Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 april; 6(4s): 519-533

"An Efficient Synthesis of Hybrid Chalcone And Acetyl Pyrazoline Derivatives As Potent Antimycobacterial And Antimicrobial Agents"

Mr. Walke Sudhir Tukaram[1*]
[1*]Bharati Vidyapeeth Deemed University sudhirwalke4561@gmail.com

Corressponding Author: Dr. Rajnikant Patel[2]
[2]PH. D (DOCTOR OF PHILOSOPHY

TABLE OF CONTENTS

Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 april; 6(4s): 519-533

CHAPTER 1:
INTRODUCTION

1.1. INTRODUCTION

The continuous increase of bacteria and viruses that are able to withstand antibiotics is a major problem for human health across the world. We must find new ways to treat these infections immediately. There is an urgent need to investigate new chemical scaffolds and hybrid molecules that may provide improved effectiveness, as many widely used antibiotics and antimycobacterial medicines are losing some of their effectiveness. To enhance the therapeutic benefits of many bioactive substances, one potential approach to drug design is to synthesize hybrid molecules that integrate their pharmacophores. Particularly in the fields of antibacterial and antimycobacterial therapies, hybrid chalcone and acetyl pyrazoline derivatives have attracted a lot of interest owing to their varied biological activities.

Chalcones are naturally occurring chemicals that belong to the flavonoid family. They have shown a broad range of biological actions, such as antibacterial, anti-inflammatory, antioxidant, anticancer, and antimalarial characteristics. They can be easily modified chemically and used to produce new derivatives due to their very adaptable α, β-unsaturated ketone system and simple chemical structure. The capacity of chalcones to bind with DNA of bacteria and fungi, block enzymes, and disrupt microbial cell membranes is the main explanation for their biological activity. Chalcones are thought to be great starting points for novel antimicrobial agents because of their structural flexibility and wide range of activities.**(Cheng et al., 2020)**

Conversely, pyrazolines are heterocyclic molecules with five carbon atoms that have shown strong biological activity, such as antibacterial, antifungal, anticancer, and anti-inflammatory properties. The capacity of pyrazoline derivatives to suppress the development of infectious microbes including mycobacteria, fungus, and bacteria has led to a surge in their popularity. Chalcone scaffolds may have their antibacterial action amplified by adding a pyrazoline component to them. In particular, acetyl pyrazoline derivatives offer promising new avenues for antimicrobial research due to their enhanced effectiveness against a wide range of resistant bacterial strains.

A novel strategy in medicinal chemistry is the production of hybrid compounds combining chalcone and acetyl pyrazoline derivatives. The goal of combining these two pharmacophores is to create more effective antimicrobials by taking advantage of their complimentary action mechanisms. The biological activity of a hybrid molecule is often greater than that of its separate components, a phenomenon known as synergistic effects. Since the tuberculosis (TB)-causing Mycobacterium tuberculosis (MTB) is showing increasing resistance to current therapies, this strategy may be very helpful in the creation of novel antimycobacterial drugs.**(Sharma & Fernandes, 2006)**

The goal of creating chalcone-pyrazoline hybrids is to improve their antibacterial capabilities by combining the best structural characteristics of the two groups of chemicals. The α, β-unsaturated ketone structure of chalcones makes them potential Michael acceptors. When these compounds react with nucleophiles, such the thiol groups found in bacterial enzymes, they block the enzyme and cause cell death. The potent microbial enzyme-binding properties of pyrazolines allow them to suppress bacterial and fungal cell growth and reproduction by interfering with critical metabolic pathways. Therefore, compounds that combine chalcones and pyrazolines may have enhanced activity against a wide variety of microbes due to their ability to interact with several microbial sites.

Research into antimycobacterial agents is piqued by hybrid chalcone and pyrazoline derivatives due to their antibacterial and antifungal characteristics. The prevalence of Mycobacterium TB strains that are resistant to many drugs, such as multidrug-resistant (MDR) and extensively drug-resistant (XDR), has made tuberculosis an even more pressing issue in global health. There is an urgent need for new antimycobacterial drugs, and hybrid molecules provide a fresh way to tackle this problem. Chalcones have antimycobacterial properties because they block enzymes that mycobacteria use to make mycolic acid, an essential component of their cell wall. Pyrazolines, which work by interfering with vital cellular functions, have also shown effectiveness against mycobacteria. Potentially effective antimycobacterial medicines that may overcome drug resistance may be produced by combining these two pharmacophores in a single hybrid molecule.

Conventional organic chemistry methods are usually sufficient to synthesize hybrid chalcone and acetyl pyrazoline derivatives. The chalcone intermediate is first formed by condensing an acetophenone derivative with a suitable aldehyde in the presence of a base. The equivalent pyrazoline is formed by cyclizing this chalcone with hydrazine or its derivatives. It is possible to further acetylate the produced hybrid compounds in order to increase their biological activity and stability. The reactions are simple, and the end products may be refined and studied using tools like mass spectrometry (MS), elemental analysis, and nuclear magnetic resonance (NMR) spectroscopy to verify their structures. **(Barua & Buragohain, 2021)**

After being synthesized, the hybrid chalcone-pyrazoline derivatives are subjected to biological assessment by means of assessing their antibacterial and antimycobacterial activities against various pathogens. One typical way to find out how much of a chemical is needed to stop a certain microbe from growing is to conduct a Minimum Inhibitory Concentration (MIC) test. A variety of bacterial and fungal strains, including Gram-positive and Gram-negative bacteria, mycobacterial species, and others, may be tested against the chemicals. Subsequent research on compounds with strong activity in these

tests might reveal their toxicological profiles, potential for use as therapeutic agents, and how they work. **(Pierce et al., 2023)**

Hybrid pyrazoline and chalcone derivatives may have additional medicinal uses beyond their antibacterial and antimycobacterial capabilities. By inhibiting essential enzymes involved in cell growth and inducing death in cancer cells, these drugs, for instance, have shown promise in anticancer research. In addition, chalcones have antioxidant characteristics that might aid in the treatment of cancer and infections by lowering oxidative stress, a factor in many illnesses. New antibacterial and antimycobacterial drugs might be developed by the synthesis of hybrid chalcone and acetyl pyrazoline derivatives. Potentially more effective against drug-resistant bacteria, these hybrid compounds combine the pharmacophores of pyrazolines and chalcones. It is critical to discover new therapeutic agents due to the worldwide increase of antimicrobial resistance and the ongoing danger of illnesses like TB. One promising approach to these issues and to the advancement of medicinal chemistry is the use of hybrid chalcone-pyrazoline derivatives. Structural optimization, in vivo effectiveness evaluation, and therapeutic application exploration should be the primary goals of future research on these molecules. **(Marinho et al., 2022)**

1.2 ANTIMICROBIAL RESISTANCE

"Antimicrobials" are a group of drugs that help keep humans, animals, and plants healthy by destroying or inhibiting the growth of infectious diseases. This category includes medications that target parasites, viruses, fungi, and bacteria. An example of a microbial phenomenon is antimicrobial resistance (AMR), which occurs when some microbes become immune to antibiotics. Disease transmission, severe illness, disability, and death are all linked to the rise of drug-resistant microbes, which make antibiotics and other antimicrobial treatments ineffective. The evolution of antibiotic resistance (AMR) is an inevitable consequence of infectious disease-related genetic changes. The overuse and improper use of antimicrobials in many medical applications, including those involving plants, animals, and humans, accelerates its development and spread. Antimicrobial medications now form the backbone of contemporary medicine. There is a real risk that life-saving treatments like cancer chemotherapy, caesarean sections, hip replacements, organ transplants, and countless more may become unavailable as drug-resistant germs continue to spread. Agricultural production drops, drug-resistant illnesses hurt plants and animals, and food security is at risk. **(Morrison & Zembower, 2020)**

The costs of antimicrobial resistance (AMR) to healthcare systems and national economies are substantial. It has a number of negative effects, including increasing the need for costly and intense treatment, decreasing the productivity of both patients and caregivers due to longer hospital admissions, and negatively impacting agricultural production. Antimicrobial resistance is a problem in any nation, regardless of its GDP. In terms of its growth, national borders make little difference. The issue is exacerbated by a number of reasons. The inadequacy of WASH (water, sanitation, and hygiene) facilities for both people and animals is one such issue. Another is the poor control of infections and diseases in homes, healthcare facilities, and farms. A further factor is the lack of affordable and high-quality vaccines, diagnostics, and medicines. Lastly, there is a lack of awareness and knowledge among the general public, as well as a violation of laws that are relevant to the issue. Both the causes and the effects of antimicrobial resistance (AMR) disproportionately affect inhabitants of low-resource settings and vulnerable communities. (Blondeau & Blondeau, 2021)

- **Mechanisms of AMR**

AMR occurs when microorganisms undergo genetic changes that enable them to survive exposure to an antimicrobial agent. These changes may occur naturally through random mutations or be acquired from other resistant organisms via horizontal gene transfer, which includes mechanisms like conjugation, transformation, and transduction. **(Christaki et al., 2020)**

The major mechanisms through which microorganisms acquire resistance include:

1. **Enzymatic Degradation**: Antimicrobial drugs may be rendered ineffective by certain bacterial enzymes. As an example, β-lactamase enzymes make penicillin and similar medicines useless by breaking down their β-lactam ring.
2. **Efflux Pumps**: Certain bacteria possess efflux pumps that actively expel antimicrobial agents from the cell, thereby reducing the concentration of the drug within the cell to sub-lethal levels.
3. **Alteration of Drug Targets**: Microorganisms may alter the structure of the molecular targets that antimicrobials are designed to attack. For example, mutations in bacterial ribosomes or enzymes can prevent antibiotics from binding effectively, thus allowing the microorganism to continue its normal function**. (Dadgostar, 2019)**
4. **Reduced Permeability**: Some bacteria can alter their cell wall or membrane to reduce the entry of antibiotics, thereby decreasing the concentration of the drug inside the cell.
5. **Bypass Mechanisms**: Microorganisms can develop alternate metabolic pathways to circumvent the effects of drugs. For example, some bacteria may bypass the steps targeted by sulfonamide antibiotics, allowing them to continue growing despite the presence of the drug.

- **Causes and Spread of AMR**

Multiple variables, many of which have some connection to human actions, contribute to AMR. The overuse and improper use of antibiotics is a major contributor. The broad use of antibiotics in agriculture, particularly in animal husbandry, to encourage growth and prevent infections in livestock, the misuse of prescribed antibiotics, and the use of antibiotics to

treat viral infections—against which antibiotics are ineffective—all fall under this category. These methods promote the propagation of resistant strains by increasing their chances of survival via natural selection. (Dadgostar, 2019)

The transmission of AMR is facilitated in large part by healthcare facilities. Hospitalized patients are at increased risk of infection, and the overuse of antibiotics in healthcare facilities has accelerated the development of bacteria that are resistant to these drugs. Resistant germs may spread among patients if infection control methods are weak.

Resistant microorganisms have been able to spread more easily across international borders due to healthcare and commerce globalization. Contaminated water sources may be a result of the incorrect disposal of pharmaceutical waste, which can lead to the spread of antibiotic-resistant bacteria from one country to another. (Tang et al., 2023)

- **Public Health Impact of AMR**

Antimicrobial resistance (AMR) is a major problem for world health because it makes illnesses harder to cure, which in turn causes people to be sick for longer, spend more money on healthcare, and die younger. Resistance is making it harder to treat common diseases that were before easy to treat with antibiotics. This includes infections of the urinary tract, pneumonia, and bloodstream. Sometimes, doctors don't have much of a choice when it comes to treating infections caused by MROs.

Antimicrobial resistance poses a risk to medical operations that depend on efficient prophylaxis against microbes. For example, when patients get infections those are resistant to currently available antibiotics, it may heighten the danger of surgical procedures, organ transplants, and cancer therapies.

Antimicrobial resistance (AMR) is among the top ten public health concerns confronting the world today, says the World Health Organization (WHO). The World Health Organization has issued a dire warning, stating that by 2050, the yearly death toll from antibiotic-resistant illnesses would top 10 million, surpassing even the death toll from cancer and other leading causes. (Salam et al., 2023)

- **Efforts to Combat AMR**

Efforts to combat AMR include promoting the judicious use of antimicrobials in both human medicine and agriculture. Public health campaigns aim to educate both healthcare providers and the public about the dangers of misuse. Hospitals and clinics are adopting antimicrobial stewardship programs, which ensure that antibiotics are only prescribed when necessary and that the correct antibiotics are used.

In the agricultural sector, several countries have imposed restrictions on the use of antibiotics as growth promoters in livestock. Global initiatives, such as the WHO's Global Action Plan on Antimicrobial Resistance, are calling for a coordinated approach to AMR, including improved infection prevention, surveillance of resistant organisms, and the development of new antimicrobials, vaccines, and diagnostic tools. (Kivumbi & Standley, 2021)

1.3 ANTIMICROBIAL AGENTS

Many different kinds of microbes may infect humans and other animals. Antimicrobial agents are medications that prevent the development of microorganisms.(Breijyeh & Karaman, 2023)

Pathogens may cause infections and illnesses, but antimicrobial drugs can stop them. It is usual to find a variety of antibacterial medications. The following are included:

1. **Antibacterial drug:** A medicine that prevents germs from causing disease is known as an antibacterial medication. Example: Zithromax.
2. **Antifungal drug:** The term "antifungal drug" describes a class of medications used to treat fungal infections. Example: Miconazole
3. **Antiviral agent:** Antiviral drugs are medications that inhibit the infectious effects of viruses. Example: Tamiflu.
4. **Antiparasitic drug:** Pharmaceutical that inhibits the development of parasites that cause disease. Example: Anthelmintics.

1.4 STRUCTURE AND PROPERTIES OF CHALCONES

Chalcone

Compound C_6H_5C (O) $CH=CHC_6H_5$ is the chemical formula for chalcone. It is a ketone that is α,β-unsaturated. Chalcones or chalconoids are collective nouns for a wide range of biologically significant chemicals. Bioactive compounds, luminous materials, and chemical building blocks, they are well-known. (Salehi et al., 2021) (Dimmock et al., 2022)

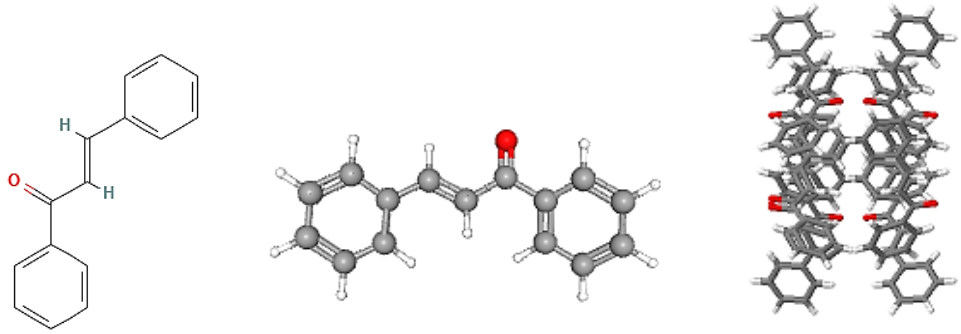


Figure 1.1: Structure of the Chalcone

• **Aromatic Rings**: Chalcones contain two phenolic (aromatic) rings (denoted as A and B). The substitution patterns on these rings can vary, leading to a wide variety of chalcone derivatives.

• **α,β-Unsaturated Carbonyl System**: The α,β-unsaturated carbonyl (C=O) structure contributes significantly to the reactivity and stability of chalcones. The double bond between the α and β carbons (C=C) allows for various chemical transformations and interactions.

The structural features of chalcones can be influenced by:

• **Substituents**: The presence of different substituents on the aromatic rings, such as hydroxyl (-OH), methoxy (-OCH_3), or halogen (-Cl, -Br), can affect their biological activity and properties.

• **Geometric Isomerism**: Chalcones can exhibit cis and trans isomerism due to the presence of the double bond, which can further influence their properties.(Hidalgo et al., 2021)

Table: 1 Chemical and Physical Properties of Chalcones (Mhaibes, 2023)

Property Name	*Property Value*	*Reference*
Molecular Weight	208.25 g/mol	Computed by PubChem 2.2 (Release 2021.10.14)
XLogP3	3.1	Computed by XLogP3 3.0 (Release 2021.10.14)
Hydrogen Bond Donor Count	0	Computed by Cactvs 3.4.8.18 (Release 2021.10.14)
Hydrogen Bond Acceptor Count	1	Computed by Cactvs 3.4.8.18 (Release 2021.10.14)
Rotatable Bond Count	3	Computed by Cactvs 3.4.8.18 (Release 2021.10.14)
Exact Mass	208.088815002 g/mol	Computed by PubChem 2.2 (Release 2021.10.14)
Monoisotopic Mass	208.088815002 g/mol	Computed by PubChem 2.2 (Release 2021.10.14)
Topological Polar Surface Area	17.1 Å²	Computed by Cactvs 3.4.8.18 (Release 2021.10.14)
Heavy Atom Count	16	Computed by PubChem
Formal Charge	0	Computed by PubChem
Complexity	242	Computed by Cactvs 3.4.8.18 (Release 2021.10.14)
Isotope Atom Count	0	Computed by PubChem
Defined Atom Stereocenter Count	0	Computed by PubChem
Undefined Atom Stereocenter Count	0	Computed by PubChem
Defined Bond Stereocenter Count	1	Computed by PubChem
Undefined Bond Stereocenter Count	0	Computed by PubChem
Covalently-Bonded Unit Count	1	Computed by PubChem
Compound is Canonicalized	Yes	Computed by PubChem (Release 2021.10.14)

1.5 HYBRID CHALCONE AND ACETYL PYRAZOLINE DERIVATIVES

Among the many physiologically active chemicals that chalcones may serve as a precursor to are pyrazolines. Incorporating pyrazoline moieties into chalcone frameworks enhances antibacterial capabilities, making these hybrids useful in combating bacterial and fungal populations that have developed resistance. Chalcones are usually formed via Claisen-Schmidt condensation, and then pyrazolines are yielded by cyclization with hydrazine derivatives. This two-step approach is common for the synthesis of these compounds. Recent studies have shown that these hybrid molecules are very important in medicinal chemistry. For example, research shows that some chalcone-pyrazoline hybrids are more effective than the individual components against Mycobacterium TB and other harmful bacteria. Many believe that structural changes that increase bioavailability and target specificity have a synergistic impact, leading to this increased activity. These hybrids make use of a wide range of synthesis tactics, from more conventional approaches like reflux conditions to more cutting-edge methods like microwave-assisted synthesis, which improves both reaction times and yields. In addition, their pharmacological profiles have been improved via the investigation of structure-activity correlations (SAR).To summarize, the effective production of hybrid chalcone and acetyl pyrazoline compounds advances our understanding of microbes and opens the door to the creation of new medicinal agents that may conquer the present

obstacles in the control of infectious diseases. Antimicrobial resistance (AMR) is a growing public health crisis that is increasingly recognized worldwide. The statistics highlighting the rise in resistant infections underscore the urgency of addressing this issue.(Roman, 2024)

1.6 STATISTICS HIGHLIGHTING THE RISE IN RESISTANT INFECTIONS

Global Statistics on Antimicrobial Resistance

1. **Infections and Deaths**:
   - According to the World Health Organization (WHO), antimicrobial resistance is responsible for approximately 1.27 million deaths annually worldwide. This number is projected to increase if no effective measures are taken.
   - A 2021 report from the Centers for Disease Control and Prevention (CDC) estimated that more than 2.8 million infections occur each year in the United States alone due to antibiotic-resistant bacteria, resulting in over 35,000 deaths.(J. Patel et al., 2023)
2. **Increase in Resistant Strains**:
   - A 2019 WHO report highlighted that, in many regions, more than 60% of bacteria that cause healthcare-associated infections are resistant to at least one antimicrobial agent.
   - The prevalence of multidrug-resistant tuberculosis (MDR-TB) has been particularly alarming. In 2021, the WHO reported that around 450,000 new cases of MDR-TB occurred globally, with only one in three people able to access proper treatment.
3. **Economic Impact**:
   - The economic burden of AMR is substantial. A 2014 study published in the journal "Health Affairs" estimated that the total annual cost of AMR in the U.S. could reach $55 billion to $163 billion due to increased healthcare expenses and lost productivity.
   - Due to healthcare expenditures and productivity losses, the European Union is anticipated to pay €1.5 billion annually in costs associated with antimicrobial resistance (AMR), according to the European Centre for Disease Prevention and Control (ECDC).
4. **Rise of Specific Resistant Infections**:
   - Methicillin-resistant Staphylococcus aureus (MRSA) has been a significant concern in both community and healthcare settings. CDC data indicates that approximately 20,000 deaths each year are associated with MRSA infections in the U.S.
   - The incidence of carbapenem-resistant Enterobacteriaceae (CRE), often termed "nightmare bacteria," has risen sharply. The CDC estimates that CRE infections affect more than 13,000 people each year in the U.S., with a mortality rate of up to 50% in patients with bloodstream infections.
5. **Children and AMR**:
   - A report from the WHO states that nearly 700,000 children under five years of age die from pneumonia caused by antibiotic-resistant bacteria each year. This statistic highlights the impact of AMR on vulnerable populations.
6. **Geographic Variability**:
   - The WHO has identified that low- and middle-income countries face a higher burden of AMR. For instance, resistance rates for E. coli and Klebsiella pneumoniae in some countries can exceed 70% for critical antibiotics.
   - The European Surveillance of Antimicrobial Consumption (ESAC) reported significant variations in antibiotic consumption across Europe, with higher consumption linked to higher rates of resistance.

1.7 IMPORTANCE OF DEVELOPING NEW ANTIMICROBIAL AGENTS TO COMBAT AMR

In order to combat antimicrobial resistance (AMR), it is essential to find novel antimicrobial agents. As resistance continues to grow, the effectiveness of existing treatments diminishes, leading to increased morbidity, mortality, and healthcare costs. Below are key points that highlight the importance of developing new antimicrobial agents:

1. ***Addressing Treatment Failures***

As AMR increases, common infections caused by previously treatable pathogens become more challenging to manage. New antimicrobial agents can restore the effectiveness of treatment options for infections caused by resistant strains, thereby reducing the rate of treatment failures and improving patient outcomes.(Huang et al., 2022)

2. ***Expanding Therapeutic Options***

The emergence of resistant strains has led to a limited arsenal of effective antibiotics. Developing new antimicrobials can diversify treatment options, enabling healthcare providers to tailor therapies to specific pathogens. This is particularly important for infections caused by multidrug-resistant organisms, which are increasingly prevalent.

3. ***Preventing the Spread of Resistance***

New antimicrobial agents can help combat resistance by providing alternative mechanisms of action that bypass existing resistance pathways. By utilizing novel compounds, healthcare providers can better manage infections, ultimately reducing the selective pressure that contributes to the emergence and spread of resistant bacteria.

4. ***Protecting Vulnerable Populations***

Certain populations, such as the elderly, immunocompromised individuals, and patients undergoing surgery or chemotherapy, are particularly vulnerable to infections. New antimicrobials are essential for protecting these high-risk groups, ensuring that infections can be effectively treated, and minimizing the risk of complications.

5. *Supporting Medical Advancements*

The effectiveness of many medical procedures, including surgeries, organ transplants, and cancer treatments, relies on the availability of effective antibiotics to prevent and treat infections. The development of new antimicrobials is essential for maintaining the safety and efficacy of these procedures, which could otherwise be jeopardized by increasing resistance.

6. *Enhancing Public Health*

New antimicrobial agents can play a critical role in controlling the spread of infectious diseases at the population level. Effective treatment options can help reduce the incidence of infections, thus lowering the overall burden of disease and improving public health outcomes.

7. *Stimulating Research and Innovation*

Investing in the development of new antimicrobials fosters innovation in the pharmaceutical industry and encourages research into alternative treatment strategies. This can lead to breakthroughs in understanding microbial biology, resistance mechanisms, and novel therapeutic approaches, including combination therapies and bacteriophage therapy.(N. Kumar et al., 2023)

8. *Mitigating Economic Burden*

The economic impact of AMR is substantial, resulting in increased healthcare costs, longer hospital stays, and lost productivity due to prolonged illness. Developing new antimicrobial agents can alleviate this burden by reducing the frequency of treatment failures, complications, and the need for more expensive interventions associated with resistant infections.

9. *Global Health Security*

AMR is a global threat that transcends national borders, making it a critical issue for global health security. Developing new antimicrobials contributes to global efforts to combat resistance, ensuring that all nations have access to effective treatments and can respond to infectious disease outbreaks effectively.

10. *Fostering International Collaboration*

The urgency of developing new antimicrobial agents has led to increased collaboration among governments, research institutions, and the private sector. These partnerships are essential for pooling resources, sharing knowledge, and accelerating the discovery and development of innovative therapies to combat AMR.

1.8 SIGNIFICANCE OF CHALCONES AND PYRAZOLINES

The medicinal chemistry and pharmacology communities have been very interested in the chalcones and pyrazolines as families of compounds because of the wide variety of biological activity and possible therapeutic uses of these molecules. In the following, we will go over the importance of pyrazolines and chalcones by outlining their structural features, biological qualities, and drug discovery relevance.(Salum et al., 2020)

Chalcones

1. Structural Characteristics

Chalcones are distinguished by their distinctive structure, which is made up of a three-carbon α,β-unsaturated carbonyl group connecting two aromatic rings. Here is a generic representation of the chemical structure of chalcones:

Aromatic Ring A – C (=O) - CH=CH - Aromatic Ring B

***Functional Groups*:** Chalcones typically contain a ketone functional group (C=O) and a double bond between the α and β carbons, contributing to their reactivity.

- *Geometric Isomerism*: Chalcones can exist in both cis and trans configurations due to the presence of the double bond, which can influence their biological activity.

2. Natural Sources and Synthetic Pathways

- *Natural Sources*: Chalcones are found in various plants, particularly in the families of *Leguminosae*, *Asteraceae*, and *Rubiaceae*. Some notable natural sources include:
  - *Sophora flavescens*: A plant used in traditional medicine.
  - *Psoralea corylifolia*: Known for its use in Ayurvedic and traditional Chinese medicine.
  - *Hibiscus sabdariffa*: Rich in flavonoids and chalcones.
- *Synthetic Pathways*: Chalcones can be synthesized through various methods, including:
  - *Aldol Condensation*: Chalcone formation by the standard route entails acetophenone-aldehyde reactions aided by a base.
  - *Claisen-Schmidt Condensation*: A more specific method involving the condensation of an aromatic aldehyde with an acetophenone under basic conditions.
  - *Enzymatic Synthesis*: Using specific enzymes to catalyze the formation of chalcones, which is considered a green chemistry approach. (Wiraswati et al., 2023)

3. Biological Activities Associated with Chalcones

Chalcones have been shown to possess a variety of biological activities, making them of great interest in medicinal chemistry:

- *Anticancer Activity*: Several chalcone derivatives have been shown to have cytotoxic effects on different types of cancer cells. They have the ability to trigger cell death, slow cell growth, and disrupt signaling pathways associated with cancer development.
- *Anti-inflammatory Effects*: To regulate inflammatory pathways, chalcones block the synthesis of cyclooxygenases (COX) and other pro-inflammatory cytokines. Because of this quality, they might be useful in the treatment of inflammatory disorders.
- *Antimicrobial Properties*: Chalcones demonstrate antimicrobial activity against bacteria, fungi, and viruses, making them valuable in developing new antimicrobial agents.
- *Antioxidant Activity*: Their ability to scavenge free radicals helps protect cells from oxidative stress, contributing to their therapeutic potential in various oxidative stress-related diseases.(Vasudha et al., 2015)

Pyrazolines

1. Overview of Pyrazolines

Pyrazolines are ring-structured heterocyclic compounds with five carbon atoms and two nitrogen atoms. The cyclization of chalcones is a common route to their synthesis. One way to depict the overall structure of pyrazolines is as:

Pyrazoline Ring: C-C-N-C-C

2. Synthesis Methods and Structural Features

- *Synthesis Methods*: Pyrazolines can be synthesized through several methods, including:
  - *Cyclization of Chalcones*: This is a common method where chalcones are reacted with hydrazines or phenylhydrazines under acidic conditions to form pyrazolines.
  - *Condensation Reactions*: Other methods involve the condensation of 1,3-dicarbonyl compounds with hydrazine derivatives.
  - *Reflux Methods*: Some synthetic approaches require refluxing reactants in solvents like ethanol or acetic acid.
- *Structural Features*: Pyrazolines often exhibit different substituents on the ring, which can significantly influence their biological properties. The presence of various functional groups allows for the optimization of their pharmacological activities. (Mantzanidou et al., 2021)

3. Pharmacological Properties and Therapeutic Applications

Pyrazolines possess various pharmacological properties, making them significant in drug development:

- *Antimicrobial Activity*: Pyrazolines have shown promising antimicrobial effects against various pathogens, including bacteria and fungi.
- *Anti-inflammatory Effects*: In the same way as chalcones suppress the synthesis of inflammatory mediators, pyrazolines have also shown anti-inflammatory capabilities.
- *Analgesic Effects*: Certain pyrazoline derivatives exhibit analgesic activity, providing pain relief in various pain models.
- *Anticancer Potential*: Pyrazolines have been reported to possess anticancer properties, with some derivatives showing cytotoxic effects on cancer cell lines by inducing apoptosis and inhibiting tumor growth.
- *Neuroprotective Properties*: Some pyrazolines demonstrate neuroprotective effects, suggesting potential applications in treating neurodegenerative diseases. (Bouyahya et al., 2021)

Pyrazoline is a kind of dihydro pyrazole that has a single endocyclic double bond. The location of the double bond determines the potential pyrazoline types, which may be one of three: (1) 1-pyrazoline, (2) 2-pyrazoline, and (3) 1, 3-pyrazoline are all types of pyrazolines, but 2-pyrazoline stands out as the most essential and appealing one for repeated research.

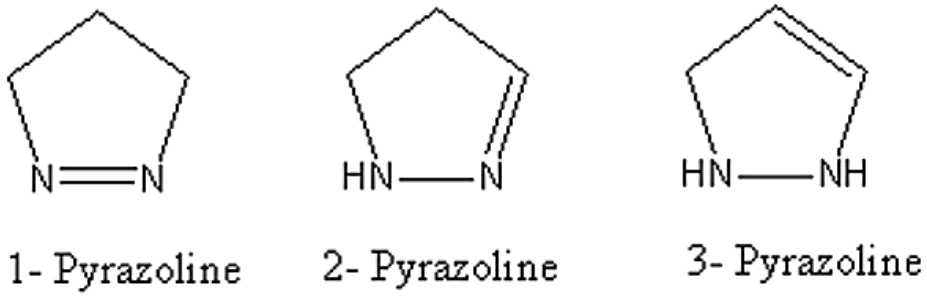


Figure: 2 Types of pyrazoline

In reality, pyrazoles may be further reduced to pyrazolines, and pyrazolidine is the simplest form of pyrazole.

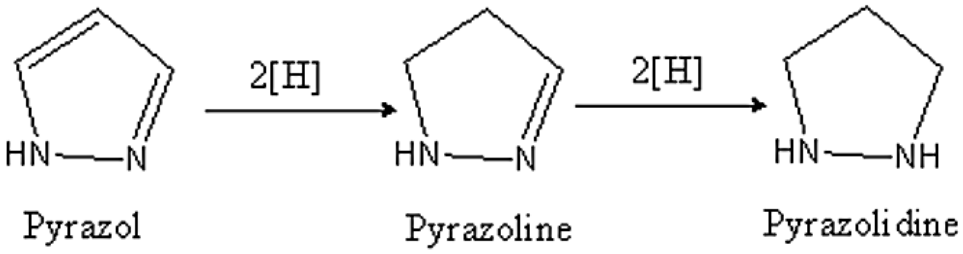


Fig. 3: Hydrogenation of pyrazole to pyrazoline then pyrazolidine

Vitamins, alkaloids, and pigments are some examples of natural items that include pyrazoline derivatives.
According to reports, pyrazoline byproducts still have many of their original therapeutic effects, including those against malaria, tuberculosis, yeast, viruses, cancer, and seizures. Celecoxib, Phenazone, Metamizole, Aminopyrine, Phenylbutazone, Sulfinpyrazone, Oxyphenbutazone, and Sulfinpyrazone are some instances of contemporary pyrazoline nucleus medications. Accessible resources such as ScienceDirect, PubMed, Scopus, and Google Scholar guided the literature search. Additional sources that were searched included books, technical reports, dissertations, and theses. The purpose of this literature review is to compile all published works over the last fifteen years

1.9 ANTIMICROBIAL ACTIVITY OF PYRAZOLINE DERIVATIVES

A variety of biological actions, including strong antibacterial capabilities, are associated with pyrazoline derivatives. The capacity of these compounds to hinder the development of several diseases, such as bacteria, fungus, and protozoa, has garnered considerable interest. These compounds are defined by the presence of a five-membered ring bearing two nitrogen atoms. The structural flexibility, ease of modification, and ability to interact with essential microbial targets are the reasons for the antibacterial activity of pyrazoline derivatives. (Dipankar et al., 2012)

1. *Mechanism of Action*

The antimicrobial activity of pyrazoline derivatives is thought to arise from multiple mechanisms:

- *Cell Membrane Disruption*: Pyrazoline derivatives can interact with microbial cell membranes, disrupting their integrity and causing cell lysis. The interaction often leads to increased permeability of the membrane, which hampers essential cellular processes.
- *Inhibition of Enzyme Function*: Some pyrazoline derivatives inhibit microbial enzymes crucial for cell wall synthesis or metabolic processes. For example, they can target bacterial DNA gyrase or topoisomerase enzymes, which are vital for DNA replication and repair.
- *Generation of Reactive Oxygen Species (ROS)*: Reactive oxygen species (ROS) are produced when pyrazoline chemicals are used. These ROS generate oxidative stress in microbial cells, which in turn causes cell death.
- *Interference with Protein Synthesis*: Pyrazolines may interfere with protein synthesis by interacting with ribosomes or other components of the translational machinery in bacteria and fungi. (Habibi et al., 2023)

2. *Antibacterial Activity*

Antibacterial activity against Gram-positive and Gram-negative bacteria has been shown in many pyrazoline derivatives, which is encouraging. Most of the time, clinically significant strains of bacteria including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis are used to test how well these chemicals work. (Chandrasekaran et al., 2020)

- *Gram-Positive Bacteria*: Bacteria with a thick peptidoglycan coating, known as Gram-positive bacteria, are highly susceptible to pyrazolines. These compounds are very powerful against these diseases because they interfere with membrane activities and impair cell wall production.
- *Gram-Negative Bacteria*: Some pyrazoline derivatives have potent antibacterial actions, especially by disrupting membranes and inhibiting enzymes, even though Gram-negative bacteria have a more intricate outer membrane structure.

3. *Antifungal Activity*

Antifungal efficacy against many harmful fungi is also shown by pyrazoline derivatives. Studies evaluating the antifungal properties of these substances often include testing them against fungal infections, such as Candida albicans, Aspergillus niger, and Trichophyton species.

• *Mode of Action*: An important component of the fungal cell membrane, ergosterol, may be hindered in its manufacture by pyrazoline derivatives. Increased membrane permeability and stunted fungal cell development may result from this disturbance. Pyrazolines are effective against fungi in part because they may cause oxidative stress in fungal cells.

4. *Structure-Activity Relationship (SAR)*

The chemical structure of pyrazoline derivatives has a significant impact on their antibacterial efficacy. They may be made much more or less effective antimicrobials by modifying the fundamental pyrazoline scaffold.

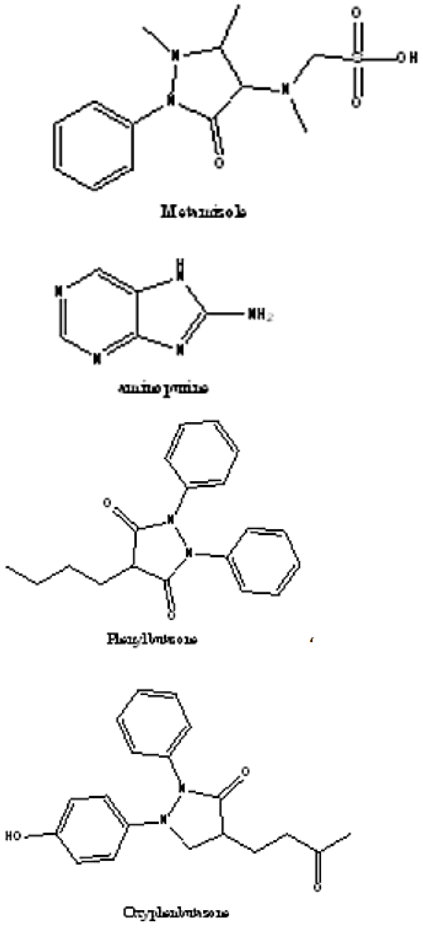
• *Substitution on the Pyrazoline Ring*: The lipophilicity and interaction of the molecule with microbial targets may be altered by inserting electron-donating or electron-withdrawing groups at certain places on the pyrazoline ring. As an example, halogenated pyrazolines are able to permeate microbial cell membranes more effectively, which leads to their enhanced antibacterial action.

• *Aromatic Substituents*: The incorporation of aromatic moieties, such as phenyl or naphthyl groups, at the C3 or C5 position of the pyrazoline ring has been shown to enhance antimicrobial properties, likely due to π-π stacking interactions with microbial proteins.

• *Hybrid Molecules*: Hybridizing pyrazoline with other bioactive scaffolds, such as chalcones, can further improve antimicrobial efficacy by creating compounds with dual mechanisms of action.

5. *Applications in Drug-Resistant Infections*

The ability of pyrazoline derivatives to fight drug-resistant bacterial and fungal strains is one of its most notable benefits. These compounds show promise as potential novel antimicrobial medication candidates since several investigations have shown that they maintain action against MRSA and other drug-resistant bacteria. Some examples of drug-resistant bacteria are Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA), which are both treated with pyrazoline derivatives. (Xuan et al., 2023)



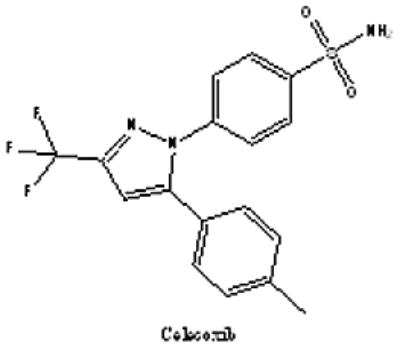


Fig. 4: Some of the modern drugs having pyrazoline nucleus

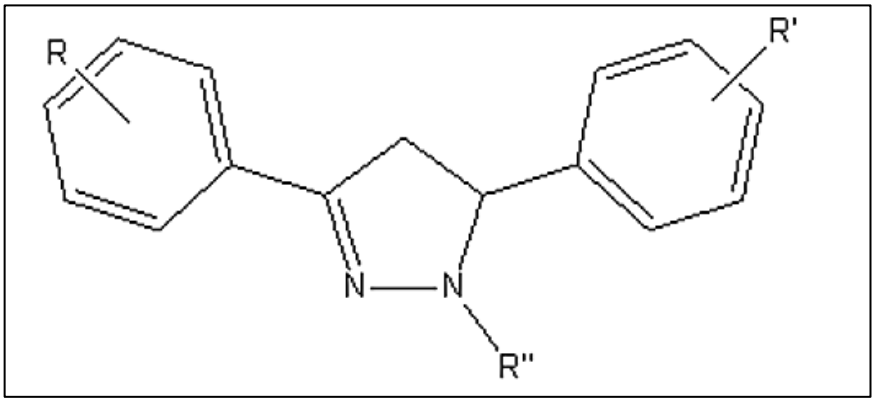


Fig. 5: N-substituted 3, 5-diphenyl pyrazoline derivatives

phenacyl bromides in ethanol were used to produce 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. The antibacterial activity of all produced derivatives was tested against the following bacteria: E. coli, S. typhimurium, S. aureus, S. faecalis, B. cereus, A. hydrophila, C. albicans, and C. glabrata.

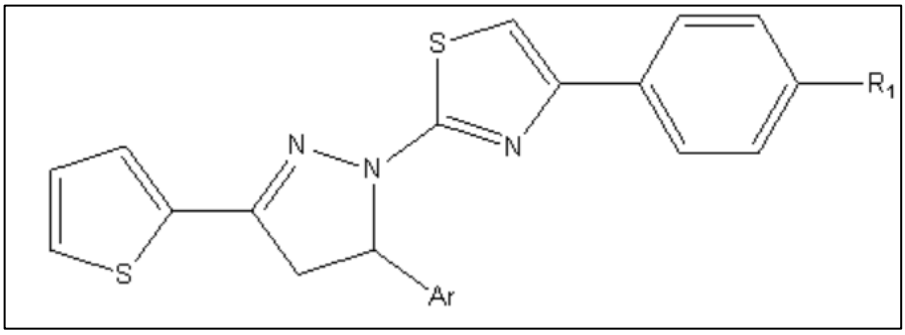


Fig. 6: Substituted 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline

The pyrazoline compounds that were substituted were produced by Kaplanciki et al. (2007). This is because 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives are produced when phenacyl bromides react with various 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines in ethanol. We tested the synthetic compound's antimicrobial activity against a variety of bacteria, including S. aureus, E. coli, B. cereus, A. hydrophila, L. monocytogenes, and C. albicans and C. glabrata.

In their 2007 publication, Kaplanciki et al. detailed the process of substituting 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines to produce novel 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. A variety of bacterial species were tested for their antibiotic activity, including E. coli, S. aureus, S. typhimurium, B. cereus, L. monocytogenes, A. hydrophila, C. albicans, and C. glabrata. The synthetic pyrazoline derivatives were also evaluated.

By condensing 4-hydroxy-3-methylacetophenone with various aromatic aldehydes in methanolic KOH, Shaharyar et al. (2007) were able to synthesis the pyrazoline derivatives as chalcones. The pyrazoline derivatives are produced when these chalcones combine with hydrazine hydrate in ethanol. Mycobacterium TB H37Rv was used to test the anti-tubercular

activity of all the produced derivatives. Mycobacterium TB drug-sensitive and drug-resistant strains were both effectively inhibited by the new heterocyclic compound's anti-tubercular action.

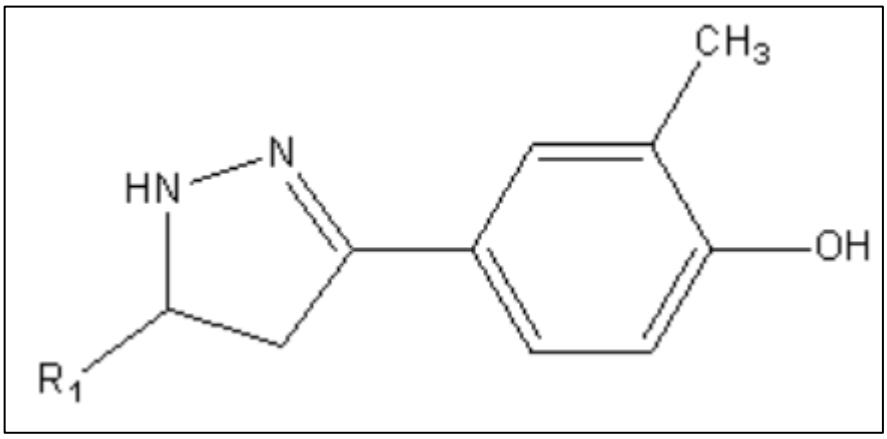


Fig. 7: 4-hydroxy phenyl pyrazoline derivatives

In their 2008 study, Munawar et al. detailed the conventional and microwave-induced reactions of quinolinyl chalcones with phenyl hydrazine, which result in a range of 2-pyrazoline derivatives. A panel of microbiologists tested each of the synthetic substances for their ability to inhibit the growth of Salmonella, Escherichia coli, Staphylococcus aureus, and Shigella dysentery. Antimicrobial activity has been shown by all synthetic variants. The antibacterial activity of substances with a chloro group substituent has been shown to be more powerful.

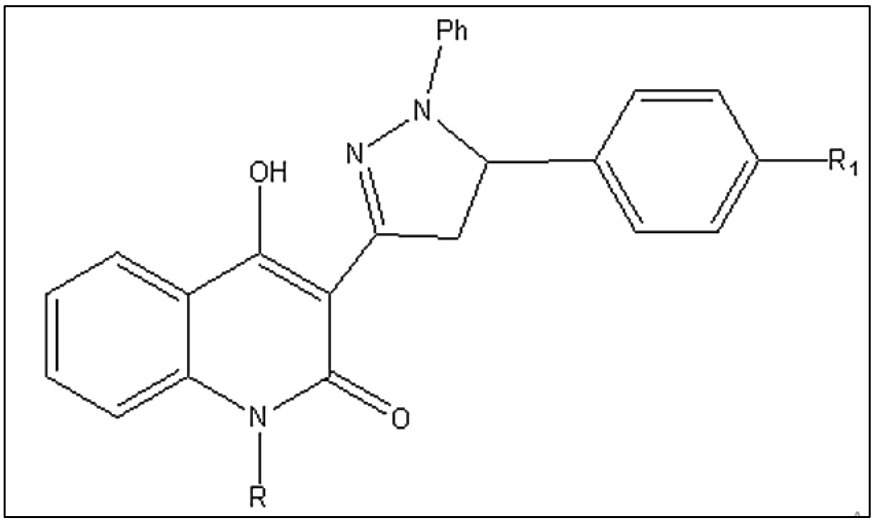


Fig. 8: Quinolinyl 2-pyrazoline derivatives

1.10 RATIONALE FOR HYBRIDIZATION IN DRUG DESIGN

1. Concept of Hybrid Drug Design in Medicinal Chemistry

Hybrid drug design is a strategic approach in medicinal chemistry that involves the combination of two or more bioactive pharmacophores into a single molecular entity. This method aims to create compounds with enhanced therapeutic efficacy, improved selectivity, and reduced side effects.

- *Mechanism of Action*: The hybridization approach leverages the individual pharmacological profiles of the parent compounds, allowing for synergistic effects where the combined action is greater than the sum of their individual effects. By targeting multiple pathways or mechanisms, hybrid drugs can potentially overcome the limitations of traditional monotherapy.
- *Drug Resistance*: Hybrid drugs can be particularly beneficial in addressing issues like drug resistance, especially in the context of antimicrobial agents. By incorporating multiple active components, hybrid compounds may effectively inhibit resistant strains of bacteria or fungi.

2. Benefits of Combining Chalcone and Pyrazoline Structures

The integration of chalcone and pyrazoline structures offers several advantages:

- *Structural Diversity*: The unique structural characteristics of chalcones and pyrazolines provide a rich platform for designing hybrid compounds with varied functional groups and substituents. This diversity can enhance the ability to fine-tune biological activity.
- *Pharmacological Synergy*: Combining these two structures can yield hybrid compounds that exhibit complementary biological properties, leading to enhanced therapeutic outcomes. For instance, the anti-inflammatory properties of chalcones can synergize with the analgesic effects of pyrazolines.
- *Optimization of Drug Properties*: The hybridization of chalcones and pyrazolines allows researchers to optimize key drug properties, such as solubility, stability, and bioavailability, which are critical for clinical efficacy. (Shalini et al., 2020)

3. Enhanced Biological Activity

Hybrid compounds often exhibit improved biological activities compared to their parent compounds. This enhancement can manifest in several ways:

- *Increased Potency*: The combination of chalcone and pyrazoline motifs can lead to hybrid molecules with increased potency against specific biological targets, such as cancer cells or infectious pathogens.
- *Target Specificity*: Hybrid drugs can be designed to target multiple receptors or enzymes, providing a broader range of therapeutic effects while minimizing off-target interactions.
- *Multifunctionality*: The ability to address multiple therapeutic targets within a single molecule can be particularly beneficial in treating complex diseases, such as cancer or infections where multiple pathways are involved.

4. Broader Spectrum of Antimicrobial Effects

One of the significant advantages of hybrid drug design is the potential for a broader spectrum of antimicrobial effects:

- *Multitarget Approach*: Hybrid compounds can target various microbial pathways, making it more challenging for pathogens to develop resistance. This multitarget strategy is especially crucial in an era of increasing antimicrobial resistance.
- *Enhanced Efficacy Against Resistant Strains*: By combining the mechanisms of action of chalcones and pyrazolines, hybrid compounds may maintain efficacy against strains that have developed resistance to traditional therapies.
- *Lower Dosage Requirements*: Improved efficacy can lead to lower dosage requirements for hybrid drugs, which may reduce the risk of side effects and toxicity. (Moulai-Hacene et al., 2020)

5. Previous Studies on Hybrid Compounds and Their Outcomes

Numerous studies have investigated the synthesis and biological evaluation of hybrid compounds derived from chalcones and pyrazolines, yielding promising results:

- *Antimicrobial Studies*: Research has shown that hybrid chalcone-pyrazoline compounds exhibit significant antibacterial and antifungal activities, often outperforming their parent compounds. For example, several studies have reported enhanced antimicrobial efficacy against resistant bacterial strains, such as Methicillin-resistant Staphylococcus aureus (MRSA).
- *Anticancer Research*: Studies have demonstrated that chalcone-pyrazoline hybrids possess potent cytotoxic effects against various cancer cell lines. These compounds can induce apoptosis through multiple pathways, including the modulation of cell cycle regulators and apoptosis-related proteins.
- *Inflammation Models*: In experimental models of inflammation, hybrid compounds have been shown to effectively reduce inflammatory markers and symptoms, highlighting their potential as anti-inflammatory agents.

1.11 THE ROLE OF CHALCONES AND PYRAZOLINES IN MEDICINAL CHEMISTRY

Chalcones and pyrazolines play a crucial role in medicinal chemistry, particularly due to their diverse biological activities and potential therapeutic applications.

Antimicrobial Properties

- ***Chalcones*:** Multiple investigations have shown that chalcone derivatives are very effective against many microorganisms, including viruses, fungi, and bacteria (both Gram-positive and Gram-negative). They usually work by destroying the cell membranes of microbes, preventing enzymes from doing their jobs, or interfering with the production of nucleic acids.
- ***Pyrazolines*:** Similarly, pyrazolines have been shown to possess antimicrobial effects. Their structural flexibility allows for the modification of various functional groups, enhancing their interactions with microbial targets. Research indicates that some pyrazoline derivatives exhibit potent activity against resistant strains, offering a promising avenue for overcoming AMR challenges.

Antimycobacterial Activity

- The resurgence of interest in chalcones and pyrazolines is also attributed to their activity against *Mycobacterium tuberculosis*, the bacterium responsible for tuberculosis (TB). The need for new antimycobacterial agents is critical, given the rising incidence of drug-resistant TB. Various chalcone and pyrazoline derivatives have demonstrated effectiveness against mycobacterial strains, contributing to the search for new treatment options.

Pharmacological Properties

- In addition to their antibacterial and antimycobacterial capabilities, pyrazolines and chalcones are known to have antioxidant, anti-inflammatory, and anticancer effects. Because of their versatility as modulators of many signaling pathways, they are promising candidates for multi-targeted treatments of complicated disorders.

1.12 RATIONALE FOR SYNTHESIS OF HYBRID CHALCONE AND ACETYL PYRAZOLINE DERIVATIVES

The rationale for synthesizing hybrid chalcone and acetyl pyrazoline derivatives stems from the growing need for new antimicrobial and antimycobacterial agents to combat drug-resistant pathogens. Both chalcones and pyrazolines have demonstrated significant biological activity, particularly against bacteria, fungi, and mycobacteria, making them attractive scaffolds for drug development.

1. Structural Versatility of Chalcones: The α,β-unsaturated carbonyl structure of chalcones gives them a wide range of biological actions, such as antibacterial, antifungal, anti-inflammatory, and anticancer capabilities. Their bioactivity may be fine-tuned because to their structural flexibility and simplicity of synthesis, which permit the inclusion of different functional groups. For this reason, chalcones are a great place to begin creating hybrid compounds that work better.

2. Biological Potency of Pyrazolines: Many studies have investigated the pharmacological effects of pyrazoline derivatives, especially those containing acetyl groups. Not only do they suppress the formation of drug-resistant strains, but they also demonstrate significant antibacterial and antimycobacterial activity. It is essential for medicinal purposes that hybrid compounds include a pyrazoline ring since it increases their bioavailability and stability.

3. Synergistic Effect of Hybrid Molecules: Hybrid molecules that combine the chalcone and pyrazoline frameworks are designed to leverage the unique bioactive properties of both moieties. The chalcone portion contributes to the inhibition of microbial enzymes and disruption of cell membranes, while the pyrazoline ring enhances the overall antimicrobial potency and pharmacokinetic profile. This combination creates a dual-action mechanism that can target multiple pathways within microbial cells, reducing the likelihood of resistance development.

4. Addressing Drug Resistance: The increasing incidence of multidrug-resistant microbial and mycobacterial strains, such as Mycobacterium tuberculosis, underscores the urgent need for novel therapeutic agents. By synthesizing hybrid chalcone-acetyl pyrazoline derivatives, the study aims to discover compounds that not only exhibit high efficacy but also circumvent common resistance mechanisms in pathogens. The dual action of these hybrid molecules could prove to be a significant advantage in overcoming the limitations of current antimicrobial therapies.

5. Potential for Drug Development: The hybrid derivatives obtained in this research have the potential to be the starting point for the creation of novel antibacterial medications due to the structural simplicity and mutability of both chalcones and pyrazolines. Future refining of these hybrid compounds' pharmacokinetics, toxicity profiles, and target selectivity might lead to their clinical usage. (M. R. Patel et al., 2011)

1.13 PROBLEM STATEMENT

Emerging as serious public health concerns, multidrug-resistant (MDR) bacteria and mycobacteria provide a substantial challenge to the present level of antimicrobial therapies. Infections caused by multidrug-resistant (MDR) bacteria, viruses, or other microbes are more difficult to treat and may raise the risk of death. Diseases caused by bacteria that are resistant to more than one medication, such as VRE and Methicillin-resistant Staphylococcus aureus (MRSA), have been identified as a major public health concern by the World Health Organization (WHO). Due to the worrisome dependence on last-resort antibiotics—which are often linked to severe side effects and restricted use—as a consequence of the ineffectiveness of traditional medicines, healthcare expenses have risen and hospital stays have been lengthened. Novel antimicrobial medicines that may successfully target these resistant organisms have not been well studied, despite progress in antibiotic development. New antibiotic research has slowed in the pharmaceutical sector because of financial motivations and the scientific complexity of fighting resistant bacteria. In addition, mycobacteria and bacteria have evolved complex resistance mechanisms, which provide challenges for existing antimicrobial medications. These mechanisms include biofilm formation and enzymatic degradation of antibiotics, both of which significantly diminish the effectiveness of drugs. Current medicines sometimes have a limited range of action, which makes them ineffective against certain infections and encourages the abuse of certain antibiotics, which makes resistance worse. This predicament highlights the critical need for new approaches to medication development to tackle diseases that have developed resistance. New classes of antibiotics with improved effectiveness, decreased resistance development potential, and broad-spectrum action are urgently needed to combat multidrug-resistant bacteria and mycobacteria. A potential route for drug development is the

research of hybrid molecules, especially those that include various structural motifs like pyrazolines and chalcones. To lessen the chances of resistance development, these chemicals may have complex action mechanisms that target many pathways inside infections. Failure to create effective new antimicrobials could cause a return of diseases that were previously controlled, putting a strain on healthcare resources and undermining the achievements of modern medicine; thus, addressing the problem of antimicrobial resistance is an economic, public health, and medical necessity. Ultimately, the present-day threats from MRSA and mycobacteria underscore significant deficiencies in the field of antimicrobial research and development, underscoring the pressing need for fresh approaches to treatment. Antimicrobial resistance is a major public health concern, and developing and testing new compounds, especially hybrid molecules, might strengthen the current toolbox of viable antibiotics.