Evaluation of Antioxidant Activity of DHPM and Imidazole Derivatives Using DPPH Radical Scavenging

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[1]*Received: 12 January 2025; Accepted: 23 March 2025; Published: 08 April 2025*

ABSTRACT

This laboratory investigates the antioxidant capacities of imidazole derivatives and DHPM using the DPPH radical scavenging assay. The concentrations of the derivatives in the ethanol solutions ranged from 0.1 to 0.4 mg/mL. Their ability to scavenge free radicals was tested by mixing 2.7 mL of each ethanolic solution with a DPPH solution that had a concentration of 40 µg/mL. The mixes were incubated in a dark environment for two hours to ensure process stability. By comparing the sample absorbance to the control at 517 nm, the percentage of DPPH radical scavenging activity was determined using a standard formula. Ascorbic acid, an antioxidant standard, was used. Because the investigated chemicals exhibited concentration-dependent radical scavenging action, the findings demonstrated their promise as natural antioxidant agents dent. To aid in the creation of pharmaceutical antioxidants, this technique offers a dependable means of assessing free radical inhibition in new compounds.

Keywords: *Antioxidants; Radical; Scavenging; Imidazole; Ethanol.*

1. Introduction

The medicinal and synthetic organic chemistry communities have taken a keen interest in imidazole and its derivatives due to its many pharmacological and biological applications. having its five-membered heterocyclic ring and two nonadjacent nitrogen atoms, the imidazole nucleus is a component of many naturally occurring and synthetically produced compounds having important therapeutic properties. Physiologically active chemicals that incorporate this heterocyclic system include histidine, histamine, biotin, and the antifungal drug ketoconazole, among many others, demonstrating its remarkable chemical and biological variety. Because of its wide range of uses in medicine, agriculture, and materials science, imidazole derivative synthesis and characterisation has become an important field of study in recent decades. Because oxidative stress is now known to play a significant role in many diseases, including cancer, heart disease, neurological disorders, and inflammation-related pathologies, it is crucial to find new imidazole-based compounds with strong antioxidant activity.

The important function of antioxidants is to protect cells and tissues from oxidative damage by neutralizing free radicals and ROS. Oxidative stress, caused by either an overabundance of free radicals or a lack of antioxidant defense systems, influences the pathophysiology of many chronic illnesses and interferes with normal physiological activities. Consequently, there has been a marked increase in the pursuit of synthetic antioxidants that are both innovative and effective. Thanks to their capacity to stabilize free radicals, bind metal ions, and contribute electrons or hydrogen atoms, imidazole derivatives have shown significant antioxidant potential. Thanks to its conjugated structure and

[1] *How to cite the article:* Prakash S., Sonune K.N (2025); Evaluation of Antioxidant Activity of DHPM and Imidazole Derivatives Using DPPH Radical Scavenging; *International Journal of Technology, Science and Engineering*; Vol 8 Issue 2; 1-7

nitrogen atoms that may form hydrogen bonds and participate in electron delocalization, the imidazole ring is an ideal electronic scaffold for the construction of powerful antioxidant compounds. Antioxidant activity of imidazole derivatives may be dramatically affected by structural changes in the nucleus, for example the addition of electron-donating or electron-withdrawing substituents.

Aldehydes, ketones, and amines are usually condensed under different reaction circumstances, with acids, bases, or metal ions serving as catalysts, in order to synthesize novel imidazole derivatives. In addition to solvent-free conditions, microwave-assisted synthesis, and ionic liquids, modern synthetic techniques now include green chemistry methodologies to increase yield, selectivity, and environmental sustainability. To characterize the compounds, which is necessary for accurately determining their structure and purity, a combination of spectroscopic methods like Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (~1H NMR), carbon-13 nuclear magnetic resonance (~13C NMR), and MS is traditionally used. These techniques guarantee the freshly synthesized compounds have the correct structural properties by providing crucial information on their functional groups, bonding patterns, and molecular structure.

Recent years have seen the synthesis of several imidazole-based molecules with outstanding antioxidant properties. Improving radical scavenging ability has been shown by substitution at several places on the imidazole ring, such as adding phenolic, methoxide, or heteroaromatic groups. These compounds are able to stabilize radical intermediates produced during oxidation-reduction processes because they include heteroatoms and conjugated π-electron systems. Antioxidant efficacy may be further enhanced by adding functional groups that facilitate hydrogen atom transfer or single-electron transfer pathways. Imidazole compounds have shown great promise as medicinal agents and additives in the food, cosmetics, and polymer sectors for preventing oxidative degradation due to their characteristics.

From a pharmacological perspective, imidazole derivatives' antioxidant activity is associated with their capacity to modify levels of endogenous antioxidant enzymes, decrease metal-induced oxidative stress, and suppress lipid peroxidation. Antioxidative actions are only one of the numerous biological activities shown by compounds derived from imidazoles. These compounds also have antifungal, antibacterial, anti-inflammatory, anticancer, and enzyme-inhibiting capabilities. They are very useful in the field of drug discovery and development due to their complex bioactivity. Researchers are able to tailor the physicochemical and biological characteristics of imidazole compounds to target certain illnesses or biochemical pathways due to the structural flexibility of the imidazole nucleus, which permits broad chemical changes.

In addition to validating the chemical structures of produced imidazole derivatives, characterisation also sheds light on structure-activity relationships (SAR), which are crucial for comprehending the impact of various substituents on antioxidant capacity. The functional groups and molecular characteristics that are responsible for the observed biological effects may be identified by systematic alteration and analysis. The capacity to scavenge radicals is improved when aromatic rings include hydroxyl and methoxide groups; however, redox potential and reactivity may be changed by substituting halogens or nitrogens. So, we may create very effective antioxidants by designing and synthesising novel imidazole derivatives according to SAR principles.

Imidazole derivative antioxidant behaviour predictions and rationalisation tools have been developed through the application of computational methods like molecular docking, density functional theory (DFT) calculations, and quantitative structure-activity relationship (QSAR) modelling. In order to back up the experimental results and direct future synthetic efforts, these methods aid in determining possible binding sites, calculating the electron density distribution, and assessing the thermodynamic feasibility of redox reactions.

To sum up, there is an important field of current pharmacological and chemical research that involves the synthesis and characterisation of novel imidazole derivatives as antioxidants. Antioxidant design relies on the imidazole ring system because of its inherent chemical diversity, its ability to engage in redox processes, and its power to stabilize radical intermediates. Further investigation into chemicals derived from imidazoles has great potential for the identification of new and powerful antioxidants that might be used as treatments for diseases caused by oxidative

stress. The chemical behavior of imidazole derivatives may be better understood and new compounds with improved biological effectiveness, stability, and safety can be developed more quickly via the combination of synthetic, spectroscopic, and computational techniques. As a result, there is a great deal of hope for the future of medicinal chemistry and associated fields as a whole in the investigation of antioxidants based on imidazoles.

2. Review of Literature

Oliveira, Ariel et al., (2025) there is mounting evidence linking free radicals to degenerative disorders like cancer, cataracts, and brain dysfunction. By intercalating with nitrogenous bases of DNA, copper (II) complexes show antioxidant activity and may operate in the therapy of various disorders by modifying or inhibiting cellular processes. Various complexes have the potential to be used in the creation of novel materials and medications for the treatment or prevention of various disorders. An imidazole-type ligand and copper (II) combination was synthesized and characterized in this study. Density functional theory (DFT) calculations performed at the B3LYP-D3(BJ)/def2-SVP level of theory demonstrated that the complex and its ligand spontaneously synthesized with a copper-nitrogen bond length of 1.988 Å. The antioxidant assays demonstrated a rapid reduction of complex radicals compared to free ligand. An IC50 value that is 1.5 times higher than that of BHT, the industry standard, shows that the chemical in question has significant antioxidant propertiess. 5 mol of Cu (3NIMDZ-H)(OH2) There may be new material applications for 2+ in the creation of antitumor drugs due to its quick kinetics characteristic for DPPH inhibition.

Gümrükçüoğlu, Nurhan. (2020) Novel Benz imidazole compounds were produced in this investigation. Mass spectroscopy, 1H and 13C nuclear magnetic resonance (NMR), and elemental analysis confirmed their structures after Thin Layer Chromatography (TLC) and melting point testing verified their purity. In order to determine if the materials have antioxidant properties, we tested them against lipid peroxidation, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and the ethoxyresorufin O-demethylase (EROD) enzyme. As compared to the reference compound, the compounds showed moderate efficacy in DPPH radical scavenging and lipid peroxidation inhibition, and they had a positive influence on EROD enzyme activity.

Abdelhamid, Antar et al., (2019). The use of pyrrolidinium hydrogen sulfate (PHS), a remarkable ionic liquid catalyst, allowed for the creation of several imidazole moieties with biological uses. A one-pot multicomponent technique was used to perform this reaction. Imidazole was created by combining ammonium acetate, several aromatic aldehydes, 1,2-diphenylethane-1,2-dione, and ethyl glycinate hydrochloride in an equimolar ratio. Among the many advantages of this method are its low reaction times, easy installation, and high yield. It is possible to crystallize and purify the imidazole's components that are produced without using chromatography, and the catalyst may be recycled. Infrared, nuclear magnetic resonance, and mass spectra have all contributed to the identification of these newly synthesized components. When tested on rats, these chemicals showed antioxidant action in vivo.

Usta, Asu et al., (2015). we synthesized and tested the antioxidant properties of many Benz imidazole derivatives in this study. These compounds include salicylic, ox diazole, thiosemicarbazide, and 1, 2, 4-triazole moieties. In order to determine if the synthetic compounds exhibited antioxidant properties, we used DPPH and ABTS•+ radical cation decolonization tests. The scavenging activity of compounds 4b, 6a, 6b, 7a, and 7b was very high

3. Materials and Methods

Antioxidant activity

DPPH radical scavenging activity

The researchers utilized their methodology to determine the DPPH radical scavenging effect. At doses of 0.1, 0.2, 0.3, and 0.4 mg/ml, aqueous solutions of each DHPM and imidazole derivative were produced. In separate test tubes that already had 2.7 ml of a 40 ρg ml-1 methanolic solution of DPPH, varying volumes (0.3 ml) of each ethanolic solution of DHPM derivative were added. After giving the combinations a good shake, they were placed in the dark for two

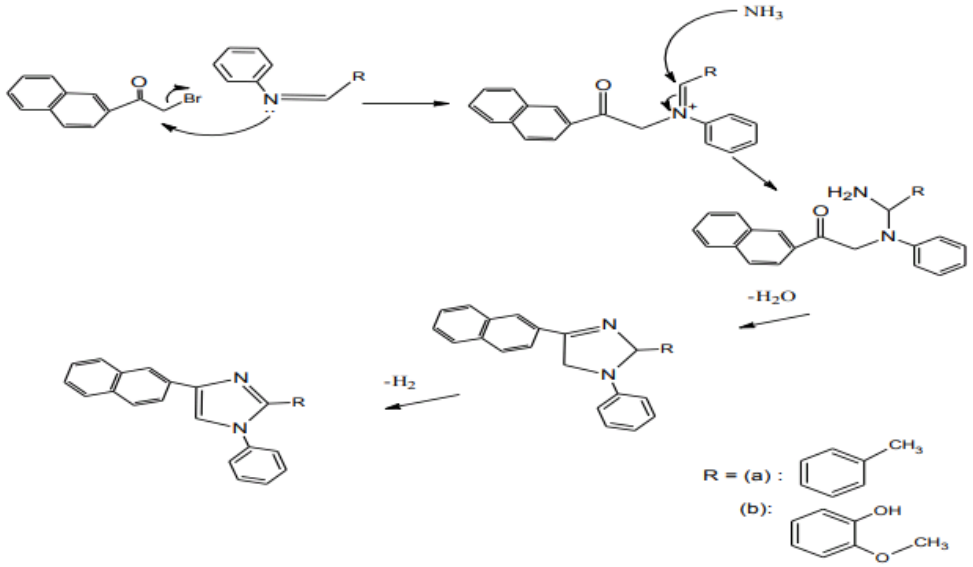
hours, or until their levels stabilized. For every sample, an absorbance reading was recorded at 517 nm. We used the following formula to determine the percentage of DPPH radical scavenging activity in the standard and the samples:

$$\% \text{ DPPH radical scavenging activity} = [1-(At/A0)] \times 100$$

Where At denotes the sample's absorbance and Ao denotes the control's absorbance. Standards made use of ascorbic acid.

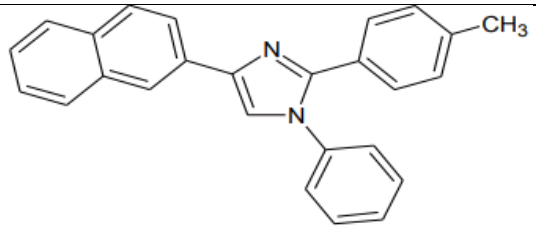
4. Results

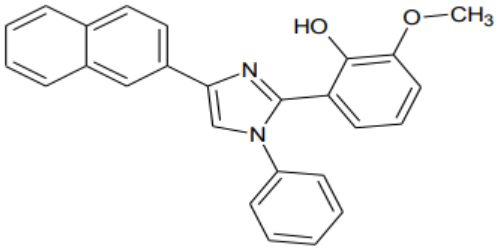
The imine and iminium intermediates are formed by condensation of the amine and aldehyde, respectively, in the preparation of imidazole's derivatives (a, b). To create the aminal intermediate, the imine is nucleophilically added to 2-bromo-2-acetonaphthone. According to Scheme (1), the dihydroimidazole intermediate may be formed via intramolecular condensation of the amine group with the neighboring carbonyl, allowing for the creation of the functionalized imidazole. Under the reaction circumstances, this intermediate might subsequently undergo air oxidation.



Secheme 1: Synthesis Mechanism of Imidazole Derivatives (a,b)

Table 1: Chemical Structures of Compounds

com.	Molecular Formula MW(g/mol)	Structural formula
a	$C_{26}H_20N_2$ 360	

b	$C_{26}H_{20}N_2O_2$ 392	

IR data and bonding

Five bands indicating aromatic, aliphatic, C=N, and C=C, C-N stretching vibrations make up the (a.b) infrared spectra of these compounds, as shown in Table (2) by the typical and KBr disk spectra. In the intervals 3050–3120, 2900–2985, 1550–1650, 1475–1600, and 1000–1350 cm–1, these bands are located, in that order.

Table 2: Fourier Transform Infrared Spectra of Imidazoles

Com.	Aromatic C-H stretching cm^{-1}	Aliphatic C-H stretching cm^{-1}	C=N stretching cm^{-1}	C=C stretching cm^{-1}	C-N stretching cm^{-1}
a	3050	2980	1610	1592	1360
b	3058	2850	1602	1468	1368

^{1}H-NMR spectral analysis

A 500 MHz NMR spectrophotometer (Bruker) was used to record the 1H-NMR of the synthesized imidazoles in DMSO-d6 and chloroform solvents, with TMS (tetramethylsilane) serving as an internal reference standard. The number of protons observed in the NMR spectra and their chemical shift (δ ppm) were in agreement with the molecule's structure. Singlet peaks with chemical shifts δ (2.50 ppm) and δ (3.38 ppm) were seen in the 1-H-NMR spectra of 2-(4-methylphenyl)-4-(naphthalen-2-yl)-1-phenyl-1H-imidazole (a) in the solvents d6-DMSO and HDO, respectively. A singlet signal for two protons (C5-H imidazole) at chemical shift δ (8.89ppm) and many peaks for aromatic protons at chemical shift δ (7.37-7.91ppm) were also seen. In its 1-H-NMR spectra, 2-methoxy-6-[4-(naphthalen-2-yl)-1-phenyl-1H-imidazol-2-yl]phenol (b) displayed two singlet peaks: one at chemical shift δ (7.25 ppm) corresponding to the chloroform solvent and another at chemical shift δ (2.46 ppm) corresponding to the methyl group. In addition, it displayed many aromatic proton peaks at chemical shift δ(6.89-7.52ppm) and a singleton peak for (C5-H imidazole) at chemical shift δ (8.66ppm).Furthermore, a signal at chemical shift δ (14.04 ppm) is associated with a hydroxyl group.

Antioxidant

Scavenging free radicals is a common method for determining antioxidant capacity. Antioxidant molecules transform DPPH-free radicals into diphenylpicryl hydrazine, a yellow chemical with a reduced absorbance at 517 nm, by attacking them with hydrogen atoms or electrons. The findings are shown in Tables (3). While all of the compounds had increased antioxidant activity, compound 2 proved to be the most efficient.

Table 3: Percentage Inhibition of Compounds

Con.	DPPH inhibition %		Ascorbic acid
	1	2	

0.2	90.50	94.30	54.30
0.3	95.80	96.20	60.10
0.4	97.10	97.40	71.40

5. Conclusion

The tremendous promise of imidazole-based chemicals in the fight against oxidative stress and associated diseases is emphasized in the current investigation into the synthesis and characterisation of novel imidazole derivatives as antioxidants. These derivatives have been successfully synthesized and their chemical integrity and desirable molecular architecture have been confirmed by structural elucidation using several spectroscopic methods. The findings show that antioxidant activity is greatly increased by making the right substitutions on the imidazole nucleus. This is mostly due to processes that include free radical stabilization, electron transfer, and hydrogen atom donation. Functional group modification is crucial for enhancing biological effectiveness, and the results show a significant correlation between the antioxidant capability of the produced compounds and their structural properties. These novel imidazole derivatives have excellent radical-scavenging capabilities and are chemically stable, making them ideal additives in the food and cosmetic sectors that may prevent oxidative deterioration. They might also be used as pharmaceutical development candidates. As a whole, the study sheds light on how to build effective antioxidant molecules using the imidazole framework, which opens up new avenues for studying and improving these compounds for medicinal uses that aim to reduce oxidative damage and strengthen cellular defenses against free radicals.

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