CHAPTER 1

INTRODUCTION

1.1 Pyrimidine Based Ligands and Copper Complexes

Pyrimidine **(1)** is an isomer of diazine which contains nitrogen atoms at positions 1 and 3 of the six-membered ring, having the molecular formula $C_4H_4N_2$.

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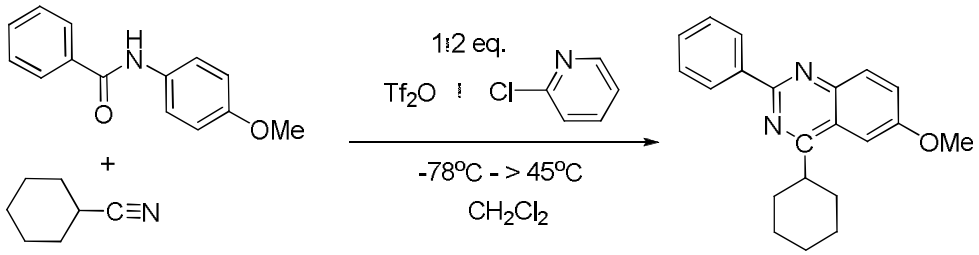
Pyrimidine is the parent heterocycle of a very important group of compounds which play a significant role in many biological systems (Saha, *et al*., 1977) and its derivatives are known as naturally occurring derivatives of the parent compound of nucleic acids and coenzymes (Gilchrist, 1997). It can also be prepared in the laboratory. A novel method of preparing pyrimidine derivatives is by reaction of certain amides with carbonitriles under electrophilic activation as shown in Scheme 1 (Movassaghi, *et al*., 2006).



Scheme 1 : Single step synthesis of pyrimidine derivatives

Some well-known pyrimidine compounds which include cytosine **(2)**, thymine **(3)** and uracil **(4)** which are present in nucleic acids, and drugs used in therapy of bacterial and viral diseases such as sulfadiazine **(5)** and thiamine (vitamin B_1) **(6)** are shown in Figure 1.1.

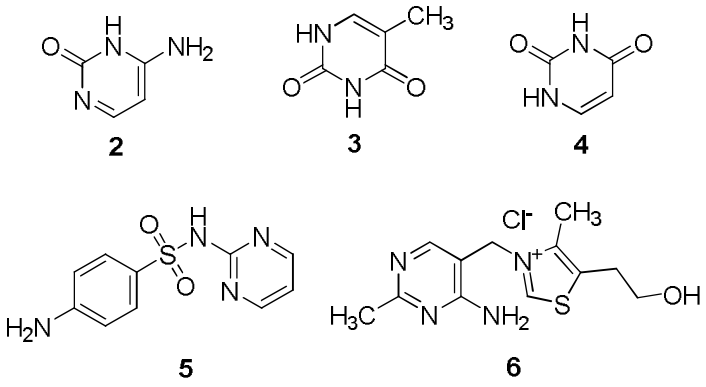


Figure 1.1 : Some well-known pyrimidine compounds

Analogues of pyrimidines are also known to act as antineoplastic agents (French, *et al*., 1966). They act as chemical mutagens, as inhibitors of nucleic acid synthesis and are consequently cancerostatic and antiviral agents. 5-Fluorouracil [5-fluoro-2,4(1H,3H)-pyrimidinedione], is an effective antineoplastic drug (Levi, *et al*., 1956, Parker, *et al*., 1990). Moreover, an analogue of pyrimidines have been reported to be used as a coronary dilator (Tenor, *et al*., 1971) and act as active herbicides for the control of broad leaf weeds in cereal crops (Jelish, *et al*., 1988, Duerr, 1991). Some pyrimidine derivatives are also associated with diverse biofunctions such as immunosuppressants and as anticonvulsant drugs (Grollman, *et al*., 1970) and has been discovered in 1935 (Birr, *et al*., 1952).

Previous studies on pyrimidine derivatives were first reported in 1909, when several 1,2,4-triazolo-[1,5-a]pyrimidine compounds were synthesized (Bulow, *et al*., 1909). Since then, other 1,2,4-triazolo-[1,5-a]pyrimidine derivatives have found use in different areas such as pharmaceutical and agricultural applications (Jelish, *et al*., 1988, Duerr, 1991). Recently, a series of 4-aminopyrimidine derivatives was identified as novel HIV inhibitors of unknown molecular target (Gadhachanda, *et al*., 2007).

It is generally accepted that a metal complex is a chemical species which contains a metal atom or ion bonded to a greater number of ions or molecules than would be expected from simple valency considerations. The ions or molecules that are bonded or coordinated with the metal are termed ligands (Houghton, 1979).

The interactions between a metal atom and the ligands can be thought of as Lewis acid-base reactions. Lewis base is a substance capable of donating one or more electron pairs. Every ligand has at least one unshared pair of valence electrons, as in pyrimidine derivatives, which have two nitrogen atoms. Therefore, ligands play the role of Lewis bases. On the other hand, a transition metal atom acts as a Lewis acid, accepting pairs of electrons from the Lewis bases (Chang, *et al*., 2005).

Transition metals have a particular tendency to form complex ions because they have more than one oxidation state. This property allows them to act effectively as Lewis acids in reactions with many molecules or ions that serve as electron donors, or as Lewis bases. The atom in a ligand that is bound directly to the metal atom is known as the donor atom. Depending on the number of donor atoms present, ligands are classified as monodentate, bidentate or polydentate (Chang, 2007).

Since pyrimidine bases are minor constituents of nucleic acids, the chemistry of pyrimidine and its compounds has been the subject of much research owing to their applications in molecular biology and medicine (Hurst, 1980). The presence of more than one heteroatom and higher ´-acidity in pyrimidine compared to that of pyridine bases play an important role in its coordination chemistry and therefore, they serve as better models in biological systems (Katritzky, *et al*., 1984, Jolibois, *et al*., 1998, Zamora, *et al*., 1997, Louloudi, *et al*., 1997). The pyrimidine ring system, present in nucleic acids, several vitamins, coenzymes and antibiotics, provides potential binding sites for metal ions such as copper(II), nickel(II), cobalt(III) and iron(III), and any information on their coordinating properties are important as a means of understanding the role of the metal ions in biological systems (Roy, *et al*., 2007).

Pyrimidine derived metal ion complexes also have been extensively studied owing to their great variety of biological activity ranging from antimalarial, antitumoral and antiviral activities, which have often been related to their chelating ability with trace metal ions (Raper, 1985, West, *et al*., 1991, West, *et al*., 1993, Casas, *et al*., 1996, Casas, *et al*., 2006). These and other recent findings have stimulated interest in pyrimidine chemistry (Constable, 1989, Lloret, *et al*., 1998).

Furthermore, mixed complexes of transition metals containing a pyrimidine group as ligands are commonly found in biological media and may have important roles in processes as catalysis of drug interaction with biomolecules (Sigel, *et al*., 1982, Rao, *et al*., 1989). It also has been suggested that the presence of metal ions in biological fluids, could have a significant effect on the therapeutic action of drugs (Kirschner, *et al*., 1966, Sornson, 1978).

The growing interest in studies of metal pyrimidine complexes has lead to investigating the bonding mode of the ligand to metal ion, as they possess divergent bonding sites. Pyrimidine has two nitrogen atoms with accessible lone pairs to bind to Lewis acids like metal ions. Furthermore, a variety of complexes of metal salts with this ligand is already known and reviewed (Salas, *et al*., 1999).

In general, pyrimidines derivatives can act as bridges between metals, but also coordinate monodentately (Albada, *et al*., 2009, Akyuz, *et al*., 2009, Balkaran, *et al*., 2009, Zhu, *et al*., 2009) and as a bridging bidentate ligand (Smith, *et al*., 1985, Smith, *et al*., 1991, Smith, *et al*., 1996, Lynch, 2000, Garcia-Raso, *et al*., 2001, Blake, *et al*., 2002).

An appreciable number of copper(II) complexes with pyrimidine derivatives are known but only a few crystal structures of these metal complexes are reported in the literature (Maldonado, *et al*., 2008). So this leads us to extend this research on selected pyrimidine derivatives and its copper(II) complexes since copper is known as one of a relatively small group of metallic elements which are essential to human health, plants and animals. These elements, along with amino and fatty acids as well as vitamins, are required for normal metabolic processes. Copper is distributed widely in the body and occurs in liver, muscle and bone. Copper metal also can be used as an anti-germ that can add to the antibacterial and antimicrobial features of buildings such as hospitals (Feder, 2008).

1.2 Fluorescence Studies

Fluorescence spectroscopy is a method widely used to study chemical sensors and its applications are rapidly expanding. Fluorescence studies offer various possibilities to study the behavior of a sensor, based upon changes in the signal intensity, the formation of excimer/exciplex compounds, energy or electron transfer processes, wavelength modification (excitation and emission) and lifetime (Pedras, *et al*., 2007). Fluorescence detection is highly sensitive and there is no longer the need for the expense and difficulties of handling radioactive tracers for most biochemical measurements (Lakowicz, 2006).

In general, fluorescence is the result of the rapid emission of light energy from a molecule which has been excited by light absorption. Moreover, molecular fluorescence spectrometry can be used for quantification of aromatic, or highly unsaturated, organic molecules present at trace concentrations, especially in biological and environmental samples. It also can be extended to a wide variety of organic and inorganic compounds via chemical labeling and derivatization procedure (Wehry, *et al*., 1966).

As with many other analytical procedures, the application of a fluorescence assay does not require knowledge of the theoretical aspects of the phenomenon. However, some information concerning the principles of fluorescence is helpful in understanding and extending its use. Fluorescence is one of several types of luminescence.

Fluorescence typically occurs from aromatic molecules and some typical fluorescent substances (fluorophores) are shown in Figure 1.2.

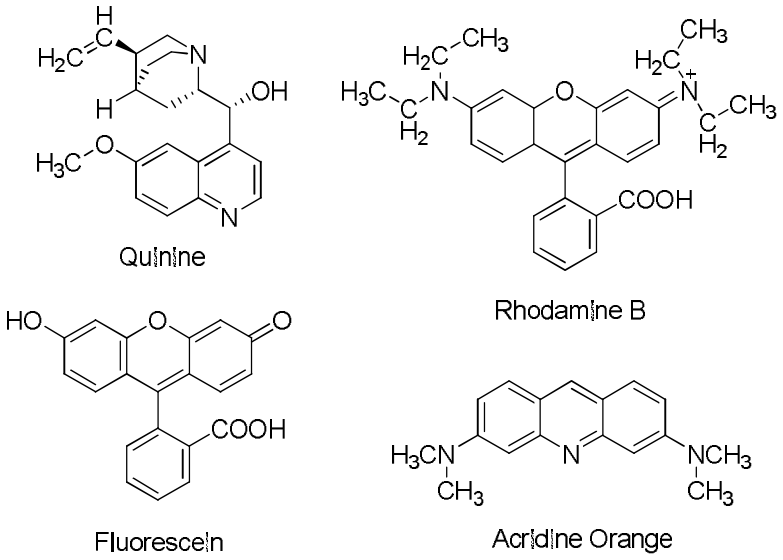


Figure 1.2 : Some typical fluorophores

Although many effective fluorescent sensors have been successfully developed for sensing alkali and alkaline earth cations (De Silva, *et al*., 1997, Valeur, *et al*., 2000, Bakirci, *et al*., 2005), there are a few examples of sensors for heavy metal ions (Meôtivier, *et al*., 2004, Matsushita, *et al*., 2005, Nolan, *et al*., 2005, Zhang, *et al*., 2005). Many heavy metal ions are known as fluorescence quenchers via enhanced spin-orbital coupling (McClure, 1952, Koike, *et al*., 1996), energy or electron transfer (Varnes, *et al*., 1972, Fabbrizzi, *et al*., 1996).

Generally, heavy metals are known to quench fluorescence. Metal ions, especially paramagnetic ions, are able to quench the fluorescence of organic ligands by enhancing the rate of same non-radiative processes that compete with fluorescence, such as

intersystem crossing. Diamagnetic non-transition metal ions are usually poor quenchers and hence form good fluorescent chelates (Provenzano, *et al*., 2004).

Although, fluorescence characteristic of nitrogen and sulfur containing heterocycles are not very well investigated, some fluorescence studies of selected 2-substituted pyrimidines (Mason, 1959), 5-substituted thiadiazolo[3,4-*d*]pyrimidines (Abdullah, 1990), 5-chlorothiazolo [5,4-*d*] pyrimidines (Abdullah, *et al*., 2004), and 6-(2-substituted-1,3,4-oxadiazol-5-yl)-2-phenylthieno[3,4-*d*]pyrimidines (Ho, *et al*., 2009), have been reported.

Earlier studies had shown that 2-fluoropyrimidine is a non-fluorescent compound (Abdullah, *et al*., 1994). When a phenoxy group is attached to a pyrimidine ring, fluorescence band was observed at 354 nm when excited at 318 nm. The nitrogen atoms in the ring favours the low lying $n \rightarrow \pi^*$ which allows phosphorescence to occur. As the result, fluorescence intensity is reduced (Abdullah, 2005).

Moreover, the effect of substituents on the fluorescence of benzene has been studied and it was found that the introduction of two nitrogen atoms into an aromatic ring system reduces fluorescence. It has been shown that the naturally occurring pyrimidine and their derivatives do not fluoresce in aqueous solution at room temperature (Xue, *et al*., 1999). Substituent with high degree of conjugation showed higher fluorescence intensity and fluoresced at a higher wavelength, whereas unconjugated substituent fluoresced at a lower wavelength (Bakar, *et al*., 2006). Introduction of polar substituents into organic chromophores causes the redistribution of electronic density in both the ground state and the excited state, which can strongly modified their absorption and fluorescence properties (Griffiths, 1976).

Previous studies also showed that the fluorescence intensity was decreased on prolong exposure to the atmosphere. The fluorescence intensity of capped sample is higher than uncapped sample. Furthermore, the fluorescence peak was also reduced with time for all compounds studied and these are believed to be due to the quenching effect of oxygen (Bakar, *et al*., 2006). Oxygen which has an unusually large diffusion coefficient could result in large quantity of oxygen diffusing into solution, and therefore quenched the fluorescence intensity of the compounds (Haroutounian, *et al*., 1988).

Investigations on the fluorescence of some heterocyclic compounds are made difficult because their fluorescence characteristics are often dependant on the nature of the solvents used and the fluorescence of crystals is especially prone to quenching even by trace amounts of impurities, because of energy transfer mechanisms (Abdullah, *et al*., 2004). In general, compounds tend to be fluorescent in polar solvents. Under these condition, the lone pair of electrons are bonded and the longest absorption wavelength is due to $\pi \rightarrow \pi^*$ transition, instead of $n \rightarrow \pi^*$. Low fluorescence intensity observed when non-polar solvents were used probably due to non-polar solvents, it cannot formed a hydrogen-bonded complex with the solute (Abdullah, *et al*., 2004).

Solvent polarity and the local environment also have profound effects on the emission spectral properties of fluorophores. Abdullah, *et al*., (2004) had studied the fluorescence characteristic of 5-chlorothiazolo [5,4-*d*] pyrimidines in various solvents and it showed that the higher fluorescence intensity were recorded in ethanol and methanol as compared to diethyl ether and other non-polar solvents was probably due to the formation of complex between 5-chlorothiazolo [5,4-*d*] pyrimidines and the solvents. This complex is probably formed through the non-bonding electron of the solute to the hydrogen atom of the solvent, forming a stable hydrogen bonded complex.

Another study for selected 2-alkylaminopyrimidine had shown the highest fluorescence peak in methanol followed by ethanol and the fluorescence intensity decreased markedly in non-polar solvent (Abdullah, *et al*., 2004).

Solvent effects shift the emission to still lower energy due to stabilization of the excited state by the polar solvent molecules. In general, only fluorophores that are themselves polar display a large sensitivity to solvent polarity. Non-polar molecules, such as aromatic hydrocarbons, are much less sensitive to solvent polarity. The theory for general solvent effects is often inadequate for explaining the detailed behavior of fluorophores in a variety of environments. This is because fluorophores often display multiple interactions with their local environment, which can shift the spectra by amounts comparable to general solvent effects (Lakowicz, 2006).

1.3 Objective of Studies

The objective of this research is to synthesize selected pyrimidine based ligands and to prepare the copper complexes of the ligands synthesized. The fluorescence-structure relationship of these pyrimidine derivatives and their copper complexes will be studied with respect to the influence of substituents in ligands, influence of solvents, quenching effect by oxygen and to study the effect on fluorescence by complexation with copper.