Research article

FURAN–PYRAZOLE HYBRIDS AS PROMISING ANTIOXIDANTS: AN IN VITRO EVALUATION

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ABSTRACT

The present study evaluates the in vitro antioxidant potential of the newly synthesized compound 3j {4-(2,6-dimethylarylazo)-1-furanyl-3,5-dimethylpyrazole} using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, with ascorbic acid as the reference standard. Compound 3j demonstrated strong free radical scavenging activity, showing higher inhibition compared to the standard under the tested conditions. The findings suggest that compound 3j possesses potent antioxidant properties, likely attributed to its unique structural features, including the furan and pyrazole rings and the dimethyl arylazo moiety. These results indicate that compound 3j is a promising candidate for further investigation as an antioxidant agent, with potential applications in the prevention and management of oxidative stress-related disorders.

Keywords: Antioxidant activity, DPPH assay, free radical scavenging, oxidative stress.

INTRODUCTION

HETEROCYCLIC CHEMISTRY

Heterocyclic compounds consist of cyclic structure with two different types of atoms in a ring. The heterocyclic rings contain one carbon atom or atom other than the carbon atom, these are known as heteroatoms. They can be either aromatic or aliphatic. Aliphatic heterocyclics consist

of amines, ethers, amides etc., whereas aromatic heterocyclics consist of heteroatom that have similar properties as that of benzene[1,2].

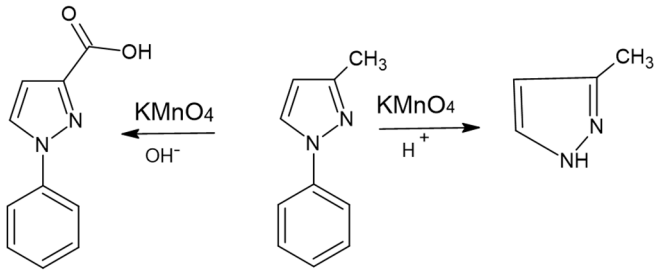
PYRAZOLE

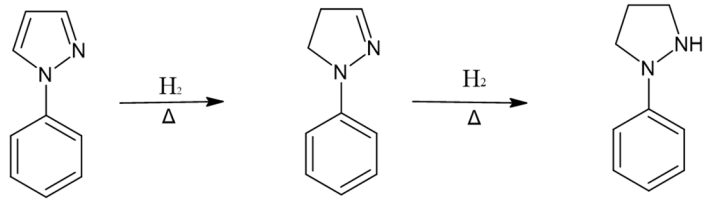
Pyrazole is a heterocyclic five membered ring compound providing broad activity and stereochemical complexity. The pyrazole ring is one of the common structural elements in many pharmaceutically active drugs. This is primarily due to its pharmacological properties. Pyrazoles can be selectively modified with different substituents to increase their broad range of activity in various sectors.

In fact, a wide range of synthetic procedure have been proved throughout the centuries. The presence of the pyrazole have shown remarkable uses in technology, drug and husbandry. These composites have Anti-bacterial, antifungal, anti-cancer, antidepressant, anti-inflammatory, anti-tuberculosis, anti-oxidant, and antiviral activities.

These compounds feature six delocalized π electrons and a planar conjugated ring structure, making them aromatic. Pyrazoline is formed via partial reduction of pyrazole, whereas pyrazolidine is obtained through full reduction[3-5].

REACTIONS OF PYRAZOLE[6]

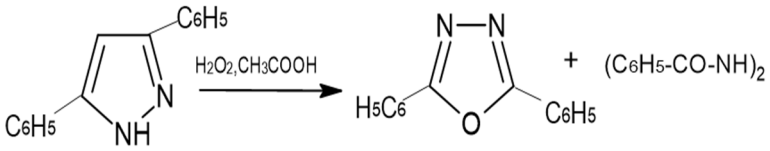
I. Oxidation Reaction

Oxidation process begins with oxidizing agents primarily targeting on the side chains.Potassium permanganate has been the most extensively used agent for side chain oxidation,generally in a neutral or alkaline media,but occassionally with sulfuric acid.
II.Reduction



Reduction processes begin with the pyrazole ring,resulting in the synthesis of pyrazolines and pyrazolidines or the removal of certain substituents.

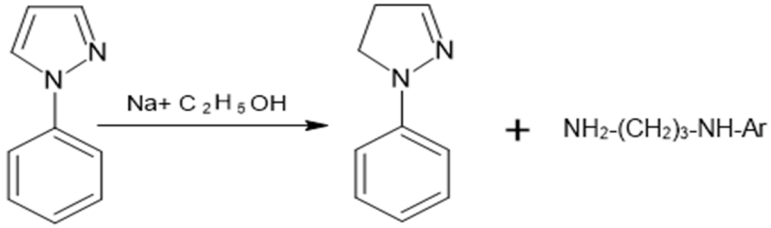
III.Cleavage of the pyrazole ring

a)Oxidative cleavage of the pyrazole



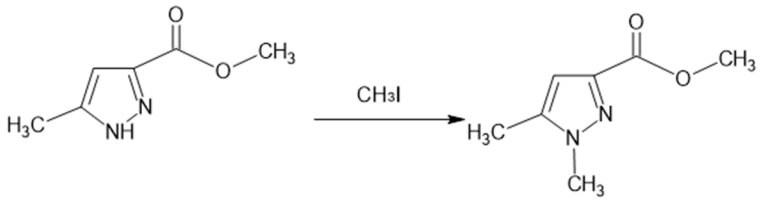
3,5-diphenyl pyrazole when oxidized,it produces an oxadiazole derivative.The latter is likely the result of dehydration of the former.

b)Reductive cleavage of the pyrazole



The reaction involves the reduction of 1-phenyl and 1-p-tolylpyrazole with sodium and alcohol produces aryl tri-methylenediamines with the corresponding pyrazolines.

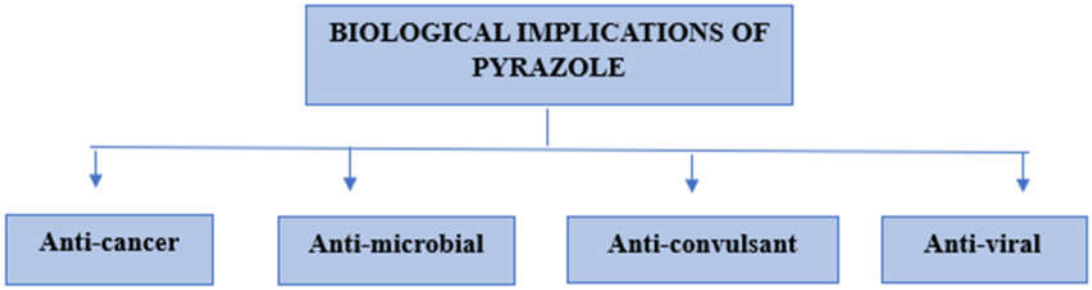
IV.Alkylation reaction



Alkylations have been carried out with alkyl halides, dialkyl sulphates and diazomethane.

BIOLOGICAL IMPLICATIONS

Pyrazoles and their analogues have numerous biological activities,including anti-bacterial,anti-convulsant, analgesic,anti-microbial, antiinflammatory,Anti-diabetic,sedative,antirheumatic, anti-cancer,and antitubercular properties.Pyrazole rings play a significant role in the pharmaceutical sector due to their various biological activities[7].



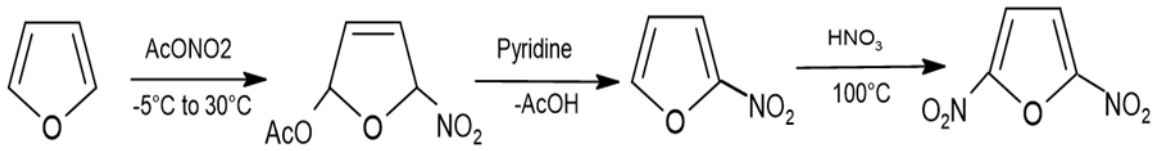
FURAN

Furan is a five membered planar ring that dissolves in most organic solvents.This is the most reactive chemical among the five membered heterocyclic compounds.It is a non-polar chemical.Furan's electrophlic substitution reactions appear in the second position.Compounds with the furan rings frequently serve as effective solvents.Some can mix with water and hexane.The existence of ether oxygen raises polarity and the capacity for hydrogen bonding.Chemically, it is the essential ring structure found in various biologically active compounds.compounds having a furan ring are commonly known as furans[8,9].

REACTIONS OF FURAN[6]

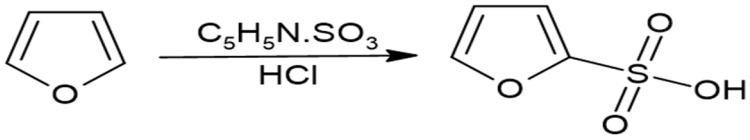
I.Electrophilic Reactions

a)Nitration



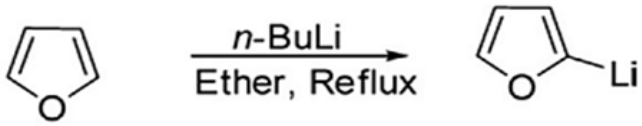
Nitration of furan with acetyl nitrate produces a secondary product,which may be removed with a mild base like pyridine to obtain 2-nitrofuran.Being heated in a waterbath with 70% nitric acid produced 2,5-dintrofuran.

b)Sulphonation



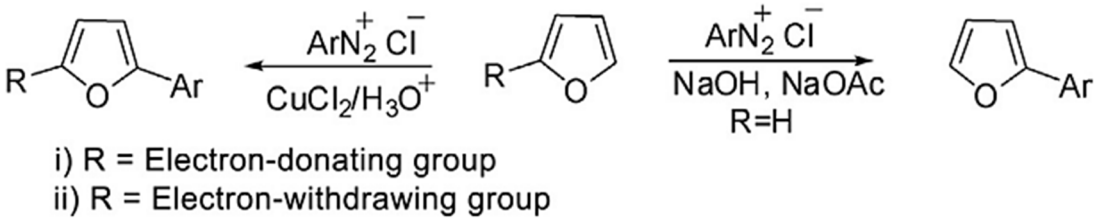
Furan-2-sulfonic acid was formed by reacting with a pyridine sulfur trioxide complex ,which was subsequently hydrolysed with acid.

II.Metalation Reaction



Furan is readily metalated using butylithium to produce 2-metalated furan.Lithiated with alkylithium generate 2-lithiofuran.

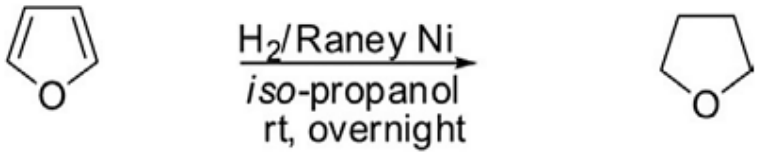
III.Coupling Reactions



i) R = Electron-donating group
ii) R = Electron-withdrawing group

At pH 8-9, arenediazonium salts react with furan to produce aryl substituted furans.It is mostly applicable regarding the parent furans.On combining with copper(II) ions the substituted furans gets arylated at pH 4-6.Electron withdrawing groups need pH 1-2.

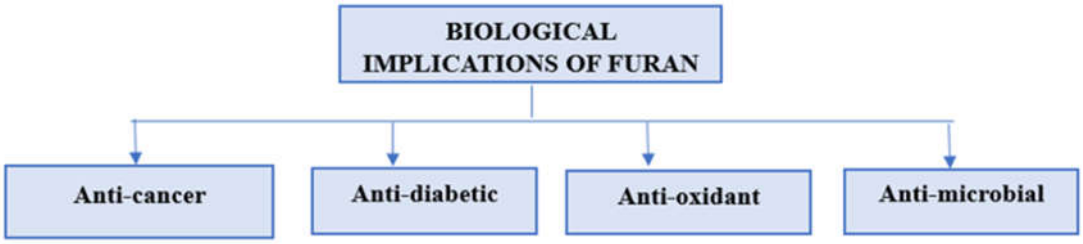
IV.Reduction Reaction



The catalytic hydrogenation of furan with different cataylsts and solvents generally yields THF. Reduction of furan using isopropanol along with Raney nickel and rhodium resulted in the formation of THF.

Biological Implications

Furan have numerous biological significance such as anti-oxidant,anti-cancer,anti-microbial and antiinflammatory properties. Furan based compounds have a tremendous possibilities in the development of new treatments for a number of ailments.



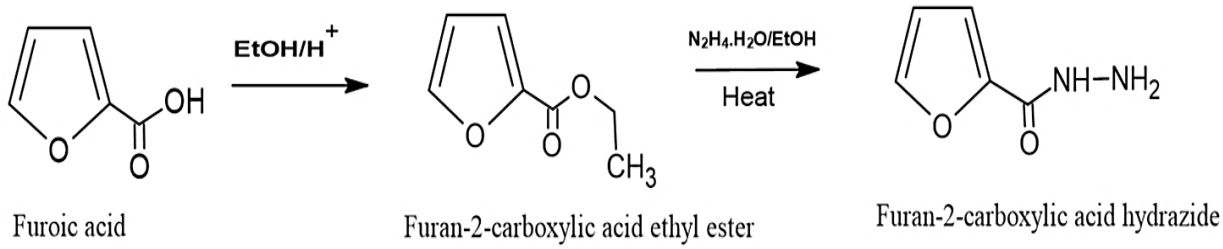
Anti-oxidant

An anti-oxidant is a chemical agent that,at low concentrations,considerably slows or suppresses the oxidation of an oxidizable substrate.Anti-oxidants are often classified into two types:primary(chainbreaking) and secondary(preventative).Secondary or preventive anti-oxidants are substances that slow the rate of oxidation.Primary anti-oxidants when present in small amount can delay or limit the initiation by interacting with lipid radicals,or decrease propagation by reacting with peroxyl or alkoxyl radicals[10,11].

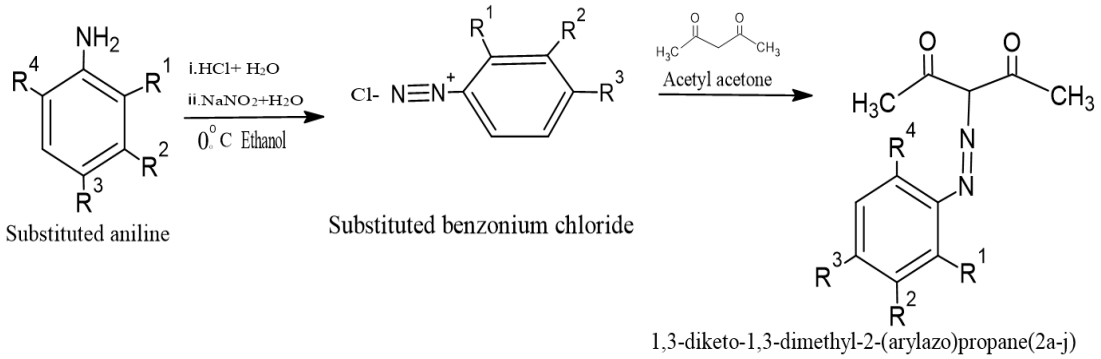
METHODS

SCHEME OF WORK

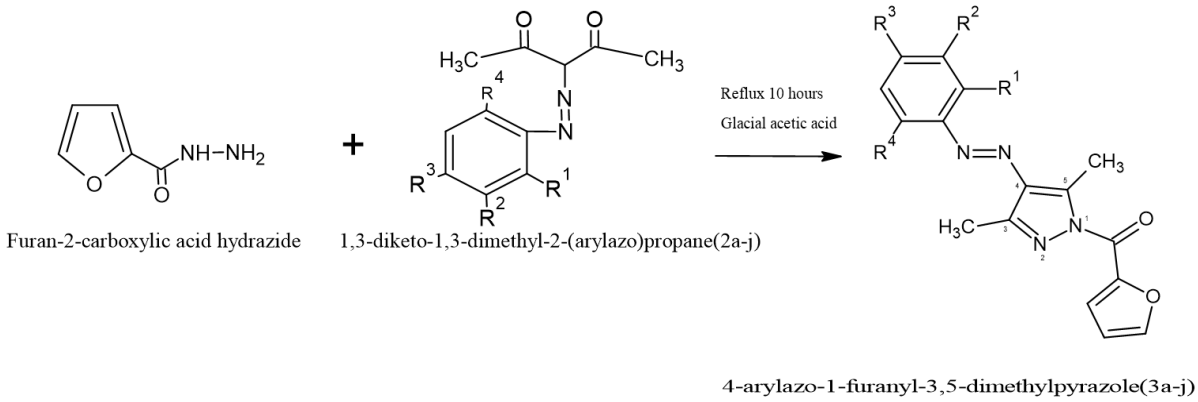
a.Synthesized Furan-2-carboxylic acid hydrazide



b.Synthesized 1,3-diketo-1,3-dimethyl-2-(arylazo)propane (2a-j)



c.Synthesized 4-arylazo-1-furanyl-3,5-dimethylpyrazole (3a-j)



Furan-2-carboxylic acid hydrazide 1,3-diketo-1,3-dimethyl-2-(arylazo)propane(2a-j)

4-arylazo-1-furanyl-3,5-dimethylpyrazole(3a-j)

Sl No.	COMPOUND CODE	R1	R2	R3	R4
1.	3a	H	H	H	H
2.	3b	H	NO_2	H	H
3.	3c	H	H	NO_2	H
4.	3d	H	Cl	H	H
5.	3e	Cl	H	H	H
6.	3f	H	H	Cl	H
7.	3g	OCH_3	H	H	H
8.	3h	H	H	OCH_3	H
9.	3i	CH_3	H	CH_3	H
10.	3j	CH_3	H	H	CH_3

PROCEDURE

General procedure for the synthesis of Furan-2-carboxylic acid hydrazide

a) Procedure for the synthesis of furan-2-carboxylic acid ethyl ester

A combination of furoic acid (11.2 grams, 0.1 mol), 60 ml of ethanol, and 1.4 ml of concentrated H_2SO_4 was refluxed for 10 hours in a round bottom flask , after refluxing it was cooled and the solution was slowly added to ice . Sufficient ammonia solution was added to

make it alkaline. The ester was isolated as oil, and the solution underwent extraction five times with 25 ml of ether,resulting ethereal extract obtained was dried using anhydrous MgSO4. The ether was evaporated and the residue was gathered. Ester's physical properties were noted;yield 76%, b.p 195°C.

b) Synthesis of furan-2-carboxylic acid hydrazide

A 1:1 mixture of ester(1) and hydrazine hydrate, along with 30ml of ethanol, was refluxed for 4-6 hours in a round-bottom flask. Surplus ethanol was removed through distillation.Acid hydrazide was separated from the product as it cooled. It was filtered and collected.Recrystallisation with methanol yielded 65% with a melting point of 71º C, according to physical data[12] .

General procedure for the synthesis of 1,3-diketo-1,3-dimethyl-2-(arylazo)propanes(2a-j)

Each aniline derivative (0.01 mol) was dissolved in a beaker filled with 8ml of hydrochloric acid and 8ml of water.The contents were mixed and was chilled to 0°C prior to the incorporation of the sodium nitrite solution (1.2 g in 3ml water).This cold diazotised solution was added drop by drop to a well-cooled and agitated combination of acetyl acetone (1ml, 0.01 mol) and sodium acetate (8g) in 25 ml of 50% ethanol. The solution obtained was filtered and after drying it was recrystallised using ethanol[13].

General procedure for the synthesis of Furan-2-carbohydrazide

2a-j derivatives (0.001 mol) obtained from acetyl acetone and Furan-2-carbohydrazide (0.001 mol) in 10 mL of glacial acetic acid were refluxed for 10 hours.After refluxing it was cooled and allowed to sit overnight. The discarded solid will be retrieved by filtering and was recrystallized using acetic acid (3a–j)[13].

BIOLOGICAL EVALUATION

Anti-oxidant study

Principle

The test sample's radical scavenging activity was carried out against DPPH . It interacts with an antioxidant molecule, which includes the donation of hydrogen and reducing DPPH.The colour change was measured at 515 nm.

Procedure

The stock solution of ascorbic acid was prepared with distilled water (1mg/ml). A freshly prepared 60µM DPPH solution in methanol was mixed with 50µl of the test substance at different concentrations . The plates were maintained in the dark, at room temperature for fifteen minutes prior to measuring the decrease in absorbance at 515 nm. The control was performed with just DPPH solution, without any extract or ascorbic acid, using 95% methanol as a blank.The experiment was conducted using the instrument Multiskan Skyhigh spectrophotometer[46].

Determined using the subsequent equation;

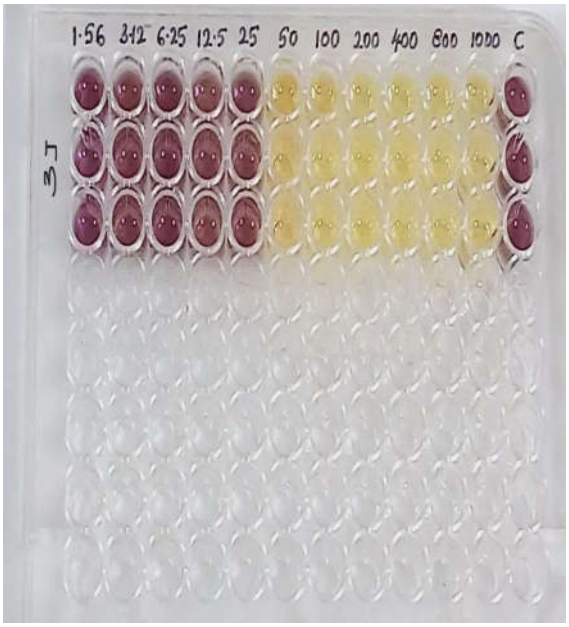
$$\% \text{ inhibition} = \frac{\text{Absorbance of control-Absorbance of test}}{\text{Absorbance of control}} \times 100$$

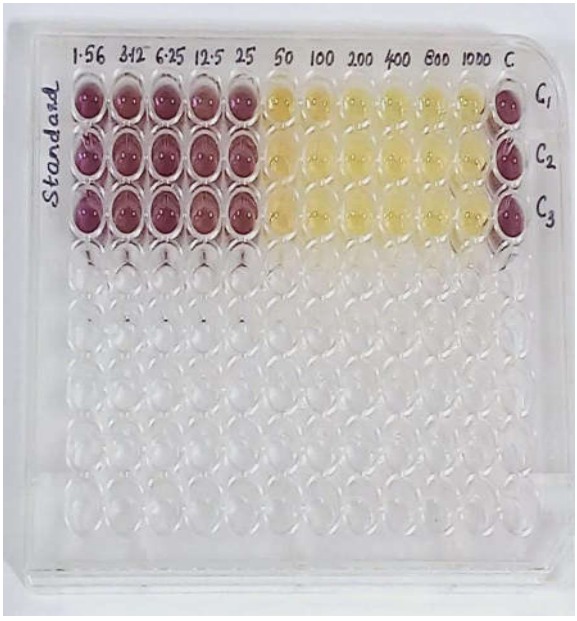
RESULTS

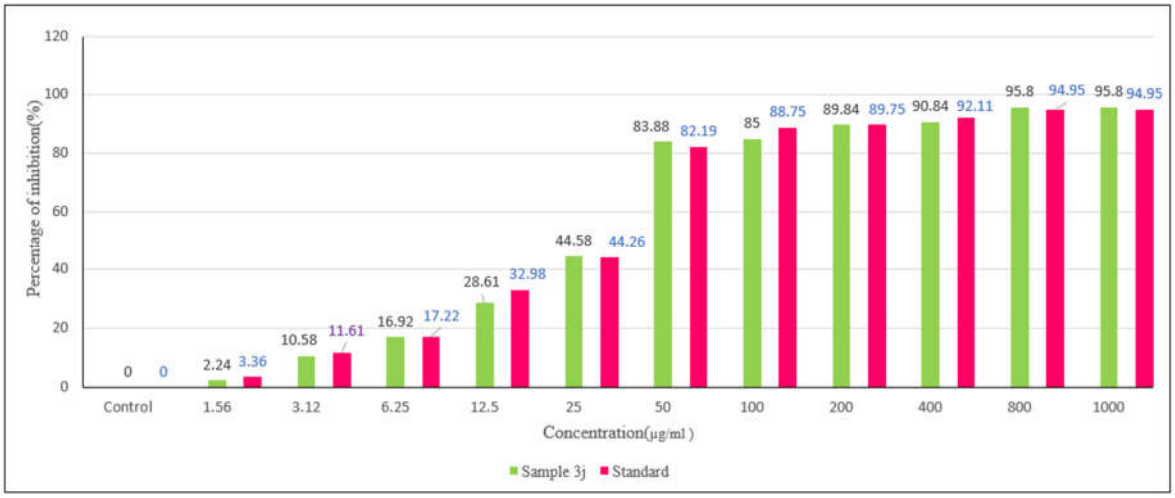
Invitro Anti-oxidant study

Invitro anti-oxidant activity of compound 3j was evaluated through the DPPH Assay method using ascorbic acid as a reference. The study was conducted at different concentrations and from the results of anti-oxidant studies ,it was observed that the compound 3j at 1000µg/ml having percentage inhibition upto 95.80% and IC_{50} value of 27.12 µg/ml .

The standard ascorbic acid at 1000 µg/ml concentration showed a percentage inhibition upto 94.95% and having an IC_{50} value of 28.19µg/ml .Therefore,it was observed that the newly synthesized compound showed a lower IC_{50} value which means stronger anti-oxidant activity. A lower IC_{50} value is indicative of higher antioxidant potency, as it requires a smaller concentration of the compound to achieve 50% inhibition of free radicals. Thus, these findings suggest that the newly synthesized compound 3j exhibits enhanced antioxidant activity compared to ascorbic acid, highlighting its potential as a potent free radical scavenger. This may be attributed to its unique structural features, which could facilitate effective electron or hydrogen atom donation to neutralize reactive species.These results encourage further exploration of compound 3j in detailed mechanistic studies and in vivo models to fully assess its therapeutic potential as an antioxidant agent.







CONCLUSION

After analyzing the results obtained from the biological activity, the possible conclusion reached is given below:

In case of anti-oxidant activity, the study were carried out by DPPH assay method,the compound 3j {4-(2,6-dimethylarylazo)-1-furanyl-3,5-dimethylpyrazole} was screened for its anti-oxidant property using ascorbic caid as standard.From the results,it was obtained that the compound 3j showed an IC_{50} value was found to be 27.12 µg/ml and that of standard Ascorbic acid was found to be 28.19 µg/ml. This enhanced activity may be attributed to its distinctive structural features, including the furan and pyrazole rings and the dimethyl arylazo moiety, which likely enhance its ability to donate hydrogen atoms or electrons to neutralize free radicals. These results suggest that compound 3j is a promising antioxidant candidate, meriting

further studies such as mechanistic investigations and in vivo evaluations to confirm its therapeutic potential against oxidative stress-related disorders.

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FUNDING

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data related to this manuscript have been disclosed within the article, and no additional datasets were generated or analyzed during the preparation of this research article.

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