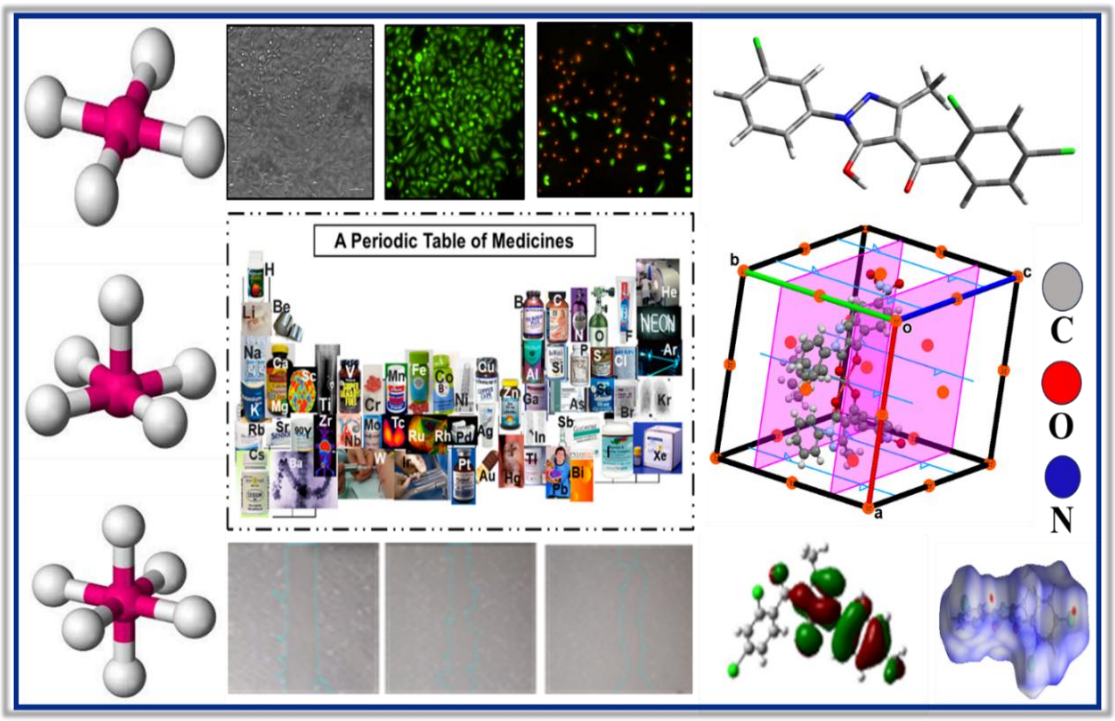
CHAPTER 1

Introduction and literature review of transition metal complexes containing a derivative of pyrazolone ligands and their coordination chemistry



1.1 Introduction

1.1.1 Transition metal chemistry and its characteristics

The d-block elements have been fundamental to our current understanding of chemistry and have significantly influenced the development of the periodic table. In **2019**, we commemorated the **150th anniversary** of Dmitri Mendeleev's publication of the first modern periodic table. The central section of the periodic table is composed of d-block elements, often referred to as transition elements or transition metals [1].

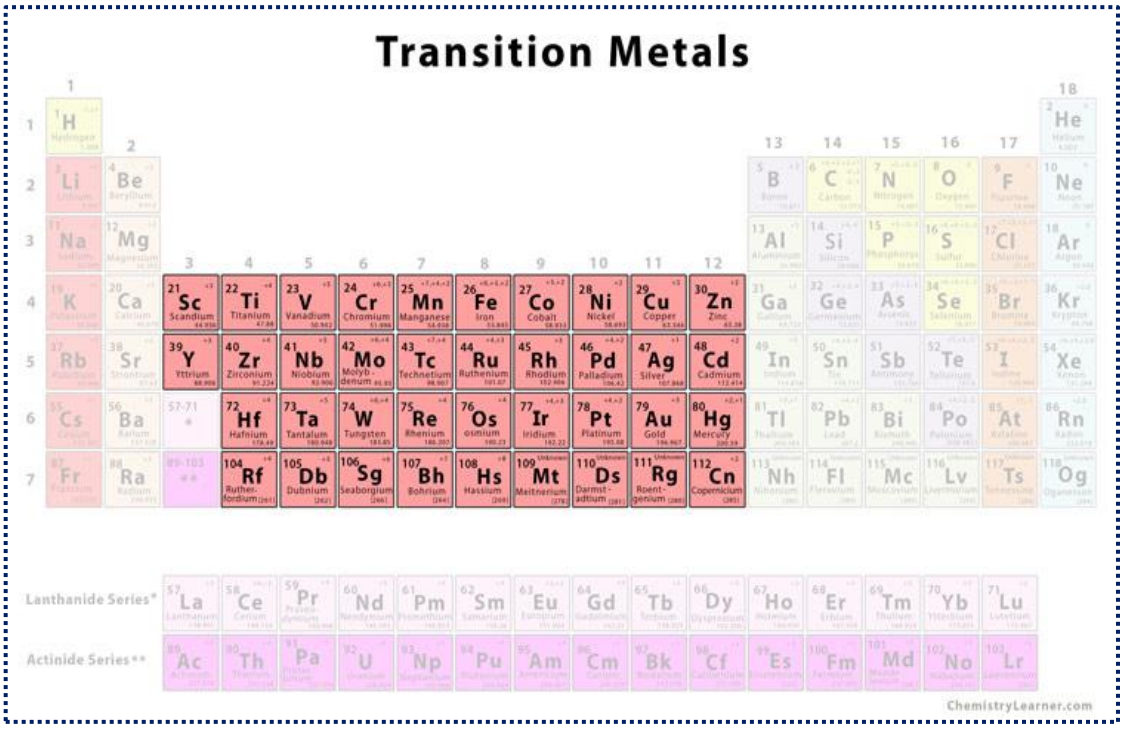


Fig.1.1. Periodic table of transition metals

The electronic configuration of Transition elements (3d elements) is characterized as,

3d elements (Z = 21 to 30): [Ar]$3d^{1-10}$ $4s^2$

Metal ions have long been acknowledged for their crucial roles in biological systems. While certain metals are essential for various physiological processes, others can be toxic. These ions are vital for numerous functions in the human body and a deficiency in specific metal ions can lead to various health issues [2]. Transition metals are highly valued for their exceptional characteristics, making them superior in numerous applications compared to other elements. For instance, copper, a vital trace metal found in Cu^{2+}, plays a significant role in metalloenzymes such as cytochrome oxidase, superoxide dismutase, and ascorbate oxidase. Similarly, manganese (Mn) is important for photosynthesis, while cobalt (Co) is an

essential component of vitamin B12 [2]. Additionally, nickel is involved in the enzyme urease. Transition metals are distinguished by their partially filled d-orbitals, which enable the formation of various coordination complexes. They display multiple oxidation states, possess distinct magnetic properties and can interact with numerous anions. Their versatility in bonding with different ligands leads to widespread applications across biological, industrial, and environmental fields [2].

These metals and certain alloys played a pivotal role in shaping both the Bronze Age and the Iron Age. Today, transition metals are abundantly present throughout the Earth in diverse quantities. They can coordinate with oxygen or nitrogen terminals in proteins through various mechanisms, contributing significantly to the structure and function of vital macromolecules in living organisms [3][4]. Recently, metal coordination compounds have gained attention for their role as antioxidants, helping to protect organisms and cells from damage caused by oxidative stress and free radicals [5]. As a key area within inorganic chemistry, coordination chemistry is experiencing rapid growth in both experimental and theoretical developments. Metal ions are essential in pharmaceuticals and are also used as diagnostic agents. Given the unique properties of metals, their advantages in drug discovery should be further harnessed to design new pharmaceuticals [2]**.**

However, elevated concentrations of metal ions are linked to several pathological conditions, including cancer. Due to these factors, metal coordination complexes have emerged as a significant area of interest in medicinal chemistry, particularly drug development [6][7]. Studies have demonstrated considerable advancements in the use of transition metal complexes as drugs for the treatment of various human diseases. Metal complexes that incorporate certain bioactive ligands often exhibit greater effectiveness in biological and pharmaceutical applications compared to the ligands in their free form [8]. The investigation of transition metal complexes and their activation strategies should progress to develop future generations of drugs capable of addressing the limitations of current therapies, such as unwanted side effects. As a result, metal-based drugs are likely to play a crucial role in advancing drug development and enhancing the quality of life for patients [2]. Some of the transition metal complexes of (Cu, Pt and Ru) which are important anticancer agents are pictured in **Fig.1.2** [9][10][11]**.**

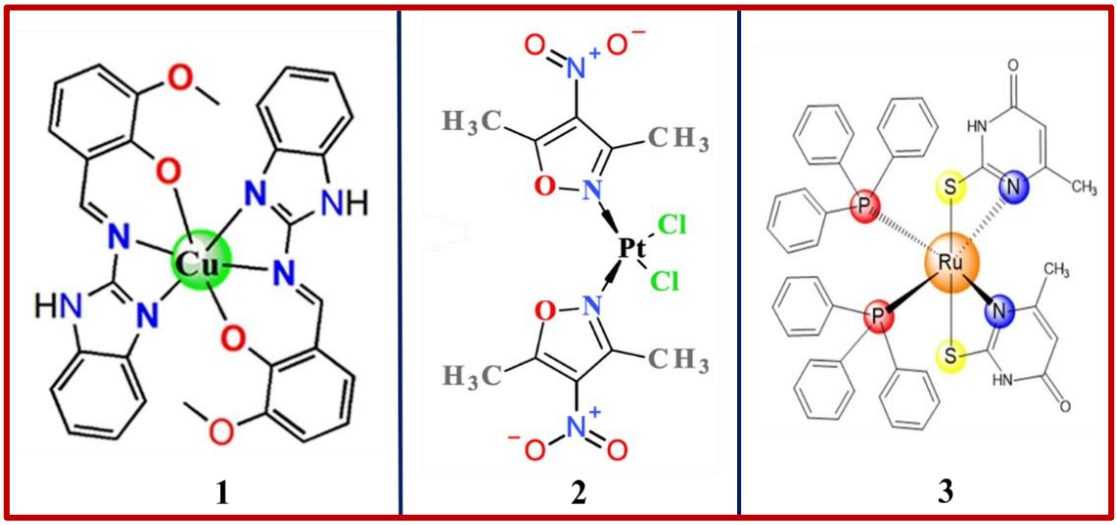


Fig.1.2. Transition metal complexes of (1) Cu, (2) Pt & (3) Ru metals as an anticancer agent [9][10][11]

In this study, we have chosen copper and nickel among the transition metals due to their unique coordination chemistry, diverse oxidation states and ability to form stable structures with various ligands.

1.1.2 Physical appearance and characteristics of Nickel and Copper metals

Axel Fredrik Cronstedt discovered nickel in 1751 in Sweden. He identified this metal from a newly found mineral near Halsingland, along with the ore known as 'kupfernickel', which was used in the production of green glass. Cronstedt managed to isolate the metal, describing it as 'hard and brittle' with only a weak magnetic attraction, ultimately naming it nickel [12][1]. Nickel is a key transition metal, with Ni(II) being more stable than its other oxidation states: Ni(0), Ni(I), Ni(III), and Ni(IV) [13]. However, It is well known that pure inorganic nickel salts or the dehydroacetic acid ligand alone are less active than nickel complexes containing Ni^{2+} [14].

Evidence of the cold working of native copper can be traced back to the 9^{th} to 7^{th} millennia BCE in regions such as Western Iran and Anatolia [15][16]. Copper is believed to be one of the first metals isolated through reductive pyrometallurgy, likely extracted from sulfide ores around 4000 BCE. Early uses of copper included its application in creating knives for household purposes [12]. After zinc and iron, copper ranks as the third most abundant trace element in the human body. This essential trace element plays a critical role as a catalyst in heme synthesis and iron absorption. The industrial significance of copper arises from its beneficial physical properties, including its appearance, ability to form alloys, resistance to corrosion, malleability, and excellent thermal and electrical conductivity [15].

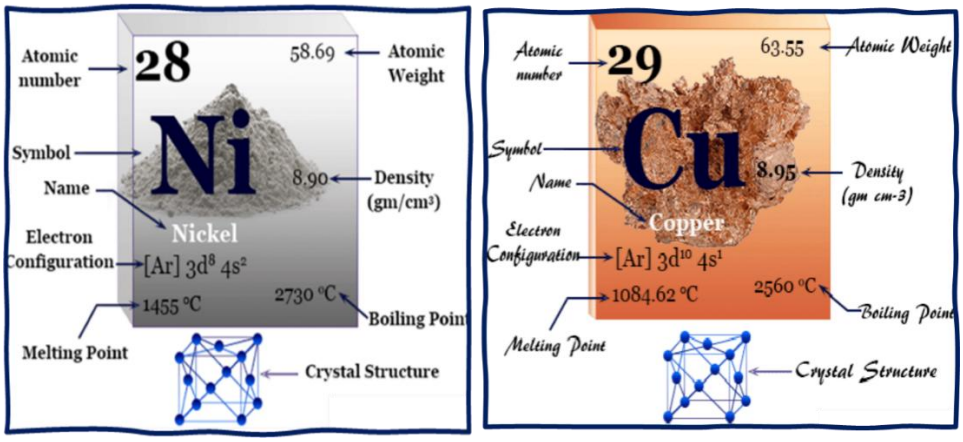


Fig.1.3. Physical appearance and characteristics of Nickel and Copper metals

1.2 A pyrazolone chemistry

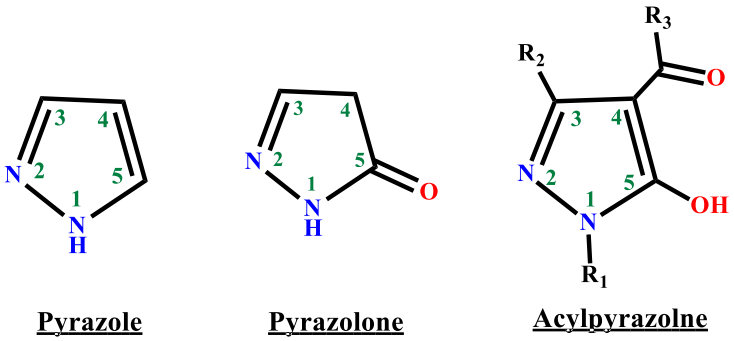


Fig.1.4. Structure of Pyrazole, Pyrazolone and Acypyrazolne [32]

Pyrazolone can be viewed as a derivative of pyrazole possessing an additional carbonyl (C=O) group. Pyrazole (1H-pyrazole or 1,2-diazole) is a group of the azole family and one of the most explored categories of researched areas. Pyrazoles possess two double bonds within their structure, giving them aromatic properties. Five-membered heterocycles that feature two adjacent nitrogen atoms are often classified based on the number of double bonds they contain [32]. These compounds are generally stable and can exhibit isomeric forms, as illustrated in **Fig.1.5.**

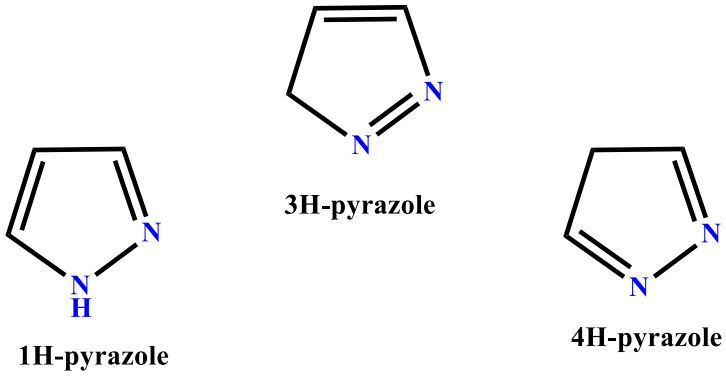


Fig.1.5. Isomeric forms of pyrazole moiety

Pyrazoles serve as core structures in various applications, including catalysis, agrochemicals, the synthesis of other compounds, and the field of medicine. Numerous applications in industries, including technology, health and agriculture, are made possible by various arrangements of the pyrazole nucleus [17][18]. Pyrazoles exhibit a wide range of biological activities, including anticancer, antibacterial, antifungal, antioxidant, and anti-inflammatory properties, among others [19][20].

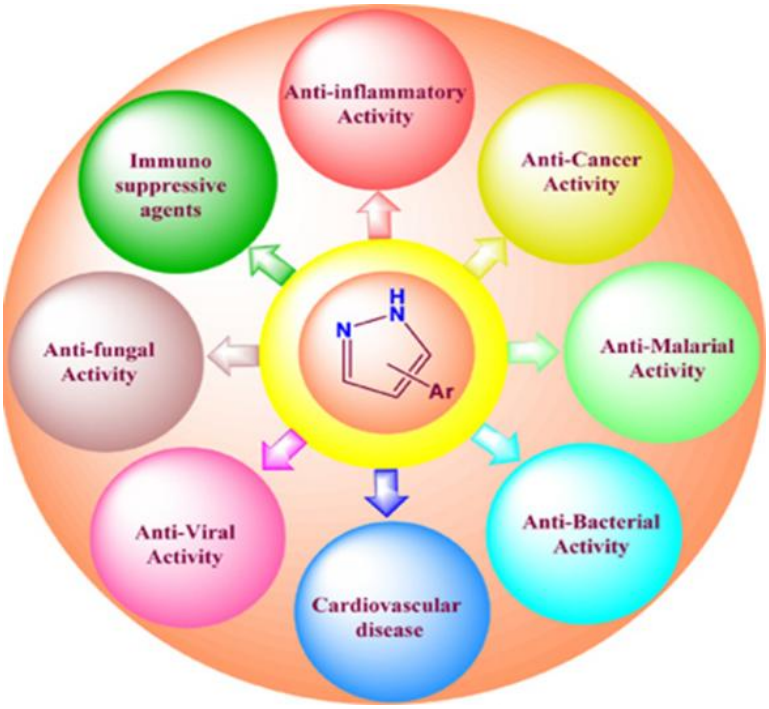


Fig.1.6. A flowchart of the application of pyrazole moiety

Compounds containing pyrazole are among the most significant groups of N-heterocycles, owing to their versatility and applicability across multiple domains. Consequently, synthesizing a wide range of pyrazole derivatives is of great interest, prompting researchers to concentrate on developing this functional scaffold and exploring novel and enhanced applications. Pyrazole derivatives are a distinct category of N-heterocyclic compounds (NHCs) characterized by a five-membered heteroaromatic ring containing two neighbouring nitrogen atoms. This structure includes one nitrogen atom resembling that of pyrrole (a proton donor) and another resembling that of pyridine (a proton acceptor) [21]. In the pharmaceutical sector, pyrazole derivatives are employed as medication ingredients or as additives in herbicides and antifungal agents due to their well-known complex properties. Recent research has focused on designing different pyrazole ligands with distinct structural properties to satisfy the individual metal-binding site's specific stereochemical needs [22].

1.2.1 Pyrazolone

Pyrazolones are a class of organic compounds known for their diverse properties and applications, particularly in medicine. These molecules can exist in various tautomeric forms. Since the discovery of antipyrine (2,3-dimethyl-1-phenyl-5-pyrazolone) by Knorr in 1883 [23][24], their analgesic and antipyretic effects have attracted considerable interest. Several drugs featuring a pyrazole core have been identified, such as the analgesic antipyrine [21]. Pyrazolones are some of the earliest synthetic pharmaceuticals, beginning with the development of antipyrine (phenazone) in the 1880s [25][26]. This spurred the development of other pyrazolones aimed at improving therapeutic outcomes, including amino antipyrine and phenylbutazone, which were synthesized in 1949 [25]. However, due to their significant toxicity in humans, their medical use has been largely restricted to veterinary treatments. Over time, research has shifted towards the development of less toxic pyrazolones, as well as compounds with antifungal, antitumor and antihyperglycemic activities [27].

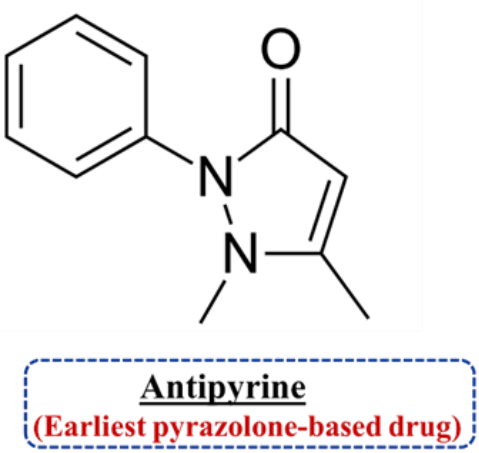


Fig.1.7. Structure of Antipyrine

Pyrazolones are characterized by two double bonds, primarily in the keto form **(Fig.1.9),** though they can also adopt the enol form [26]. These compounds feature a 5-membered heterocyclic structure with two adjacent nitrogen atoms and can be considered derivatives of pyrazole with an added carbonyl (C=O) group. This heterocycle can be found in a variety of well-known drugs from various categories that have a range of therapeutic effects [28]. Pyrazolones have significant commercial value, particularly in the production of analgesics and dyes [23]. Their first synthesis of 3-methyl-1-phenyl-5-pyrazolone was achieved by Ludwig Knorr in 1883 through a condensation reaction involving ethyl acetoacetate and phenylhydrazine [24].

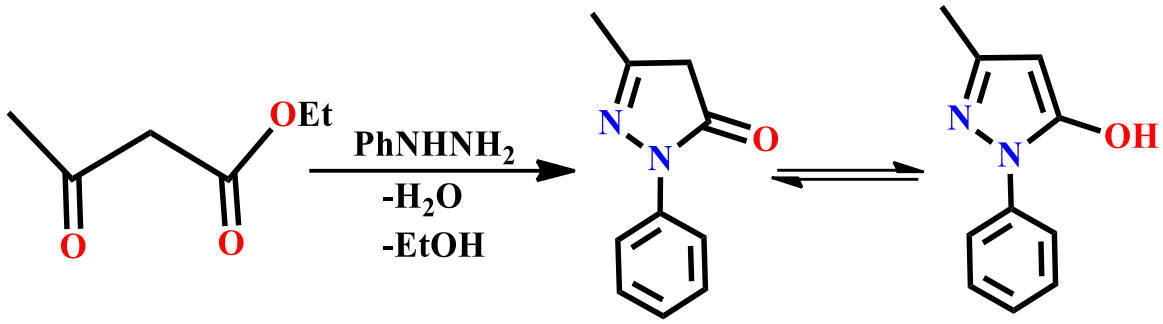


Fig.1.8. Synthetic route of 3-methyl-1-phenyl-5-pyrazolone

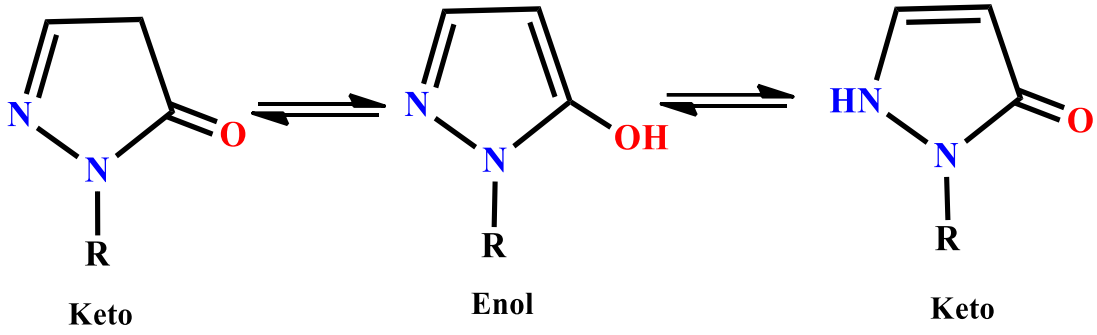


Fig.1.9. Keto and enol forms of pyrazolone

Numerous molecules with great potential for breakthroughs in medicinal chemistry, coordination chemistry, and functional materials are produced using the well-known synthon pyrazolone [29]. Pyrazolone-based ligands have gained increasing attention for their roles in catalysis, biological applications, sensors, functional materials, and pigments for dyes. Since the 1980s, numerous metal complexes incorporating pyrazolone-based ligands have been studied, and several have demonstrated notable properties, including catalytic activity, anticancer, antioxidant, antifungal, and antimicrobial activities, as well as improved NLO, photoluminescence, and optical properties compared to simple pyrazolone ligands [30]. Various pyrazolone derivatives, including **1.** Antipyrine, **2.** Aminophenazone, **3.** Propyphenazone, **4**. Metamizole, **5.** Phenylbutazone, **6.** Edaravone is commonly used as a nonsteroidal anti-inflammatory drug (NSAIDs) known for its strong antipyretic and analgesic effects **(Fig.1.11)** [31][32][33][34]**.**

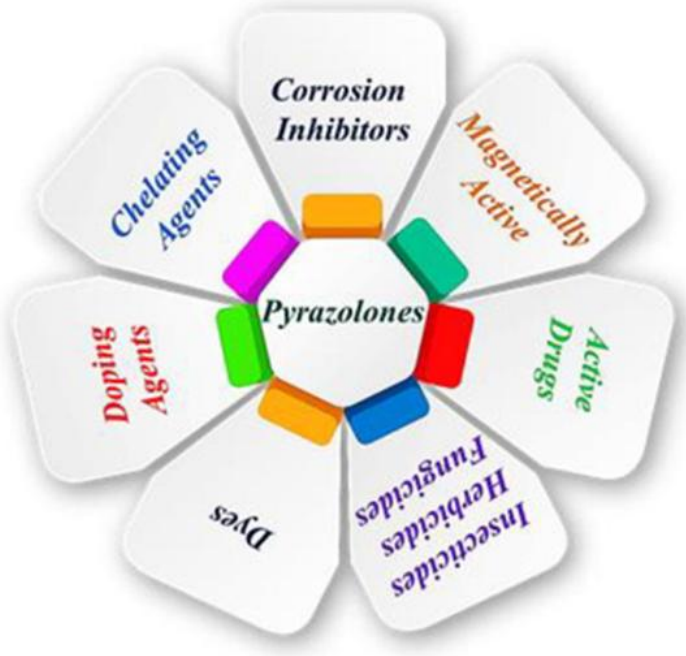


Fig.1.10. Applications of pyrazolone [35]

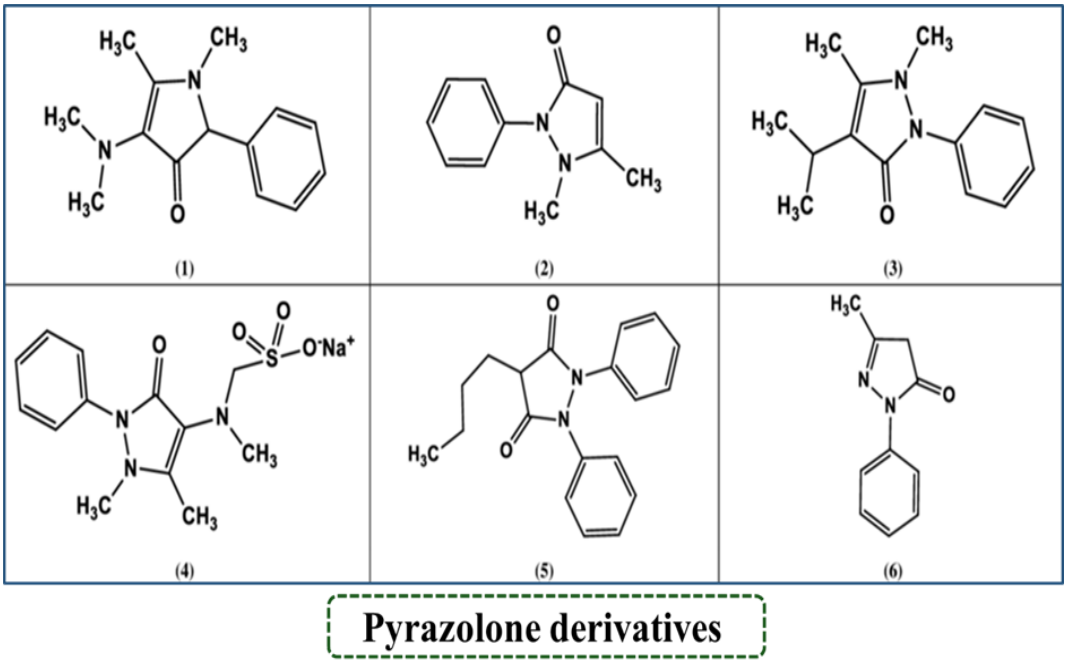


Fig.1.11. The well-known derivatives of pyrazolone

However, pyrazolones are known to cause allergic reactions, with reported side effects such as anaphylaxis, skin reactions, and agranulocytosis [33][36]. The discovery of these characteristics prompted researchers to create a derivative of pyrazolone compounds that behaved similarly but had superior medicinal effects [37]. The pyrazolone derivative 4-acyl-pyrazolone, with its numerous electron-rich donor centres and keto-enol tautomerism, may form a range of coordination compounds [38].

1.2.2 Acylpyrazolone

Acylpyazolones are organic compounds that contain a pyrazolone ring with an acyl group attached at the fourth carbon (C-4) position. Acylpyrazolone is an expansion of a well-known pyrazolone derivative, Edaravone (a 6th derivative shown in **Fig.1.11**), also known as 3-methyl-1-phenyl-2-pyrazoline-5-one. Acylation at the 4th position of Edaravone results in the formation of acylpyrazolone. Acylpyrazolone was first synthesized at the end of the nineteenth century and subsequently found to be used as a chelating agent [39][29][30].

By 1959, Jensen and coworkers outlined a one-step synthesis method that involves acylating the C-4 position of the pyrazole ring in a basic dioxane solution with calcium hydroxide at reflux temperature. The resulting calcium complex remains stable under alkaline conditions, preventing undesired side reactions. Afterwards, treating the mixture with an acidic aqueous solution yields acylpyrazolone (AP) as a solid, water-insoluble powder with a high yield. **Fig.1.12** depicts the standard synthesis route for 4-acyl-5-pyrazolone, which enables the formation of chelating ligands tailored for specific metal ion coordination. This method has been used successfully for the preparation of various 4-acyl substituted pyrazolones [40]. The 4-acyl-5-pyrazolones represent an intriguing class of β-diketones, where a pyrazole ring is integrated with a chelating functional group [39].

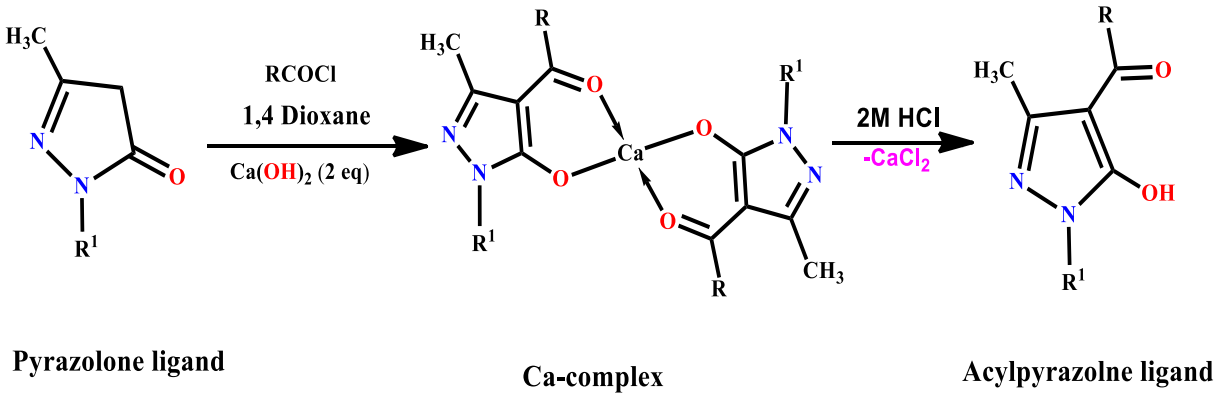


Fig.1.12. Synthetic route for 4-acyl-5-pyrazolone

The acylpyrazolones can exist in various tautomeric forms, both in solution and in the solid state, as illustrated in **Fig.1.13** [41][42]**.** Numerous investigations have looked into the tautomerism of 4 -acylpyrazolones. A quick (compared to NMR timeframe) inter-conversion of the OH and NH forms in solution results in averaged sets of signals [39].

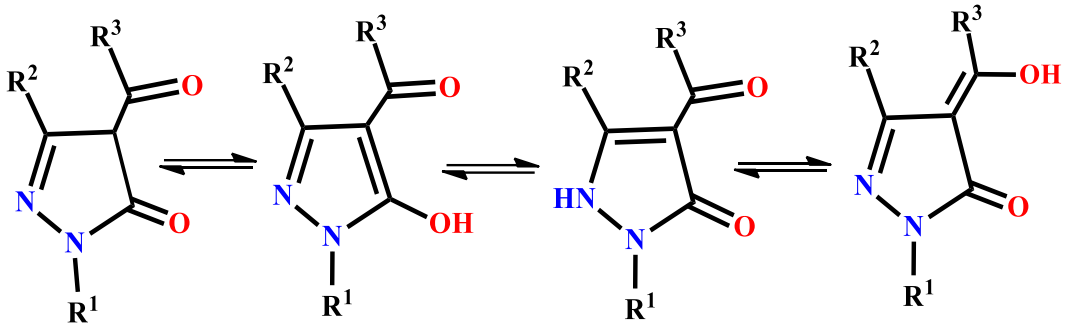


Fig.1.13. Tautomerism of acylpyrazolone

The spectroscopic analysis and characterization of various acylpyrazolone ligands have been extensively studied using methods such as FTIR, UV–Vis, ^{1}H and ^{13}C NMR, Mass spectrometry, and ab initio calculations. These investigations primarily focus on understanding the factors that affect the equilibrium between their tautomeric forms. The X-ray crystallographic analysis of several acylpyrazolone ligands has been conducted, revealing that when crystallized from chloroform, they adopt the enol form with an intramolecular O–H···O hydrogen bonding system [29][41].

1.2.3 Application of acylpyrazolone

These compounds were used in analytical chemistry to determine and isolate many metal ions. Chelation by acylpyrazolonates is facilitated by a lower pK_a value than those of conventional dicarbonyl ligands, leading to greater separation efficacy of intense colours due to electronic transitions. These compounds are distinguished by their participation in diverse chemical reactions and unique structural properties, making them significant in medicinal and synthetic chemistry [40].

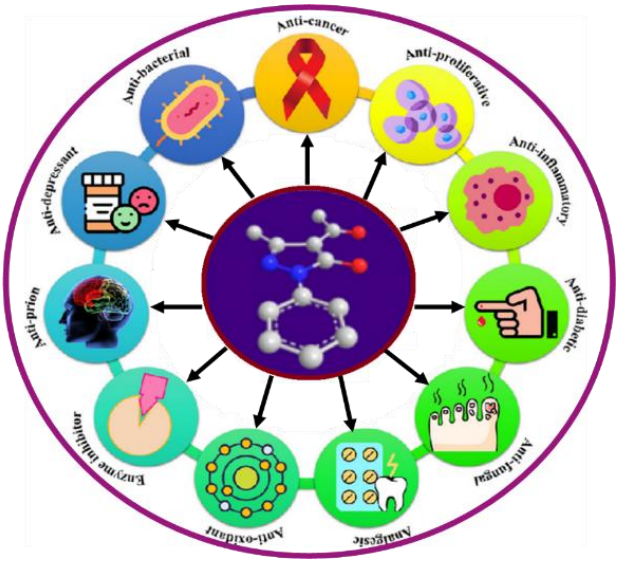


Fig.1.14. A flowchart of the application of Acylpyrazolone ligand

Acylpyrazolones and their derivatives are known for their significant biological activity, which is attributed to their unique structural framework. These compounds find utility in general chemistry, biochemistry, and medicinal chemistry [40][41][43]. Due to their significant pharmacological and biological applications, acylpyrazolones have gained recognition as an increasingly important class of heterocycles [44]. Being the heterocyclic β-diketones, they can form mono- and multinuclear complexes. Additionally, acylpyrazolone ligands have been used for complexation with various transition metals [30]. They can develop a variety of coordination compounds because of the keto-enol tautomerism and multiple electron-rich donor centres. 4-Acyl pyrazolones have gained considerable attention among researchers due to their simple synthesis through the acylation of substituted pyrazolones and corresponding acid chlorides [45][39]. Recently, there has been growing interest in their complexes within medicinal chemistry due to their ease of synthesis, stability, versatility, and significant biological activity [46].

1.3 Transition metals complexes based on a derivative of pyrazolone ligand

1.3.1 A brief review: Work done on Cu(II) and Ni(II) complexes

In terms of biological and pharmacological applications, metal complexes with certain bioactive ligands can prove more efficient than those ligands with their free-state [47]. Ligand selection during the synthesis and design of drug molecules has always become an important tool [48]. The biological potency of the ligand is increased by the formation of metal complexes. One of the key advantages of acylpyrazolone ligands is their ability to form stable complexes with a diverse array of elements, including main group and transition metals, as well as lanthanides and actinides [49]. This versatility allows for their application in numerous fields. Acylpyrazolones exhibit intriguing coordination modes in their neutral form. The enol tautomer can coordinate to a metal centre as a chelating bidentate ligand through its oxygen atom [29].

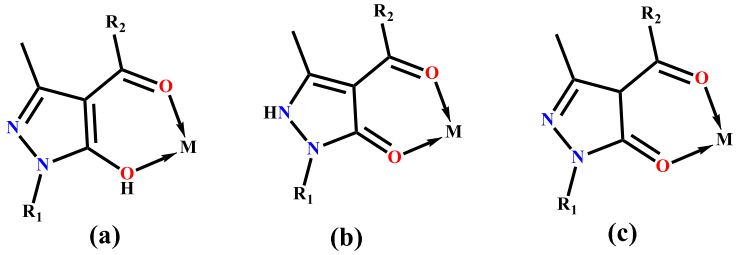


Fig.1.15. Coordination modes of neutral Acylpyrazolones in complexes [39]

We selected the biocompatible metal ion Cu from the group of metal ions because of its significance in biological living systems. Transition metal copper-based complex is very favourable and very advantageous. Copper complexes are renowned for their efficiency in cancer treatment because of their cytotoxic effect on tumour cells [50][29]. As a result, efforts have been made over the past several decades to advance the synthesis and characterization of Cu(II) compounds to better understand the role of copper active sites in various biological processes. Further research is being conducted on these complexes due to their significance in bioinorganic chemistry [51].

Okafor has done some of the earliest publications on the Cu(II) complexes of acyl pyrazolonates. He developed novel complexes and studied them, revealing the Cu(II) complexes' mononuclear formation and bidentate characteristics of the pyrazolone ligands acylpyrazolone based copper metal complexes have strong bio-activity [52]. Even recent reviews by Marchetti and colleagues have explored the various applications of pyrazolone-based transition metal complexes, as well as the properties and uses of acylpyrazolone ligands and their corresponding transition metal complexes [29][30][53]**.**

A series of copper complexes based on pyrazolone derivatives were synthesized as pictured as follows. The first synthesis of copper acylpyrazolonates involved the $[Cu(Q^{Ph})_2]$ compound, produced by combining $Cu(OAc)_2 \cdot H_2O$ with HQPh ligand [54]. Structural studies have also been conducted on the derivatives $[Cu(Q^{Ph})_2]$ complex-**1** and $[Cu(Q^{Ph})_2(MeOH)_2]$ complex-**2 (Fig.1.16)** [29]**.** The coordination geometry around Cu(II) exhibits a slightly distorted square-planar structure in complex-**1**, while in the latter, the geometry adopts a distorted octahedral configuration in complex-**2.** A number of $[Cu(Q)_2]$ and $[Cu(Q)_2L]$ derivatives (Q= pyrazolone moiety, Q^{Ph}, Q^{Me}, Q^{CF3} , Q^{CCl3} , Q^{PhpBr} etc and L = bipy or phen) were reported [55][56][57]. Copper compounds exhibit a transition between Cu(II) and Cu(I) ions, which can generate superoxide and hydroxyl radicals and cause cell death; they are potentially cytotoxic [29]. In this scenario, the $[Cu(Q)(PR_3)_2]$ complex-**3** (R = Ph, Cy, Bn, Ph-p-Me) complexes were synthesized when $[Cu(Q)_2]$ interacts with an excess of PR_3, a reduction from Cu(II) to Cu(I) was observed and $[Cu(Q)(PR_3)_2]$ (Q= pyrazolone moiety , R = Ph, Cy, Bn, Ph-p-Me) complexes were synthesized [56]. The crystal structure of $[Cu(QnPe)_2(H_2O)]$ complex-**4** shows a square-pyramidal geometry, as depicted in **Fig.1.16.**

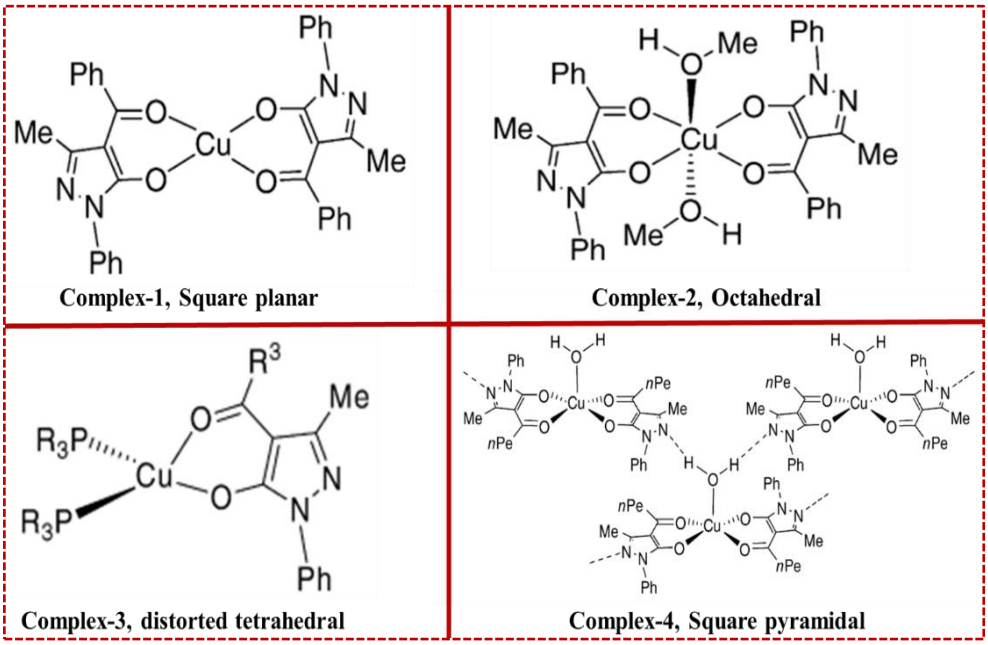


Fig.1.16. Four, five and six coordinated copper acylpyrazolone complexes

A Cu(I) complex (complex-**5**) was synthesized. In addition to complex-**5,** complex-**6** was also prepared. In complex-**6**, the ligand Q^{py,CF_3} chelates the copper metal through the nitrogen atoms of the pyrazole and pyridine rings. Single crystal analysis revealed distorted tetrahedral copper environments in both complexes **(Fig. 1.17)** [58]**.** A complex-**7** that adopts the anticipated square planar geometry was prepared, featuring two ligands in an anti-configuration. It demonstrates antibacterial activity against Gram-positive bacteria (B. subtilis) but is ineffective against Gram-negative bacteria (E. coli) [59].

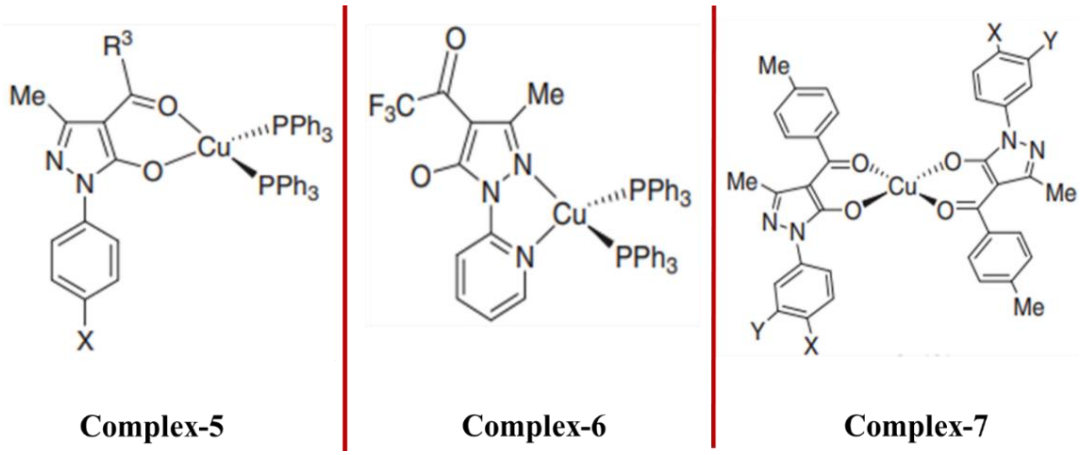
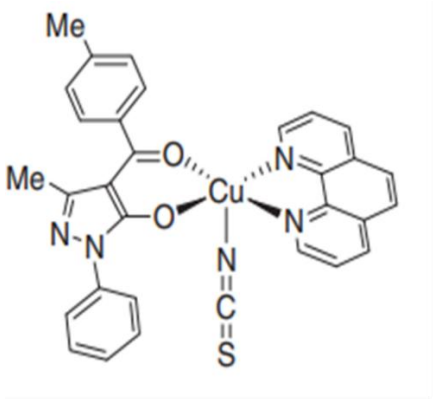


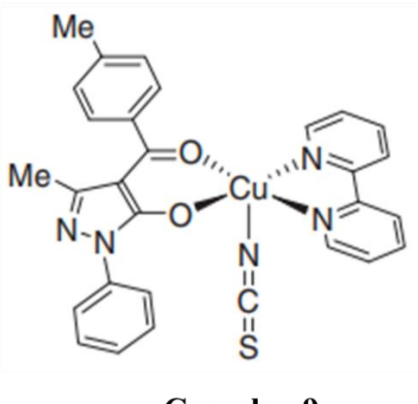
Fig.1.17. Four coordinated copper acylpyrazolone complexes

The mixed-ligand Cu(II) complexes (complex-**8** and complex-**9**) were synthesized with slightly distorted square-pyramidal structures **(Fig. 1.18).** A cytotoxicity assay was performed against the human lung cancer cell line (A549). Their interaction with calf thymus DNA occurs through intercalation. They also interact with bovine serum albumin and exhibit

cytotoxicity against both the human lung cancer cell line (A549) and non-cancerous rat cardiomyoblast (H9C2) cell lines [60].



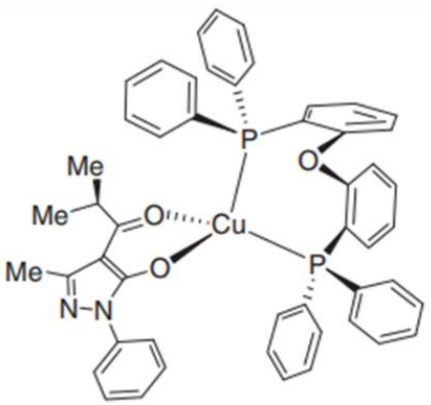
Complex-8



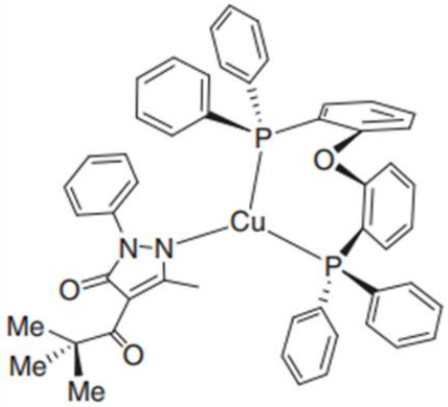
Complex-9

Fig.1.18. Distorted square pyramidal copper acylpyrazolone complexes

In 2015, Bochkarev et al. synthesized two Cu(II) complexes (complex-**10** and complex-**11**). The single crystal data revealed a classic tetrahedral environment around copper atoms in both complexes **(Fig. 1.19)** [39]**.** By Jadeja et al. in 2017, The ternary mixed-ligand Cu(II) complexes (complex-**12** and complex-**13**) were synthesized using 4-acyl pyrazolone as the primary ligand, 2,2'-bipyridyl or 1,10-phenanthroline as the secondary ligand, and $Cu(NO_3)_2$ as the metal salt in a 1:1:1 molar ratio. The inhibitory effects of the complexes were tested on human lung carcinoma (A549) and human lung epithelial (L132) cell lines using the MTT assay. A gene expression study was conducted to examine the expression levels of Bax (pro-apoptotic) and BCl2 (anti-apoptotic) genes. The synthesized complexes showed significant effectiveness against the mentioned cancer cells [61].



Complex-10



Complex-11

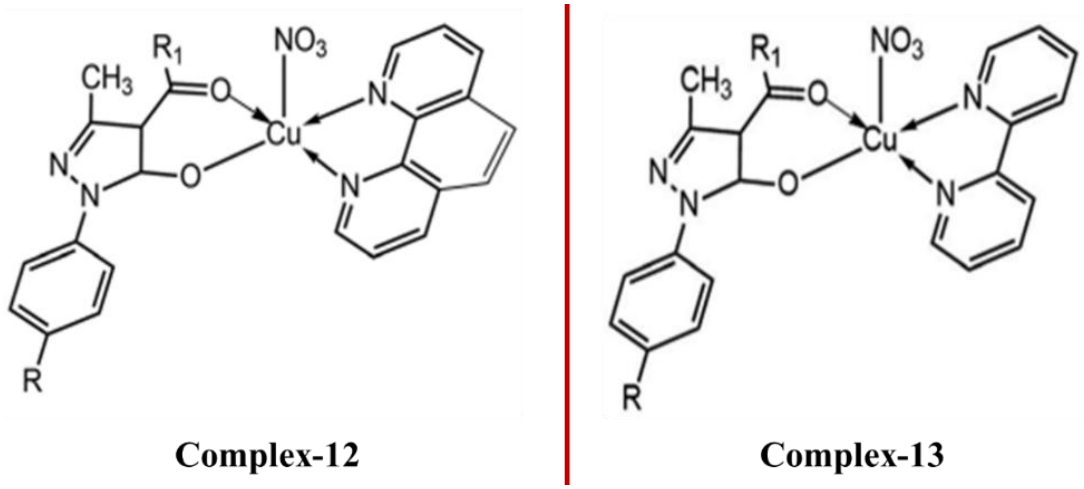


Fig.1.19. Four and five coordinated copper acylpyrazolone complexes

A mononuclear Cu(II) complex (complex-**14**) was synthesized using a 4-acyl pyrazolone ligand in 2018 by Nakum et al. X-ray diffraction analysis revealed the distorted square planar geometry of the complex [62]. The mononuclear distorted square planar Cu(II) complexes (complex-**15** and complex-**16**) were prepared by Bagdatli et al. in 2019. In the synthesis of both complexes, a 4-Aroyl-5-pyrazolone ligand was used.

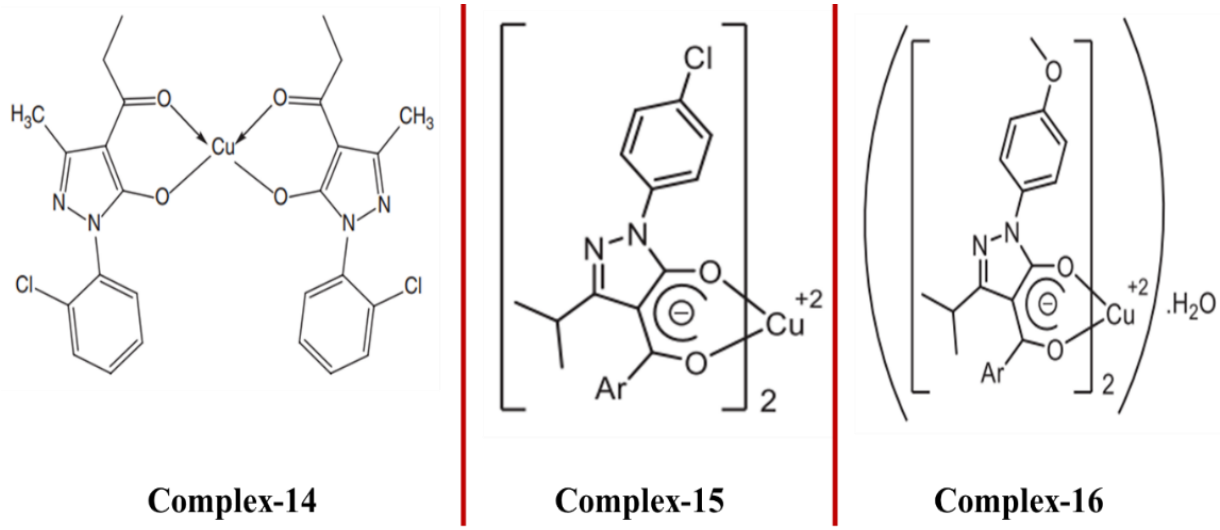
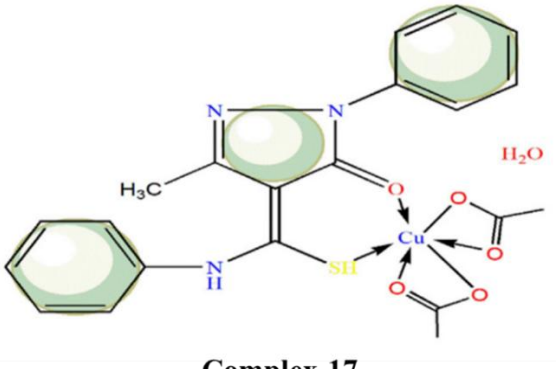


Fig.1.20. Distorted copper(II) square planar complexes

The distorted octahedral Cu(II)-L4 complex-**17** was synthesized using a pyrazole derivative in 2021 along with Cu(II)-L4 complex and complexes Cu(II)-L3 and Cu(II)-L5 were also synthesized. The Cu(II) complexes were evaluated for *in vitro* anticancer activity against the MCF-7 breast cancer cell line and showed significant effectiveness [63].



Complex-17

Fig.1.21. Six coordinated copper acylpyrazolone complex

This study continues the investigations on copper metal complexes by examining a wider range of ligands and evaluating their biological activities. By extending the scope, it seeks to offer deeper insights into their structural and anticancer properties, addressing the gaps identified in previous research. The findings aim to advance our understanding of how ligand diversity affects these complexes' biological efficacy and structural characteristics. Making physiologically relevant transition metal complexes is a key objective of this study due to the wide spectrum of biological activities of copper complexes based on pyrazolone derivatives. In the upcoming chapters, Chapters **3** and **4**, we describe the synthesis of eight novel Cu(II) complexes-four square pyramidal and four square planar. Additionally, all synthesized complexes have been characterized using various techniques. Importantly, we have also conducted biological studies.

Although Cu(II) complexes derived from pyrazolone derivatives are extensively researched for their biological effects, Ni(II) complexes featuring similar ligands are also quite significant. These Ni(II) pyrazolone complexes exhibit distinctive structural and electronic characteristics, enhancing their potential applications in areas such as catalysis, materials science and medicine. Ni(II) complexes can adopt various geometries, including square planar, tetrahedral, trigonal bipyramidal, and octahedral, with octahedral and square planar being the most common. The distinct chemical and physical properties of each Ni(II) complex, determined by its specific ligand, make their study fascinating and challenging [13]. The first known compounds of Ni(II) 4-aminoalkylidene-5-pyrazolone were found to be dihydrate octahedral paramagnetic with two unpaired electrons [30].

Recent interest has emerged in exploring how the structure and coordination of various complexes influence magneto-structural correlations, particularly within mixed organic-inorganic coordination complexes. Although research in this area has been ongoing for years, there is a renewed emphasis on this class of complexes due to their diverse

applications, including antimicrobial activity, molecular sensing and superconductivity. To investigate these correlations, researchers utilize a wide variety of metal ions and ligands, comparing their magnetic and coordination properties. Ni(II) is particularly noteworthy because of its unique magnetic characteristics. In both octahedral and tetrahedral geometries, the Ni(II) ion has a spin state of S = 1 and displays single-ion anisotropy, which has been documented in isolated Ni(II) complexes as well as in coordination polymers, such as linear chains [64]**.**

A series of Ni complexes have been synthesized in the past, as detailed below, and the pursuit of developing Ni(II) pyrazolone-based complexes still continues. This ongoing research aims to enhance our understanding of their properties and potential applications. The Ni(II) complexes (complex-**1** and complex-**2**) were synthesized. Both structures exist in an octahedral geometry with the solvent molecules in apical positions and the two ligands in anti-configuration [65]. A six-coordinated Ni(II) complexes (complex-**3** and complex-**4**) were prepared. Single crystal revealed the distorted octahedral geometry. The nickel ion is located at an inversion centre and features a slightly distorted octahedral coordination environment in both complexes. This ligand arrangement influences the electronic characteristics of the Ni(II) centre, potentially impacting its reactivity and coordination dynamics. The distortion from a perfect octahedron is likely due to steric interactions among the ligands, which may significantly affect the stability and overall properties of the compound. Upon irradiation with ultraviolet light at 312 nm, a complex-**3** exhibits a colour change to yellow, demonstrating its photochromic properties in the solid state. Additionally, the fluorescent emission observed in complex-**3** suggests its potential application as a luminescent material [66].

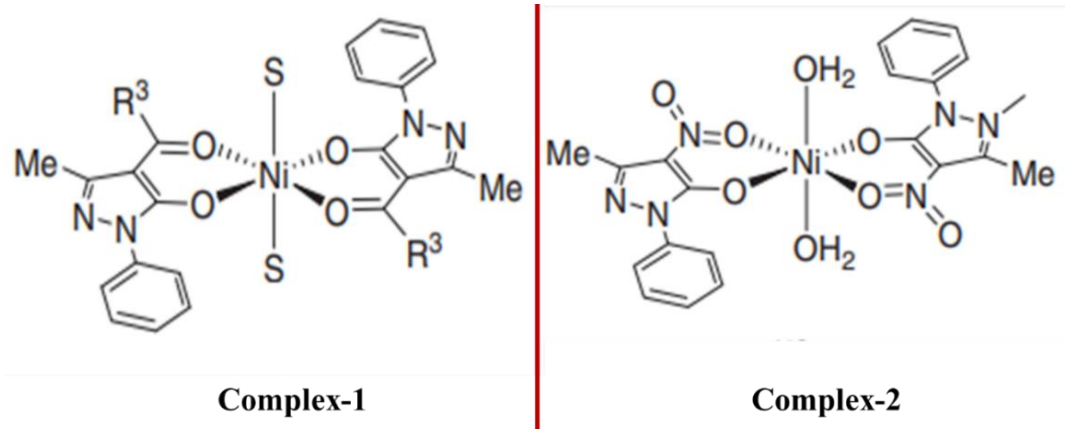


Fig.1.22. Octahedral Ni(II) acylpyrazolone complexes

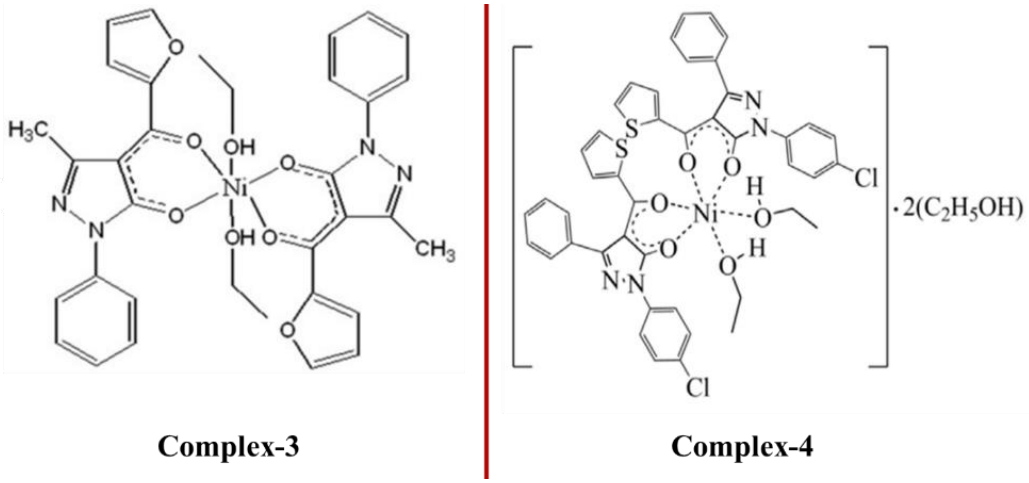


Fig.1.23. Distorted octahedral Ni(II) acylpyrazolone complexes

Besides our work on copper complexes, we have successfully synthesized three novel octahedral Ni(II) complexes, followed by a comprehensive characterization of these compounds. Chapter **5** offers an in-depth examination of the findings and methodologies utilized throughout our research. It provides extensive details on the synthesis, characterization, and analysis of the nickel complexes, facilitating a comprehensive understanding of their properties and potential applications.

1.4 *In vitro* Anticancer activity

Cancer is defined by the uncontrolled proliferation of cells, driven by transformed cells that evolve through natural selection. In the mid-19th century, Rudolf Virchow's 'cellular theory' of disease proposed that all diseases, including cancer, stem from alterations at the cellular level. This insight helped establish cancer as a condition characterized by abnormal cell growth. Before the incorporation of evolutionary concepts, views on cancer during the 18th to 20th centuries evolved alongside the discovery of cells. In multicellular organisms, the process of cell proliferation is tightly regulated. The various types of cancer, including those affecting the blood, lungs, colon and rectum, prostate, skin, breast, uterus, thyroid, and lymphatic system, among others [67].

Chemotherapy is commonly used as a systemic treatment for cancer patients, often in combination with surgery or radiotherapy. To tackle issues such as drug resistance, researchers are focusing on developing new metal-based anticancer agents, which show promise as a therapeutic strategy [50]. Interest in metal complexes for cancer therapy dates back to the unexpected discovery of cisplatin as an agent that inhibits tumour cell growth made by Rosenberg and his team in the 1960s. The role of pyrazoles in cancer treatment was

first explored by Sakai et al., who reported the anticancer properties of dichloro-bis(pyrazole) platinum(II). Although cisplatin is highly effective against a range of cancers, its toxicity remains a significant concern. As a result, researchers are actively exploring alternative compounds with lower toxicity [18].

Heterocyclic compounds have the potential to serve as scaffolds for pharmacophores, leading to the development of selective and effective anticancer agents. Specifically, nitrogen-containing heterocycles are fundamental structures in numerous approved anticancer medications. As a result, complexes derived from pyrazole are considered a promising option for creating highly bioactive compounds with excellent pharmacological properties. The research focused on synthesizing inorganic coordination compounds, highlighting the significant bioactivity of transition metal centres and can be utilized to develop innovative anticancer agents that operate through mechanisms distinct from those of free, uncoordinated ligands. Metallic elements are crucial in medicine and nutrition, playing key roles in various biological processes and providing unique chemical properties not typically found in free organic compounds [68]. Due to these factors, transition metal complexes of copper have become one of the most intriguing and attractive areas in medicinal chemistry [2]. In this study, we primarily focus on lung cancer and neuroblastoma cancer.

1.4.1 MTT assay

Various methods have been developed to assess the chemosensitivity of tumour cell lines. However, many of these assays face practical challenges that limit the number of variables that can be tested. Drug effects on cell growth can also be evaluated by directly counting cells in liquid culture. However, large-scale manual counting using trypan blue exclusion is highly tedious and time-consuming. Therefore, alternative rapid assays to assess cell viability, providing insights into tumour cell sensitivity to anticancer agents, are highly sought after. A fast and efficient colourimetric assay, the MTT assay, proves valuable for tracking the development of multidrug-resistant cells in culture. This method can assess cytotoxicity, cell proliferation, or activation. It utilizes a rapid colourimetric assay based on the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), which specifically measures viable cells and can be analyzed using a multiwell spectrophotometer (ELISA reader). This assay is quantitative and adaptable, substantially improving over traditional approaches commonly used for proliferation and cytotoxicity measurements.

The primary benefit of the MTT assay lies in its simplicity, enabling the examination of numerous parameters in a relatively short period [69][70]. A pictogram illustrating this assay is provided in **Fig.1.24.**

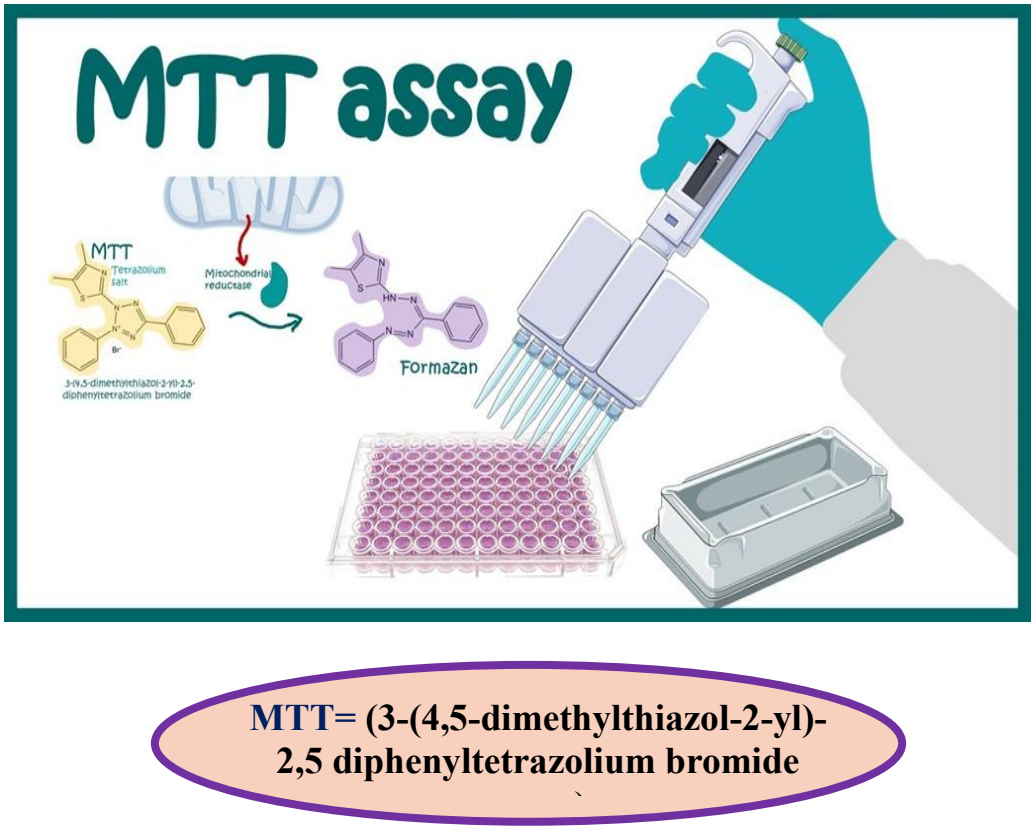


Fig.1.24. A pictogram of MTT assay

In the upcoming chapters (Chapters **3** and **4**), we describe how the MTT assay has been adapted to evaluate the impact of antineoplastic agents on NCI-H23 (lung adenocarcinoma), HepG2 (hepatocellular carcinoma), and SH-SY5Y (neuroblastoma) cancer cells. A detailed methodology will be provided in those chapters.

1.4.2 Scratch assay/Wound healing assay

Cancer continues to be one of the primary causes of mortality globally, affecting millions each year and highlighting the pressing need for effective therapeutic strategies. A promising method for investigating the behaviour of cancer cells is the scratch assay, an adaptable *in vitro* technique that simulates wound healing to evaluate cell motility and proliferation. The purpose of the scratch assay is to evaluate the cell migration through an *in vitro* method. Additionally, this method can be employed to assess the effectiveness of therapeutic compounds before their clinical application. Cell migration is a critical process

in developing and maintaining multicellular organisms, and its dysregulation is associated with various pathological conditions, such as cancer and atherosclerosis [71][72]. Migration can occur in different forms, including the movement of single cells and groups or sheets of cells from one area to another [73]. There are two primary categories of migration: single-cell migration and collective-cell migration. *In vitro* assays are fundamental for studying cell migration, as they enable researchers to quantify the migratory ability of cells under controlled conditions [73][74]. The scratch or wound healing assay is particularly popular due to its straightforward design and affordability [74][75]. Cell migration is crucial in various physiological processes, including embryonic development, wound healing, tumour invasion, neoangiogenesis and metastasis [76]. A scratch assay consists of cultivating a cell monolayer until it reaches confluence in a multiwell plate. This technique relies on the principle that when an artificial gap, referred to as a "scratch/wound", is created in a confluent cell monolayer, the cells at the margin of the gap will migrate toward the open area. This movement continues until the scratch is closed and new cell-cell contacts are reestablished. The process of cell migration is monitored by observing the re-colonization of the scratched area to quantify the extent of cell movement [75]. A detailed methodology will be explained in Chapters **3a** and **3b.** The pictogram of this assay is illustrated in **Fig.1.25.**

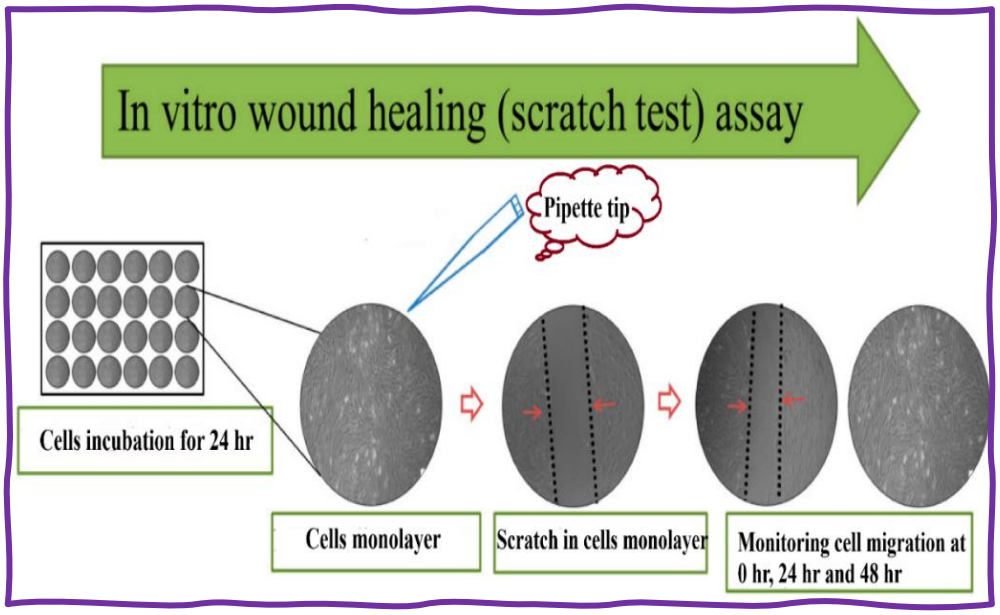


Fig.1.25. A pictogram of Scratch/wound healing assay

1.4.3 Live-dead assay/Cell death analysis

The Live/Dead assay is a common laboratory technique used in cell biology and toxicology to assess cell viability, distinguishing between live and dead cells within a

population. The method relies on membrane integrity to differentiate the two: live cells have intact membranes that allow selective uptake of specific dyes, while dead cells, with compromised membranes, allow entry of dyes that healthy cells typically exclude. Accurate and rapid estimation of cell viability is crucial for evaluating the effects of drugs, chemical or physical stimuli, and other factors on cell function. Common methods for assessing cell viability often involve mixing cells with reagents that convert a substrate into a coloured or fluorescent product. For example, membrane integrity is frequently used as an indicator to distinguish live cells from dead ones, with specific dyes being employed to separate the two populations [77].

Fluorescent dyes are often employed in this assay, with live cells emitting green fluorescence and dead cells emitting red. This assay is critical for studying the cytotoxic effects of drugs, environmental stressors, or therapies on cells. Additionally, it has significant applications in tissue engineering, cancer research, and regenerative medicine, where understanding cell survival and death is vital for advancing treatments.

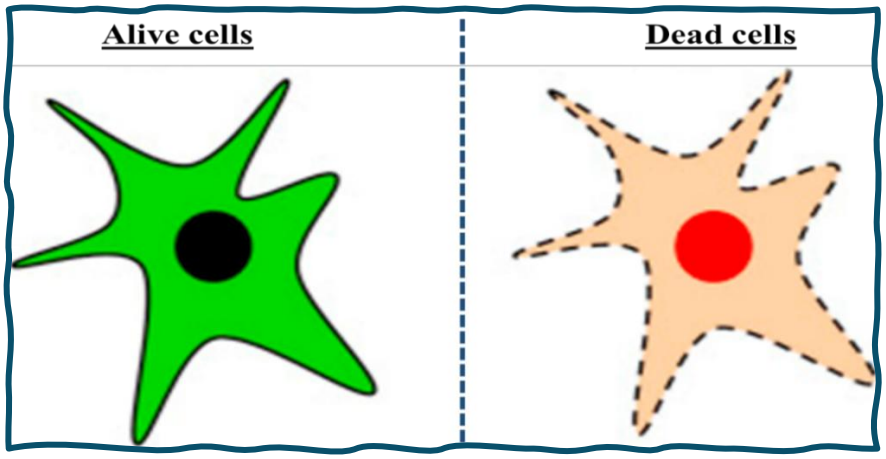


Fig.1.26. Alive and dead cells image

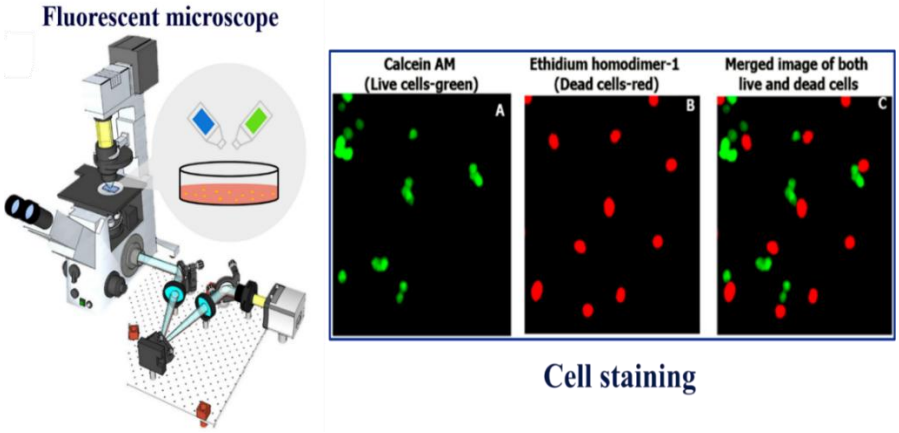


Fig.1.27. Image of cell staining using dye and fluorescent microscope

1.4.4 Gene Expression Study

Real-time PCR has proven to be a reliable method for measuring gene expression levels. The use of real-time PCR for genetic analysis and RNA quantification is increasing [78]. A more detailed discussion on gene expression studies will be presented in Chapter **4a.**

Following this introduction, we will outline the methodology utilized in the live-dead assay, scratch assay and gene expression study, present our results and discuss their significance for the advancement of new cancer therapies. Based on existing literature, we synthesized and characterized acyl pyrazolone ligands and their transition metal complexes using copper and nickel metals. Comprehensive characterizations were carried out using various spectroscopic techniques. The specifics of all synthesized compounds will be discussed in the following chapters **(Chapters 2-5).**

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