Vol 6, Issue 1, January 2026 www.ijesti.com E-ISSN: 2582-9734
International Journal of Engineering, Science, Technology and Innovation (IJESTI)

Evaluation of DPPH and Hydroxyl Radical Scavenging Activity of Novel Benzothiazole–Thiazolidinone Derivatives

Wadnere Kishor Eknath [1]

[1] Research Scholar, Department of Chemistry, Mansarovar Global University, Sehore, M.P., India.

Dr. Pushpendra Tiwari [2]

[2] Supervisor, Department of Chemistry, Mansarovar Global University, Sehore, M.P., India.

ABSTRACT

A series of benzothiazole–thiazolidinone derivatives were synthesized via a multistep reaction pathway. The antioxidant properties of the synthesized derivatives were evaluated using DPPH and hydroxyl radical scavenging assays (HRSA) at concentrations ranging from 50 to 250 µg/mL, with ascorbic acid serving as the reference standard. Among the derivatives, 7b demonstrated the strongest antioxidant potential, achieving 93.16 ± 0.83% in the DPPH assay and 94.10 ± 2.35% in HRSA at the highest concentration tested, approaching the activity of the standard. Compounds 7a and 7e showed moderate activity, whereas 7c and 7d were comparatively less effective, likely due to electron-withdrawing substituents limiting hydrogen-donating ability. the study highlights that hydroxy-substituted benzothiazole–thiazolidinone derivatives, particularly 7b, possess significant free radical scavenging activity and may serve as promising candidates for further pharmacological and therapeutic development.

Keywords: *Benzothiazole, Radical scavenging, Antioxidant, Electron, Ascorbic Acid.*

I. INTRODUCTION

Novel antioxidant molecules that might mitigate the harmful effects of free radicals in biological systems are of great interest due to the rising frequency of illnesses associated with oxidative stress. The partial reduction of oxygen during cellular metabolism gives birth to free radicals, especially reactive oxygen species (ROS), which are very reactive molecules. When it comes to these radicals, hydroxyl radicals (•OH) are at the top of the list for destructiveness and reactivity. They can damage cells and play a role in the development of many diseases and disorders, such as cancer, neurodegenerative disorders, heart problems, and aging-related anomalies. For the same reason, many researchers use stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and other free radicals as model systems to assess the antioxidant capabilities of both man-made and naturally occurring chemicals. Because of the therapeutic potential and the need to strengthen cellular defense systems against oxidative damage, the search for powerful antioxidants has therefore become an important topic in medicinal chemistry.

A new family of heterocyclic chemicals called benzothiazole derivatives has recently arisen; these compounds have anticancer, antibacterial, antifungal, anti-inflammatory, and antioxidant capabilities, among many others. The benzothiazole moiety has an electron-rich system that can stabilize free radicals through delocalization and resonance processes. It is defined by the fusion of a benzene ring with a thiazole ring. The synthesis of hybrid compounds with superior biological profiles has been achieved by extensive structural alteration of the benzothiazole framework, which has been shown to increase its pharmacological activity and specificity. Thiazolidinone scaffolds integrated into benzothiazole derivatives constitute one such strategy. Antimicrobial, anticancer, anti-inflammatory, and antioxidant effects are just a few of the many pharmacological benefits associated with thiazolidinones, a class of

compounds characterized by a five-membered heterocyclic ring containing sulfur and nitrogen atoms. Therefore, there has been a lot of interest in the idea of combining benzothiazole and thiazolidinone units into one molecular entity, since this might lead to synergistic effects that increase the free radical scavenging ability of both compounds.

One of the most important ways to determine if novel chemical entities have medicinal potential is to test their antioxidant activity. When it comes to testing a compound's ability to neutralize free radicals, the DPPH and hydroxyl radical scavenging tests are two of the most trusted and extensively used options. A stable nitrogen-centered radical that appears very violet in solution is utilized in the DPPH test. When DPPH comes into contact with an antioxidant, it forms its non-radical form by a single-electron reduction. This process causes a drop in absorbance, which may be detected quantitatively using spectrophotometry. The simplicity, repeatability, and sensitivity of this test make it ideal for the quick screening of freshly synthesized compounds for their antioxidant potential. Conversely, substances are evaluated for their capacity to scavenge extremely reactive hydroxyl radicals produced by the Fenton reaction or other in vitro techniques in the hydroxyl radical scavenging experiment. The ability of a substance to prevent hydroxyl radical-induced oxidative changes in biomolecules is indicative of its possible therapeutic use in reducing oxidative stress. These tests are vital for characterizing antioxidant capabilities and give complementary insights into substances' radical scavenging capacity.

Critical parameters impacting the biological performance of benzothiazole-thiazolidinone compounds include their solubility, stability, and pharmacokinetic characteristics, in addition to their chemical qualities. To maximize the chemicals' protective effects against oxidative damage, it is important to optimize these characteristics so that they interact with free radicals efficiently under physiological settings. To further aid in the identification of active sites within the molecules and to offer mechanistic insights into radical scavenging processes, in vitro antioxidant experiments are integrated with computer modeling and molecular docking investigations. By bringing together different fields, we can learn more about how molecular changes affect free radical neutralization and speed up the process of finding novel antioxidants.

Learning about DPPH and its ability to scavenge hydroxyl radicals is important for reasons beyond simple academic interest; oxidative stress is associated with numerous diseases, including those of the nervous system (such as Alzheimer's and Parkinson's), the heart (such as atherosclerosis), the inflammatory system, diabetes, and cancer. Strong antioxidant chemicals, such as benzothiazole-thiazolidinone derivatives, can reduce the harmful effects of reactive oxygen species (ROS), making them potential therapeutic agents or building blocks for new medications. Additionally, these molecules might discover uses in the pharmaceutical, nutraceutical, and cosmetic fields, where antioxidants are valued for their ability to preserve and prolong the shelf life of products. Therefore, exploring such compounds offers a potential way to create multifunctional medicines that can tackle oxidative stress and the diseases it causes.

II. REVIEW OF LITERATURE

Koshelev, Vladimir et al., (2023) To create novel powerful antioxidants, this study employed a class of thiosemicarbazones that had phenol fragments as building blocks. The final products, thiazole and thiazolidinone, have a heterocyclic fragment in addition to the phenol substituent. A potential energy scan and NOESY NMR spectroscopy data were utilized to ascertain the most stable conformation of thiosemicarbazone. When thiosemicarbazones interacted with various bromoketones, such as bromodimedone, bromoacetophenone, and bromoacetylcoumarin, a variety of thiazole compounds were extracted. Results ranged from 71% to 94%. With respectable yields ranging from 82% to 95%,

thiazolidinone derivatives were produced by reacting thiosemicarbazones with chloroacetic acid or maleic anhydride. In vitro antioxidant activities were assessed for each product using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) to estimate radical cation scavenging activity and the ferricyanide/Prussian blue technique to quantify ferric reducing capacity. When compared to 4-methyl-2,6-di-tert-butylphenol, the antioxidant activity of the majority of the synthetic compounds tested was higher; however, derivatives containing a 2,6-di-tert-butylphenol fragment exhibited the greatest activity.

Karaca, Şevval et al., (2022) A novel strategy for treating Alzheimer's disease (AD) involves the use of dual acetylcholinesterase (AChE)-monoamine oxidase B (MAO-B) inhibitors. Fourteen novel benzothiazoles (4a-4n) were conceptualized and produced in this study. All compounds were tested for their inhibitory potentials of AChE, butyrylcholinesterase (BChE), MAO-A, and MAO-B utilizing the in vitro fluorometric technique in biological activity investigations. Furthermore, an in vitro kit-based technique was used to assess the inhibitory effects of active substances on amyloid beta (Aβ)-aggregation. Results from the biological assay demonstrated that compounds 4a, 4d, 4f, 4h, 4k, and 4m inhibited the AChE and MAO-B enzymes enough. To inhibit the AChE and MAO-B enzymes, respectively, Compound 4f showed IC50 values of 23.4 ± 1.1 nM and 40.3 ± 1.7 nM. New evidence suggests that compound 4f may block the action of AChE and MAO-B enzymes and halt the buildup of beta amyloid plaques in the brains of Alzheimer's disease patients. Furthermore, the results of the in silico investigations corroborate the biological activity that was found. Both enzymes' active sites were strongly interacted with by compound 4f. Particularly encouraging and fascinating is the observation that chemical 4f interacts with flavin adenine dinucleotide (FAD) in the active site of the MAO-B enzyme.

Khan, Shoaib et al., (2022) The study's main candidates against α-amylase and α-glucosidase enzymes were discovered to be thiazolidinone-based benzothiazole derivatives 1–15, which were produced via a stepwise procedure. With an IC50 range of 2.10 ± 0.70 to 37.50 ± 0.70 μM for α-amylase and 3.20 ± 0.05 to 39.40 ± 0.80 μM for α-glucosidase, nearly all of the derivatives exhibited good to outstanding activity. The normal medication acarbose had an activity level of 9.10 ± 0.10 μM, whereas other analogues, including 4 (2.40 ± 0.70 and 3.50 ± 0.70 μM), 5 (2.30 ± 0.05 and 4.80 ± 0.10 μM), and 6 (2.10 ± 0.70 and 3.20 ± 0.70 μM), had an activity level that was twice as high. As an added bonus, every chemical now has a known structure-activity relationship (SAR). A study using molecular docking was conducted to investigate the binding interactions with α-amylase and α-glucosidase enzymes.

Cabrera-Pérez, Laura et al., (2016) There is mounting evidence that the antioxidant properties of benzothiazoles and thioureas can render reactive chemical entities inactive. Compound 1, (E)-5-((benzo[d]thiazol-2-ylimino)(methylthio)methylamino)-2-hydroxybenzoic acid, and compound 2, (S,E)-2-((benzo[d]thiazol-2-ylimino)(methylthio)methylamino)-3-(4-hydroxyphenyl) propanoic acid, were both developed and produced within this context as benzothiazole-isothiourea derivatives. The free radical scavenging ability of both compounds was evaluated in vitro using the 2,2'-diphenyl-1-picrylhydrazyl radical reduction and Fenton reaction. Compound 1 showed the greatest scavenging activity, according to the data. Therefore, it was tested in vitro utilizing the first stage of the acetaminophen-induced hepatotoxicity paradigm. Compound 1 was shown to enhance reduced glutathione content and decrease malondialdehyde levels, as mentioned before. It also has the ability to defend against the reactive intermediate N-acetyl-p-benzoquinoneimine and inhibit cytochrome P450.

Maddila, Suresh et al., (2015) The 5-((10H-phenothiazin-10yl)methyl) amino acid series The multi-step procedure used to manufacture chemical (1) yielded -4-(substitutedbenzylideneamino)-4H-1,2,4-triazole-3-thiol derivatives (6a-i). Upon condensation with a variety of appropriate aldehydes in the presence of H2SO4, the essential intermediate (5) yielded a range of title compounds (6a-i). By analyzing their elemental composition and utilizing IR, 1H-NMR, 13C-NMR, and MS spectra, the structures of new

compounds were defined. In vitro antioxidant activity was assessed for all of these new compounds using DPPH, nitric oxide, and hydrogen peroxide radical scavenging tests. Due to the presence of electron-releasing groups, compounds 6d, 6e, and 6i exhibited strong antioxidant activity.

III. MATERIAL AND METHODS

Chemicals and Reagents

The compounds 2-aminobenzenethiol and 5-aminosalicylic acid were purchased from Loba Chemie Pvt. Ltd. in Mumbai, India. A source of ethanol was Sigma-Aldrich in India. To conduct this study, a variety of chemicals and reagents were procured from CDH (Central Drug House) in New Delhi, India, or Loba Chemie Pvt. Ltd. in the same city. These included hydrochloric acid, sulfuric acid, potassium hydroxide, benzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, chlorobenzaldehyde, hydroxybenzaldehyde, and chloroacetyl chloride. The compounds were utilized in their original, unrefined form.

Uncorrected melting point apparatus was used to find the open capillary technique melting points of all of the synthetic substances.

Synthesis of Benzothiazole–Thiazolidinone Derivatives

The synthesis of the target compounds was carried out through a sequential multistep reaction pathway involving the formation of key intermediate compounds. Initially, 4-amino-2-(benzo[d]thiazol-2-yl)phenol was synthesized, which was subsequently converted into N-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)-2-chloroacetamide. This intermediate was further cyclized to yield (E)-2-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenylimino)thiazolidin-4-one. The third intermediate was then reacted with various aromatic aldehydes through a condensation reaction to obtain the desired substituted benzylidene derivatives.

The final synthesized compounds obtained from this reaction sequence included (2E,5E)-2-((3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)imino)-5-benzylidenethiazolidin-4-one (7a) and its substituted analogues, namely (2E,5E)-2-((3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)imino)-5-(4-hydroxybenzylidene)thiazolidin-4-one (7b), (2E,5E)-2-((3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)imino)-5-(4-chlorobenzylidene)thiazolidin-4-one (7c), and (2E,5E)-2-((3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)imino)-5-(4-nitrobenzylidene)thiazolidin-4-one (7d).

Evaluation of Antioxidant Action

DPPH Scavenging Assay: The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test was used to assess the antioxidant effect of the produced compounds. We used the stable radical DPPH to evaluate the hydrogen-donating and radical-scavenging capabilities of the produced compounds as a measure of their free radical scavenging activity. The DMSO was used to create the test samples (10-100 μL), which were then combined with 1.0 mL of DPPH solution and methanol until a final volume of 4 mL was reached. A visible spectrophotometer was used to measure the absorbance of the final solution at 517 nm. The chemical that used as a reference was ascorbic acid. Increased free radical scavenging activity was demonstrated by a lower absorbance of the reaction mixture.

Hydroxy Radical Scavenging Activity: A mixture of 1 mL of iron EDTA solution, 0.5 mL of EDTA solution, 1 mL of DMSO, and 0.5 mL of ascorbic acid was added to test solutions with different concentrations (50, 100, 150, 200, and 250 µg/mL). We incubated the mixture in a water bath set to boil at 80 to 90°C for 15 minutes. The reaction mixture was then incubated at room temperature for 15 minutes after adding 1 mL of ice-cold TCA and 3 mL of Nash reagent. At 412 nm, the absorbance was measured.

The data were presented as mean values ± standard deviations and made sure to execute all experiments in triplicate.

IV. RESULTS AND DISCUSSION

Table 1: Comparative DPPH Antioxidant Activity of Synthesized Benzothiazole Derivatives

Test Compound	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Ascorbic Acid (Standard)	95.12 ± 0.68	–	–	–	–
7a	20.35 ± 0.42	32.18 ± 0.57	48.92 ± 0.81	64.37 ± 0.69	78.54 ± 0.74
7b	38.74 ± 0.59	56.83 ± 0.88	70.46 ± 0.95	84.29 ± 0.61	93.16 ± 0.83
7c	15.62 ± 0.71	24.87 ± 0.65	36.94 ± 0.79	51.38 ± 0.72	63.25 ± 0.68
7d	10.48 ± 0.54	18.36 ± 0.61	27.82 ± 0.84	39.91 ± 0.56	52.44 ± 0.73
7e	22.91 ± 0.63	34.76 ± 0.91	49.58 ± 0.74	65.82 ± 0.69	77.36 ± 0.88

Table 1 shows that all the benzothiazole derivatives that were produced have antioxidant activity that is concentration dependent. This shows that they might be used as free radical scavengers. The compound with the highest scavenging activity was 7b, which showed a steady rise from 38.74 ± 0.59% at 50 µg/mL to 93.16 ± 0.83% at 250 µg/mL. This increased activity was comparable to that of ascorbic acid, the standard antioxidant, which showed 95.12 ± 0.68% scavenging at the lowest concentration. The inclusion of electron-donating hydroxy groups in 7b is responsible for its higher activity, since they stabilize free radicals and promote the donation of hydrogen atoms. The antioxidant capacity of compounds 7a and 7e was modest, and their scavenging activity increased gradually from around 20-23% at 50 µg/mL to 78-77% at 250 µg/mL. When tested at their maximum concentrations, compounds 7c and 7d showed much reduced scavenging effects, with concentrations of 63.25% and 52.44%, respectively.

Table 2: Comparative Hydroxyl Radical Scavenging (%) of Synthesized Benzothiazole Derivatives

Test Compound	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Ascorbic Acid (Standard)	89.20 ± 0.56	–	–	–	–
7a	22.10 ± 0.72	28.45 ± 1.03	50.32 ± 1.03	67.84 ± 0.70	84.50 ± 1.91
7b	37.25 ± 1.91	53.10 ± 0.67	69.05 ± 0.84	89.50 ± 1.90	94.10 ± 2.35
7c	15.20 ± 0.72	19.05 ± 0.70	31.20 ± 1.03	46.00 ± 1.63	61.50 ± 2.03
7d	10.75 ± 0.72	14.50 ± 0.36	22.00 ± 1.46	33.50 ± 0.63	45.75 ± 1.03
7e	17.50 ± 0.36	21.50 ± 1.51	36.00 ± 1.03	51.20 ± 0.70	65.50 ± 0.63

Table 2 displays the hydroxyl radical scavenging activity (HRSA) of the synthesized benzothiazole-thiazolidinone derivatives. All of the compounds exhibit an increase in antioxidant potential that is concentration dependent. Compound 7b had the greatest scavenging activity among the derivatives, with values ranging from 37.25 ± 1.91% at 50 µg/mL to 94.10 ± 2.35% at 250 µg/mL. This value is very near to that of the standard antioxidant, ascorbic acid, which showed 89.20 ± 0.56% at the lowest dosage that was tested. The existence of hydroxy substituents that donate electrons, which stabilize hydroxyl radicals and make hydrogen donation easier, is probably responsible for this increased activity. The structural properties of compounds 7a and 7e appear to encourage radical neutralization to a large degree, as their

scavenging percentages increased progressively from around 22–23% at 50 µg/mL to 84–65% at 250 µg/mL, indicating moderate action. On the other hand, at the maximum concentration, 7c scavenged 61.50% of hydroxyl radicals while 7d scavenged 45.75 percent.

V. CONCLUSION

The antioxidant capacity of a variety of benzothiazole-thiazolidinone compounds was assessed in this work, which also effectively synthesized them. In both DPPH and hydroxyl radical experiments, all of the compounds showed free radical scavenging activity that was concentration dependant. The presence of electron-donating hydroxy groups likely explained why compound 7b, out of all the produced derivatives, was the most effective antioxidant, showing activity similar to that of the gold standard, ascorbic acid. Substituents that take electrons out of a molecule decrease its antioxidant efficacy; compounds 7a and 7e showed moderate activity, while compounds 7c and 7d showed comparatively low activity. These findings demonstrate how structural characteristics significantly affect the capacity to scavenge free radicals. In summary, the study confirms that hydroxy-substituted benzothiazole-thiazolidinone compounds, namely 7b, show potential as antioxidants and necessitate more research into their medicinal and pharmacological uses.

REFERENCES

1. V. Koshelev, O. Snastina, S. Vorobyev, A. Stupnikova, and L. Ivanova, "Synthesis and antioxidant activity of novel thiazole and thiazolidinone derivatives with phenolic fragments," *Appl. Sci.*, vol. 13, no. 24, pp. 1–14, 2023.
2. Ş. Karaca, D. Osmaniye, B. Sağlık, S. Levent, S. Ilgin, Y. Özkay, A. C. Karaburun, Z. Kaplancıklı, and N. Gundogdu-Karaburun, "Synthesis of novel benzothiazole derivatives and investigation of their enzyme inhibitory effects against Alzheimer's disease," *RSC Adv.*, vol. 12, no. 36, pp. 23626–23636, 2022.
3. S. Khan, M. Khan, W. Rehman, M. Shah, R. Hussain, L. Rasheed, Y. Khan, A. Dera, R. Pashameah, E. Alzahrani, and A.-E. Farouk, "Design, synthesis, in silico testing, and in vitro evaluation of thiazolidinone-based benzothiazole derivatives as inhibitors of α-amylase and α-glucosidase," *Pharmaceuticals*, vol. 15, no. 10, pp. 1–17, 2022.
4. R. Rajalakshmi, R. Santhi, and T. Elakkiya, "Synthesis, characterization, biological evaluation and molecular docking studies of some oxazinyl-thiazolidinone derivatives," *Asian J. Chem.*, vol. 32, no. 9, pp. 2125–2129, 2020.
5. M. Haroun, C. Tratrat, K. Kositzi, E. Tsolaki, A. Petrou, B. Aldhubiab, M. Attimarad, S. Harsha, A. Geronikaki, K. N. Venugopala, H. S. Elsewedy, M. Sokovic, J. Glamoclija, and A. Ciric, "New benzothiazole-based thiazolidinones as potent antimicrobial agents: Design, synthesis and biological evaluation," *Curr. Top. Med. Chem.*, vol. 18, no. 1, pp. 75–87, 2018.
6. L. Cabrera-Pérez, I. Padilla-Martínez, A. Cruz, J. Mendieta-Wejebe, F. Tamay-Cach, and M. Rosales-Hernández, "Evaluation of a new benzothiazole derivative with antioxidant activity in the initial phase of acetaminophen toxicity," *Arab. J. Chem.*, vol. 12, no. 8, pp. 1–12, 2016.
7. S. Maddila, M. Momin, S. Gorle, L. Palakondu, and S. Jonnalagadda, "Synthesis and antioxidant evaluation of novel phenothiazine linked substitutedbenzylideneamino-1,2,4-triazole derivatives," *J. Chil. Chem. Soc.*, vol. 60, no. 2, pp. 2919–2923, 2015.
8. R. Keri, M. Patil, S. Patil, and S. Budagumpi, "A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry," *Eur. J. Med. Chem.*, vol. 89, no. 1, pp. 207–251, 2014.

9. P. Prabhu, P. Theivendren, S. Shastry, S. Arumugam, and S. Pande, "Synthesis and anticancer evaluation of 2–phenyl thiazolidinone substituted 2–phenyl benzothiazole–6–carboxylic acid derivatives," *J. Saudi Chem. Soc.*, vol. 13, no. 1, pp. 1–5, 2012.
10. D. Havrylyuk, L. Mosula, B. Zimenkovsky, O. Vasylenko, A. Gzella, and R. Lesyk, "Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety," *Eur. J. Med. Chem.*, vol. 45, no. 11, pp. 5012–5021, 2010.
11. D. Cressier, C. Prouillac, P. Hernandez, C. Amourette, M. Diserbo, C. Lion, and G. Rima, "Synthesis, antioxidant properties and radioprotective effects of new benzothiazoles and thiadiazoles," *Bioorg. Med. Chem.*, vol. 17, no. 14, pp. 5275–5284, 2009.
12. G. Kılcıgil, C. Kus, T. Coban, B. Can Eke, S. Ozbey, and M. Iscan, "Synthesis, antioxidant and radical scavenging activities of novel benzimidazoles," *J. Enzyme Inhib. Med. Chem.*, vol. 20, no. 5, pp. 503–514, 2005.