T.C.
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SYNTHESIS AND BIOLOGICAL ACTIVITY STUDIES OF SOME 3-ARYL-2-(4-(SUBSTITUTEDPHENYL)THIAZOL-2-YL)ACRYLONITRILE DERIVATIVES

Pharm. Shukurah ANAS, MS

Pharmaceutical Chemistry Program

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This thesis study has been approved and accepted as a Master dissertation in "Pharmaceutical Chemistry Program" by the assessment committee, whose members are listed below, on 03.04.2023.

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ETHICAL DECLARATION

In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with supervisor (Assoc. Prof. Keriman ÖZADALI SARI) and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

(Signature)

Shukurah ANAS

ACKNOWLEDGEMENT

Firstly, I would like to thank my supervisor, Assoc. Prof. Keriman ÖZADALI SARI, for her invaluable guidance and support throughout the research process. Her expertise, encouragement, and constructive criticism have been instrumental in shaping my research.

I would also like to express my appreciation to Prof. Dr. NESRİN GÖKHAN KELEKÇİ, Head of Department, for providing me with the necessary resources and facilities to carry out my research work.

I am also thankful to Siva Krishna VAGOLU and Assoc. Prof. Ceren ÖZKUL KOÇAK for their contribution towards the biologic activity aspect of my research.

Additionally, I would like to express my appreciation to the faculty staff and research assistants, who provided their valuable support and assistance throughout my research journey.

I cannot forget to thank my parents, Alhaji Anas ADAM and Hajia Sadatu ADAMS, and my siblings for their unwavering love, encouragement, and support throughout my academic journey.

Finally, I would like to acknowledge my supportive friends, especially Dr. Rashid ALHASSAN, for their encouragement and for always being there to lend a helping hand and my girl Koko, who has been a constant source of comfort and joy. Thank you all once again for your support and encouragement.

ABSTRACT

Anas, S., Synthesis and Biological Activity Studies of Some 3-Aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile Derivatives, Hacettepe University Graduate School of Health Sciences, Faculty of Pharmacy Department of Pharmaceutical Chemistry, Master of Science Thesis, Ankara, 2023. In this study, the synthesis of 16 novel derivatives of 3-aryl-2-(4-(substituted phenyl)thiazol-2-yl)acrylonitrile (**1**-**16**), including two compounds (**14** and **15**) previously reported in the literature, was carried out and their structures were elucidated using $^{1}H/^{13}C$-NMR, IR, and HRMS methods. The antimycobacterial activities of the synthesized compounds were investigated against *Mycobacterium tuberculosis* H37Rv strain, but none of the compounds showed MIC values lower than 50 μM. Additionally, the antibacterial activities of the target compounds against various gram-positive and gram-negative bacteria, and their antifungal activities against Candida species were evaluated using microdilution methods, with ciprofloxacin and fluconazole used as reference drugs.

Among the tested compounds, 2-(4-(2,4-dichlorophenyl)thiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (**10**) was found to be the most promising antimicrobial agent, showing an MIC value of 32 μg/ml against *C. parapsilosis*. Based on these findings, compound **10** was identified as a lead compound for obtaining more active derivatives.

Key Words: Antimycobacterial, antimicrobial, antibacterial, antifungal, tuberculosis, acrylonitrile, thiazole.

ÖZET

Anas, S., Bazı 3-Aril-2-(4-(sübstitüefenil)tiyazol-2-il)akrilonitril Türevlerinin Sentezi ve Biyolojik Aktivite Çalışmaları, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, Eczacılık Fakültesi, Farmasötik Kimya Anabilim Dalı, Yüksek Lisans Tezi, Ankara, 2023. Bu çalışmada, 2 tanesi literatürde kayıtlı (**14** ve **15**) olmak üzere 16 adet yeni 3-aril-2-(4-(sübstitüefenil)tiyazol-2-il)akrilonitril türevinin (**1-16**) sentezi yapılmış ve yapıları $^{1}H/^{13}C$-NMR, IR ve HRMS yöntemleri ile aydınlatılmıştır. Sentezlenen bileşiklerin antimikobakteriyel aktiviteleri *Mycobacterium tuberculosis* H37Rv suşuna karşı incelenmiş ancak bileşiklerden hiçbiri 50 μM' dan daha düşük MİK değeri göstermemiştir. Buna ek olarak, hedef bileşiklerin çeşitli gram-pozitif ve gram-negatif bakterilere karşı antibakteriyel ve Candida türlerine karşı antifungal aktiviteleri mikrodilüsyon yöntemleri kullanılarak incelenmiş, referans ilaçlar olarak siprofloksasin ve flukonazol kullanılmıştır.

Test edilen bileşikler arasında, 2-(4-(2,4-diklorofenil)tiyazol-2-il)-3-(4-(trifluorometil)fenil)akrilonitril (**10**), tüm mikroorganizmalar içinde *C. parapsilosis*'e karşı MİK = 32 μg/ml değeri ile en umut verici antimikrobiyal aktivite gösteren bileşik olmuştur. Bu bulgulara dayanarak, 10 numaralı bileşik daha aktif bileşikler elde etmek için öncü bir bileşik olarak belirlenmiştir.

Anahtar Kelimeler: Antimikobakteriyel, antimikrobiyal, antibakteriyel, antifungal, tüberküloz, akrilonitril, tiyazol

CONTENT

ABBREVIATIONS

3D-QSAR	Three-Dimensional Quantitative Structure-Activity Relationship
5-LOX	5-Lipoxygenase
AChE	Acetylcholinesterase
AIBN	Azobisisobutyronitrile
AML	Acute Myeloid Leukemia
AR	Antibiotic Resistance
BCG	Bacillus Calmette-Guérin
CD	Centers For Disease Control and Prevention
CoMSIA	Comparative Similarity Indices Analysis
COVID-19	Coronavirus Disease 2019
DABCO	1,4-Diazabicyclo[2.2.2]Octane
DCM	Dichloromethane
DHFR	Dihydrofolate Reductase
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DR-TB	Drug-Resistant Tuberculosis
E	Ethambutol
FQs	Fluoroquinolones
H	Isoniazid
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome
IC_{50}	Half-maximal inhibitory concentration
IFIs	Invasive Fungal Infections
IMP	Imipenem
INH	Isoniazid
IR	Infrared
KOtBu	Potassium tert-Butoxide
LA	Lewis Acid

LAT	Lysine-Aminotransferase
LTBI	Latent Tuberculosis Infection
MA	Mycolic Acid
MDR-TB	Multidrug-Resistant TB
MIC	Minimum Inhibition Concentration
MRP	Meropenem
MRSA	Methicillin-Resistant Staphylococcus Aureus
Mtb	*Mycobacterium tuberculosis*
NaSCN	Sodium thiocyanate
NBS	N-Bromosuccinimide
NCTS	N-Cyano-N-Phenyl-p-Methylbenzenesulfonamide
NH	Nitrile Hydratase
NMR	Nuclear Magnetic Resonance
NSAID	Nonsteroidal Anti-Inflammatory Drug
NTS	Nitrilase
PAN	Polyacrylonitriles
PAS	P-Aminosalicylic Acid
PBP	Penicillin Binding Protein
pre-XDR-TB	Pre-Extensively Drug-Resistant TB
***p*TsOH**	*p*-Toluenesulfonic Acid
PZA	Pyrazinamide
R	Rifampicin
RR-TB	Rifampicin-Resistant
SLDs	Second-Line Drugs
TB	Tuberculosis
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
WHO	The World Health Organization
XDR-TB	Extensively Drug-Resistant TB
Z	Pyrazinamide

FIGURES

TABLES

1. INTRODUCTION

Tuberculosis (TB) is a communicable disease that ranks as a primary contributor to morbidity and mortality on a global scale. *Mycobacterium tuberculosis* (Mtb), the causative agent of TB, is a bacillus whose genetically identical descendants trace back to the Paleolithic era in East Africa 3.3 million years ago. This bacterial pathogen emerged as an epidemic in Europe and North America during the 18th and 19th centuries (1, 2). Despite a decrease in tuberculosis (TB) prevalence in developed countries over the last century, it remains a persistent challenge in developing nations due to its severe social consequences (3). Prior to the emergence of coronavirus disease 2019 (COVID-19), TB was the leading infectious disease responsible for mortality, surpassing human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS). The latest annual report on TB from the World Health Organization (WHO) reveals that in 2021, an estimated 106 million individuals were afflicted with the disease, compared to 101 million in 2020, and that 16 million individuals died from TB in 2021, an increase from 15 million in 2020 (3).

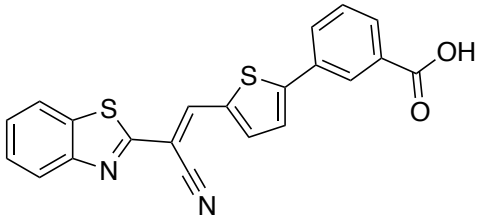
Isoniazid (H) (1952), pyrazinamide (Z) (1954), ethambutol (E) (1963), and rifampicin (R) were the first-line drugs used to treat tuberculosis, but their success was severely impacted by the emergence of drug resistance (4, 5). Drug-resistant tuberculosis (DR-TB) poses a continuing global health threat, leading to increased morbidity and mortality, negative health outcomes, escalated treatment expenses, among other consequences (6). WHO categorizes drug-resistant TB into five main types: isoniazid-resistant TB, rifampicin-resistant (RR)-TB, and multidrug-resistant TB (MDR-TB), pre-extensively drug-resistant TB (pre-XDR-TB), which is MDR-TB with resistance to a fluoroquinolone, and extensively drug-resistant TB (XDR-TB), which is TB resistant to any fluoroquinolone and at least one of the second-line injectables, such as amikacin, capreomycin, or kanamycin (7). More recently, there have been concerns about the emergence of global resistance jeopardizing the effectiveness of novel MDR-TB treatment regimens, such as bedaquiline, a WHO-approved group A drug for treating MDR-TB.

This underscores the ongoing need for developing new antituberculosis agents, improving access to diagnosis, and implementing effective treatment regimens for all forms of DR-TB (8).

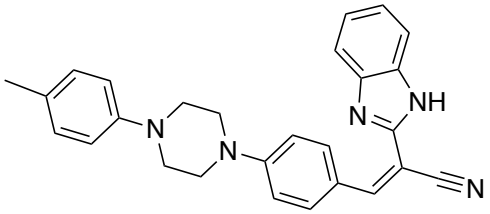
Bacterial and fungal infections can lead to a range of severe illnesses, and antibiotic resistance (AR) is a significant global healthcare challenge, with developing nations often experiencing a greater impact (9). In 2013, the Centers for Disease Control and Prevention (CDC) published a list of emerging antibiotic resistance threats, which was updated in 2019 based on the latest national estimates of death and infection caused by 18 antimicrobial-resistant bacteria and fungi. The list is structured according to the level of urgency, with some microorganisms classified as Serious Threats. These include Drug-resistant Candida Species, extended-spectrum β-lactamase-producing Enterobacterales (ESBL-E), Methicillin-resistant Staphylococcus aureus (MRSA), and Drug-resistant Streptococcus pneumoniae, among others.

The nitrile group is a vital functional group that can be found in both pharmaceuticals and natural products. Over the last few decades, the FDA has approved over 30 nitrile-containing pharmaceuticals for the treatment of a wide range of clinical conditions (10). Acrylonitrile, which is composed of a nitrile group attached to a vinylic moiety, is a promising pharmacophore that continues to pique the interest of medicinal chemistry researchers. Acrylonitriles are crucial building blocks for the synthesis of many biologically active compounds because of their wide range of biological activities (11-16), as well as their chemical importance, which includes improving the solubility of compounds and the potential for the formation of hydrogen bonds or hydrophobic interactions (17). The acrylonitrile moiety is present in a number of drug molecules that have been marketed, including entacapone (18), rilpivirine (19), teriflunomide (20), Luliconazole (21) and others. Among the numerous studies on the biological and pharmacological activities (such as cancer (22-26), antibacterial (11, 14, 27), antiparasitic (16, 28, 29) , analgesic (30, 31), antialzheimer (32, 33) etc.) of acrylonitrile derivatives carrying heterocyclic rings, their antimycobacterial activity has steered extensive studies

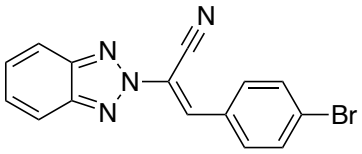
for their potential as an anti-TB drug candidate (15, 17, 34, 35).



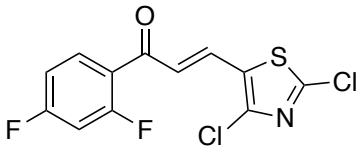
MIC=64.43 μg/mL against *M. Tuberculosis* (15)



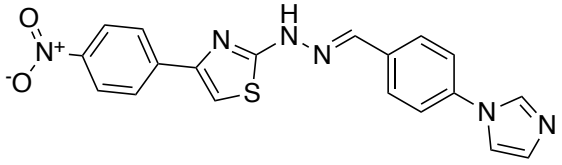
MIC=0.78 μg/mL against *M. Tuberculosis* (34)



MIC=6.25 μg/mL against *M. Tuberculosis* and MIC=12.5 μg/mL against M. avium (36)



MIC=2.43 μg/mL against *M. Tuberculosis (50)*



MIC=1.03 μg/mL against *M. Tuberculosis* (51)

Figure 1.1. Chemical structures of acrylonitrile and thiazole derivatives possessing antimycobacterial activity.

In a study evaluating the efficacy of acrylonitrile-containing compounds against dormant-phase *Mycobacterium* at a concentration of 10 μg/ml, some of the compounds exhibited a bacterial log reduction of 2.9-fold. Notably, they displayed greater potency compared to first-line TB drugs isoniazid (1.2 log fold), rifampicin (1.3 log fold), and moxifloxacin (15) (**Figure 1.1**).

In a different investigation, a compound carrying acrylonitrile structure was discovered to have greater effectiveness against Mtb than ethambutol (MIC = 1.56 g/ml) with a MIC of 0.78 g/ml. The bacterial count of dormant forms of mycobacterium was significantly reduced by this compound antibiotic, decreasing it by 2.8 log fold,

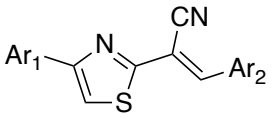
outperforming frequently prescribed first-line medications isoniazid, ciprofloxacin, rifampicin, and moxifloxacin (34) (**Figure 1.1**).

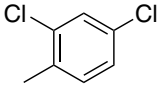
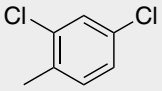
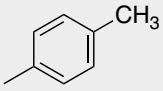
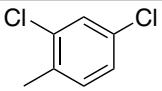
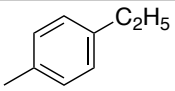
A substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitrile derivative was discovered to have excellent inhibitory effects against both *M. avium* and *M. tuberculosis*. In particular, it showed 93% and 99% inhibitory efficacy against *M. avium* and *M. tuberculosis*, respectively, at a MIC = 12.5 g/ml (36) (**Figure 1.1**).

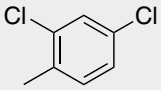
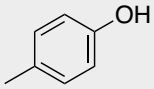
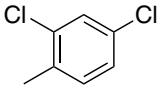
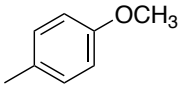
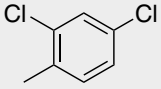
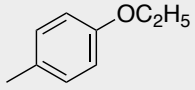
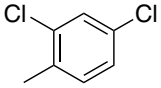
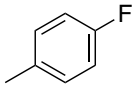
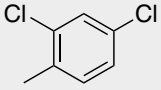
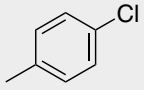
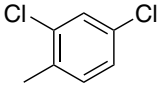
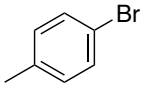
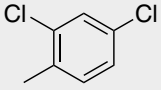
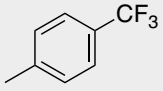
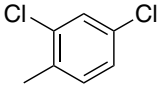
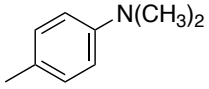
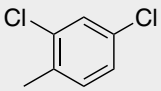
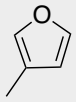
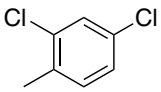
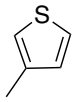
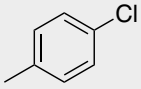
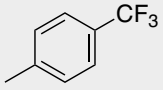
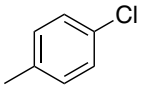
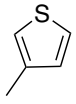
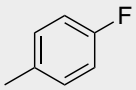
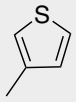
Thiazole has played a variety including its use as a pharmacophore, spacer, or bioisosteric scaffold. Thiazole derivatives demonstrating promising biological activities such as anticancer (37-39), antiviral (40-42), antialzheimer (43, 44), antidiabetic (45, 46), antioxidant (47-49) etc. and in more recent investigations, thiazole has also demonstrated various antitubercular (15, 35, 50, 51) (**Figure 1.1**) and antimicrobial activities (52-59).

On the basis of these findings, we combined the thiazole ring and acrylonitrile moiety to develop a new series of 3-aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile derivatives (**1-16**) derivatives and tested their antimycobacterial and antimicrobial activities (**Table 1.1**).

Table 1.1. The structure of the synthesized compounds (**1-16**).



Compounds	Ar_1	Ar_2
1		
2		
3		

4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		

2. LITERATURE REVIEW

2.1 Acrylonitrile Derivatives

Acrylonitrile, also known as 2-propenenitrile, propenenitrile, acrylic acid nitrile, propylene nitrile, vinyl cyanide, or propenoic acid nitrile, is an organic compound consisting of a vinyl moiety attached to a nitrile. It has a chemical formula of CH_2CHCN and a molecular mass of 53.064 g/mol.

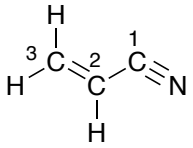


Figure 2.1. Chemical formula of acrylonitrile

2.1.1 General Synthesis of Acrylonitrile

Unlike most organic compounds, acrylonitrile, which is often referred to as α, β-unsaturated in literature, is not found naturally on Earth, necessitating the use of various synthetic procedures and materials (60). Historically, large-scale production of acrylonitrile was done using the ethylene cyanohydrin process, which involved the based-catalyzed addition of HCN to ethylene oxide in the liquid phase at 60˚C (60). Later in 1970, this method was discontinued and was replaced by the newly discovered propylene ammoxidation process, also known as the SOHIO process. Although this method is known to produce a high yield of acrylonitrile at a lower cost, the toxic chemicals and harsh reaction conditions required, combined with the high demand for acrylonitrile, prompted the development of new synthesis strategies (61). The general synthesis of acrylonitrile can be grouped into the main categories listed below (62, 63) (**Figure 2.2**).

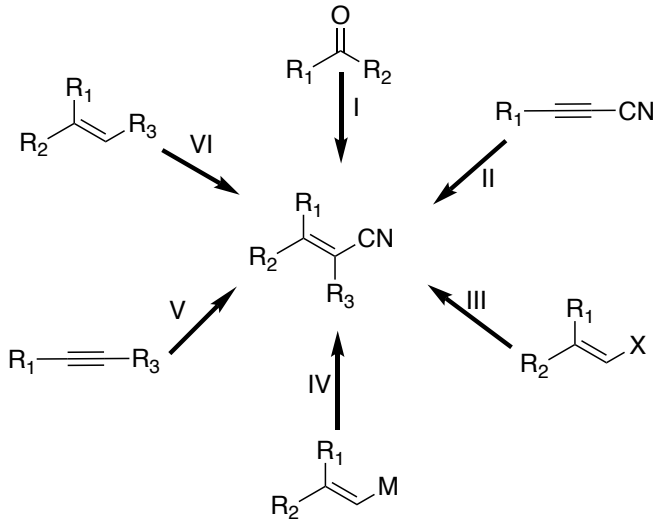


Figure 2.2. Synthesis Routes of Acrylonitrile Derivatives

Condensation of Carbonyl Compounds and Nitriles (I)

The availability of carbonyl precursor in aldehydes and ketones makes this approach the most used method in synthesizing acrylonitrile. In this method, a base is used as a nucleophile to eliminate an acidic proton of a nitrile compound, making it less stable and reactive to attack the carbonyl compound to form an enol. This reaction is a type of aldol condensation reaction known as Knoevenagel condensation. The enol intermediate eventually forms an α,β-unsaturated nitrile in a base medium.

Nadaf et al employed a multi-step synthetic methodology to prepare 1,2-disubstituted imidazoles in their investigation. The experimental procedure employed piperidine as the base and ethanol as the solvent. The reaction involved the Knoevenagel condensation of 1-methyl-1*H*-imidazole-2-carbaldehyde and 2-(4-nitrophenyl) acetonitrile, which yielded a condensed product, namely 3-(1-methyl-1*H*-imidazol-2-yl)-2-(4-nitrophenyl)acrylonitrile. This synthetic approach has been demonstrated to be uncomplicated, effective, and takes place under gentle conditions (17) (**Figure 2.3**).

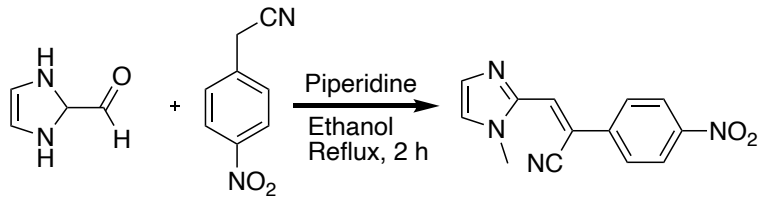


Figure 2.3. Synthesis of acrylonitrile by Knoevenagel condensation of 1-methyl-1*H*-imidazole-2-carbaldehyde and 2-(4-nitrophenyl)acetonitrile

Kavitha et al further developed a discovery by synthesizing 2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenylacrylonitrile through a one-pot synthesis involving 3-(2-bromoacetyl)-2*H*-chromen-2-one (2), 2-cyanothioacetamide, and benzaldehyde. They utilized various base mediums such as piperidine, piperazine, *N*-methylpiperazine, *N*-ethylpiperazine, morpholine, triethylamine, triphenylphosphine, ammonium acetate, sulfamic acid, and *L*-proline. Results showed that *L*-proline was the most effective catalyst, and methanol and ethanol were the optimal solvents for the Knoevenagel condensation. This study revealed that *L*-proline and polar protic solvents were the best choices for the catalyst and solvent, respectively. Furthermore, the one-pot synthesis method is an environmentally friendly alternative and allows for improved chemical reaction efficiency and reduced reaction time. The results were extended to different heterocyclic and heteryl aldehydes, yielding good product yields, although longer completion times were observed for heteryl aldehydes possibly due to electronic factors (35) (**Figure 2.4**).

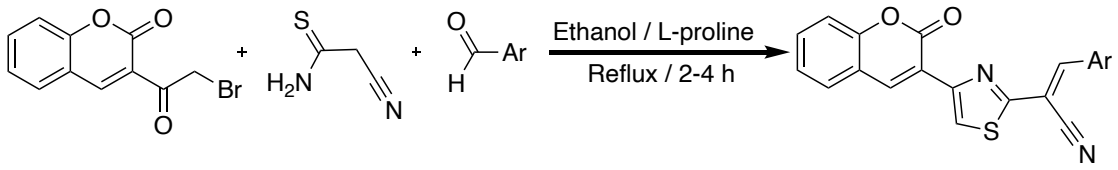


Figure 2.4. One-pot synthesis of acrylonitrile using 2-cyanothioacetamide and benzaldehyde

A study conducted by A. Lattanzi et al. described the use of a mild base, lithium hydroxide, and activated 4 Å molecular sieves to develop stereoselective α,β-unsaturated

esters. The researchers attributed the shorter reaction time to the activated 4 Å MS. The predominant E-stereoselectivity observed was attributed to the directing effect of the hydroxyl group present in α-hydroxy ketones. The results showed that LiOH was more effective than commonly used bases such as LDA, LiHMDS, and NaH in this type of reaction (64) (**Figure 2.5**).

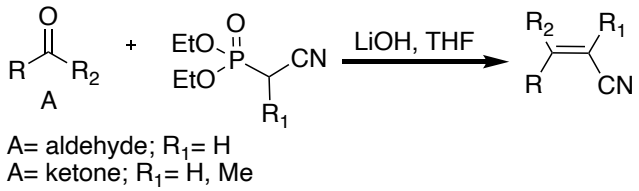


Figure 2.5. Synthesis of stereoselective acrylonitrile from aldehydes and ketones

Tamioka et al. conducted a study to synthesize stereoselective α,β-disubstituted acrylonitriles using two different approaches. The first approach, known as the linear approach, involved the one-pot olefination of a substituted acetonitrile using bis(diisopropylamino)chloroborane reagent with 2 equiv of a lithiated RCH_2CN, followed by the addition of benzaldehyde, resulting in fair to good yield with (Z)-stereoselectivity. However, this approach required the use of expensive and/or not readily available precious nitrile RCH_2CN, making it less desirable. To overcome this limitation, the authors proposed an alternative approach, called the divergent approach, which took advantage of the nature of an α-boryl carbanion. This approach involved the reaction of a carbanion with an alkyl halide (RX) to form an alkylated intermediate, which could then be treated with a base and an aldehyde to provide acrylonitriles. This approach, starting from simple acetonitrile (CH_3CN), was found to be more flexible and versatile in accessing various acrylonitrile derivatives (65) (**Figure 2.6**).

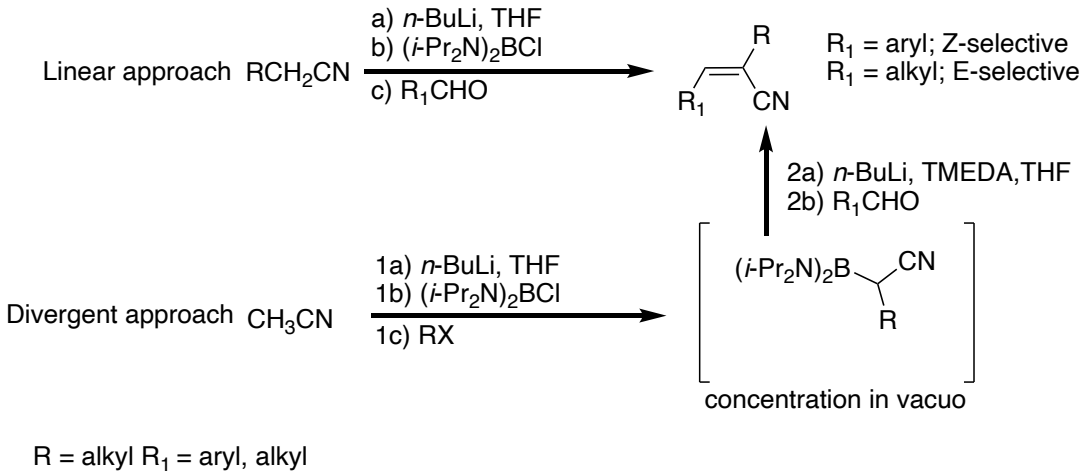


Figure 2.6. One-pot synthesis of α,β-disubstituted acrylonitrile from substituted and non-substituted acetonitrile

Conjugate Additions or Reductions of Alkyne-Nitriles (II)

Another popular method for producing acrylonitrile is the conjugate addition or reduction of alkyne-nitriles. Primarily, Grignard reagents were the first organometallics reported to interact conjugately with unsaturated nitriles.

In a study by Kleijn et al., it has been demonstrated that dialkylargentates, such as $R_2AgMgCl$, exhibit a high propensity to react with enynyl nitriles when tetrahydrofuran (THF) is present. Upon undergoing protolysis, the intermediate product yields a considerable amount of trans-2,4-alkadienenitriles. However, substitution of the Grignard reagent with RCu reagents results in the production of a Z-isomer (66) (**Figure 2.7**).

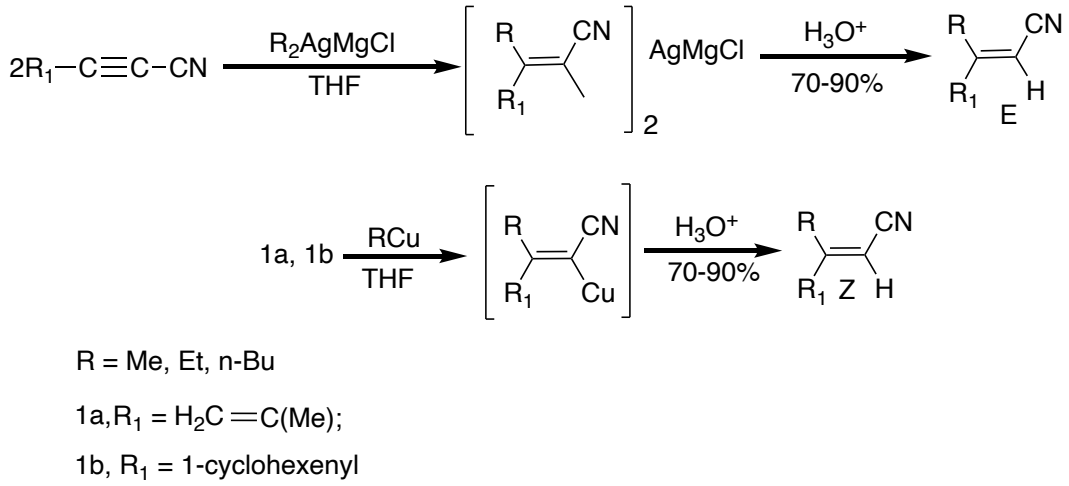


Figure 2.7. Synthesis of acrylonitrile by addition of dialkylargentates to enynenitriles

Fleming et al. conducted a recent investigation into the stereoselective chelation-controlled conjugate addition of Grignard reagents to γ-hydroxy-alkyne-nitriles. The reaction process begins with the deprotonation of the γ-hydroxy-alkyne-nitriles by *t*-BuMgCl, followed by the stepwise conjugate addition of a second Grignard that leads to the formation of a cyclic magnesium chelate. It is postulated that the cyclic magnesium chelate generates a more reactive ate complex capable of alkylating both aliphatic and aromatic aldehydes when *t*-BuLi is added. The chelation-controlled conjugate addition and alkylation are shown to simplify the production of tri- and tetra-substituted acrylonitrile (67) (**Figure 2.8**).

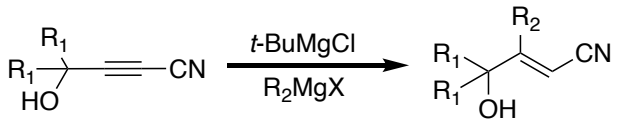


Figure 2.8. Synthesis of acrylonitrile from γ-hydroxy-alkyne-nitriles

Michon et al. documented that the intermolecular hydroamination of 3-phenylpropiolonitrile, an internal alkyne, with pyrazole proceeded with high regio- and stereoselectivity in the presence of gold(I) catalyst under solvent-free conditions. The

outcome was the formation of two (*Z*)-regioisomers, with a 9/1 ratio and a high degree of isolation (68) (**Figure 2.9**).

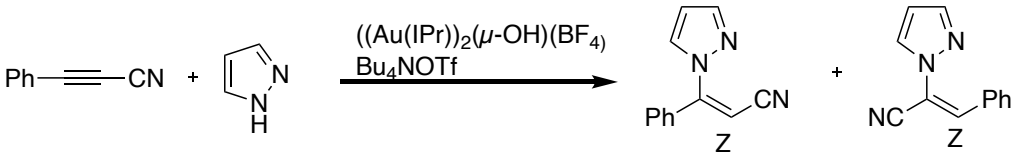


Figure 2.9. Synthesis of acrylonitrile by hydroamination of 3-phenylpropiolonitrile

Cyanide coupling of vinyl halides (III)

The transition-metal-catalyzed cyanation reaction employing KCN, NaCN, or $K_4[Fe(CN)_6$ represents a highly attractive and contemporary approach for synthesizing organic cyanides. This method has been utilized in recent developments for coupling reactions of C—CN bonds, including aryl and vinyl halides and pseudohalides, arylboronic acids, as well as alkene, alkyne, and aromatic hydrocarbons (69).

The pioneering study by Murahashi et al. presented the first instance of palladium-catalyzed vinylnitrile synthesis from vinyl halides employing potassium cyanide-crown ether for cyanidation (70). Li et al. also contributed to this field with a report on a microwave-assisted coupling reaction utilizing palladium-catalyzed arylvinyl bromides and potassium ferrocyanide, resulting in the production of highly stereoselective α,β-unsaturated nitriles. The procedure is straightforward, expeditious, and affords a product that is easily separable (71) (**Figure 2.10**).

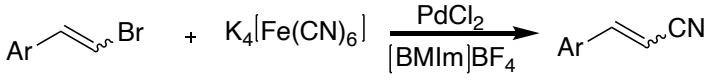


Figure 2.10. Synthesis of acrylonitrile from arylvinyl bromides

Sakakibara et al. conducted a reaction between 1-bromo-2-ethoxyethene, a vinyl halide, and alkali cyanide KCN in DMF for a duration of 4 hours, utilizing Nickel catalyst to produce 3-ethoxyacrylonitrile. In this study, various solvent systems were investigated

to determine the most effective system for the reaction. The results indicated that nearly all of the procedures tested produced high yields and high stereoselectivity at 50 °C when utilizing the KCN-DMF system with intermediate cyanide solubility. The KCN in hexamethyl phosphoric triamide (HMPA) and KCN-MeCN systems with low cyanide solubility accelerated the coupling of the halides to inhibit the cyanation, whereas the NaCN-DMF and NaCN-HMPA systems with high cyanide solubility yielded the lowest yield because the presence of excess cyanide ion inhibited the reaction (72) (**Figure 2.11**).

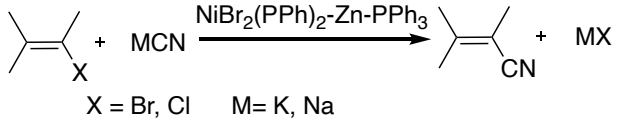


Figure 2.11. Synthesis of acrylonitrile from vinyl halide and an alkali cyanide

Despite its advantages, the transition-metal-catalyzed cyanation reaction suffers from several drawbacks. Cyanation sources commonly utilized in this reaction are hazardous and generate toxic HCN gas. Additionally, the reaction leads to stoichiometric quantities of metal waste. Another issue that needs to be considered is the need for careful control of the concentration of the reaction mixture to minimize the formation of inactive cyano transition metal complexes in situ that can cause catalyst poisoning (73).

Cyanation of vinyl anions (IV)

In a study conducted by Wang et al., the cyanation of vinylsilanes was achieved using ammonium iodide and DMF as the combined source of nitrogen and carbon atoms for the introduction of the cyano group. The reaction was observed to occur in two distinct steps. The organosilanes were initially converted to their iodo intermediates, followed by the formation of cyanation complexes. The researchers found that the cyanation proceeded smoothly, resulting in the production of the desired acrylonitrile products (74) (**Figure 2.12**).

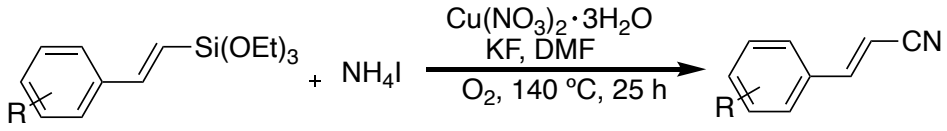


Figure 2.12. Synthesis of acrylonitrile by copper-mediated cyanation of vinylsilanes

Cyanation of Alkynes (V)

The direct cyanation of alkynes is considered a highly efficient and straightforward approach for the synthesis of acrylonitrile. The metal-catalyzed direct addition of X-CN bonds, where X represents C, B, Br, or other similar bonds, into alkynes has been the subject of extensive research over the years due to its conceptual novelty, product utility, and high degree of regio- and stereoselectivity (75-77) (**Figure 2.13**).

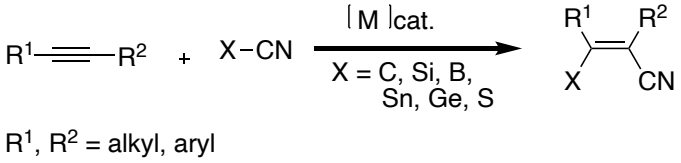


Figure 2.13. Synthesis of acrylonitrile by cyanation of alkynes

Cheng et al. investigated a Pd-catalyzed three-component arylcyanation of internal alkynes with aryl bromides and $K_4[Fe(CN)_6]$, which provides a direct and stereoselective method for synthesizing fully substituted, α,β-unsaturated nitriles from simple starting materials. The reaction was compatible with both diphenylacetylene and dialkyl-substituted alkynes, yielding the desired product in satisfactory yields. This method is considered attractive due to the utilization of readily available internal alkynes, aryl bromides, and an environmentally friendly cyanation reagent (75) (**Figure 2.14**).

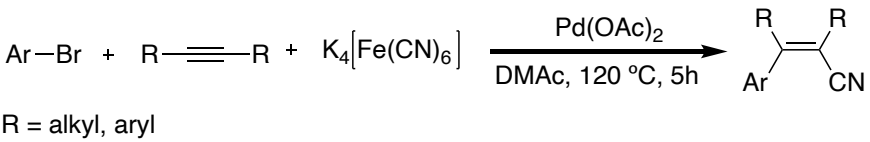


Figure 2.14. Synthesis of acrylonitrile from palladium-catalyzed cyanation of alkyne

Nakao employed the nickel/Lewis acid (LA) cooperative catalysis strategy in carbocyanation reactions that involve the cleavage of C-CN bonds in nitriles, utilizing both the organic and cyano groups. The reaction relied solely on a nickel catalyst and was limited to the use of aryl and allylcyanides as nitrile substrates. However, the introduction of LA cocatalysts significantly accelerated the rate of arylcyanation and extended the range of nitriles employed in the reaction to include alkynyl, alkenyl, and alkylcyanides. The result of these reactions was the creation of a variety of highly stereo- and regioselective acrylonitrile molecules (78) (**Figure 2.15**).

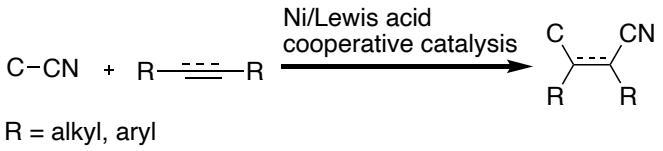


Figure 2.15. Synthesis of acrylonitrile Nickel/LewisAcid-Catalyzed Carbocyanation of alkyne

Sakata has reported a regio- and stereoselective iodocyanation and dicyanation reaction of alkynes with cyanogen iodide, catalyzed by copper. This method allowed for the formation of complex acrylonitrile structures with well-controlled regio- and stereoselectivity. The reaction mechanism, including the stepwise processes of diiodide formation, selective monocyanation, and second cyanation, was elucidated. Furthermore, it was noted that the selectivity of the products could be altered by modifying the reaction conditions (79) (**Figure 2.16**).

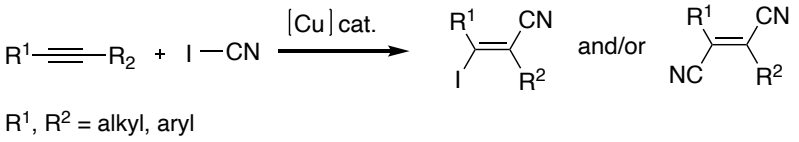


Figure 2.16. Synthesis of acrylonitrile copper-catalyzed iodocyanation and dicyanation of alkyne

Cyanation of alkene (VI)

In the direct C(sp^2)-H cyanation of alkenes with directing groups, the rhodium(III)-catalyzed C-H reaction is a valuable alternative to other transition metals due to its substrate scope and functional group compatibility (80, 81)**.**

A practical method for the synthesis of alkenyl nitriles has been developed using Rh(III)-catalyzed direct vinylic C-H cyanation reaction with *N*-cyano-*N*-phenyl-p-methylbenzenesulfonamide as a cyanation reagent for acrylonitrile compound synthesis. This new C-H cyanation process accommodates both acrylamides and ketoximes as substrates (80) (**Figure 2.17**).

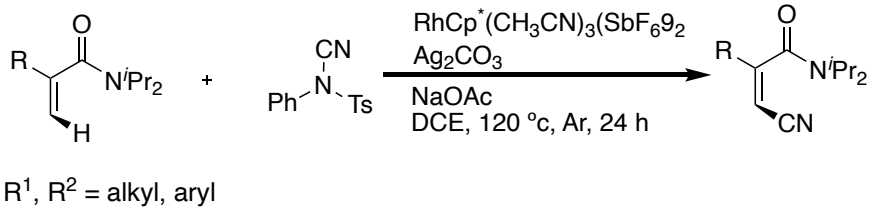


Figure 2.17. Synthesis of acrylonitrile form Rh(III)-catalyzed cyanation of alkene

Wang et al. conducted a study on transition metal-free cyanation of alkenes using aryl(biscyano)iodine(III) reagent as the activator and trimethylsilyl cyanide as the source of cyanide. The researchers aimed to expand the approach previously mentioned by exploring a new mechanism involving the electrophilic activation of the alkene by cyano iodine(III) species generated in situ from a [bis(trifluoroacetoxy)iodo]arene. The study found that the method could be successfully applied to noncyclic 1,1- and 1,2-

disubstituted alkenes, achieving high stereoselectivity and proving to be a highly useful approach (82) (**Figure 2.18**).

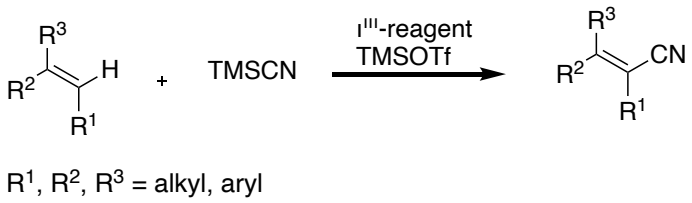


Figure 2.18. Synthesis of acrylonitrile by direct metal-free cyanation of alkene

2.1.2 Chemical Properties of Acrylonitriles

Acrylonitrile is an important chemical intermediate due to its versatile functionality. Its double bond and nitrile group make it amenable to a diverse range of reactions. The nitrile group can undergo hydrolysis, hydrogenation, esterification, or reduction, while the double bond can engage in reactions including polymerization, copolymerization, cyanoethylation, cyclization, and halogenation.

Reaction of the Nitrile group

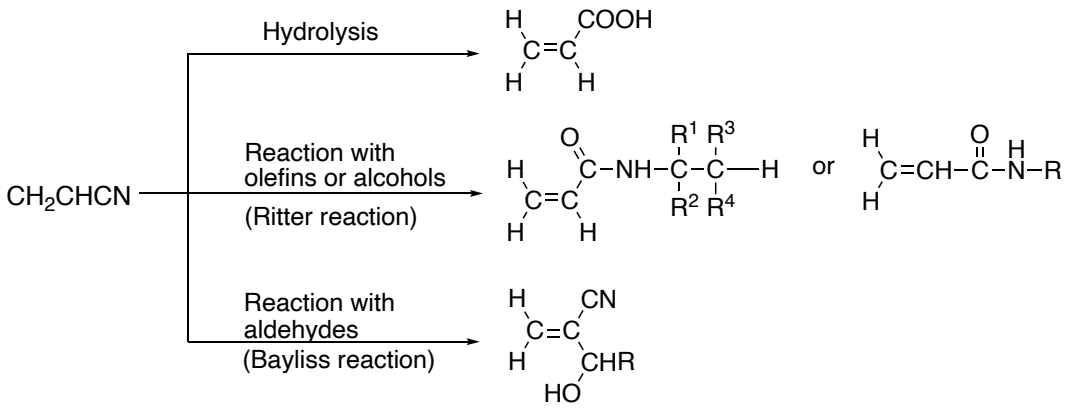


Figure 2.19. Reactions of nitrile group of acrylonitrile

i) Hydrolysis Reaction of Nitrile Group

The nitrile group present in acrylonitrile can be converted to the corresponding carboxylic acid through the action of strong acids and bases. This chemical reaction has diverse applications, such as in the synthesis of acrylamide, biodegradation of acrylonitrile, and treatment of acrylonitrile wastewater (83).

Dong et al. conducted a one-pot selective conversion of acrylonitrile to acrylic acid in a hydrothermal system utilizing NaOH as a catalyst. This process involved the hydrolysis of acrylonitrile to acrylamide, which was further hydrolyzed to form acrylic acid. The experimental parameters, including the initial concentration of acrylonitrile, reaction temperature, reaction time, and amount of alkali, significantly influenced the yield of acrylic acid. The optimal conditions for obtaining the highest yield of acrylic acid (55%) were found to be an initial acrylonitrile concentration of 3x103mg/L, a reaction temperature of 300°C, and a reaction time of 90 seconds with a 1.0M NaOH catalyst (83) (**Figure 2.20**).

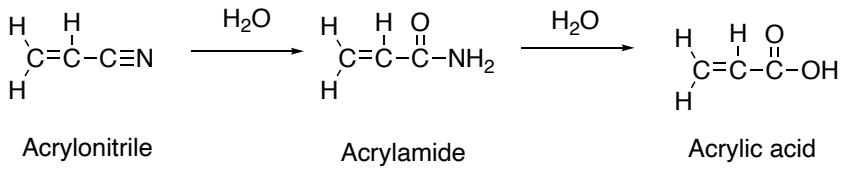


Figure 2.20. Reaction mechanism for the synthesis of acrylic acid from acrylonitrile.

Another study describes a novel method for enzymatic acrylamide synthesis. The process is defined using immobilized *Pseudomonas chlororaphis* RPZ-18 cells in a system containing concentrated acrylonitrile solutions as the substrate. The hydrolysis product is an acrylamide with has a high chemical purity. However, the hydrolysis reaction may be restricted to solely produce acrylamide by way of the catalytic activity of the nitrile hydratase (NH) component of nitrilase (NTS) (specifically the first component), following destruction of the amidase component in the same strain (84) (**Figure 2.21**).

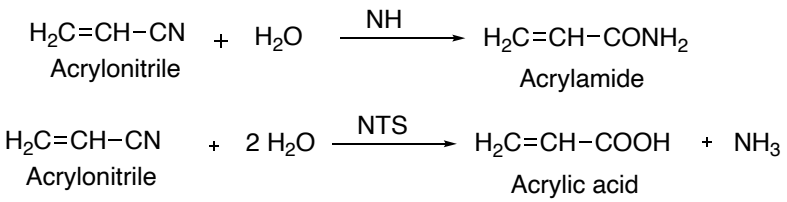


Figure 2.21. Enzymatic hydrolysis of acrylonitrile in *Pseudomonas chlororaphis*

ii) Reaction of Acrylonitrile with Olefins and Alcohols

The chemical transformation of acrylonitrile's nitrile group through reaction with alcohols or olefins, referred to as The Ritter reaction, generates carboxylic amides, including the production of acrylamide (85).

This reaction offers an efficient method for the synthesis of amides that feature secondary or tertiary alkyl groups on the nitrogen atom. In a recent study aimed at developing a practical method for preparing a mildewcide, *N*-cyclohexylisothiazolone, the starting compound was synthesized using the Ritter reaction of acrylonitrile with cyclohexanol in the presence of concentrated sulfuric acid. Subsequently, the intermediate product was subjected to thiolation with thiourea, which yielded acrylamide (85, 86) (**Figure 2.22**).

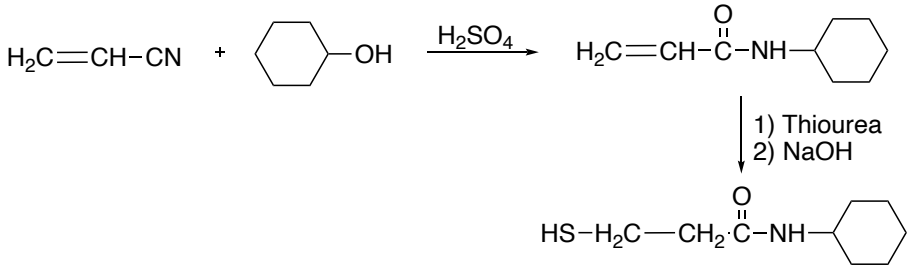


Figure 2.22. Ritter reaction of acrylonitrile with cyclohexanol

Kazantsev et al. conducted a similar experiment in which *N*-Alkylacrylamides were synthesized from acrylonitrile and commercial olefin fractions in the presence of sulfuric acid using the Ritter reaction. It was also established that, the main and side

reactions are affected by temperature, reactant ratio, and sulfuric acid concentration. The reaction is widely assumed to follow the scheme illustrated in **Figure 2.23** (86).

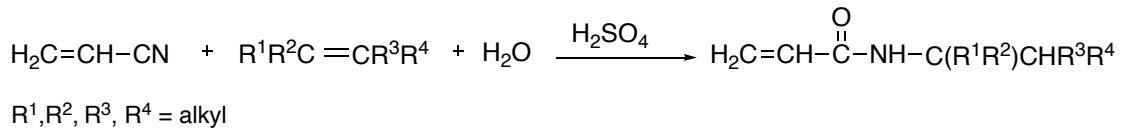


Figure 2.23. Ritter reaction of acrylonitrile with olefins

iii) Reaction of Acrylonitrile with Aldehydes

Acrylonitriles have also been observed to react with aldehydes and ketones in a base catalyzed reaction known as the Bayliss-Hillman reaction (87, 88), This is shown in a study by Hill and Isaacs. In the study, a good yield of 2-cyanobut-1-en-3-ol (R = Me) is obtained when an acrylonitrile was subjected to acetaldehyde in the presence of a base catalyst, 1,4-diazabicyclo[2.2.2]octane (DABCO) (88) (**Figure 2.24**).

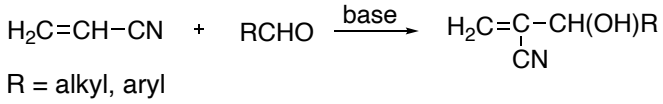


Figure 2.24. Reaction of acrylonitrile with aldehyde

Reaction of the Double Bond of Acrylonitrile

i) Diels Alder and Related Reactions of Acrylonitrile

The Diels-Alder reaction involves the chemical reaction between a conjugated diene and a substituted alkene, called the dienophile, resulting in a substituted cyclohexene derivative. This cycloaddition reaction is well-established and extensively researched in organic chemistry (89) (**Figure 2.25**).

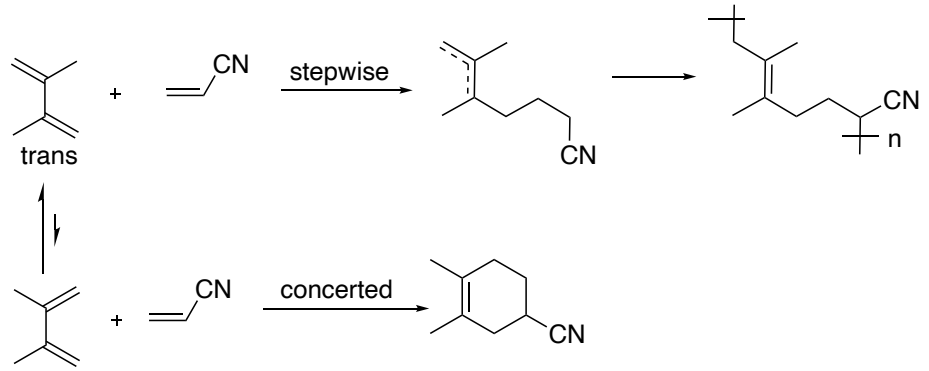


Figure 2.25. Diels-Alder reaction

A study by James et al. provides an example of this reaction and examines the concerted and stepwise pathways for the reactions between (*E*)-1,3-pentadiene and acrylonitrile. The reaction generates a Diels-Alder adduct product, comprising a mixture of the two expected regioisomers (90) (**Figure 2.26**).

Figure 2.26. Diels-Alder reaction between (*E*)-1,3-pentadiene and acrylonitrile.

ii) Hydrogenation and Hydration Reactions of Acrylonitrile

The catalytic hydrogenation of acrylonitrile leads to the production of propionitrile (91). This reaction can be catalyzed by several transition metals, including Rh, Ni, and Pd (92-95). The presence of C=C and C≡N bonds in acrylonitrile makes controlling the selectivity of hydrogenation crucial in producing propionitrile. Nickel-based catalysts, such as the nickel boride catalyst (96) and Ni-B/SiO_2 amorphous catalyst (97) have shown high efficiency in selectively hydrogenating acrylonitrile. Acrylonitrile, upon undergoing further hydrogenation, leads to the formation of propylamine and other byproducts, such as 3-(propylamino)propanenitrile and 3-(dipropylamino)propanenitrile, which are created via addition and disproportionation reactions as shown in **Figure 2.27** (95).

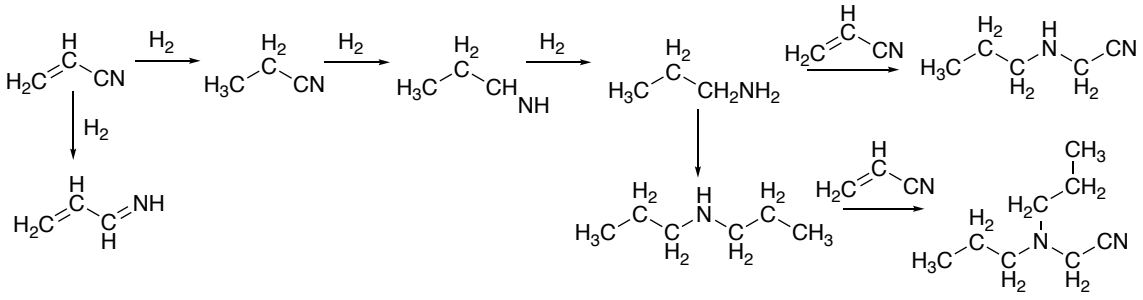


Figure 2.27. Mechanism of hydrogenation of acrylonitrile.

The process of selectively hydrating acrylonitrile to produce acrylamide has been the subject of various studies involving different metal oxide catalysts, with MnO_2, CuO, and Co_3O_4 found to exhibit high levels of activity and selectivity. The catalytic activities are significantly influenced by the preparation method, and the degree of hydration activity displayed by MnO_2 is directly related to the quantity of phenol adsorption. The hydration of acrylonitrile results in the production of both acrylamide and ethylenecyano-hydrine by addition to the C=N and C=C bond in acrylonitrile, respectively (98) (**Figure2.28**).

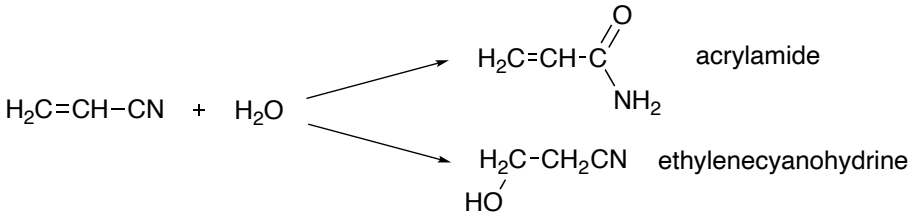


Figure 2.28. Hydration of acrylonitrile

iii) Halogenation of Acylonitrile

Several studies have reported on the halogenation of acrylonitrile via heat or UV light (99-102).

The addition of a halogen molecule produces 2,3-dihalopropionitriles at low temperatures. In the absence of UV light, an increase in temperature results in a second addition of halogen to produce 2,2,3-trihalopropionitrile (102) (**Figure 2.29**).

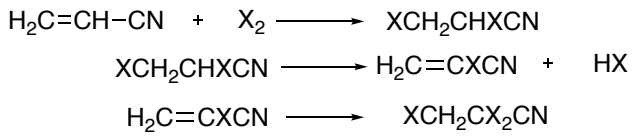


Figure 2.29. Halogenation of acrylonitrile

In the presence of ultraviolet light, the main product of the reaction is 2,3-dichloropropionitrile until significant amounts of hydrogen chloride are formed, at which point side reactions dominate, with 3-chloropropionitrile and 2,2,3-trichloropropionitrile as the main products. It was proposed that hydrogen chloride is formed first during the free-radical photochlorination process, but that once formed, it catalyzes a parallel ionic reaction pathway that produces most, if not all of the 3-chloropropionitrile and 2,2,3-trichloropropionitrile by-products(101, 102) (**Figure 2.30**).

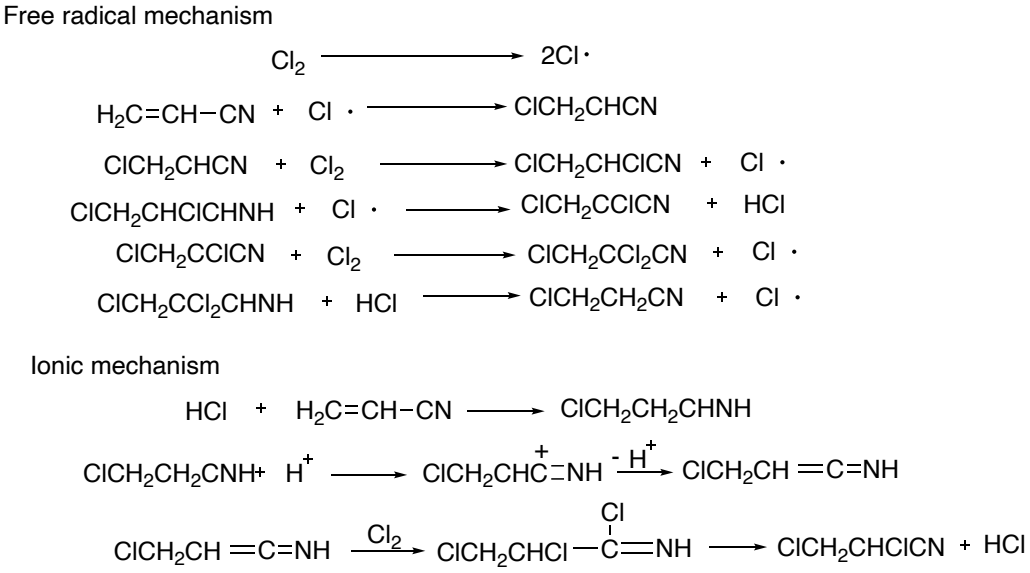


Figure 2.30. Proposed mechanisms of acrylonitrile chlorination

iv) Reaction of Acrylonitrile with Diazo compounds

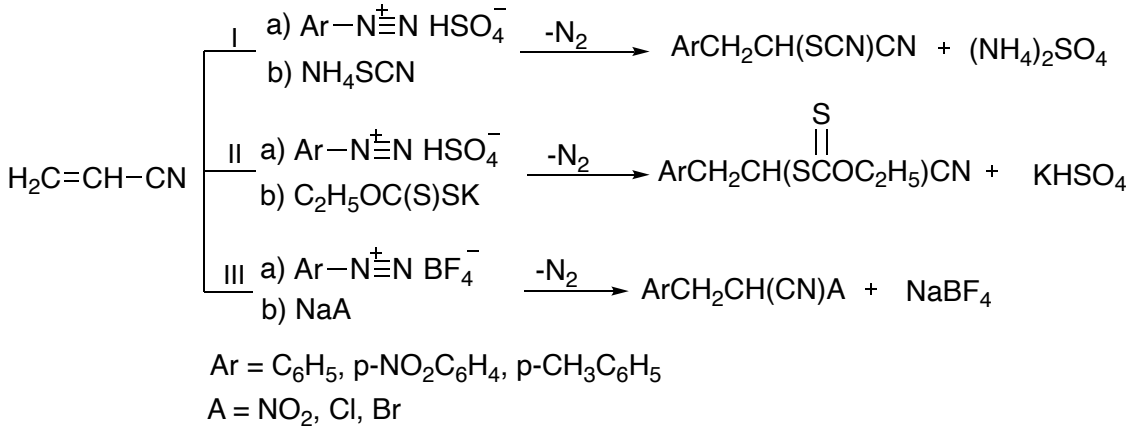


Figure 2.31. Reactions of acrylonitrile with various diazonium salts

Naidan et al. conducted an exploration on the process of acrylonitrile thiocyanatoarylation, wherein they observed that the combination of benzenediazonium sulfates and acrylonitrile in an aqueous acetone medium, in the presence of thiocyanate ions, resulted in a reaction wherein the unsaturated compound underwent addition with thiocyanato- and arene-sulfonyl groups. This reaction occurred over a range of temperatures from -16 to -20 °C (103) (**Figure 2.31** I).

Moreover, under the influence of copper(I)ethylxanthate and potassium xanthate, benzene- and *p*-nitrobenzene-diazonium sulfates react with acrylonitrile in an aqueous acetone solution at 5-10 °C. The reaction occurs with the elimination of nitrogen and produces α-(ethoxythiocarbonylthio)-β-phenylpropionitrile (51%) and α-(ethoxythiocarbonylthio)-β-(p-nitrophenyl)propionitrile (40%) (104) (**Figure 2.31** II).

Acrylonitriles react with diazonium salts in the presence of copper salts in a reaction known as the Meerwein reaction. In a review paper, various reactions of aromatic diazonium salts with diene and monounsaturated compounds in the presence of nucleophiles are described. It was discovered that in an acetone-water reaction medium, arylchloro(bromo)ethanes are formed in 25%-75% yield when arenediazonium

tetrafluoroborates interact with acrylonitrile in the presence of sodium nitrite, chloride, and bromide and catalytic amounts of copper salts (105) (**Figure 2.31** III).

v) Reactions with alcohol and carbon monoxide

In the presence of a hydrogenation catalyst such as cobalt or ruthenium, acrylonitrile can react with a mixture of carbon monoxide and hydrogen and a saturated alcohol to produce cyanopropionaldehyde acetals. This reaction is most effective when using saturated primary and secondary monohydric and dihydric alcohols such as methanol, ethanol, ethylene glycol, and propylene glycol. At least two alcoholic hydroxyl groups are required for each acrylonitrile ethylenic bond. The reaction is carried out by heating the reactants at temperatures and pressures of 100-200°C and above 600 atmospheres, and the catalyst of choice is typically selected from the VIIIth group of metals in the periodic table (106) (**Figure 3.32**).

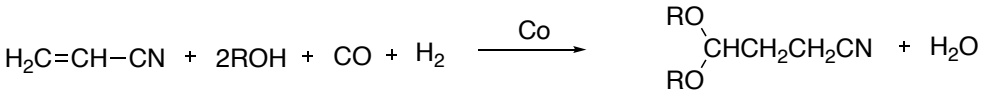


Figure 2.32. Reaction of acrylonitrile with carbon monoxide and hydrogen and a saturated alcohol

Thiyagarajan et al. describe a low-cost, and easily obtainable potassium *tert*-butoxide (KOtBu) catalyzed Michael addition reaction with acrylonitrile as a typical Michael acceptor. This catalytic protocol is said to be compatible with a diverse range of heteroatom nucleophiles, including aliphatic and aromatic primary and secondary alcohols, thiols, and amines with acrylonitrile, substituted acrylonitrile, acrylamide, and acrylic esters. The general ionic mechanism for Michael addition reactions begins with the deprotonation of an acidic proton from the Michael donor by a base, which results in the formation of nucleophilic intermediate I. Nucleophile I conjugates with Michael acceptor acrylonitrile to form intermediate II, which provides Michael addition product upon hydrogen abstraction from protonated base (107) (**Figure 2.33**).

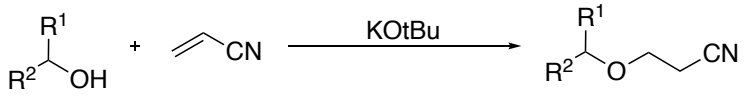


Figure 2.33. Reaction of acrylonitrile with alcohol

Cyanoethylation of Acrylonitrile

The reactive double bond of acrylonitrile readily undergoes cyanoethylation by a range of organic and inorganic compounds possessing labile hydrogen atoms. This reaction results in the introduction of a cyanoethyl (-CH_2-CH_2-CN) group in place of the original double bond. Cyanoethylation is a well-known reaction and is analogous to a Michael reaction (108) (**Figure 2.34**).

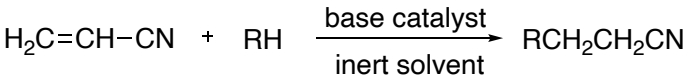


Figure 2.34. Cyanoethylation of acrylonitrile

Reactive hydrogen atoms are typically present in the compounds involved in cyanoethylation of acrylonitriles, example ammonia, water, alcohols, phenols, ketones, carboxylic acid, esters, oximes, mercaptans, etc. Although some amines may necessitate an acidic catalyst for cyanoethylation, it is generally required to have an alkaline catalyst, comprising hydroxides, alkoxides, hydrides, etc. The most effective catalysts for cyanoethylation are highly basic quarternary ammonium hydroxides, such as benzyltrimethylammonium hydroxide. Inert solvents, including benzene, dioxane, pyridine, and acetonitrile, are commonly used, and the reactions are often exothermic, making cooling necessary to prevent excessive polymerization (108).

Polymerization of Acrylonitrile

Acrylonitrile is a monomer with a high degree of reactivity and can undergo polymerization with various vinyl monomers under diverse conditions. Polyacrylonitriles (PAN) have extensive applications in the pharmaceutical industry, including their use as

antioxidants, emulsifying agents, insecticides, solvents, surface coatings, cross-linking agents, and catalyst treatments for medicines (109).

Polymerization of acrylonitrile can occur through either homopolymerization or copolymerization. However, due to the difficulty in melting and processing acrylonitrile homopolymer, it is often copolymerized to achieve the desired thermal stability, melt flow, and physical properties (109). Homopolymerization can be achieved rapidly by using initiators such as radiation, free radicals (110), anionic initiators including metal alkyls and alkali metal alkoxides, and azo compounds such as 2,2'-azobis(isobutyronitrile) at moderate temperatures below 100 °C in solvents such as DMSO, DMF, or an aqueous solution of NaSCN (111).

According to research conducted by Shi and Jiang, lithium amides have been demonstrated as effective anionic initiators for anionic polymerization of acrylonitrile to produce high molecular weight polyacrylonitrile. Different types of lithium amides derived from diisopropylamine, diethylamine, hexamethyldisilazane, dicyclohexylamine, and 2,2,6,6-tetramethylpiperidine were used as initiators, and polyacrylonitrile with weight-average molecular weights ranging from 1.02×10^6 g/mol to 1.23×10^6 g/mol (Mw/Mn = 1.9-2.2) were obtained. The homopolymerization of acrylonitrile was carried out in *N*,*N*-dimethylformamide with minimal side reactions. (112) (**Figure 2.35**).

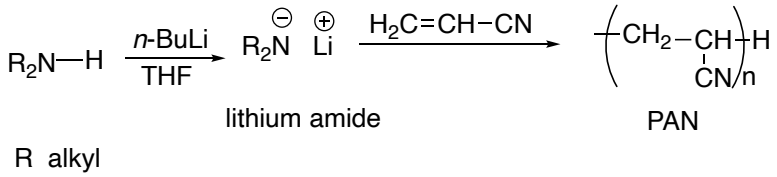


Figure 2.35. Homoplymerization of acrylonitrile

In contrast, copolymerization refers to the incorporation of electron-donating monomer units into the polymer structure, either randomly or using specific strategies such as combining a potent acceptor monomer with a potent donor monomer to obtain alternating equimolar copolymers. Other methods employed in the synthesis of

acrylonitrile block copolymers include ultrasonic, radiation, and chemical techniques, such as the use of polymer ions, polymer radicals, and organometallic initiators (109).

Atlas et al. conducted an investigation aimed at enhancing the dielectric properties of poly(ATRIF), which is a homopolymer made from 2,2,2-trifluoroethyl acrylate (ATRIF). The strategy involved increasing the polarity of poly(ATRIF) by adding monomers with a high dipole moment, such as acrylonitrile. Copolymers that had both C-CN and C-F substituents were produced by copolymerizing acrylonitrile and 2,2,2-trifluoroethyl acrylate (ATRIF) with azobisisobutyronitrile (AIBN) as the initiator in acetonitrile solution at 70°C using free radical polymerization (113) (**Figure 2.36**).

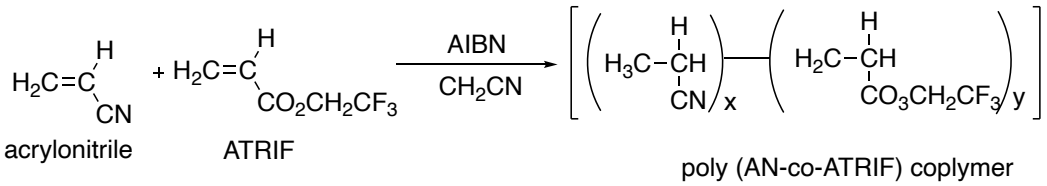


Figure 2.36. Copolymerization of acrylonitrile with ATRIF

2.1.3 Spectral Properties of Acrylonitrile Derivatives

Acrylonitrile has several characteristic peaks in its infrared (IR) spectrum and nuclear magnetic resonance (NMR) spectrum.

IR Spectra

The IR spectrum of acrylonitrile is characterized by the presence of several characteristic peaks. A strong absorption band at around 2150-2259 cm^{-1}, which corresponds to the triple bond between the carbon and nitrogen atoms in the molecule. A strong absorption band at around 1620-1450 cm^{-1} corresponding to the C=C stretching vibration and then C-H stretch vibration exhibits medium absorption band at around 700-800 cm^{-1} in the fingerprint region (17, 34, 36, 114, 115)

In the FT-IR characterization of the Pyridine-Carbazole Acrylonitrile Derivatives produced by Pérez-Gutiérrez et al., all three synthesized compounds displayed similar characteristics. While the C=C vibrations of the acrylonitrile molecular double bond peaked around 1628–1629 cm^{-1}, the bands at 745–744 cm^{-1} were attributed to the C–H vibrations of the double bond, and the bands at 2210–2213 cm^{-1} were caused by the C≡N stretching vibrations (115).

The target compounds, 2-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-(4-substitutedpiperazin-1-yl)phenyl)acrylonitrile, revealed absorption bands between 2230 and 2250 cm^{-1} due to C≡N stretching in the IR spectra of newly synthesized benzimidazole-acrylonitrile derivatives by Sirim et al (34).

In new 2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-arylacrylonitrile compounds, Kavitha et al. observed a strong, sharp adsorption band extending between 2156 cm^{-1} and 2336 cm^{-1} that corresponded to the C≡N group (35).

^{1}H-NMR Spectra

Due to the presence of the vinyl group (CH_2=CH-) and nitrile group, acrylonitrile displays a distinctive NMR spectrum. The nature of the substituents and their positioning with respect to the vinyl proton determine the ^{1}H-NMR spectrum characteristics of the olefinic proton present on the 2,3-substituted acrylonitrile moiety.

The ^{1}H-NMR spectra of 2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-arylacrylonitrile compounds dissolved in DMSO-d_6 with tetramethylsilane (TMS) as an internal standard using a 400 MHz spectrometer revealed that the singlet in the downfield regions around 8.17-8.55 ppm corresponding to vinylic proton were the primary evidence for the structures of the prepared compounds (35).

Similarly, the olefinic proton found in 3-(naphthalen-1-yl)-2-(4-(naphthalen-1-yl(phenyl)amino)-phenyl)acrylonitrile also exhibited a singlet at 8.10 ppm (115).

In another study, the presence of a singlet at 7.88 ppm in the ^{1}H-NMR spectrum of (2'-hydroxy)-4-((naphthalen-1-yl)methyleneamino)phenyl)-3-(1-methyl-1H-imidazol-2-yl)acrylonitrile corresponded to the olefinic proton (17).

Under similar conditions, the target compounds created by Sirim et al., one of them being 2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-phenylpiperazin-1-yl)phenyl)acrylonitrile, showed a proton (C_3-H) on the acrylonitrile moiety as a singlet at about 8.15 ppm (34).

By using NMR techniques, it is challenging to determine the configuration of the double bond in acrylonitrile due to the steric and electrical repulsions between the aromatic and heteroaromatic groups (116). Nevertheless, research has revealed that the *Z* isomer is less stable for these molecules than the *E* isomer (117).

^{13}C-NMR Spectra

The ^{13}C-NMR spectrum of an alpha, beta-substituted acrylonitrile moiety typically exhibits 3 major signals in the range. The chemical shifts of the carbons are influenced by the nature of the substituents and the electronic environment of the carbon atoms.

Benzimidazole-acrylonitrile hybrid derivatives synthesized by Sirim et al exhibited very distinctive peaks around 152 ppm, corresponding to the C_3 of the acrylonitrile moiety. Peaks of the C_2 of acrylonitrile appeared around 95 ppm. While the carbon atom of the nitrile group was not very distinctive, it is expected to have appeared in the aromatic region between 110-129 ppm (34).

In a similar trend, a series of (*E*)-2-(benzo[*d*]thiazol-2-yl)-3-arylacrylonitriles synthesized by Trilleras et al. were distinguished by chemical shifts ranging from 143.2 to 146.9 ppm for C_3 and 102.2 to 109.5 ppm for C_2 as a result of the highly polarized carbon-carbon double bond (C_2-C_3). The peak of the CN showed up around 115.6 to 117.3 as expected. The solvent of choice was deuterochloroform and a spectrometer with a frequency of 100 MHz was used (118).

2.1.4 Biological Activities of Acrylonitrile Derivatives

Anticancer Activity

Resveratrol, a natural polyphenolic compound, has been studied for its potential anticancer properties (119, 120). *In vitro* and in vivo studies have shown its chemo-preventive and chemotherapeutic effects, leading to the synthesis and evaluation of its analogs for potential anticancer properties (121-123). A recent study focused on the synthesis of (Z)-benzothiophene acrylonitrile resveratrol derivatives, which exhibited cytotoxic activity in human cancer cell lines with GI_{50} values between 10-100 nM, possibly through their interaction with tubulin. This makes them a potential treatment target for advanced prostate cancer. Moreover, these compounds were found to reduce the growth of cells resistant to P-glycoprotein (23) (**Figure 2.37 A**).

This study synthesized and evaluated a series of novel compounds related to resveratrol for their anticancer activity against human cancer cell lines, including acute myeloid leukemia (AML) cells. The compounds included diarylacrylonitrile and trans-stilbene analogues with a cyano group and various aromatic substituents to enhance their activity. Molecular docking studies identified two highly effective compounds that shared a binding site on the α,β-tubulin heterodimer, with one having greater binding affinity than the other. Microtubule depolymerization assays confirmed that the more potent compound was better at inhibiting tubulin polymerization in AML cells. Analysis of the binding cavity residues showed minor differences in van der Waals contacts between the compounds and colchicine. The most potent trans-stilbene analogues had better tubulin binding properties than their corresponding cis-stilbene analogues (24) (**Figure 2.37 B**).Özen et al. synthesized phenylacrylonitrile derivatives and evaluated their potential as anticancer agents against MCF-7 cell lines. The researchers found that compounds with meta-substituted phenyl rings containing methyl, trifluoromethyl, and chloro groups and para-substituted methyl and nitro groups demonstrated the most potent anti-cancer effects at a concentration of 100 μM. Lower doses of the compounds also showed a dose-dependent decrease in cell viability. In addition, the study revealed that meta-substituted

compounds were more effective in inhibiting cell viability compared to para-substituted compounds (25) (**Figure 2.37 C**).

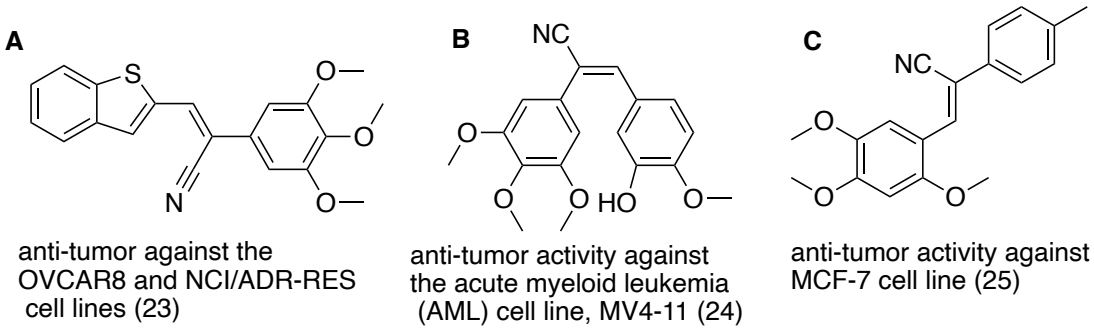


Figure 2.37. Some anti-cancer acrylonitrile derivatives in literature

Antimicrobial Activity

The study conducted by AlNeyadi et al. focused on synthesizing and testing a new series of benzazole acrylonitrile-based compounds for their antimicrobial properties against bacterial strains, including those that are typically resistant to antibiotics. The results showed that one compound, (*E*)-2-(benzo[*d*]thiazol-2′-yl)-3-(2″,4″-diaminopyrimidin-5″-yl)acrylonitrile, exhibited strong antibacterial activity against all bacterial strains tested, with a minimum inhibitory concentration (MIC) value of 1.0 g/ml. Docking studies revealed that the compound's mechanism of action involved irreversible inhibition of the penicillin binding protein (PBP) enzyme, which is necessary for bacterial cell wall synthesis. Additionally, the compound was found to enhance the antibacterial activity of amoxicillin, a commonly used penicillin antibiotic, which could potentially help overcome bacterial resistance (11) (**Figure 2.38 A**).

The antibacterial activity of 2,6-disubstituted heteroaryl acrylonitrile was examined on three different bacteria cultures, namely *Enterococcus hirae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, in a study looking at the structure-activity relationships of novel heteroaryl-acrylonitriles as cytotoxic and antibacterial agents. *S. epidermidis* was the most sensitive, while all of the chemicals tested had almost no effect

on *E. hirae*. The best activity in the two Staphylococcus strains was demonstrated by compound 2-(benzimidazol-2-yl)-3-(5-nitrothiophen-2-yl) acrylonitrile and its imidazo[4,5-*b*]pyridine analogue (124) (**Figure 2.38 B**).

Perin et al. investigated the antibacterial effects of novel benzazole-derived acrylonitriles with bicyclic heteroaromatic rings on a panel of Gram-positive and Gram-negative bacterial strains. Per collected data, only one acrylonitrile, (*E*)-2-(1*H*-benzo[*d*]imidazol-2-yl)-3-(benzofuran-2-yl) acrylonitrile, showed moderate efficacy against *S. aureus* ATCC 29213 (MIC 16 μM) and *S. pneumoniae* ATCC 49619 (MIC 32 μM) bacterial strains (14) (**Figure 2.38 C**).

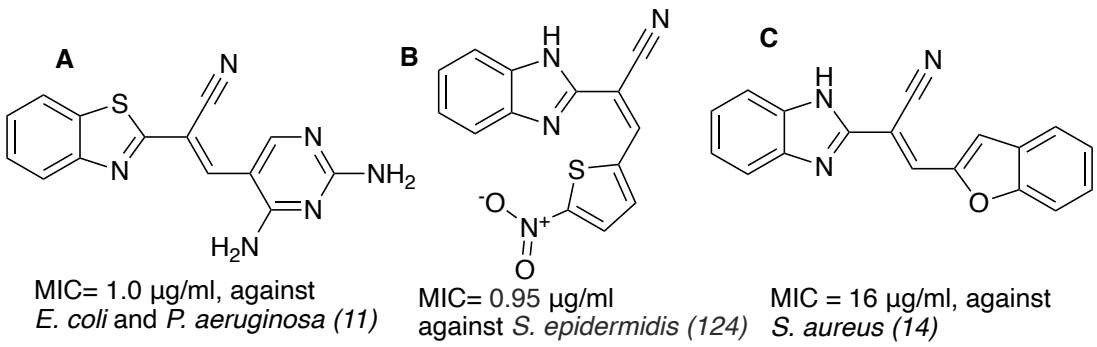


Figure 2.38. Some antibacterial acrylonitrile derivatives in literature

Antiviral Activity

The study focused on the benzimidazole acrylonitrile derivatives that target the Vif-A3G interaction and can be used for the treatment of HIV-1. The analysis showed that *N,N*-alkyl substitution or p-hydroxyl coupled with m-methoxy substitution of ring A, benzimidazole substructures comprising rings B and C, and an alkene linker between rings A and B were necessary for the anti-HIV-1 activity of the compounds. The compound 2-(1*H*-benzimidazol-2-yl)-3-(4-diethylaminophenyl)acrylonitrile demonstrated the highest potency, with IC_{50} values of 3.45 nM in the anti-HIV-1 replication assay in H9 cells. This compound also showed acceptable acute toxicity, suggesting that it has potential as a

starting point for developing more potent inhibitory compounds for the treatment of HIV-1 (125) (**Figure 2.39**).

Forero et al. conducted a bio-guided screening of *Euphorbiaceae* species against influenza A virus (FLUAV) to investigate the usefulness of bioactive compounds present in medicinal herbs in the treatment or prophylactic treatment of influenza. It was discovered that *Codiaeum variegatum* had significant anti-FLUAV activity. They isolated a cyanoglucoside with the chemical formula 2-(3,4,5)-trihydroxy-6-hydroxymethyltetrahydropyran-2-yloxymethyl)acrylonitrile from *C. variegatum*, which has been reported to have antiviral activity in previous studies (126) (**Figure 2.39**).

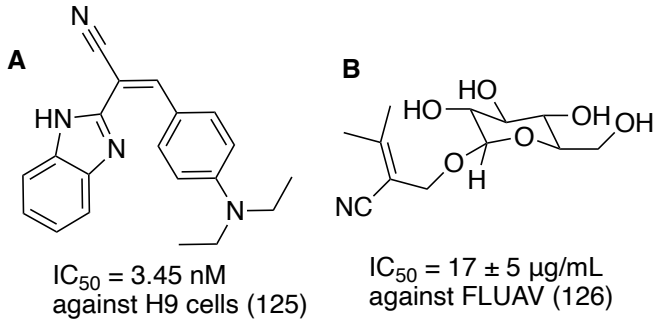
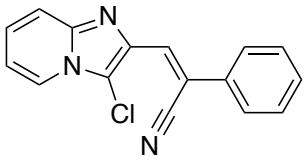


Figure 2.39. Some antiviral acrylonitrile derivatives in literature

Antifungal Activity

In recent years, the use of the acrylonitrile chain in the development of new nitrile-containing drugs has become increasingly popular due to its attractive properties. The imidazo[1,2-*a*]pyridine hybrid molecule, which has shown excellent antifungal activity when connected to functional groups or other rings, has also recently been incorporated into the structure of new topical antifungal azoles such as luliconazole and lanoconazole (127, 128). N'Guessan et. al. developed new anti-Candida imidazo[1,2-*a*]pyridinylarylacrylonitriles, and found that the addition of a chlorine atom at position 3 of imidazo[1,2-*a*]pyridine improved the antifungal activity against three strains of *Candida* (*C. albicans*, *C. glabrata*, and *C. tropicalis*)**.** (*Z*)-3-(3-chloroimidazo[1,2-*a*] pyridin-2-yl)-2-phenylacrylonitrile was found to have the best antimycotic profile with

MICs ranging from 1.4 to 357.5 μM. However, the displacement of the nitrile from one position to another along the phenylacrylonitrile chain did not improve antifungal activity. Standards used in this experiment were fluconazole and ketoconazole with MIC values ranging from 0.64-326.5 μM and 23.52-188.17 μM respectively (129) (**Figure 2.40**).



MIC= 1.4 μg/ml
against *C. tropicalis*

Figure 2.40. Antifungal acrylonitrile derivative

Antiparasitic Activity

Bethencourt-Estrella et al. conducted a study in which they examined a variety of acrylonitriles for their *in vitro* activity against *Leishmania amazonensis*, a parasitic protozoa that causes Leishmaniasis. The results indicated that the acrylonitriles tested were more selective than miltefosine, the standard medication, with a range of activity between 0.57±0.10 and 363.38±20.24 μM against *L. amazonensis* promastigotes (29) (**Figure 2.41 A**).

Sharma et al. synthesized and evaluated benzimidazole acrylonitrile derivatives as potential antimalarial agents with a dual receptor mechanism to overcome drug resistance. The compounds showed significant antimalarial activity, inhibited the production of hemozoin and falcipain-2 enzymes, and had acceptable cytotoxicity limits. These enzymes are required for the growth of *Plasmodium falciparum*, the parasite that causes malaria (16) (**Figure 2.41 B**).

Bethencourt-Estrella et al. conducted a study to evaluate the anti-Trypanosoma cruzi activity of newly synthesized acrylonitriles. The Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. The acrylonitrile derivatives investigated

displayed trypanocidal activity against *T. cruzi*, while exhibiting only moderate cytotoxicity toward murine macrophages. Further investigations revealed that the synthesized acrylonitriles may induce programmed cell death in *T. cruzi*, as evidenced by changes in mitochondrial membrane potential, ATP levels, reactive oxygen species accumulation, and chromatin condensation in *Trypanosoma cruzi* epimastigotes 24 hours after treatment (28) (**Figure 2.41 C**).

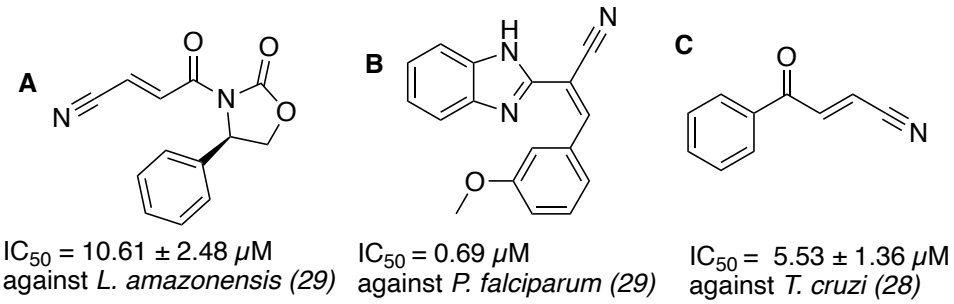


Figure 2.41. Some antiparasitic acrylonitrile derivatives in literature

AChE Inhibitory Activity

Acrylonitriles have also been the subject of several studies investigating their potential as inhibitors of acetylcholinesterase (AChE) (32, 130, 131).

de la Torre et al. designed and tested a series of compounds called (*E*)-2-(benzo[*d*]thiazol-2-yl)-3-heteroarylacrylonitrile derivatives as inhibitors of AChE. They found that the presence of a [5-(4-chlorophenyl)furan-2-yl] substituent at position 3 of the acrylonitrile scaffold led to the most potent inhibitor (*E*)-2-(Benzo[*d*]thiazol-2-yl)-3-[5-(4-chlorophenyl)furan-2-yl]acrylonitrile (IC_{50} = 64 μM). Molecular modeling analysis revealed that hydrogen bond between the nitrogen atom of the acrylonitrile moiety and the backbone nitrogen of Phe288 to at the active site of AChE (130) (**Figure 2.42 A**).

In another study, Parveen et al synthesized a series of (*Z*)-acrylonitrile analogues to evaluate their AChE inhibitory potential. The study revealed that all of the compounds synthesized exhibited significant AChE inhibitory activity. Compound (*Z*)-3-(3′,4′,5′-

Trimethoxyphenyl)-2-(4′′-nitrophenyl)-acrylonitrile, which contained a 3,4,5-trimethoxy-substituted benzene moiety (ring A), displayed the strongest AChE inhibition with an IC_{50} value of 0.20 μM. The research findings indicate that the (Z)-isomer is more stable than the (*E*)-isomer, as confirmed by the crystallization method used to assess their relative stability (32) (**Figure 2.42 B**)

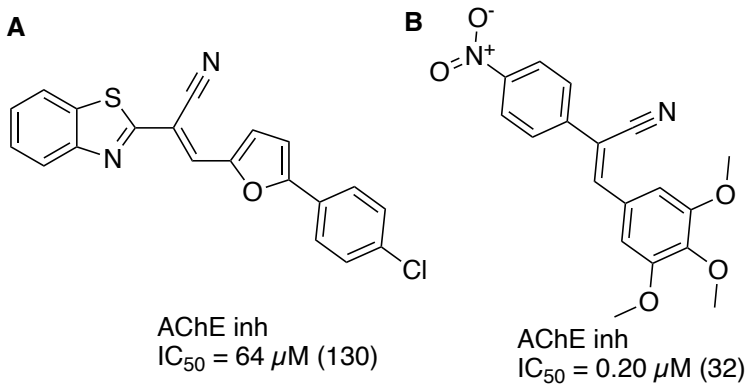
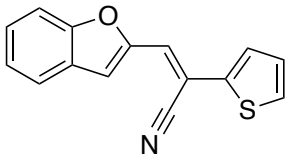


Figure 2.42. Acrylonitriles derivatives with AChE inhibiting activity in literature

Although recent literature has identified the heteroaryl-acrylonitrile scaffold as a promising framework for the development of novel AChE inhibitors (AChEIs) (32, 130), here is no established theory on the structure-activity relationship of E/Z-heteroaryl-acrylonitrile derivatives as AChEIs. To address this gap, de-la-Torre et al. used a comparative similarity indices analysis (CoMSIA) to conduct a three-dimensional quantitative structure-activity relationship (3D-QSAR) analysis on both literature-reported and newly synthesized heteroaryl-acrylonitrile derivatives. The results of this analysis revealed that the electrostatic characteristics of the substituents at positions 2 and 3 on the acrylonitrile scaffold, as well as the presence of hydrogen-bond donor groups, were critical determinants of the inhibitory activity of these compounds. Additionally, the CoMSIA results demonstrated that the electrostatic and hydrogen-bond donor fields were most strongly associated with AChE inhibitory activity. These findings provide a more solid theoretical foundation for predicting the affinities of heteroaryl-acrylonitriles, and offer insight into the structural design, creation, and synthesis of novel and highly selective AChEIs in the future(131).

Antioxidant Activity

Several studies have examined the potential antioxidant activity of compounds containing an acrylonitrile moiety (31, 132-134). In this regard, (*E*)-3-(benzofuran-2-yl)-2-(thiophen-2-yl)acrylonitrile (TACNBNF) was found to exhibit noteworthy antioxidant activity (63.47%) in an *in vitro* antioxidant research. The study evaluated the scavenging capacity of the compound for free radical production using the DPPH assay, with D-ascorbic acid (Vitamin C) as the control antioxidant agent. At a concentration of 500mg/ml, the compound exhibited strong antioxidant activity (63.47%), while D-ascorbic acid (Vitamin C) showed 94.89% (133) (**Figure 2.43**).



antioxidant
DPPH scavenging% = 63.47%

Figure 2.43. Molecular structure of TACNBNF

Anti-inflammatory Activity

In a reported study, newly synthesized acrylonitrile derivatives such as 3-(4-hydroxyphenyl)-2-(6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepin-3-yl)acrylonitrile, exhibited significant analgesic and anti-inflammatory activity in mice with carrageenan-induced paw edema and acetic acid-induced writhing's compared to ketorolac, a nonsteroidal anti-inflammatory drug (NSAID) used to treat pain (30) (**Figure 2.44 A**).

A similar study conducted by Sivaramakarthikeyan et al. investigated the potential anti-inflammatory effects of benzimidazole-tethered pyrazoles using protein denaturation as the evaluation method. The study found that all molecules tested exhibited significant anti-inflammatory activity, with the compound containing para-nitrophenyl moiety on the pyrazole ring (*E*)-2-(1*H*-benzo[*d*]imidazol-2-yl)-3-(3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)acrylonitrile, showing the highest activity (95% inhibition) compared to

other evaluated molecules. Moreover, its activity was superior to that of diclofenac sodium, which is a commonly used anti-inflammatory drug and exhibited a 90% inhibition of protein denaturation (31) (F**igure 2.44 B**).

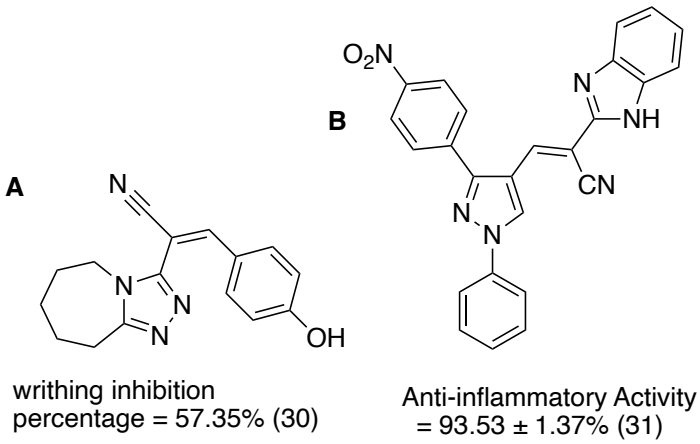


Figure 2.44. Acrylonitrile derivatives with anti-inflammatory activities

Antimycobacterial Activity

Acrylonitriles have been extensively studied as potential antituberculosis drugs. Although some promising findings have been reported, the use of acrylonitriles as antituberculosis drugs is still in the early stages of development and requires further investigation.

Sanna et al. synthesized and evaluated the antitubercular efficacy of 22 acrylonitrile derivatives, finding that several compounds demonstrated potent activity against Mtb. The researchers observed that the position and nature of substituents on the benzotriazole ring significantly affected the activity of the compounds. Further investigation revealed that one of the most active derivatives, (*E*)-2-(2*H*-benzo[*d*][1,2,3]triazol-2-yl)-3-(4-bromophenyl)acrylonitrile had an MIC of 6.25 g/ml against *M. tuberculosis* H37Rv and *M. avium*. These findings suggest that acrylonitrile derivatives could be promising leads for developing new antituberculosis drugs, but

additional research is necessary to optimize their therapeutic potential (36) (**Figure 2.45 A**)

Reshma et al. have developed a series of twenty-two compounds and tested their effectiveness against both replicative and non-replicating bacterial stages. (*E*)-4-(5-(2-(Benzo[*d*]thiazol-2-yl)-2-cyanovinyl)thiophen-2-yl)isophthalicacid was reported to be an exceptionally potent compound that is effective against latent tuberculosis, with a Lysine-aminotransferase (LAT) IC_{50} of 2.62 μM and a considerable log reduction of 2.9 and 2.3 log fold against nutrient-starved and biofilm-forming mycobacteria, respectively (15) (**Figure 2.45 B**). Through genetic expression profiling and highlights, Lysine-aminotransferase (LAT) has been identified as a potential therapeutic target for the treatment of latent tuberculosis (15).

Sirim et al. synthesized and evaluated several benzimidazole-acrylonitrile hybrid derivatives for their antimycobacterial activity against Mtb H37Rv *in vitro*. Among the compounds tested, 2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(4-methylphenyl)piperazin-1-yl)phenyl)acrylonitrile exhibited the highest efficacy with a MIC of 0.78 μg/ml against Mtb. Furthermore, this compound was found to be more potent than first-line drugs including isoniazid, ciprofloxacin, rifampicin, and moxifloxacin, demonstrating a 2.8 log fold reduction in bacterial count of dormant forms of *Mycobacterium* (34) (**Figure 2.45 C**).

Nadaf et al. investigated the antimycobacterial activity of (2*Z*)-2-((*E*)-4-(benzylideneamino)phenyl)-3-(1-methyl-1*H*-imidazol-2-yl)acrylonitrile derivatives against Mtb H37Rv. The compounds containing hydroxy benzaldehyde and salicyaldehyde substituents demonstrated the highest antimycobacterial activity, with a MIC of the three most potent compounds between 0.2 and 0.4 g/ml, while the compound lacking a hydroxyl group showed the lowest activity (17) (**Figure 2.45 D**).

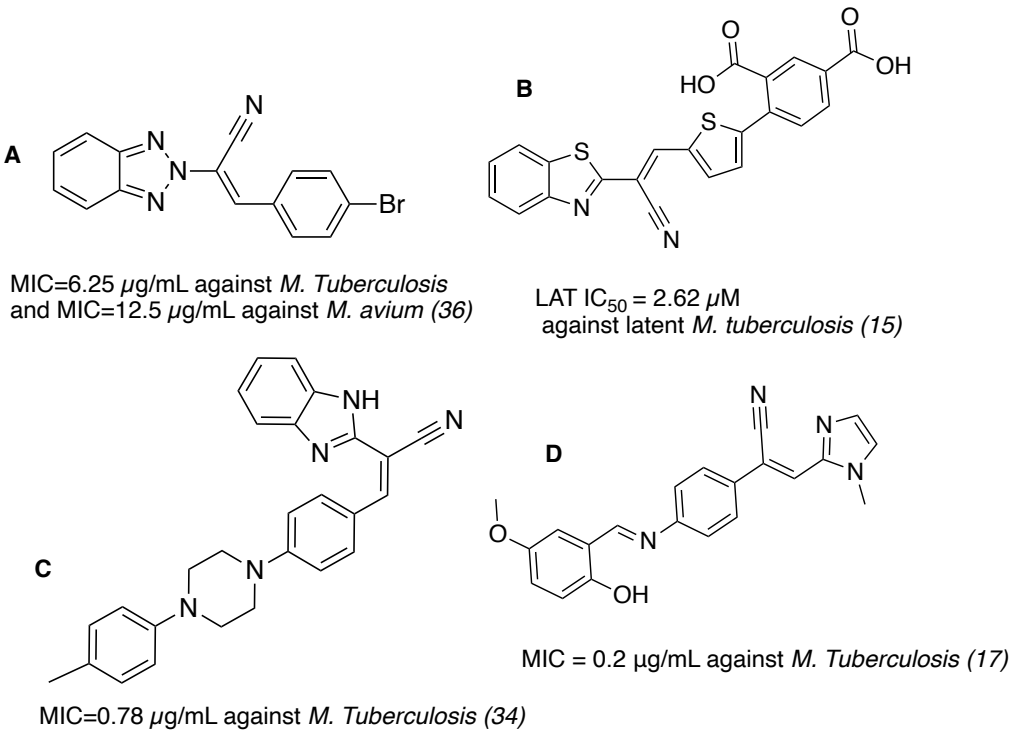


Figure 2.45. Antimycobacterial acrylonitrile derivatives in literature

2.2 Thiazole Derivatives

Thiazole, a heterocyclic compound containing sulfur and nitrogen heteroatoms, was discovered by Hantzsch and Weber in 1887 (135). It is a vital and versatile component of numerous natural products and biologically active heterocyclic compounds. Various functionally diverse thiazole analogs are considered key azole frameworks found in many natural products (35). The thiazole functional group is frequently incorporated as a fundamental component in the structure of therapeutic agents such as ritonavir, penicillin-G, tiazofurin, abafungin, sulfathiazole, sulfazole, bleomycin, pramipexole, febuxostat, and vitamin-B, among others (136).

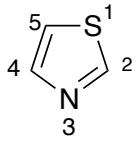


Figure 2.46. Chemical structure or thiazole

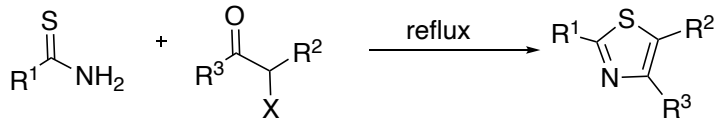
2.2.1 Synthesis of Thiazoles Derivatives

Various pathways have been explored in the development of the thiazole and its derivatives. The synthesis of thiazoles was initiated by Hofmann and Hantzsch employing two principal synthetic routes: addition reaction and cyclization reaction utilizing diverse catalysts and techniques (137).

Hantzsch-Thiazole Synthesis

The process involves addition of α-halogeno ketones and *N*-monosubstituted thioureas in a neutral solvent, yielding only 2-(*N*-substitutedamino)thiazoles. This method provides a simple, quick, and environmentally friendly method for the solvent-free synthesis of 2-aminothiazoles (138, 139) (**Figure 2.47**).

The study by Özkay et al. involved synthesizing hydrazone derivatives of thiazole to assess their anticholinesterase activities. The synthetic approach involved reacting pyrrole-2-carboxaldehydes with thiosemicarbazide in ethanol and then condensing the resulting thiosemicarbazones with α-bromoacetophenone derivatives using the Hantzsch reaction, ultimately yielding 1-substituted pyrrole-2-carboxaldehyde (4-(4-substituted phenyl)-1,3-thiazol-2-yl) hydrazones (140) (**Figure 2.47**).



X = Cl, Br, I, OMs, OTf

R^1 = NH-alkyl, NH-aryl, CH_2CN, NHN=CH-aryl
R^2 = H, alkyl
R^3 = alkyl, aryl

Figure 2.47. Synthesis of thiazole using Hantzsch-Thiazole synthesis.

Cook-Heilbron Thiazole Synthesis

Cook-Heilbron synthesis, which was discovered by Cook and Heilbron, can also be used to synthesize the thiazole ring by using -aminonitriles or -aminoamides and carbon disulfide as reactants. Under mild conditions, aminonitrile is reacted with salt and esters of thioacids, carbon disulfide, or isothiocyanates to produce 5-aminothiazoles, with substitution occurring at position 2 via the reaction of aminonitrile with salt and esters of thioacids, carbon disulfide, or isothiocyanates (141, 142). **Figure 2.48 A** depicts an example of the Cook-Heilbron reaction involving aminonitriles with carbon disulfide to form 5-amino-2-mercapto-thiazoles (141).

Castagnolo et al. described a domino alkylation-cyclization reaction for synthesizing 2-aminothiazoles from a variety of substituted propargyl bromides and thiourea derivatives as starting materials. The reaction was carried out in the presence of a stoichiometric amount of potassium carbonate and DMF as a solvent, and was microwave irradiated at a temperature of 130°C for 10 minutes, consisting of two 5-minute cycles (143) (**Figure 2.48 B**)

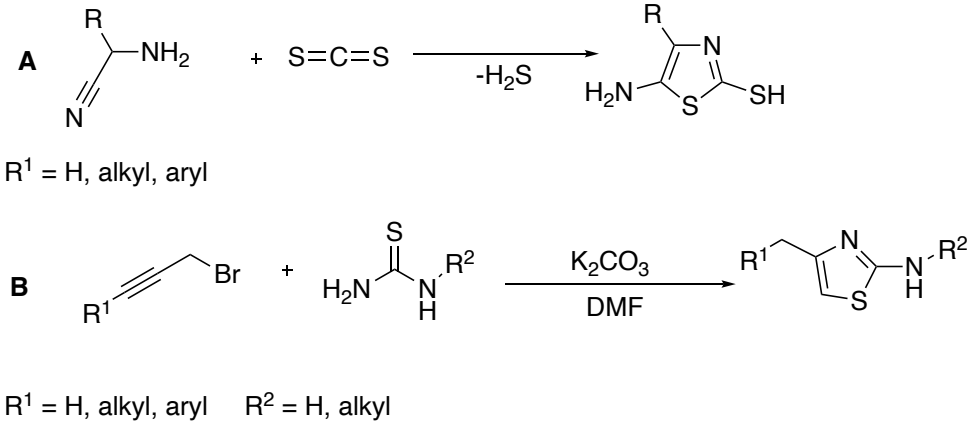


Figure 2.48. Synthesis of thiazole using Cook-Heilbron synthesis

Gabriel synthesis

The Gabriel synthesis is an alternative synthetic technique for synthesizing thiazole derivatives that involves the closure of the thiazole ring via a reaction between acylamino-ketone and phosphorus pentasulfide, resulting in 2,5-disubstituted thiazole derivatives (144). In a study by Kotadiya, phosphorus pentasulfide was heated with *N*-(2-oxopropyl)acetamide to produce the desired compound, 2,5-dimethylthiazole (145) (**Figure 2.49**).

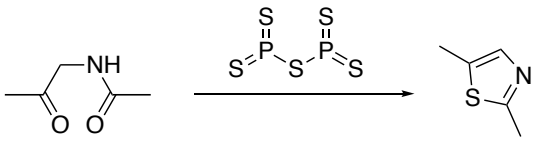


Figure 2.49. Synthesis of thiazole using Gabriel synthesis

2.2.2 Biological activities of Thiazole Derivatives.

Antimicrobial Activity

Despite significant advances in science and the availability of various antibacterial and antifungal drugs, infectious diseases continue to pose a challenge to healthcare systems worldwide due to the emergence of bacterial resistance to existing drugs. Thiazole

derivatives have been discovered to have antimicrobial activity against an array of microorganisms (146-148).

A series of new thiazole derivatives were recently synthesized and examined for antimicrobial activity against several bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, as well as the fungus *Candida albicans*. These derivatives demonstrated broad-spectrum antimicrobial activity with moderate antifungal activity, making them a promising candidate for further development as antimicrobial agents (146) (**Figure 2.50 A**).

In a study, a group of researchers synthesized and characterized a series of Schiff bases that incorporate both 2,4-disubstituted thiazole and cyclobutane rings, as well as hydrazone moieties within the same molecule. These Schiff bases were evaluated for their antibacterial and antifungal activities against various microorganisms, and the results showed a MIC value of 16 µg/ml against C. tropicalis and Bacillus subtilis, indicating their potential as antimicrobial agents (149) (**Figure 2.50 B**).

Bikobo et al. also conducted an antimicrobial activity screening on novel thiazole derivatives against gram-positive and gram-negative bacteria as well as fungi. The results indicated that these derivatives exhibit antibacterial and antifungal properties and demonstrated superior inhibitory activity against *S. aureu*s when compared to the reference drug spectinomycin (150) (**Figure 2.50 C**).

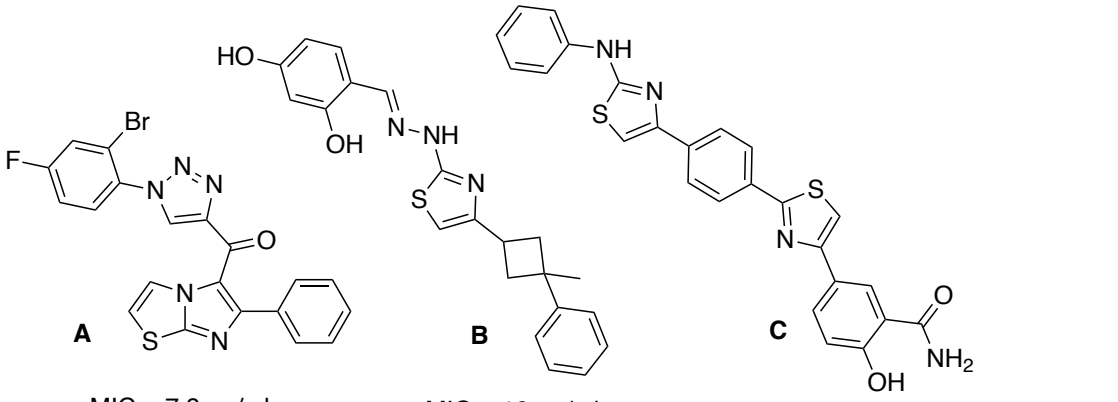


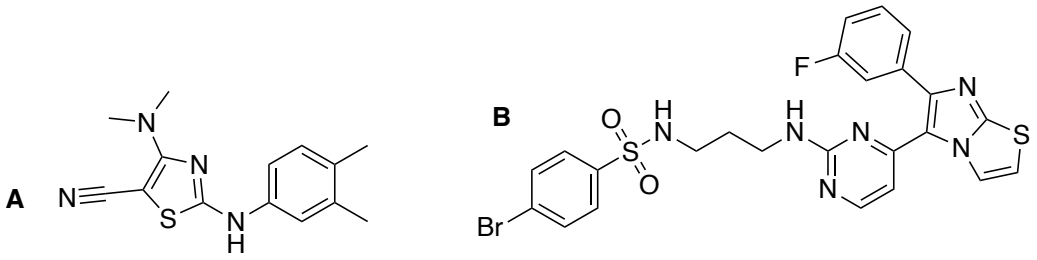
Figure 2.50. Antimicrobial thiazole derivatives in literature

Anticancer Activity

According to published studies, thiazole-based compounds can suppress the progression and proliferation of cancer cells through multiple mechanisms, thereby targeting multiple therapeutic pathways simultaneously. As a result, it is possible to create a diverse range of molecules based on the thiazole-based scaffold with various functions (151-153).

A recent study documents the inhibit Bcl-2 jurkat and A-431 cells by a range of novel thaizole-based compounds. A recent study found that a variety of novel thaizole-based compounds inhibit Bcl-2 jurkat and A-431 cells. At concentrations ranging from 32 to 52 μM, some of the molecules were found to be effective against cell lines while sparing normal cells. Apoptosis in cancer cells may be brought on by the compounds' interaction with the Bcl-2 proteins, according to in silico studies that confirmed this interaction (152) (**Figure 2.51 A**).

Abdel-Maksoud et al. conducted a study to evaluate the effectiveness of imidazo[2,1-*b*]thiazole derivatives in inhibiting the growth of cancer cells, specifically melanoma and colon cancer cells. The researchers synthesized and tested a new series of these derivatives and found that they exhibited strong inhibitory effects against two melanoma cell lines and two colon cancer cell lines. Additionally, the compounds displayed significant enzyme activity against WTBRAF, V600EBRAF, and CRAF mutation, and showed stronger molecular interactions with the BRAF active site compared to the native ligand dabrafenib (153). V600EBRAF mutation, is the most common mutation in BRAF and is present in several cancer types, including thyroid cancer, melanoma, and colorectal cancer (154) (**Figure 2.51 B**).



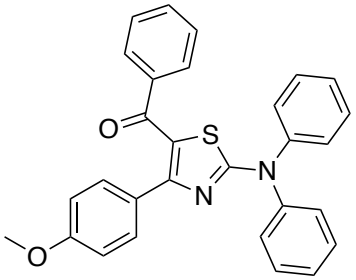
IC_{50} = 34.77 μM against Jurkat Bcl-2
IC_{50} = 34.31 μM against A-431 (152)

IC_{50} = 9.3 nM against ^{V600E}BRAF (154)

Figure 2.51. Anticancer thiazole derivatives in literature

Antiinflammatory Activity

A new class of enzyme 5-lipoxygenase (5-LOX) inhibitors was designed using the pharmacophore modeling technique and tested for inhibitory potential against the 5-LOX enzyme *in vitro*. The results showed that these inhibitors had an IC_{50} value of 0.9±0.1 μM, which was higher than the IC_{50} value of the commercially available drug Zileuton (IC_{50} = 1.5±0.3 μM) (155) (**Figure 2.52**).



5-LOX inhibitor IC_{50} = 0.9 μM

Figure 2.52. Antiinflammatory thiazole derivative

Antimycobacterial Activity

Numerous studies on the synthesis and evaluation of thiazole derivatives for their ability to combat Mtb, the causative agent of tuberculosis, have been conducted (41, 156-158).

Novel 1,2,3-triazole derivatives with thiazole and pyrazole moieties were tested *in vitro* for antimycobacterial activity against both dormant and active strains of Mtb H37Ra. The screening results revealed that these derivatives had moderate to good antitubercular activity against Mtb H37Ra dormant and active strains (158) (**Figure 2.53 A**).

In a study by E. Gürsoy et al., a series of novel derivatives of imidazo[2,1-*b*]thiazole, including acyl-hydrazone and spirothiazolidinone, synthesized and assessed for their antiviral and antimycobacterial activity against Mtb H37Rv strain showed positive results with MIC values equal to or less than 10 µg/ml (41) (**Figure 2.53 B**).

A recent study also detailed the synthesis, characterization, and evaluation of various 5-methyl-4-thiazolidinone derivatives for their potential *in vitro* antimycobacterial activities against the Mtb H37Rv strain. Among the synthesized compounds, the thiazolidinone compound identified as 2-(4-ethoxyphenyl)-5-methyl-3-(phenylamino)thiazolidin-4-one exhibited notable antimycobacterial activity, with a MIC of 12.5 µg/ml against Mtb (159) (**Figure 2.53 C**).

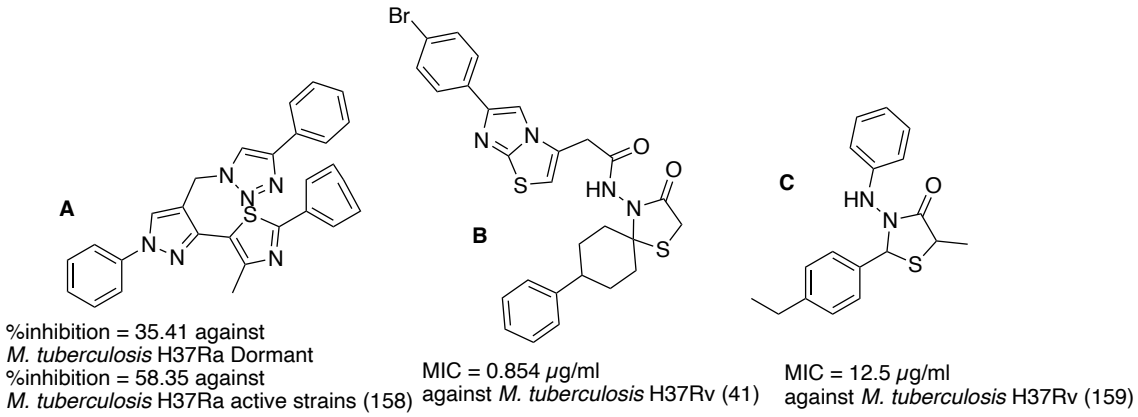


Figure 2.53. Antimycobacterial thiazole derivatives in literature

2.3 Tuberculosis, Vaccine and Treatment

Tuberculosis (TB) remains a significant global public health issue, caused by the infectious agent Mtb. While mycobacteria have supposedly existed for over 150 million years, the first isolation of the bacillus was achieved by the famous German scientist Robert Koch in March 1882 as Tubercle bacillus and was named Mtb a year later (2).

TB has a global presence, yet its incidence is considerably higher in developing countries, primarily due to poverty, malnutrition, HIV infection, smoking, diabetes, and drug resistance being major determinants of the worldwide TB epidemic (160). In 2021, the WHO identified the regions of South-East Asia (45%), Africa (23%), and the Western Pacific (18%) as having the highest incidence of new TB cases, whereas the Eastern Mediterranean (8.1%), the Americas (2.9%), and Europe (2.2%) had the lowest incidence (3). In 2021, it is estimated that there were 10.6 million new cases of TB and 1.6 million TB-related deaths (3).

Mtb is primarily transmitted through the dissemination of airborne droplet nuclei, which are generated through the coughing, sneezing, shouting, or singing of an infected individual (161). These particles exhibit a size range of 1-5 μm and can linger in the air for extended periods, leading to the widespread distribution of the bacterium within a given room or building.

The inhalation of droplet nuclei containing Mtb is the primary mode of infection in susceptible individuals (161, 162). After being exposed to Mtb through aerosol transmission, the immune system may exhibit resistance or clear the bacillus in its early stages (163). In the absence of this, the immune response limits further multiplication of the bacilli within 2 weeks to 3 months after initial infection, resulting in latent tuberculosis infection (LTBI) (161). The bacilli can survive in the body for decades during this stage. Individuals with LTBI are asymptomatic and non-infectious (161, 163). However, if the infection progresses to the symptomatic or active tuberculosis stage, it can result in pulmonary disease, which can lead to further transmission of the disease (163).

Mtb is an intracellular pathogen that can survive in the human body due to its complex cell envelope structure (164-166). TB is a contagious disease caused by Mtb that affects various parts of the body, with the lungs being the most commonly affected (167). TB infection is influenced by environmental factors and is prevalent in congested living conditions, healthcare settings, and households with active TB cases (168). The progression of TB is suppressed by the host immune system, making immunocompromising conditions and lifestyle factors such as smoking and heavy alcohol use risk factors for developing active TB (169, 170). Symptoms of TB include persistent

cough, fatigue, weight loss, fever, night sweats, chest pain, difficulty breathing, chills, and in some cases, cancer (171).

Tuberculosis Vaccine

According to research, natural human defenses against tuberculosis exist, suggesting the possibility of discovering and developing a preventive vaccine (172). Evidence supporting this includes accounts of individuals who have cleared their Mtb infection after becoming infected and later testing negative for the disease (173-175).

Although a vast majority of individuals infected with Mtb can control the infection without developing active tuberculosis, the ongoing tuberculosis epidemic highlights the inability of many people to generate adequate immune control of the infection (175). This underscores the necessity of developing a vaccine against TB.

The Bacillus Calmette-Guérin (BCG) vaccine, developed in 1921, is a weakened form of Mycobacterium bovis, the main cause of bovine tuberculosis, and is the only tuberculosis vaccine approved for worldwide use. It is the most widely used vaccine globally, with over four billion doses administered since its introduction (176).Typically, the BCG vaccine is administered to infants shortly after birth or when they first interact with health services in many countries. Although not universally administered, it is recommended for individuals with a high risk of TB exposure, including healthcare workers and individuals residing with someone with active TB (177).

Despite its limitations, the BCG vaccine continues to be a valuable tool in the fight against TB, and research is being conducted to develop more effective vaccines (172). In September 2022, the WHO identified 16 vaccine candidates for TB in various stages of clinical trials, including four in phase I, eight in phase II, and four in phase III. These vaccine candidates aimed to prevent TB infection and disease, as well as improve treatment outcomes (3).

Tuberculosis Treatment

Following the identification of tuberculosis, several decades were dedicated to the development of therapies and pharmaceuticals to combat this ailment. Notably, this

included the confinement of individuals afflicted with tuberculosis to specialized healthcare facilities known as sanatoriums, as well as the application of Thoracic Tuberculin Surgery. These treatments persisted as the primary therapeutic strategies for tuberculosis until the introduction of antibiotics in the middle of the 20th century, which marked a significant decrease in their usage (1).

In the mid-twentieth century, the development of antibiotics began with the discovery of sulfonamides by Gerhard Domagk in 1935 and streptomycin by Schatz, Bugie, and Waksman in 1944. Streptomycin was initially declared as the most effective antituberculosis agent, but drug resistance was a major challenge. However, the discovery of *p*-aminosalicylic acid (PAS) in the same year proved to be beneficial in reducing drug resistance when used in combination with streptomycin. Subsequently, the introduction of first-line drugs like isoniazid (1952), pyrazinamide (1954), ethambutol, and rifampin (1963) ushered in a new era of medication for tuberculosis treatment, with rifampin being the most effective and widely used drug due to its high efficacy and patient compliance (4).

The emergence of drug resistance among patients treated with isoniazid monotherapy prompted the development of multidrug tuberculosis treatment regimens. For different drugs, chromosomal mutations in bacteria occur spontaneously and at varying frequencies. To combat this, a four-drug regimen for newly diagnosed patients was proposed, with rifampicin and isoniazid as core components. This novel approach revolutionized tuberculosis treatment by drastically reducing treatment duration to from six to eight months, paving the way for short-course chemotherapy (5). The triumph of Rifampicin-containing short-course chemotherapy in the treatment of isoniazid-resistant TB was not long-lasting.

In the 1990s, the effectiveness of this therapy was significantly challenged by the emergence of multi-drug-resistant TB (MDR-TB), which is characterized by the bacterial resistance to at least isoniazid and rifampicin (178). Multi-drug-resistant TB (MDR-TB) has become a major challenge to TB control efforts. However, MDR-TB can still be cured using second-line drugs (SLDs) with a low risk of long-term relapse. Nevertheless, SLDs are less potent and more harmful than the primary drugs used for TB treatment. For

instance, fluoroquinolones such as ciprofloxacin and ofloxacin are often used as SLDs (179). In 2014, the global incidence of extensively drug-resistant (XDR) tuberculosis was reported, characterized by resistance to multiple drugs, including fluoroquinolone and at least one second-line injectable drug such as amikacin, capreomycin, or kanamycin.

Similar to 2020, in 2021, among 3.5 million individuals diagnosed with bacteriologically confirmed pulmonary TB, 71% underwent rifampicin resistance testing, an increase from 61% in 2019 and 50% in 2018. Of these, 141 953 cases of MDR/RR-TB and 25 038 cases of pre-XDR-TB or XDR-TB were found (3). These grim statistics emphasize the dire need for a combined strategy of continuous research and development of new anti-tuberculosis molecules for all forms of DR-TB, as well as ensuring improved access to both diagnosis and effective treatments.

The categorization of anti-TB drugs has traditionally been divided into first- and second-line drugs, with isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin being the primary first-line drugs. However, in 2014, the WHO developed a new classification system that grouped TB drugs into five categories based on efficacy, experience of use, safety, and drug class (180).

Group 1. First-line oral drugs; isoniazid, rifampicin, ethambutol, pyrazinamide, rifabutin and rifapentine.

Group 2. Injectable anti-TB drugs (injectable agents or parental agents); streptomycin, kanamycin, amikacin and capreomycin.

Group 3. Fluoroquinolones (FQs); levofloxacin, moxifloxacin and gatifloxacin.

Group 4. Oral bacteriostatic second-line anti-TB drugs; ethionamide, prothionamide, cycloserine, terizidone, para-aminosalicylic acid and para-aminosalicylate sodium.

Group 5. anti-TB drugs with limited data on efficacy and/or long-term safety, including new anti-TB agents; bedaquiline, delamanid, linezolid, clofazimine, amoxicillin/clavulanate, imipenem/cilastatin, meropenem, high-dose isoniazid, thioacetazone and clarithromycin.

WHO updated the classification of medicines used in treating multidrug-resistant and rifampicin-resistant tuberculosis (MDR/RR-TB) in the 2022 operational handbook on tuberculosis. The updated classification was based on evidence from the 2018 WHO

guidelines on DR-TB treatment which categorizes TB medicines for MDR/RR-TB treatment into three groups (A, B, and C) according to drug class and the level of certainty in their effectiveness and safety. This classification considers the balance between benefits and risks associated with each drug (181).

Group A: Include all three medicines; levofloxacin or moxifloxacin, bedaquiline and linezolid.

Group B: Add one or both medicines; clofazimine and cycloserine or terizidone.

Group C: Add to complete the regimen, and when medicines from Groups A and B cannot be used; ethambutol, delamanid, pyrazinamide, imipenem/cilastatin or meropenem, amikacin or streptomycin, ethionamide or protionamides and *p*-aminosalicylic acid.

Isoniazid

Isoniazid (INH or H) is an effective drug used to treat TB and as a preventative measure for people who have latent Mtb infection (182). INH is a prodrug that enters MTB cytoplasm via passive diffusion and is activated by catalase-peroxidase (KatG) **(Figure 2.54)**. Active INH products kill only actively dividing bacteria by targeting specific enzymes (InhA and KasA). INH is bacteriostatic initially but becomes bactericidal after 24 hours. INH inhibits mycolic acid (MA) synthesis, which is essential for mycobacteria's acid-fastness and cell envelope impermeability. MAs are mycobacteria-specific and a selective target for anti-TB drugs (183).

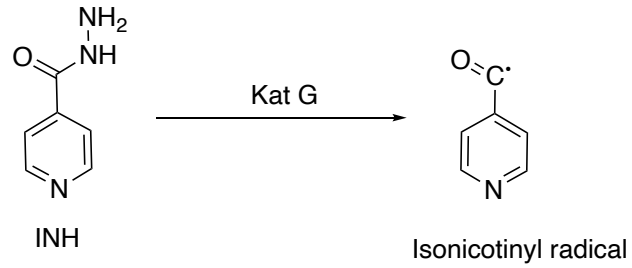


Figure 2.54. INH activation to isonicotinyl radical

Rifampicin

Rifampicin, a modified form of rifamycin, has been an effective anti-tuberculosis drug since its introduction in 1972, owing to its high sterilizing activity against Mtb (184). Rifampicin, along with isoniazid and pyrazinamide, has been an important component of short-course TB chemotherapy (185). The enzyme DNA-dependent RNA polymerase (RNAP) is required for transcription. The 400 kDa RNAP core enzyme's -subunit binds to RIF near the RNA/DNA channel to inhibit RNA chain elongation. As a result, the -subunit of bacterial RNAP is physically blocked (186) (**Figure 2.55**).

Ethambutol

Ethambutol is a bacteriostatic drug that inhibits bacterial multiplication by interfering with arabinogalactan biosynthesis in the cell wall (187). According to research, ethambutol acts synergistically with isoniazid against Mtb by binding to a TetR transcriptional regulator, which increases the sensitivity of the targeted inhA gene. This gene encodes an essential enoyl-acyl carrier protein reductase, which is required for the integrity of the bacterial cell wall (188) (**Figure 2.55**).

Pyrazinamide

PZA is a derivative of pyrazine-2-carboxylic acid that exhibits superior penetration capabilities across biological membranes (189). Its antibacterial efficacy is dependent on pyrazinamidase, an enzyme found in PZA-susceptible tubercle bacilli and absent in resistant strains. This enzyme converts pyrazinamide to pyrazinoic acid, which has demonstrated antituberculosis activity *in vitro* (190) (**Figure 2.55**).

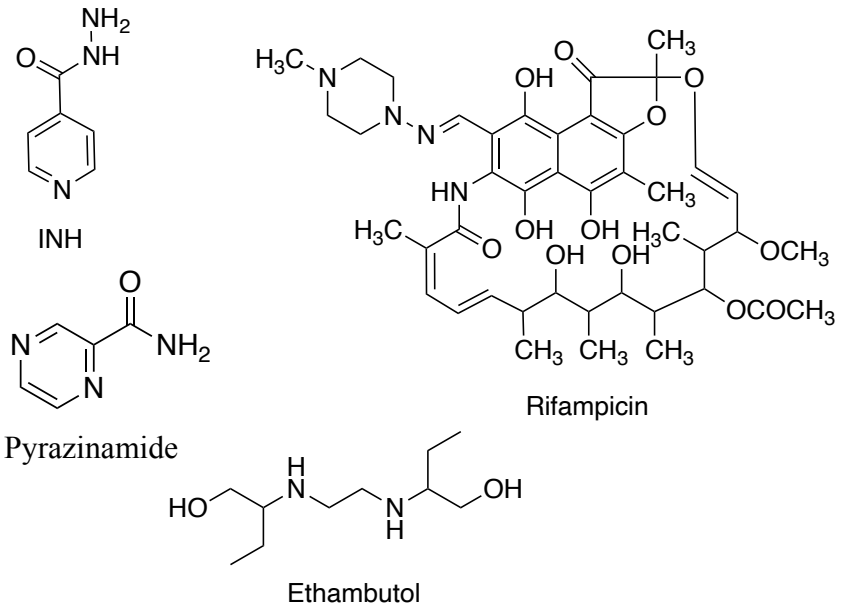


Figure 2.55. Molecular formulars of first-line oral antituberculosis drugs

Rifabutin

Rifabutin, an analog of rifampicin, is commonly employed in patients co-infected with HIV due to its reduced potential for drug interactions with antiretroviral agents, compared to rifampicin. The drug is also effective in treating atypical mycobacterial infections, and in some instances, has shown efficacy against rifamycin-resistant strains (191). Like rifampicin, Rifabutin functions via inhibition of the β-subunit-dependent DNA–RNA polymerase, thereby suppressing DNA formation by Mtb (192) (**Figure 2.56**).

Rifapentine

Rifapentine, also a rifampicin derivative with a cyclopentyl substitution, was synthesized in 1965 and was granted approval for use in the United States in 1998. The drug exhibits bactericidal activity against Mtb by impeding bacterial RNA polymerase, which disrupts the transcription of mRNA from DNA templates (193) (**Figure 2.56**).

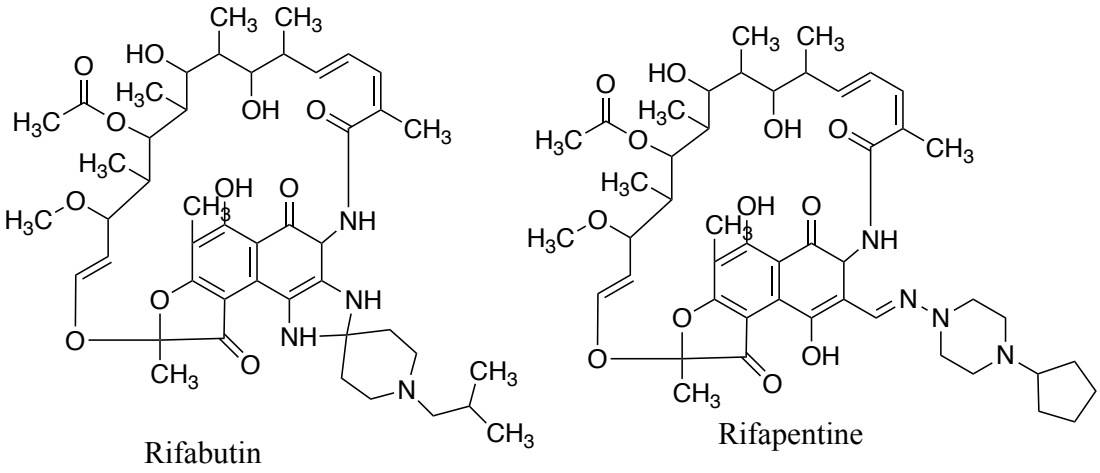


Figure 2.56. Molecular structures of rifampicin analogues

Aminoglycosides

For over half a century, aminoglycosides, specifically streptomycin, have demonstrated efficacy against tuberculosis. Kanamycin and amikacin, and capreomycin, are crucial secondary antibiotics used to manage patients with multidrug-resistant tuberculosis (MDR-TB). These drugs are administered by injection and operate by influencing the 30S ribosomal subunit, leading to a frame shift mutation and/or hindering RNA translation (194, 195) (**Figure 2.57**).

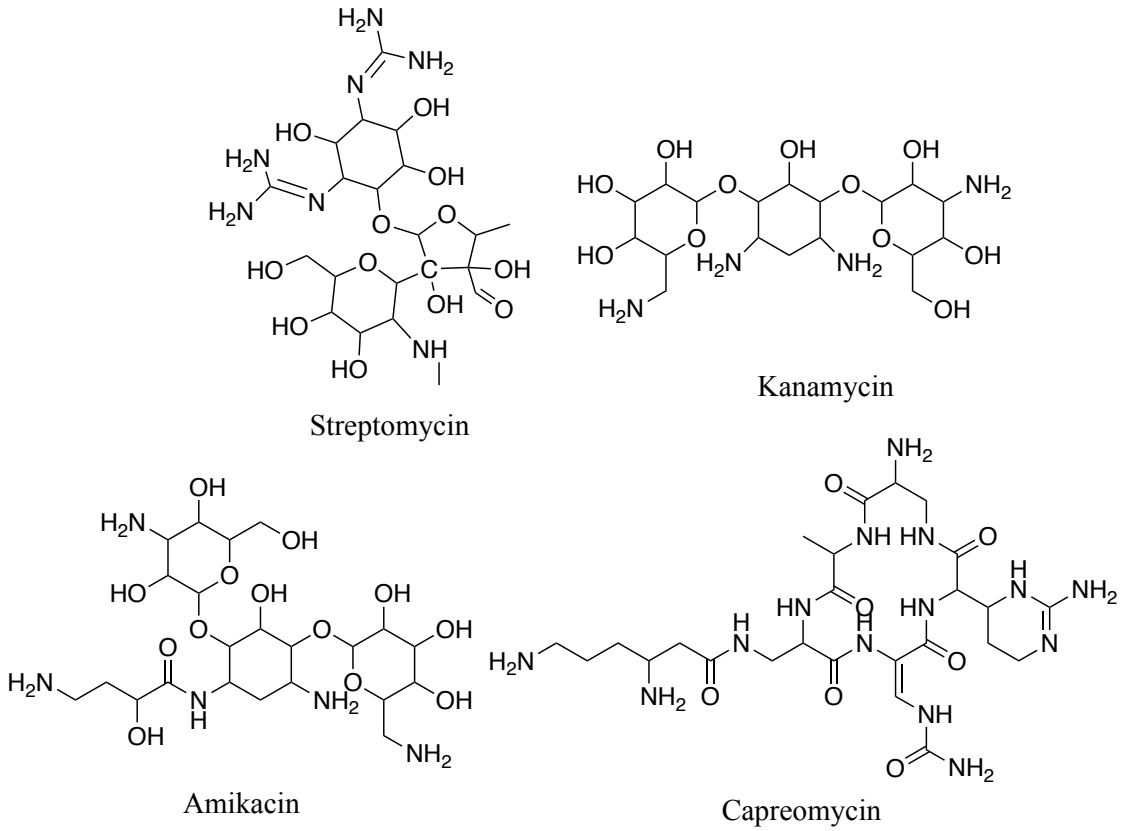


Figure 2.57. Molecular structures of aminoglycosides

Fluoroquinolones

Fluoroquinolones such as levofloxacin, moxifloxacin and gatifloxacin are antibiotics that inhibit bacterial DNA synthesis They inhibit two DNA topoisomerases, DNA gyrase and topoisomerase IV, which are required for replication but are absent in human cells, making them both specific and bactericidal. DNA topoisomerases are essential in the separation of bacterial DNA double-helix by inserting an additional strand of DNA through the break and resealing the previously separated strands (196) (**Figure 2.58**).

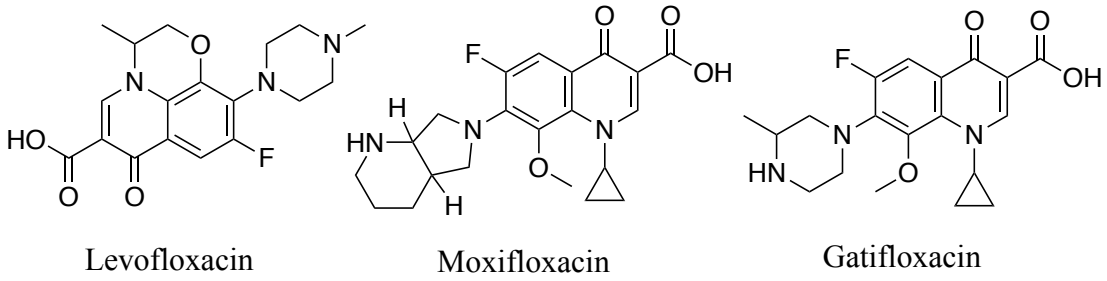


Figure 2.58. Molecular structures of fluoroquinolones

Second-line Antituberculosis Drugs

Second-line antituberculosis drugs include ethioamides, protionamides cycloserine or its derivative terizidone, and *p*-aminosalicylic acid (PAS), a structural analog of the folate precursor PABA, is one of the oldest antitubercular drugs, having entered clinical use in 1944. PAS is a prodrug that activates the folate biosynthetic pathway in Mtb, eventually inhibiting dihydrofolate reductase (DHFR) (197). Thioamide drugs are thought to inhibit mycolic acid biosynthesis while ethionamide, like isoniazid, is an inhA inhibitor. Cycloserine and terizidone, a cycloserine double molecule, on the other hand inhibits the incorporation of alanine into the mycobacterial cell wall by inhibiting specific enzymes competitively (198) (**Figure 2.59**).

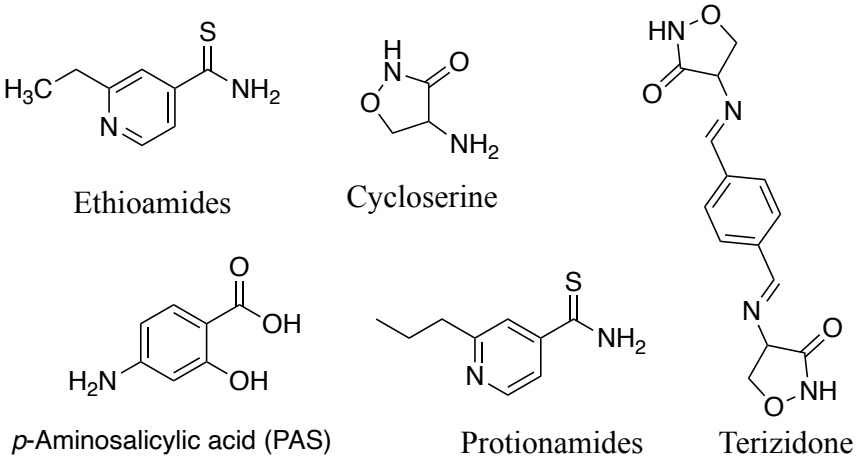


Figure 2.59. Molecular structures of second line antituberculosis drugs

Beta-lactam Antibiotics

Beta-lactam antibiotics, including co-amoxiclav (Amoxicillin/clavulanic acid) and synthetic carbapenems such as imipenem (IMP) and Meropenem (MRP), are capable of targeting several transpeptidases or penicillin-binding proteins that participate in the process of bacterial cell-wall synthesis. The effectiveness of these antibiotics can be enhanced by the presence of a beta-lactamase inhibitor (157, 198, 199) (**Figure 2.60**).

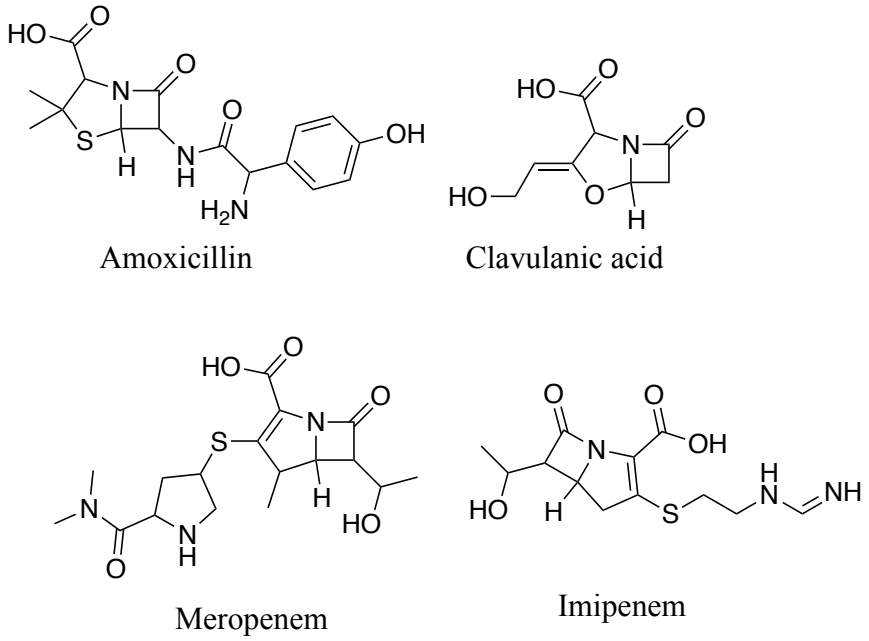


Figure 2.60. Molecular structures of beta-lactam antibiotics

Bedaquiline

On December 28, 2012, the FDA granted accelerated approval to SIRTURO™ (bedaquiline) Tablets for the treatment of multi-drug-resistant TB (MDR-TB) in combination therapy for adults (200). Bedaquiline, also known as TMC207, is a diarylquinoline drug (**Figure 2.61**) that targets the energy metabolism of mycobacteria. *Mycobacteria* require ATP for survival, and ATP production through ATP synthase is essential for both active and dormant, replicating and non-replicating, extracellular and

intracellular, and fermenting and non-fermenting mycobacteria. Bedaquiline selectively inhibits the mycobacterial ATP synthase complex (200, 201).

Delamanid

Delamanid (**Figure 2.61**) is a potential anti-TB drug that exhibits mycobacteria-specific bactericidal activity without affecting gram-positive or gram-negative bacteria or intestinal flora (202). It is a prodrug that is activated by the enzyme deazaflavin-dependent nitroreductase of Mtb. Delamanid inhibits the synthesis of methoxy and keto mycolic acids (MA) through the mycobacteria F420 system, generating nitrous oxide, thus preventing the synthesis of MA, which is crucial for the survival of mycobacteria (203).

Linezolid

Linezolid is an oxazolidinone antibiotic (**Figure 2.61**) that inhibits protein synthesis by binding to 23S ribosomal RNA of the bacterial 50S subunit, which prevents the formation of a functional 70S initiation complex (204). It is approved for the treatment of resistant gram-positive bacteria and has also shown effectiveness against DR-TB (205). The latest WHO guidelines for drug-resistant tuberculosis recommend linezolid as a core second-line medicine in the MDR-TB regimen (206).

Clofazimine

Clofazimine's (**Figure 2.61**) antimicrobial mechanism is not fully understood, but it is suggested that it mainly acts on the outer membrane of mycobacteria by targeting the respiratory chain and ion transporters. This inhibition is thought to occur through phenazine molecules, which act as auto-oxidizable electron acceptors. Consequently, the respiratory system oxidizes Clofazimine instead of NADH, leading to a reduction in ATP availability for cellular processes (207).

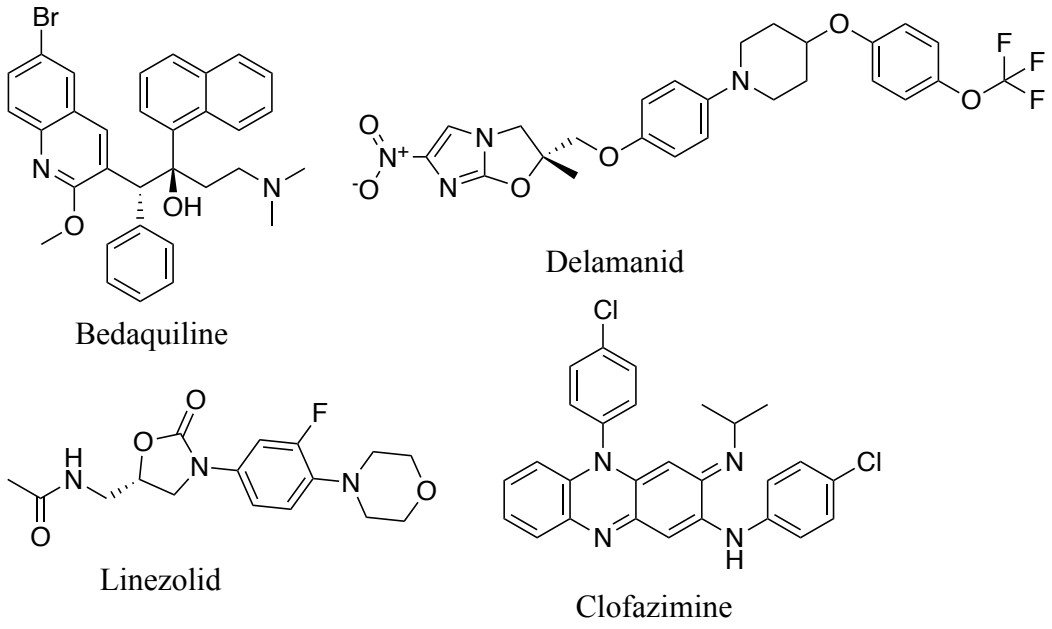


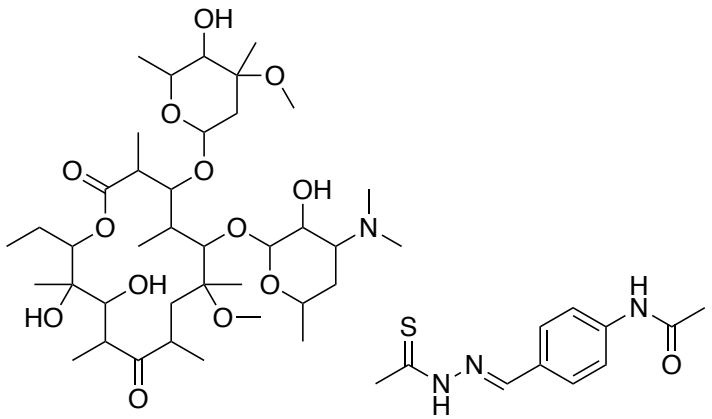
Figure 2.61. Molecular structures of some multi-drug-resistant TB (MDR-TB) drugs

Clarithromycin

Clarithromycin (**Figure 2.62**) is a second-generation macrolide that exerts its antimicrobial action by impeding protein synthesis in a broad range of bacteria through binding to the large ribosomal subunit and inhibiting the formation of the 50S particle in rapidly dividing cells (208). Despite its effectiveness against other mycobacterial infections, the clinical usage of clarithromycin against Mtb is restricted due to the intrinsic resistance of this bacterium to macrolides (209).

Thioacetazone

Thioacetazone (**Figure 2.62**) is a prodrug that is activated via S-oxidation of its thiocarbonyl moiety by the flavin-containing monooxygenase EthA. It inhibits mycolic acid biosynthesis when activated by binding to the HadA component of the HadABC dehydratase complex (210). It also inhibits mycolic acid cyclopropanation, which is required for cell envelope permeability, host immunomodulation, and Mtb persistence (211, 212).



Clarithromycin Thioacetazone

Figure 2.62. Molecular structures of clarithromycin and thioacetazone

2.4 Infections and Antimicrobial Treatments

Infection is defined as the incursion of pathogenic agents into living tissue, leading to their proliferation and production of toxins, as well as eliciting a response from the host tissue. Diseases that arise from the presence of infectious agents are categorized as infectious diseases. The causative agents of infections comprise a wide range of biological entities, including viruses, viroid, prions, bacteria, fungi, nematodes (hookworms, pinworms), arthropods (lice, fleas, ticks), and other macro-parasites such as tapeworms. Pharmacological interventions employed in the treatment of infectious diseases are typically classified as antiviral, antibiotic, antifungal, antiprotozoal, and anthelmintic agents (213).

Bacterial Infections and Antibiotics

Bacteria are prokaryotic unicellular microorganisms. They are well-known for their adaptability and versatility, as they can live in a variety of environments ranging from soil and water to the human body. Bacteria are also known for their metabolic diversity, which enables them to perform a variety of functions such as decomposition, nitrogen fixation, and fermentation (214). Bacterial cells are relatively simple, lacking a nucleus and other membrane-bound organelles. Their genetic material, on the other hand,

is contained in a single circular chromosome, along with smaller plasmids that may contain additional genetic information. Bacterial cell envelopes are generally made up of a cell membrane, a cell wall, and sometimes an outer capsule or slime layer. The cell wall provides structural support and protection, and it is made up of proteins (214, 215).

Bacteria have evolved several mechanisms for genetic material exchange, including horizontal gene transfer and recombination. This enables them to rapidly acquire new characteristic features that may be favorable in specific environments, such as antibiotic resistance or the ability to degrade specific pollutants. Some bacteria can also form biofilms, which are complex communities of cells that adhere to surfaces and provide protection against environmental stressors (216).

While many bacteria are harmless or even beneficial to humans and other organisms, some are pathogenic. Pathogenic bacteria can cause a variety of symptoms and complications by producing toxins or invading host tissues (217). However, advancements in microbiology have enabled the development of antibiotics and other treatments that can aid in the treatment of bacterial infections.

Antibiotics are classified into several groups or classes, each with its own mechanism of action and spectrum of activity. They work by disrupting the life cycle or cellular processes of bacteria, which ultimately leads to their death. The general mechanism of action of antibiotics involves targeting specific components of bacterial cells, such as their cell walls, membranes, proteins, and DNA (218).

The thiazole group has been reported by some studies on molecular docking to contributes to the antibiotic's potency and specificity against bacterial cells due to its interaction with the enzyme's active site by orienting its bulky imine moieties (219, 220). Among the various types of antibiotics are examples of known antibiotics that contain a thiazole group (**Figure 2.63**).

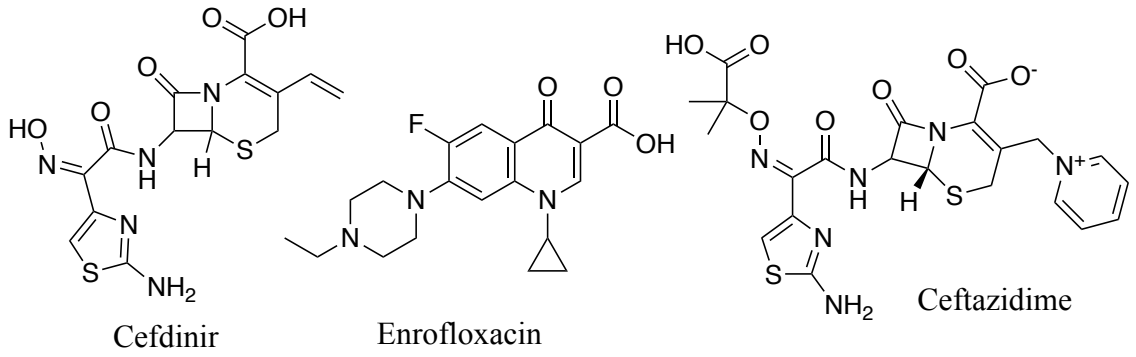


Figure 2.63. Molecular structures of some thiazole-containing antibiotic

Fungal Infections and Antifungals

Fungi are eukaryotic organisms that can be found in almost all environments, including soil, water, air, and on other living organisms. Fungi can reproduce both sexually and asexually, depending on the species. They produce spores, which are small, lightweight structures that can be carried by wind, water, or other organisms. Fungi obtain nutrients by absorbing them from their surroundings, usually from dead or decaying matter (221).

Although fungi are integral to the ecosystem, but some species can cause infections in humans and animals. Fungal infections, or mycoses, can affect various parts of the body and are caused by several species of fungi, including yeasts and molds. Fungi can enter the body through different routes such as the skin, inhalation, ingestion, or contact with contaminated surfaces(222). Factors such as weakened immune systems, diabetes, cancer, antibiotic use, and exposure to contaminated environments can increase the risk of developing a fungal infection (223).

Fungal infections can be classified into four categories: cutaneous, subcutaneous, systemic, and opportunistic infections. Cutaneous infections involve the deeper layers of skin and can be triggered by yeasts and dermatophytes, with examples including tinea versicolor and candidiasis. Candidiasis and pityriasis versicolor currently affect 20%-25% of the global population, but their prevalence is increasing (224). Subcutaneous infections, on the other hand, affect underlying tissues and muscles, and are caused by fungi present in soil, with examples including sporotrichosis and mycetoma. Mycetoma is a chronic

infectious disease that can be caused by various fungi species (eumycetoma) or aerobic filamentous bacteria (actinomycetoma), while sporotrichosis is also a chronic subcutaneous mycosis caused by a group of dimorphic fungi from the genus Sporothrix. Both mycetoma and sporotrichosis have a global distribution, especially in tropical areas (225). Systemic infections also known as invasive fungal infections, are caused by fungi entering the body through inhalation or ingestion and can affect various organs and tissues. *Candida*, *Aspergillus*, and *Cryptococcus* species are the most common causes of invasive fungal infections (IFIs), which pose a significant clinical challenge due to the associated morbidity and mortality (226).

Candida species are the primary cause of mycoses in humans, with candidiasis being an opportunistic infection that can spread throughout the body in those with weakened immune systems (227). Candida albicans is the most common cause of systemic candidiasis, which has a high mortality rate. However, non-albicans Candida species, such as *C. dubliniensis*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, are becoming more prevalent and are associated with high mortality rates and drug resistance (228, 229). Bloodstream or invasive infections have a high mortality rate and can have a considerable impact on hospital operations, making them a major healthcare concern worldwide. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species*, *Streptococcus pneumoniae*, and *Salmonella* species are the main pathogens responsible for these illnesses. Conversely, the use of empiric and broad-spectrum antibiotics to treat infections brought on by these bacteria has the potential to harm patients and medical professionals due to the evolution of resistance (230).

Antifungal drugs are categorized based on their impact into two groups known as fungistatic and fungicidal. Fungistatic substances impede the growth of fungi without killing them, unlike fungicides. However, their mechanisms of action determine their classification into various subgroups (231).

The azoles are a type of antifungal agent which includes drugs like fluconazole and itraconazole. Azoles block the activity of the enzyme lanosterol demethylase, which

inhibits the synthesis of ergosterol, a key component of the fungal cell membrane. This inhibition causes toxic sterols to accumulate in the fungal cell membrane, impairing membrane function and eventually leading to cell death (232). Azoles are the most common antifungal drugs used in clinics, partly because pharmaceutical companies and medicinal scientific centers have focused their attention on developing them (232). Azoles are generally well tolerated by patients, but they can cause significant drug-drug interactions and the emergence of drug-resistant fungal strains (233) (**Figure 2.64**).

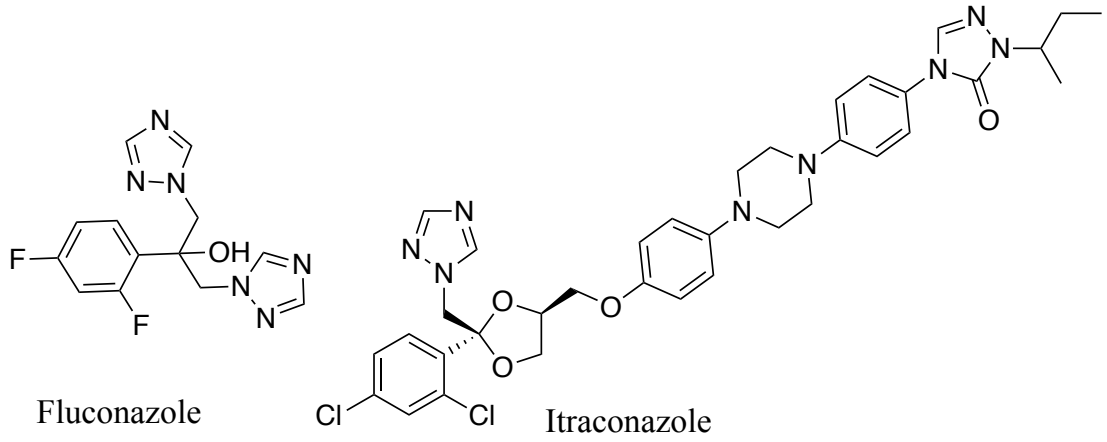


Figure 2.64. Molecular structures of some examples of azoles

3. METHOD AND MATERIALS

3.1 Chemical Studies

3.1.1 Materials

2-Cyanoacetamide, Lawesson's reagent, 2,2',4'-trichloroacetophenone (**b**), *N*-bromosuccinimide (NBS), *p*-toluenesulfonic acid (*p*TsOH), 4'-chloroacetophenone, 4'-fluoroacetophenone, benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 4-(trifluoromethyl)benzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-fluorobenzaldehyde, 4-ethylbenzaldehyde, 4-ethoxybenzaldehyde, 4-(dimethylamino)benzaldehyde, 4-hydroxybenzaldehyde, thiophene-2-carbaldehyde, furan-2-carbaldehyde, and *L*-proline used in our studies are products of *Sigma* company. Solvents used for the thin layer chromatography (TLC) system are *n*-hexane and ethyl acetate.

3.1.2 General Synthesis Procedure

Synthesis of 2-Cyanothioacetamide (a)

The synthethic route for the thionation 2-cyanoacetamide is outlined in scheme 1 as described in published methodology (234). A mixture 2-cyanoacetamide (119 mmol) and Lawesson's reagent (61.1 mmol) in 180 ml of tetrahydrofuran (THF) was stirred at room temperature overnight. The precipitated solid was filtered and air dried. The resulting crude compound was used for the subsequent step without further purification (83% yield) (**Figure 3.1**).

Synthesis of 2-Bromoacetophenone Derivatives (c and d)

To a solution of NBS (4.28 g, 24 mmol, 2.4 equiv) and *p*TsOH (0.4 g, 2 mmol, 0.2 equiv) in dichloromethane (DCM) (10 ml), a solution of substituted acetophenone (20 mmol) in DCM (8 ml) was added in drops at 0° C. Afterward, the reaction mixture was heated at reflux for 4 hours. After the completion of the reaction was confirmed by thin

layer chromatography (TLC), H_2O (20 ml) was added and the organic layer was separated, followed by double extraction of the aqueous layer with DCM (30 ml). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (20 ml) and brine (20 ml) to remove excess water, dried over anhydrous Na_2SO_4, and concentrated under low pressure. The solid obtained **c** and **d** was used for the following step without further purification (85-89% yield) (235) (**Figure 3.1**).

Synthesis of 3-Aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile Derivatives (1-16)

A solution comprising 1 mmol of **a**, 2-haloacetophenone derivatives **b-d** (1 mmol), appropriate aldehydes (1 mmol), 10 mol % *L*-proline, and 3-5 mL of ethanol was subjected to reflux on an oil bath for 2-5 hours. The progress of the reaction was monitored using TLC. Upon completion of the reaction, the resulting solid was filtered, washed with cold ethanol, and subjected to recrystallization from a suitable solvent to yield pure target compound **1-16** (35) (**Figure 3.1**).

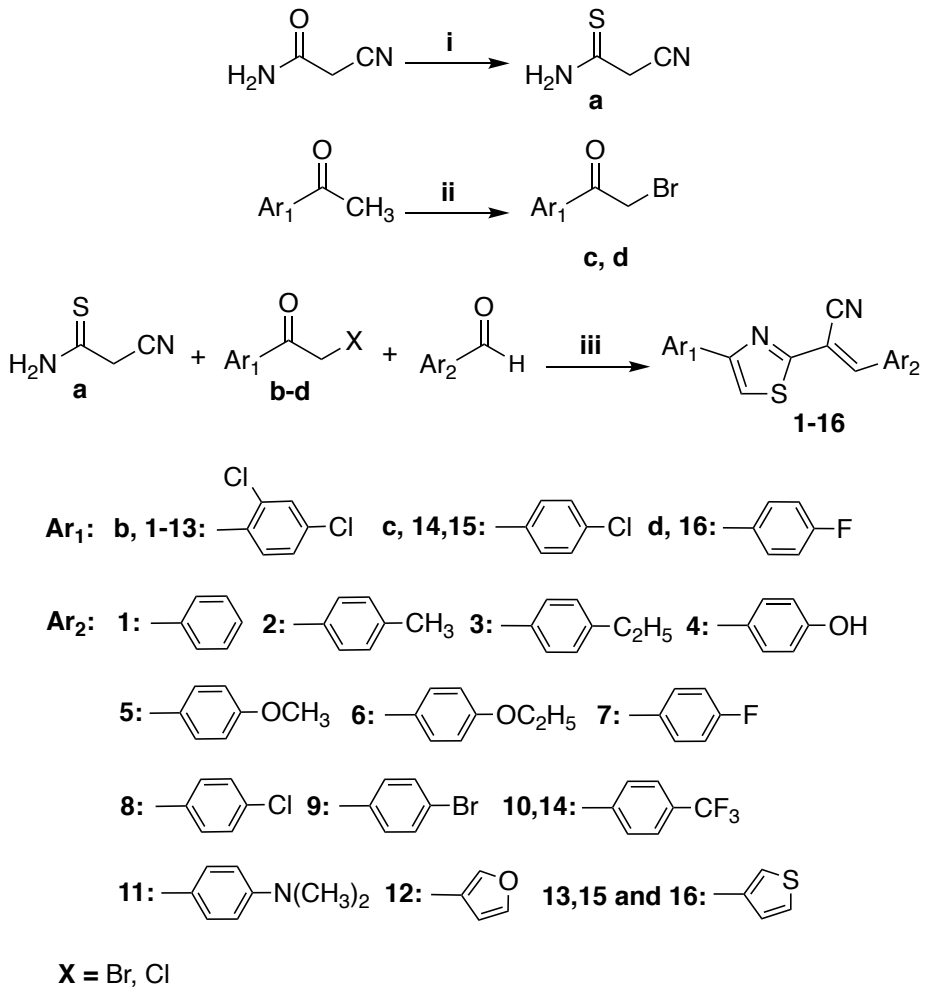


Figure 3.1. Synthesis strategy of target compounds **1-16**. Reagents and conditions: (i) Lawesson's reagent, THF (ii) NBS, *p*TsOH, DCM, H_2O; (iii) *L*-proline, EtOH.

3.1.3 Analytical Methods

Melting Points

Melting points of synthesized molecules were determined by the Thomas Hoover Capillary Melting Point Apparatus device and recorded values are uncorrected.

3.1.4 Spectroscopic Methods

IR Spectra

The IR spectra of the synthesized compounds were obtained, and the wave number was evaluated on the (cm^{-1}) scale using the Perkin Elmer FT-IR System Spectrum BX spectrophotometer, Reduced Total Reflection (ATR) apparatus MIRacleTM PIKE Technologies, zinc selenide (ZnSe crystal) at Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Chemistry Research Laboratory.

^{1}H-NMR and ^{13}C-NMR Spectra

^{1}H-NMR and ^{13}C-NMR spectra of the compounds were obtained in either using Varian Mercury (Agilent) 400 MHz and Bruker Avance Neo 500 MHz spectrometer devices in deuterated dimethylsulfoxide (DMSO-d_6, Merck) or deuterated chloroform $CDCl_3$ solution at Ankara University Faculty of Pharmacy Central Laboratory, results were evaluated on the δ (ppm) scale and coupling constants, *J*, were reported in hertz.

Mass Spectra

The HRMS spectra of the synthesized compounds were measured at Hacettepe University Advanced Technologies Research and Application Center (HUNITEK) using the Bruker Daltonics maXis II ETD nLC/LC-QTOF-Mass Spectrometer device.

3.2 Biological Activity Studies

Studies on the antimycobacterial activities of the synthesized compounds were carried out in the Microbiology Department of the University of Oslo (Oslo, Norway). Antimicrobial activity studies were carried out in the Hacettepe University Antimycobacterial Research Laboratory (Sihhiye, Ankara, Turkey).

3.2.1 *In vitro* Antimycobacterial Activity Studies

Material and Method

The inoculum was derived from fresh Löwnstein-Jensen medium, which was suspended in 7H9-S medium supplemented with oleic acid, albumin, dextrose, and catalase (OADC), and then adjusted to an OD590 of 1.0 and diluted 1:20. An inoculum of 100 µl was used for each drug concentration tested. To prepare the drug stock solutions, each drug was thawed and diluted in 7H9-S at four times the final highest concentration. Serial two-fold dilutions of each drug were then made directly in a sterile 96-well microtiter plate, using 100 µl 7H9-S. A growth control without antibiotics and a sterile control were included on each plate. Sterile water was added to the perimeter wells to prevent evaporation during incubation. The plate was sealed, covered, and incubated at 37°C under normal atmosphere. After seven days of incubation, alamar blue solution was added to each well and the plate was re-incubated overnight. The MIC was determined as the lowest concentration of drug that prevented a change in color from blue (oxidized) to pink (reduced), indicating bacterial growth (236).

3.2.2 *In vitro* Antimicrobial Activity Studies

Material and Method

The *in vitro* antibacterial activity of test substances was evaluated against Gram-positive *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, Gram-negative *E. coli* ATCC 25922, and the antifungal activity against *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 90018, and *C. krusei* ATCC 6258 using the liquid microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) protocol (237, 238). Sixteen test substances were prepared in dimethyl sulfoxide at a concentration of 4096 µg/ml. For the evaluation of antibacterial activity, Mueller-Hinton Broth (MHB; Difco) was added to 96-well U-bottom microplates, and the test substances were added to the first wells, followed by serial dilutions ranging from 1024 µg/ml to 0.5 µg/ml.

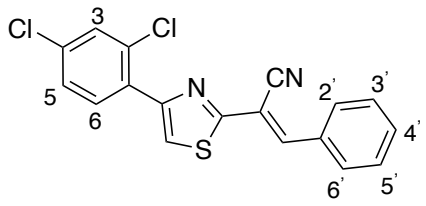
Ciprofloxacin was used as a control antibiotic. Bacterial suspensions were prepared in sterile PBS at a density of 0.5 McFarland turbidity standard from fresh cultures of standard bacterial strains on Tryptic Soy Agar (TSA, Merck). Bacterial suspensions were added to each well at a bacterial density of 5X105 cfu/ml.

Serial dilutions of test substances to be tested for antifungal activity were prepared by adding RPMI-1640 medium to microplates. Candida spp. stock cultures were produced on Sabouraud Dextrose Agar (SDA, Merck) medium, and the next day, fungal suspensions were prepared at a 0.5 McFarland standard turbidity and added to each well at a concentration of 0.5-2.5x105 cfu/mL. Fluconazole was used as the control antifungal.

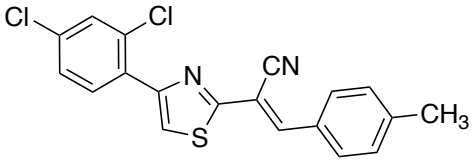
Only growth controls containing the relevant microorganism and sterility controls containing only medium were added to each plate. Inoculated microplates were incubated at 35°C for 24 hours, and the lowest concentration of test substance that inhibited microorganism growth in the wells was determined as the MIC.

4. RESULTS

4.1 Chemical Studies

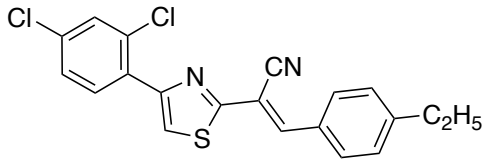


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-phenylacrylonitrile (1). Yield 35.8%, mp. 150 °C; FT-IR spectrums (cm^{-1}); ν 3134 (aromatic C-H), 2212 (C≡N stretch), 1590, 1571, 1556 (C=N and C=C stretch), 1478, 1446 (C=C stretch), 1373, 1357, 1301 (C-N stretch), 1038 (=C-H stretch), 859, 750, 684 (monosubstituted benzene and 1,2,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.31 (1H; s; =CH-), 8.27 (1H; s; thiazole), 8.01-8.00 (2H; m; H-2',6'), 7.96 (1H; d; H-6, *J* = 8.4 Hz), 7.72 (1H; d; H-3, *J* = 2.0 Hz), 7.57-7.53 (4H; m; H-3', 4', 5' and H-5) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.65 (thiazole C_2), 150.62 (thiazole C_4), 145.76 (acrylonitrile C_3), 133.61, 132.66, 132.36, 131.95, 131.86, 131.09, 129.77 (2C), 129.73, 129.11 (2C) and 127.64 (aromatic C), 121.23 (C≡N), 116.15 (thiazole C_5), 104.71 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{18}H_{10}Cl_2N_2S$, 378.9839; found $[M+Na]^+$ 378.9830, $[M+Na+2]^+$ 380.9801.

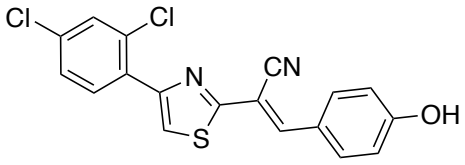


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(*p*-tolyl)acrylonitrile (2). Yield 25.0%, mp. 150 °C; FT-IR spectrums (cm^{-1}); ν 3037, (aromatic C-H stretch), 2922 (aliphatic C-H stretch), 2228 (C≡N stretch), 1595, 1557 (C=N and C=C stretch), 1474, 1451 (C=C stretch), 1375 (C-N stretch), 1192 (CH_3 bend), 1107, 1037 (=C-H stretch), 954, 858, 808, 746 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends,

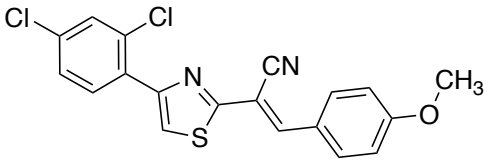
C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.26 (1H; s; thiazole), 8.25 (1H; s; =CH-), 7.91-7.97 (3H; m; H-6 and H-2',6'), 7.73 (1H; d; H-3, J = 1.6 Hz), 7.55 (1H; dd; H-5, J_1 = 2.2, J_2 = 8.2 Hz), 7.37 (2H; d; H-3',5', J = 8.4 Hz), 2.37 (3H; s; -CH_3), ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.87 (thiazole C_2), 150.51 (thiazole C_4), 145.70, 142.52 (acrylonitrile C_3), 133.55, 132.66, 131.91, 131.10, 129.90 (2C), 129.75 (2C), 129.70, 129.66 and 127.64 (aromatic C), 121.02 (C≡N), 116.36 (thiazole C_5), 103.41 (acrylonitrile C_2), 21.17 (CH_3); HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{19}H_{12}Cl_2N_2S$, 392.9996 found $[M+Na]^+$ 392.9988, $[M+Na+2]^+$ 394.9959.



2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-ethylphenyl)acrylonitrile (3). Yield 31.0%, mp. 134 °C; FT-IR spectrums (cm^{-1}); ν 3151 (aromatic C-H stretch), 2962 (aliphatic C-H stretch), 2213 (C≡N stretch), 1589, 1557 (C=N stretch), 1485, 1450 (C=C stretch), 1372 (C-N stretch), 1300, 1182 (CH_2 and CH_3 stretch), 1038 (=C-H stretch), 860, 827, 743 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.28 (1H; s; thiazole), 8.27 (1H; s; =CH-), 7.94-7.98 (3H; m; H-6 and H-2', 6'), 7.74 (1H; d; H-3, J = 2 Hz), 7.55 (1H; dd; H-5, J_1 = 2.0, J_2 = 8.0 Hz), 7.40 (2H; d; H-3',5', J = 8.4 Hz), 2.67 (2H; q; -CH_2, J = 7.6 Hz), 1.19 (3H; t, CH_3, J = 7.6 Hz) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.87 (thiazole C_2), 150.52 (thiazole C_4), 148.57, 145.70 (acrylonitrile C_3), 133.56, 132.66, 131.91, 131.10, 130.00 (2C), 129.91, 129.74, 128.58 (2C) and 127.65 (aromatic C), 121.04 (C≡N), 116.36, (thiazole C_5) 103.48 (acrylonitrile C_2), 28.16 (CH_2), 15.00 (CH_3) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calculated for $C_{20}H_{14}Cl_2N_2S$, 407.0152; found $[M+Na]^+$ 407.0145, $[M+Na+2]^+$ 409.0116.

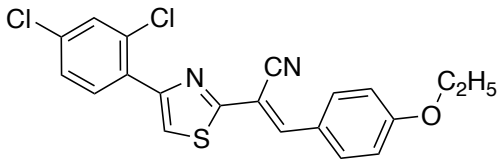


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-hydroxyphenyl)acrylonitrile (4). Yield 77.2%, mp. 235 °C; FT-IR spectrums (cm^{-1}); ν 3331 (OH stretch), 3141 (aromatic C-H stretch), 2222 (C≡N stretch), 1610, 1585, 1557 (C=N stretch), 1516, 1473, 1438 (C=C stretch), 1372, 1356 (C-N stretch), 1289 (C-O stretch), 1049 (=C-H stretch), 856, 830, 760 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.56 (1H; s; -OH), 8.23 (1H; s; thiazole), 8.17 (1H; s; =CH-), 7.94-7.98 (3H; m; H-6 and H-2',6'), 7.75 (1H; d; H-3, J = 2 Hz), 7.55 (1H; dd; H-5, J_1 = 2.4, J_2 = 8.4 Hz), 6.93 (2H; d; H-3',5', J = 8.4 Hz) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{18}H_{10}Cl_2N_2OS$, 394.9789; found $[M+Na]^+$ 394.9781, $[M+Na+2]^+$ 396.9753.

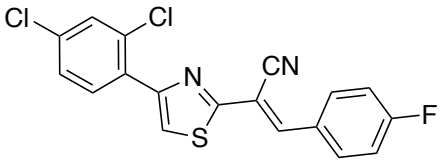


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-methoxyphenyl)acrylonitrile (5). Yield 34.0%, mp. 163 °C; FT-IR spectrums (cm^{-1}); ν 3037, (aromatic C-H stretch), 2843 (aliphatic C-H stretch), 2215 (C≡N stretch), 1580, (C=N stretch), 1512, 1474, 1446 (C=C stretch), 1357, 1308 (C-N stretch), 1263, 1171, 1040 (C-O stretch), 1028, 858, 825, 815, 754 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.23 (1H; s; thiazole), 8.21 (1H; s; =CH-), 8.03 (2H; d; H-2',6', J = 9.2 Hz), 7.96 (1H; d; H-6, J = 8.4 Hz), 7.72 (1H; d; H-3, J = 2.4 Hz), 7.55 (1H; dd; H-5, J_1 = 2.4, J_2 = 8.4 Hz), 7.11 (2H; d; H-3',5', J = 8.8 Hz), 3.83 (3H; s; -OCH_3) ppm;^{13}C-NMR (100 MHz, DMSO-d_6) δ 162.34, 162.31 (thiazole C_2), 150.43 (thiazole C_4), 145.40 (acrylonitrile C_3), 133.57, 132.74, 132.25 (2C), 131.95, 131.18, 129.83, 127.721 and 124.98 (aromatic C), 120.68 (C≡N), 116.83 (thiazole C_5), 114.83 (2C),

101.32 (acrylonitrile C_2), 55.63 (CH_3) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{19}H_{12}Cl_2N_2OS$, 408.9945; found $[M+Na]^+$ 408.9937, $[M+Na+2]^+$ 410.9908.

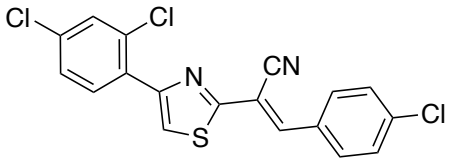


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-ethoxyphenyl)acrylonitrile (6). Yield 55.7%, mp. 161 °C; FT-IR spectrums (cm^{-1}); ν 2985, (aromatic C-H stretch), 2938, 2884 (aliphatic C-H stretch), 2225 (C≡N stretch), 1590, (C=N stretch), 1512, 1475, 1453 (C=C stretch), 1395, 1311 (C-N stretch), 1260 (C-O stretch), 1182, 1104 (CH_2 and CH_3 stretch), 1048, 1034, 821,787 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.24 (1H; s; thiazole), 8.23 (1H; s; =CH-), 8.04 (2H; d; H-2',6', J = 8.4 Hz), 7.98 (1H; d; H-6, J = 8.4 Hz), 7.75 (1H; d; H-3, J = 1.6 Hz), 7.56 (1H; dd; H-5, J_1 = 8.8, J_2 = 2 Hz), 7.12 (2H; d; H-3',5', J = 8.4 Hz), 4.15 (2H; q; -OCH_2, J = 7.2 Hz), 1.36 (3H; t; CH_3, J = 7.2 Hz) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 162.24, 161.59 (thiazole C_2), 150.42 (thiazole C_4), 145.35 (acrylonitrile C_3), 133.50, 132.65, 132.16 (2C), 131.90, 131.18,129.71, 127.61 and 124.75 (aromatic C), 120.51 (C≡N), 116.72 (thiazole C_5), 115.12 (2C), 101.16 (acrylonitrile C_2). 63.58 (CH_2), 14.35 (CH_3) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{20}H_{14}Cl_2N_2OS$, 423.0102; found $[M+Na]^+$ 423.0090, $[M+Na+2]^+$ 425.0062.

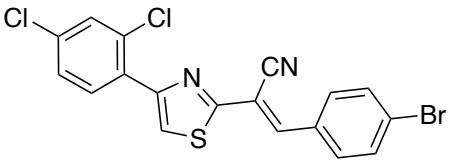


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-fluorophenyl)acrylonitrile (7). Yield 22.7 %, mp. 161 °C; FT-IR spectrums (cm^{-1}); ν 3137 (aromatic C-H stretch), 2210 (C≡N stretch), 1594, 1581 (C=N stretch), 1506, 1480 (C=C stretch), 1373, 1301 (C-N stretch) 1239 (C-F stretch), 1160, 1038, 861, 820, 789, 751 (1,2,4-disubstituted benzene

and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.18 (1H; s; thiazole), 8.02-7.99 (3H; m; H-6 and H-2',6'), 7.95 (1H; s; =CH-), 7.51 (1H; d; H-3, J = 2 Hz), 7.36 (1H; dd; H-5, J_1 = 8.2, J_2 = 2.2 Hz), 7.21-7.17 (2H; m; H-3',5') ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{18}H_9Cl_2FN_2S$, 396.9745; found $[M+Na]^+$ 396.9745.

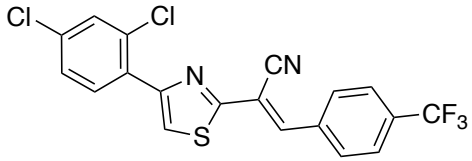


3-(4-Chlorophenyl)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)acrylonitrile (8). Yield 34.2%, mp. 188 °C; FT-IR spectrums (cm^{-1}); ν 3124 (aromatic C-H stretch), 2229 (C≡N stretch), 1588 (C=N stretch), 1494, 1475, 1450 (C=C stretch), 1376 (C-N stretch), 1146, 1098 1038, 959, 819, 752 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.36 (1H; s; thiazole), 8.33 (1H; s; =CH-), 8.06 (2H; d; H-2',6', J = 8.4 Hz), 7.99 (1H; d; H-6, J = 8.4 Hz), 7.78 (1H; d; H-3, J = 2.4 Hz), 7.67 (2H; d; H-3',5', J = 8.2 Hz), 7.59 (1H; dd; H-5, J_1 = 8.6, J_2 = 2.4 Hz) ppm; HRMS (Q-TOF): *m/z* $[M+Na+2]^+$ calcd for $C_{18}H_9Cl_3N_2S$, 412,9450; found $[M+Na+2]^+$ 414.9411, $[M+Na+4]^+$ 416.9383.

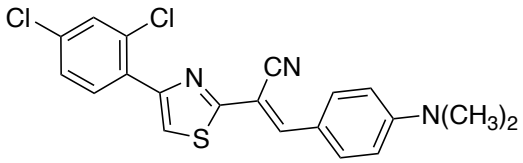


3-(4-Bromophenyl)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)acrylonitrile (9). Yield 40.0 %, mp. 191 °C; FT-IR spectrums (cm^{-1}); ν 3132 (aromatic C-H stretch), 2229 (C≡N stretch), 1583 (C=N stretch), 1490, 1474, 1449 (C=C stretch), 1373 (C-N stretch), 1037,1106, 1080, 957, 906, 860, 813, 755 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch, C-Br stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.30 (2H; s; =CH-, thiazole), 7.94 -7.97 (m; 3H; H-6 and H-2',6'),

7.74-7.79 (3H; m; H-3 and H-3',5'), 7.56 (1H; dd; H-5, J_1 = 8.4 Hz) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{18}H_9Cl_2BrN_2S$, 456.8945; found $[M+Na]^+$456.8940, $[M+Na+2]^+$ 458.8916, $[M+Na+4]^+$ 460.8889.

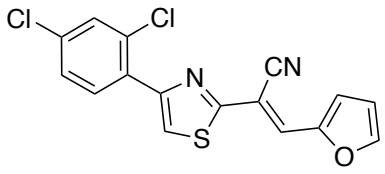


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (10). Yield 25.1%, mp. 153 °C; FT-IR spectrums (cm^{-1}); ν 3152 (aromatic C-H stretch), 2224 (C≡N stretch), 1586 (C=N stretch), 1474, 1450 (C=C stretch), 1322, (C-N stretch), 1256, 1156, 1118(C-F stretch), 1071, 952, 830, 753 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.44 (1H; s; thiazole), 8.35 (1H; s; =CH-), 8.18 (2H; d; H-2',6', J = 7.76 Hz), 7.92-7.97 (3H; m; H-6 and 3',5'), 7.75 (1H; d; H-3, J = 2 Hz), 7.56 (1H; dd; H-5, J_1 = 8.4, J_2 = 2.4 Hz), ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.16 (thiazole C_2), 150.77 (thiazole C_4), 144.02 (acrylonitrile C_3), 136.31, 136.33, 133.71, 132.69, 131.98, 131.01, 130.72, 130.33 (2C), 129.80, 127.72, 125.96 and 125.91 (aromatic C), 122.05 (C≡N), 115.68 (thiazole C_5), 107 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{19}H_9Cl_2F_3N_2S$, 446.9713; found $[M+Na]^+$ 446.9705, $[M+Na+2]^+$ 448.9676.

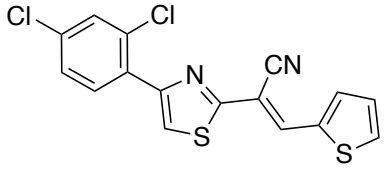


2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(4-(dimethylamino)phenyl)acrylonitrile (11). Yield 94.9%, mp. 166 °C; FT-IR spectrums (cm^{-1}); ν 2909 (aromatic C-H stretch), 2218 (C≡N stretch), 1580, 1524 (C=N stretch), 1472,1450 (C=C stretch), 1365, 1327 (CH_3 stretch), 1197, 1167 (C-N stretch), 1100,

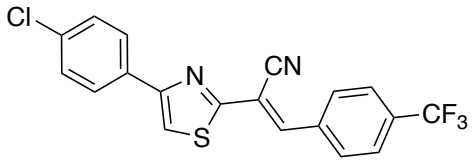
1035,956, 811, 785 (1,2,4-disubstituted benzene and 1,4-disubstituted benzene C-H bends, C-S stretch, C-Cl stretch). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.12 (1H; s; thiazole), 8.04 (1H; s; =CH-), 7.97 (1H; d; H-6, J = 8.4 Hz), 7.92 (2H; m; H-2',6'), 7.71 (1H; d; H-3, J = 2.4 Hz), 7.53 (1H; dd; H-5, J_1 = 8.8, J_2 = 2.4 Hz), 6.83-6.80 (2H; m; H-3',5'), 3.04 (6H; s; -N(CH_3)$_2$), ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 163.28 (thiazole C_2), 152.64, 150.18 (thiazole C_2), 145.54 (acrylonitrile C_3), 133.34, 132.67, 132.30 (2C), 131.85, 131.34, 129.70, 127.57, 119.29, 119.23 (C≡N), 117.85 (2C), 111.67 (thiazole C_5), 96.02 (acrylonitrile C_2), 39.52 (2 CH_3 and DMSO-d_6) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{20}H_{15}Cl_2N_3S$, $[M+Na]^+$ 422.0261; found $[M+Na]^+$ 422.0256, $[M+Na+2]^+$ 424.0227.



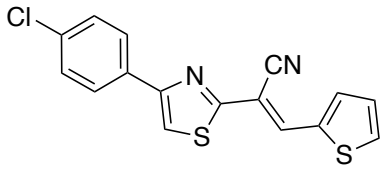
2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(furan-2-yl)acrylonitrile (12). Yield 98.0%, mp. 179 °C; FT-IR spectrums (cm^{-1}); ν 3210 (aromatic C-H stretch), 2600 (C≡N stretch), 1650 (C=N stretch), 1440 (C=C stretch), 1350, 1300, (C-N stretch), 1200,1150 (C-O stretch), 867, 750, 700 (1,2,4-trisubstituted benzene C-H bends, and C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.26 (1H; s; thiazole), 8.15 (1H; s; =CH-), 8.14 (1H; d; H-5', J = 1.6 Hz), 7.98 (1H; d; H-6, J = 8.4 Hz), 7.75 (1H; d; H-3, J = 2.4 Hz), 7.56 (1H; dd; H-5, J_1 = 8.4, J_2 = 2.4 Hz), 7.37 (1H; d; H-3', J = 3.2 Hz), 6.83 (1H; dd; H-4', J_1 = 3.6, J_2 = 1.6 Hz) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.56 (thiazole C_2), 150.68 (thiazole C_4), 148.70 (acrylonitrile C_3), 148.33, 133.63, 132.75, 131.95, 131.13, 130.46, 129.83, 127.71, 121.35, 121.06, 116.21 (C≡N), 113.92 (thiazole C_5), 99.50 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{16}H_8Cl_2N_2OS$, 368.9632; found $[M+Na]^+$ 368.9623, $[M+Na+2]^+$ 370.9594, $[M+Na+4]^+$ 372.9565.



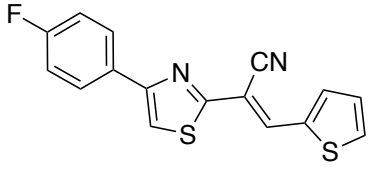
2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-3-(thiophen-2-yl)acrylonitrile (13). Yield 94.1%, mp. 169 °C; FT-IR spectrums (cm^{-1}); ν 3088 (aromatic C-H stretch), 2218 (C≡N stretch), 1580 (C=N stretch), 1470, 1416 (C=C stretch), 1252, 1189, (C-N stretch), 1105, 1037, 863,721 (1,2,4-disubstituted benzene C-H bends, C-S stretch and C-Cl stretch); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.59 (1H; s; =CH-), 8.28 (1H; s; thiazole), 8.09 (1H; d; H-5', J = 5.2 Hz), 7.99 (1H; d; H-6, J = 8.0 Hz), 7.97 (1H; d; H-3', J = 3.6 Hz), 7.77 (1H; d; H-3, J = 2 Hz), 7.58 (1H; dd; H-5, J_1 = 8.4, J_2 = 2.4 Hz), 7.33 (1H; dd; H-4', J_1 = 4.8, J_2 = 3.2 Hz) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.54 (thiazole C_2), 150.56 (thiazole C_4), 138.69 (acrylonitrile C_3), 137.46, 136.42, 134.35, 133.61, 132.73, 131.95, 131.13, 129.82, 128.57, 127.72, 120.93 (C≡N), 116.45 (thiazole C_5), 100.54 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{16}H_8Cl_2N_2S_2$, 384.9404; found $[M+Na]^+$ 384.9396, $[M+Na+2]^+$ 386.0366, $[M+Na+4]^+$ 388.9336.



2-(4-(4-Chlorophenyl)thiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (14). Yield 43.5%, mp. 166 °C; FT-IR spectrums (cm^{-1}); ν 3106 (aromatic C-H stretch), 2225 (C≡N stretch), 1618 (C=N stretch), 1480, 1455 (C=C stretch), 1320(C-N stretch), 1241, 1216 1116, (C-F stretch), 1068, 828,748 (1,4-disubstituted benzene C-H bends, C-S stretch and C-Cl stretch); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.29 (1H; s; thiazole), 8.07 (2H; d; H-2',6', J = 8 Hz), 7.90 (2H; d; H-2,6, J = 8.8 Hz), 7.76 (2H; d; H-3',5' J = 7.6 Hz), 7.60 (1H; s; =CH-), 7.43 (2H; d; H-3,5, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.78 (thiazole C_2), 156.02 (thiazole C_4), 142.27 (acrylonitrile C_3), 135.74, 134.72, 133.02, 132.70, 132.01, 130.09 (2C), 129.11(2C), 127.80 (2C), 126.15, 124.90, 122.19, 116.16 (C≡N), 115.08 (thiazole C_5), 107.23 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{19}H_{10}ClF_3N_2S$, 413.0103; found $[M+Na]^+$ 413.0100, $[M+Na+2]^+$ 415.0072.



2-(4-(4-Chlorophenyl)thiazol-2-yl)-3-(thiophen-2-yl)acrylonitrile (15). Yield 66.9%, mp. 173 °C; FT-IR spectrums (cm^{-1}); ν 3103 (aromatic C-H stretch), 2157 (C≡N stretch), 1582 (C=N stretch), 1476, 1418 (C=C bend stretch 1312 (C-N stretch), 1191 1090, 1055, 830, 723, 721 (1,4-disubstituted benzene C-H bends, C-S stretch and C-Cl stretch); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.59 (1H; s; =CH-), 8.29 (1H; s; thiazole), 8.08 (1H; d; H-5', J = 4 Hz), 8.06-8.03 (2H; m; H-2,6), 7.97 (1H; d; H-3', J = 2.7 Hz), 7.56-7.53 (2H; m; H-3,5), 7.33 (1H; dd; H-4', J_1 = 4, J_2 = 3 Hz) ppm; ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.72 (thiazole C_2), 154.47 (thiazole C_4), 138.82 (acrylonitrile C_3), 137.77, 136.94, 134.70, 133.54, 132.72, 129.37(2C), 129.05 and 128.40 (2C) (aromatic C), 117.00 (C≡N), 116.79 (thiazole C_5), 101.15 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{16}H_9ClN_2S_2$, 350.9793; found $[M+Na]^+$ 350.9783, $[M+Na+2]^+$ 352.9752.



2-(4-(4-Fluorophenyl)thiazol-2-yl)-3-(thiophen-2-yl)acrylonitrile (16). Yield 60.1 %, mp. 172 °C; FT-IR spectrums (cm^{-1}); ν 3103 (aromatic C-H stretch), 2157 (C≡N stretch), 1582, 1526 (C=N stretch), 1479 (C=C stretch), 1228 (C-N stretch), 1188 (C-F stretch), 832, 754, 722 (1,4-disubstituted benzene C-H bends, C-S stretch and C-Cl stretch); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.59 (1H; s; =CH-), 8.22 (1H; s; thiazole), 8.06-8.08 (3H; m; H-2,6 and H-5'), 7.96-7.97 (1H; d; H-3', J = 2.7 Hz), 7.30-7.34 (3H; m; H-3,5 and H-4'), ppm; ^{13}C-NMR (125 MHz, DMSO-d_6) δ 163.66, 162.57 (thiazole C_2), 161.71, 154.73 (thiazole C_4), 138.69 (acrylonitrile C_3), 137.71, 136.95, 134.64, 130.51, 129.04 (2C), 128.86 (2C) and 128.79 (aromatic C), 117.03 (C≡N), 116.32, 116.15, 115.90

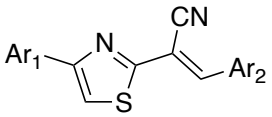
(thiazole C_5), 101.20 (acrylonitrile C_2) ppm; HRMS (Q-TOF): *m/z* $[M+Na]^+$ calcd for $C_{16}H_9FN_2S_2$, 335.0089; found $[M+Na]^+$ 335.0083.

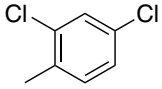
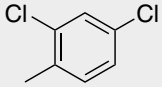
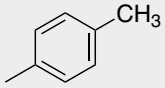
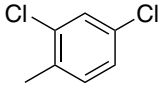
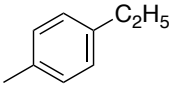
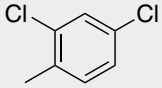
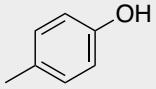
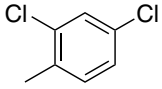
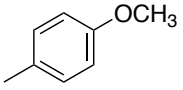
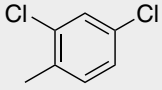
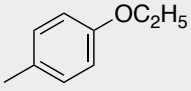
4.2 Biological Activity Studies

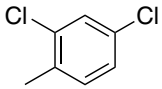
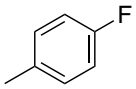
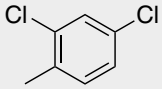
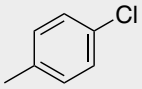
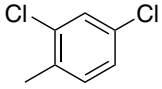
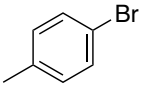
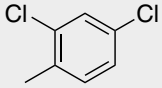
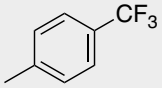
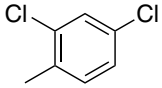
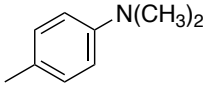
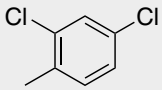
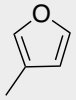
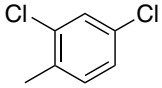
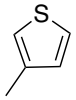
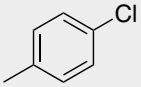
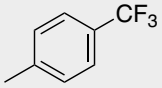
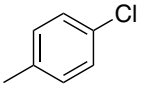
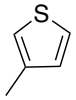
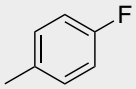
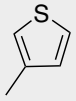
4.2.1 *In vitro* Antimycobacterial Activity Studies

The *in vitro* antimycobacterial activities of synthesized compounds **1-16** against Mtb H37Rv strain were tested using the MABA method. Isoniazid used as standard compound in activity studies. The MIC values of the compounds are given in **Table 4.1** in µg/ml.

Table 4.1. Antimycobacterial activity results for synthesized compounds (**1-16**)



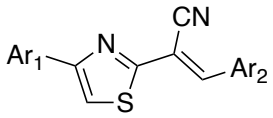
Compounds	Ar_1	Ar_2	MIC (µg/ml)
1			>50
2			>50
3			>50
4			>50
5			>50
6			>50

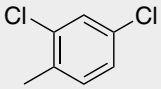
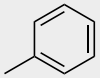
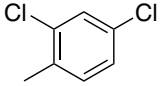
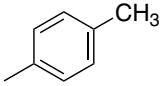
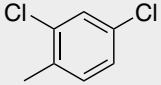
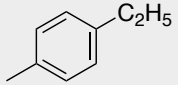
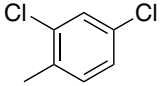
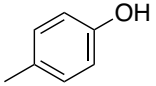
7			>50
8			>50
9			>50
10			>50
11			>50
12			>50
13			>50
14			>50
15			>50
16			>50

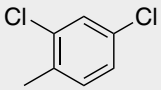
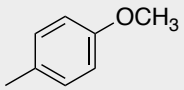
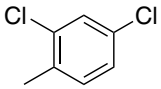
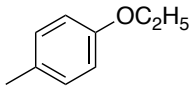
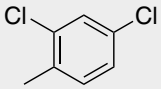
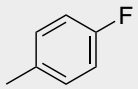
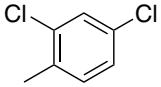
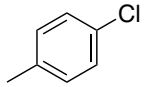
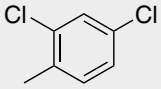
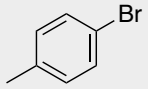
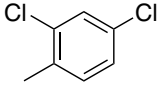
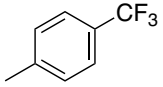
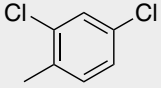
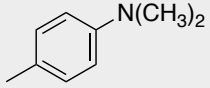
4.2.2 *In vitro* Antimicrobial Activity Studies

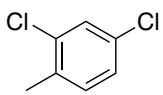
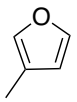
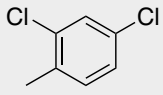
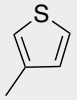
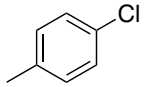
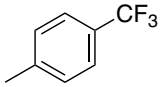
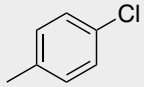
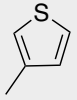
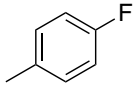
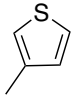
The antibacterial and antifungal activities of compound **1-16** were tested against Gram-positive *S. aureus*, *E. faecalis*, Gram-negative *E. coli*, *C. albicans*, *C. parapsilosis*, and *C. krusei* by measuring the MIC values, and all the results are summarized in **Table 4.2.** where ciprofloxacin and fluconazole were used as a control antibiotic and control antifungal respectively.

Table 4.2. *In-vitro* antimicrobial activity result for synthesized compounds (**1-16**)



			S. aureus	*E. faecalis*	*E. coli*	*C. albicans*	*C. parapsilosis*	*C. krusei*
Comp.	**Ar_1**	**Ar_2**	**ATCC 29213**	**ATCC 29212**	**ATCC 25922**	**ATCC 90028**	**ATCC 90018**	**ATCC 6258**
1			1024	1024	1024	256	256	128
2			1024	1024	>1024	512	128	256
3			1024	1024	1024	128	**64**	256
4			1024	512	1024	256	256	256

5			1024	1024	512	256	256	256
6			1024	1024	512	**64**	512	**64**
7			1024	1024	1024	128	256	512
8			1024	>1024	1024	128	512	128
9			1024	512	256	128	256	128
10			1024	512	512	128	**32**	128
11			>1024	>1024	1024	256	256	128

12			1024	1024	1024	256	256	128
13			1024	1024	1024	256	256	128
14			1024	1024	1024	256	128	128
15			1024	1024	512	256	128	256
16			>1024	1024	512	256	128	256
CPFX	-	-	0.25	1	0.015	NA	NA	NA
FLZ	-	-	NA	NA	NA	0.25	0.25	4

CPFX: Ciprofloxacin

FLZ: Fluconazole

5. DISCUSSION

In this study, a total of 16 derivatives of 3-aryl-2-(4-substituted-2-thiazole)acrylonitrile (**1-16**), two of which (compound **14** and **15**) had been previously reported in literature, were synthesized and screened for their antituberculosis and antimicrobial activities. In view of the considerable structural resemblance of these thiazole acrylonitrile group compounds to similar molecules with documented antimicrobial activity in literature, the inquiry into the antibacterial and antifungal activities of these compounds was incorporated into the thesis. The structures of compounds **1-16** were authenticated through analyses of data obtained from IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry, followed by an investigation into their biological activities.

5.1 Chemical Studies

5.1.1 General Synthesis Procedures

Synthesis of 2-Cyanothioacetamide (a)

The thionation of 2-cyanoacetamide using Lawesson's reagent resulted in a 50% yield of 2-cyanoethanethioamide. Lawesson's reagent is a thionating agent that is known for its mild and convenient conversion of ketones, esters, and amides into thioketones, thioesters, and thioamides, respectively, in high yields. The mechanism of thionation using Lawesson's regent is illustrated in **Figure 5.2** (239).

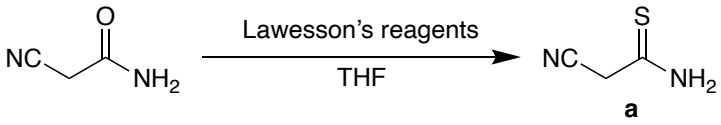


Figure 5.1. Thionation of 2-cyanoacetamide using Lawesson's reagent

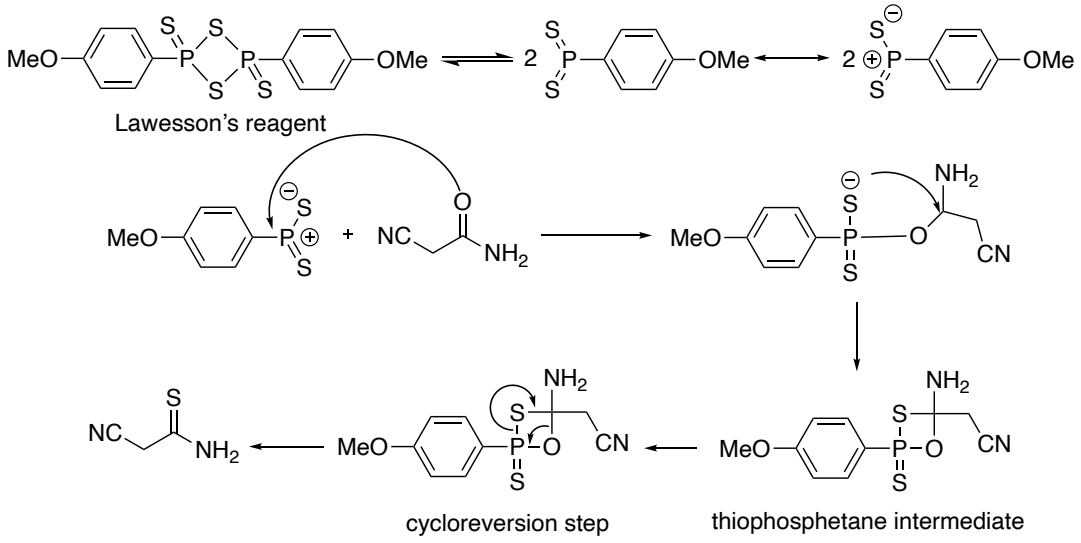


Figure 5.2. Mechanism of the thionation reaction of 2-cyanoacetamide using Lawesson's reagent

Synthesis of 2-Bromoacetophenone Derivatives (c and d)

The procedure for synthesizing substituted 2-bromoacetophenones (**c** and **d**) involves bromination of substituted acetophenones using NBS and *p*TsOH in DCM. The resulting solid product after extraction, **c** and **d** is obtained in 80-94% yield. Proposed mechanism is illustrated in **Figure 5.3** (240). 2,2',4'-Trichloroacetophenone (**b**) was obtained from commercial source.

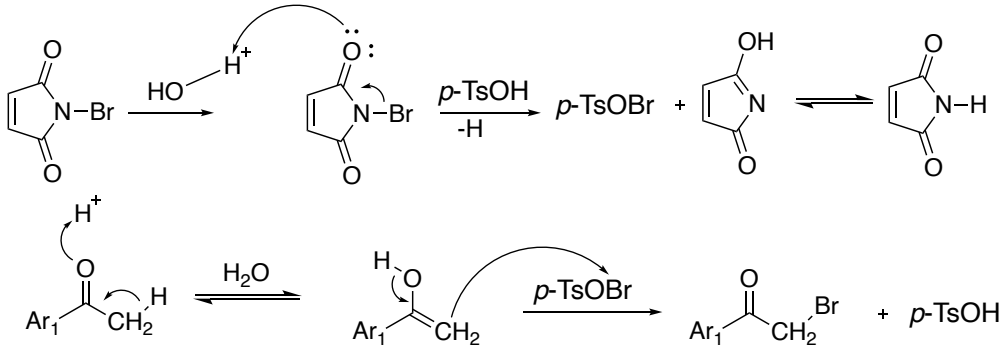


Figure 5.3. Plausible mechanism for α-bromination of acetophenones

Synthesis of 3-Aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile Derivatives (1-16)

The title compound, 3-aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile derivatives **1-16** were formed in a one-pot reaction. In the first step, 2-haloacetophenone derivatives reacts with 2-cyanothioacetamide (**a**) in a Hantzsch thiazole synthesis (**Figure 5.4**), followed by a Knoevenagel condensation of the thiazole intermediate with aldehyde in the presence of *L*-proline as catalyst (**Figure 5.5**). The reaction produced the desired compounds with 22.7-94.1% yield and melting points between 134 °C and 191°C, which is consistent with the melting points of the only two unoriginal compounds in the series (**14** and **15**) reported the literature (241, 242).

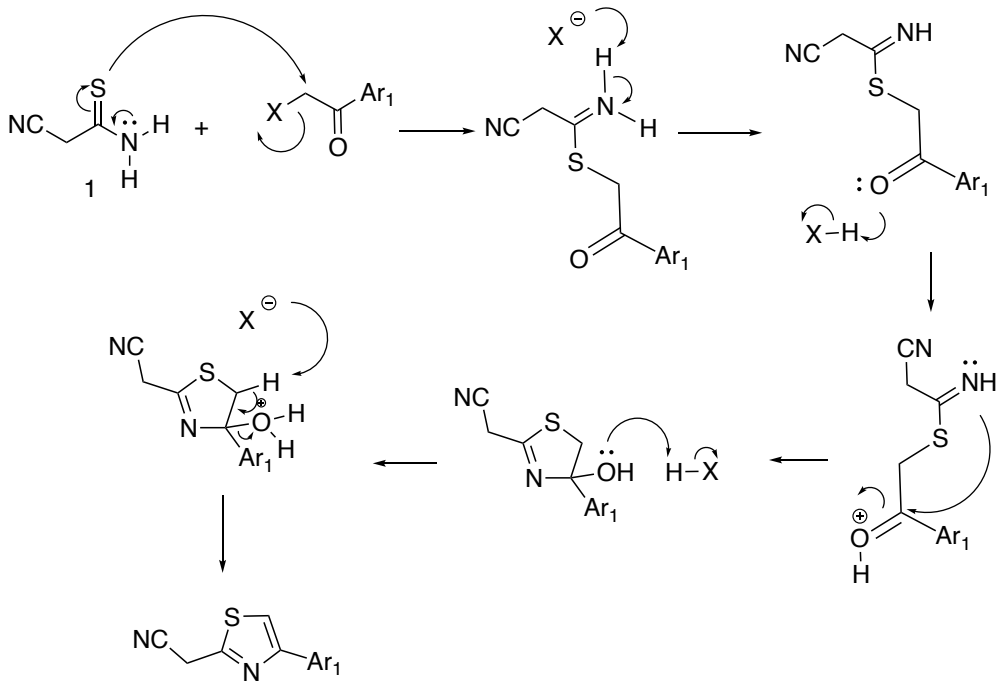


Figure 5.4. Mechanism of Hantzsch thiazole synthesis

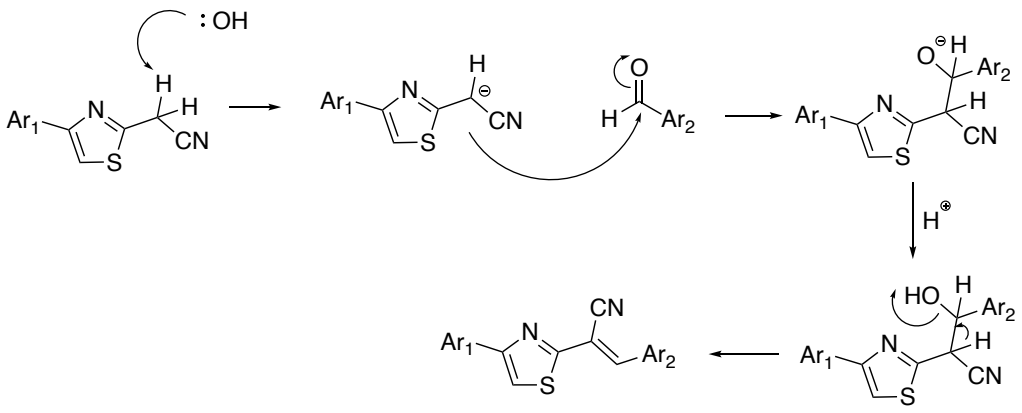


Figure 5.5. Mechanism of Knoevenagel condensation

Using a one-pot method, 2-haloacetophenones, 2-cyanothioacetamide, aldehydes and *L*-proline as a catalyst were initially suspended in ethanol. As the reaction proceeds, the intermediate products become soluble in ethanol and the suspended starting materials decrease. Towards the end of the reaction, the thiazole acrylonitrile derivatives form and start to precipitate. Reactions lasting approximately 4 hours have a yield ranges from 22.7% to 98% for 2-(4-(2,4-dichlorophenyl)thiazole acrylonitrile derivatives and from 43.5% to 66.9% for 2-(4-(4-chlorophenyl)thiazole acrylonitrile derivatives. Overall, compounds **11**, **12**, and **13** were obtained with the highest yields of 94.9%, 98.0%, and 94.1%, respectively. The low yield of compound **7** and **10** is likely due to a byproduct formed during the reaction that cannot be isolated. This product is detected by thin layer chromatography during the reaction, and the final compounds were generally different shades of yellow powder and exhibited interesting fluorescence behavior under UV light.

5.1.2 Characterization of the Structures of Synthesized Compounds

IR Spectra

The FT-IR analysis of the synthesized compounds 3-aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile derivatives **(1-16)** revealed the presence of stretching frequencies in the 2900-3188 cm^{-1} range, which are associated with aromatic group C-H stretch. Similar characteristic bands were observed in the 2157-2229 cm^{-1}

region, which can be attributed to the C≡N stretch, as previously documented in studies on similar compounds (34, 36, 241, 242). Stretching bands were observed in the 1580-1650 cm^{-1} and 1311-1357 cm^{-1} region, corresponding to the C=N and C-N stretches of the thiazole moiety, respectively, while substituted benzene C-H bend, C-S stretch, and C-Cl stretch were assigned bands in the 867-721 cm^{-1} range. Notably, the absence of stretching bands related to aldehyde carbonyl and NH_2 functionalities confirmed the formation of the compounds (17). An example of an IR spectrum of the target compounds, Compound **5** is given in **Figure 5.6**.

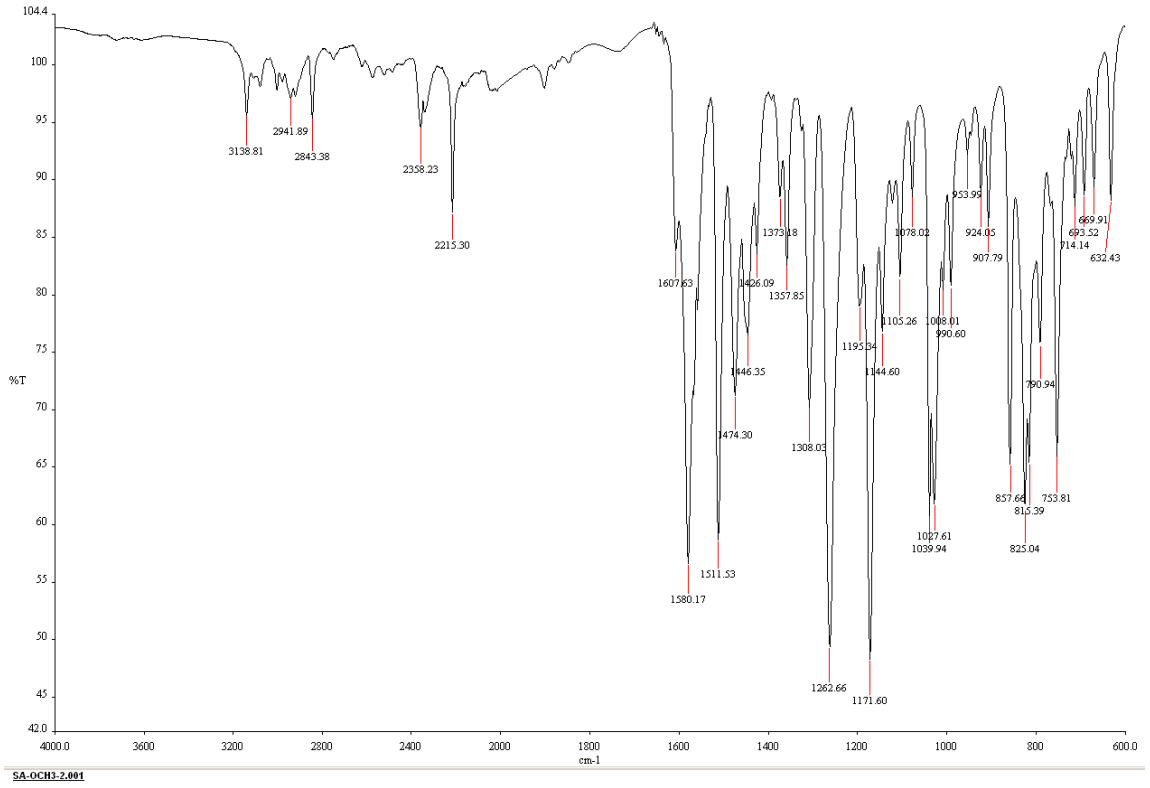


Figure 5.6. IR spectrum of compound **5**.

^{1}H-NMR spectra

The ^{1}H-NMR spectra of the synthesized compounds demonstrate the appearance of singlets within the range of 7.95-8.59 ppm corresponding to the proton of the CH=C(CN) moiety of the acrylonitrile fragment, and within the range of 8.06-8.44 ppm

for the H_5 atom of thiazole. These protons affirm the formation of the compound (242). ^{1}H-NMR spectrum of compound **5** is shown in **Figure 5.7**.

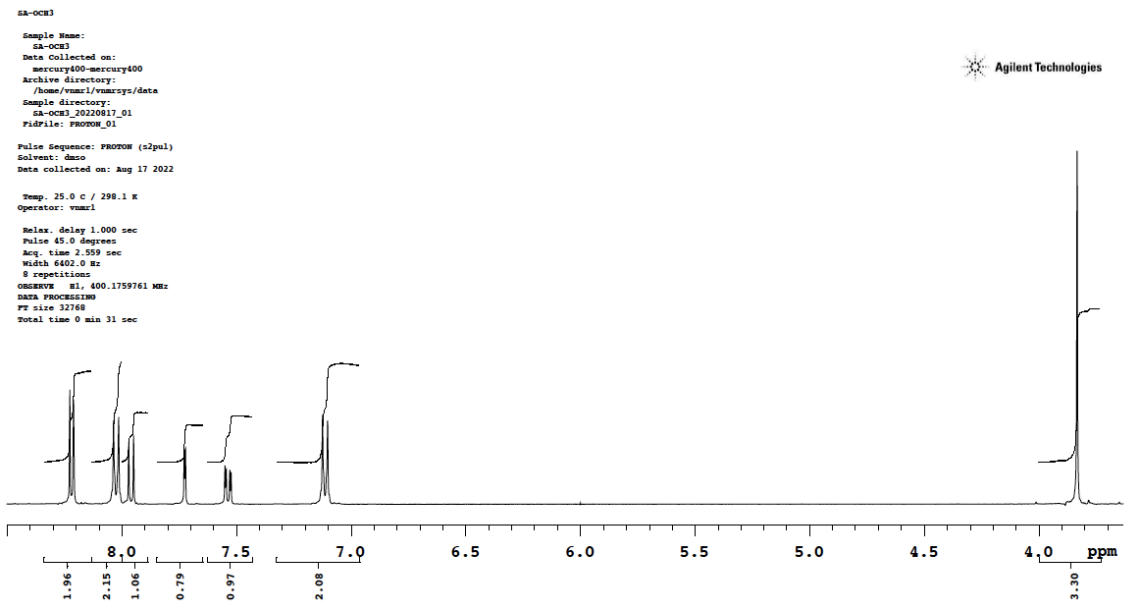


Figure 5.7. ^{1}H-NMR spectrum of compound **5**

^{13}C-NMR spectra

In the data obtained from the ^{13}C-NMR spectra, the chemical shifts of the =CH moiety in the acrylonitrile fragment is observed within the 138.69-148.70 ppm range. Meanwhile, the C_2 and C_4 atoms of the thiazole ring exhibit resonance in the 161.16-163.28 and 150.18-156.02 ppm regions, respectively. Additionally, the C_5 atom of the thiazole ring displays signals in the 111.67-116.83 ppm range, while the peak corresponding to the C≡N functional group appears as expected in the aromatic region around 117.03-122.05 ppm (242). ^{13}C-NMR spectrum of compound 5 is shown in **Figure 5.8**.

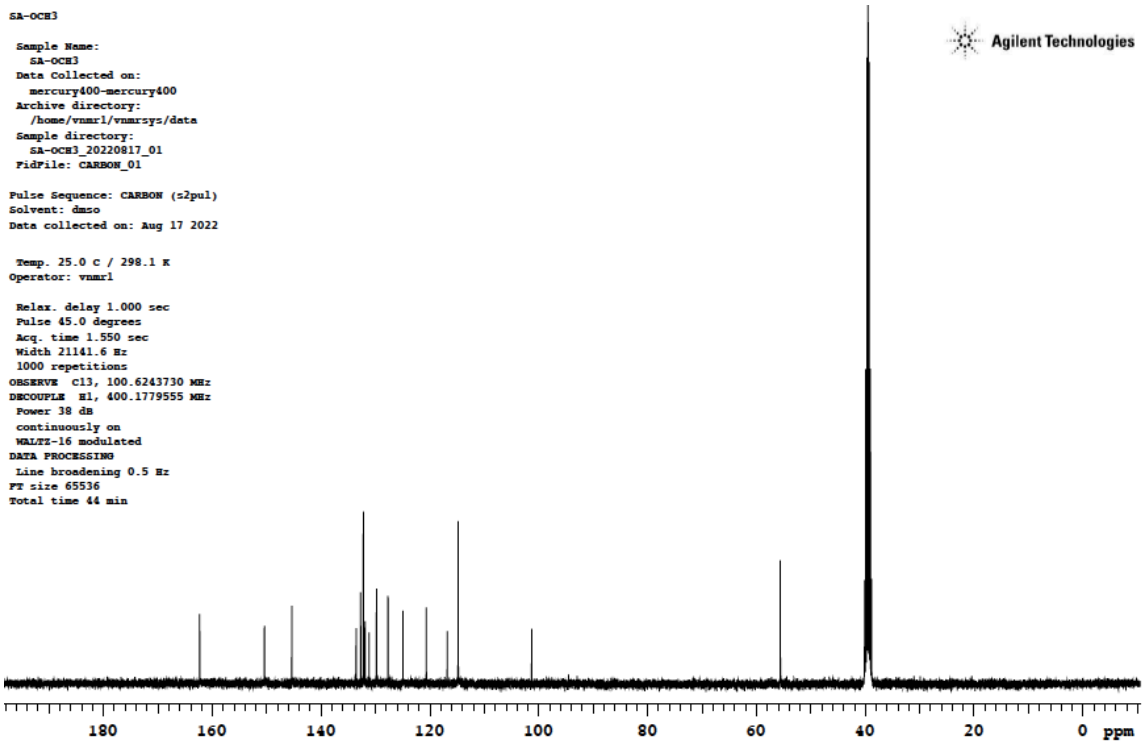


Figure 5.8. ^{13}C-NMR spectrum of compound **5**.

Mass spectra

Mass spectra of compounds were recorded using nLC/LC-QTOF, it is commonly observed that the $[M+Na]^+$, $[M+Na+2]^+$and/or $[M+Na+4]^+$ peaks serves as the predominant peaks throughout the series due to the presence of chloride and bromide isotopes. **Figure 5.9** shows the mass spectrum for compound **5**.

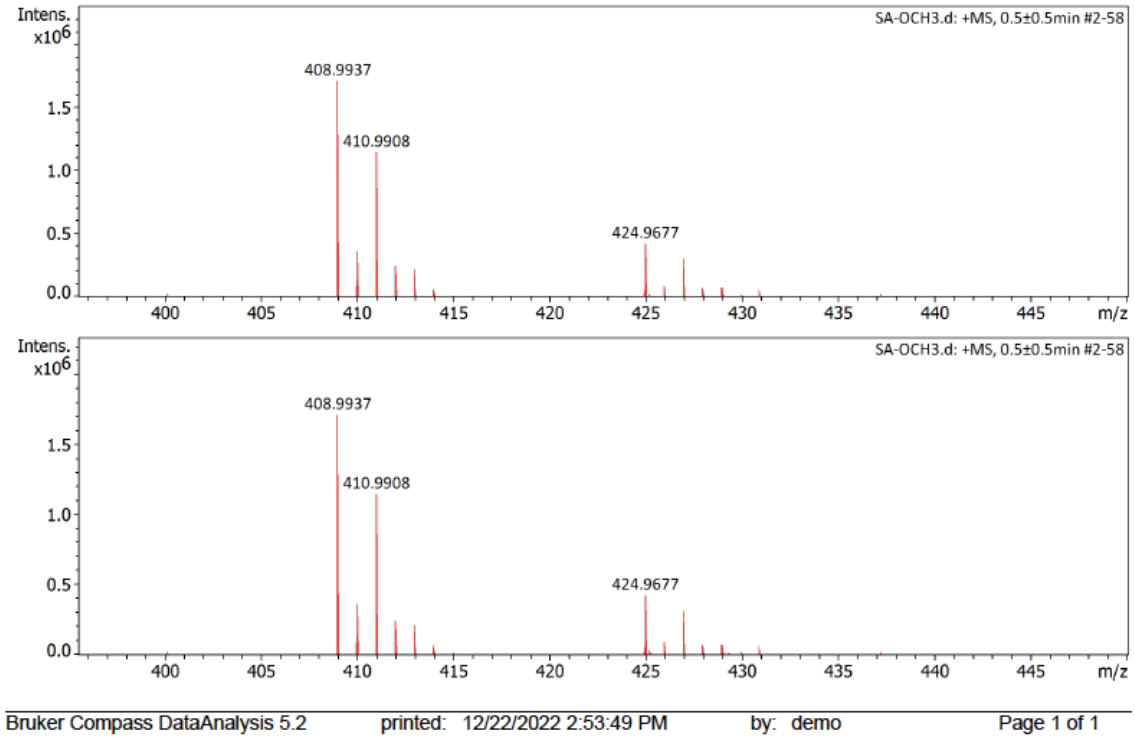


Figure 5.9. Mass spectrum of compound **5**.

5.2 Biological Activity Studies

5.2.1 Antimycobacterial Activity

In this study, the *in vitro* antimycobacterial activity of **1-16** against Mtb H37Rv were evaluated using the MABA technique, with isoniazid as the reference compound. The outcomes were assessed depending on the MIC in µg/ml. The findings showed that none of the compounds exhibited significant anti-mycobacterial activity against Mtb. Although the synthesis of compounds **14** and **15** have been previously documented, their bioactivities were not investigated. Therefore, additional research and optimization is necessary to elucidate the outcomes of this study.

5.2.2 Antimicrobial Activity

The MIC values of all the final compounds were determined by testing them against gram-positive, gram-negative, and fungal microorganisms. Compounds **1-16** were tested against standard strains of gram-positive *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212, gram-negative *E. coli* ATCC 25922, and Candida species *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 90018, and *C. krusei* ATCC 6258. It has been reported in literature that certain acrylonitrile and thiazole derivatives exhibit both antifungal and antibacterial effects (48, 59, 63, 137). To this effect, the compounds originally designed for antituberculosis purposes were screened for both antibacterial and antifungal activity.

In our antimicrobial activity studies, the microdilution method was employed due to its precision, speed, ease of use, and cost-effectiveness. The CLSI principles were also adhered to. In this regard, the compounds were tested alongside reference drug compound, and controls of the culture media and solvents used were established.

Antibacterial Activity

This investigation aimed to assess the antibacterial effectiveness of compounds **1-16** against standard bacteria, with ciprofloxacin as a control drug. The findings indicate that all compounds displayed MIC values greater than or equal to 1024 µg/ml against *S. aureus*, while ciprofloxacin had a lower MIC value of 0.25 µg/ml. Compounds **4**, **9**, and **10** were the most active against *E. faecalis* with a MIC of 512 µg/ml, in comparison to ciprofloxacin, which had a MIC of 1 µg/ml. Compound **9 (**3-(4-bromophenyl)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)acrylonitrile) exhibited the highest activity against *E. coli* with a MIC of 256 µg/ml, while compounds **5**, **6**, **10**, **15**, and **16** demonstrated a MIC of 512 µg/ml, compared to the standard MIC of 0.015 µg/ml. In the absence of a structure-activity relationship study, there was no discernible relationship between the structure of the compounds and their activity, but compounds with Ar_1 and Ar_2 as 2,4-disubstituted benzene and 4-substituted benzene groups respectively showed relatively low MIC values,

indicating higher activity against the tested bacteria as opposed to their counterparts. However, all compounds displayed much lower activities than ciprofloxacin, implying that they have no substantial antibacterial effects.

Antifungal Activity

The antifungal activities were assessed for the 16 compounds against standard fungi. The most effective compounds were found to be **10** (2-(4-(2,4-dichlorophenyl)thiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile), **6** (2-(4-(2,4-dichlorophenyl)thiazol-2-yl)-3-(4-ethoxyphenyl)acrylonitrile), and **3** (2-(4-(2,4-dichlorophenyl)thiazol-2-yl)-3-(4-ethylphenyl)acrylonitrile), with MIC values of 32 µg/ml against *C. parapsilosis*, 64 µg/ml against both *C. albicans* and *C. krusei*, and 64 µg/ml against *C. parapsilosis*, respectively. Most of the compounds showed effective concentration within the range of 128-256 µg/ml against all yeast strains, except for **2**, **7**, **6**, and **8**, which recorded the highest MIC values of 512 µg/ml. Compounds **2** and **7** exhibited the least activity against *C. albicans* and *C. krusei*, respectively, whereas compounds **6** and **8** showed the least activity against *C. parapsilosis*.

Compound **10** was found to be the most potent compound among all 16 compounds, with an MIC value of 32 µg/ml against *C. parapsilosis*. Although the compounds were observed to exhibit more activity towards candida strains than gram-positive and negative bacteria, the structures of the compounds and their activity could not be established without a structure-activity relationship study. Similar to the antibacterial activity results, compounds with low MIC values had Ar_1 being 2,4-disubstituted benzene and Ar_2 being 4-substituted benzene groups. But since all the activities were much lower than those of fluconazole, it's not possible to conclude that they have any antifungal effects that are appreciably better than fluconazole.

6. CONCLUSION

In summary, this study involved the synthesis and characterization of 16 derivatives of 3-aryl-2-(4-substituted-2-thiazole)acrylonitrile derivatives (**1-16**), two of which (Compound **14** and **15**) had been previously reported in literature. The structures of these compounds were determined using various analytical techniques including IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The antimycobacterial activities of the compounds were compared against Mtb H37Rv *in vitro*, with isoniazid used as a reference drug. Additionally, their antibacterial and antifungal activities were assessed against gram-positive and gram-negative bacteria and yeast, respectively, using ciprofloxacin and fluconazole as reference drugs. The results of the study showed that none of the compounds had antimycobacterial activity, however, compound **10** carrying the substituent 4-(trifluoromethyl)phenyl on the third carbon of the acrylonitrile moiety, exhibited relatively good antimicrobial potency against all microorganisms tested, with the highest antifungal activity observed against *C. parapsilosis* (MIC=32 µg/ml) and lower MIC values against *C. albicans* and *C. krusei* (MIC=128 µg/ml), as well as the lowest MIC of 512 µg/ml against both *E. faecalis* and *E. coli*. There was no clear relationship between the structure of the compounds and their activity, although compounds with 2,4-disubstituted phenyl and 4-substituted phenyl groups as Ar_1 and Ar_2, respectively, showed relatively low MIC values. While the results were not as anticipated, the study yielded valuable insights that can guide future research and improve our knowledge of the topic.

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8. APPENDIX

APPENDIX-1: Turnitin originality report

Synthesis and Biological Activity Studies of Some 3-Aryl-2-(4-(substitutedphenyl)thiazol-2-yl)acrylonitrile Derivatives

ORİJİNALLİK RAPORU

%28	%16	%24	%9
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

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7	Naoki Sakata, Kohei Sasakura, Gaku Matsushita, Kazuhiro Okamoto, Kouichi Ohe. "Copper-Catalyzed Regio- and Stereoselective Iodocyanation and Dicyanation of Alkynes with Cyanogen Iodide", Organic Letters, 2017 Yayın	<%1

APPENDIX-2: Digital Turnitin originality report

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Dijital Makbuz

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Gönderinizin ilk sayfası aşağıda gönderilmektedir.

Gönderen:	Shukurah Anas
Ödev başlığı:	Synthesis and biological activity studies of some 3-aryl-2-(4-(...
Gönderi Başlığı:	Synthesis and Biological Activity Studies of Some 3-Aryl-2-(4-...
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Dosya boyutu:	981.22K
Sayfa sayısı:	99
Kelime sayısı:	22,373
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Gönderim Tarihi:	12-Nis-2023 02:24ÖÖ (UTC+0300)
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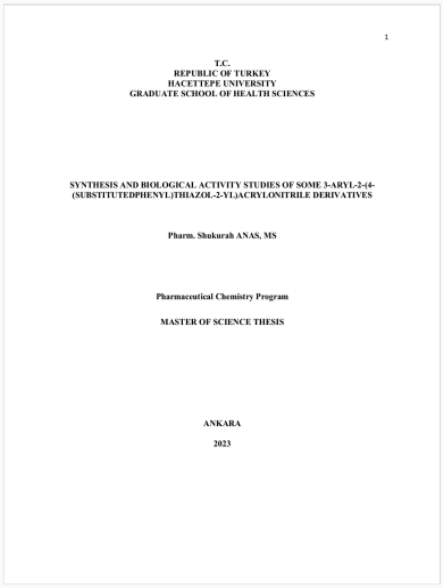

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SYNTHESIS AND BIOLOGICAL ACTIVITY STUDIES OF SOME 3-ARYL-2-(4-(SUBSTITUTEDPHENYL)THIAZOL-2-YL)ACRYLONITRILE DERIVATIVES

Pharm. Shukurah ANAS, MS

Pharmaceutical Chemistry Program

MASTER OF SCIENCE THESIS

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9. CURRICULUM VITAE