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SYNTHESIS, STRUCTURE AND *IN VITRO* ANTICANCER STUDIES OF DINUCLEAR SILVER(I)-*N*-HETEROCYCLIC CARBENE COMPLEXES DERIVED FROM XYLYL LINKED *BIS*-BENZIMIDAZOLIUM SALTS

By

MUHAMMAD ADNAN IQBAL

Thesis submitted in fulfillment of the requirements

for the degree of

Doctor of Philosophy

January 2014

DEDICATION

I dedicate this thesis to:

My beloved brother ***Engineer Burhan Iqbal*** *in the honor of his unconditional financial and moral support with legitimate guidance throughout my career that has now brought me to this stage of success and prosperity.*

"Thanks a lot my brother"

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

NHC	*N*-heterocyclic carbene
Ar	Arene
DMSO	Dimethyl sulfoxide
THF	Tetrahydrofuran
DCM	Dichloromethane
OAc	Acetate
h	Hour(s)
Anal.	Analysis
Calc.	Calculated
^{n}J	Nuclear spin-spin coupling constant through bonds
Å	Angstrom
h	Crystallographic index
α	Crystallographic unit-cell angle between axes *b* and *c*
β	Crystallographic unit-cell angle between axes *a* and *c*
a	Crystallographic unit cell axis *a*
b	Crystallographic unit cell axis *b*
c	Crystallographic unit cell axis *c*
HIFBS	Heat inactivated foetal bovine serum
PBS	Phosphate buffer saline
PS	Penicillin/streptomycin
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
µl	Microliter
OD	Optical density
DPPH	2,2-diphenyl-1-picrylhydrazyl
PMA	Phorbol 12-myristate 13-acetate
LPS	Lipopolysachharide
ELISA	Enzyme linked immunosorbant assay
IL-1	Interleukin-1
TNF-α	Tumor necrosis factor-α
COX	Cyclooxygenase
IC_{50}	Concentration of test substance to achieve 50% inhibition

RPMI	Roswell Park Memorial Institute (media was name on the name of developer, More et al., at Roswell Park Memorial Institute)
Mes	Mesityl
dppe	1,2-Bis(diphenylphosphino)ethane
RT	Room temperature
ORTEP	Oak Ridge Thermal Ellipsoid Plot
cod	1,5-cyclooctadiene
qnt	quintet
br.m	broad multiplet

SINTESIS, STRUKTUR DAN KAJIAN *IN VITRO* ANTIKANSER KOMPLEKS DINUKLEAR ARGENTUM *N*-HETEROSIKLIK KARBENA DIPEROLEHI DARI KAITAN XYLYL GARAM *BIS*-BENZIMIDAZOLIUM

ABSTRAK

Kajian ini menerangkan tentang sintesis tiga siri garam bis-benzimidazolium sebagai prekusor *N*-heterosiklik karbena (NHC) dan kompleks mereka dengan ion argentum(I) untuk mendapatkan komplek argentum(I)-NHC. Setiap siri garam adalah terbitan samada daripada *para-, meta-*, atau *orto*-xilin sistem berangkai yang mempunyai gantian etil-desil, benzil dan *i*-propil di kedudukan 3 pada cincin benzimidazolium. Kesemua garam dan kompleks telah dicirikan oleh spektroskopi (FT-IR, ^{1}H and ^{13}C NMR), analisis unsur (CHN) dan teknik pembelauan sinar-X kristal tunggal. Semua kompleks telah disediakan melalui tindakbalas *in situ* diantara Ag_2O dengan garam bis-benzimidazolium yang sepadan dan telah diuji dengan sel kanser kolon manusia. Bagaimana pun hanya garam dan kompleks terpilih telah diuji selanjutnya dengan promielositik akut leukemia dan sel leukemia mielogenus abadi. Garam dan kompleks ini menunjukkan potensi aktiviti antikanser menentang kesemua sel kanser yang diuji. Walaubagaimana pun, kompleks terbukti mempunya sitotoksiksiti lebih tinggi berbanding dengan garamnya. Selanjutnya, aktiviti anti-radang bagi *N*-heksil gantian *para*-xylyl berangkai garam bis-benzimidazolium dan kompleksnya telah diuji. Memandangkan kanser dan keradangan adalah berkait antara satu sama lain, maka kita mencadangkan bahawa dadah yang mempunyai potensi antikanser mungkin juga mempunyai potensi terhadap anti-radang. Menariknya, kedua-dua sebatian ini terbukti mempunyai aktiviti anti-radang. Selain itu, tindakan mekanisma telah diterokai dan didapati sebatian inhibitor

siklooksigenase-1 dan siklooksigenase-2 serta sitokin (interleukin-1, α-faktor nekrosis tumor, and nitrik oksida) adalah penghalang. Dalam semua kes didapati kompleks mempunyai hasil yang lebih baik dan berganda berbanding dengan garam masing-masing.

SYNTHESIS, STRUCTURE AND *IN VITRO* ANTICANCER STUDIES OF DINUCLEAR SILVER(I)-*N*-HETEROCYCLIC CARBENE COMPLEXES DERIVED FROM XYLYL LINKED *BIS*-BENZIMIDAZOLIUM SALTS

ABSTRACT

The current study was aimed to synthesize three series of *bis*-benzimidazolium salts (**12**-**44**) as stable *N*-heterocyclic carbene (NHC) precursors and their complexation with silver(I) ions in order to obtain dinuclear silver(I)-NHC complexes (**45**-**77**). Each series of salts was derived either from *para*-, *meta*-, or *ortho*-xylene linked systems having ethyl-decyl, benzyl and *i*-propyl substituents at number 3-position of benzimidazolium ring. The salts and complexes were characterized by spectroscopy (FT-IR, ^{1}H and ^{13}C NMR), elemental analysis (CHN) and single crystal X-ray diffraction techniques. All the complexes were prepared by *in situ* reaction of Ag_2O with the corresponding bis-benzimidazolium salts and were tested against human colon cancer cells. Selected salts (**12**-**14**, **18**-**20**, **21**) and respective complexes (**45**-**47**, **29**-**31**, **54**) were further tested against acute promyelocytic leukaemia and immortalized myelogenous leukaemia cells. The compounds showed potential anticancer activity against all the tested cancer cell lines. Moreover, complexes exhibited higher cytotoxicity compared to respective salts. The anticancer potential of compounds increased with the increase in chain length at position 3-nitrogen. Furthermore, considering the triangular relationship among cancer, inflammation and oxidation, selected compounds were further tested for possible anti-oxidant and anti-inflammatory activities. The tested compounds did not show anti-oxidant behaviour however, proved to have anti-inflammatory activity comparable to the standards used. Additionally, the anti-inflammatory mechanism of action was explored and the compounds were found cyclooxygenase-1 and

cyclooxygenase-2 inhibitors as well as cytokines (interleukin-1, tumor necrosis factor-α, and nitric oxide) blockers. In all cases the complexes were found to have many fold better results compared to the respective salts concluding that silver imparts an important role against cancer and inflammation.

CHAPTER ONE

INTRODUCTION

1.1 The *N*-heterocyclic carbenes

N-heterocyclic carbenes (NHCs) are versatile class of ligands which have various types based on ring size, starting from the carbenes derived from four membered *N*-heterocycles and extend upto seven membered *N*-heterocycles (Figure 1.1) (Hahn and Jahnke, 2008), among which perhaps five membered NHCs are the most widely studied carbenes (Bates et al., 2009).

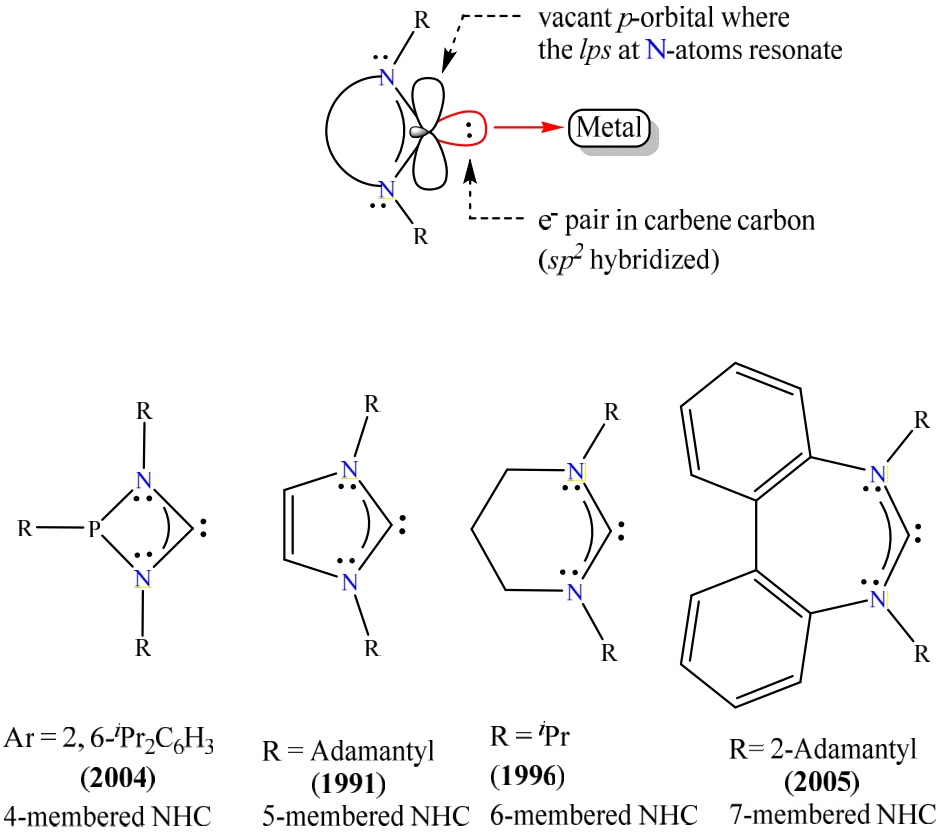


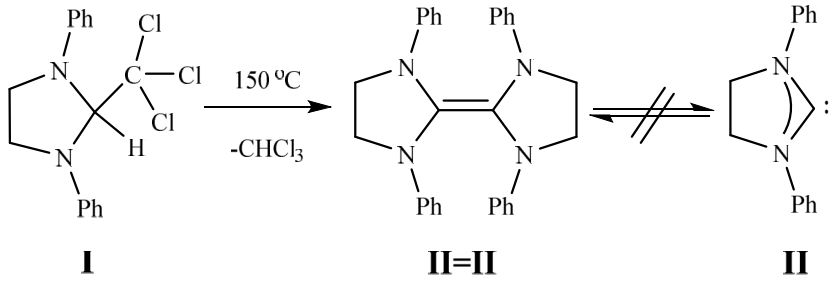
Figure 1.1: Types of NHCs based on ring size with year of discovery. Orbital view of electron pair in a sp^2-hybridized carbon with vacant *p*-orbital and π-donor N-atoms.

In these carbenes a divalent carbon moiety is flanked by two π-donor nitrogen atoms. The strong σ-donating and week π-accepting properties of NHCs have

rendered them as excellent ligands not only for d-block elements (Arnold and Pearson, 2007) but also for f-block elements (Arnold and Casely, 2009a; Arnold and Casely, 2009b; Evans, 2007; Evans et al., 1981).

1.2.1 Discovery of NHCs (Background)

Discussion about NHCs was initiated by Wanzlick in 1960 by his report on the α-elimination of chloroform from **I** to get free NHC **II** (Wanzlick and Schikora, 1960). However, Wanzlick could never isolate **II** and always obtained its dimer **II=II** (Hahn and Jahnke, 2008) (Figure 1.2). Wanzlick also tried the cleavage of dimeric entatraamine according to **II=II** → 2 × **IV** using cross-metathesis method but failed (Lemal et al., 1964; Winberg et al., 1965).

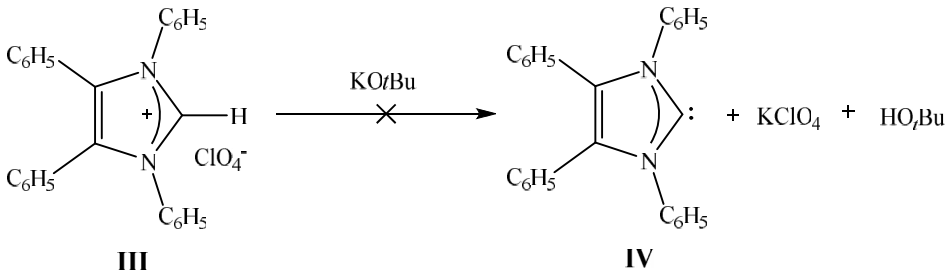


I **II=II** **II**

Figure 1.2: Formation of dimeric entatraamine **II=II** instead of free NHC **II**.

Around 1970, Wanzlick decided to change the starting material for the generation of free NHC. He tried to prepare the free carbene **IV** by deprotonation of 1,3,4,5-tetraphenylimidazolium perchlorate **III** with KO*t*Bu (Schönherr and Wanzlick, 1970) (Figure 1.3), because at that time it was already known that azolium cations react in presence of base-catalyzed medium (Fild et al., 1964; Olofson et al., 1964; Staab et al., 1964). The free carbene again could not be isolated. However, generation of free NHC **IV** by this method was detected as the intermediate product

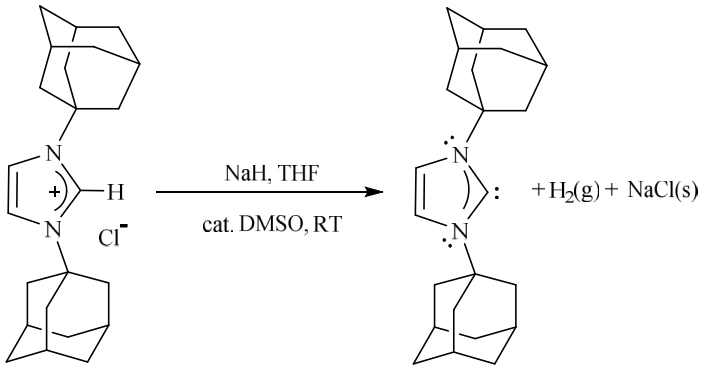
from the identification of some of its reaction products with water or mercuric acetate (Schönherr and Wanzlick, 1970).



III IV

Figure 1.3: Attempt to synthesize free carbene **IV** from azolium salt **III**.

Up to this point, a concept had already developed that a NHC can be synthesized but only as an intermediate and can't be isolated as a stable product for further laboratory or commercial use. However, Arduengo believed that the intermediate carbene must not be as unstable as was being assumed by scientific community (Arduengo and Krafczyk, 1998). Finally in 1991, Arduengo and co-workers synthesized and isolated the first crystallographically elucidated NHC **VI** from an azolium salt **V** in presence of sodium hydride and catalytic amount of DMSO using THF as reaction medium (Figure 1.4) (Arduengo et al., 1991). This carbene was found to have a unique stability at room temperature in the absence of oxygen and moisture. Later on, in 1998 Arduengo et al., also prepared, isolated, and crystallographically characterized carbene **IV** (Figure 1.3) that Wanzlick could not isolate in 1968 (Arduengo III et al., 1998).



V **VI**

Figure 1.4: Arduengo's first stable *N*-heterocyclic carbene **VI**.

The discovery of first stable carbene **VI** led to significant interest in the field of carbene chemistry. To date, different methods for the syntheses of NHCs have been reported, which include the desulfuration of imidazole-2(3H)-thiones (Kuhn and Kratz, 1993) and methanol elimination by thermolysis of 5-methoxy-1,3,4-triphenyl-4,5-dihydro-1H-1,2,4-triazoles (Figure 1.5) (Enders et al., 1995; Hahn and Jahnke, 2008).

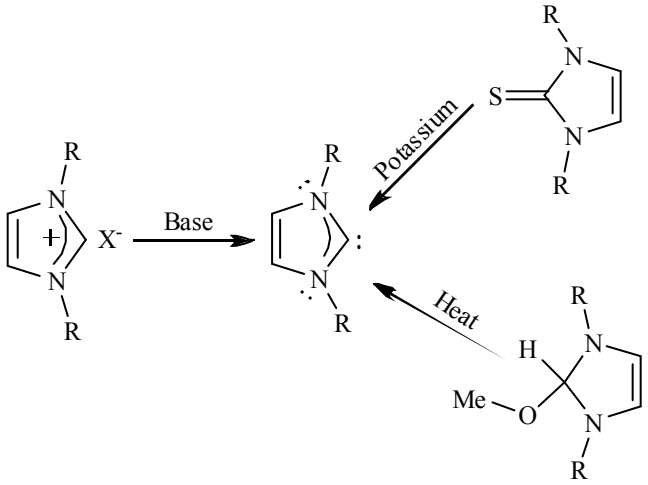


Figure 1.5: The reported methodologies for syntheses of the free NHCs.

1.2.2 The stability of NHCs

Initially, many researchers thought that the unique stability of the NHC, synthesized by Arduengo, was due to the bulky *N*-adamantyl substituents which prevent the dimerisation of the carbene due to steric hindrance. However, Arduengo's syntheses of another stable carbene with *N*-methyl substituents proved otherwise (Arduengo et al., 1992). Arduengo justified that the electronic contributions are the main stabilizing factors which involve electron donation from the adjacent nitrogen atoms to the vacant *p*(π) orbital of the carbene carbon (Figure 1.6).

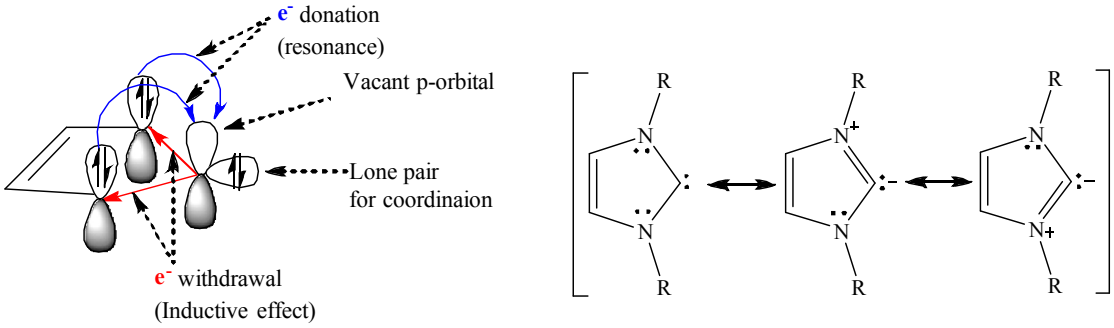


Figure 1.6: Orbital (**a**) and resonance (**b**) representations of electronic stabilization in imidazole-2-ylidenes.

Hence the role of *N*-substituents is unique, for example the π-donor substituents increase *p*π character of the singlet carbene (see Figure 1.7) by transferring π electrons to the empty *p*-orbitals of the carbene carbon (Bourissou et al., 1999) and consequently, the bulky substituents on the nitrogen atoms contribute to the stability of the carbenes (Bourissou et al., 1999; Hahn et al., 2000). Furthermore, due to higher electronegativity of nitrogen as compare to carbon atom,

charge density is considered to be inductively withdrawn through the σ-framework, stabilizing the carbene lone pair (Arduengo et al., 1992).

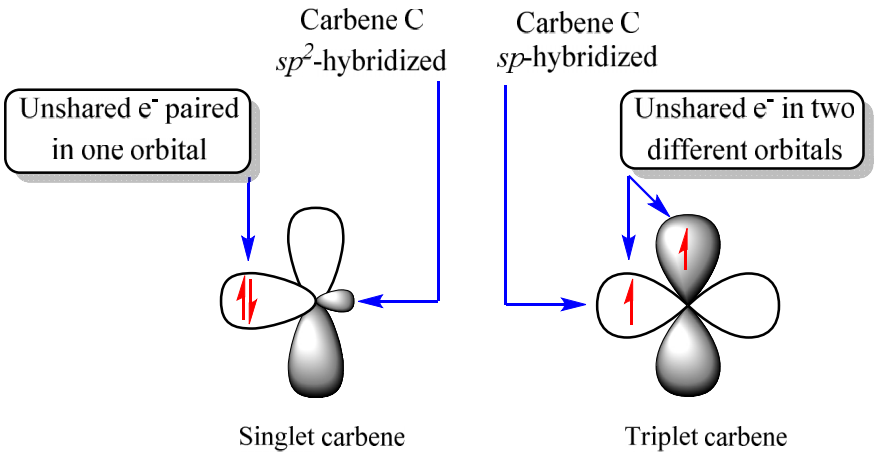


Figure 1.7: The orbital representation of singlet and triplet carbenes. In singlet carbenes unshared electrons are present in the same orbital whereas in triplet carbenes these electrons are present in two different orbitals.

1.3 Syntheses of azolium salts: the NHC precursors

Azolium salts are generally synthesized by two common synthetic routes (Weskamp et al., 2000):

1.3.1 Nucleophilic substitution starting at the azole heterocycle.

In this route, azole (imidazole, benzimidazole, triazole etc) heterocycle is first reacted with a strong base (eg., KOH, NaOH) to get potassium or sodium azolide that is subsequently reacted with one equivalent of alkyl or aryl halide in appropriate solvent to collect the 1-alkyl or 1-aryl azole (Herrmann, 2002; Starikova et al., 2003b). 1-substituted azole is then reacted with one equivalent of alkyl or aryl halide of interest at position 3-nitrogen (Figure 1.8). This method allows the syntheses of unsymmetrical imidazolium salts by stepwise alkylation.

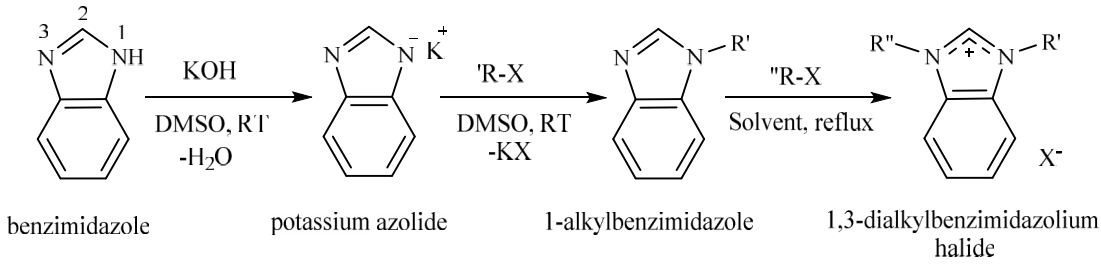


Figure 1.8: General representation of syntheses of benzimidazolium salts by neucleophilic substitution.

1.3.2 Multi-components reaction, building up the heterocycle with the appropriate substituents in one step.

In this route, primary amines, glyoxal, and formaldehyde are reacted in the presence of Brønsted acid as one pot reaction (Figure 1.9) (Böhm et al., 2000; Herrmann et al., 1996).

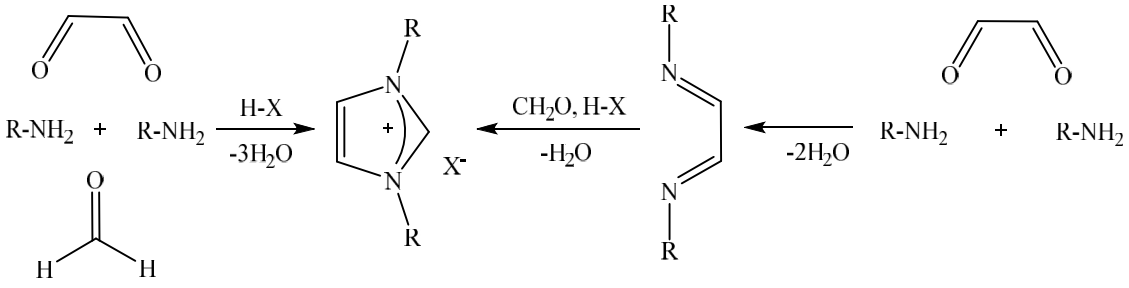


Figure 1.9: General representation of syntheses of imidazolium salts by multi-components reaction.

This flexible route is suitable for the syntheses of symmetrical 1,3-substituted azolium salts. However, unsymmetrical substituted azolium salts can also be synthesized by combining a multicomponent cyclization with *N*-alkylation reaction (Gridnev and Mihaltseva, 1994). According to this method, an initial cyclization at pH 1 yields an *N*-alkylazolium salt that is subsequently alkylated at second nitrogen atom in presence of base to give the asymmetrically substituted derivative (Figure 1.10).

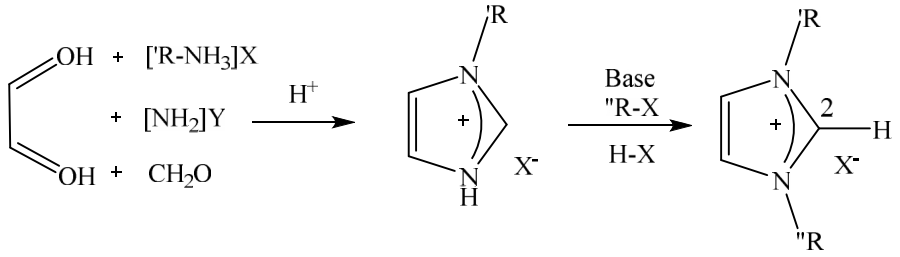


Figure 1.10: General representation of multicomponent cyclization and *N*-alkylation.

The 1,3-alkyl/aryl-substitutions facilitate the lone pairs at nitrogen atoms to resonate on NCN framework. This phenomenon makes position 2-hydrogen acidic to be easily removed by a suitable base (Figure 1.10). In the current study, syntheses of *bis*-benzimidazolium salts were carried out according to the route 1.3.1 (page 6) with minor modifications.

1.4 Syntheses of NHC complexes (Discovery of M-NHC complexes)

Although, the first stable *N*-heterocyclic carbene was only isolated in 1991, its complexation with metals (Cr and Hg) was already achieved 22 years earlier by Öfele and Wanzlick (Öfele, 1968; Wanzlick and Schönherr, 1968) independently.

Öfele and co-workers were actually trying to synthesize some dihydro complexes by heating the hydropentacarbonyl chromium heterocyclic salt. They found that there is an unusual reaction when imidazolium salts used, which lead to the formation of an *N*-heterocyclic carbene chromium complex shown in Figure 1.11.

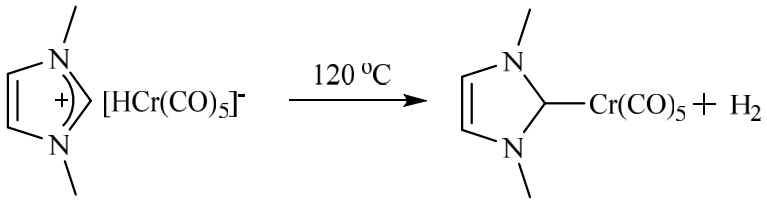


Figure 1.11: Öfele's chromium-NHC complex.

At the same time, Wanzlick and Schönherr synthesized a mercury(II)-NHC complex by a direct reaction between 1,3-diphenylimidazolium perchlorate and mercury(II) acetate (Figure 1.12) in DMSO. In this reaction, the acetate ions play the main role that is the *in situ* deprotonation of the imidazolium salt and release of the acetic acid to form the complex. Later on, the metal acetate route was adopted as one of the general routes for the syntheses of several transition metal-NHC complexes.

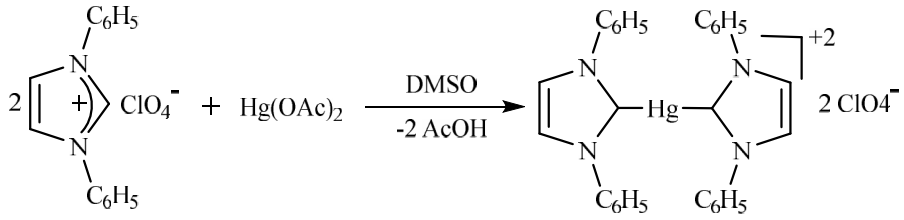


Figure 1.12: Wanzlick's mercury(II)-NHC complex.

Since the discovery of Öfele's Cr(III)-NHC and Wanzlick's Hg(II)-NHC complexes that , NHCs have been widely synthesized and used in organometallic and inorganic chemistry. The first comprehensive review about the synthetic methods of free NHCs and their coordination chemistry was compiled by Herrmann and Köcher about 15 years ago (Herrmann and Köcher, 1997) and the latest one by Hahn and Jahnke about 5 years ago (Hahn and Jahnke, 2008) which additionally describes the types of NHCs. Catalytic applications of this class have been reviewed by Herrmann (Herrmann, 2002), Crudden (Crudden and Allen, 2004), and Glorius (Glorius, 2007) whereas biological applications by K-Nebioglu (Kascatan-Nebioglu et al., 2007), Garrison (Garrison and Youngs, 2005), and recently by Teyssot (Teyssot et al., 2009a). Several methods have been explored for the preparation of NHC complexes (Enders and Gielen, 2001; Peris and Crabtree, 2003; Weskamp et al., 2000). To date, NHCs have been incorporated to almost all the transition metals of periodic table

using different synthetic routes (Arnold and Pearson, 2007; Evans, 2007). A brief review by Weskamp describes various synthetic routes for bonding transition metals to NHCs (Weskamp et al., 2000).

1.5 Syntheses of silver(I)-NHC complexes

Syntheses of silver(I)-NHC complexes have been reported mainly by four routes: **(1.5.1)** generation of free carbene from azolium salt and subsequent reaction with a silver salt, (Arduengo et al., 1993; Caballero et al., 2001a; Chung, 2002; Fox et al., 2002), **(1.5.2)** *In situ* reaction of azolium salts with basic silver reagents, (Arnold, 2002; Chianese et al., 2004; Danopoulos et al., 2003; Guerret et al., 2000; Guerret et al., 1997; Herrmann et al., 2004; Hu et al., 2004; Mayr et al., 2004; Melaiye et al., 2005; Tulloch et al., 2000; Wanniarachchi et al., 2004), **(1.5.3)** *in situ* reaction of azolium salts with a base in presence of silver salt, (Wang and Lin, 1998), **(1.5.4)** transmetallation from a tungsten NHC to silver (Ku et al., 1999).

1.5.1 Free carbene route

The first silver(I) *N*-heterocyclic carbene complex was synthesized by this route in 1993 (Figure 1.13) (Arduengo et al., 1993). According to this method, deprotonation of azolium salts was accomplished by a strong base (like KH or KO*t*Bu) and free NHC was subsequently reacted with the silver source. Following Arduengo, several silver(I)-NHC complexes have been reported using this method (Caballero et al., 2001a; Caballero et al., 2001b; Chung, 2002; Fox et al., 2002). Currently, this method is not popular due to strict reaction conditions and decomposition of some specific azolium salts (Caballero et al., 2001a; Caballero et

al., 2001b; César et al., 2002; Chen et al., 2002; Guerret et al., 2000; McGuinness and Cavell, 2000; Wang et al., 2004).

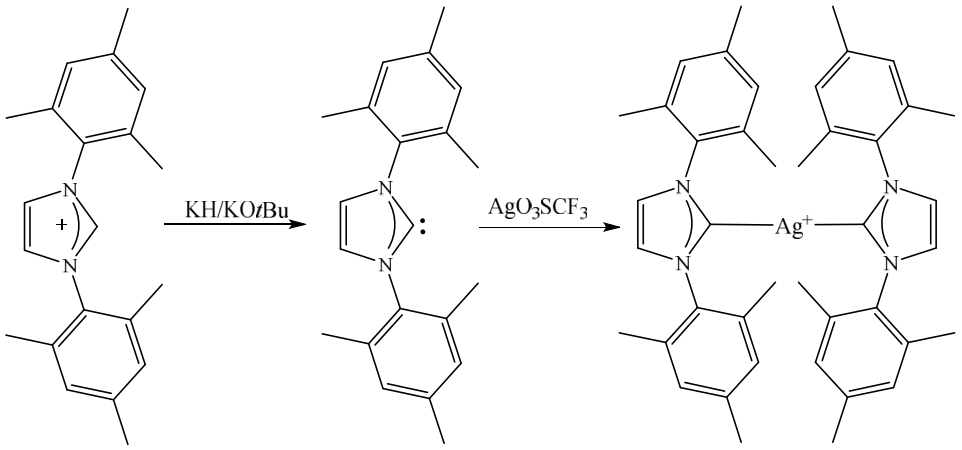
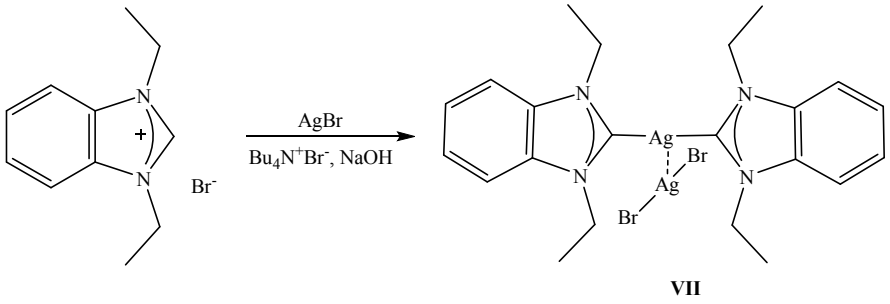


Figure 1.13: Syntheses of first silver *N*-heterocyclic carbene complex (1993).

1.5.2 Base in the presence of a silver salt

This method was first reported by Wang and Lin in 1998 (Wang and Lin, 1998). These researchers used a basic phase transfer catalyst to synthesize **VII** from benzimidazolium bromide in presence of AgBr (Figure 1.14). However, this procedure was proved unsuccessful for other imidazolium salts (Tulloch et al., 2000).



VII

Figure 1.14: Wang and Lin's method of base in presence of silver salt.

1.5.3 Transmetallation to silver(I)-NHC complexes

This method was proposed by Liu and co-workers in 1998 (Liu et al., 1998). However, transmetallation from tungsten(0) *N*-heterocyclic carbene complexes to silver was achieved in 1999 (Ku et al., 1999; Liu and Reddy, 1999). Although the claimed compounds could neither be isolated nor structurally characterized, however spectroscopic evidences indicated the successful transformations. This method was largely abandoned because silver NHC complexes generated from this method were found to be sensitive to moisture, which is not the characteristic of silver NHC complexes. Later on, transmetallation from other metal centers (Mo(0), Cr(0), Rh(I), Pd(II), Pt(II)) was suggested but sufficient spectroscopic or structural evidences couldn't be generated to substantiate this assertion (Chen et al., 2001; Liu and Reddy, 1999).

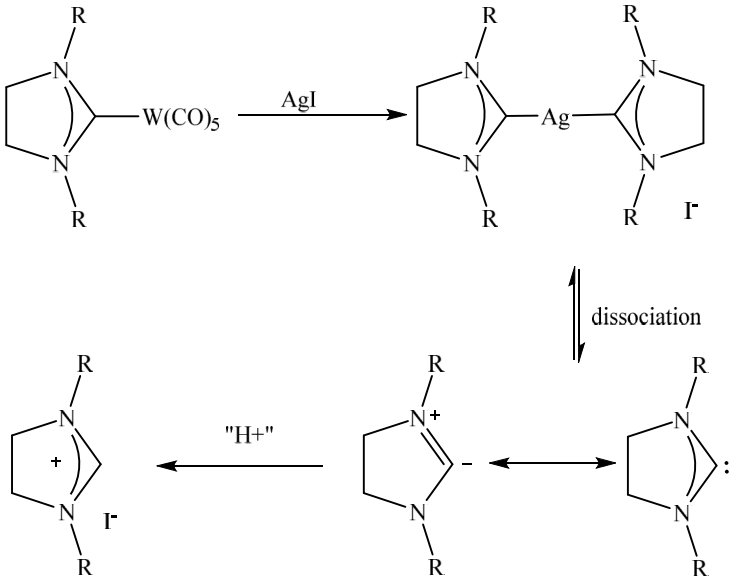


Figure 1.15: Transmetallation from tungsten(0) NHC to Ag and dissociation of silver complexes.

1.5.4 "Silver Base" route

This is the most widely used method for the syntheses of silver-NHC complexes. In this method deprotonation and coordination phenomenon occurs spontaneously in a single step.

This method was first reported by Wang and Lin in 1998 using Ag_2O (Figure 1.16) (Wang and Lin, 1998). A detailed mechanism for these reactions has been described by Hayes and co-workers (Hayes et al., 2007).

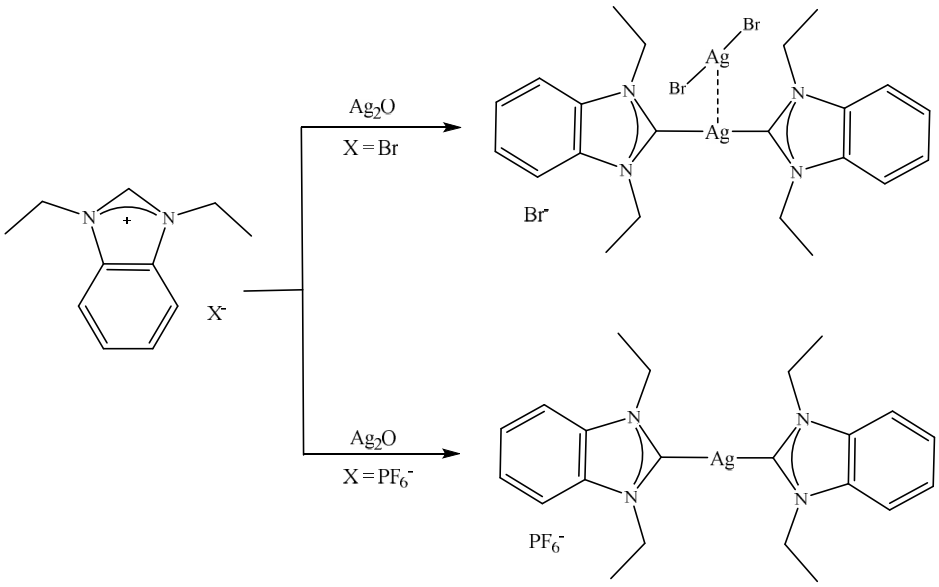
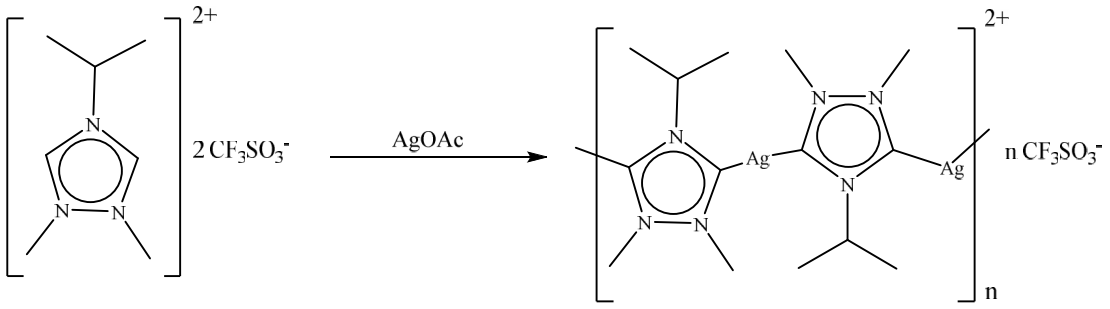


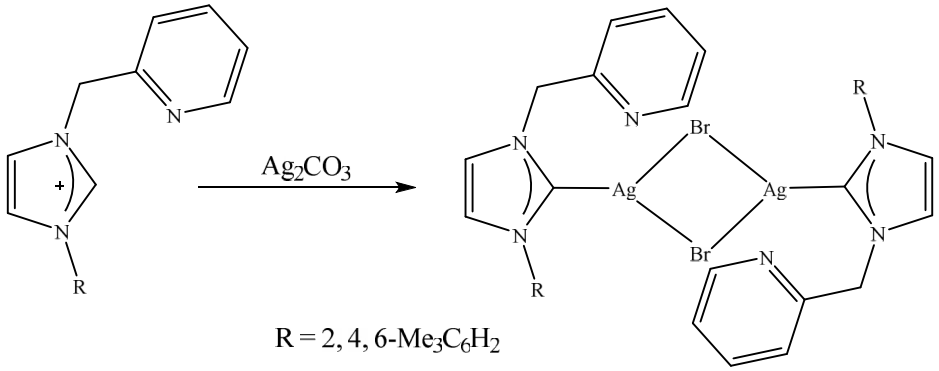
Figure 1.16: Syntheses of silver *N*-heterocyclic carbene complexes using silver oxide.

Other silver bases such as AgOAc and Ag_2CO_3 have also been reported successfully (Figure 1.17). For example, the use of AgOAc was first introduced by Bertrand and colleagues by reporting the first silver NHC polymer (Scheme 1.15a) (Guerret et al., 2000; Guerret et al., 1997). Tulloch et al., reported a series of silver NHC complexes of structure shown in Scheme 1.15b by using Ag_2CO_3 as metallation agent (Tulloch et al., 2000). The use of Ag_2CO_3 was also successfully employed by

others (Van Veldhuizen et al., 2002). However, reaction durations using this reagent were reported to be longer than reaction times using Ag_2O (Tulloch et al., 2000).



(**a**)



(**b**)

Figure 1.17: (**a**) Syntheses of silver-NHC polymer using AgOAc and (**b**) Syntheses of bridging complexes using Ag_2CO_3.

Literature shows that in this route the most frequently used "metal base" is silver oxide (Baker et al., 2005; Bonnet et al., 2003; Catalano et al., 2004; Chianese et al., 2004; Chiu et al., 2005; De Frémont et al., 2005; Frøseth et al., 2005; Herrmann et al., 2004; Hu et al., 2004; Lee et al., 2004; Liu et al., 2003; Mata et al., 2004; Mayr et al., 2004; Melaiye et al., 2004; Quezada et al., 2004; Ramírez et al., 2005; Ramnial et al., 2003; Sentman et al., 2005; Simons et al., 2003; Tulloch et al., 2000; Wang and Lin, 1998; Wanniarachchi et al., 2004) because the reactions can be easily monitored by the uptake of unreacted silver oxide and found to be faster than using other bases.

The reactions of silver oxide with azolium salts have been carried out successfully in solvents, such as dichloromethane, 1,2-dichloromethane, DMSO, acetone, methanol, acetonitrile, DMF, and water. Hence, in present study "Silver Base" route was adopted with minor modifications.

1.6 Applications of silver(I)-NHC complexes

1.6.1 Ligand transfer chemistry

Many NHC complexes are synthesized directly from the azolium salts by deprotonation and subsequently reacting with the desired metals. However, many ligands fail to metalate directly. Many researchers used silver(I)-NHC complexes for the purpose of transferring metal center to the other metals of interest. Transmetallation reactions using silver(I)-NHC can be carried out under aerobic conditions and even in the presence of moisture. To the date, transmetallation using silver(I)-NHC complexes has been successfully achieved for a variety of metals: Au(I), Cu(I), Cu(II), Ni(II), Pd(II), Pt(II), Rh(I), Rh(III), Ir(I), Ir(III), Ru(II), Ru(III), and Ru (IV). The metal to which silver(I)-NHC complexes are most widely transferred is perhaps palladium, where a variety of Pd reagents have been used for transmetallation: $Pd(cod)Cl_2$, (César et al., 2002; Danopoulos et al., 2003; Frøseth et al., 2003; Magill et al., 2001; Tulloch et al., 2003) $Pd(cod)Br_2$, (Tulloch et al., 2003) $Pd(cod)CH_3Cl$, (McGuinness and Cavell, 2000; Neilson et al., 2012; Tulloch et al., 2003) $PdCl_2$, (Chiu et al., 2005; Lee et al., 2004; Lee et al., 2005) $PdCl_2(PhCN)_2$ (Simons et al., 2003).

1.6.2 Catalysis

Silver(I)-NHC complexes have also been studied for their catalytic potential. However, compared to the other M-NHC complexes (M = Pd, Ru, Ni, Ir, Rh) (Herrmann, 2002) very few studies have been reported for silver(I)-NHC complexes. Ramirez and co-workers were the first to study the catalytic activity of silver(I)-NHC complexes (Ramírez et al., 2005). They reported the catalytic use of silver complexes for diboration reactions with terminal and internal alkenes. Sentman et al., and Bonnet et al., studied catalytic activity of silver(I)-NHC complexes for ring opening polymerization of L-lactide (Bonnet et al., 2004; Sentman et al., 2004). This area is not that widespread for this class of compounds.

1.6.3 Biological

1.6.3.1 Silver a medicinally important metal

Silver is a medicinally valuable metal. Early civilizations used this metal to purify and store water (Russell and Hugo, 1994). Silver nitrate was frequently used as antimicrobial long before 18th century and it was well recognized as an antiseptic in wound care for more than 200 years (Lansdown, 2004). In the 19th century, another useful medicinal application of silver compounds was explored i.e., at very low concentrations, silver compounds were found to kill certain microorganisms (Russell and Hugo, 1994; V. Von Nageli, 1893). In 1881, Créde introduced the use of 1% silver nitrate solution to prevent the eye infections in newborns (Lansdown, 2004). The method is still in use today. The use of colloidal silver solutions to avoid the irritation associated with silver nitrate was introduced in early 20th century (Gibbs, 1999; Lansdown, 2002b). This method remained valid until 1940s. The silver compounds gradually lost interest following the discovery of penicillin and few other

new antibiotics (Lansdown, 2004). Then in 1965, the use of 0.5% silver nitrate solution for the treatment of burn wounds by Moyer (Moyer et al., 1965) re-established the medicinal interest of silver. Moreover in 1968, the discovery of silver sulfadiazine was the true revival of silver antibiotics (Fox, 1968).

In the 21st century, organic and inorganic silver compounds have been introduced in wound dressings (Graham, 2005; Tomaselli, 2006). Silver has been utilized in several different kinds of wound dressings including nylon fabrics, meshes, biodegradable colognes, low adherent materials, carbon fibers, and hydrofiber alginates (Graham, 2005; Lansdown, 2004). These silver containing dressings have been used in the treatment of acute and chronic wounds, leg ulcers and several degrees of burn wounds (Graham, 2005).

The pure silver metal is inactive; whereas, in the presence of moisture, silver readily ionizes to generate silver cations, which then show antimicrobial activity (Lansdown, 2002a; Lansdown, 2004). Furthermore, the activity of silver cations in living systems depend on their bioavailability (Silver, 2003) that is further dependent on delivery methods, solubility, ionization of silver sources and the presence of biologically compatible ligands (Melaiye et al., 2004; Silver, 2003).

1.6.3.2 Benzimidazole: a medicinally important heterocyclic moiety

Benzimidazole is an aromatic organic compound, composed of benzene ring fused with imidazole (Figure 1.18). Its empirical formula and molecular weight is $C_7H_6N_2$ and 118.14 g/mol respectively. Benzimidazole is a medicinally important heterocyclic moiety. For example, *N*-ribosyl-dimethylbenzimidazole is a naturally occurring derivative of benzimidazole which serves as an axial ligand for cobalt in vitamin B_{12} (Barker et al., 1960). This makes benzimidazole a biologically accepted pharmacophore in the field of medicinal chemistry as its derivatives are structural isosters of naturally occurring nucleotides, which makes them compatible with the biopolymers of the living systems (Narasimhan et al., 2012).

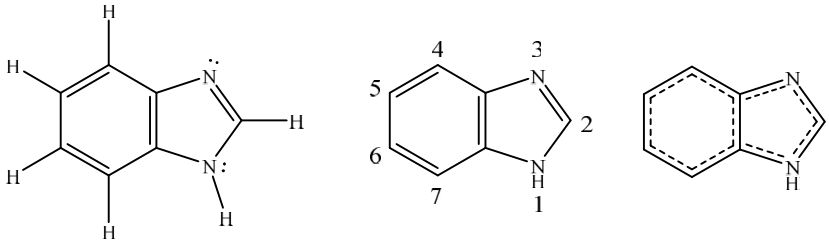
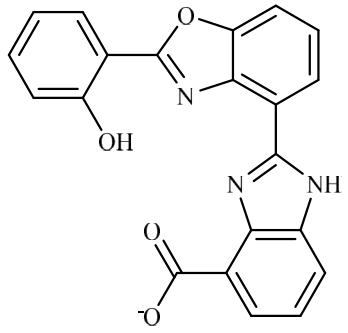


Figure 1.18: Representation of benzimidazole moiety and numbering.

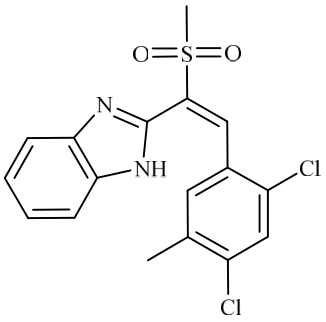
This biological compatibility of benzimidazole has created interest in researchers to synthesize its organic derivatives and to screen them for biological activities. To the date, a number of benzimidazole derivatives have been successfully synthesized and tested as protein kinase CK2 inhibitors (Andrzejewska et al., 2003; Pagano et al., 2004), tyrosine kinase inhibitors (Zien et al., 2003), and topisomerase inhibitors (McBride et al., 2006; Pinar et al., 2004). Benzimidazole was also found active against a wide variety of pathogenic viruses, for example benzimidazoles have shown good results as antiviral (De Clercq and Naesens, 2006; Starcevic et al., 2007),

antihepatitic (Ishida et al., 2006; Li et al., 2007), anti-HIV (Middleton et al., 2004), antienteroviral (Ramla et al., 2007a), anti-RSV (Andries et al., 2003), antibacterial (Goker et al., 2005; He et al., 2003; Kumar et al., 2002), antifungal (Goker et al., 2002; Kawasaki et al., 2003), antianthelmintic (Mavrova et al., 2006), antiamoebic (Lopez-Vallejo et al., 2007; Sondhi et al., 2002), antiprotozoal (Andrzejewska et al., 2004; Ismail et al., 2004), and antimycobacterial (Klimesova et al., 2002) agents. Benzimidazoles have also been tested for diabetes (Minoura et al., 2004), hypertension (Estrada-Soto et al., 2006), inflammation (Mader et al., 2008; Snow et al., 2007) and allergy (Nakano et al., 2000).

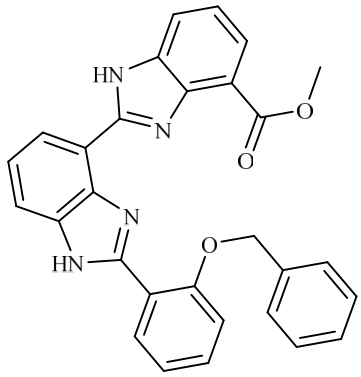
In the last decade, very few benzimidazole derivatives were tested against cancer. For example, compound **VIII**, as shown in Figure 1.19, was tested against MCF-7 (breast cancer), HL-60 (Leukemia), HT-29 (Human colon carcinoma), and PC-3 (Prostate cancer) whereas **IX** against HT-29 only (Kumar et al., 2002; Vedula et al., 2003). Compound **X** was tested against A-549 (Human lungs carcinoma), BFTC-905 (Bladder carcinoma), RD (Human embryo rhabdomyosarcoma), MES-SA (Human sarcoma carcinoma), and HeLa (Cervical cancer) cancer cell lines showed better results than UK-1 analogues (Huang et al., 2006). Compound **XI** was tested against Epstein-Barr virus early antigen (EBV-EA) and was found potentially active (Ramla et al., 2007b).



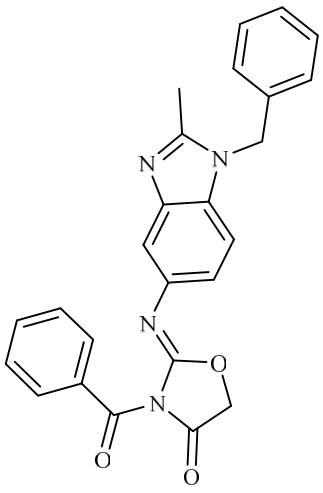
VIII



IX



X



XI

Figure 1.19: Benzimidazole derivatives tested against various types of cancer.

However, on the basis of literature, *it was found that benzimidazolium salts have never been tested against any type of cancer. This research also fills up this area of gap.*

1.6.3.3 Cancer and metal-based drugs

Cancer is the uncontrolled growth of abnormal cells in a living system that initially induces tumor (Figure 1.20). If not treated properly, a tumor invades in the nearby parts of the body and hampers the normal functions of other cells. This

process continues which leads to a number of ailments and in some cases death. A detailed introduction of cancer is beyond the scope of this thesis.

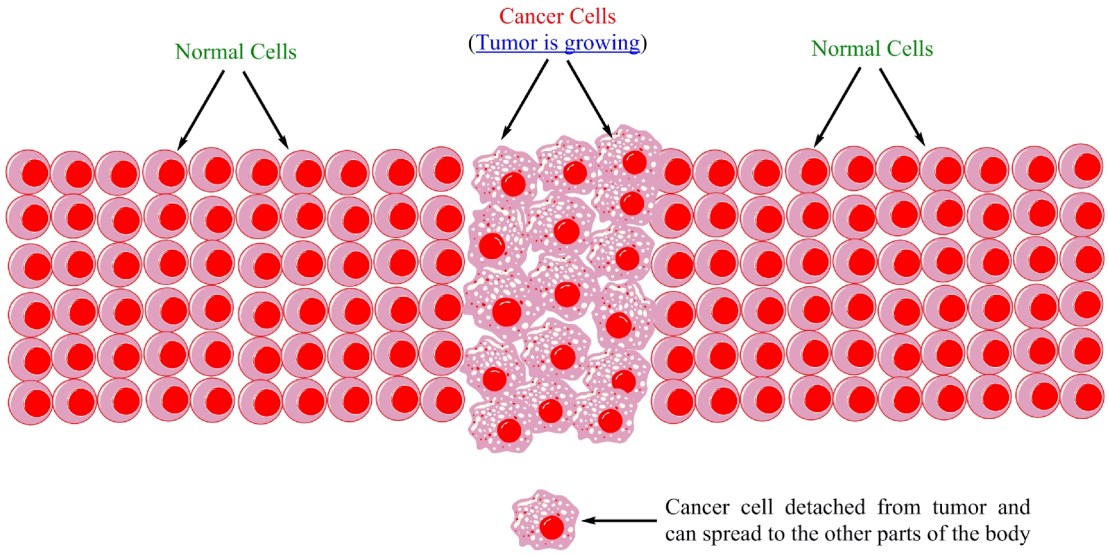


Figure 1.20: Diagram showing malignant tumor.

Beside the fact that cancer is curable at initial stages, it has however become one of the most fatal diseases as the mortality rate due to cancer is increasing worldwide. According to the global cancer statistic report, in 2002, 10.9 million new cancer cases were registered all around the world and 6.7 million patients died, whereas within the next five years (2008) this number increased to 12.6 million new cases and 7.5 million deaths. A rapid development in anti-cancer drug discovery has become substantial (Parkin et al., 2005).

Different therapeutic options like chemotherapy, radiotherapy and surgery are now extensively used to cure different types of cancer. In chemotherapy, the exploration of cisplatin (Figure 1.21) as an anticancer agent by Rosenberg and co-workers is perhaps the first historical example of metal-based anticancer drugs

(Rosenberg et al., 1965; Rosenberg et al., 1967; Rosenberg et al., 1969). However, the severe side effects (nephrotoxicity, neurotoxicity, and ototoxicity) confined its applications. In addition, it's poor solubility in water, chemical incompatibility to thiols, and natural or developed resistance of some tumours towards cisplatin further decreased its medicinal worth (Lippert, 1999). However, discovery of cisplatin provided an opportunity to further explore the metal-based anticancer drugs. Later on, a number of cisplatin analogous compounds were synthesized and biologically evaluated but only few of them proved to have pharmacological advantages over cisplatin and are being used worldwide for tumor therapy (Figure 1.21) (Wheate et al., 2010).

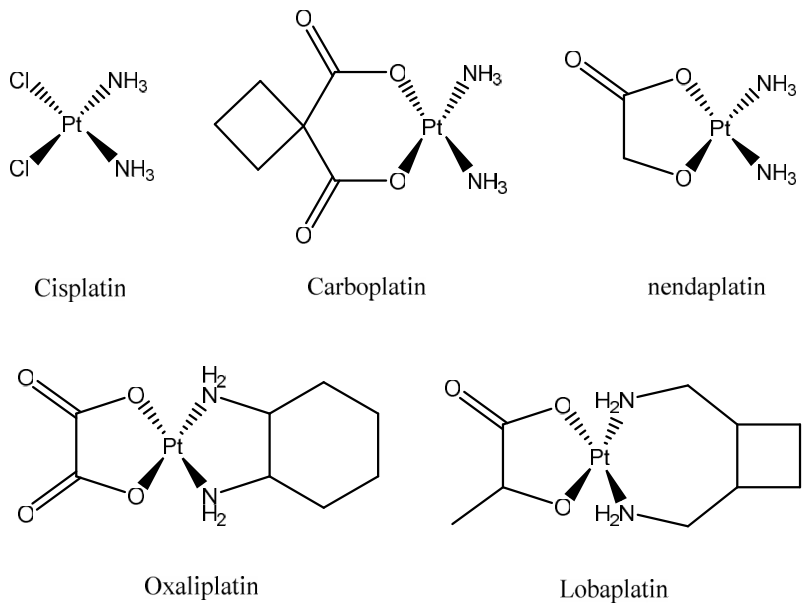


Figure 1.21: Worldwide approved platinum-based anticancer drugs.

Platinum based drugs are now so frequently used that only oxaliplatin is expected to cross 2 billion Euros business in the next two years (Berners-Price, 2011). This drug was recently approved to be used as a second line therapy in metastatic colorectal cancer and showed minor side affects so far which can be

treated symptomatically. In addition to platinum, a wide range of transition-metal drugs have been at various stages of development (Alessio, 2011; Gasser et al., 2011; Hannon, 2007).

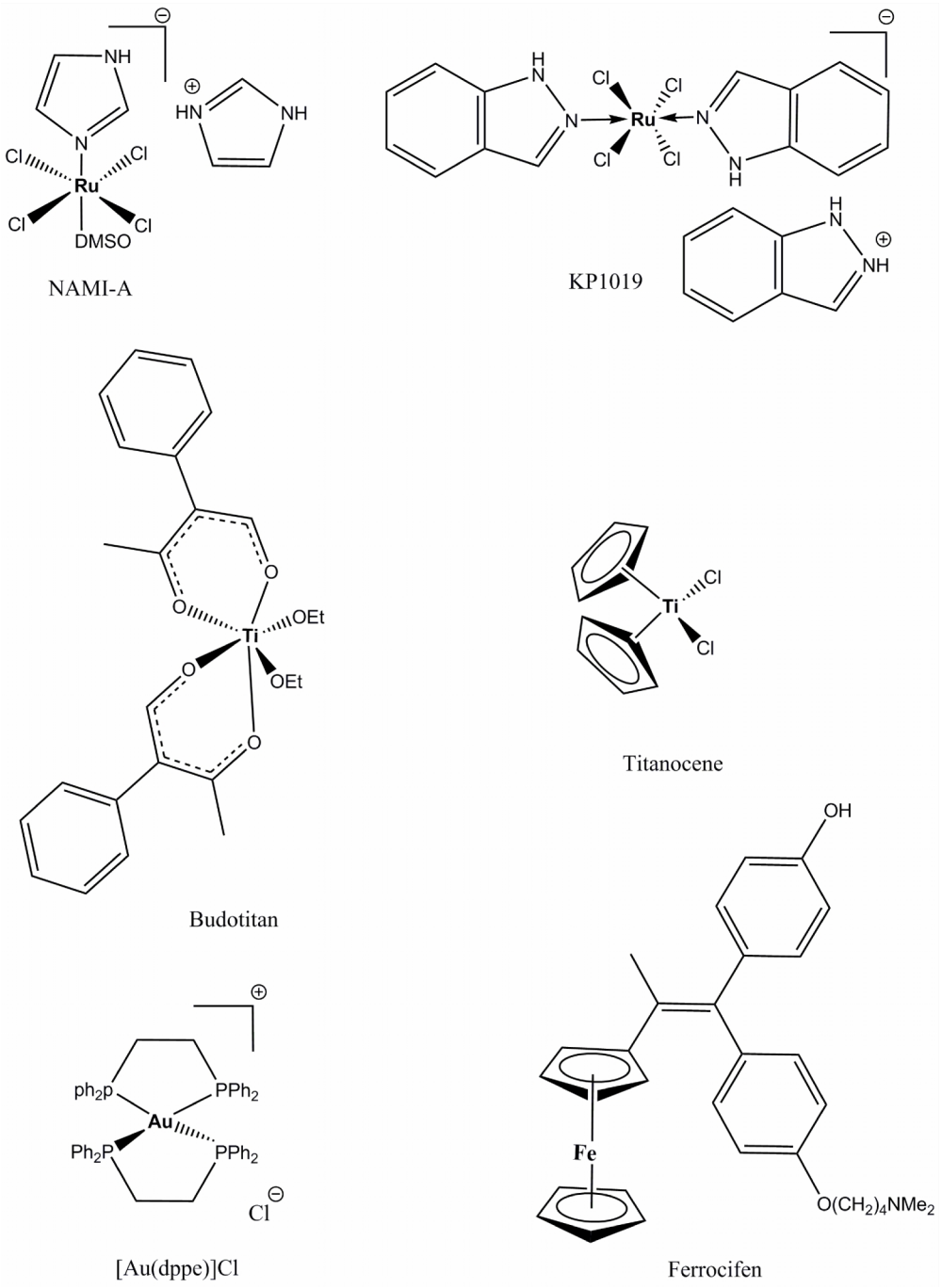


Figure 1.22: Examples of some transition metal complexes having potential anticancer properties.

For example (Figure 1.22), two ruthenium based drugs, NAMI-A and KP1019 have been in preclinical and clinical phase I and II (Hartinger et al., 2008; Rademaker-Lakhai et al., 2004); two titanium based drugs, Budotitane and Titatocene have also been reported in phase I and II respectively (Abeysinghe and Harding, 2007); an iron based drug, Ferrocifen is in its preclinical formulation studies (Vessières et al., 2005); a gold complex, $[Au(dppe)]^{+}$ was subjected to preclinical studies in late 1986. However, none of these could pass all the stages of clinical development until today.

Recently, metal-NHCs appeared as a rapidly growing field of research in medicinal chemistry. This is also evident from several recent research reports, reviews and patents (Chardon et al., 2012; Eloy et al., 2012; Gasser and Metzler-Nolte, 2012; Gautier and Cisnetti, 2012; Haque et al., 2013; Liu and Gust, 2013; Medvetz et al., 2008a; Monteiro et al., 2012; Oehninger et al., 2013; Teyssot et al., 2009a; Wang et al., 2011).

1.6.3.4 Silver(I)-NHC complexes as potential anti-cancer metallodrugs

Silver(I)-NHC complexes have been widely tested for their antimicrobial activities (Kascatan-Nebioglu et al., 2007). However, they were rarely studied for their anticancer activity (Teyssot et al., 2009a), until recently.

In this regard, Youngs, Tacke and Gautier's research teams are at the forefront. Youngs recently reported anticancer properties of a series of Ag(I)-NHC complexes of structures NHC-Ag-OAc and NHC-Ag-NHC on various cell lines (Medvetz et al., 2008b; Siciliano et al., 2011). These complexes were found to be efficient on OVCAR-3 (ovarian cancer) and MB157 (breast cancer) cells whereas no