Chapter 1

Introduction & Literature Review

1.1 Introduction of Heterocyclic compounds

Heterocyclic compounds are organic chemical compounds with atoms joined in rings that include at least one element other than carbon. The term "heterocyclic" indicates at least one ring structure, while the prefix "hetero-" refers to the noncarbon atoms, known as heteroatoms, in the ring. Heterocyclic compounds have a similar structure to cyclic organic compounds that only contain carbon atoms in the rings, such as cyclopropane or benzene. However, heteroatoms give heterocyclic compounds different physical and chemical properties compared to their all-carbon-ring counterparts.[1] An inorganic heterocycle is identified when the ring lacks a carbon atom, and **Figure 1** showcases various examples.

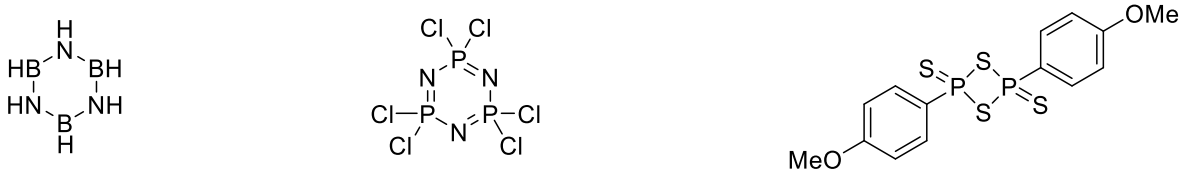
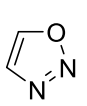


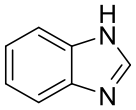
Figure 1

If a molecule has at least one carbon atom as a ring atom, then it is classified as an organic heterocyclic compound as shown in **Figure 2**. In this scenario, the term "heteroatoms" is used to describe all non-carbon atoms in the ring. The determination of the ring size not only depends on the type of atoms within it but also on the total number of atoms present. The minimum size for a ring is three members. Among the rings, the five- and six membered heterocycles are the most important. Heterocycles with seven, eight, nine, and larger numbers of members are all possible, as there is no maximum limit for the ring size. For heterocyclic compounds, it is worth noting that the N-atom reigns supreme as the most prevalent heteroatom, with the O- and S-atoms coming in as close contenders in terms of importance. Heterocycles that contain elements such as selenium (Se), tellurium (Te), phosphorus (P), arsenic (As), antimony (Sb), bismuth (Bi), silicon (Si), germanium (Ge), tin (Sn), lead (Pb), or boron (B) are not as common as those with more conventional atoms like nitrogen, sulfur and oxygen. This is because these elements have unique properties and

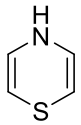
pose specific challenges, making their incorporation into heterocyclic structures less frequent.[2]



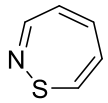
1,2,3-oxadiazole



1*H*-benzo[*d*]imidazole



4*H*-1,4-thiazine



1,2-thiazepine

Figure 2

The fusion of a benzene ring with a heterocyclic ring gives rise to benzo-fused heterocyclic compounds, which form a significant and varied subclass of heterocycles. The fusion of these compounds results in a distinctive combination of properties and reactivities, making them extremely valuable in fields such as medicinal chemistry, organic synthesis, and materials science.[3] When a benzene ring is fused with a heterocyclic ring, it forms a conjugated system that frequently enhances the stability and electronic properties of the compounds. This conjugation has the potential to impact chemical reactivity and physical properties, such as melting point, solubility, and spectral characteristics, by increasing aromaticity. The biological activity of benzo-fused heterocycles can be influenced by their extended π-system, affecting their interaction with biological targets.[4]

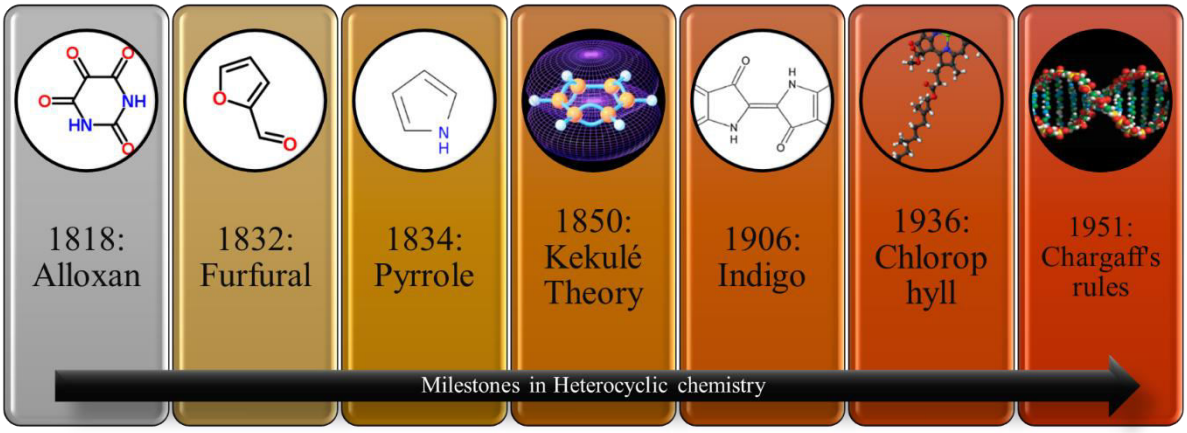
1.2 Historical background of Heterocyclic compounds

The study and application of heterocyclic compounds have played a crucial role in the advancement of organic chemistry and medicinal science, with their rich and storied history serving as a testament to their profound impact. Since its establishment, heterocyclic chemistry, which focuses on the analysis of compounds with heterocyclic rings, has undergone significant progress, fueled by scientific curiosity and practical necessities.

1.2.1 Early discoveries and development in Heterocyclic chemistry

The early 19th century marks the origins of heterocyclic chemistry **(Figure 3)**. In 1818, Luigi Valentino Brugnatelli accomplished the first significant milestone by synthesizing alloxan from uric acid.[5] Johann Wolfgang Döbereiner, a prominent figure in the scientific community, contributed noteworthily to this area of research. In 1832, he successfully isolated furfural, an oxygen-containing heterocycle, by employing sulfuric

acid in the treatment of starch.[6] Following this, in the year 1834, Friedrich Ferdinand Runge was able to obtain pyrrole, also known as "fiery oil," through the process of dry distillation of bones.[7] These initial findings served as a catalyst for a more extensive investigation into the composition and formation of heterocycles. During the mid-19th century, researchers made significant advancements in the identification of heterocyclic compounds. Notably, compounds such as thiophene, which contains sulfur, and pyrrole, another heterocycle with nitrogen, were discovered. In the field of organic chemistry, August Kekulé played a crucial role in the 1850s by providing valuable insights into the structural aspects of these compounds.[8] He specifically emphasized the significance of ring structures in understanding organic molecules. In the year 1882, two renowned chemists by the names of Adolf von Baeyer and Viggo Drewsen successfully synthesized indigo dye.[9] This remarkable achievement not only revolutionized the field of synthetic chemistry but also had a profound impact on the agricultural industry, causing significant disruption. This event served as a turning point, emphasizing the tremendous possibilities that synthetic heterocyclic compounds hold. Throughout the early 20th century, the field underwent continuous evolution, marked by significant developments. In the 1930s, there were further advancements in understanding petroleum's biological origin and the natural occurrence of heterocyclic compounds. This was exemplified by Alfred Treibs' isolation of chlorophyll derivatives from crude oil in 1936.[10] Another significant discovery occurred in the mid-



20th century, specifically in 1951, with the formulation of Chargaff's rules.[11] The significance of heterocyclic chemicals, including purines and pyrimidines, in the genetic code was underscored by Erwin Chargaff's research. This further emphasized the crucial role that these compounds hold in the realms of biology and genetics.[12]

Figure 3

The historical development of heterocyclic chemistry has played a central role in advancing the field of organic chemistry, and its applications have been wide-ranging. Heterocyclic compounds, with their rich history of early discoveries and continuous advancements in modern synthetic techniques, have always been at the forefront of scientific innovation and have proven to be incredibly useful in practical applications.

1.2.2 The Evolution of Synthetic Heterocycles in the Pharmaceutical Industry

The integration of heterocyclic compounds into the pharmaceutical industry was a major development that occurred throughout the 20th century. The discovery of penicillin in 1928 by Alexander Fleming,[13] and the subsequent elucidation and synthesis of its β-lactam ring by Dorothy Hodgkin and her colleagues, served as a remarkable milestone in highlighting the medicinal capabilities of heterocyclic compounds.[14] Penicillin's success served as a reminder of how crucial heterocyclic frameworks are in the process of designing and developing drugs. During this period, numerous heterocyclic drugs were developed, including sulfa drugs[15] (containing a sulfonamide group), barbiturates[16] (based on a barbituric acid scaffold), and a variety of alkaloid-derived therapeutics. The discovery of these compounds revolutionized medicine, providing effective treatments for a wide range of diseases and conditions.

1.2.3 Innovative Progress and Applications of Heterocyclic Compounds

Throughout the latter half of the 20th century and well into the 21st century, the field of heterocyclic chemistry has witnessed significant advancements, primarily fueled by innovative developments in synthetic techniques, computational chemistry, and an enhanced comprehension of biological systems. The advancements made in recent years have significantly broadened the range of applications and usefulness of heterocyclic compounds across multiple disciplines.

1.2.3.1 Innovations in Synthetic Techniques

Modern synthetic methods have revolutionized the construction of complex heterocyclic structures. The development of transition metal-catalyzed cross-coupling

reactions stands out as one of the most significant advancements in the field. The Suzuki-Miyaura, Heck, and Stille couplings are examples of reactions that have made the synthesis of heterocycles much easier. These reactions facilitate the formation of carbon-carbon and carbon-heteroatom bonds in mild conditions, effectively streamlining the process. For example:

→ **Suzuki-Miyaura Coupling:** Akira Suzuki and Norio Miyaura developed this reaction in the 1979.[17] It enables the efficient formation of biaryl compounds, which play a vital role in pharmaceuticals and agrochemicals.

→ **Heck Reaction:** Palladium-catalyzed coupling of alkenes with aryl halides, developed by Richard F. Heck in the 1972, has allowed for the synthesis of complex alkenes and aromatic heterocycles.[18]

→ **Stille Coupling:** In the late 1970s, John K. Stille discovered a reaction that allows for the coupling of organostannanes with organic halides. This method is highly versatile and useful for constructing heterocyclic frameworks.[19]

1.2.3.2 Expanded Applications Beyond Pharmaceuticals

The application of heterocyclic compounds has expanded beyond pharmaceuticals into various industries:

⇒ **Agrochemicals:** Heterocyclic compounds play a crucial role in creating pesticides, herbicides, and fungicides. One example of a widely used agricultural fungicide is azoxystrobin, which contains a pyrimidine ring. It is used to safeguard crops against fungal diseases.[20]

⇒ **Dyes and Pigments:** The vibrant colors and stability of heterocycles like phthalocyanines[21] and azo compounds make them highly popular as dyes and pigments.[22] These compounds can be found in textiles, inks, and coatings.

⇒ **Polymers and Materials:** The development of advanced materials is closely tied to the use of heterocyclic compounds. Electronic devices, like organic semiconductors[23], LEDs[24], and solar cells[25], use polyheterocycles such as polythiophene and poly-pyrrole.

⇒ **Medicinal Chemistry:** Complex heterocyclic structures are commonly incorporated in modern drugs to improve effectiveness and specificity. One compelling example to consider is imatinib[26], a tyrosine kinase inhibitor that plays a crucial role in combating chronic myeloid leukemia. This drug stands out because of its unique composition, featuring a piperazine ring that is intricately linked to other heterocyclic components. This serves as a clear testament to the remarkable therapeutic potential that heterocycles possess.

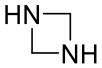
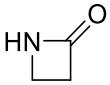
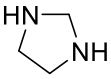
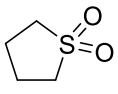
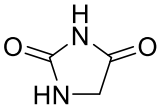
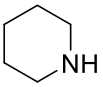
1.3 Classification of Heterocyclic compounds

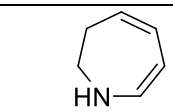
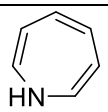
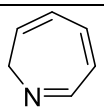
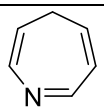
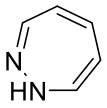
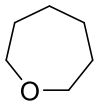
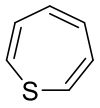
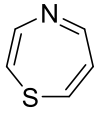
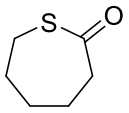
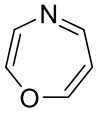
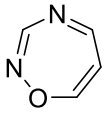
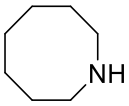
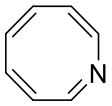
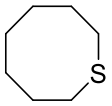
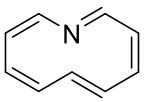
Heterocyclic compounds, which are recognized as a cornerstone of organic chemistry, can be categorized into different groups using various criteria. These criteria include the size of the ring, the type and number of heteroatoms present, as well as the presence or absence of aromaticity. The importance of understanding these classifications cannot be overstated, as it is key to fully appreciating the diversity and functionality of heterocyclic compounds in **various applications.**

1.3.1 Classification by Ring Size

A commonly used method to categorize heterocyclic compounds is based on the count of atoms forming the ring, which provides a clear classification, as depicted in **Table 1**. The focus of this classification is to showcase the wide range of structural variations within these compounds and to underscore the influence that ring size has on their overall properties. Various significant properties of heterocyclic compounds, including ring strain, stability, reactivity, and electronic properties, depend on the size of the ring. Therefore, accurately determining the size becomes crucial in understanding these compounds. The presence of significant ring strain is commonly observed in smaller rings, such as those with three or four members, leading to an increase in their reactivity. When the ring size increases, the strain decreases, which in turn leads to the formation of more stable compounds that are less reactive. Larger rings and fused systems offer even greater complexity and functionality, making them crucial in advanced applications.

Table 1: Classification of Heterocyclic compound based on ring sizes

Ring size	Examples & Structures
Three-membered rings	 aziridine  oxirane  thiirane  2H-azirine
Four-membered rings	 azetidine  1,3-diazetidine  2,3-dihydroazete  azete  azetidine-2one  oxetane  2H-oxete  thietane  2H-thiete
Five-membered rings	 pyrrolidine  1H-pyrrole  pyrazolidine  pyrazole  imidazolidine  tetrahydrofuran  furan  tetrahydrothiophene  thiophene  oxazole  imidazole  thiazole  1,2,5-oxadiazole  1,3,4-thiadiazole  1,3,4-triazole  sulfolane  hydantoin
Six-membered rings	 piperidine  pyridine  pyrimidine  pyrazine  1,3,5-triazine  tetrahydropyran  4H-pyran  1,4-dioxane  1,4-dioxine  pyrylium thiane 4H-thiopyran 1,3,5-trithiane morpholine 4H-1,4-thiazine

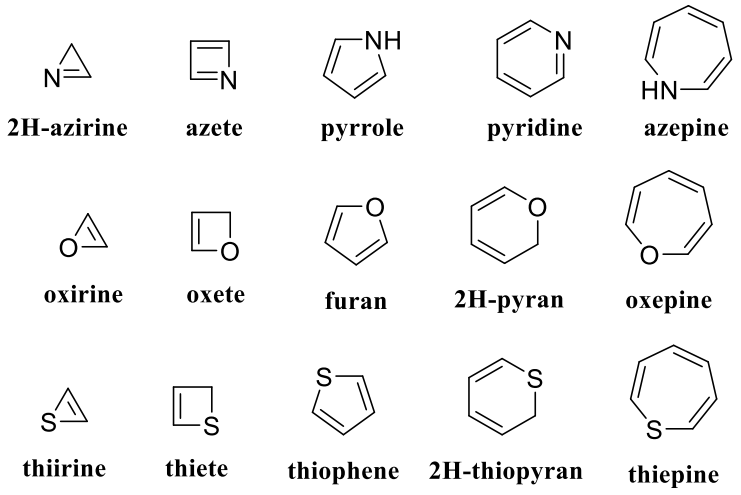
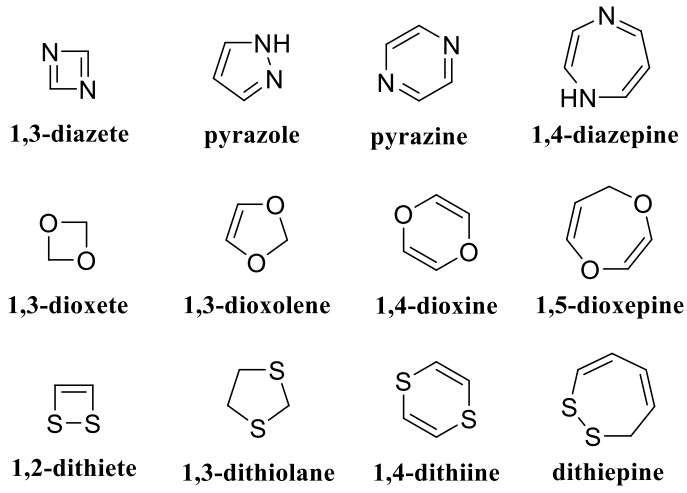
Seven-membered rings	 **2,3,-dihydroazepine**  **azepine**  **2H-azepine**  **4H-azepine**  **1,2-diazepine**  **oxepane**  **thiepine**  **1,4-thiazepine**  **2-Thiepanones**  **1,4-oxazepine**  **1,2,4-oxadiazepine**
Larger rings	 **azocane**  **azocine**  **thiocane**  **azonane**  **azecine**

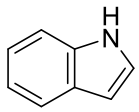
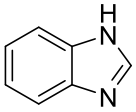
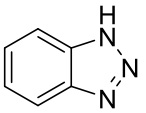
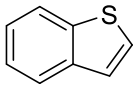
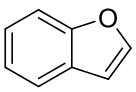
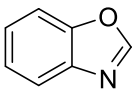
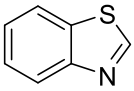
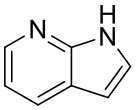
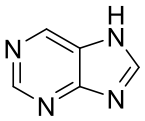
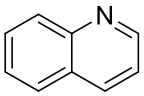
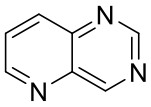
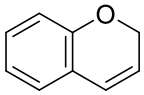
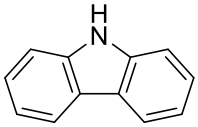
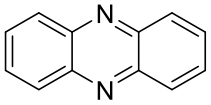
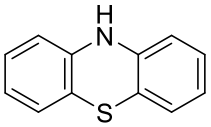
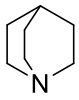
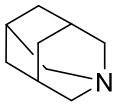
1.3.2 Classification by Type and Number of Heteroatoms

Heterocyclic compounds can also be classified by considering the type and quantity of heteroatoms that are present within the ring structure. Understanding the reactivity, electronic properties, and potential applications of these compounds becomes easier with the help of this classification system. The presence of nitrogen, oxygen, and sulfur as heteroatoms in carbon-based rings imparts unique chemical characteristics that set them apart from rings that consist only of carbon atoms. The presence and type of heteroatoms affect the compound's involvement in chemical reactions, interaction with biological systems, and suitability for industrial uses.

Table 2: Classification of Heterocycles by Type and Number of Heteroatoms

Types & no. of hetero atoms	**Examples & structures**

Monocyclic Heterocycles with One Heteroatom	
Monocyclic Heterocycles with Multiple Heteroatoms	

Bicyclic and Polycyclic Heterocycles	 **indole**  **benzimidazole**  **benzotriazole**  **benzo[*b*]thiophene**  **benzofuran**  **benzoxazole**  **benzothiazole**  **7-azaindole**  **purine**  **quinoline**  **pyrido[3,2-d]pyrimidine**  **2H-chromene**  **carbazole**  **phenazine**  **phenothiazine**  **2-azaadamantane**  **quinuclidine**  **1-azaadamantane**

1.3.3 Classification by Aromaticity

Understanding the concept of aromaticity is essential in heterocyclic chemistry, as it significantly shapes the stability, reactivity, and electronic properties of these compounds. Heterocyclic compounds based on aromaticity can be categorized into two primary groups: aromatic and non-aromatic. The conjugated π-electron systems found in aromatic heterocycles adhere to Hückel's rule. According to this rule, a molecule can be classified as aromatic if it possesses a total of (4n+2) π-electrons, where n represents a non-negative integer. This rule aids in identifying highly stable compounds with delocalized π-electrons across the ring structure. Conversely, non-aromatic heterocycles deviate from Hückel's rule, thereby lacking the exceptional stability that is characteristic of aromatic compounds. As a result, these heterocycles display alternative reactivity patterns and offer a wide range of applications.

(1) Aromatic Heterocycles

The distinguishing features of aromatic heterocycles include their planar structure, conjugated π-electron system, and adherence to Hückel's rule, which contribute to their distinct characteristics. The presence of these properties in aromatic heterocycles is of utmost importance because of the significant stability and unique reactivity they confer, making them vital components in various chemical and biological processes. Pyridine, furan, thiophene, imidazole, pyrimidine, indole, quinoline, and benzothiazole are all well-known examples of aromatic heterocycles. The aromatic nature of these compounds provides stability, enabling them to engage in various chemical reactions. This quality makes them extremely valuable in fields like medicinal chemistry and materials science.

(2) Non-aromatic Heterocycles

The absence of a fully conjugated π-electron system or the violation of Hückel's rule characterizes non-aromatic heterocycles. These compounds do not exhibit the specific stability that is typically associated with aromaticity, leading to different chemical properties and reactivity. Piperidine, tetrahydrofuran, morpholine, aziridine, oxirane, azetidine, and oxetane are examples of non-aromatic heterocycles. In organic synthesis, these compounds are frequently used as

intermediates and serve as foundational units for constructing more complex molecules. Their non-aromatic properties make them more reactive and give them unique applications not found in aromatic substances.

1.4 Overview of Indole, Benzothiazole and Triazoles

Indole, benzothiazole, and triazole are three important categories of heterocyclic compounds. They each have distinct structures and find extensive use in diverse fields, particularly in medicinal chemistry. The distinct chemical properties and biological activities of these compounds are attributed to the heteroatoms (such as nitrogen, sulfur, and oxygen) present in their ring structures. Indole is a fundamental structure in many natural products and pharmaceuticals, playing a crucial role in biological processes and therapeutic applications. The unique structure of benzothiazole, with its fused benzene and thiazole ring, allows for diverse pharmacological activities and applications in materials science, like the production of dyes and polymers. Triazole is a chemical compound can exist in two forms: 1,2,3-triazole and 1,2,4-triazole. These five-membered rings are extensively employed in the synthesis of antifungal, antiviral, and antibacterial agents.

1.4.1 Indole: Structure, Synthesis, and Significance in biological term

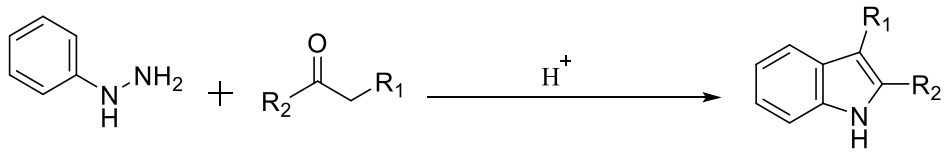
The structure of indole involves a benzene ring and a pyrrole ring fused together, creating a bicyclic heterocycle. Indole plays a crucial role in organic chemistry because of its unique electronic properties and ability to provide substantial stability. Indole, which was found in the 1866 by Adolf von Baeyer, gets its name from the combination of "indigo" and "oleum," signifying its connection to indigo dye.[27] It serves as a vital structural motif in many natural products and biologically active compounds, including tryptophan, an essential amino acid, serotonin, a neurotransmitter, and melatonin, a hormone.[28] Several alkaloids contain indole derivatives, which are known for their diverse biological activities.[29] The presence of aromaticity in both rings adds to the overall stability of the indole structure[30], making it an ideal choice for different chemical reactions. Indole derivatives are widely used in medicinal chemistry, showcasing their versatility. Due to their extensive range of biological activities, such as anti-cancer, antiviral, anti-inflammatory, antimicrobial, anti-HIV, and anticholinesterase properties, these compounds serve as vital building blocks in many drugs.[31]

Methods of Synthesis of Indole

Several synthetic routes have been developed for the construction of the indole ring system, each offering unique advantages:

(1) Fischer Indole Synthesis:

The Fischer indole synthesis is a chemical process that generates the aromatic heterocycle indole **(Figure 4)** using a (substituted) phenyl hydrazine, an aldehyde or ketone, and acidic conditions. Brønsted acids like HCl, H_2SO_4, polyphosphoric acid, and p-toluene sulfonic acid, or Lewis's acids such as boron trifluoride, zinc chloride, and aluminium chloride, can catalyse this reaction.[32]

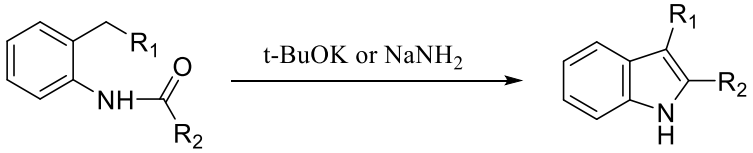


Fischer Indole Synthesis

Figure 4

(2) Madelung Synthesis:

By utilizing strong base at high temperature, the Madelung synthesis generates indoles through the intramolecular cyclization of N-phenylamides. Commonly used reaction conditions involve the use of sodium or potassium alkoxide as a base in hexane or tetrahydrofuran solvents, at temperatures ranging from 200 to 400 °C. The synthesis process also involves a hydrolysis step. The significance of the Madelung synthesis lies in its ability to generate indoles through a base-catalysed thermal cyclization of N-acyl-o-toluidine.[33]



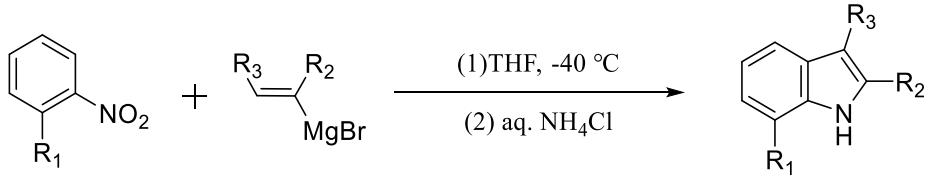
Madelung Indole Synthesis

Figure 5

(3) Bartoli Indole Synthesis:

In the Bartoli indole synthesis, ortho-substituted nitroarenes and nitroso arenes react with vinyl Grignard reagents, resulting in the formation of substituted indoles.

The reaction typically fails without ortho substitution next to the nitro group, and larger ortho substituents usually lead to better reaction yields. The steric hindrance caused by the ortho group plays a role in enabling the required [3,3]-sigma tropic rearrangement for product formation. When reacting with nitroarenes, it is necessary to use three equivalents of the vinyl Grignard reagent for full conversion, whereas only two equivalents are needed for nitroso arenes.[34,35]



Bartoli Indole Synthesis

Figure 6

Biological Importance of Indole

Indole and its derivatives are essential for various biological processes. For example, tryptophan, which is an essential amino acid, acts as a precursor to serotonin and melatonin. These two compounds are crucial for regulating mood, sleep, and various physiological processes.[36] Moreover, IAA, also known as indole-3-acetic acid, plays a vital role in plant growth and development.[37] Indole derivatives serve as important scaffolds in drug discovery because of their wide range of pharmacological activities. These compounds have gained recognition for their notable applications, such as serving as anticancer agents like vincristine, as well as playing a role in antiviral drug such as umifenovir.[38,39] The presence of indole-based compounds is vital in the development of drugs that specifically target the central nervous system, including antidepressants and antipsychotics.[40,41] In addition to their biological importance, indole derivatives are used in different industrial sectors for synthesizing dyes, perfumes, and agricultural chemicals.

1.4.2 Benzothiazole: Structure, Synthesis, and Significance in biological term

Benzothiazole is a bicyclic heterocycle that combines a benzene ring with a thiazole ring. The thiazole ring consists of one nitrogen atom and one sulfur atom. The distinct chemical properties of benzothiazole, resulting from its unique structure, make it a valuable

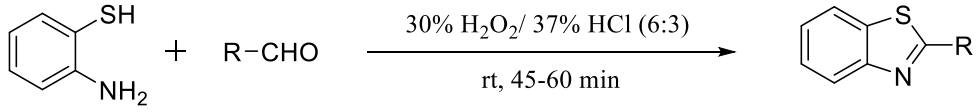
and versatile compound in various fields, with a particular focus on medicinal and materials chemistry.[42]

Methods of Synthesis of Benzothiazole

A wide range of reactions are available for the production of the benzothiazole moiety, from which some reactions described here for synthesizing benzothiazole. Each method has its own specific advantages, depending on the desired derivatives and the required conditions.

(1) Condensation of 2-Aminothiophenol with Aldehydes:

Guo *et al*. synthesized a range of benzothiazole compounds with various substituents by condensing 2-aminothiophenol with aldehydes and their derivatives.[43] They used a catalyst mixture of H_2O_2/HCl in ethanol at room temperature for one hour **(Figure 7)**. The most effective ratio for the coupling in the comparative study was determined to be 1:1:6:3 of 2-aminothiophenol, aromatic aldehyde, H_2O_2, and HCl. Additionally, the method described here enables the synthesis of benzothiazoles with high yields using aldehydes that have substituents either electron donating or withdrawing. This procedure offers several advantages, including a quick reaction time, easy and fast isolation of the products, and excellent yields.

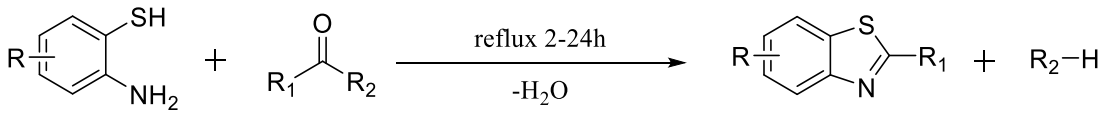


Condensation with aldehyde

Figure 7

(2) Condensation of 2-Aminothiophenol with Ketones:

Elderfield and colleagues studied the reaction between ortho-aminobenzenethiol and its derivatives with various ketones, resulting in the formation of 2,2-disubstituted benzothiazolines (Figure 8).[44] Notably, when benzothiazoline is subjected to pyrolysis under reflux conditions, it can undergo a reaction that leads to the production of a 2-substituted benzothiazole while simultaneously eliminating hydrocarbons.

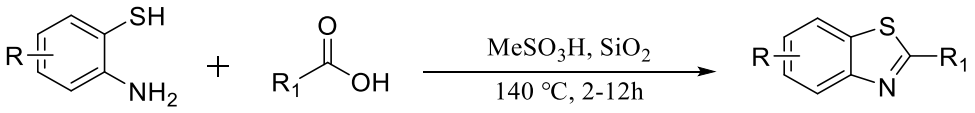


Condensation with keton

Figure 8

(3) Condensation of 2-Aminobenzenethiol with Acids:

A high-yielding method for synthesizing a series of 2-substituted benzothiazole compounds was investigated by Sharghi *et al*.[45] The approach involved condensing 2-aminobenzenethiol with various aliphatic or aromatic carboxylic acids, as shown in **Figure 9**. A novel combination of methane sulfonic acid and silica gel was created as a fast medium for the condensation reaction of aromatic and aliphatic carboxylic acids with 2-aminothiophenol, resulting in the synthesis of 2-substituted benzothiazoles. This method offers benefits such as simplicity, use of diverse carboxylic acids, and easy handling of reaction conditions.

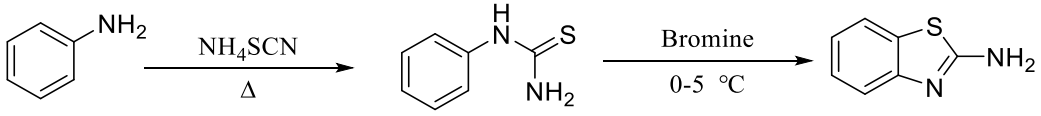


Condensation with acid

Figure 9

(4) Cyclization reaction of phenyl thiourea:

Starting from aniline, R. Chikhale and co-workers successfully synthesized 2-aminobenzothiazole **(Figure 10)**.[46] When aniline is combined with potassium thiocyanate and hydrochloric acid, it results in the production of phenyl thiourea with a good yield. Under controlled conditions of temperature (0-5 ℃) and in the presence of bromine, phenyl thiourea can undergo cyclisation, leading to the production of 2-aminobenzothiazole. The application of this method allows for the production of different benzothiazole analogues by utilizing various aniline derivatives.



Cyclization reaction of phenyl thiourea using bromine

Figure 10

Biological Significance of Benzothiazole

The broad spectrum of biological activities exhibited by benzothiazole and its derivatives highlights their significance as scaffolds in the discovery and development of drugs. Their distinctive composition enables them to engage with a range of biological targets, resulting in a wide range of pharmacological effects. Benzothiazole derivatives have significant anticancer activity by interfering with DNA replication and protein synthesis, making them potential chemotherapeutic agents.[47] Strong antimicrobial properties are found in many derivatives of benzothiazole, which effectively combat various bacterial, fungal, and viral infections.[48,49] Additionally, these compounds exhibit anti-inflammatory effects by inhibiting the synthesis of pro-inflammatory cytokines and mediators, thus reducing inflammation.[50] Activity on the central nervous system, including anticonvulsant and antidepressant effects, is exhibited by certain benzothiazole compounds.[51,52] This makes them potential candidates to treat neurological disorders. The broad biological significance of benzothiazole emphasizes its importance in the creation of novel therapeutic agents for diverse diseases and conditions.

1.4.3 Triazole: Structure, Synthesis, and Medicinal importance

Triazole is a heterocyclic compound comprising a five-membered ring with three nitrogen atoms. The significance of triazoles lies in their diverse biological activities and robust chemical properties, making them a crucial class of compounds in medicinal and industrial chemistry. Triazoles can be categorized into two primary types, namely 1,2,3-triazole and 1,2,4-triazole, based on the positions of nitrogen atoms in the ring. Each type displays distinct characteristics and has diverse applications.

[A] 1,2,3-Triazole:

The structure of 1,2,3-Triazole is characterized by a five-membered ring and three nitrogen atoms positioned at the 1st, 2nd, and 3rd positions. The positioning of nitrogen atoms gives rise to particular electronic and structural attributes in the molecule. Due to the aromatic nature of the 1,2,3-triazole ring, it exhibits stability and resistance against

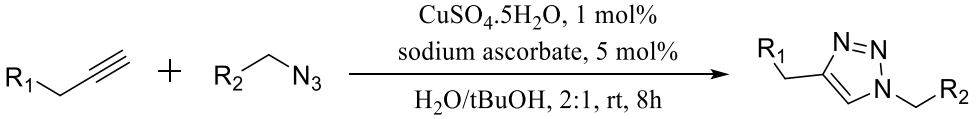
different chemical reactions. The triazole ring possesses a high electron density, which grants it exceptional versatility in a wide range of biological and chemical applications.

Synthesis methods of 1,2,3-Triazole

There are multiple synthetic methods available for the synthesis of 1,2,3-Triazole, and some of these methods involve the use of sodium azido, trimethylsilyl azido, alkyl/aryl azido, hydrazone, sulphonamide, hydrazine, and diazo-compounds as raw materials to provide nitrogen atoms for the creation of 1,2,3-Triazole.

(1) Synthesis of 1,2,3-triazole using substituted azide as a nitrogen source:

According to the findings presented by Rostovtsev Vsevolod V *et al*., when a mixture of substituted propargyl and azide derivatives was subjected to a reaction with 5 mol% of sodium ascorbate and 1 mol% of copper sulphate in a 2:1 ratio of water to tertbutyl alcohol, it led to the successful synthesis of a 1,4-disubstituted triazole compound.[53] Remarkably, this reaction exhibited exceptional efficiency and regioselectivity, resulting in a yield of 91% after being stirred for a duration of eight hours at room temperature. The simplicity, mild reaction conditions, and high yield of the desired triazole product are the key factors that make this method highly favoured.



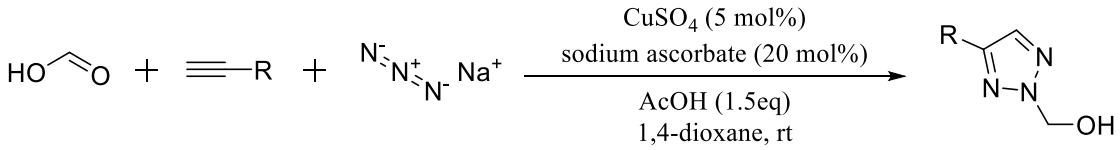
1,2,3-Triazole synthesis using azide derivtives as nitrogen source

Figure 11

(2) Sodium azide as nitrogen source for preparation of 1,2,3-triazole:

Kalisiak and the coauthor demonstrated a highly efficient approach to synthesize 2-hydroxymethyl-2H-1,2,3-triazoles.[54] This involved a three-component synthetic pathway, where terminal alkyne, NaN_3, and formaldehyde were reacted together in the presence of a $CuSO_4$, in one pot. By employing copper as a catalyst, equal amounts of acetic acid were added, and 1,4-dioxane was used as the solvent. The range of yields at room temperature was between 67% and 95%. The ability to work

with different substrates and functional groups makes it possible to create a wide range of target products.

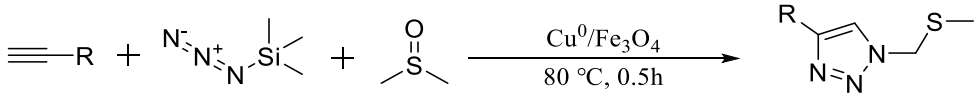


1,2,3-Triazole synthesis using sodium azide as nitrogen source

Figure 12

(3) Use of trimethylsilyl azide as source of nitrogen for synthesis 1,2,3-triazole:

A novel approach to synthesizing 1,2,3-triazole was introduced by Kumar Tiwari *et al*., involving the reaction of alkynes, $TMSN_3$, and dimethyl sulfoxide.[55] In this method, nano-Cu^0/Fe_3O_4 was used as an efficient catalyst, enabling the regioselective synthesis of 1,2,3-triazoles with a high yield of 96% in a one-pot tandem reaction. The reaction demonstrates compatibility with various alkynes, including aromatic, aliphatic, heteroaromatic, and poly aromatic compounds. The wide range of substrates and simple operation offered a convenient and appealing route for triazole synthesis. By using magnetic retrieval, the catalyst in the reaction could be reused six times, experiencing no notable loss in its activity.

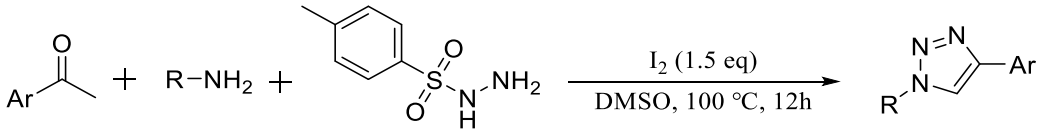


1,2,3-Triazole synthesis using trimethylsilyl azide as nitrogen source

Figure 13

(4) The role of hydrazine in synthesizing 1,2,3-triazole as a nitrogen source:

Zhang *et al.* demonstrated the nonmetal-mediated synthesis of 1,4-disubstituted-1,2,3-triazole via a three-component reaction involving aniline, aromatic ketone, and tosyl hydrazide.[56] This method allows for preparing different 1,4-disubstituted-1,2,3-triazoles, achieving high yields (75%–92%) through the successive formation of C-N and N-N bonds. The reaction conditions were mild, obviating the need for metal reagents and azide to incorporate nitrogen atoms.



1,2,3-Triazole synthesis using hydrazine as nitrogen source

Figure 14

The Role of 1,2,3-Triazole in Biological Contexts

The extensive biological activities displayed by 1,2,3-triazole derivatives have positioned them as valuable components in the realm of pharmaceutical research and development. Their notable qualities include antifungal, antibacterial, and antiviral properties, leading to the development of many drugs based on the 1,2,3-triazole scaffold that are in clinical use or in the process of being developed.[57,58] The 1,2,3-triazole moiety can be found in popular antifungal agents, such as fluconazole and itraconazole.[59] Furthermore, the potential anticancer and anti-inflammatory properties of 1,2,3-triazoles are being investigated, making them promising contenders for new therapeutic drugs.[60,61] Incorporating the 1,2,3-triazole ring into bio-conjugates and molecular probes enhances the development of novel diagnostic and therapeutic tools because of its robustness.

[B] 1,2,4-Triazole:

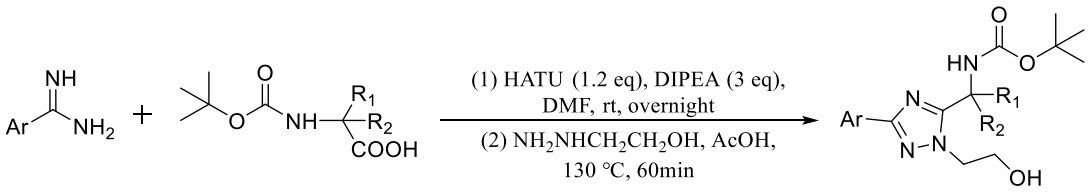
Another example of a five-membered ring with nitrogen atoms at positions 1, 2, and 4 is 1,2,4-triazole. This arrangement, similar to 1,2,3-triazole, produces an aromatic ring system, which plays a crucial role in the compound's stability and exceptional electronic properties. The positioning of nitrogen atoms in 1,2,4-triazole plays a crucial role in determining its hydrogen bonding capabilities and overall reactivity, setting it apart from the 1,2,3-triazole isomer.

Techniques for the production of 1,2,4-Triazole

Amidines, imidates, amidrazones, aryl diazoniums, and hydrazones are the main synthesis methods that can be used as raw materials. These methods provide nitrogen atoms which are essential for synthesizing 1,2,4-triazole derivatives.

(1) Synthesis of 1,2,4-triazole by use of amidine:

The reactivity of nucleophilic nitrogen atoms in amidine made it a popular choice as an organic catalyst and ligand for creating nitrogen-carbon bonds. A highly efficient technique has been developed by Castanedo *et al.* using a one-pot, two-step process to produce 1,3,5-trisubstituted-1,2,4-triazole with good yield.[62] The sequence commenced by the in-situ formation of an amide through the reaction between a carboxylic acid and amidine. Aniline underwent a subsequent reaction with a monosubstituted hydrazine, resulting in the formation of a cyclized trisubstituted triazole. High regioselectivity and good functional group tolerance are among the advantages of employing this method.

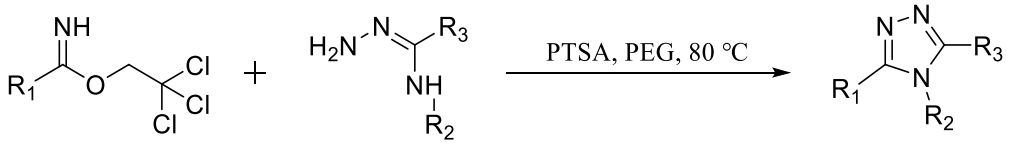


1,3,5-Trisubstituted 1,2,4-triazole synthesis using amidine

Figure 15

(2) Imidates as a nitrogen source for preparing 1,2,4-triazole

A series of 3,4,5-trisubstituted-1,2,4-triazoles were synthesized by Mangarao et al. using 2,2,2-trichloroethyl imidate, polyethylene glycol (PEG) as a solvent, and p-Toluenesulfonic acid (PTSA) as the catalyst under mild conditions.[63] By employing PTSA as the catalyst and using PEG as the medium, a facile, effective, and eco-friendly method was developed for synthesizing 1,2,4-triazole from 2,2,2-trichloroethyl imidate. The reactions proceeded smoothly under mild conditions, leading to the formation of the desired 1,2,4-triazoles with excellent yields.



1,2,4-triazole synthesis utilizing imidate for source of nitrogen

Figure 16

(3) Utilization of amidrazone for synthesis 1,2,4-triazole:

Vidavalur *et al*. demonstrated the synthesis of 3,4,5-trisubstituted-1,2,4-triazole in polyethylene glycol (PEG) through the employment of ceric ammonium nitrate

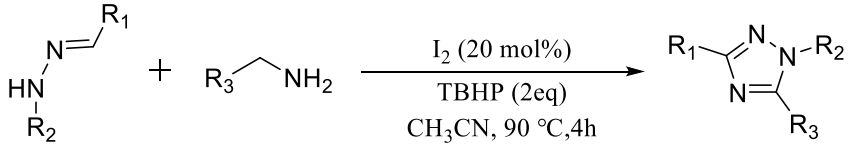
(CAN) by oxidative cyclization, yielding highly favourable outcomes.[64] In this reaction, CAN served as both an oxidant and a Lewis acid. Benzaldehyde was introduced to form a diazizone intermediate after the activation of an amino nitrogen atom. Lastly, the 1-(sulfur)-phosphonated 5-amino-1,2,4-triazole framework was synthesized via the oxidation cyclization reaction. The operational procedures for the reaction conditions were relatively simple.

Amidrazone mediated synthesis of 1,2,4-triazole

Figure 17

(4) Using amidrazone for the synthesis of 1,2,4-triazole:

Hydrazones were a favoured choice in many approaches to synthesizing fused 1,2,4-triazole as they served as effective precursors for nitrogen heterocycles. The presence of highly reactive nitrogen pairs and SP-carbon atoms are notable characteristics of these compounds. Triazoles can be synthesized by the reaction of hydrazone with different amines. A synthetic method was developed by Chen et al. that converts hydrazone to 1,2,4-triazole with high yield using amines and I_2/TBHP.[65] The synthesis of multiple compounds was achieved by incorporating a diverse range of functional groups into hydrazone and amine fragments. The method is characterized by easily accessible raw materials, simple operating conditions, and a wide range of substrates.



Hydrazone as source of nitrogen for synthesis of 1,2,4-triazole

Figure 18

Medicinal applications of 1,2,4-Triazole

1,2,4-Triazole derivatives have gained widespread recognition for their therapeutic potential, specifically as antibacterial, antifungal, and anticancer agents.[66,67,68] Drugs like

voriconazole and posaconazole, which are employed in the treatment of fungal infections, consist of the 1,2,4-triazole group.[69,70] Moreover, the antiviral properties of 1,2,4-triazoles are being studied, making them potential candidates for the development of antiviral treatments.[71] Apart from their antimicrobial effects, 1,2,4-triazole derivatives are being investigated for their potential applications in treating neurological disorders, inflammation, and for protection of blood-brain barrier.[72] The 1,2,4-triazole scaffold exhibits remarkable versatility, facilitating extensive chemical modification and enabling the development of molecules possessing improved biological activity and specificity.

1.5 Advanced Computational Techniques in Medicinal Chemistry

Computational chemistry is a multidisciplinary area that employs computer simulations to address chemical issues, combining concepts from chemistry, physics, and mathematics to simulate and forecast the behaviour of molecules and chemical reactions.[73] The study of heterocyclic compounds, which exhibit a wide range of biological activities and possess structural diversity, is an important area in chemical research where computational methods play a crucial role. Computational techniques, such as Quantitative Structure-Activity Relationship (QSAR), molecular docking, and molecular dynamics simulations, allow researchers to explore the properties and interactions of heterocyclic compounds at the molecular level, providing insights that complement experimental data. Through the utilization of computational chemistry, our research aims to harness its capabilities in predicting the behaviour of novel heterocyclic structures, identifying promising drug candidates, and comprehending the mechanisms of action of these compounds. This plays a pivotal role in expediting the discovery and development of new therapeutic agents.

1.5.1 Quantitative Structure-Activity Relationship (QSAR)

QSAR, which stands for Quantitative Structure-Activity Relationship, is a computational technique that utilizes statistical methods to establish a relationship between the chemical structure of compounds and their biological activities.[74] Through the analysis of the relationship between molecular descriptors (quantitative representations of molecular properties) and biological activity data, QSAR models can accurately predict the

activity of novel compounds, provide guidance in the design of new molecules, and optimize those that already exist.[75]

Principles and mechanism

QSAR comprises various crucial steps that can be described as follows[76]:

(1) **Data Collection:** Gathering a dataset of chemical compounds that are known to exhibit specific biological activities.
(2) **Descriptor Calculation:** Calculating molecular descriptors that provide quantitative representations of diverse compound properties, including electronic, hydrophobic, and steric features.
(3) **Model Building:** Developing a model that establishes a correlation between molecular descriptors and biological activity using statistical techniques, such as regression analysis or machine learning.
(4) **Validation:** To ensure the model's reliability, a separate set of compounds (validation set) is used to evaluate its predictive power.
(5) **Prediction:** Applying the approved model to make predictions about the activity of untested or novel compounds.

Applications

QSAR finds multiple significant applications in drug discovery and development:

- **Drug Design:** By analysing the chemical structure, QSAR models can predict the biological activity of drug candidates.[77]
- **Lead Optimization:** Lead compounds can be optimized through QSAR, which identifies structural modifications to improve activity and decrease toxicity.[78]
- **Environmental Chemistry:** QSAR helps forecast the environmental effects and toxicity of chemicals, assisting in regulatory evaluations.[79]

Challenges and Limitations

Although QSAR is helpful, it has its limitations, such as:

- **Quality of Data:** The accuracy of QSAR models is contingent upon the quantity and quality of data employed in their creation.[80]

- **Complexity of Biological Systems:** It is possible for QSAR models to fall short in capturing the full complexity of biological interactions, resulting in potential inaccuracies.[81]
- **Applicability Domain:** The reliability of QSAR models is limited to the chemical space covered by the training data.[82]

1.5.2 Molecular Docking

Molecular docking, a computational technique, is employed to predict the preferred orientation of one molecule to a second molecule. This prediction is made with the purpose of forming a stable complex when the two molecules are bound to each other. In drug discovery and design, this method is widely employed to anticipate the interaction between potential drug candidates and their biological targets, including proteins or nucleic acids.[83]

Principles and Mechanism

To perform molecular docking, the following crucial steps are involved[83]:

(1) Preparation of the Target and Ligand: The target (usually a protein) and the ligand (a potential drug molecule) are both prepared in three-dimensional structures. To ensure the desired outcome, it may require performing tasks such as cleaning up the structures, adding hydrogen atoms, and verifying the protonation states.

(2) Binding Site Identification: Identifying the binding site on the target, where the ligand is expected to bind, is crucial. The information can be obtained through either experimental data or by making predictions using computational tools.

(3) Docking Simulation: Through the use of algorithms, the ligand is effectively inserted into the binding site of the target, with various orientations and conformations being explored. The evaluation of ligand-target interactions often involves the use of scoring functions, which consider various factors, including hydrogen bonding, hydrophobic interactions, and electrostatic interactions.

(4) Scoring and Ranking: The scoring of docking results predicts the binding strength between the ligand and the target. The poses with the highest scores are examined to identify potential candidates for additional research.

Applications

The applications of molecular docking extend to drug discovery and several other fields:

- **Drug Design:** Docking aids in the identification and optimization of potential drug candidates by predicting how they bind to biological targets.[84]
- **Protein-Protein Interactions:** Docking serves as a valuable tool in examining protein interactions, enabling a better understanding of biological pathways and mechanisms.[85]
- **Virtual Screening:** The drug discovery process can be significantly sped up by virtually screening extensive compound libraries to identify potential binders to a target.[86]

Challenges and Limitations

While molecular docking is helpful, it also has limitations, such as[87]:

- **Accuracy of Scoring Functions:** Simplifications in the models used by scoring functions can lead to inaccurate predictions of binding affinities.
- **Flexibility of Molecules:** The ligand and the target are commonly considered as inflexible entities, which can cannot account for significant structural changes during the binding process.

1.5.3 Molecular Dynamics Simulation

The process of molecular dynamics (MD) simulation involves using computer algorithms to simulate the movement of atoms and molecules in a physical system. The detailed behaviour of molecular systems, including proteins, nucleic acids, membranes, and small molecules, can be extensively studied through MD simulations. These simulations involve solving Newton's equations of motion for a system of interacting particles.[88]

Principles and Mechanism

The following steps are involved in performing molecular dynamics simulations[89]:

(1) **Initialization:** The starting positions and velocities of the atoms in the system are defined. This task often entails the utilization of experimental structures sourced from databases, such as the Protein Data Bank (PDB).

(2) **Force Field Application:** The system is subjected to a force field that includes the potential energy function and parameters for atomic interactions, such as bond lengths, angles, and van der Waals forces.

(3) **Energy Minimization:** To eliminate steric clashes or unfavourable atomic interactions, the system undergoes energy minimization before starting the MD simulation. By implementing this method, the system's potential energy is lowered to a local minimum, ensuring a stable starting point for the simulation.

(4) **Equilibration:** To achieve the desired temperature and pressure, the system is adjusted to a state of equilibrium. Usually, this process is divided into two steps.

- **NVT Run (Canonical Ensemble):** The system remains balanced with a constant number of particles, volume, and temperature. Throughout this run, the system's temperature is carefully regulated using a thermostat, allowing it to steadily reach the desired temperature with minimal deviations.
- **NPT Run (Isothermal-Isobaric Ensemble):** The system reaches equilibrium with a constant number of particles, pressure, and temperature. To achieve the desired pressure and temperature conditions, a barostat is employed in this run to regulate the system's pressure and volume. This step is of utmost importance for simulating systems under realistic physiological conditions.

(5) **Production MD Simulation:** The production MD simulation takes place after the system has reached equilibrium. Numerical techniques, like the Verlet algorithm or the leapfrog algorithm, are employed to integrate Newton's equations of motion over time. This step calculates the positions and velocities of the atoms at every time step, facilitating the dynamic evolution of the system.

(6) **Simulation and Data Collection:** Throughout a predetermined timeframe, the simulation gathers information on the positions, velocities, and energies of atoms. The collected data can be employed to analyse different aspects of the system, such as changes in shape, energy interactions, and dynamic behaviours.

Applications

The field of chemistry and biology extensively utilizes molecular dynamics simulations due to their wide range of applications:

- **Understanding Protein Dynamics:** Molecular dynamics simulations offer valuable information about the structural changes and dynamic behaviour of proteins, which play a vital role in their functionality.[90]
- **Drug Binding Mechanisms:** Simulations are useful in understanding the interaction between drugs and their targets, including how they bind and cause conformational changes.[91]
- **Biomolecular Interactions:** The study of biomolecular interactions, such as protein-ligand, protein-protein, and protein-DNA interactions, can be conducted through molecular dynamics (MD).[92]
- **Material Properties:** The use of MD simulations allows for the exploration of material properties at the molecular level, spanning polymers, nanomaterials, and crystals.[93]

Challenges and Limitations

Molecular dynamics simulations encounter various challenges such as[94]:

- **Computational Cost:** The computational demands of MD simulations are high, particularly when dealing with large systems and long simulation times.
- **Accuracy of Force Fields:** The quality of the simulation is determined by the force field utilized. While modern force fields are highly developed, accurately representing certain interactions may still pose challenges.
- **Time Scale Limitations:** Current computational resources often make it impractical to simulate processes that take a long time, such as milliseconds to seconds, which in turn limits the study of slow processes.

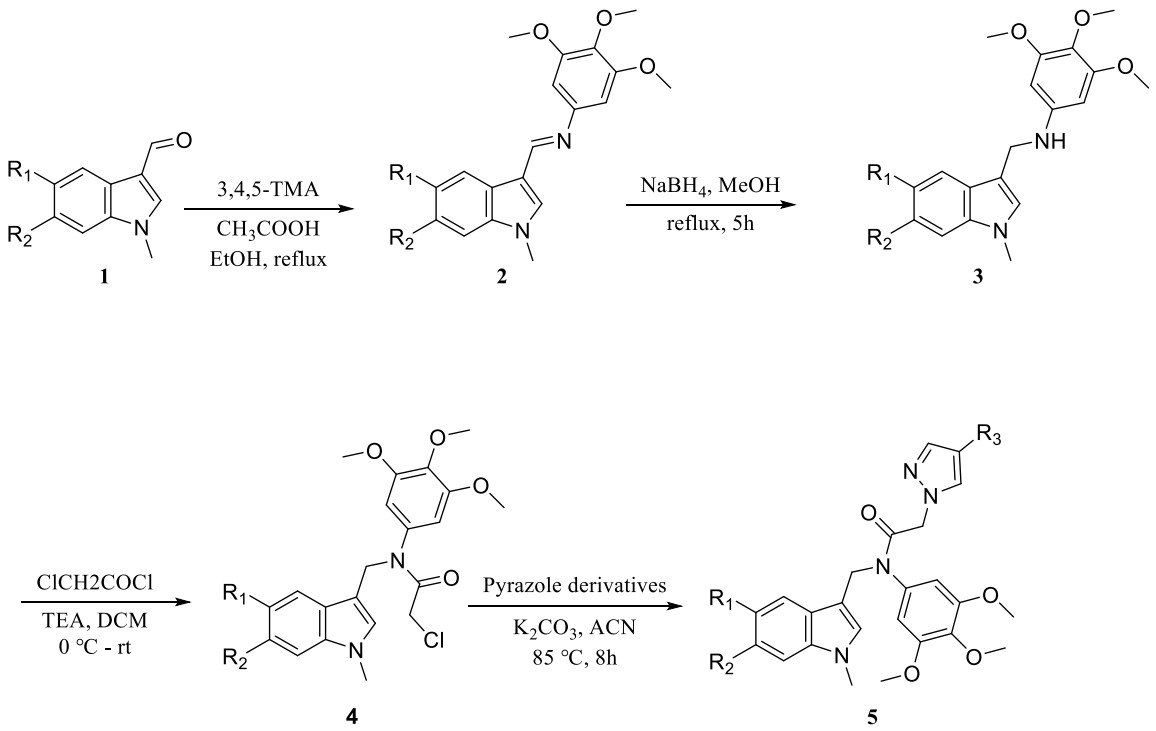
1.6 Literature review

This literature review aims to summarize research on heterocyclic compounds, particularly indole, benzothiazole, 1,2,4-triazole, and 1,2,3-triazole. The review will explore indole's synthesis pathways and biological significance. Following this, we will

examine benzothiazole, focusing on its synthetic strategies and pharmacological applications. The review will cover the synthesis and biological activities of 1,2,4-triazole and 1,2,3-triazole separately. This literature review will highlight the relevance of heterocyclic compounds in ongoing research and their potential for future applications.

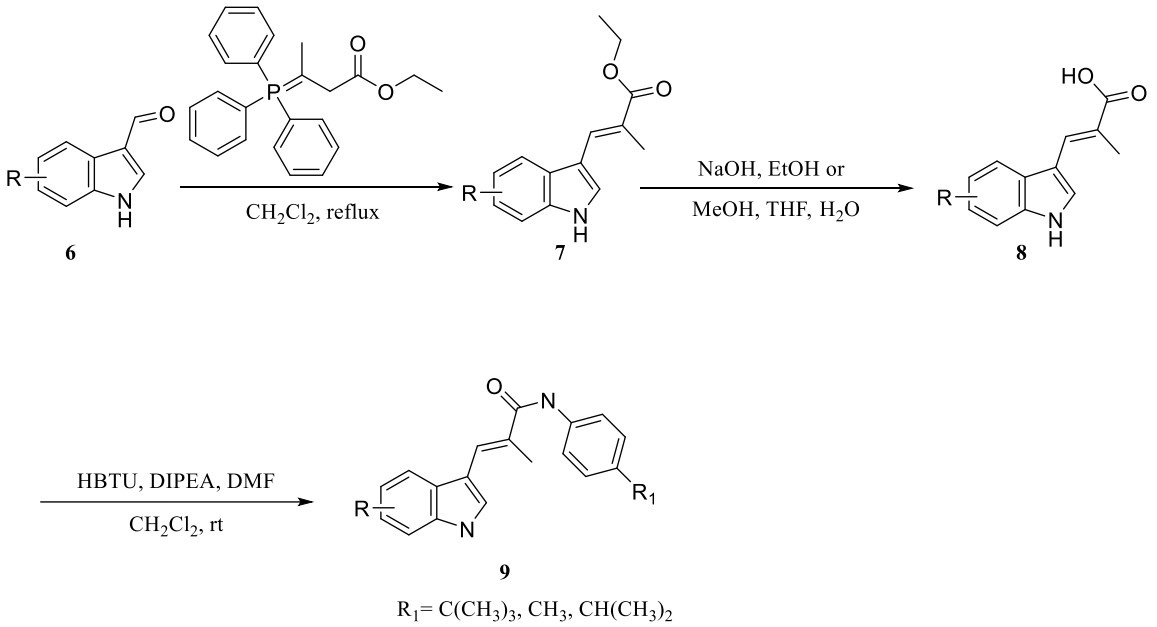
1.6.1 Comprehensive Approaches to the Synthesis and Biological Evaluation of Substituted Indole Scaffolds

The provided reaction **(Scheme 1.1)** outlines the synthesis of pyrazole derivatives incorporating an indole scaffold work by A. Ren *et al.*[95] In the first step, the indole compound **1** is reacted with 3,4,5- trimethoxy aniline (TMA) in the presence of Ac. acid at reflux resulting in the formation of compound **2**. The compound **2** is reduced using sodium borohydride ($NaBH_4$) in methanol, which selectively reduces the imine group, leading to the formation of the corresponding amine compound **3**. After reduction, the chloroacetyl chloride in the presence of triethylamine (TEA) and dichloromethane (DCM) at 0°C, formed the acylated indole intermediate compound **4**. In the final step, compound **4** is reacted with a pyrazole derivative using K_2CO_3 in ACN, yielding the target pyrazole-indole compound **5.** These compounds have shown remarkable effectiveness in inhibiting tumour growth in different cell lines. The inhibitory effects of a chloro derivative were potent against HeLa, MCF-7, and HT-29 tumor cell lines, with IC_{50} values at low micromolar levels. Additionally, this compound triggered programmed cell death, halted cell cycle progression, and prevented tubulin assembly, which aligns with colchicine's mode of action. It shows potential as a tubulin polymerization inhibitor, making it a promising candidate for further development.



Scheme 1.1

G. Jin *et al.*[96] detailed a method for synthesizing a series of indole derivatives **(Scheme 1.2)** aimed at inhibiting HCV replication. Initially, compound **6** underwent a reaction with a (Carbethoxyethylidene)triphenyl phosphorane under reflux to produce compound **7**. This intermediate was subsequently hydrolyzed using NaOH in a mixture of EtOH or THF, MeOH and water, resulting in compound **8** with a terminal acid group. Further reactions involved coupling with alkyl-substituted anilines to produce compound **9**, highlighting variations in N-substitution patterns. Biological evaluation of these compounds, including a cyano (CN) substituted derivative, revealed promising antiviral activities against HCV. G. Jin *et al.* discovered that this CN-substituted derivative displayed potent inhibition of HCV replication with an EC50 of 1.1 μM and an EC90 of 2.1 μM, while maintaining a CC50 of 61.8 μM. It effectively reduced viral RNA and protein levels in a dose- and time-dependent manner. Furthermore, resistance mapping and enzymatic studies identified HCV NS5B RNA polymerase as the target of this derivative, underscoring its potential as a lead compound for developing new HCV therapies.



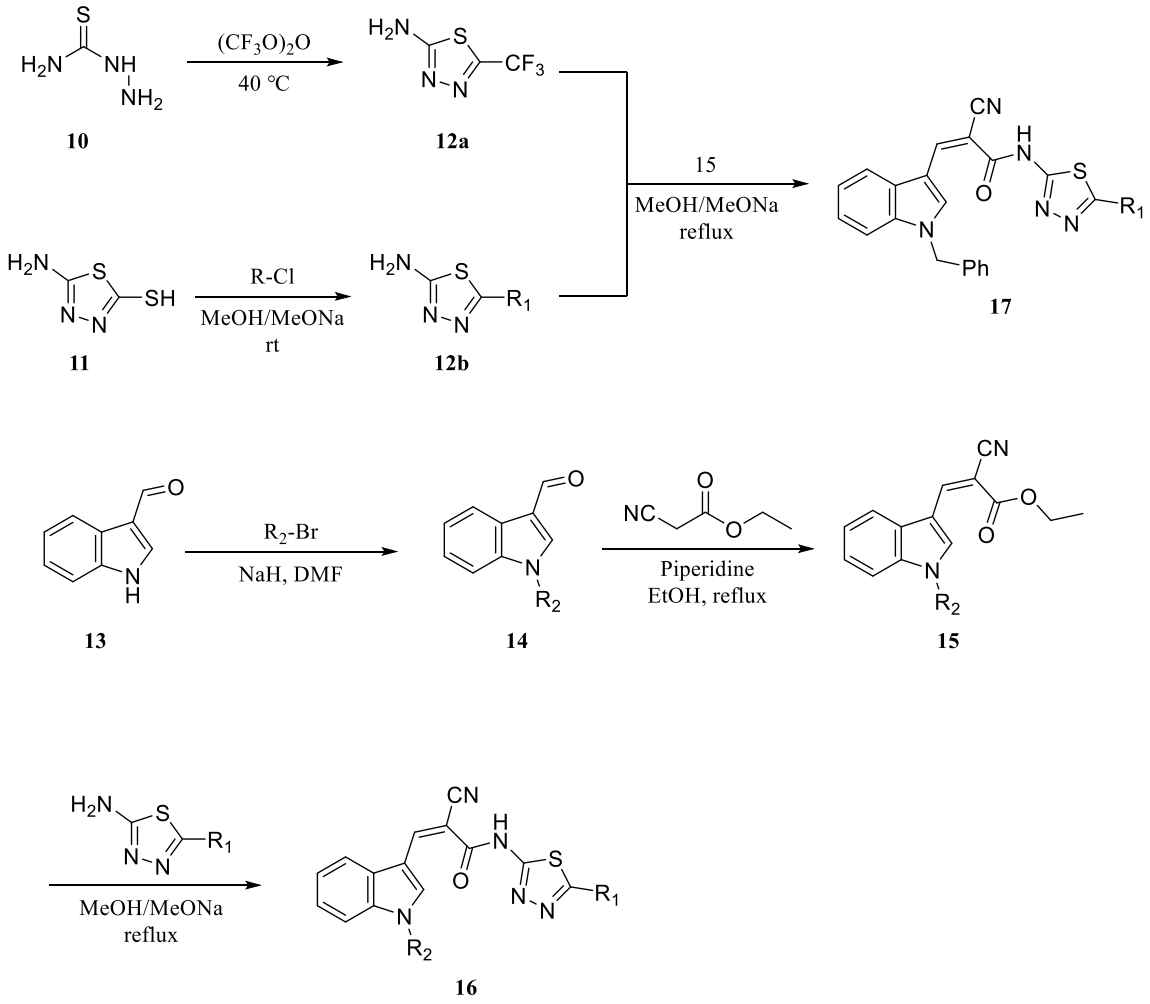
Scheme 1.2

H. Liu *et al.*[97] describe the synthesis of thiadiazol acrylamide analogues **16** and **17**, as outlined in **Scheme 1.3**. The amino thiadiazoles **12a** and **12b** were efficiently synthesized through cyclo dehydration involving thiosemicarbazide **10** and trifluoroacetic anhydride for compound **12a**, or by alkylating amino thiadiazoles **11** under basic conditions for compounds **12b**. Additionally, various N-protected indolaldehydes **14** were condensed with ethyl cyanoacetate to produce fragments **15**. The subsequent refluxing of these two sets of intermediates **(12a, 12b and 15)** in a MeONa/MeOH mixture facilitated the formation of the desired thiadiazol acetamides **16** and **17**.

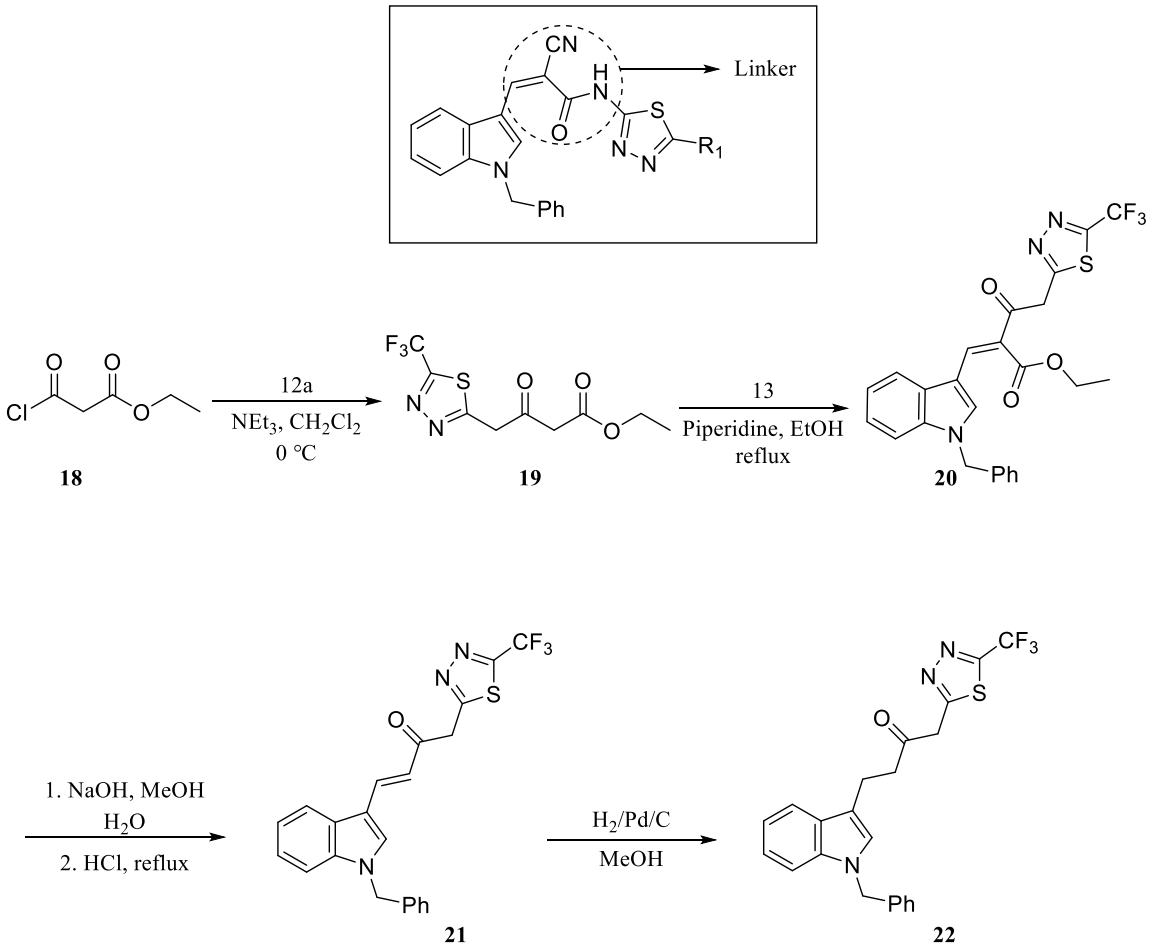
In their investigation of the impact of the linker in thiadiazoloacrylamides on biological activity, H. Liu *et al.* conducted additional chemical modifications. The synthesis of compounds **21** and **22** is depicted in Synthetic **Scheme 1.4**. The process began with the intermediate **12a**, which was coupled with acryl chlorides **18**. This was followed by a dehydration reaction with N-protected indolaldehyde **13** to produce thiadiazoloacrylamide **20**. The target compound **21** was then synthesized through the decarboxylation of compound **20**. In a subsequent step, compound **21** was hydrogenated to produce compound **22**.

Biological evaluation revealed that among the synthesized compounds, the one with a fluoro substituent at the R_2 position exhibited the most potent activity, with an IC_{50}

of 2.24 µM in in vitro DENV2 NS2B-NS3pro assays. Due to concerns about the potential toxicity associated with the strong electro-affinity of the linker, further chemical optimization was undertaken to modify the enamide fragment. This led to the synthesis of the denitrile product 21 and its hydrogenated olefin counterpart 22. Contrary to expectations, both of these compounds were found to be inactive, suggesting that the enamide moiety plays a crucial role in the inhibitory efficacy against NS2B/NS3pro.



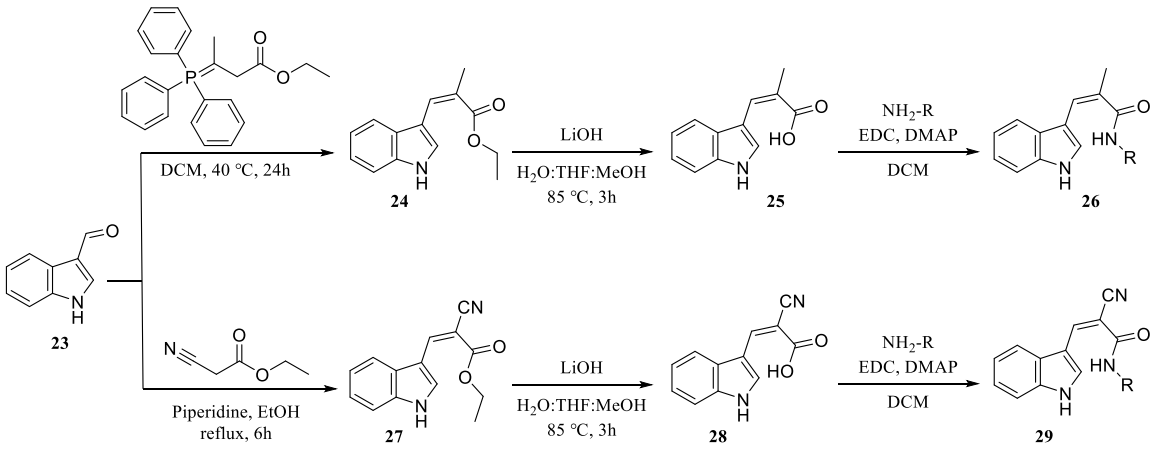
Scheme 1.3



Scheme 1.4

M. Hawash *et al.*[98] described the synthesis of indole-2-methyl acrylamide analogs, utilizing indole-3-carbaldehyde **23** as the starting point for a series of reactions outlined in **Scheme 1.5**. Initially, indole-3-carbaldehyde was subjected to a Wittig reaction with (1-carbethoxyethylidene) triphenyl phosphorane, resulting in the formation of the ethyl ester **24**. Subsequently, a Knoevenagel condensation of the same aldehyde **23** with ethyl cyanoacetate led to the creation of Ethyl (E)-2-cyano-3-(1H-indol-3-yl) acrylate **27**. Both ester derivatives, **24** and **27**, were then hydrolyzed using lithium hydroxide (LiOH) in a mixture of THF and methanol, producing key intermediates **25** and **28**, respectively. The next step involved converting these intermediates into amide derivatives **26** and **29** through a reaction with the appropriate anilines, facilitated by the use of EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and DMAP (4-Dimethylaminopyridine) in DCM.

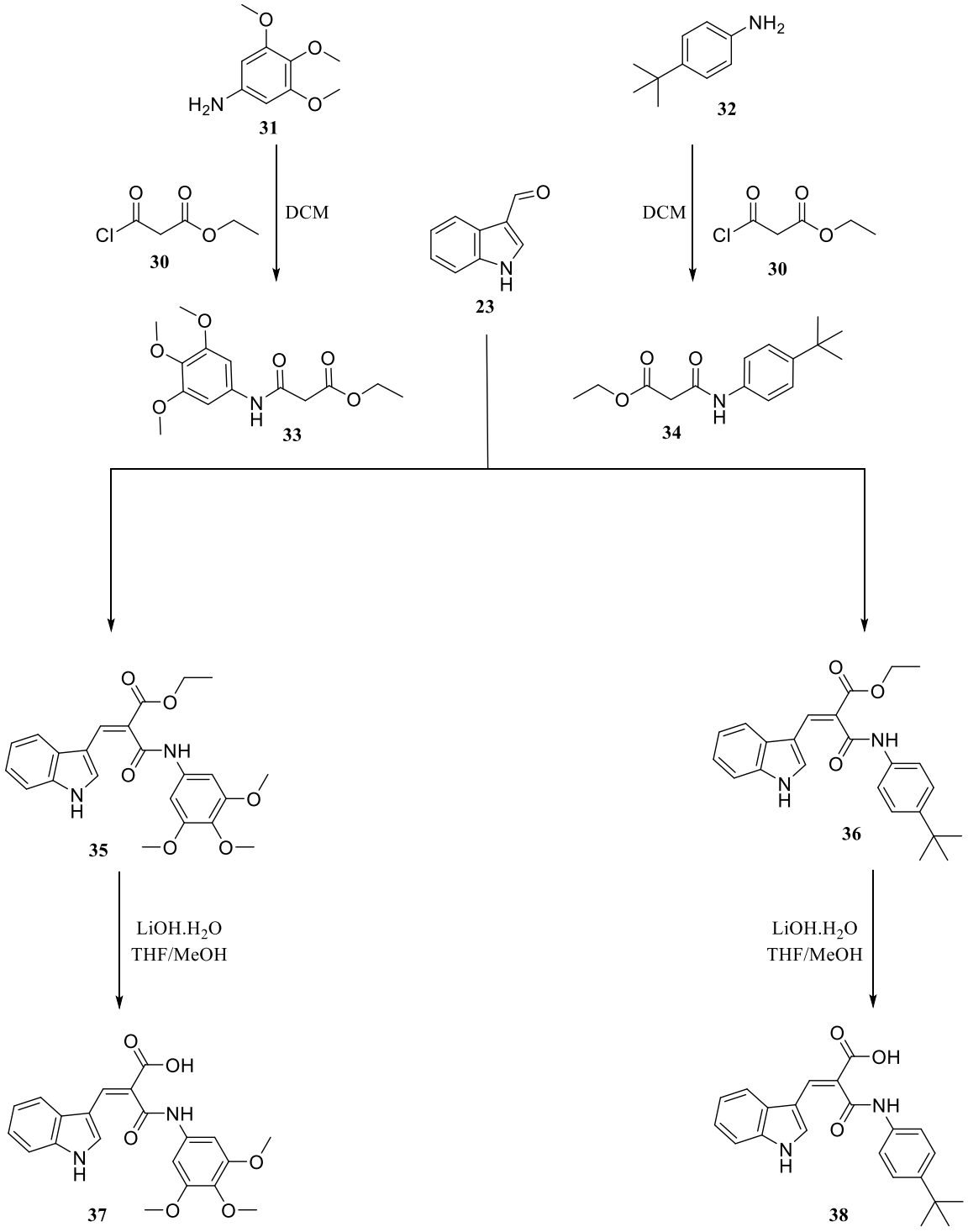
This series of reactions efficiently prepared the (E)-2-substituted-(1H-indole-3-yl)acrylic acids **25** and **28** into their corresponding amide derivatives.



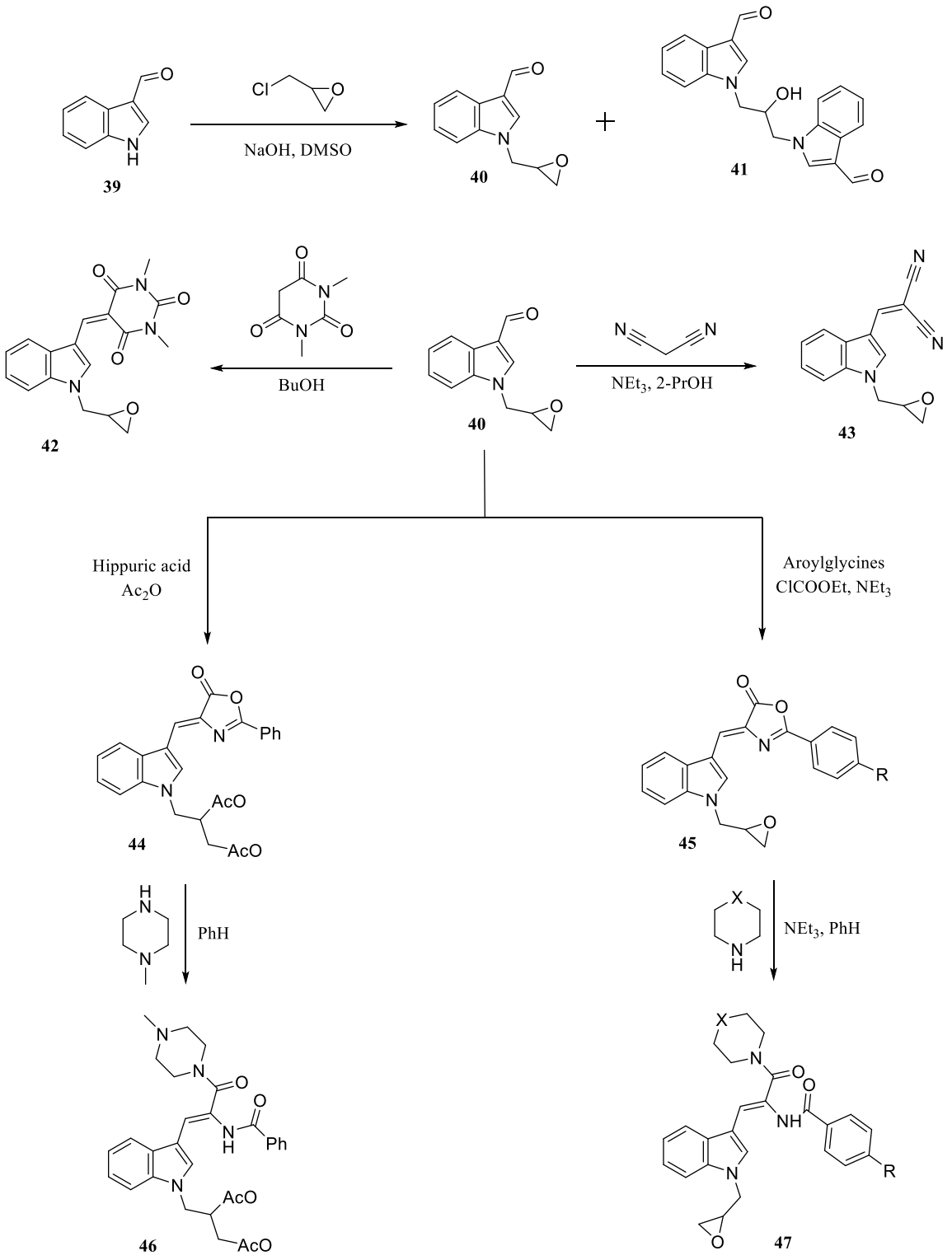
Scheme 1.5

In the synthesis of derivatives containing -COOEt and -COOH groups in the intermediate chain, specific aniline derivatives **31** and **32** were initially reacted with ethyl malonyl chloride in DCM, yielding compounds **33** and **34**. These products, identified as ethyl 3-oxo-3-((substituted phenyl)amino)propanoates, were subsequently condensed with compound **23**, using piperidine as a catalyst in a solvent-free environment, to produce compounds **35** and **36**. Following this, the ester group in the intermediates was hydrolyzed using lithium hydroxide monohydrate ($LiOH.H_2O$) in a mixture of THF, MeOH and water to obtain compounds **37** and **38**, as depicted in **Scheme 1.6**.

Continuing from the synthesis, M. Hawash *et al.* initially assessed the in vitro antiproliferative activities of these compounds, specifically targeting cancer cell lines with an emphasis on hepatocellular carcinoma. The study found that five of the compounds exhibited moderate antitumor effects. Further investigations into their mechanism revealed that one compound, distinguished by a cyano group and a 3,4,5-trimethoxy amide substitution, acted as an inhibitor of tubulin polymerization. Moreover, analysis of the cell cycle demonstrated that this compound induced G2/M-phase arrest in Huh7 cells. The findings suggested that polar substitutions on the indole acrylamide scaffold could increase the compounds' efficacy in inhibiting tubulin polymerization. Despite these insights, variations in the inhibition mechanisms among the compounds were noted, as no consistent shift in bioactivity related to the substitutions was observed across the cancer cell lines.


31
32
DCM
DCM
30
30
23
33
34
35
36
LiOH.H2O
THF/MeOH
LiOH.H2O
THF/MeOH
37
38

Scheme 1.6

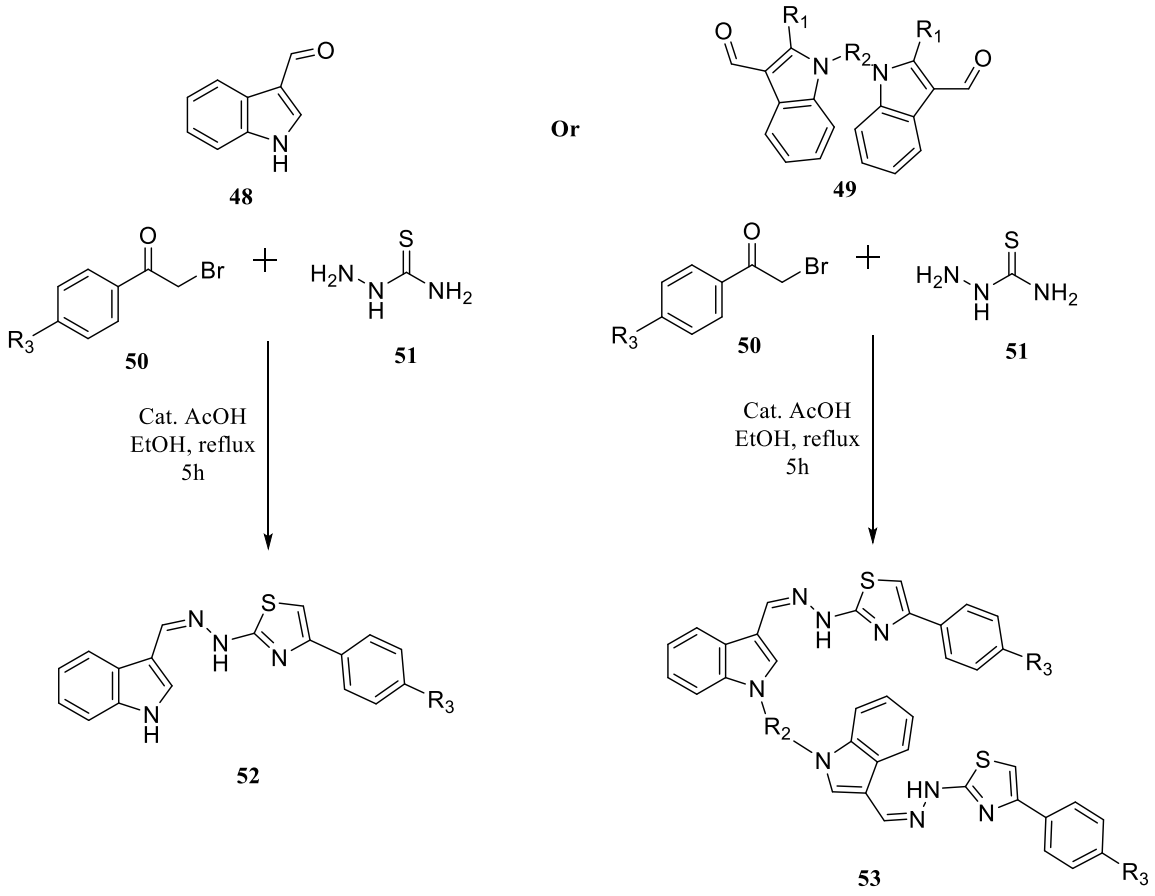


Scheme 1.7

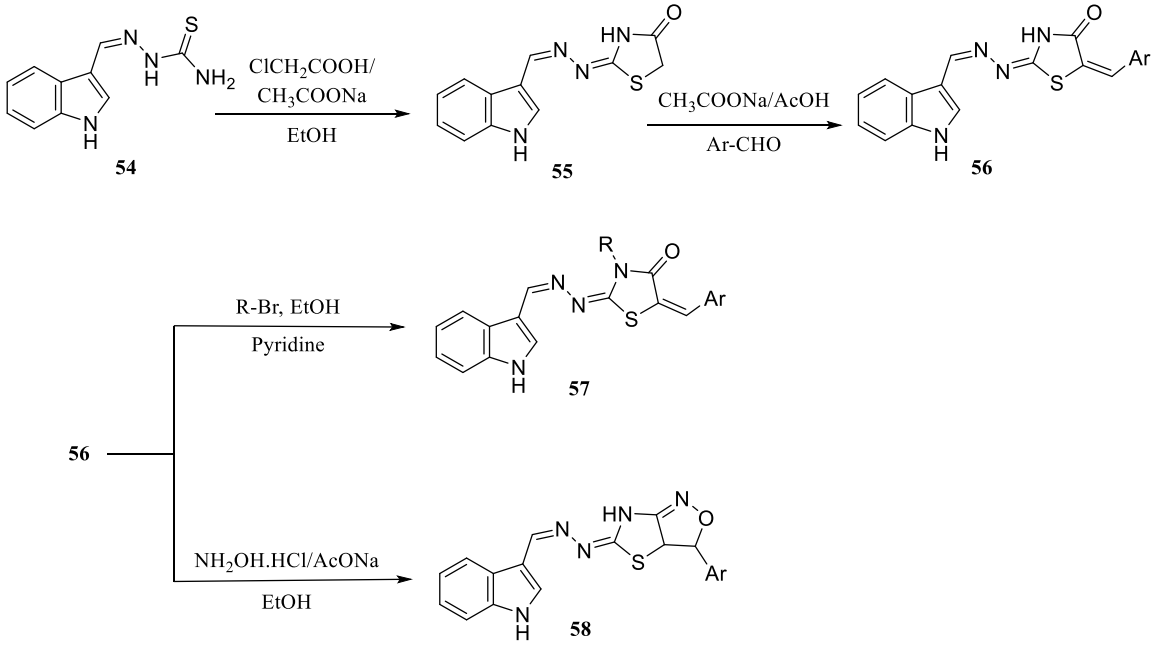
The K. F. Suzdalev *et al.*[99] detail the synthesis of 1-(oxiran-2-ylmethyl)-1H-indole-3-carbaldehyde **40** and its subsequent reactions with active methylene compounds. They

began by reacting compound **39** with epichlorohydrin in the presence of NaOH, which yielded the anticipated epoxide compound **40** and a secondary compound **41** characterized by dual indole units. This secondary formation occurred due to the opening of the oxirane ring in compound **40**, triggered by the N-indolyl anion in the reaction mix. Further reactions involved compound **40** and active methylene compounds such as 1,3-dimethylbarbituric acid and malononitrile, leading to the production of condensation products **42** and **43**, respectively. Additionally, efforts to synthesize oxazolone **44** via the traditional Erlenmeyer-Plöchl reaction wherein compound **39** was heated with hippuric acid in acetic anhydride resulted in both oxazolone ring formation and bisacylation of the epoxy fragment. An adapted Erlenmeyer-Plöchl reaction was employed to prepare oxazolones **45**, using the starting aldehyde **40**, aroylglycines, and ethyl chloroformate in the presence of triethylamine. Finally, the oxazolones **44** and **45** were subjected to reactions with cyclic amines such as N-methyl piperazine or morpholine, yielding the amides **46** and **47**, respectively, as a result of oxazolone ring opening.

O. Mahmoodi Nosrat *et al*.[100] in their publication describe a synthesis procedure involving the reaction of one equivalent of indole-3-carbaldehyde **48** or half-equivalent of bisaldehydes **49** with one equivalent of phenacyl bromide **50** and thiosemicarbazide **51**, along with a few drops of acetic acid in ethanol. This reaction, conducted at reflux temperature, successfully produced the target compounds **52** and **53**, both yielding satisfactorily as shown in **Scheme 1.8**. To identify the optimal conditions for the reaction, the initial tests involved reacting compound **48**, **51**, and compound **50** in either absolute ethanol or ethanol (95%) with a catalytic amount of acetic acid, both under reflux conditions. When conducted in absolute ethanol, the reaction yielded only 55% of the desired product after 12 hours. However, by adding a catalytic amount of acetic acid to the ethanol (95%), the yield significantly increased to 89% in just 7 hours. Following synthesis, all compounds were subjected to in vitro antibacterial testing against both Gram-positive bacteria (*Bacillus subtilis* and *Micrococcus luteus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Salmonella enteritis*). Notably, several of these compounds demonstrated significant antibacterial efficacy. Particularly, the bis-compounds featuring a methoxy (OCH_3) donating group showed notable activity against the Gram-positive strains.

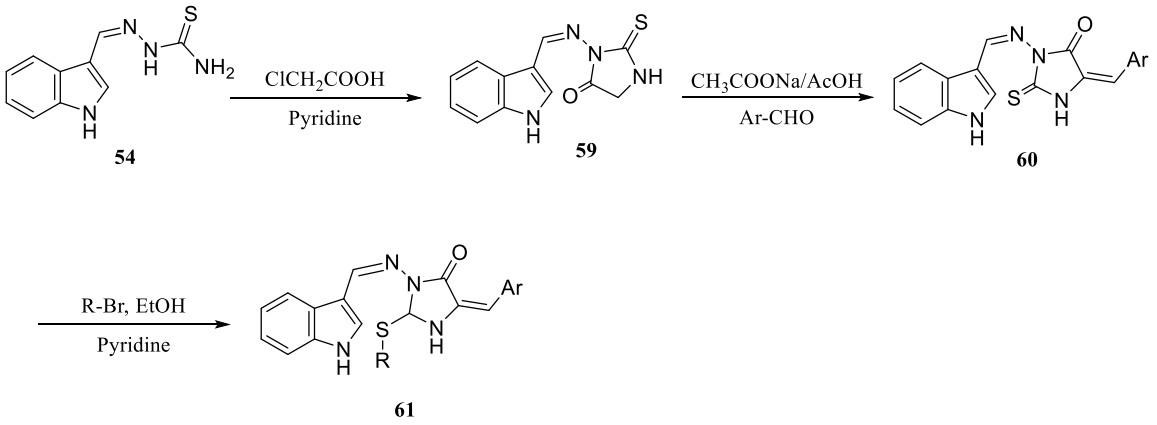


Scheme 1.8



Scheme 1.9

B. Dinesh *et al.*[101] detailed the preparation of 3-thiazolidin-4-one-2-yl-methylene hydrazido-1H-indole **55 (Scheme 1.9)** through the reaction of indole-3-carbaldehyde thiosemicarbazone **54** with chloroacetic acid in ethanol, supplemented with anhydrous sodium acetate. Conversely, 3-[2-thioxo-imidazolin-4-one-3-yl-imino methylene]-1H-indole **59 (Scheme 1.10)** was synthesized using a similar method but in the presence of pyridine instead of sodium acetate. Subsequent reactions of compounds **55** and **59** with aromatic aldehydes, using acetic acid and sodium acetate, led to the formation of the arylidene derivatives **56** and **60**, respectively. Further synthesis involved the base-catalyzed reaction of **56** and **60** with phthalimidoxy ethyl bromide resulting in the production of 3-[(5-arylidene-3-N-ethoxyphthalimido-1,3-thiazolidin-4-one-2-yl)methylene hydrazide]-1H-indoles **57** and 3-[5-arylidene-2-(2-ethoxyphthalimido-thiol)imidazolin-4-one-3-yl imino methylene]-1H-indoles **61**. Concurrently, the cyclocondensation of **56** with hydroxylamine hydrochloride in the presence of sodium acetate yielded 3-[(3-aryl-[4,6]thiazolidino[4,5-c]isoxazolin-5(6H)-yl]methylene hydrazido-1H-indoles **58**. All the synthesized compounds were screened for the antimicrobial evaluation which shows the substitution of fluoro group enhances the antimicrobial activity.

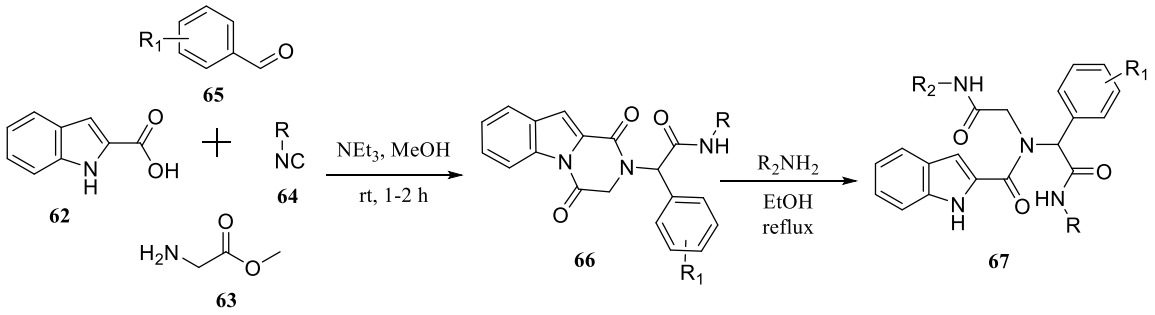


54 59 60 61

Scheme 1.10

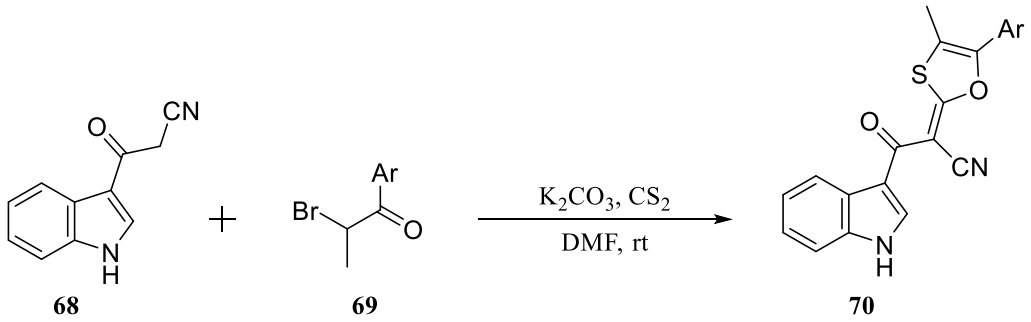
P. Shashi *et al.*[102] initiated their synthesis by adding triethylamine to a methanol solution of amino ester hydrochloride **63**, which was then stirred at room temperature for 10 minutes **(Scheme 1.11)**. Subsequent additions to this mixture included corresponding aldehyde **65**, 1H-indole-2-carboxylic acid **62**, and isocyanide **64**, sequentially, resulting in the formation of indole-fused diketopiperazines **66** with yields ranging from good to excellent. In the next step, compound **66** was refluxed with various amines in ethanol,

producing highly functionalized carboxamides **67**, also in good to excellent yields. Notably, the final products were achieved through a regioselective ring-opening of the diketopiperazine unit **66** via an intermolecular transamidation reaction under mild conditions. Some of the synthesized compounds were notable for showing significant in vitro antileishmanial activity and were subsequently assessed for in vivo efficacy via intraperitoneal administration. Administered at a dosage of 50 mg/kg/day for five consecutive days, these compounds demonstrated inhibition rates of 70.0%, 63.5%, and 63.4% against Leishmania amastigotes on the seventh day post-treatment in a hamster model of visceral leishmaniasis.



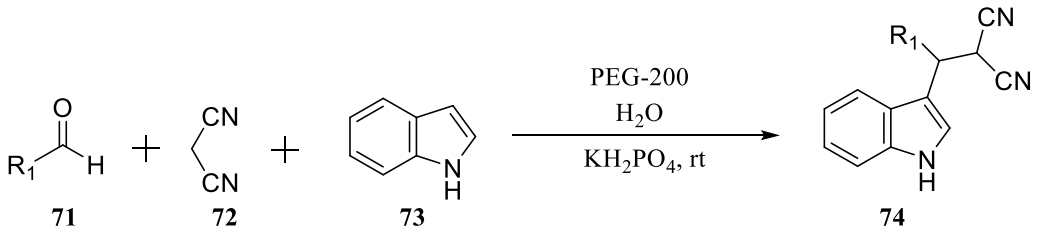
Scheme 1.11

J.-Y. Liu *et al.*[103] devised a series of new multicomponent domino reactions (MDRs) that facilitate the synthesis of various functionalized ring structures, which are of significant interest in chemical and pharmaceutical fields. The reaction proceeded by an indol-3-yl substituted β-oxopropanenitrile **68**, carbon disulfide, and α-bromo propiophenones **69** which were solubilized in DMF in the presence of K_2CO_3 under mild conditions. This particular setup led to the formation of fully substituted (Z)-1,3-oxathioles **70** in good amount of yield.

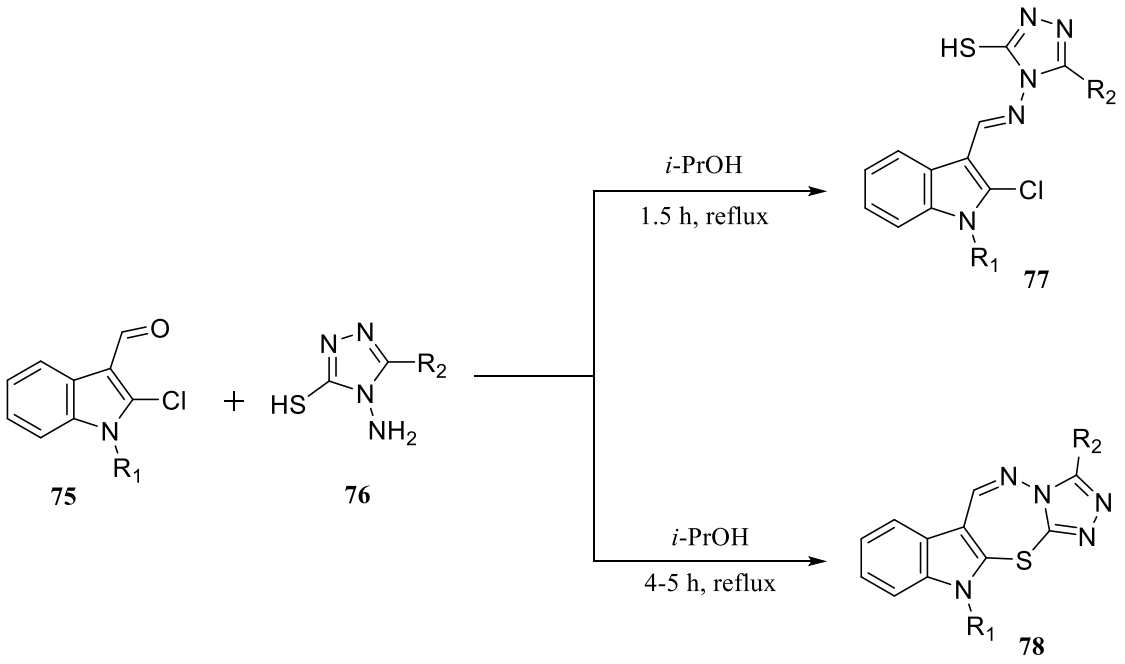


Scheme 1.12

L. Wang *et al.*[104] developed a sustainable three-component reaction that combines indoles, aldehydes, and malononitrile in water, facilitated by polyethylene glycol (PEG-200), to produce 3-indole derivatives with good to excellent yields **(Scheme 1.13)**. In their methodology, KH_2PO_4, PEG-200, water, aldehyde **71**, malononitrile **72**, indole **73**, and a magnetic stir bar were placed into a 10 mL vial, which was then sealed. This reaction mixture was stirred at room temperature, leading to the synthesis of the final compounds **74**.



Scheme 1.13

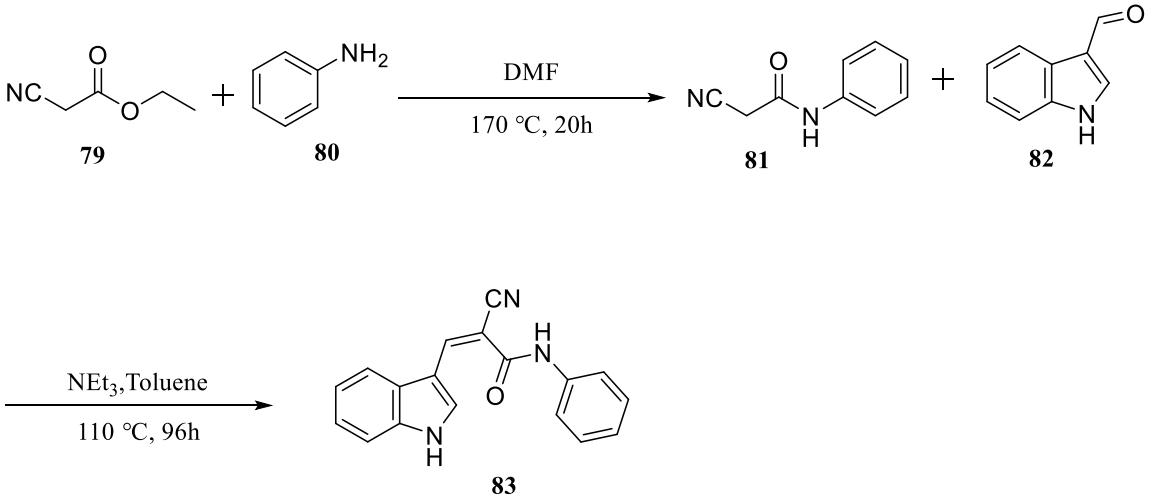


Scheme 1.14

N. I. Vikrishchuk *et al.*[105] documented a reaction where 1-alkyl-2-chloro-1H-indole-3-carbaldehydes **75** were combined with 4-amino-5-alkyl(aryl)-4H-1,2,4-triazole-3-thiols **76 (Scheme 1.14)**, leading to the formation of new heterocyclic compounds known as triazolo(thiadiazepino)indoles **78**. The formation of this heterocyclic system occurs

through the cyclization of the intermediate 5-alkyl-4-[indol-3-yl(methylideneamino)]-4H-1,2,4-triazole-3-thiols **77**. In the process, reacting compound **75** with **76** under reflux conditions for 1.5 hours produced intermediate molecule **77**. However, extending the reaction duration to 4–5 hours led to the formation of triazolo(thiodiazepino)indoles **78**. This longer reaction likely facilitates the cyclization of intermediates 77 with the concurrent elimination of a hydrogen chloride molecule.

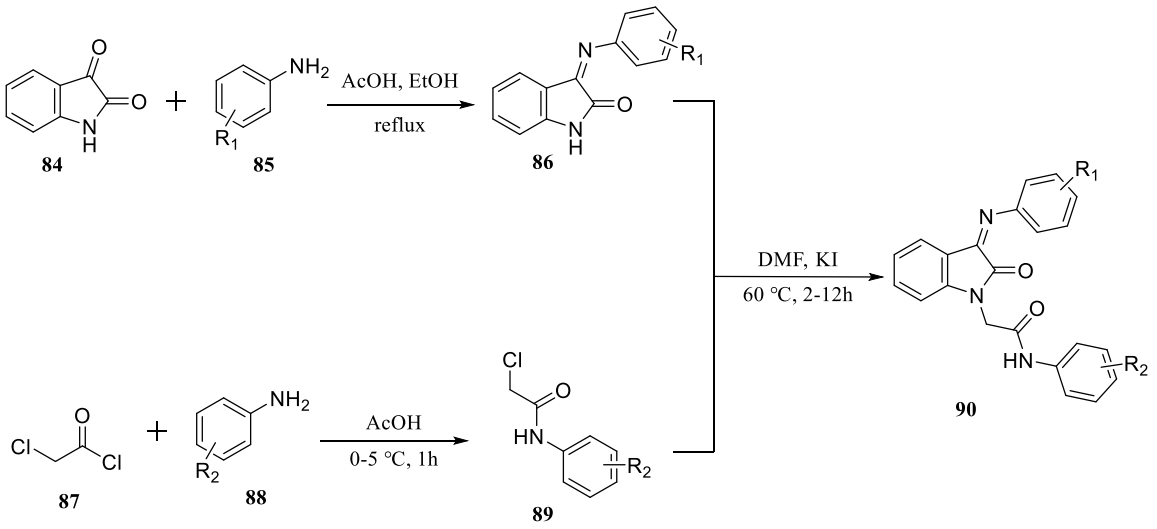
P. Silva *et al*.[106] detailed the synthesis of the titled compound **83 (Scheme 1.15)** by initially performing an amidation reaction between aniline **80** and ethyl 2-cyanoacetate **79**. This step was followed by a Knoevenagel-type condensation with indole aldehyde **82**, using equimolar amounts. The condensation occurred under the influence of a basic catalyst such as NEt_3, which facilitated the formation of a carbanion. This carbanion then underwent a nucleophilic attack on the carbonyl carbon of the indole aldehyde. The reaction sequence concluded with the elimination of water, leading to the creation of a carbon-carbon double bond.



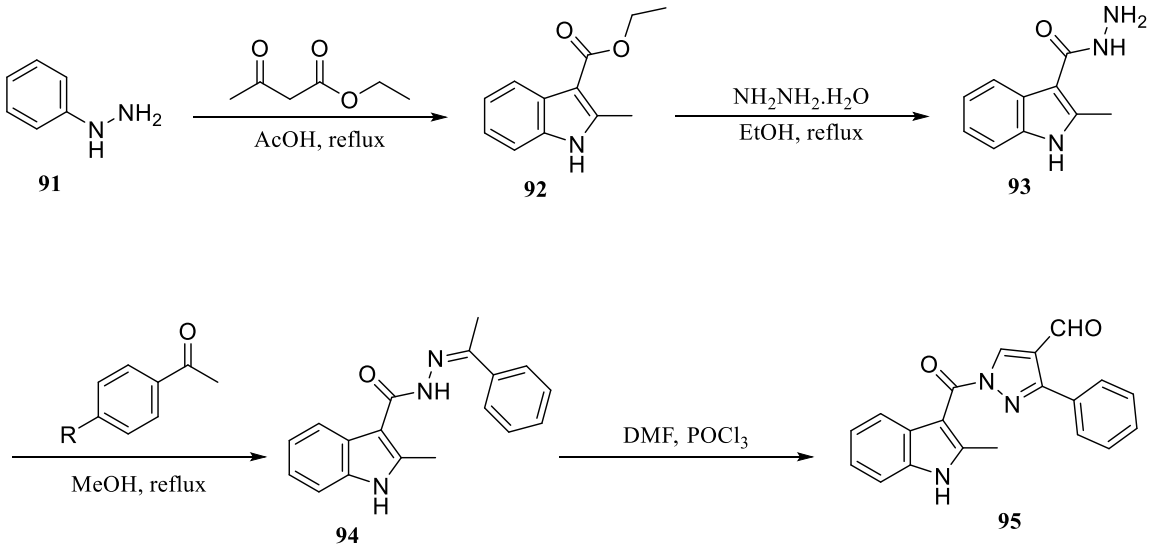
Scheme 1.15

B. Debnath *et al*.[107] reported the synthesis of 2-oxo-3-(arylimino)indolin-1-yl)-N-aryl acetamide derivatives, along with their evaluation for antibacterial and antifungal effectiveness against various pathogenic microorganisms. Notably, the derivatives containing a methoxy group demonstrated the highest activity. The synthesis of the analogs of compound **90** was conducted according to the steps outlined in **Scheme 1.16**. Initially, 1,3-dihydro-indol-2-one **86** was synthesized by the reaction of compound **84** with

substituted anilines **85**. Following this, substituted anilines **88** and chloroacetyl chloride **87** were reacted in the presence of glacial acetic acid under ice-cold conditions, producing chloroanilides **89**. These chloroanilides **89** were then reacted with Schiff bases of compound **86** to yield the target compounds **90**.



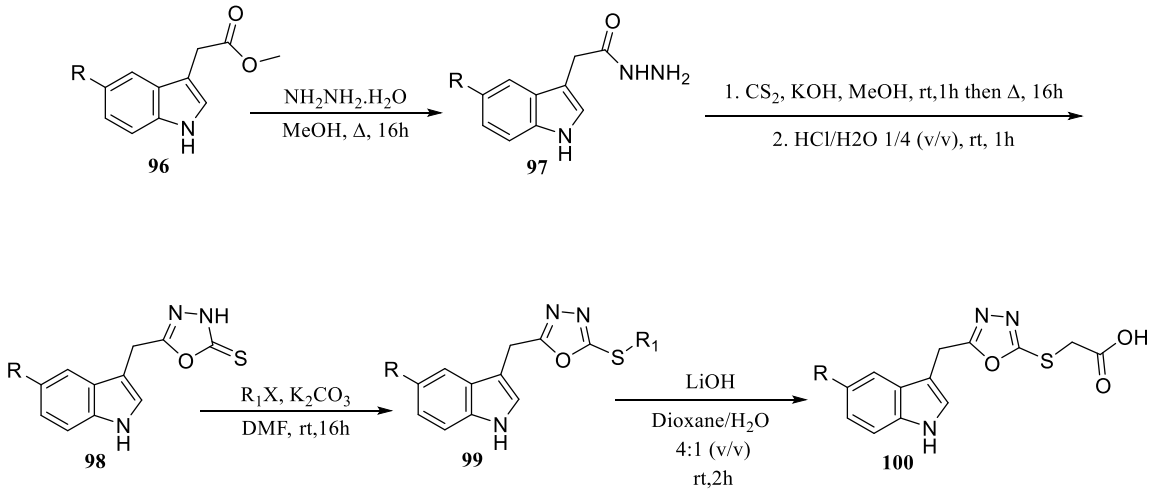
Scheme 1.16



Scheme 1.17

P. Bharath Rathna Kumar *et al.*[108] detailed the synthesis of 1-[(2-methyl-1H-indol-3-yl) carbonyl]-3-substituted phenyl-1H-pyrazole-4-carbaldehyde derivatives **95 (Scheme 1.17)**. This was achieved by first reacting phenyl hydrazine **91** with a combination of ethyl acetoacetate and glacial acetic acid to produce ethyl-2-indole-3-carboxylate **92**. This

intermediate, upon reaction with hydrazine hydrate, yielded product **93**. Subsequently, product **93** was condensed with various acetophenones in methanol in the presence of glacial acetic acid, resulting in the formation of hydrazones **94**. These hydrazones **94** were then treated with Vilsmeier-Haack reagent, leading to the formation of the final 1-[(2-methyl-1H-indol-3-yl) carbonyl]-3-substituted phenyl-1H-pyrazole-4-carbaldehyde derivatives **95**.



Scheme 1.18

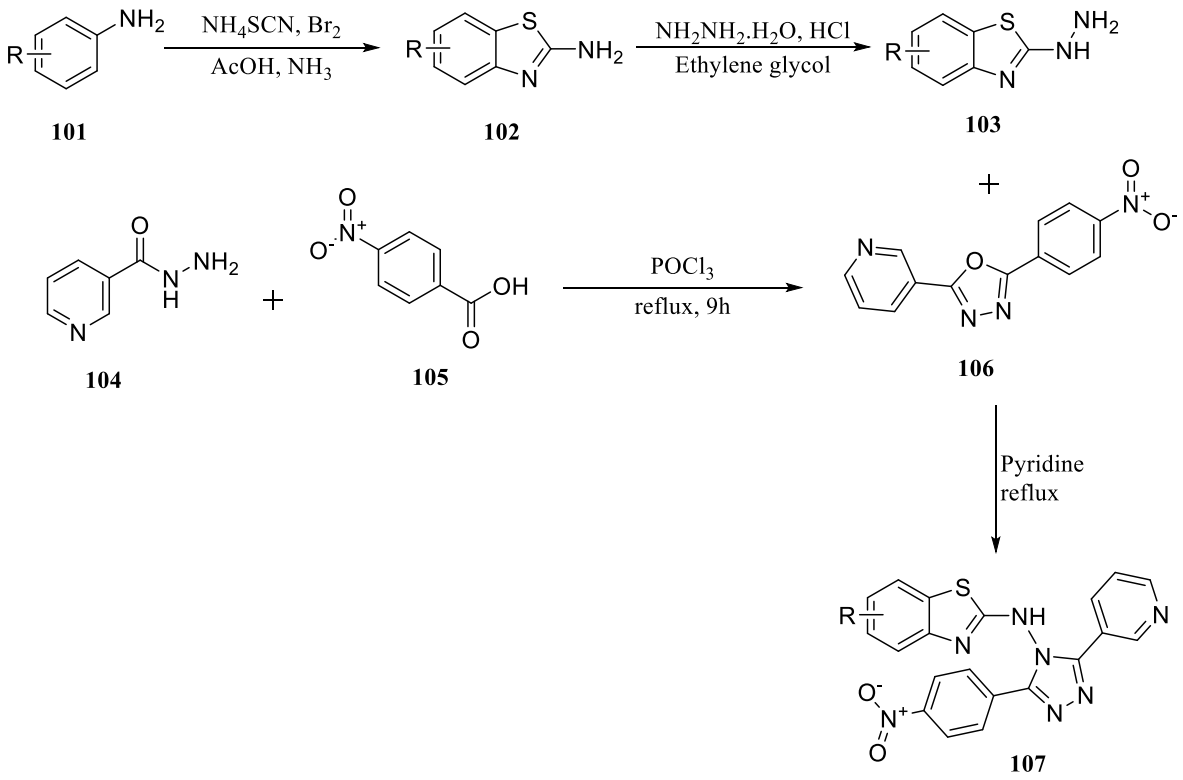
M. Iškauskienė *et al.*[109] reported on the synthesis of 2-[(1H-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles **(Scheme 1.18)**, starting with the conversion of ester **96** to hydrazide **97**, achieving yields between 80–90% by using hydrazine hydrate in methanol. This hydrazide **97** was then treated with carbon disulfide in the presence of methanolic potassium hydroxide, and subsequently acidified with dilute hydrochloric acid to produce the 1,3,4-oxadiazole derivative **98**. The next phase involved the S-alkylation of these 1,3,4-oxadiazole derivatives **98**. These alkylation reactions were successfully carried out at room temperature using K_2CO_3 as a base, yielding the desired product **99** in high yields. The esters **99** were then hydrolyzed under basic conditions to form the corresponding carboxylic acids **100**.

Further, all S-alkylated derivatives were evaluated for their protective effects both in vitro and in vivo. Compounds with a methyl group on the indole ring and propyl, butyl, or benzyl groups on the sulfhydryl were found to protect Friedreich's ataxia fibroblasts from glutathione depletion caused by the γ-glutamylcysteine synthetase inhibitor,

buthionine sulfoximine. Additionally, two of the active compounds notably improved the survival of Caenorhabditis elegans under oxidative stress induced by juglone.

1.6.2 In-Depth Analysis of the Synthesis Routes and Biological Activities of Benzothiazole and Triazole Conjugates

In a significant study, Patel N.B. *et al.*[110] reported the synthesis and in vitro antimicrobial and antitubercular activity of a various series of 3-(3-pyridyl)-5-(4-nitrophenyl)-4-(N-substituted-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole **(Scheme 1.19)**. The antimicrobial activity of these compounds was assessed against bacteria and fungi. In addition, the Lowenstein-Jensen agar method was used to assess their antitubercular activity against M. tuberculosis H37Rv. The synthesized compounds exhibited good to moderate antimicrobial and antitubercular properties.

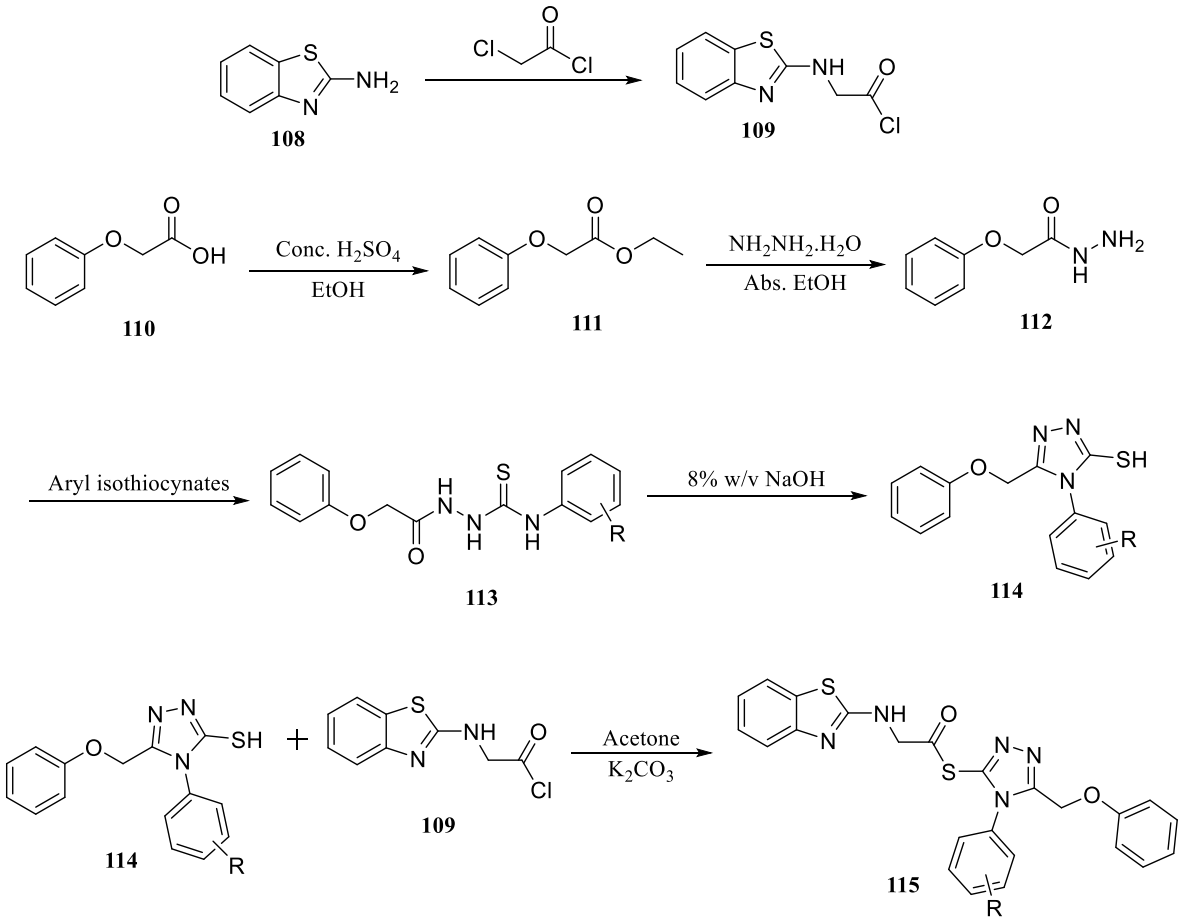


Scheme 1.19

Treating Compound **102** with hydrazine hydrate, concentrated hydrochloric acid, and ethylene glycol results in the formation of 2-hydrazino-1,3-benzothiazole **103**. Nicotinoyl hydrazide **104** undergoes intermolecular cyclization with 4-nitro benzoic acid to yield 2-(3-pyridyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **106** in the presence of $POCl_3$.

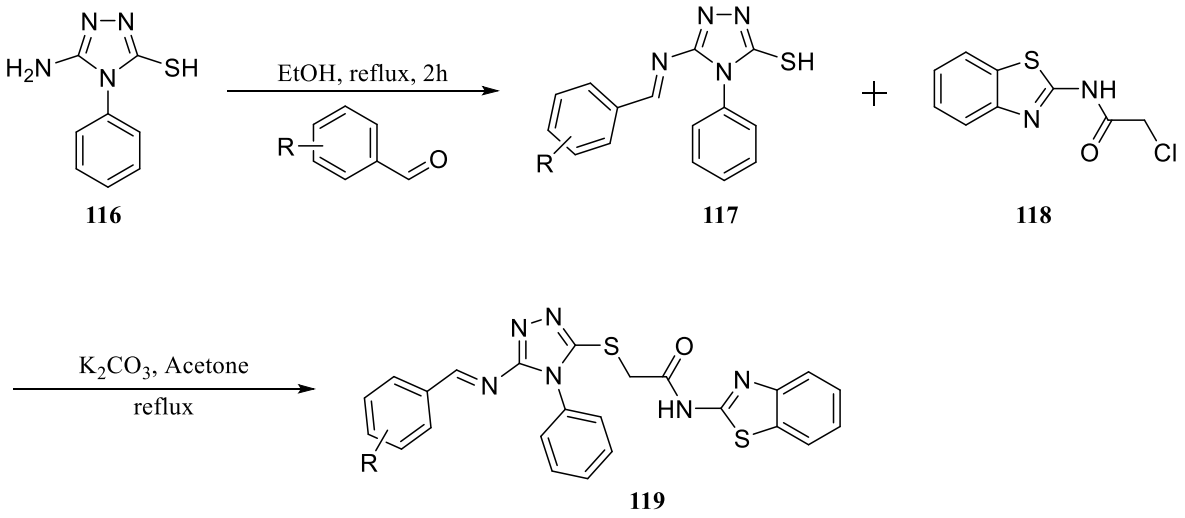
The condensation of **106** with various substituted **103** derivatives in pyridine yields final compound **107**.

T. Sana *et al*.[111] synthesized a series of novel N-(benzothiazol/oxazol-2-yl)-2-[(5-(phenoxymethyl)-4-aryl-4H-1,2,4-triazol-3-yl)thio] acetamide derivatives and investigated their in vitro anti-inflammatory activity and p38α MAP kinase inhibition **(Scheme 1.20)**. The compounds with potential in vitro activities were tested in vivo for their anti-inflammatory effects using the carrageenan-induced rat paw edema model. The compound with 4-fluoro substitution had the highest activity, inhibiting edema by 84.43%. The synthesis pathway involved stirring 2-aminobenzothiazole with chloroacetyl chloride in dichloromethane and triethylamine to prepare compound **109**. Phenoxy acetic acid was esterified to its ethyl ester, then treated with hydrazine hydrate in absolute ethanol to synthesize 2-phenoxyacetohydrazide **112**. Compound **112** reacted with aryl isothiocyanates in ethanol to produce 2-(2-phenoxyacetyl)-N-phenylhydrazinecarbothioamides **113**. Reaction of compound **113** with 8% w/v NaOH yielded derivatives **114**. Finally, N-(benzothiazol/benzoxazol-2-yl)-2-[(5-(phenoxymethyl)-4-aryl-4H-1,2,4-triazol-3-yl)thio]acetamides **115** were synthesized by coupling compounds **109** with **114**.



Scheme 1.20

Al-Masoudi Najim A. *et al.*[112] reported the synthesis of new Schiff base ligands derived from 5-amino-4-phenyl-4H-1,2,4-triazole-3-thiol **116** and substituted benzaldehydes via a condensation reaction **(Scheme 1.21)**. The key intermediate, compound **118**, was synthesized by reacting chloroacetyl chloride with 2-aminobenzothiazole. This intermediate was then coupled with compound **117** to yield the benzothiazole derivative **119**, using anhydrous K_2CO_3 in acetone. The newly designed and synthesized Schiff base ligands were evaluated for their anti-HIV-1 and HIV-2 activity by examining their inhibition of HIV-induced cytopathogenicity in MT-4 cells. Notably, the compound with a 4-chloro substitution emerged as the most active inhibitor in cell culture against both HIV-1 and HIV-2, providing a promising lead for further optimization in anti-HIV drug development.



Scheme 1.21

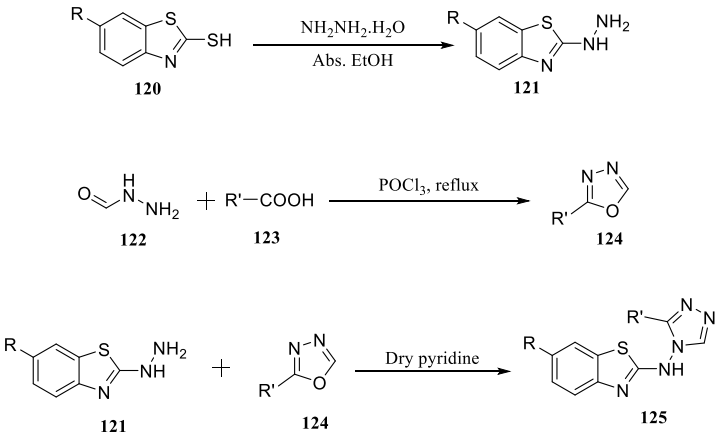
Tariq Sana *et al.*[113] reported the synthesis of a new series of N-[3-(substituted-4H-1,2,4-triazol-4-yl)] benzo[d]thiazol-2-amines **(Scheme 1.22)**, which were subsequently evaluated for their in vitro anti-inflammatory activity using the BSA anti-denaturation assay and p38α MAPK inhibition. Among the compounds tested for in vivo anti-inflammatory activity, the one with a 2,6-dichloro substitution demonstrated the highest efficacy, achieving 85.31% inhibition compared to 83.68% for the reference drug diclofenac sodium.

The synthesis pathway began with preparing 2-hydrazinylbenzothiazole **121** by refluxing 2-mercaptobenzothiazole **120** with hydrazine hydrate in ethanol. This was followed by intermolecular cyclization of formic acid hydrazide **122** with substituted acids **123** in the presence of $POCl_3$, resulting in the formation of the corresponding oxadiazole **124**. The last step involved the condensation of oxadiazole **124** with 2-hydrazinylbenzothiazole **121** to yield the target compound **125**.

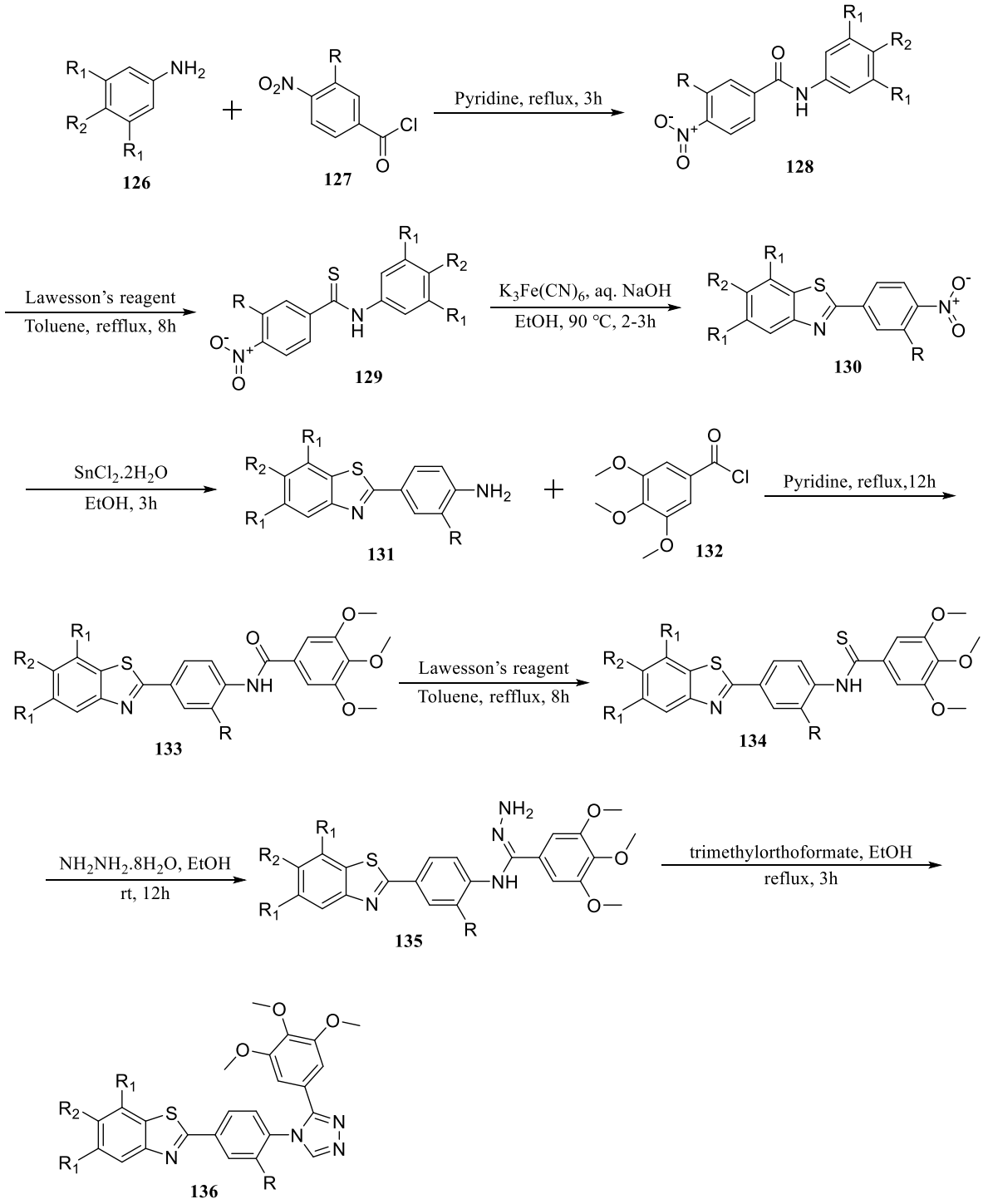
Subba Rao A. V. *et al.*[114] synthesized a series of colchicine site-binding tubulin inhibitors by modifying the combretastatin pharmacophore and evaluated their antiproliferative activity on selected cancer cell lines. SAR analysis revealed that the most potent compounds, substituted with methoxy and fluoro groups, exhibited antiproliferative effects comparable to combretastatin (CA-4). Mitotic cell cycle arrest in the G2/M phase showed disruption of microtubule dynamics, which was confirmed by tubulin

polymerization assays and immunocytochemistry studies at the cellular level. Western blot analysis revealed that these compounds led to the accumulation of tubulin in the soluble fraction. The colchicine competitive binding assay and molecular docking studies suggested these mimics bind to the colchicine site of tubulin, similar to CA-4. Additionally, the triggering of apoptotic cell death after mitotic arrest was investigated using Hoechst staining, Annexin-V-FITC assay, mitochondrial membrane potential assessment, ROS generation measurement, and caspase-3 activation analysis.

The synthesis of various substituted 2-(4-aminophenyl) benzothiazole precursors **131** was achieved via Jacobson's thioanilide radical cyclization. Initially, substituted anilines **126** reacted with substituted p-nitrobenzoylchloride **127** in pyridine to form benzanilides **128**, which were then converted to thiobenzanilides **129** using Lawesson's reagent. Cyclization using Jacobson's method with potassium ferricyanide and NaOH yielded nitrobenzothiazole derivatives **130**, which were reduced with stannous chloride to get the corresponding substituted 2-(4-aminophenyl) benzothiazoles **131**, as shown in **Scheme 1.23**. These intermediates were then coupled with trimethoxy benzoyl chloride (132) to form trimethoxy-substituted benzothiazole amides **133**. Subsequent conversion of the carbonyl functionality to thiocarbonyl groups using Lawesson's reagent in refluxing toluene produced thioamide **134**. Reaction of thioamide with hydrazine hydrate yielded amidrazones **135**, which were used in the next step without purification. Finally, intramolecular cyclization with trimethyl orthoformate under reflux conditions and catalytic H_2SO_4 furnished the desired 1,2,4-triazoles **136**.



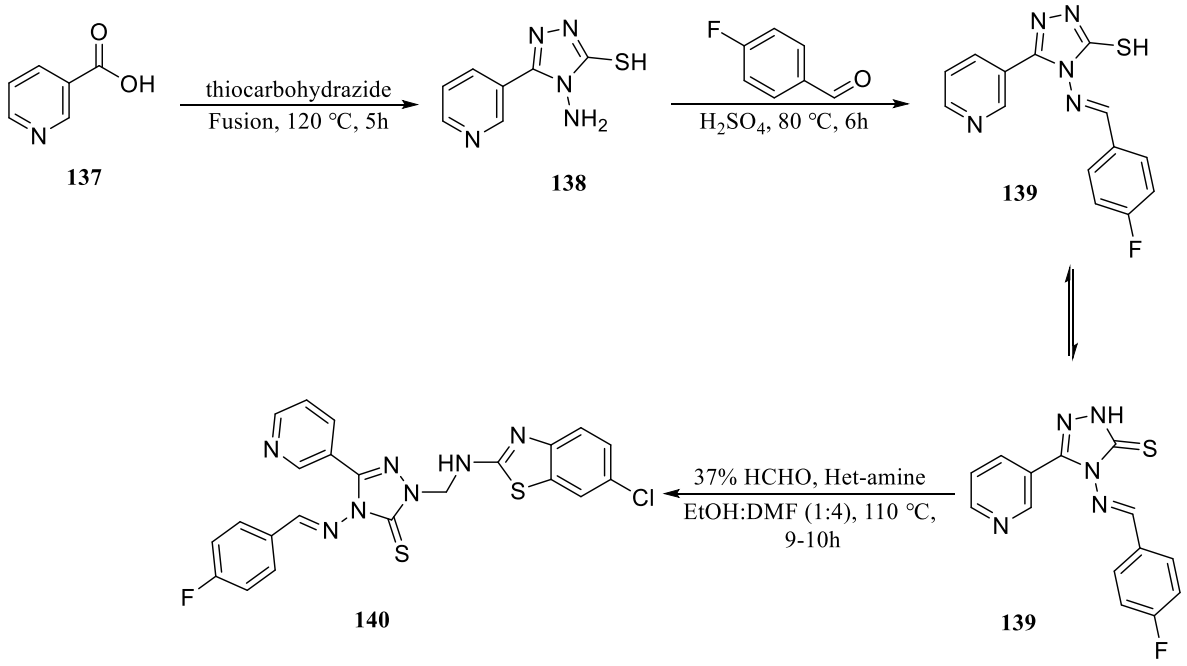
Scheme 1.22



Scheme 1.23

Patel Vatsal M. *et al.*[115] reported the synthesis of a series of novel triazole analogues using the N-Mannich reaction through a conventional synthetic route. These compounds were assessed for their antimicrobial, antituberculosis, and antiprotozoal activities. The key

precursor, 4-((4-fluorobenzylidene)amino)-5-(pyridine-4-yl)-4H-1,2,4-triazole-3-thiol **139**, required for the synthesis of the target compounds **140**, was obtained by reacting 4-fluorobenzaldehyde with 4-amino-(5-pyridine-4-yl)-4H-1,2,4-triazole-3-thiol **138** in the presence of a catalytic amount of conc. H_2SO_4. The intermediate **138** was prepared from isonicotinic acid using the fusion method. The synthetic route for compound **140** is outlined in **Scheme 1.24**. The key intermediate **139**, when reacted with various heterocyclic amines and 37% formaldehyde in a mixture of EtOH (1:4), afforded the desired compounds **140** via a conventional synthetic approach.

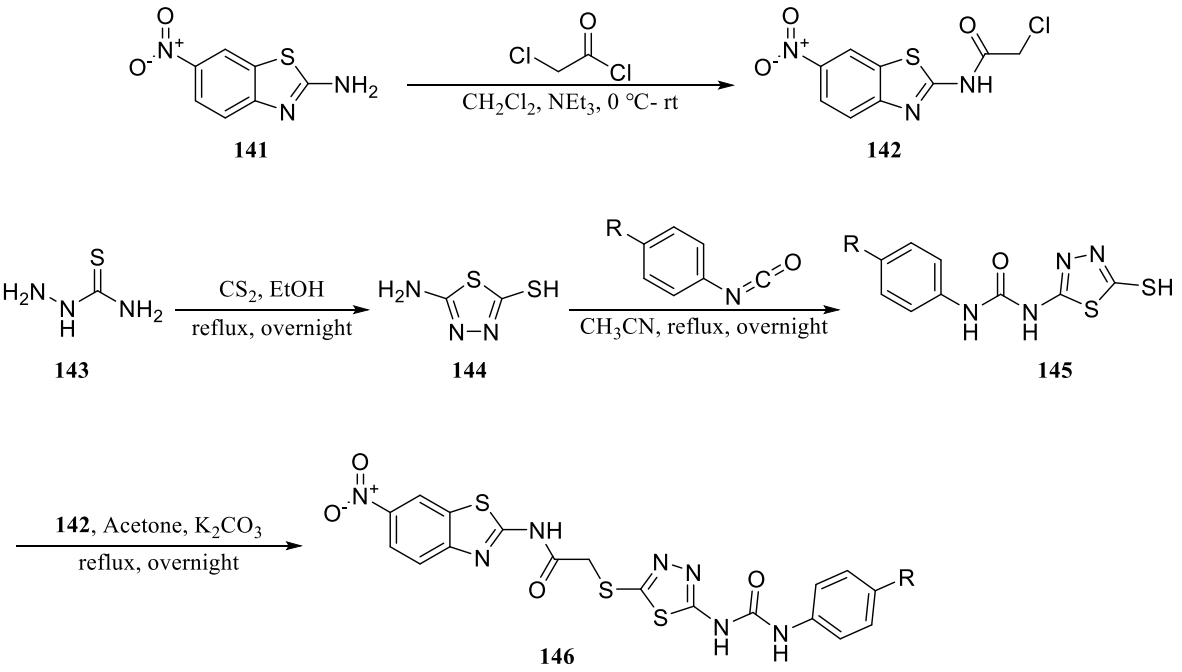


137 138 139 139 140

Scheme 1.24

Al-Sanea Mohammad M. *et al.*[116] synthesized a new series of 2-aminobenzothiazole hybrids linked to 1,3,4-thiadiazole aryl urea moieties. The in vitro antitumor effects of these new hybrids were evaluated against three cancer cell lines: HCT-116, HEPG-2, and MCF-7, using Sorafenib (SOR) as a reference drug. The synthesis of the new 1,3,4-thiadiazole analogues **146** is illustrated in **Scheme 1.25**. The process began with 6-nitrobenzo[d]thiazol-2-amine **141**, which underwent acetylation with chloroacetyl chloride and triethylamine as a base to yield the chloride derivative **142**, a key precursor for the subsequent nucleophilic substitution reaction. The key intermediates **145** were obtained by refluxing the 2-thiol derivative **144** with various 4-substituted phenyl

isocyanate derivatives in acetonitrile. These urea derivatives **145** then reacted with the chloride derivative **142** to produce the target benzothiazole-thiadiazole aryl urea hybrids **146**.

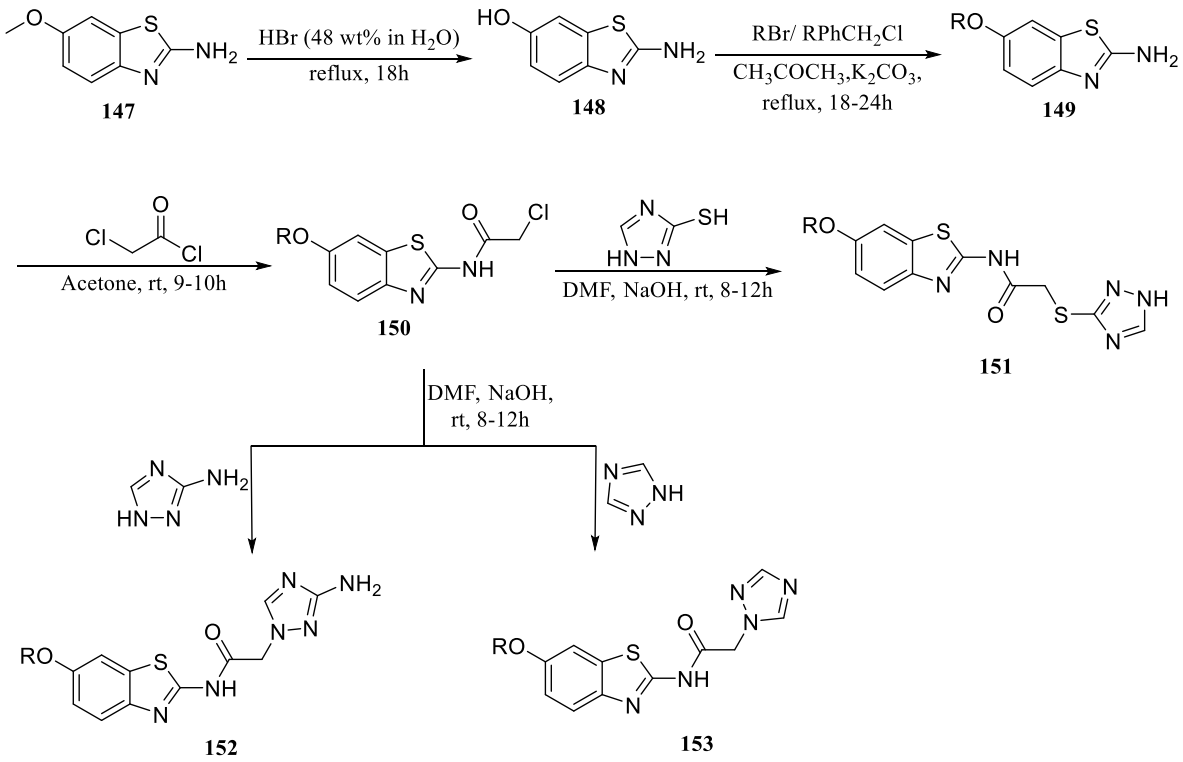


Scheme 1.25

Liu Da-Chuan *et al.*[117] reported the synthesis and evaluation of new benzothiazoles with mercapto-triazole and other heterocycle substituents for their anticonvulsant activity and neurotoxicity. These evaluations were conducted using the maximal electroshock (MES), subcutaneous pentylenetetrazol (scPTZ), and rotarod neurotoxicity (TOX) tests. Among the synthesized compounds, 151 derivatives demonstrated the most potent anticonvulsant activity, with ED_{50} values of 50.8 mg/kg and 54.8 mg/kg in the MES test, and 76.0 mg/kg and 52.8 mg/kg in the scPTZ seizures test, respectively.

The synthesis of all target compounds is outlined in **Scheme 1.26**. Initially, compounds **149** were treated with chloroacetyl chloride at room temperature in acetone to yield derivatives **150**. These derivatives **150** then reacted with various azoles, including 1N-1,2,4-triazole-3-thiol, triazole, and 3-amino-1,2,4-triazole, in refluxing DMF in the presence of NaOH. This reaction resulted in the substitution of the 2-chlorine atom by these heterocycles, producing the corresponding compounds: 2-((1H-1,2,4-triazol-3-yl)thio)-N-

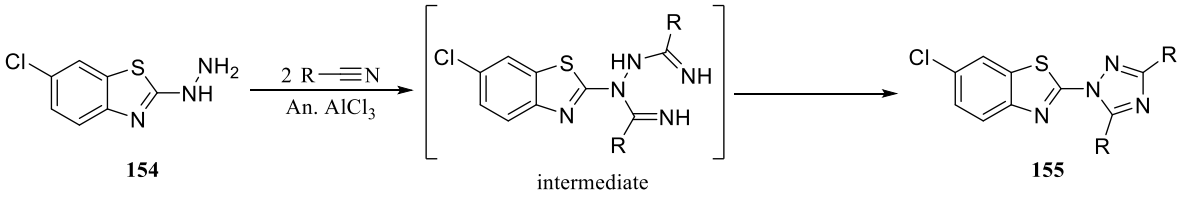
(6-alkoxybenzo[d]thiazol-2-yl)acetamide **151**, N-(6-alkoxybenzo[d]thiazol-2-yl)-2-(1H-1,2,4-triazol-1-yl)acetamide **153**, and 2-(3-amino-1H-1,2,4-triazol-1-yl)-N-(6-alkoxybenzo[d] thiazol-2-yl)acetamide **152**.



147 **148** **149** **150** **151** **152** **153**

Scheme 1.26

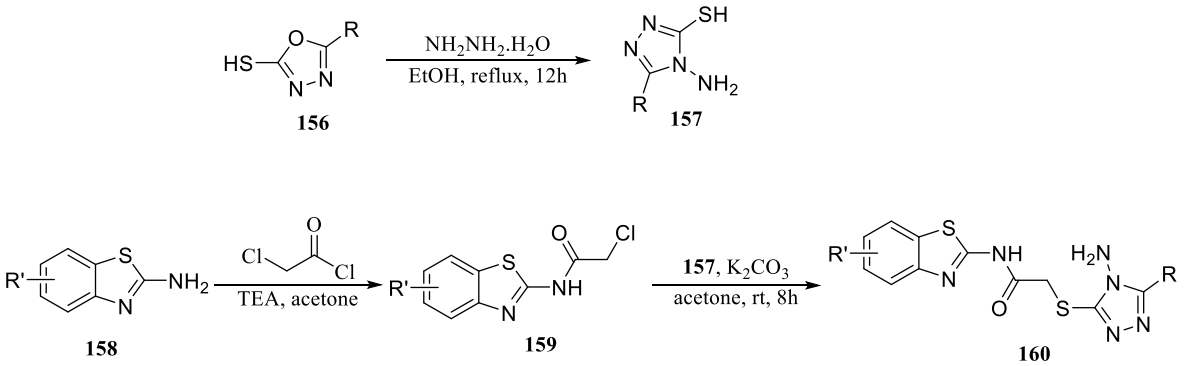
Kuberkar Sharad V. *et al*.[118] reported the synthesis of 1-(6-chloro-1,3-benzothiazol-2-yl)-3,5-dimethyl-1H-1,2,4-triazole derivatives **155**. The key intermediate, 6-chloro-2-hydrazine-1,3-benzothiazole **154**, was heated with anhydrous aluminium chloride in the presence of two moles of acetonitrile and its derivatives (benzonitrile and p-tolunitrile) in an oil bath. The reaction conditions varied depending on the nitrile used: for acetonitrile, the temperature was maintained at 130–140 °C, while for benzonitrile and p-tolunitrile, the temperature was increased to 160–170 °C. The reaction was carried out for three hours, resulting in the formation of the desired **155** derivatives, as illustrated in **Scheme 1.27**.



Scheme 1.27

Sever Belgin *et al.*[119] reported the synthesis and evaluation of 2-[(4-amino-5-aryl-4H-1,2,4-triazol-3-yl)thio]-N-(benzothiazol-2-yl)acetamide derivatives for their inhibitory activity against aldose reductase (AR). Among the synthesized compounds, those with pyridyl and nitro substitutions exhibited the most potent AR inhibitory activity.

The synthesis of the desired triazoles **160** is illustrated in **Scheme 1.28**. Initially, 4-amino-5-aryl-4H-1,2,4-triazole-3-thiol **157** was synthesized by reacting 5-aryl-1,3,4-oxadiazole-2-thiol **156** with hydrazine hydrate. Concurrently, 2-chloro-N-(benzothiazol-2-yl)acetamide derivatives **159** were prepared by reacting 2-aminobenzothiazoles derivatives **158** with chloroacetyl chloride in the presence of triethylamine. The final step involved a nucleophilic substitution reaction between compounds **157** and **159** in the presence of K_2CO_3, resulting in the formation of the target compounds **160**.

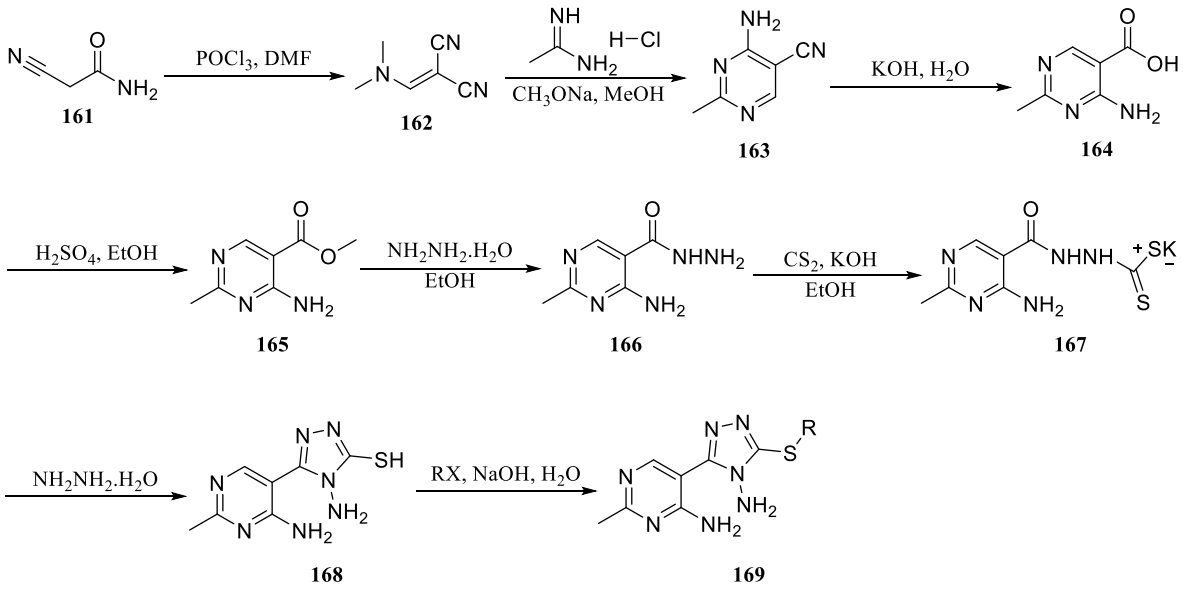


Scheme 1.28

Wu Wen-Neng *et al.*[120] reported the synthesis and evaluation of a series of novel 1,2,4-triazole derivatives containing a pyrimidine moiety for their fungicidal activities. Preliminary biological tests indicated that some of the synthesized compounds exhibited moderate to good fungicidal activity against the tested plant pathogenic fungi compared to the commercial agent Pyrimethanil. Notably, compounds with 2,4-dichloro and 3,4-

dichloro substitutions exhibited excellent antifungal activity against Phomopsis sp., with half-maximal effective concentration (EC_{50}) values of 25.4 µg/mL and 31.6 µg/mL, respectively.

The synthetic route began with 2-cyanoacetamide **161**, which was reacted with $POCl_3$ and DMF to form intermediate **162**. This intermediate was then converted to 4-amino-2-methylpyrimidine-5-carbonitrile **163**. The next step involved reacting compound **163** with KOH in water to obtain 4-amino-2-methylpyrimidine-5-carboxylic acid **164**. Subsequent esterification using H_2SO_4 and ethanol produced the corresponding ester, which was further reacted with hydrazine hydrate to yield the hydrazide product **166**. Upon reaction with CS_2 in the presence of KOH and methanol, potassium 2-(4-amino-2-methylpyrimidine-5-carbonyl)hydrazine-1-carbodithioate salt **167** was formed. This salt was then treated with hydrazine hydrate to obtain compound **168**. Various derivatives were prepared by reacting compound **168** with different halogen derivatives in the presence of NaOH and water.

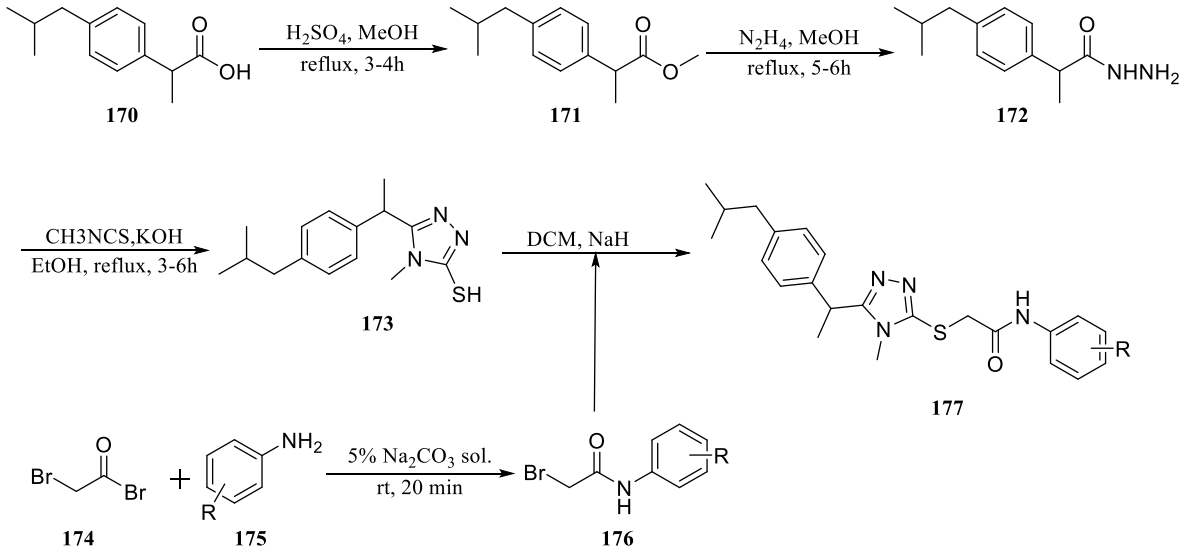


Scheme 1.29

Akhter Naheed *et al.*[121] reported the synthesis of structural hybrids of 1,2,4-triazole and acetamides through chemical modification of 2-(4-isobutylphenyl) propanoic acid **170**. These derivatives were evaluated for their anti-liver carcinoma effects against the HepG2 cell line. Among all the synthesized compounds, the derivative with two methyl groups at

the ortho-position exhibited the highest anti-proliferative activity, with an IC_{50} value of 16.782 µg/mL. This compound also demonstrated low toxicity, with a value of 1.19 ± 0.02%.

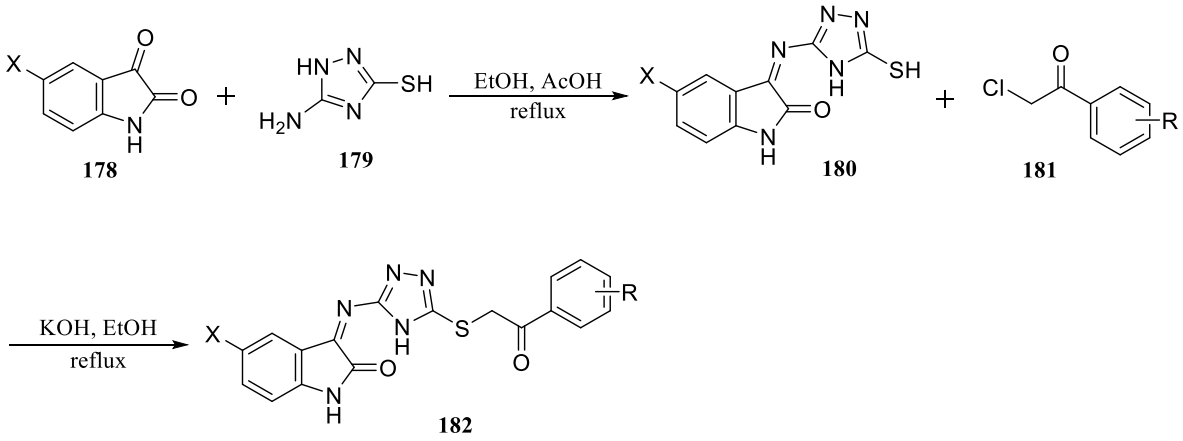
The synthesis pathway began with the Fischer esterification of compound **170**, which was refluxed with absolute methanol at 76 °C for 3–4 hours to produce compound **171**. This ester was then refluxed with hydrazine hydrate in methanol at 76 °C for 3–4 hours to yield 2-(4-isobutylphenyl)propane hydrazide **172**. Subsequently, compound **172** was converted into 5-(1-(4-isobutylphenyl)ethyl)-1,2,4-triazole-2-thiol **173** by heating it slowly at 225 °C for 3–6 hours with methyl isothiocyanate in 10% KOH and absolute ethanol. The reaction mixture was then acidified to pH 4–5 with con. HCl, leading to the precipitation of compound **173**, which was isolated by filtration. Finally, at room temperature, compound **173** was treated with various N-arylated aralkyl/alkyl/aryl 2-bromoacetamides **176** in the presence of DCM and NaH as catalysts, resulting in the formation of the final compounds **177**.



Scheme 1.30

Kiani Amir *et al.*[122] investigated the antiangiogenic effects of isatin-1,2,4-triazole conjugates. The synthesized and characterized compounds, (Z)-3-((5-(benzylthio)-4H-1,2,4-triazol-3-yl)imino)-5-haloindolin-2-one macromolecules **182**, were evaluated for their cytotoxicity using the MTT assay in vitro against U87MG (human glioblastoma

astrocytoma) and A2780 (human ovarian carcinoma) cell lines. The synthesis pathway, depicted in **Scheme 1.31**, began with the reaction of isatin derivatives with 5-amino-1H-1,2,4-triazole-3-thiol **179** in the presence of acetic acid in ethanol under reflux conditions, yielding (Z)-3-((5-mercapto-4H-1,2,4-triazol-3-yl)imino)indolin-2-one **180**. Subsequently, compound **180** was reacted with various phenacyl chloride derivatives to produce the target macromolecules **182**.



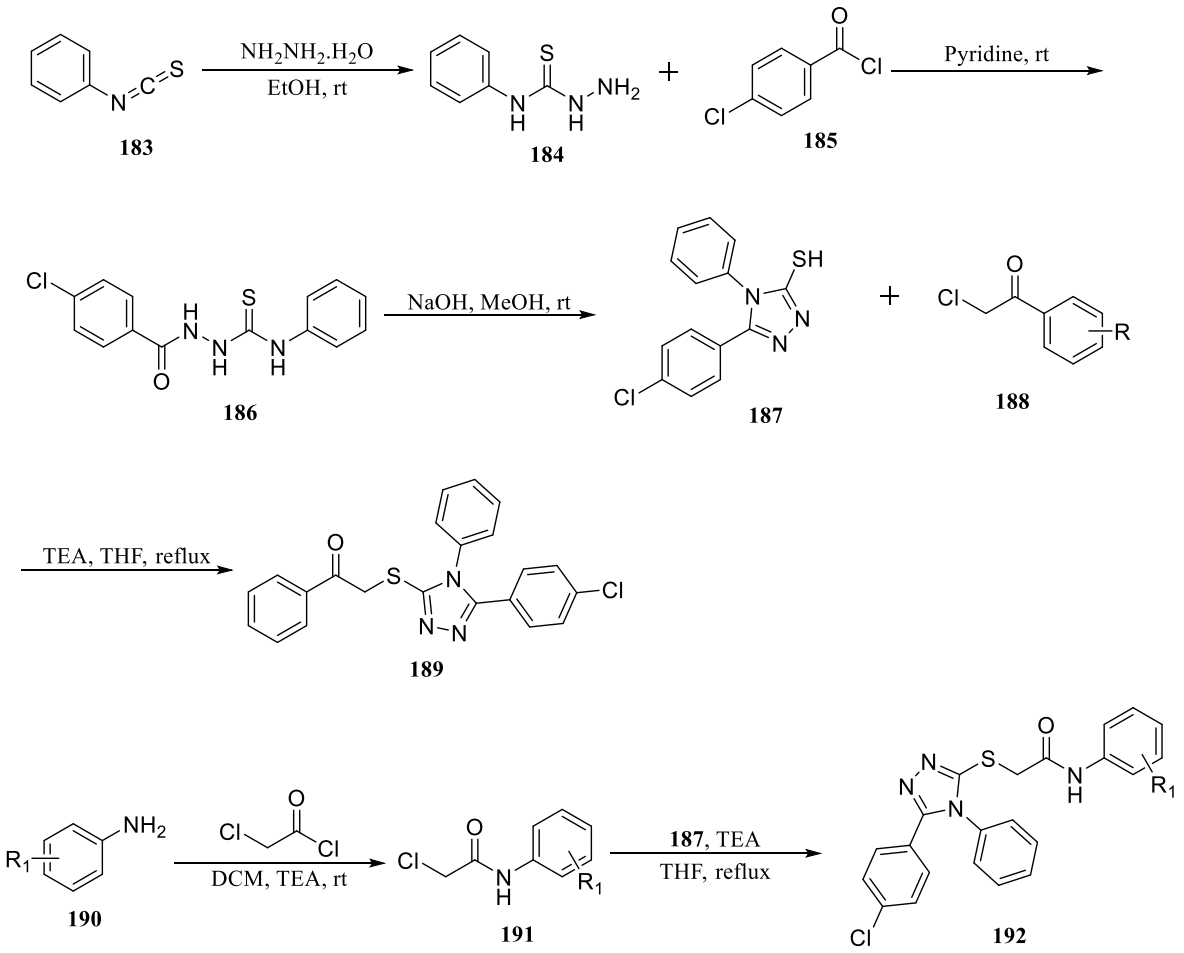
178 179 180 181 182

Scheme 1.31

Patel Krupa R. *et al*.[123] reported the discovery of a novel series of 1,2,4-triazole-3-thiol molecules using a structure-based design approach. In silico models indicated that 5-(4-chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol derivatives have drug-like properties and can mimic critical binding residues of p53. These compounds were tested for their in vitro antiproliferative activity against A549, U87, and HL60 cell lines, with twelve out of sixteen showing significant inhibitory activity in the micromolar range.

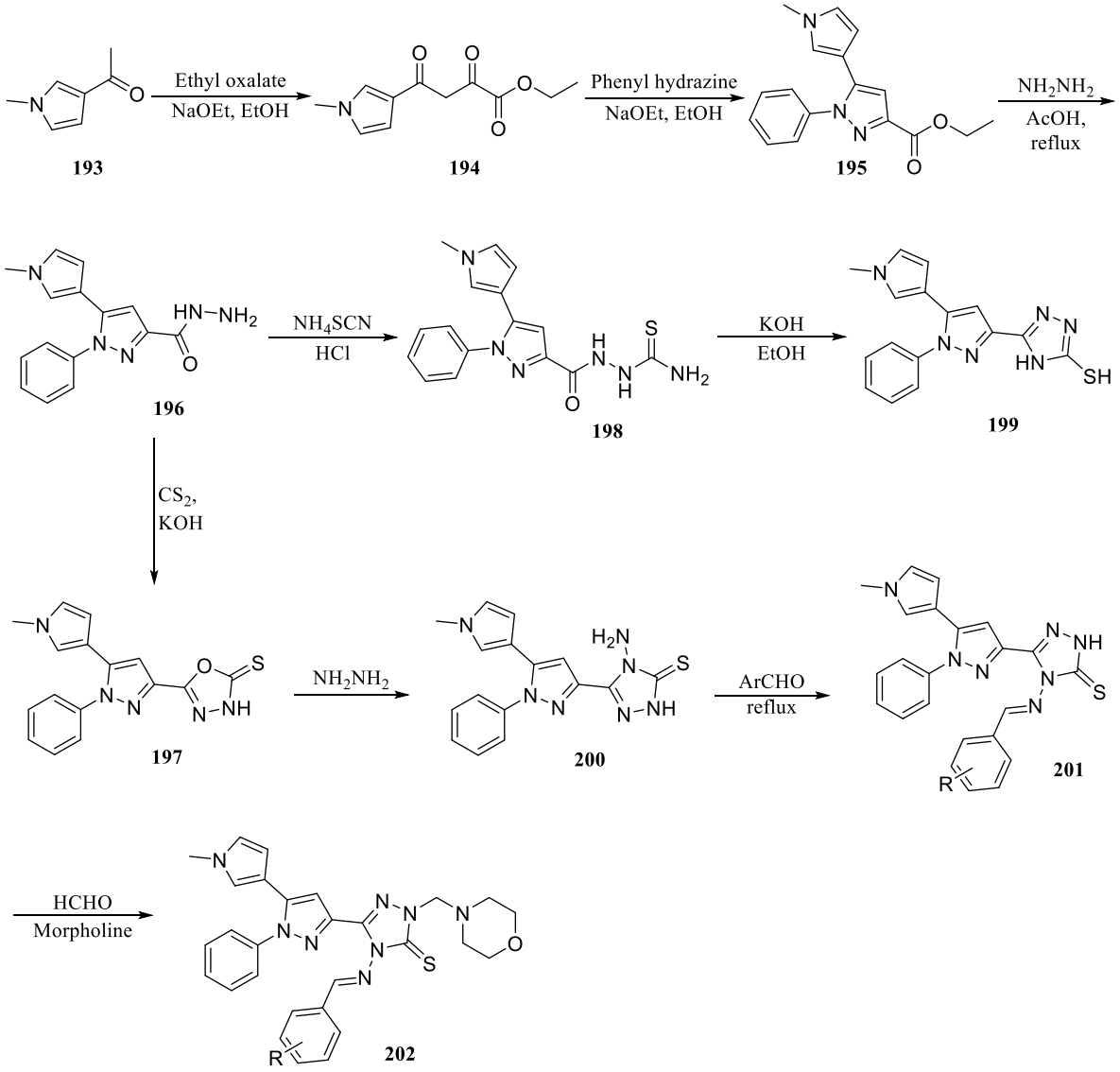
The synthesis pathway, outlined in **Scheme 1.32**, began with isothiocyanatobenzene **183** reacting with hydrazine hydrate to yield N-phenylhydrazinecarbothioamides **184**. This intermediate was then treated with 4-chlorobenzoyl chloride in pyridine to form 2-(4-chlorobenzoyl)-N-phenylhydrazinecarbothioamides **186**. Intramolecular condensation of **186** in refluxing methanolic NaOH produced the desired scaffold, 5-(4-chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol **187**. Derivatization of compound **187** was conducted via two methods. The first method involved reacting with various phenacyl chloride derivatives to obtain

products **189**. The second method involved synthesizing different chloroacetamide derivatives, which were then reacted with **187** to produce products **192**. Both methods were performed in THF with triethylamine under reflux conditions.



Scheme 1.32

Abdelrehim El-Sayed M. *et al.*[124] reported the synthesis of novel compounds, [1,3,4]oxadiazole **197** and triazole-3-thione **199**, which were screened for cytotoxic activity against human colon carcinoma cell lines. The compound [1,2,4]triazole-3-thiol **200** exhibited cytotoxic activity comparable to the standard drug Vinblastine.



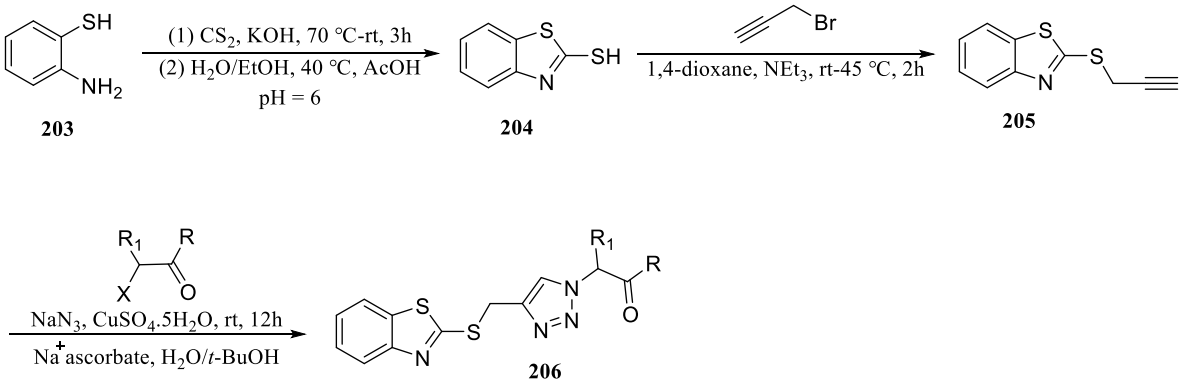
Scheme 1.33

The synthetic route, depicted in **Scheme 1.33**, began with the reaction of 3-acetyl-1-methylpyrrole **193** with diethyl oxalate in the presence of NaOEt, yielding the ethyl 2,4-dioxobutanoate derivative **194**. Cyclocondensation of compound **194** with phenyl hydrazine in AcOH under reflux conditions produced ethyl-5-(1-methyl-1H-pyrrol-3-yl)-1-phenyl-1H-pyrazole-3-carboxylate **195**. Subsequent reaction of compound **195** with hydrazine hydrate in EtOH under reflux resulted in the carboxylic acid hydrazide **196** in good yield. Heterocyclization of compound **196** with CS_2 in ethanolic KOH yielded the novel substituted [1,3,4]oxadiazole-2-thione **197**. Alternatively, the acid hydrazide **196** was reacted with NH_4SCN in the presence of HCl under reflux to produce thiosemicarbazide

198, which was further refluxed in ethanolic KOH to obtain the [1,2,4]triazole-3-thione derivative **199**. Hydrazinolysis of compound **197** on the oxygen atom of oxadiazole led to the formation of 4-amino-5-[5-(1-methyl-1H-pyrrol-3-yl)-1-phenyl-1H-pyrazol-3-yl]-4H-[1,2,4]triazole-3-thiol **200**. Condensation of compound **200** with various aromatic aldehydes resulted in the formation of Schiff bases **201**. Subsequent reaction of compounds **201** with morpholine and formaldehyde afforded the corresponding Mannich bases **202**.

Mirjafary Zohreh *et al*.[125] synthesized a new series of 1,2,3-triazole derivatives **206** based on benzothiazole through a 1,3-dipolar cycloaddition reaction between S-propargyl mercaptobenzothiazole and α-halo esters/amides, achieving moderate to good yields. The synthesized compounds were evaluated for anti-inflammatory activity via COX-2 inhibition, antifungal activity via CYP51 inhibition, and anti-tuberculosis activity targeting proteins ENR, DPRE1, pks13, and thymidylate kinase using molecular docking studies. ADMET analysis indicated that phenyl and m-toluidine substituted compounds were the most promising drug-like candidates among the six synthesized molecules.

The synthesis began with the reaction of 2-aminothiophenol **203** with CS_2 to produce 2-mercaptobenzothiazole **204**, as shown in **Scheme 1.34**. This compound was then propargylated using propargyl bromide at 45 °C in dioxane in the presence of Et_3N. The azide intermediates were formed in situ by reacting the corresponding α-halo esters/amides with sodium azide. Finally, the desired 1,2,3-triazoles **206** were synthesized via an azide-alkyne [3 + 2] cycloaddition reaction at room temperature.

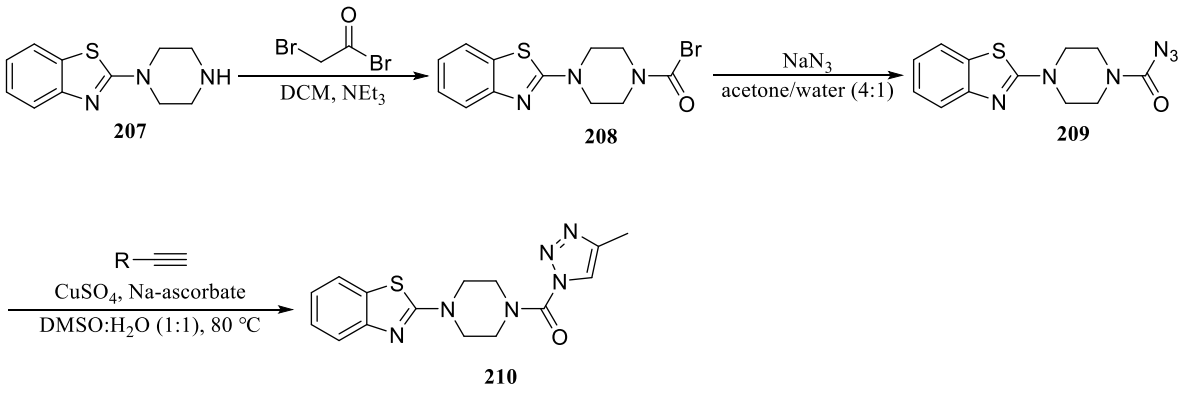


203 204 205 206

Scheme 1.34

Aouad Mohamed R. *et al.*[126] designed and synthesized novel regioselective 1,2,3-triazole-based benzothiazole-piperazine conjugates using the click chemistry approach. These newly created 1,2,3-triazole hybrids were evaluated for their antiproliferative activity against four selected human cancer cell lines: MCF7, T47D, HCT116, and Caco2. The majority of the synthesized compounds demonstrated moderate to potent activity against all the examined cancer cell lines. The synthetic protocols for these bioactive compounds are depicted in **Scheme 1.35**.

The precursor, 2-azido-1-(4-(benzo[d]thiazol-2-yl)piperazin-1-yl)ethanone **209**, required for the 1,3-dipolar cycloaddition reaction, was synthesized by first acylating 2-(piperazin-1-yl)benzo[d]thiazole **207** with bromoacetyl bromide in the presence of triethylamine in dichloromethane at room temperature, resulting in the intermediate bromoacetylpiperazine **208**. This intermediate was then treated with sodium azide in a mixture of acetone and water (4:1), yielding the targeted azide **209**. The 1,3-dipolar cycloaddition reaction of the synthesized azide **209** with commercially available functionalized alkynes was carried out in the presence of copper sulphate and sodium ascorbate as catalysts, using a DMSO/H_2O (1:1) solvent system. This reaction produced novel benzothiazole-piperazine-1,2,3-triazole hybrids **210** with different hydroxylated and/or ester-based alkyl side chains.

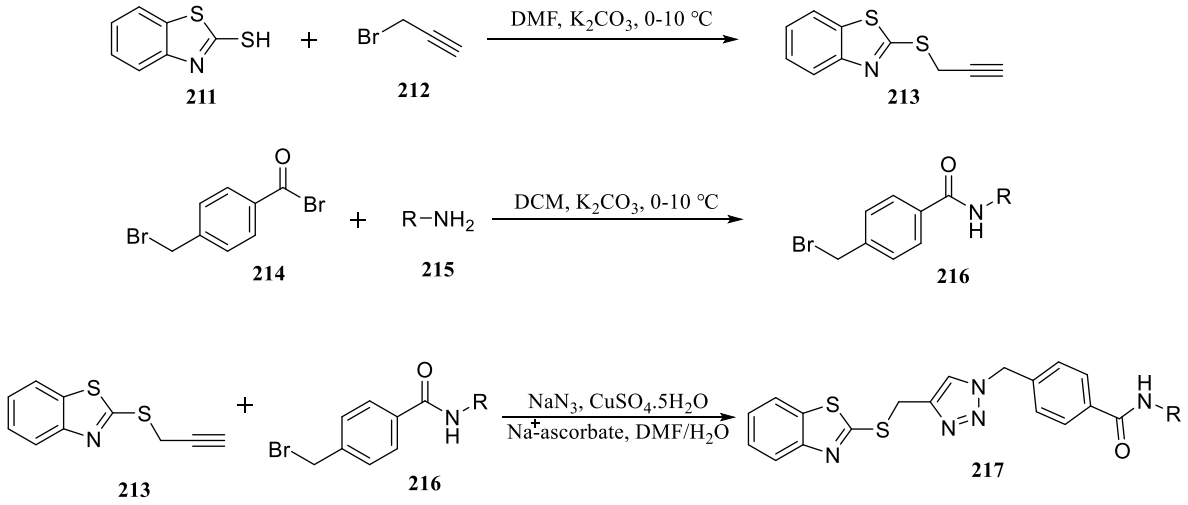


Scheme 1.35

Kaushik C. P. *et al.*[127] reported the synthesis of a series of benzothiazole linked 1,4-disubstituted 1,2,3-triazoles through a copper(I) catalyzed azide-alkyne cycloaddition reaction. These triazole derivatives were evaluated for their in vitro antibacterial activities

against two Gram-positive bacteria and two Gram-negative bacteria using the serial dilution technique, demonstrating moderate to good activity. Among the synthesized triazoles, the compound with a 4-Br substitution exhibited the most promising antibacterial activity.

The synthetic pathway for the benzothiazole-containing triazoles **217** is illustrated in **Scheme 1.36**. The terminal alkyne 2-(prop-2-yn-1-ylthio)benzothiazole **213** was synthesized from the propargylation of 2-mercaptobenzothiazole **211** in DMF with propargyl bromide **212** in the presence of K_2CO_3. Concurrently, 4-(bromomethyl)-N-aryl benzamide derivatives **216** were synthesized by reacting 4-(bromomethyl)benzoyl bromide **214** with aromatic amines **215** using K_2CO_3. Finally, derivatives **216** and **213** were dissolved in DMF, followed by the addition of aqueous NaN_3, a catalytic amount of $CuSO_4.5H_2O$, and sodium ascorbate. The mixture was stirred for 6-10 hours at 25-40 °C to obtain the targeted 1,4-disubstituted 1,2,3-triazoles **217**.



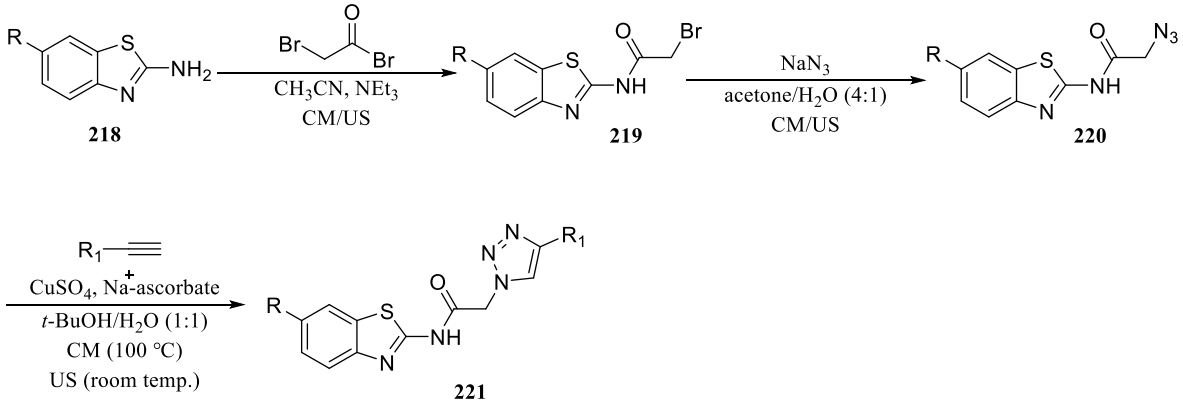
211 212 213 214 215 216 213 216 217

Scheme 1.36

Rezki Nadjet[128] reported the synthesis of N-(benzo[d]thiazol-2-yl)-2-(4-substituted-1H-1,2,3-triazol-1-yl)acetamides **221** via a 1,3-dipolar cycloaddition reaction between 2-azido-N-(benzo[d]thiazol-2-yl)acetamide derivatives **220** and various alkynes, performed both with and without ultrasound irradiation. The compounds were screened for antimicrobial activity against three Gram-positive bacteria, three Gram-negative bacteria,

and two fungal strains. Most compounds showed promising antimicrobial activity with a MIC range of 4–16 μg/mL.

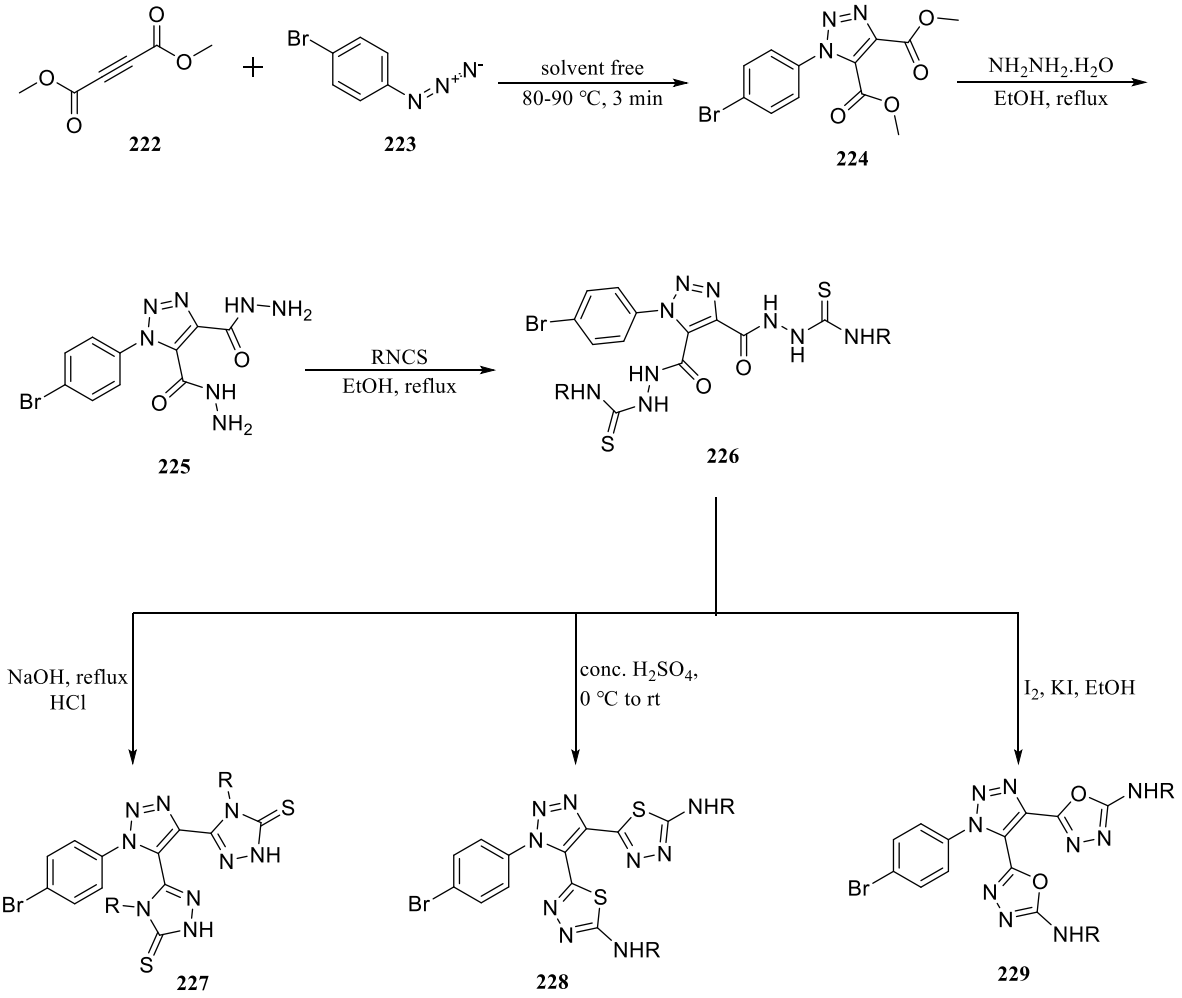
The synthesis began with 2-aminobenzothiazole derivatives **218**, as depicted in **Scheme 1.37**. Compounds **219** were obtained by acylating unsubstituted or substituted amino benzothiazole **218** with bromoacetyl bromide in the presence of triethylamine in acetonitrile at room temperature. Using ultrasound irradiation, the same products **219** were produced more efficiently, with higher yields and shorter reaction times (1 hour). Next, compounds **219** were treated with sodium azide in a mixture of acetone and water (4:1, v/v) at room temperature for 24 hours, yielding azidobenzothiazoles **220**. Ultrasound irradiation significantly accelerated this reaction, reducing the required time to 2–3 hours. Finally, 1,3-dipolar cycloadditions between azidobenzothiazoles **220** and various terminal alkynes were conducted under conventional heating at 100 °C for 6–10 hours with copper sulfate and sodium ascorbate as catalysts, in a *t*-BuOH/H_2O (1:1, v/v) solvent system. Using ultrasound irradiation, the reaction time was reduced to 3–6 hours, with comparable yields.



Scheme 1.37

Aouad Mohamed Reda *et al.*[129] reported the design and synthesis of novel 1,2,3-triazole-based scaffolds, including 1,2,4-triazole, 1,3,4-oxadiazole, and 1,3,4-thiadiazole. Docking studies indicated that these 1,2,3-triazole derivatives exhibited strong binding affinity (-6.0 to -8.8 kcal/mol) to protease active sites, suggesting potential antiviral activity against COVID-19.

The synthesis of dimethyl 1-(4-bromophenyl)-1H-1,2,3-triazole-4,5-dicarboxylate **224** involved a solvent-free 1,3-dipolar cycloaddition of dimethylacetylenedicarboxylate **222** and p-bromoazidobenzene **223** at 80–90 °C for 3 minutes (**Scheme 1.38**). The resulting ester **224** was treated with hydrazine hydrate in refluxing ethanol for 4 hours to produce bis-acid hydrazide **225**. This hydrazide was then refluxed with phenyl, ethyl, and methyl isothiocyanate in ethanol for 6 hours to yield bis-acid thiosemicarbazide **226**. Compound **226** underwent base-assisted intramolecular cyclization in 10% NaOH, forming 1,2,3-triazole-bis(1,2,4-triazole-3-thione) **227**. Dehydrative cyclization with sulfuric acid at 0 °C produced bis-(2-aryl/alkylamino-1,3,4-thiadiazoles) **228**, and oxidative cyclization with I_2/KI in ethanol yielded 1,2,3-triazole-bis-(2-aryl/alkylamino-1,3,4-oxadiazoles) **229**.

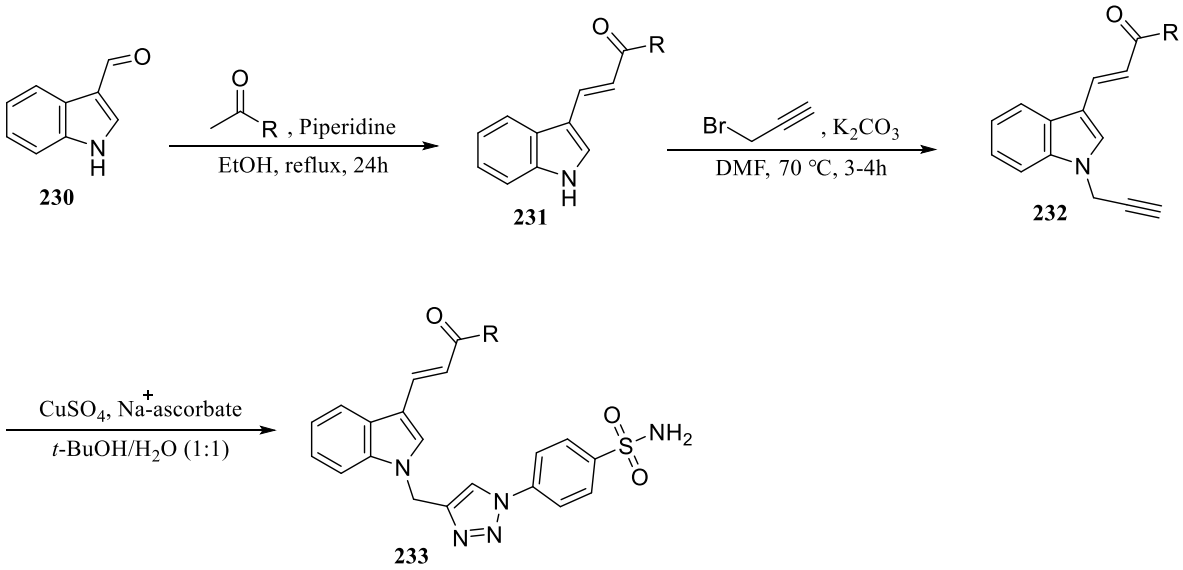


Scheme 1.38

1.6.3 Review of Indole-Based Triazole and Benzothiazole Hybrids: Synthesis and Bioactivity Insights

Singh Priti *et al.*[130] reported the synthesis of a novel series of indolyl chalcones incorporating benzenesulfonamide-1,2,3-triazole **233** via a click chemistry reaction. These compounds were evaluated for their inhibitory activity against a panel of human carbonic anhydrases (hCAs). Many of the newly synthesized compounds demonstrated noteworthy inhibition constants in the nanomolar range, with some derivatives exhibiting greater potency than the standard drug acetazolamide (AAZ) against the hCA I isoform. Specifically, compounds with 4-Br (18.8 nM), 3-OCH_3 (38.3 nM), and 4-CH_3 (50.4 nM) were found to be 13, 6, and 5 times more potent than AAZ, respectively.

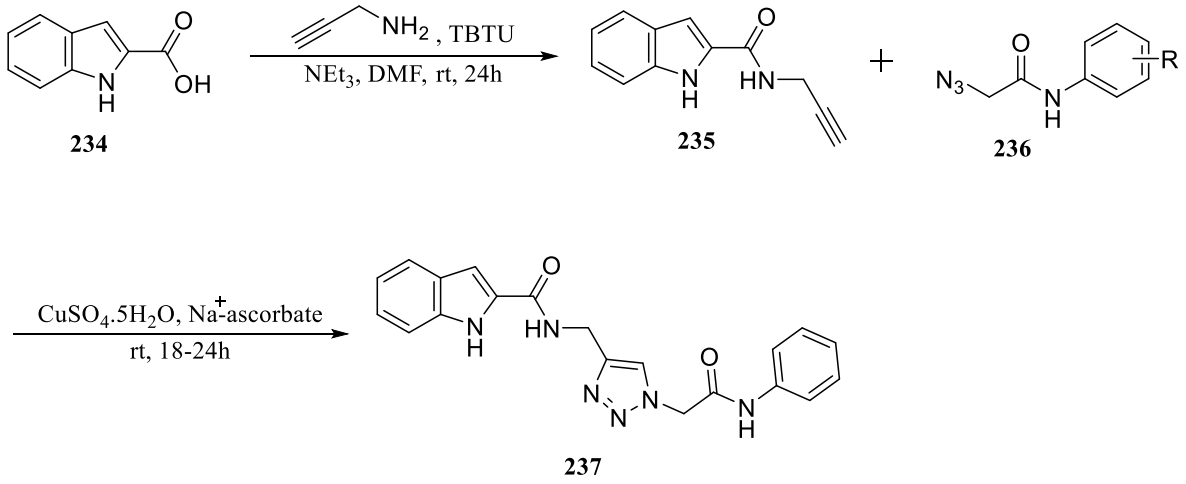
The synthetic strategy for these target compounds is illustrated in **Scheme 1.39**. The chalcones **231** were synthesized through a Knoevenagel condensation by reacting indole-3-carboxaldehyde with various acetophenones, using piperidine as the base and ethanol as the solvent. The resulting chalcones **231** were then propargylated with propargyl bromide and anhydrous K_2CO_3 in dry DMF to yield intermediates **232**. Concurrently, benzene sulphonamide azide was prepared from sulphanilamide via a diazotization reaction using concentrated HCl, $NaNO_2$, and NaN_3. This azide was then subjected to a click reaction with the substituted intermediates **232** in the presence of $CuSO_4$ and sodium ascorbate, using *t*-BuOH and H_2O (1:1) as the solvent mixture, to afford the **233**.



Scheme 1.39

Sayahi Mohammad Hossein *et al.*[131] designed and synthesized a novel series of N-phenylacetamide-1,2,3-triazole-indole-2-carboxamide derivatives **237** as potent α-glucosidase inhibitors. These compounds, which incorporate indole and carboxamide-1,2,3-triazole-N-phenylacetamide moieties, were synthesized using a click chemistry reaction and subsequently evaluated against yeast α-glucosidase. All the synthesized compounds exhibited superior potency compared to the standard inhibitor acarbose. Notably, the compound with a 4-Br substitution showed the highest inhibitory activity, demonstrating approximately 28 times greater inhibition than the standard inhibitor.

The synthetic route for these derivatives is detailed in **Scheme 1.40**. N-(prop-2-yn-1-yl)-1H-indole-2-carboxamide **235** was synthesized by reacting 1H-indole-2-carboxylic acid **234** with propargylamine in the presence of TBTU and Et_3N. The click reaction between compound **235** and azide derivatives **236** yielded the target compounds **237**. This method provided an efficient synthesis of N-phenylacetamide-1,2,3-triazole-indole-2-carboxamide derivatives with significant α-glucosidase inhibitory activity.

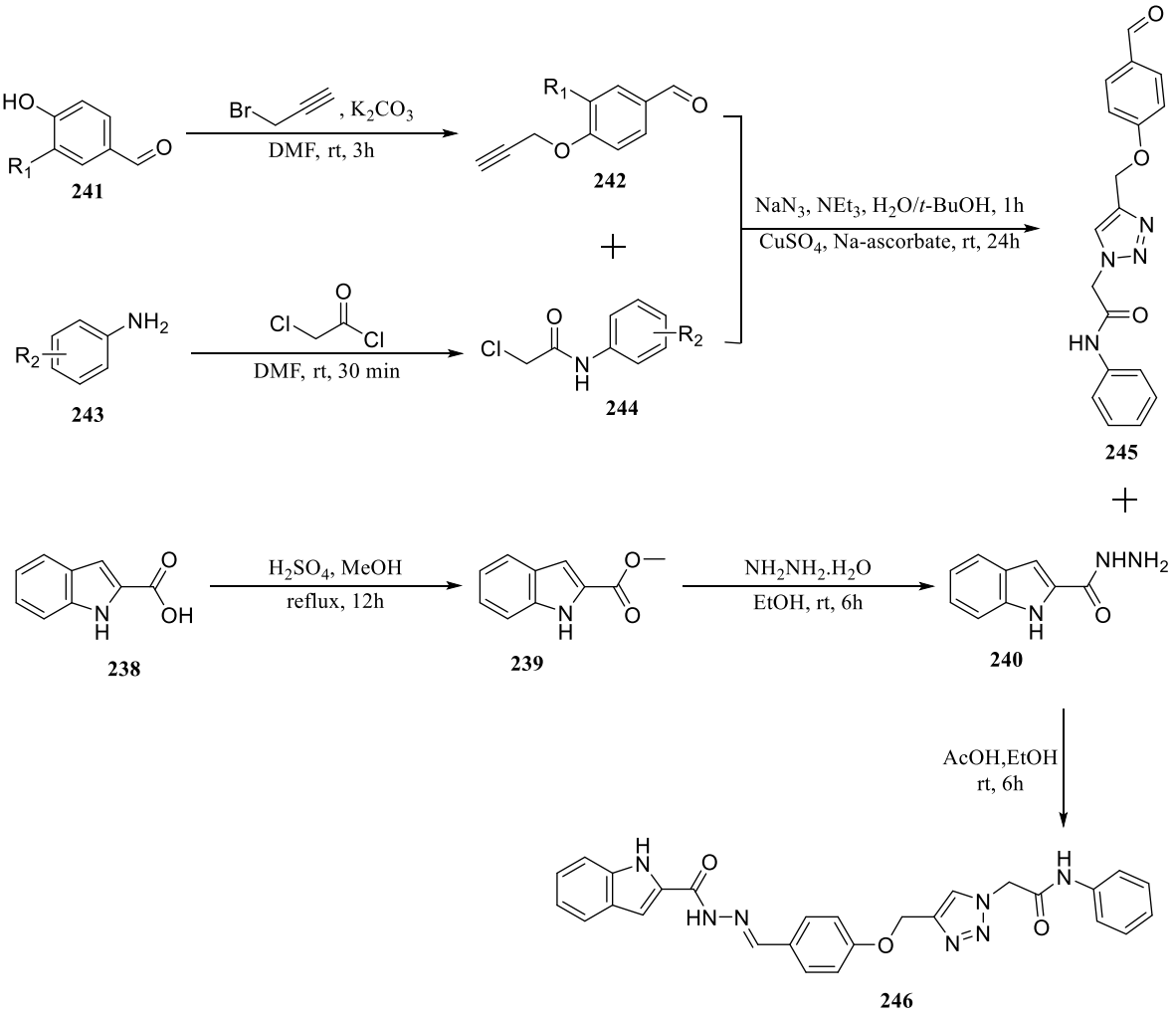


Scheme 1.40

Emadi Mehdi *et al.*[132] designed and synthesized a new series of indole-carbohydrazide-phenoxy-1,2,3-triazole-N-phenylacetamide hybrids **246** as potent α-glucosidase inhibitors. These compounds were evaluated for their α-glucosidase inhibitory activity, showing superior potency compared to acarbose, the standard inhibitor. Notably,

the compound with a 4-OMe substitution exhibited an IC_{50} of 6.31 μM against MCF-7 cells, making it 118.8 times more potent than acarbose.

The synthesis, outlined in **Scheme 1.41**, began with converting indole-2-carboxylic acid **238** to methyl 1H-indole-2-carboxylate **239** using sulfuric acid in methanol, followed by conversion to 1H-indole-2-carbohydrazide **240** with hydrazine in ethanol. Concurrently, 4-(prop-2-ynyloxy)benzaldehydes **242** were synthesized from 4-hydroxy-benzaldehyde **241** and propargyl bromide with K_2CO_3 in DMF. N-phenyl-2-chloroacetamide derivatives **244** were prepared by reacting aniline derivatives **243** with chloroacetyl chloride in DMF. These intermediates (242 and 244) were further reacted: various chloride derivatives **244** were reacted with sodium azide in a mixture of H_2O and *t*-BuOH (1:1) in the presence of Et_3N at room temperature. Subsequently, a mixture of compounds **242**, sodium ascorbate, and $CuSO_4 \cdot 5H_2O$ was added to the azide mixture, and the reaction was continued at room temperature to yield the 1,2,3-triazole derivatives **245**. Finally, 1H-indole-2-carbohydrazide **240** was reacted with 1,2,3-triazole derivatives **245** in acetic acid/ethanol to obtain the target compounds **246**.

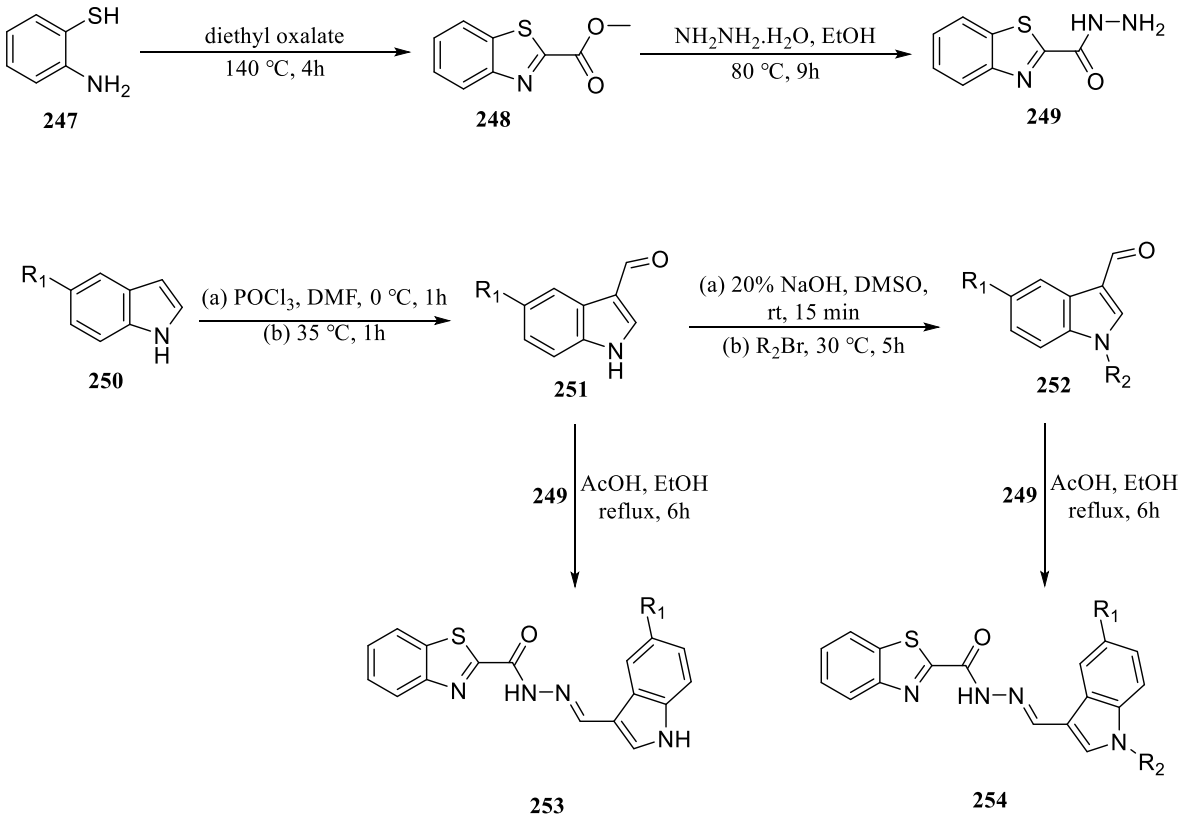


Scheme 1.41

Liu Kai *et al.*[133] reported the design, synthesis, and evaluation of a series of new N-acyl hydrazone derivatives containing benzothiazole and indole moieties for their in vitro antiproliferative activity against the Hep G2 cancer cell line. One compound, lacking any substitution, exhibited outstanding antiproliferative activity with an IC_{50} value of 0.78 μM against Hep G2. Additionally, it was noted that C-5 substitutions on the indole ring might play a crucial role in enhancing cytotoxic activities. The synthetic method for these linked N-acyl hydrazone derivatives (**253** and **254**) is illustrated in **Scheme 1.42**.

The synthesis began with the condensation of compound **247** with diethyl oxalate to produce compound **248**, which was then reacted with hydrazine to obtain the target hydrazides **249**. Under Vilsmeier-Haack conditions (DMF-$POCl_3$), compounds **250** were

transformed into the corresponding 3-carboxaldehyde functionalized indoles **251**. The N-ethyl, N-isopropyl, and N-phenylsulfonyl derivatives of indole-3-carboxaldehyde **252** were synthesized by reacting compound **251** with bromoethane, 2-bromopropane, or benzene sulfonyl chloride in the presence of NaOH in a mixed solvent of DMSO and water. This reaction, utilizing DMSO and water as a mixed solvent, achieved unprecedented high yields. Finally, the condensation of compound **249** with either compounds **251** or **252** resulted in the formation of the target compounds (**253** and **254**).

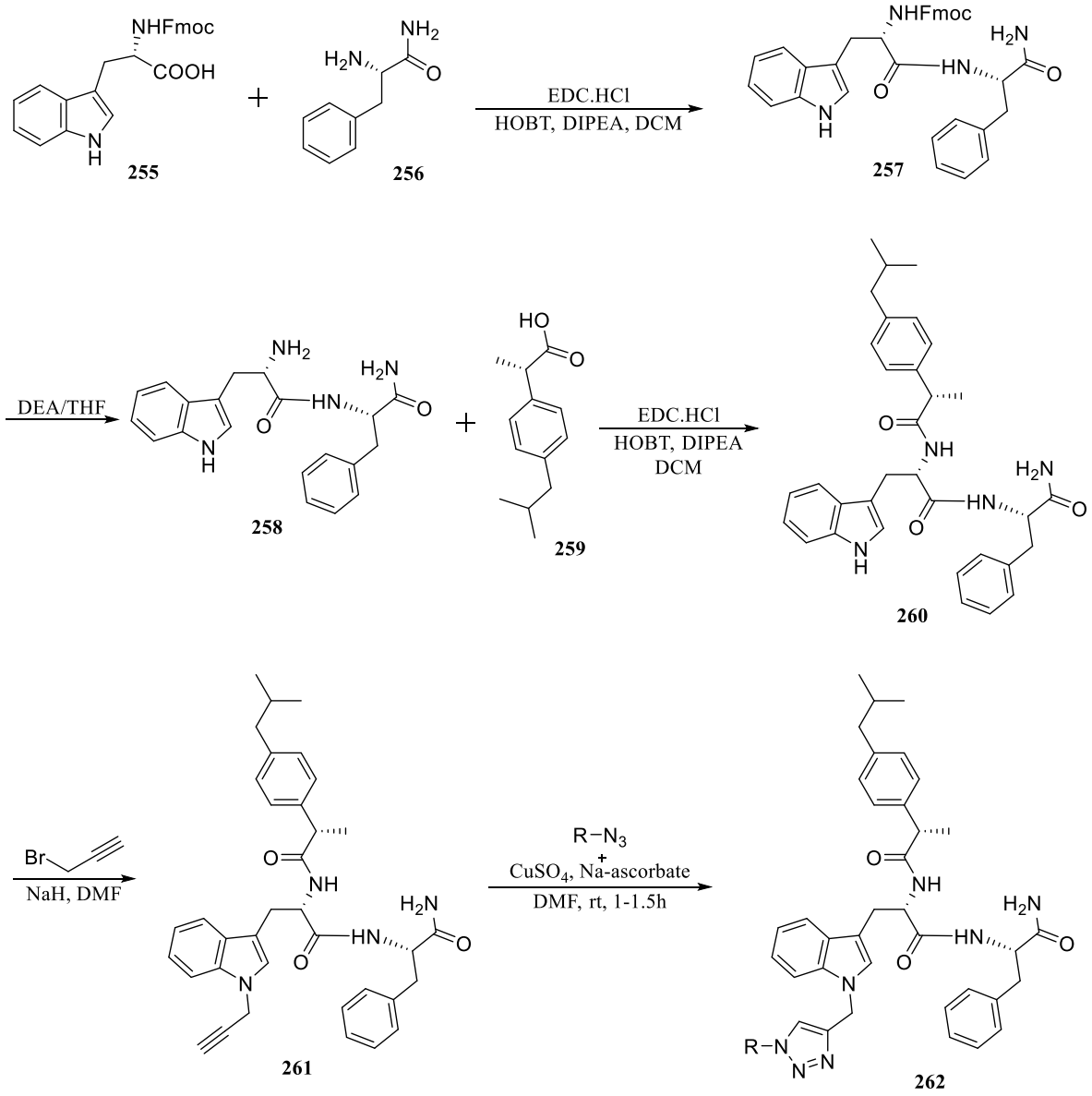


Scheme 1.42

Suryapeta Srinivas *et al.*[134] reported the design and synthesis of a series of new indole-triazole-peptide conjugates as potential dual-activity agents for antibacterial and anticancer applications. Most compounds exhibited moderate to notable antibacterial activity, with the compound featuring a 2-Cl substitution demonstrating good activity against all tested strains. The cytotoxic activities of some synthesized compounds were evaluated using an MTT assay on the human lung cancer cell line A549. Among the

promising compounds, those with 2,6-dimethyl, 2-Cl, and 2-NO_2 substitutions showed low IC_{50} values. The synthetic route for the target compounds **262** is depicted in **Scheme 1.43**.

The synthesis process began with the reaction of S-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(1H-indole-3-yl)propanoic acid **255** and (2S)-2-amino-3-phenylpropanamide **256** in the presence of coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and hydroxyl benzotriazole (HOBT), along with N,N-diisopropylethylamine (DIPEA) in DCM. This reaction yielded the peptide **257** as a white solid. Deprotection of the Fmoc group in compound **257** with diethylamine in THF afforded the desired amine **258**. The amine **258** was then coupled with ibuprofen **259** using EDC·HCl, HOBT, and DIPEA in DCM to produce compound **260**. The regioselective N-propargylation of the indole ring in compound **260** was achieved using propargyl bromide in the presence of NaH in DMF, resulting in the terminal alkyne **261**. Finally, the terminal alkyne **261** was coupled with a series of azides in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate at room temperature using DMF as a solvent, yielding the target molecules **262**.


NHFmoc
COOH
255
NH2
H2N
O
256
EDC.HCl
HOBT, DIPEA, DCM
NHFmoc
H2N
HN
O
257
DEA/THF
258
HO
259
EDC.HCl
HOBT, DIPEA
DCM
260
Br
NaH, DMF
261
R−N3
+
CuSO4, Na-ascorbate
DMF, rt, 1-1.5h
262

Scheme 1.43