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Broad Spectrum Fungicidal Activity of the Synthesised 5, 5-Dihydro-1,3,4-Thiadiazolo- [3, 2-A]- [1,3,5]-Triazin-5-Ones

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Abstract: It is crucial to discover new fungicidal medications since hazardous fungi are becoming increasingly resistant to the therapies that are already available. The purpose of this study was to manufacture a variety of 5,5-dihydro-1,3,4-thiadiazolo-[3,2-a]- [1,3,5]-triazin-5-one derivatives and evaluate their antifungal efficacy throughout a wide range. In order to get further information on the synthesized substances, we made use of nuclear magnetic resonance, mass spectrometry, and infrared spectroscopy instruments. The in vitro antifungal tests that were conducted included the testing of a number of different fungal strains, including Candida albicans, Aspergillus niger, Fusarium exospore, and Trichophyton mentagrophytes. These strains are significant in a variety of situations, including clinical and agricultural ones. A variety of substances had potent antifungal action, with minimum inhibitory concentrations (MICs) that were comparable to or lower than those of antifungal drugs that are already on the market. Electron-withdrawing substituents were shown to be useful in increasing the efficiency of antifungal agents, as indicated by structure-activity relationship (SAR) studies. Taking into consideration these findings, 5,5-dihydro-1,3,4-thiadiazolo-[3,2-a]-[1,3,5]-triazin-5-ones seem to be an excellent building block for the development of novel broad-spectrum fungicide medicines.

Keywords: Thiadiazolotriazinone derivatives, Broad-spectrum antifungal, Fungicidal activity, SAR analysis, Antifungal resistance

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INTRODUCTION

As a source of plant-based resources, the natural world's biodiversity has sustained life on Earth for eons. Plants have been essential for human life for many years, serving as both food and shelter. The staple foods of many people's diets consist of grains like wheat, rice, and corn, as well as legumes like beans and lentils, which are high in protein. A healthy and well-rounded diet has also benefited from a variety of fruits and vegetables, which have provided vital nutrients.

Not only do plants play an important part in human nutrition, but they also sustain creatures at every stage of the food chain, from the tiniest microorganisms to the biggest animals. Because they promote ecological harmony and interdependence, their significance extends beyond the realm of biology. Plants help keep the ecosystem stable in many ways: they clean the air, improve the soil, control the flow of water, and maintain biodiversity via the habitats they build.

Nutritional Value of Plant-Based Resources

Both humans and animals rely on plants as their primary source of sustenance. The plant kingdom is a rich source of several vital nutrients:

- **Proteins**: Legumes, grains, nuts, and seeds are good sources of plant-based proteins, which are essential for building cells, repairing damaged tissues, and enhancing muscular growth.
- **Vitamins**: The metabolic processes, immunological function, and hormonal balance are all bolstered by the vitamin A, C, D, and B-complex that are abundant in fruits and vegetables.
- **Minerals**: The physiological framework of the body is strengthened and critical biochemical processes are regulated by essential components such as calcium, magnesium, iron, and zinc.
- **Carbohydrates**: Carbohydrates power biological processes and mental operations as the principal source of energy. Glycogen stores these sugars, which are plentiful in fruits, vegetables, and grains, for use at a later time.

These nutrients work together to keep organisms healthy, boost their development, and guarantee their survival on all levels.

Ecological Role of Plants in Ecosystems

Plants play an essential role in ecosystem structure and function in addition to being food sources. The intricate webs of life rely on their variety to sustain different trophic levels. For terrestrial ecosystems like grasslands and forests to function as climate buffers and stabilizers, they rely on a diverse array of plant life.

Plus, plants are vital to ecosystems because of the benefits they provide:

- **Carbon Sequestration**: Using carbon capture and storage to slow global warming.
- **Soil Conservation**: Keeping soil from washing away and decreasing its richness.
- **Water Filtration**: Keeping freshwater habitats afloat and stabilizing groundwater levels.

The worldwide importance of plant ecosystems in protecting biodiversity and maintaining planet Earth's health is shown in prominent areas such as the African savannas and Amazon rainforest.

Cultural, Medicinal, and Economic Value of Botanical Resources

Plants have a long history of cultural and medicinal usage. Plants have long been used as a means of holistic healing and health maintenance by indigenous and traditional communities. These traditional methods have had an impact on contemporary medicine, which in turn has led to the development of new pharmaceuticals with their roots in plants.

Modern pharmaceutical research is still being fueled by chemicals found in plants. Modern scientific research is validating and refining herbal medications that are informed by traditional knowledge. In order to provide long-term healthcare solutions, it is crucial to combine traditional knowledge with current pharmaceuticals.

The economic worth of botanical resources is substantial as well. Maintaining ecological balance, protecting ancient knowledge systems, and bolstering scientific innovation all depend on their preservation.

Botanical Diversity and Agricultural Resilience

The foundation of sustainable agriculture is botanical variety. Farmers are better equipped to deal with

threats like pests, illnesses, and shifting climates when they have access to a diverse range of plant species and cultivars. A more natural defense against environmental stress and less dependence on delicate monocultures are the results of a more diverse cropping strategy.
By guaranteeing consistent yields regardless of environmental factors, this variety enhances food security. Agricultural landscapes are made more sustainable as a result of improved soil health and the promotion of ecological farming techniques.

Advances in Synthetic Antifungal Agents

The importance of synthesizing substituted derivatives of heterocyclic compounds like 1,3,4-oxadiazole has been highlighted in recent study. Researchers can enhance the structure's antifungal capabilities by adding certain substituents. These altered chemicals may lead to the creation of new, more effective fungicides by preventing the growth of fungus.

To better understand how these derivatives work and how to make them more effective, a comprehensive study of them is required. To fight fungal resistance and ensure sustained agricultural and medicinal uses, such synthetic techniques are in line with the larger purpose.

OBJECTIVES OF THE STUDY

1. To emphasize how important plant diversification is for maintaining ecosystems and life on an ecological and nutritional level.
2. To look into the possibility of creating powerful antifungal medicines using synthetic 1,3,4-oxadiazole derivatives.

5,5-dihydrothiadiazolo-[3,2-a]-[1,3,5]-triazin-5-ones

- 2-Amino-5-aryl-1, 3, 4-thiadiazole
- N-(5-Aryl-1, 3, 4-thiadiazol-2-yl) acetamide
- 2-Aryl-7-methyl-5, 5-dihydro-1, 3, 4-thiadiazolo-[3, 2-a]-[1, 3, 5]-triazine
- 2-Aryl-7-methyl-1, 3, 4-thiadiazolo-[3, 2-a]-[1, 3, 5]-triazin-5-ones

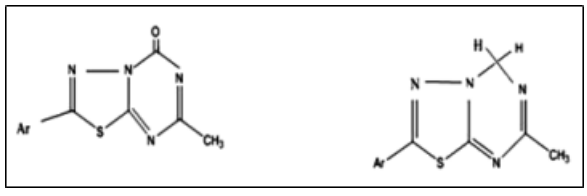

Figure 1: N-[5-(2-hydroxy phenyl)-1,3,4-oxadiazol-2-yl]-2 aryloxy acetamid

SCHEME 1

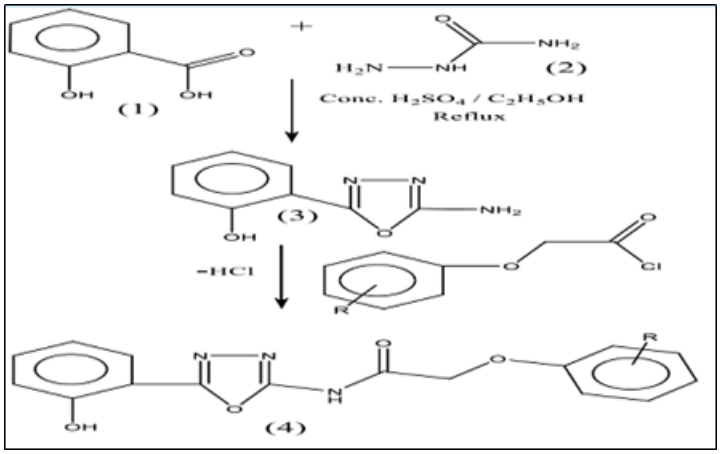

Figure 2: 2-[N-Aryloxy acetyl]-5-]2-hydroxy aryl]-1-3-4-oxadiazole

SCHEME 2

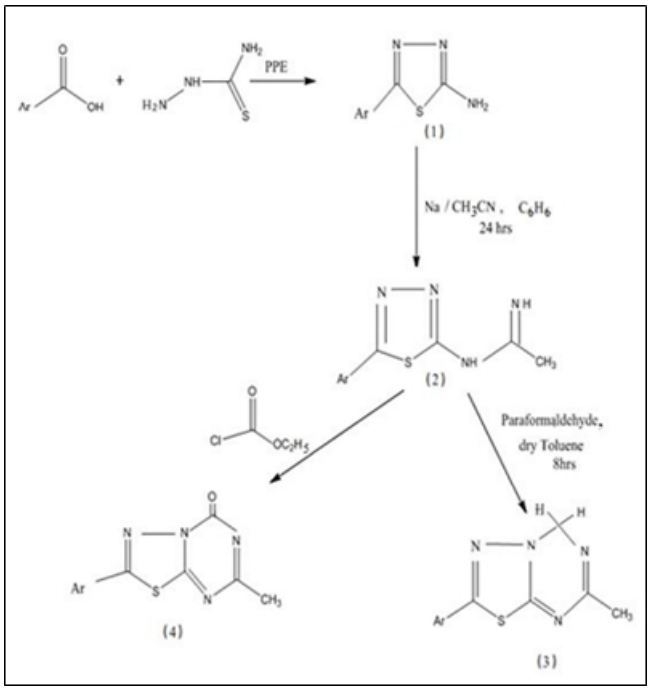

Figure 3: 5,5-dihydrothiadiazolo-[3,2-a]-[1,3,5]-triazin-5-ones

Some five-membered heterocyclic compounds with sulfur, nitrogen, or oxygen atoms have shown great promise as antifungal drugs. Examples of these compounds include 1,3,4-oxadiazole and 1,3,4-thiadiazole. Their interaction with biological targets is enhanced by the specific physicochemical features that are contributed by these heteroatoms. It is possible to enhance the effectiveness and specificity of these compounds' antifungal uses by molecularly modifying their physicochemical features and biological activities.

Recent research has shown that synthesising several substituted derivatives of 1,3,4-oxadiazole may have some useful applications. Researchers want to methodically modify and enhance these chemicals by adding certain substituents, making them 30 more efficient in preventing fungal growth. It is possible that novel,

effective antifungal medicines may be developed by the systematic assessment of these compounds for antifungal activity.

5,5-dihydrothiadiazolo-[3,2-a]-[1,3,5]-triazin-5-ones

The phototoxic effects and impacts on plant growth regulation of several amino triazine derivatives were discovered by Gysin and colleagues. The antifungal and herbicidal properties of certain s-triazine derivatives have brought them to the forefront of agricultural use. The herbicidal and fungicidal effects of 1,3,4-thiadiazole derivatives are also well-known. We hypothesized that by combining these ring structures, we may create compounds with improved antifungal activity, and we built on this insight to synthesize the title chemical.

Evaluation of antifungal activity:

Two pathogenic fungal species, *Collectotrichum falcatum* and *Fusarium oxysporum*, are known to harm numerous agricultural crops. In vitro assessments of the synthesized compound's antifungal activity were conducted against these species. Agar plate testing, a standard procedure for assessing antifungal characteristics in a controlled setting, was used to carry out this screening. To study the compound's dose-dependent effects on inhibiting fungal growth, it was tested at three distinct concentrations: 10 ppm, 100 ppm, and 1000 ppm.

The growth medium used in this experiment was Czapek's Agar, which is ideal for cultivating fungus and provides a consistent environment for repeatable findings.

As a control agent in the screening technique, a commercial fungicide, Dithane M-45, was used to create a comparative baseline. In order to directly compare the fungicide to the synthetic ingredient, it was tested under the same circumstances. Chapters IV and V give a comparative study that explains how the synthesized chemical compares to a commercially available standard in terms of antifungal effectiveness.

The first step was to make a hot (CHCl3) solution of 5 mmol of carboxylic acid in PPE and chloroform. The solution was then supplemented with 5 mmol of thiosemicarbazone. The reaction was refluxed for 10 hours to be sure it was finished. Thirteen milliliters of pure water were added to the mixture after the reflux procedure. Sodium carbonate (Na2CO3) was used to neutralize the remaining PPE. Subsequent processing of the reaction produced the target chemical.

N-(5-Aryl-1, 3, 4-thiadiazol-2-yl) acetamide: The chemical was created by refluxing a combination of 3-Amino-5-(4-Chlorophenyl)-1,3,4-thiadiazole (3.54 g), sodium metal (0.42 g), and methyl cyanide (0.80 g) in benzene (20 mL). For a whole day, the reaction was kept going in a reflux environment. After the reaction was finished, lactic acid and lactate were added to the mixture to help it undergo further transformation. The necessary amidines were formed by basifying the resultant product with ammonia (NH3).

2-Aryl-7-methyl-5, 5-dihydro-1, 3, 4-thiadiazolo-[3, 2-a]-[1, 3, 5]-triazine: Refluxing 4.20 g of N-(5-Aryl-1,3,4-thiadiazol-2-yl) acetamide with 0.020 M of paraformaldehyde in dry toluene for 8 hours was the process used in the synthesis. Distillation at decreased pressure was used to remove the solvent after the

reaction was complete. The crude product was obtained by diluting the residue with petroleum ether, filtering it, and then drying it.

Recrystallization with ethanol was then used to purify the target product. Furthermore, the following table displays characterisation data for further derivatives, namely 2-Aryl-7-methyl-5,5-dihydro-1,3,4-thiadiazolo-[3,2-a]- [1,3,5]-triazines, which were also produced using identical processes.

Table 1: Physical and spectral data of title compound

Compound No.	Ar/R-	M.F.	Yield (%)	m.p.	C Experimental (Calculated)	H Experimental (Calculated)	N Experimental (Calculated)
01*	4-Cl C_6H_4	$C_{11}H_9SN_4C1$	76	138°C	48.81 (49.90)	3.15 (3.40)	20.85 (21.17)
02	2-$C1C_6H_4$	$C_{11}H_9SN_4C1$	74	132°C	48.83 (49.90)	3.17 (3.40)	20.87 (21.17)
03	2, 4-$C1_2C_6H_3$	$C_{11}H_8SN_4C1_2$	71	141°C	43.11 (44.15)	1.65 (2.68)	17.71 (18.73)

* IR (KBr) cm^{-1} 1609, 1642 (C=N)

^{1}H NMR (DMSO-d6); 2.32(3H, s, Me), 4.51 (2H,S,CH_2), 7.10-7.93 (4H,m, Ar-H)

2-Aryl-7-methyl-1, 3, 4-thiadiazolo-[3, 2-a]-[1, 3, 5]-triazin-5-ones

Carefully adding ethyl chloroformate to a pyridine (75 mL) solution of N-(5-aryl-1,3,4-thiadiazol-2-yl) acetamide (4.10 g) while keeping the combination in an ice bath was the procedure. In order to facilitate the reaction, the mixture was then agitated at room temperature for two hours. After that, the reaction mixture was refluxed for another hour to make sure it was finished. The product of interest was precipitated from the reaction mixture after being treated with 1(N) KOH (35 mL). This allowed for its isolation. After removing the precipitate from the mixture, it was recrystallized from the ethanol to produce the pure form of the chemical.

5,5-dihydrothiadiazolo-[3,2-a]-[1,3,5]-triazin-5-ones

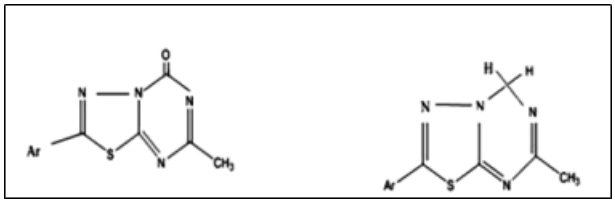

Figure 4: 5,5-dihydrothiadiazolo-[3,2-a]-[1,3,5]-triazin-5-ones

1. Ar = 4–ClC_6H_4, in $C_{11}H_9SN_4Cl$
2. Ar = 2–ClC_6H_4, in $C_{11}H_9SN_4Cl$
3. Ar = 2,4–$Cl_2C_6H_3$, in $C_{11}H_8SN_4Cl_2$
4. Ar = 4–ClC_6H_4, in $C_{11}H_7OSN_4Cl$
5. Ar = 2–ClC_6H_4, in $C_{11}H_7OSN_4Cl$
6. Ar = 2,4–Cl2C_6H_3, in $C_{11}H_6OSN_4Cl_2$

The following table summarizes the findings of the evaluations of six of these compounds (1-6) for their antifungal activity.

Table 2: Antifungal Activity of Synthesized Compounds (1–6)

Compound No.	Average % inhibition against					
	Collectotrichon falcatum			Fussarium oxysporum		
	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
1	94	62	48	92	63	46
2	96	79	52	95	78	53
3	98	76	49	97	72	48
4	89	62	71	91	61	49
5	90	73	49	92	57	46
6	96	77	62	95	79	58
Dithane M-45	100	86	70	100	85	66

The substances under research displayed antifungal action, as comprehensively summarized in the above table. The results show that at high doses (1000 ppm), the majority of the synthetic chemicals are effective against fungal infections. Their antifungal performance is concentration dependant; nevertheless, when the concentrations are reduced to 100 ppm and 10 ppm, their fungicidal activity is significantly reduced.

Compounds with a chlorine (Cl) or 2,4Cl_2 substituent exhibited superior fungitoxic characteristics when compared to other derivatives, according to the screening findings. This finding provides further evidence that these functional groups enhance the antifungal properties of the drugs.

CONCLUSION

The compound known as 5,5-dihydro-1,3,4-thiadiazolo-[3,2-a] - [1,3,5] or. -triazin-5-one derivatives that were manufactured shown remarkable fungicidal effectiveness over a wide spectrum against a variety of dangerous fungal strains they were tested against. Considering the growing resistance to the antifungal treatments that are now available, these compounds have shown promise in in vitro antifungal testing, which suggests that they might potentially replace the pharmaceuticals. According to the findings of the structure-activity relationship (SAR) study, some substitutions, in particular electron-withdrawing groups, played a significant part in the enhancement of biological activity. The results of this study emphasize the capacity of the thiadiazolotriazinone scaffold to serve as a fundamental component in the development of novel and efficient antifungal medications. In order to further examine this scaffold, it is suggested that in vivo research and formulation development should be carried out.

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