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Evaluation of Some Novel 1, 5- Diphenyl-2, 4-Disubstituted-1H-Imidazole for the Antioxidant Activity.

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1H-Imidazole, Ferric reducing antioxidant power (FRAP), DPPH method, Antioxidant activity.

Abstract

Numerous diseases, including diabetes, atherosclerosis, cancer, and neurological and cardiovascular diseases, are caused by oxidative stress. Therapeutic antioxidants are promising options for preventing and treating illnesses caused by oxidative stress. We present the design, Antioxidant Activity of Some 1, 5- Diphenyl-2, 4-Disubstituted- 1H-Imidazole by two methods Ferric reducing antioxidant power (FRAP) and DPPH method.

1. Introduction

In order to make adenosine triphosphate, which cells embrace as a resource of energy, oxidation entails the transfer of electrons from one atom to another. Free radicals like oxygen and nitrogen are produced in huge quantities when there is an imbalance in the flow of electrons. Oxidative stress, which results from the buildup of these free radical overloads and damages vital molecules, DNA, proteins, and lipids, is what causes chronic diseases including autoimmune disorders, cancer, diabetes, ageing, and neurological and cardiovascular conditions. Antioxidants are substances, both natural and artificial, that can stop the oxidation of other molecules by halting the chain reaction and preventing the creation of free radicals.

The imidazole core structure is used as a significant scaffold because of its high antioxidant activity and free-radical scavenging capacity. The imidazole molecule has distinct qualities that make it useful for free-radical scavenger activity via a variety of defensive mechanisms, such as its ability to bind and form complexes.

Histidine, histamine, and natural nucleotides are just a few examples of biological substances that include the heterocyclic aromatic chemical imidazole (recognized as 1,3-diaza-2,4-cyclopentadiene). Compounds containing imidazoles have been reported to have a wide variety of biological properties, including those of antifungal, anti-TB, antibiotics cytotoxic, antioxidant, anti-inflammatory, and NSAID. [1–4].

They represent a significant class of heterocyclic compounds due to their wide range of medical and physiological functions.

Several antioxidant techniques used to demonstrate antioxidant activity of imidazole molecules that have been di-, tri-, and tetra-substituted [5-7]. In order to neutralize oxidative stress, which develops after reactive oxygen species (ROS) overwhelm the body's built-in antioxidant defence system, this trait is helpful. Researchers are very interested in compounds having antioxidant properties since oxidative stress has been linked to many chronic and degenerative human illnesses, including cardiovascular, neurological, and neoplastic disorders [8,9].

The most prevalent form of dementia in the elderly, Alzheimer's disease (AD) [10], is one of the neurodegenerative illnesses in which oxidative stress has been related to underlying causes. AChEI

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(Acetylcholinesterase inhibitors) are prospective drugs for management of Alzheimer's disease patients since cholinergic insufficiency is significantly associated to disease development [12]. Imidazole-containing substances have also undergone encouraging AChEI testing [13].

Many imidazole derivatives with tri-substitutions have been reported in the literature, including those with alkyl and aryl groups; the aryl groups often display heterocyclic features. Trisubstituted imidazoles have generally been synthesised a lot lately, either to provide novel synthetic methodologic alternatives or to research certain biological aspects [4]. We describe the current study in light of the substantial body of research on pharmacological activity of imidazole derivatives and previously mentioned SAR approach.

2. Materials and Methods

General synthetic method

All the desired novel series of 1, 5- Diphenyl-2, 4-Disubstituted- 1H-Imidazole were synthesized as described in Scheme 1 belowand screened for In vivo Antioxidant Property.

Scheme:

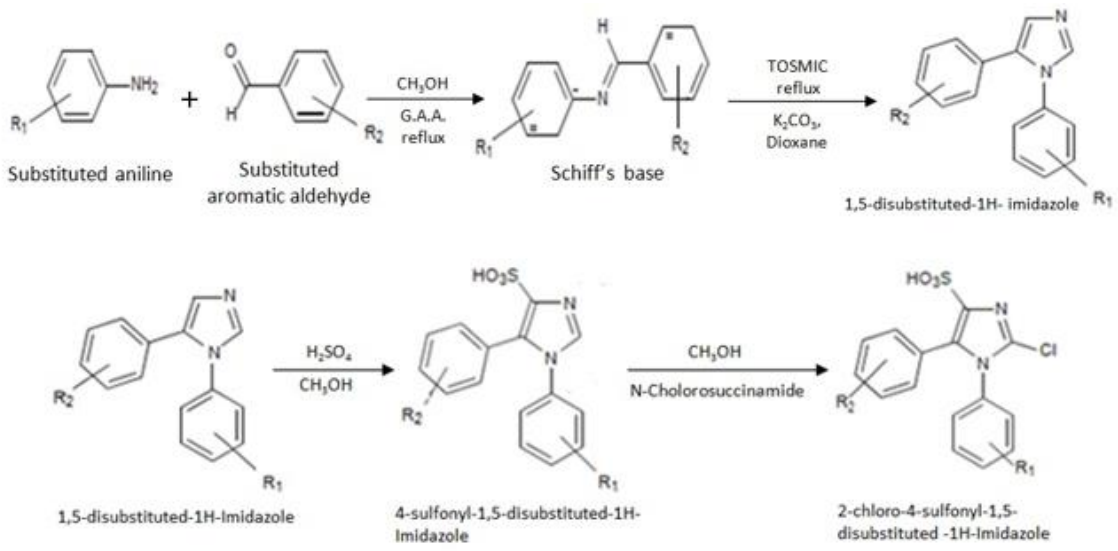
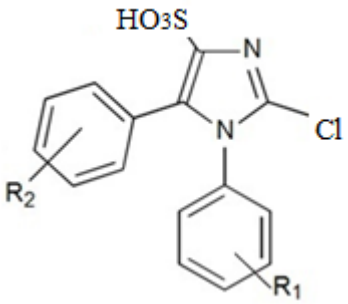


Figure 1: The Scheme for reaction.



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Sr. No.	Compound	R1	R2
1	I	2-NO2,4-Cl	Benzaldehyde
2	II	2,4-dinitro	Benzaldehyde
3	III	2-NO2,4-Cl	2-Cl
4	IV	2,4-dinitro	4-Cl
5	V	2-Cl	2-NO2
6	I-F	2-Br	2-Cl
7	I-G	2-Cl,5-Cl	2-Cl
8	I-H	2-Cl,5-Cl	4-OH

Table 1

DPPH antioxidant activity

By using the DPPH technique, the free radical scavenger action was assessed. DPPH is frequently utilized for evaluation of a substance's limit to function as a hydrogen donor or free radical scavenger. The DPPH method can be used to determine the total antioxidant capacity of a sample, regardless of whether the sample is solid, liquid, or gaseous. There is a large absorption maximum with a purple colour at 517 nm, caused by the odd electron in the DPPH free radical. When the DPPH radical's odd electron pairs with a hydrogen atom from an antioxidant, the resulting reduced DPPH (1,1-diphenyl-2-picrylhydrazine) changes colour from purple to yellow due to a decrease in the DPPH radical's molar absorptivity. The reduction of DPPH by an antioxidant is shown structurally and chemically in Figure 2[14].

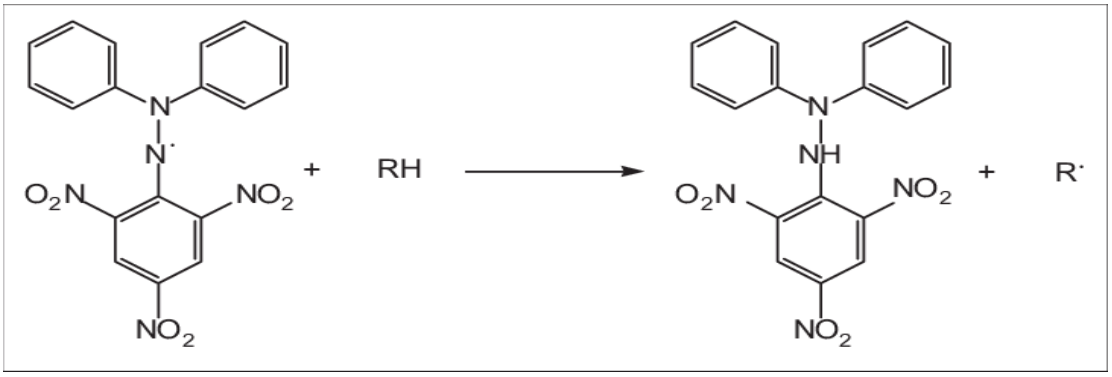

Figure 2: Structure of DPPH and its reduction

Antioxidant activity

Ferric reducing antioxidant power (FRAP) method.

Recently synthesised compounds can be evaluated quantitatively for their antioxidant properties using the FRAP assay [15]. The FRAP reagent was made by combining 2.5 millilitres of 10 millimetres ferric TPTZ, 2.5 millilitres of 20 millimetres FeCl3, and 25 millilitres of a 0.3 millimetre acetate buffer at a pH of 3.6. Before usage, the mixture was freshly made and heated to 37 °C. Ferrous 2,4,6-tripyridyl-s-triazine (blue in colour) is produced when the antioxidants react with the colourless ferric tripyridyl triazine complex. Different dosages (50, 75, and 100 g/ml) of synthesised compounds were mixed with the FRAP reagent in 1 ml of DMSO to measure antioxidant activity. The substance was incubated at 50 °C for 20 minutes.A precise volume of the solution was added to a cuvette, and a Shimadzu UV spectrometer was used to detect the absorbance at 593 nm. Additionally calibrated was the absorbance standard. BHA and TBHQ served as references. By simply inserting absorbance in the calculation, the antioxidant capacity of 1H Imidazole derivatives at different doses was calculated.

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$$\text{FRAP value of (sample) } \mu m = \frac{\text{Absorbance (sample)} \times \text{FRAP value of standard } \mu m}{\text{Absorbance (Standard)}}$$

DPPH method

When assessing the capacity of antioxidant components to scavenge free radicals, the stable free cradical DPPH is frequently used [16]. The DPPH free radical has a prolonged violet hue in methanol, but when combined with antioxidants or reducing agents, it turns colourless or yellow. Radicals can be transformed into a stable diamagnetic molecule by receiving the antioxidant's odd electron or proton. (yellow). A solution of synthesised compounds was created in methanol (100 g/ml) to test the antioxidant activity.

Concurrently, a DPPH solution was prepared in another container; this solution had the highest absorbance at 517 nm because it contained stable 1,1-diphenyl-2-picryl hydrazyl free radicals. (The colour violet). Four millilitres of DPPH solution were added to four millilitres of each test chemical, and the mixture was left at room temperature for thirty minutes. The Shimadzu UV spectrometer was used to determine the absorbance at 517 nm. Additionally, the absorbances of the standard and blank were calibrated. Tocopherol was used as a benchmark. By simply replacing absorbance in the method above, the antioxidant activity of 1H-imidazole derivatives was calculated.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

3. Result & Discussion

Antioxidant activity

Ferric reducing antioxidant power (FRAP) method.

The majority of the compounds demonstrated excellent antioxidant action. Some of the studied compounds' antioxidant levels are much closer to those of conventional medicines. Table 2 displays the synthesised compounds' antioxidant activity.

Table 2: FRAP antioxidant activity results.

Compounds	Ferric ion (Fe3+) reducing antioxidant power in nm		
	50 µg/ml	75 µg/ml	100 µg/m
I-a	0.8040	0.8200	0.8560
I-b	0.7570	0.7640	0.7820
I-c	0.7380	0.7590	0.7810
I-d	0.6710	0.6900	0.7410
I-e	0.6580	0.6730	0.7160
I-f	0.8140	0.8370	0.8630
I-g	0.4020	0.4250	0.4400
I-h	0.7920	0.8130	0.8500
BHA	0.9420	1.1360	1.3520
TBHQ	0.9560	1.2570	1.3940

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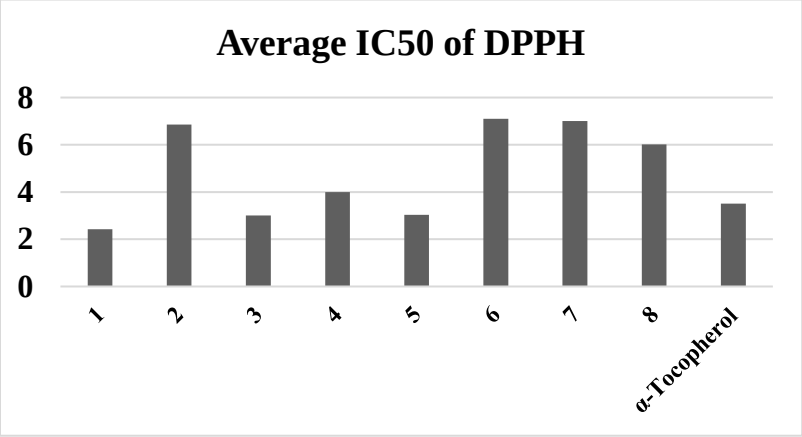
DPPH method

Because of its high reliability and short analysis time, DPPH is an ideal approach for gauging the antioxidant activity of newly synthesised compounds. The outcome shows that decreasing the absorbance results in a shorter analytical time and more accurate results, because of electron movement. The majority of molecules possessed significant antioxidant effects.Table 3 displays the synthetic chemicals' anti-oxidant activity.

Compound	0 mins	30 mins	1 hr	2 hr	Average IC50
I-a	3.14	3.42	4.3	4.1	3.74
I-b	11.7	5.88	5.07	4.8	6.8625
I-c	3.5	3.8	4.1	3.2	3.65
I-d	2.2	3.63	3.5	3.7	3.2575
I-e	2.29	2.58	3.32	3.96	3.04
I-f	9.11	6.71	6.65	5.92	7.1
I-g	2.98	5.32	5.87	6.06	5.0575
I-h	1.43	3.24	4.5	4.2	3.3425
α-Tocopherol	3.99	3.82	3.03	3.19	3.5075

Table -3 antioxidant activity results.



Effects of α-Tocopherol & the synthetic compounds

4. Conclusion:

The majority of the synthesized compounds showed potential antioxidant activity and radical scavenging capacity by reducing ferric tripyridyl triazine complex. As a standard medicine, some of the evaluated Imidazole compounds were found to have significant antioxidant action.

Studies on antioxidants show that these compounds perform much better than conventional α -tocopherol in terms of antioxidant performance. Antioxidant's IC50 values at various doses and DPPH elimination features are shown in graph.

Table 2 displays the IC50 values of the synthesized compounds. compounds for DPPH radical scavenging capabilities. The average scavenging effect declined in

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the following order, as seen in Figure 3 and Table 2: I-h > I-c > I-a > I-e > I-d.

The -tocopherol standard demonstrated efficient radical scavenging abilities, whereas samples I-b, I-f, and I-g did not. With an IC50 value, samples I-e, I-d, and I-H had the greatest scanning capacity. Compounds I-e, I-d, I-c, and I-a have excellent activity when compared to the standard according to the ferric reducing antioxidant (FRAP) technique, but compounds I-b, I-d, and I-g have poor activity. In relation to this, it can be asserted that these new compounds are perfect for facilitating the creation of fresh antioxidants.

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