containing heteroatom nuclei have recently received a lot of attention due to their wide chemotherapeutic properties. The 1, 3, 4-Oxadiazole isomer is typically lower lipophilicity (log D) than its isomeric partner [13]. 1, 3, 4-Oxadiazole and its derivatives have demonstrated a wide array of biological activities, including anti-tubercular, anti-diabetic, anti-inflammatory, analgesic, anticancer, antimalarial, anti-allergic agent, antibacterial, anticonvulsant, GSK-3 inhibitors with *in-vivo* antidepressant and vasodilator activities [14].

1, 3, 4-oxadiazole derivatives have higher water solubility, reduced lipophilicity, and better metabolic stability than other isomeric oxadiazole. This nucleus can also be utilized as a bioisostere by replacing certain functions such as esters, amides, and carbamates. The aromatic structure of oxadiazole, together with its planar geometry, allows this moiety to function as a flat aromatic linker, providing suitable orientation to bind at COX-2 enzymes, reducing inflammation without creating ulcerogenic effects [15]. The chemistry of heterocycles and benzothiazole-fused bicyclic complexes, which have a biological profile of interest and are known to have anti-inflammatory, antioxidant, and anticancer properties, have both played important roles in this context [16]. Many medications with distinct biological characteristics, including viozan, probenazole, ethoxazolamide, and riluzole, are based on benzothiazole. Dyes, medications, and polymer chemistry are just a few of the areas of chemical study that have consistently shown interest in benzothiazole derivatives. Numerous benzothiazoles, including those with antituberculous, antiproliferative, antibacterial, anthelmintic, antioxidant, and antimicrobial properties, have been patented [17]. The primary goal of the research is to evaluate the *in-vitro* reducing power of selected derivatives of 1, 3, 4-oxadiazole–benzothiazole (C3 and V3 Compounds) using the Oyaizu reducing power method.

TABLE 1: LIST OF COMPOUNDS PROCURED

Compound Code	Compound description
C3	 3',4'-oxadiazol-2'-yl amino-6-fluoro-7-p-nitro anilino (1,3)-benzothiazole
V_3	 2[5'-(o-hydroxy p-methoxy phenyl)-1',3,'4'-oxadiazol-2'-yl amino]-6-fluoro-7-p-nitro anilino (1,3)-benzothiazole

MATERIALS AND METHODS: All chemicals and reagents utilized in the present study were of analytical reagent (AR) quality. Potassium ferricyanide ($K_3[Fe(CN)_6]$), ferric chloride ($FeCl_3$), Trichloroacetic acid (CCl_3COOH), and potassium dihydrogen phosphate were acquired from Merck Life Science Pvt. Ltd., India. The standard antioxidant, ascorbic acid, was acquired from Mumbai, India's Himedia Laboratories Pvt. Ltd. Distilled and deionized water was utilized throughout the experiment. The synthesized 1,3,4-oxadiazole–benzothiazole derivatives (compound C3 and compound V3) were procured from Postgraduate department of Pharmaceutical Chemistry, SCS College of Pharmacy Harapanahalli characterized using ^1H NMR and LC–MS analysis, as reported in earlier work [18], Phosphate buffer (0.2 M, pH 6.6) was newly generated using conventional laboratory techniques. Before being used, every piece of glassware was properly cleaned and dried. A UV-visible spectrophotometer with matching quartz cuvettes (1 cm path length) was used to measure absorbance. The centrifuge, analytical balance, and

temperature-controlled water bath were utilized for precise weighing, sample separation, and incubation, respectively. Experiment was carried out in the year 2025. The complete experimental investigation was carried out at the S.C.S. College of Pharmacy's Postgraduate Department of Pharmacology in Harapanahalli, Davanagere, 583131, Karnataka, India.

Preparation of Test Solutions: Dimethyl-formamide (DMF) was used to make stock solutions of the test compounds (compound C3 and compound V3) at a concentration of 1 mg/mL. To ensure that the compounds were fully dissolved, the solutions were sonicated for 10 minutes. The stock solution was serially diluted to reach the required concentrations (20-120 µg/mL) for testing. When the generated solutions were visually inspected to ensure 100% solubility and clarity; there was no turbidity or precipitation at any concentration. To avoid interference in absorbance measurements, all solutions were freshly prepared before the experiment and, if necessary, filtered to remove any undissolved particles.

Oyaizu Reducing Power Assay:

Principle: The reducing antioxidant power assay is based on the idea that an increase in absorbance equals an increase in antioxidant activity. Reduction is the gain of electrons, whereas oxidation is their loss [19]. A reducing agent (or reductant) contributes electrons to aid in the reduction of another chemical, whereas an oxidizing agent (or oxidant) absorbs electrons, resulting in the oxidation of another reactant. Because oxidation and reduction are interconnected, they occur concurrently within a system [20]. In the Fe^{3+}- reducing assay, reductants facilitate the conversion of Fe^{3+} to Fe^{2+}. A bioactive compound's reducing potential is measured by monitoring the direct reduction of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$. When free Fe^{3+} is added to the reduced product, it produces a vivid blue-colored complex known as Perl's Prussian blue, $Fe_4[Fe(CN)_6]^3$, with a strong absorbance of 700 nm. The Fe^{3+}-reducing test thus employs an electron transfer process with a ferric salt serving as the oxidant [5].

Procedure: Oyaizu was the first to assess antioxidants capacity for reduction [21]. Phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide ($[K_3Fe(CN)_6]$) (1%), as well as different concentrations of compound C3, compound V3and standard (ascorbic acid) were prepared in 1 mL of distilled water. For 20 minutes, the resultant mixture was incubated at 50 °C. After adding 2.5 mL of 10% Trichloroacetic acid (TCA), the mixture was centrifuged for 10 minutes at 3000 rpm. Following centrifugation, the top layer (2.5 mL) was gathered and combined with 0.5 mL of 0.1% $FeCl_3$ and distilled water. The absorbance of the final solution was measured at 700 nm with a spectrophotometer. A higher absorbance value suggested that the examined materials had a stronger reductive potential [22]. Distilled and deionized water is the best solution for measuring reducing power. Each experiment was carried out in triplicate.

Statistical Analysis: The data is presented as mean ± standard deviation (SD), and all trials were done in triplicate (n=3). The statistical study employed one-way ANOVA and Tukey's post-hoc test to determine the significance of group differences. A statistically significant result was considered as **p < 0.05.

RESULTS:

TABLE 2: REDUCING POWER OF COMPOUND C3 AND COMPOUND V3 WITH STATISTICAL SIGNIFICANCE

Concentration (µg/mL)	Ascorbic acid (% increase in absorbance)	Compound C3 (% increase in absorbance)	Compound V3 (% increase in absorbance)
20	41.84±0.02	18.40± 0.01*	12.87±0.01*
40	91.48±0.03	21.89± 0.02*	15.84±0.02*
60	142.19±0.02	24.87±0.01**	21.78±0.01*
80	197.87±0.03	28.85±0.02**	25.74±0.01**
100	225.53±0.02	29.35±0.01**	27.72±0.02**
120	244.33±0.03	32.33±0.02**	34.65±0.01**

Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test.*p < 0.05, **p < 0.01 compared to standard (ascorbic acid). The percentage increase in absorbance was calculated relative to the control (containing all the reagents except test and standard compounds) using the formula:

% increase in absorbance = [(Abs of Test − Abs of Control) / Abs of Control] × 100

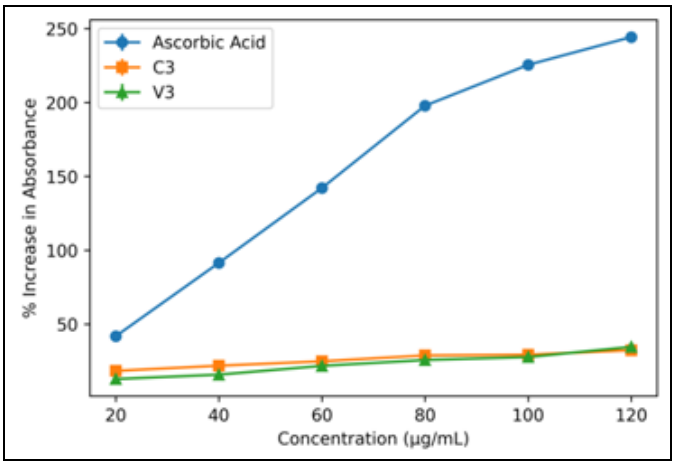


FIG. 1: REDUCING POWER OF COMPOUND C3 AND COMPOUNDV3 COMPARED WITH ASCORBIC ACID. The reducing power was expressed as percentage increase in absorbance at 700 nm. Values are presented as mean ± SD (n=3).

DISCUSSION: The Oyaizu reducing power method for antioxidant activity is based on the theory that antioxidants use electron donors from antioxidant compounds to change the yellow ferric complex (Fe^{3+}) into the bluish green ferrous complex (Fe^{2+}). A UV-Vis spectrophotometer with a wavelength of 700 nm is then used to measure the color change in order to determine the absorbance value [23]. The results of the present study show that both compounds have concentration-dependent reducing power, as indicated by the steady increase in percentage increase in absorbance with increasing concentrations of the test compounds. This pattern indicates that the studied compounds have electron-donating properties, which is an important feature of antioxidant molecules. When compared to the standard antioxidant (ascorbic acid), the test compounds had lower reducing activity but both derivatives displayed detectable reducing potential within the studied concentration range. Compound C3 had greater percentage increase in absorbance values than compound V3, indicating a better reducing capability. This discrepancy in activity could be due to structural changes in the oxadiazole-benzothiazole scaffold, which can affect electron distribution and redox behaviour. These compounds may act as antioxidants by contributing electrons by contributing electrons and stabilizing free radical intermediates. The concentration range of 20-120 µg/mL was established after considering preliminary solubility and test results. Within this range, the test compounds demonstrated a clear concentration-dependent increase in reducing power, allowing them to be compared to the standard. The results show concentration-dependent reducing activity; however, IC_{50} values were not computed since the halfway impact was not obtained at the tested concentrations. However, the current work is restricted to a single chemical assay that does not fully characterize antioxidant behaviour in biological systems. To better comprehend these compounds' antioxidant capabilities and determine their biological significance, more study involving acute toxicity, *in-vivo* studies, as well as additional antioxidant tests like DPPH, ABTS, or ORAC, would be necessary.

CONCLUSION: In the present study, it was observed that 2(5'-substituted phenyl) 1', 3', 4' oxadiazol-2'-yl amino)-6-fluoro-7-substituted (1, 3)-benzothiazole derivatives have demonstrated concentration-dependent increase in the reducing property. The results show that compound C3 and compound V3 have a moderate reducing potential, which may contribute to antioxidant potential, even if the reducing activity of the test compounds was lower than that of the standard antioxidant (Ascorbic acid). However, these results should be interpreted with caution because the current study is limited to a single *in-vitro* chemical experiment. Further studies, including multiple antioxidant assays, acute toxicity assessments, and *in-vivo* studies, are required to determine the biological importance and potential applications of these compounds.

ACKNOWLEDGEMENT: The authors express their sincere gratitude to His Holiness Shri Varasadyojatha Shivacharya Swamiji, President of TMAE Society, Harapanahalli; Principal & Vice Principal of S.C.S. College of Pharmacy, Harapanahalli, for their continuous encouragement and support throughout this work.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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How to cite this article:
Pasarad AC, Chandana CM, Durganjali and Shashidhar VK: Antioxidant potential of selected 1, 3, 4-oxadiazole–benzothiazole derivatives: an *in-vitro* study. Int J Pharm Sci & Res 2026; 17(7): 2140-44. doi: 10.13040/IJPSR.0975-8232.17(7).2140-44.