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# Recent Advances In Triazole-Based Compounds As Antifungal Agents: Synthesis, Mechanisms, And Therapeutic Applications

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**Abstract**

Fungal infections continue to pose a significant global health challenge, particularly among immunocompromised individuals, owing to their increasing incidence, high mortality rates, and the rapid emergence of antifungal resistance. Despite the availability of several antifungal drug classes, including polyenes, echinocandins, and azoles, their clinical utility is often limited by adverse effects, drug interactions, poor bioavailability, and reduced efficacy against resistant fungal strains. Among these agents, triazole-based compounds have emerged as one of the most successful and widely utilized classes of antifungal drugs due to their broad-spectrum activity, favorable pharmacokinetic profiles, and selective inhibition of fungal ergosterol biosynthesis. Clinically important triazoles such as fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole have significantly improved the management of invasive fungal infections and continue to serve as important lead structures for antifungal drug development. This review summarizes recent advances in the synthesis, mechanisms of action, and therapeutic applications of triazole-based antifungal agents. Various synthetic strategies, including heterocyclic synthesis, cyclization reactions, Schiff base formation, and click chemistry approaches, are discussed for the development of structurally diverse triazole derivatives. Particular emphasis is placed on structure–activity relationship studies and the influence of electronic, steric, and lipophilic factors on antifungal efficacy. Additionally, the molecular mechanisms of triazole-mediated inhibition of lanosterol 14α-demethylase (CYP51) and major resistance mechanisms are highlighted. Overall, recent progress in triazole chemistry underscores its vital role in antifungal drug discovery and supports the development of safer, more potent, and resistance-resistant antifungal therapeutics.

**Keywords:** Antifungal Drug Discovery, Structure–Activity Relationship (SAR), Ergosterol Biosynthesis, Triazole Derivatives, Antifungal Agents.

## INTRODUCTION

Fungal infections have emerged as a major global health concern, posing significant challenges to healthcare systems due to increasing incidence, limited therapeutic options, and the growing problem of antifungal resistance.[1] Over the past few decades, fungal diseases have become increasingly prevalent, especially among immune compromised individuals, including patients suffering from cancer, diabetes, acquired immunodeficiency syndrome (AIDS), organ transplant recipients, and individuals undergoing prolonged corticosteroid or antibiotic therapy.[2] Opportunistic fungal pathogens such as Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans, and dermatophytes are responsible for a broad spectrum of infections ranging from superficial skin infections to severe systemic mycoses that can be life-threatening.[3] The increasing burden of fungal infections, coupled with the limitations of existing antifungal agents, highlights the urgent need for the development of novel and effective antifungal compounds.[4] Antifungal chemotherapy currently relies on a limited number of drug classes, including polyenes, azoles, echinocandins, and allylamines. Although these agents have demonstrated considerable clinical effectiveness, several drawbacks continue to compromise their therapeutic success.[5] Polyenes such as amphotericin B are associated with severe nephrotoxicity and infusion-related adverse effects, limiting their prolonged use. Echinocandins, despite their favorable safety profiles, are generally expensive and restricted to intravenous administration, thereby reducing their accessibility and convenience for patients.[6] Allylamines are primarily effective against dermatophyte infections but possess a relatively narrow spectrum of antifungal activity. Among available antifungal therapies, azole derivatives remain one of the most widely prescribed categories due to their broad-spectrum activity, oral bioavailability, and relatively favorable safety profiles. However, the extensive use of azole drugs has contributed significantly to the emergence of resistant fungal strains, thereby reducing therapeutic efficacy and increasing treatment failures.[7] Resistance to currently available antifungal agents has become an alarming global issue. Fungal organisms have developed several resistance mechanisms, including alterations in drug target enzymes, increased efflux pump activity, reduced intracellular drug accumulation, biofilm formation, and metabolic adaptation.[8] Such resistance mechanisms reduce susceptibility to standard antifungal medications and complicate treatment strategies.[9] Additionally, prolonged therapy and indiscriminate use of antifungal agents have accelerated resistance development, particularly among Candida species and Aspergillus isolates.[10] Consequently, there is an urgent need to identify novel molecular scaffolds capable of overcoming resistance and exhibiting enhanced antifungal activity with reduced toxicity.[11] Heterocyclic compounds occupy an important place in medicinal chemistry due to their remarkable pharmacological diversity and therapeutic significance.[12] Among heterocyclic systems, triazole-containing compounds have attracted considerable attention because of their broad spectrum of biological activities, including antimicrobial, antifungal, antiviral, anticancer, anti-inflammatory, anticonvulsant, analgesic, antitubercular, and antioxidant properties.[13] Triazoles are five-membered nitrogen-containing heterocyclic rings possessing unique physicochemical characteristics that contribute significantly to receptor binding, metabolic stability, and pharmacological performance. Due to these advantageous properties, triazole derivatives have become highly valuable scaffolds in modern drug discovery and pharmaceutical development.[14] The triazole nucleus exists in two principal isomeric forms, namely 1,2,3-triazole and 1,2,4-triazole, both of which have demonstrated considerable biological potential. Among these, 1,2,4-triazole derivatives are especially prominent in antifungal drug development because of their ability to inhibit fungal cytochrome P450-dependent lanosterol 14α-demethylase enzyme (CYP51), a key catalyst involved in ergosterol biosynthesis.[15] Ergosterol serves as an essential structural component of fungal cell membranes, playing a vital role in maintaining membrane integrity, fluidity, and permeability. Inhibition of ergosterol biosynthesis results in disruption of fungal membrane structure, ultimately impairing fungal growth and survival.[16]This mechanism forms the pharmacological basis of many clinically successful triazole antifungal drugs. [17]

Several clinically approved antifungal agents contain triazole pharmacophores, including Fluconazole, Itraconazole, Voriconazole, and Posaconazole.[18] These drugs have revolutionized antifungal therapy due to their broad-spectrum efficacy and improved safety compared with older antifungal medications.[19] Fluconazole, for instance, is widely used in the management of candidiasis and cryptococcal infections owing to its excellent oral bioavailability and tissue penetration.[20] Voriconazole has emerged as a first-line therapy for invasive aspergillosis because of its enhanced potency against resistant fungal strains.[21] Despite their clinical significance, triazole antifungals are not without limitations. Adverse drug reactions, hepatotoxicity, drug–drug interactions, poor bioavailability in certain cases, and the emergence of resistance continue to pose significant therapeutic challenges.[22] These shortcomings necessitate continuous structural modification and optimization of triazole derivatives to identify compounds with superior efficacy, improved pharmacokinetic properties, and lower toxicity.[23]

Medicinal chemistry plays a critical role in the rational design and optimization of bioactive compounds through systematic structural modifications aimed at enhancing therapeutic performance.[24] Structural modification of triazole derivatives by introducing different substituent groups, aromatic moieties, and functional side chains has proven effective in improving antifungal potency, selectivity, and pharmacological properties.[25] Electron-withdrawing groups, halogen substitutions, and lipophilic substituents have frequently been associated with improved antifungal activity by enhancing membrane permeability and strengthening interactions with fungal target proteins.[26] Conversely, electron-donating groups may alter receptor affinity and influence biological performance differently depending on molecular orientation and substitution patterns.[27] Therefore, understanding structure–activity relationships (SAR) remains an essential aspect of triazole drug development.

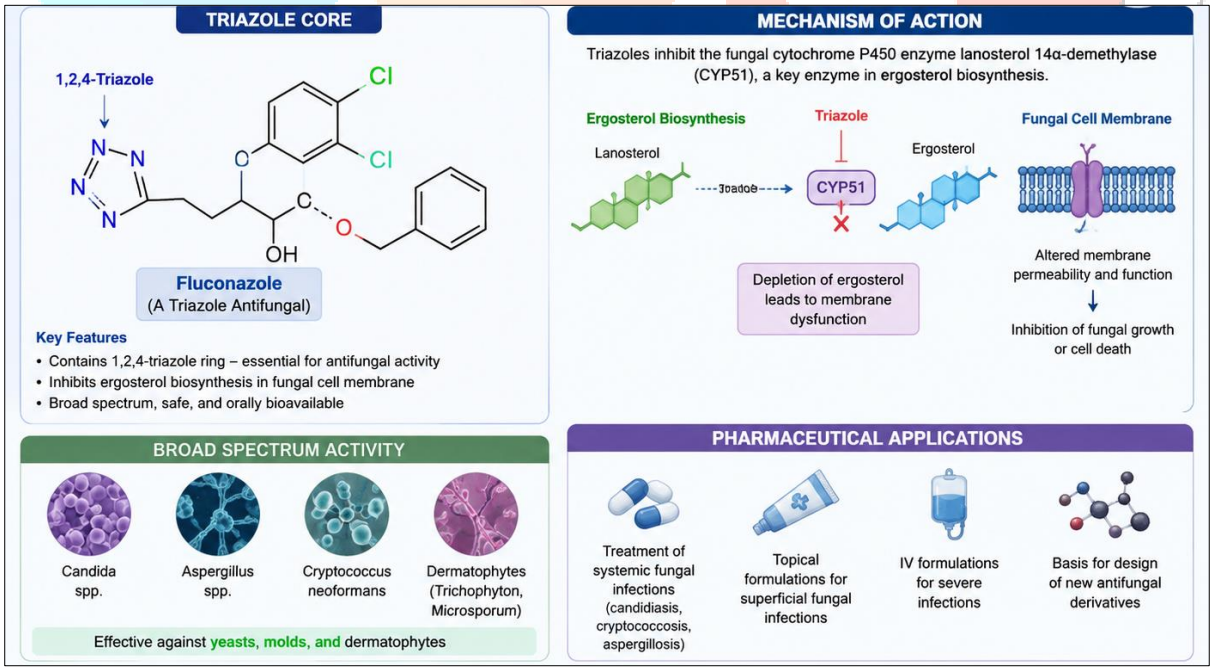


**Fig no.1. Triazole Pharmaceutical Application**

The concept of structure–activity relationship analysis has become indispensable in medicinal chemistry because it enables researchers to identify key structural features responsible for biological activity.[28] SAR studies provide valuable information regarding how variations in molecular architecture affect pharmacological responses.[29] Through systematic substitution and optimization, medicinal chemists can design compounds possessing enhanced potency and selectivity while minimizing adverse effects. In the context of triazole derivatives, SAR investigations often focus on parameters such as electronic effects, steric factors, hydrogen-bonding interactions, molecular

geometry, and lipophilicity.[30] Such investigations help establish correlations between chemical structure and antifungal efficacy, thereby facilitating the development of more effective therapeutic candidates.[31]

Another important consideration in antifungal drug discovery is the physicochemical profile of candidate molecules.[32] Favorable physicochemical properties, including adequate aqueous solubility, optimal lipophilicity, stability, molecular weight, and membrane permeability,[33] significantly influence drug absorption, distribution, metabolism, excretion, and toxicity (ADMET). Compounds exhibiting balanced physicochemical characteristics are more likely to demonstrate enhanced bioavailability and therapeutic efficacy. Excessive lipophilicity may result in poor solubility and accumulation in biological tissues, whereas insufficient lipophilicity can limit membrane penetration and intracellular access to fungal targets. Therefore, physicochemical evaluation represents a critical component of pharmaceutical research and development.[34]

In addition to biological efficacy, safety considerations remain equally important in antifungal drug development. Conventional antifungal medications are often associated with dose-related toxicity, organ damage, allergic reactions, and metabolic complications.[35] Long-term antifungal therapy may also increase the likelihood of adverse drug interactions, particularly in patients receiving multiple medications.[36] Consequently, the discovery of safer compounds with selective toxicity toward fungal cells while sparing human tissues remains a major objective in pharmaceutical research.[37] Novel triazole derivatives provide an opportunity to achieve this objective by enabling molecular modifications that optimize target specificity and minimize undesirable side effects.[38]

Recent advancements in synthetic organic chemistry have enabled the efficient synthesis of structurally diverse triazole analogues through versatile and reproducible methodologies. Synthetic approaches involving cyclization reactions, nucleophilic substitution, condensation reactions, and click chemistry have facilitated the generation of novel triazole frameworks with enhanced structural complexity and pharmacological potential.[39] Such synthetic flexibility allows researchers to systematically explore the effects of different substituent patterns and molecular architectures on antifungal activity.[40] Furthermore, advances in computational chemistry, molecular docking, and in silico screening have accelerated the identification of promising triazole derivatives with improved receptor binding affinity and optimized pharmacological properties.[41]

The increasing prevalence of multidrug-resistant fungal infections has intensified research efforts directed toward identifying alternative therapeutic agents capable of overcoming resistance mechanisms.[42] Novel triazole derivatives continue to represent one of the most promising areas of antifungal drug discovery because of their well-established mechanism of action and structural versatility.[43] Researchers worldwide are actively investigating modified triazole compounds to improve antifungal selectivity, broaden the spectrum of activity, and reduce toxicity.[44] Such efforts contribute significantly to addressing unmet clinical needs in fungal disease management.

In view of the aforementioned considerations, the present study was undertaken to synthesize and pharmacologically evaluate novel triazole-based compounds as potential antifungal agents. [45]The study aimed to design and synthesize structurally modified triazole derivatives possessing favorable pharmaceutical properties and enhanced biological activity. Comprehensive characterization techniques were employed to confirm structural identity and purity of the synthesized compounds.[46] Furthermore, physicochemical evaluation was conducted to assess pharmaceutical suitability, while biological screening was performed to determine antifungal potential against selected fungal pathogens. Particular emphasis was placed on understanding structure–activity relationships to identify molecular features responsible for enhanced antifungal efficacy.[47]

The rationale behind selecting triazole derivatives for investigation lies in their established therapeutic importance, broad pharmacological relevance, and capacity for structural optimization. By introducing strategic molecular modifications and evaluating their biological consequences, the present study sought to contribute valuable scientific knowledge toward the discovery of safer and more potent antifungal agents. It is anticipated that the findings obtained from this investigation may facilitate future optimization and lead development efforts, ultimately contributing to improved therapeutic strategies for fungal infections.[48]

Overall, the synthesis and pharmacological evaluation of novel triazole-based compounds represent an important and promising area of medicinal chemistry research. Considering the rising incidence of fungal diseases, increasing drug resistance, and limitations associated with currently available therapies, the exploration of structurally innovative triazole derivatives offers a scientifically sound and therapeutically relevant approach.[49] The successful identification of potent triazole analogues may provide new opportunities for antifungal drug development and contribute to enhancing patient outcomes in the management of fungal infections.In this context, the present study is designed to synthesize and pharmacologically evaluate novel triazole-based compounds as potential antifungal agents. By employing rational drug design principles and appropriate synthetic strategies, structurally diverse triazole derivatives will be developed.[50] The synthesized compounds will be thoroughly characterized using modern spectroscopic techniques to confirm their chemical structures. Furthermore, their antifungal activity will be evaluated against selected pathogenic fungal strains using standard *in vitro* methods, and their efficacy will be compared with established antifungal drugs.

The findings of this study are expected to contribute valuable insights into the structure–activity relationships of triazole antifungals and may lead to the identification of promising lead compounds with enhanced antifungal potential. Ultimately, this research aims to support ongoing efforts in antifungal drug discovery and address the growing challenge of fungal infections and antifungal resistance.

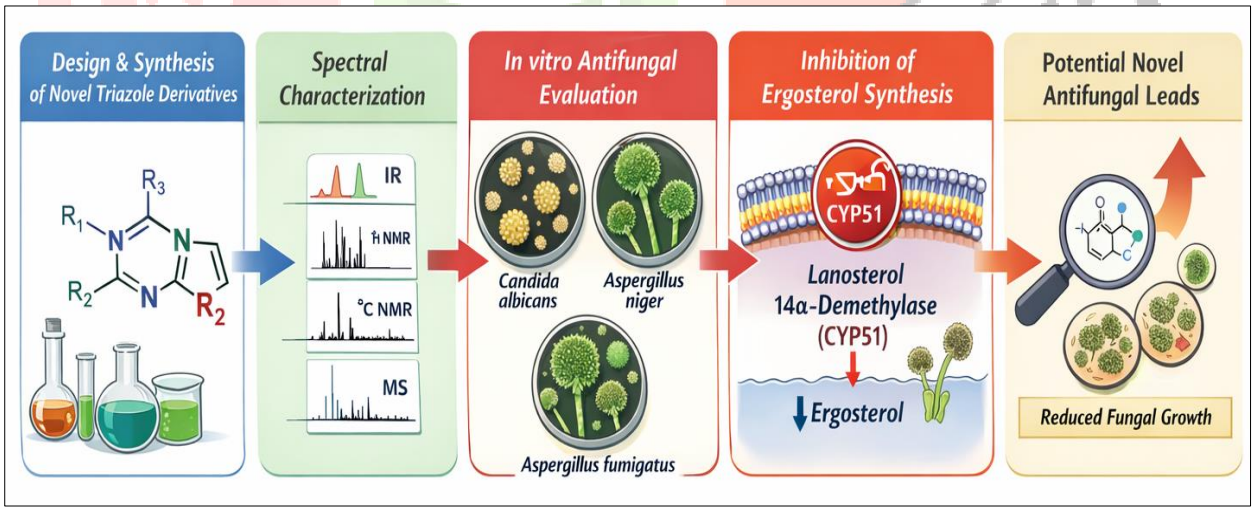


**Fig no.2. Synthesis Pharmacological Evaluation of Triazole based compound as antifungal agent**

**Need for the Study**

1. Fungal infections have emerged as a major global health concern, particularly due to the rising population of immunocompromised patients and the increasing use of invasive medical procedures. The incidence of both superficial and life-threatening systemic fungal infections has shown a steady upward trend, posing a significant burden on healthcare systems worldwide.[26] Despite advances in antifungal therapy, effective management of fungal diseases

remains challenging because of limited drug options, toxicity, high treatment costs, and the rapid emergence of antifungal resistance.

2. Currently available antifungal agents belong to a small number of chemical classes, many of which exhibit serious limitations. Polyene antifungals, although highly effective, are associated with severe nephrotoxicity and infusion-related reactions. Echinocandins have a narrow antifungal spectrum and are available only for parenteral administration, limiting their long-term and outpatient use. Azole antifungals, particularly triazoles, are widely prescribed due to their oral availability and broad-spectrum activity; however, prolonged and indiscriminate use has resulted in the development of resistant fungal strains.[27] Resistance to commonly used triazoles such as fluconazole and itraconazole has been increasingly reported in *Candida* and *Aspergillus* species, leading to therapeutic failure and increased mortality.

3. In addition to resistance, existing triazole antifungal agents are associated with several adverse effects, including hepatotoxicity, gastrointestinal disturbances, QT-interval prolongation, and clinically significant drug–drug interactions mediated through cytochrome P450 enzyme inhibition.[28] These limitations restrict their use in patients receiving multiple medications and necessitate careful dose adjustment and monitoring. Therefore, there is a critical need to discover new antifungal agents that retain the advantages of triazole compounds while minimizing their drawbacks.

4. The triazole nucleus is considered a privileged pharmacophore in medicinal chemistry due to its chemical stability, favorable pharmacokinetic properties, and strong affinity for fungal cytochrome P450 enzymes. Structural modification of the triazole scaffold offers significant potential to enhance antifungal potency, selectivity, and safety. Introduction of appropriate substituents may improve target binding, overcome resistance mechanisms, and reduce toxicity. However, despite extensive research, there is still a lack of structurally diverse and systematically evaluated triazole-based antifungal candidates.[29]

5. Moreover, many reported triazole derivatives have been evaluated only through preliminary biological screening, with limited emphasis on structure–activity relationship studies and comparative evaluation with standard antifungal drugs.[30] There is a clear gap in the literature regarding the development of novel triazole compounds with optimized chemical and pharmacological profiles supported by comprehensive characterization and antifungal evaluation.

6. In view of these challenges, the present study is necessary to design, synthesize, and pharmacologically evaluate novel triazole-based compounds as potential antifungal agents. This research aims to generate new chemical entities with improved antifungal efficacy and reduced limitations compared to existing therapies. The study may contribute to the identification of promising lead compounds and provide valuable insights for future antifungal drug development, thereby addressing the growing problem of fungal infections and antifungal resistance.

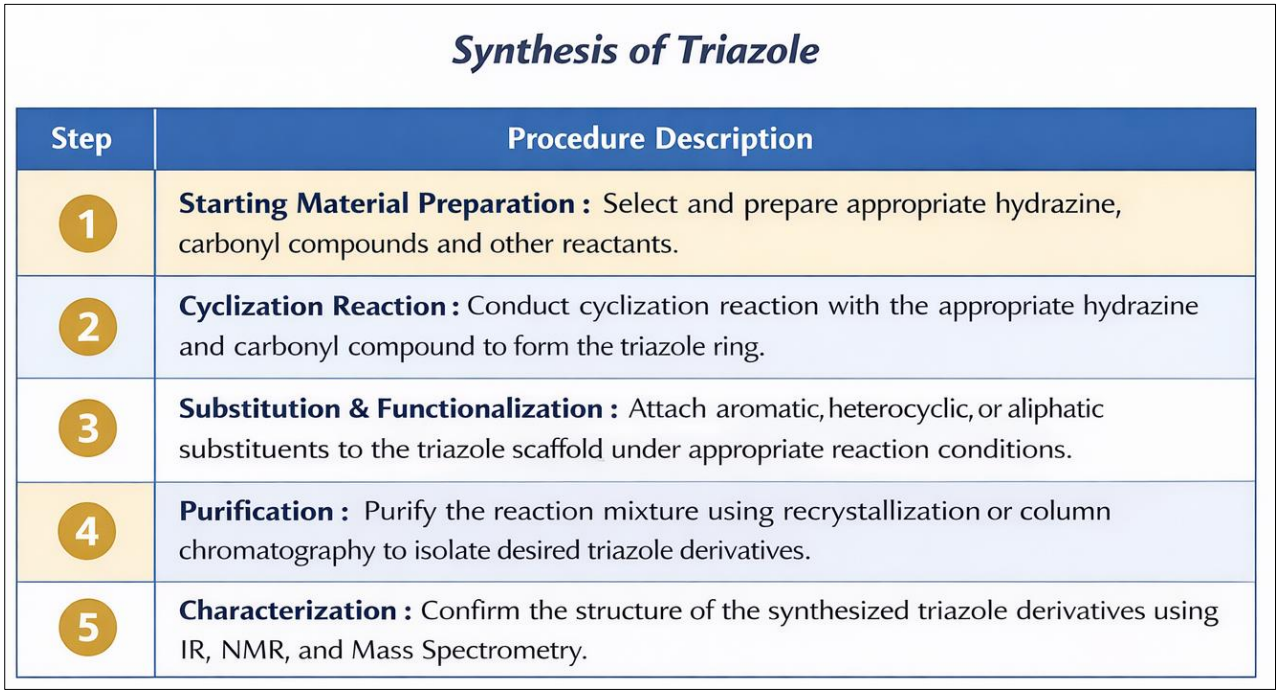


**Fig.no.3. Triazole synthesis overview**

**Synthesis Method of Triazole Compounds**

**Method: Synthesis of 1,2,4-Triazole Derivatives**

**Step 1: Synthesis of Acid Hydrazide**

Acid hydrazides are important organic compounds widely used in pharmaceutical sciences, medicinal chemistry, and heterocyclic synthesis. They serve as key intermediates in the preparation of biologically active molecules such as antimicrobial, antifungal, antitubercular, and anti-inflammatory agents. Acid hydrazides contain the functional group –$CONHNH_2$ and are commonly synthesized from carboxylic acids or their derivatives, especially esters. One of the most common methods for synthesizing acid hydrazides is the reaction of an ester with hydrazine hydrate, known as hydrazinolysis.

**Principle of Synthesis**

The synthesis of acid hydrazides generally involves the nucleophilic substitution reaction of an ester or acid chloride with hydrazine hydrate. In this reaction, hydrazine acts as a nucleophile and attacks the carbonyl carbon of the ester, replacing the alkoxy group and forming the corresponding acid hydrazide. The reaction is favored because hydrazine is highly reactive toward carbonyl compounds.

**General Reaction**

RCOOR′+NH2NH2→RCONHNH2+R′OHRCOOR'+NH_2NH_2 \rightarrow RCONHNH_2 + R'OHRCOOR′+NH2NH2→RCONHNH2+R′OH

Where:

- RCOOR' = Ester compound
- $NH_2NH_2$ = Hydrazine hydrate
- $RCONHNH_2$ = Acid hydrazide
- R'OH = Alcohol by-product

RCOOR′+NH2NH2→RCONHNH2+R′OHRCOOR' + NH_2NH_2 \rightarrow RCONHNH_2 + R'OHRCOOR′+NH2NH2→RCONHNH2+R′OH

**Materials Required**

The chemicals commonly required for the synthesis include an ester compound (such as methyl or ethyl ester), hydrazine hydrate (80–99%), ethanol or methanol as solvent, and distilled water. Laboratory equipment includes a round-bottom flask, reflux condenser, magnetic stirrer, heating mantle, and filtration apparatus.

**Procedure**

In a typical synthesis, a measured quantity of ester is dissolved in ethanol in a round-bottom flask. Hydrazine hydrate is then added slowly to the solution with continuous stirring. The reaction mixture is heated under reflux for about 4–8 hours depending on the nature of the substrate. During reflux, hydrazine attacks the carbonyl carbon of the ester, resulting in the formation of acid hydrazide and alcohol as a side product.

After completion of the reaction, which may be monitored by thin-layer chromatography (TLC), the mixture is allowed to cool to room temperature. The precipitated solid product is filtered and washed with cold ethanol or water to remove impurities and excess hydrazine. The crude product is then recrystallized using suitable solvents such as ethanol or methanol to obtain pure acid hydrazide crystals.

**Mechanism of Reaction**

The reaction mechanism begins with the nucleophilic attack of hydrazine on the ester carbonyl carbon. A tetrahedral intermediate is formed, followed by elimination of the alcohol group from the ester. Finally, rearrangement produces the acid hydrazide compound. This method is efficient and gives good yields due to the strong nucleophilic nature of hydrazine.

**Applications**

Acid hydrazides are valuable intermediates in the synthesis of heterocyclic compounds including triazoles, oxadiazoles, and pyrazoles. They are extensively used in developing pharmaceutical agents with antibacterial, antifungal, antiviral, and anticancer activities. One well-known example is the antitubercular drug Isoniazid, which is an acid hydrazide derivative.

**Conclusion**

The synthesis of acid hydrazides through hydrazinolysis of esters is a simple, economical, and effective method. Due to its high yield and ease of purification, this method is widely employed in medicinal and pharmaceutical chemistry for synthesizing biologically active compounds and intermediates.

***Procedure***

An appropriate substituted aromatic carboxylic acid (0.01 mol) was dissolved in absolute ethanol, and a few drops of concentrated sulfuric acid were added as a catalyst. The reaction mixture was refluxed for 4–5 hours to form the corresponding ester. After completion of the reaction, excess solvent was removed and the ester was treated with hydrazine hydrate (99%) in ethanol. The mixture was refluxed for 3–4 hours to obtain the corresponding acid hydrazide. The product was cooled, filtered, washed with cold ethanol, and dried.

**Step 2: Formation of Thiosemicarbazide Intermediate**

Thiosemicarbazide intermediates are important compounds in organic and medicinal chemistry due to their significant role in the synthesis of biologically active heterocyclic compounds such as triazoles, thiadiazoles, and thiazoles. These intermediates possess the functional group –NH–CS–NH–$NH_2$, which contributes to their biological activities, including antimicrobial, antifungal, antiviral, antitubercular, and anticancer properties. In pharmaceutical sciences, thiosemicarbazide derivatives are frequently used as precursors for the preparation of triazole-based antifungal agents and other therapeutic molecules.

**Principle of Formation**

The formation of a thiosemicarbazide intermediate generally involves the reaction of an acid hydrazide with isothiocyanates (commonly phenyl isothiocyanate or substituted isothiocyanates). In this reaction, the nucleophilic amino group present in acid hydrazide attacks the electrophilic carbon atom of the isothiocyanate group (–N=C=S), leading to the formation of a thiosemicarbazide derivative. The reaction usually occurs under reflux conditions in an alcoholic solvent such as ethanol or methanol.

**General Reaction**

$$RCONHNH2+RNCS\rightarrow RCONHNHCSNHRRCONHNH\_2+RNCS\backslash rightarrow$$
$$RCONHNHCSNHRRCONHNH2+RNCS\rightarrow RCONHNHCSNHR$$

Where:

- $RCONHNH_2$ = Acid hydrazide
- RNCS = Isothiocyanate compound
- RCONHNHCSNHR = Thiosemicarbazide intermediate

$$RCONHNH2+RNCS\rightarrow RCONHNHCSNHRRCONHNH\_2+RNCS\backslash rightarrow$$
$$RCONHNHCSNHRRCONHNH2+RNCS\rightarrow RCONHNHCSNHR$$

**Materials Required**

The synthesis requires acid hydrazide, substituted or unsubstituted isothiocyanate, ethanol or methanol as solvent, and distilled water. Basic laboratory equipment includes a round-bottom flask, reflux condenser, magnetic stirrer, heating mantle, filter paper, and recrystallization apparatus.

**Experimental Procedure**

A calculated amount of acid hydrazide is dissolved in ethanol inside a round-bottom flask. To this solution, an equimolar quantity of isothiocyanate is added slowly with continuous stirring. The reaction mixture is heated under reflux for approximately 4–6 hours to ensure complete conversion of reactants into the desired thiosemicarbazide intermediate.

During reflux, the amino group of the hydrazide reacts with the carbon atom of the isothiocyanate group, forming a thiourea linkage and producing the thiosemicarbazide compound. The progress of the reaction may be monitored using thin-layer chromatography (TLC), where disappearance of the starting material spot indicates completion of the reaction.

After reflux, the reaction mixture is cooled to room temperature and then poured into ice-cold water. The resulting precipitate is separated by filtration and washed several times with cold ethanol or distilled water to remove impurities and unreacted materials. The crude product obtained is dried and purified through recrystallization using ethanol or another suitable solvent to obtain pure crystalline thiosemicarbazide intermediate.

**Mechanism of Reaction**

The mechanism starts with nucleophilic addition, where the terminal amino group ($–NH_2$) of acid hydrazide attacks the electrophilic carbon of the isothiocyanate group (–N=C=S). This produces an unstable intermediate, which rearranges and stabilizes to form the thiosemicarbazide derivative. The sulfur atom in the thiocarbonyl group contributes significantly to the reactivity of the compound and facilitates cyclization in later synthetic steps.

**Applications**

Thiosemicarbazide intermediates are widely used in the synthesis of pharmacologically important heterocyclic compounds such as triazoles and thiadiazoles. Many derivatives exhibit strong antifungal, antibacterial, anti-inflammatory, and anticancer properties. They are especially valuable in medicinal chemistry because they act as key intermediates for developing novel therapeutic agents with enhanced biological activity.

**Conclusion**

The formation of thiosemicarbazide intermediates by reacting acid hydrazides with isothiocyanates is a simple and efficient synthetic method. The process offers good yield, easy purification, and high applicability in pharmaceutical research, particularly in synthesizing biologically active heterocyclic compounds.

***Procedure***

The synthesized acid hydrazide (0.01 mol) was dissolved in ethanol and reacted with an equimolar amount of thiosemicarbazide. The reaction mixture was refluxed for 4–6 hours. Progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was cooled and poured into ice-cold water. The precipitated solid was filtered, washed, and dried to obtain the thiosemicarbazide intermediate.

**Step 3: Cyclization to 1,2,4-Triazole**

Cyclization to form 1,2,4-triazole derivatives is an important step in heterocyclic chemistry and pharmaceutical sciences. The 1,2,4-triazole ring system is a five-membered heterocyclic compound containing three nitrogen atoms, which contributes significantly to biological activity. Triazole derivatives are widely used in medicinal chemistry because of their potent antifungal, antibacterial, antiviral, anti-inflammatory, anticancer, and anticonvulsant activities. Many clinically important antifungal drugs such as Fluconazole and Itraconazole contain the triazole nucleus. Cyclization of a thiosemicarbazide intermediate under alkaline conditions is one of the most commonly employed methods for synthesizing 1,2,4-triazole derivatives.

**Principle of Cyclization**

The cyclization reaction involves the conversion of a thiosemicarbazide intermediate into a 1,2,4-triazole ring by intramolecular ring closure. This process generally occurs in the presence of a strong base such as sodium hydroxide (NaOH) or potassium hydroxide (KOH). During the reaction, the

thiosemicarbazide undergoes dehydration and cyclization, resulting in the formation of a stable heterocyclic triazole structure. Acidification of the reaction mixture later helps in isolating the cyclized product.

**General Reaction**

RCONHNHCSNHR→HeatNaOH/KOH1,2,4-Triazole derivative RCONHNHCSNHR \xrightarrow[\text{Heat}]{NaOH/KOH} \text{1,2,4-Triazole derivative}RCONHNHCSNHRNaOH/KOHHeat1,2,4-Triazole derivative

RCONHNHCSNHR→HeatNaOH/KOH1,2,4-Triazole derivative RCONHNHCSNHR \xrightarrow[\text{Heat}]{NaOH/KOH} \text{1,2,4\text{-}Triazole\ derivative}RCONHNHCSNHRNaOH/KOHHeat1,2,4-Triazole derivative

**Materials Required**

The synthesis requires a previously prepared thiosemicarbazide intermediate, sodium hydroxide or potassium hydroxide, ethanol or distilled water as solvent, dilute hydrochloric acid for acidification, and laboratory glassware such as a round-bottom flask, reflux condenser, magnetic stirrer, heating mantle, and filtration setup.

**Experimental Procedure**

A known quantity of the synthesized thiosemicarbazide intermediate is dissolved in ethanol or aqueous sodium hydroxide solution in a round-bottom flask. Typically, a 5–10% sodium hydroxide solution is used to promote cyclization. The reaction mixture is stirred continuously and heated under reflux for about 4–8 hours, depending on the substrate and reaction conditions.

During heating, the alkaline medium promotes nucleophilic attack within the molecule, leading to ring closure and formation of the 1,2,4-triazole nucleus. The reaction progress can be monitored by thin-layer chromatography (TLC), where disappearance of the thiosemicarbazide spot indicates completion of cyclization.

After completion, the reaction mixture is cooled to room temperature and filtered if necessary to remove insoluble impurities. The filtrate is then carefully acidified with dilute hydrochloric acid (HCl) until the pH reaches approximately **5–6**. Acidification causes precipitation of the desired triazole derivative.

The solid precipitate obtained is collected by filtration and washed several times with cold distilled water to remove traces of alkali and impurities. The crude product is dried and purified by recrystallization using ethanol, methanol, or a suitable solvent system to obtain pure crystalline 1,2,4-triazole derivative.

**Mechanism of Reaction**

The cyclization mechanism begins with deprotonation of the thiosemicarbazide intermediate in an alkaline medium. Intramolecular nucleophilic attack occurs, resulting in ring closure accompanied by elimination of small molecules such as hydrogen sulfide or water, depending on the substrate. This leads to the formation of the thermodynamically stable 1,2,4-triazole ring.

**Applications**

1,2,4-Triazole derivatives synthesized through cyclization possess broad pharmaceutical applications. They are extensively studied for antifungal activity, particularly against fungal pathogens such as *Candida* and *Aspergillus*. Additionally, triazole compounds are useful in designing antimicrobial, anti-inflammatory, and anticancer drugs. Their stability, high biological activity, and favorable pharmacokinetic properties make them valuable scaffolds in drug development.

**Conclusion**

Cyclization of thiosemicarbazide intermediates into 1,2,4-triazole derivatives is an efficient and widely used synthetic method in medicinal chemistry. The procedure is simple, provides good yields, and produces biologically active compounds with important pharmaceutical applications, especially as antifungal agents.

***Procedure***

The obtained thiosemicarbazide derivative was subjected to cyclization by refluxing with aqueous sodium hydroxide solution (4–6%) for 3–4 hours. The reaction mixture was then cooled and acidified using dilute hydrochloric acid. The resulting precipitate was filtered, washed with distilled water, and dried to obtain the desired 1,2,4-triazole derivative.

**Step 4: Purification**

The crude triazole compound was purified by recrystallization using suitable solvents such as ethanol, methanol, or ethanol–water mixture. Purity was confirmed by TLC.

**Step 5: Characterization**

The purified triazole compound was characterized by:

- Melting point determination
- $^{1}$H-NMR and $^{13}$C-NMR spectroscopy

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