SYNTHESIS, STRUCTURE AND ANTIMICROBIAL PROPERTIES OF NEW SILVER(I)- AND PALLADIUM(II)-*N*-HETEROCYCLIC CARBENE COMPLEXES DERIVED FROM [BENZ]IMIDAZOL-2-YLIDENES

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UNIVERSITI SAINS MALAYSIA
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By

PATRICK O. ASEKUNOWO

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to

- *The memory of my father*.
- *My late sister who passed on a love of reading and respect for education*.
- *My loving mother*.
- *My lovely wife and children*.

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LIST OF ABBREVIATIONS

NHC	*N*-heterocyclic carbene
[Benz]imidazole	Imidazole and benzimidazole
Benzimi	Benzimidazolium
Ar	Arene
Aliph	Aliphatic
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethylsulfoxide
DCM	Dichloromethane
THF	Tetrahydrofuran
NaH	Sodium hydride
h	Hour
MIC	Minimum inhibitory concentration
5FU	5-Fluorouracil
Anal.	Analysis
Calc.	Calculated
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*

NaOAc	Sodium acetate
Et_3N	Triethyl amine
t-BuOk	Potassium tertiary butoxide
COD	1,5-Cyclooctadiene
KHMDS	Potassium hexamethyldisilazide
NMR	Nuclear magnetic resonance spectroscopy
XRD	X-ray diffraction
OD	Optical density
ORTEP	Oak Ridge Thermal Ellipsoid Plot
Mes	Mesityl

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Attended occupational Safety and Health Course; Universiti Sains Malaysia. Penang, Malaysia, February 2013.

SINTESIS, STRUKTUR DAN CIRI ANTIMIKROB KOMPLEKS ARGENTUM(I)- DAN PALLADIUM(II)-*N*-HETEROSIKLIK KARBENA BARU TERBITAN [BENZ]IMIDAZOL-2-ILIDENA

ABSTRAK

Tesis ini melaporkan sintesis, struktur dan ciri antimikrob pelbagai kompleks argentum (I)- dan palladium(II)-NHC mengandungi ligan [benz]imidazolin-2-ilidena. Hasil penemuan penyelidikan ini dibentangkan dalam empat bab. Dua jenis garam [benz]imidazolium dengan gantian yang berlainan telah disediakan. Jenis yang pertama ialah garam mono-[benz]imidazolium (**1-15**) yang berteraskan eter, nitril dan alil. Kesemua garam mudah diubah dengan menggunakan gantian akil/akil aril pada posisi ketiga pada gelang [benz]imidazolium. Manakala jenis yang kedua ialah garam bis-[benz]imidazolium (**16-25**) terbitan sistem terangkai propil, piridina dan para-xilena. Kompleks disediakan melalui tindakbalas *in situ* Ag_2O dengan garam [benz]imidazolium sepadan secara mempelbagaikan nisbah molar logam kepada garam, menghasilkan dua puluh lima kompleks Ag(I)-NHC (**26-50**) yang baru. Struktur krystal sinaran-X bagi lima belas kompleks telah ditentukan dan menunjukkan variasi dalam corak stuktur merentangi siri dengan spesies mononuklear dan dinuklear. Garam dan kompleks telah dicirikan dengan kaedah spektroskopi (FTIR, ^{1}H dan ^{13}C NMR), analisis unsur (CHN) dan kaedah pembelauan sinaran-X kristal tunggal. Kesan daripada gantian yang berbeza pada kompleks ke atas aktiviti antibakteria telah diuji. Efikasi antibakteria bagi sebatian telah diperiksa dengan menggunakan *Staphylococcus aureus* (ATCC 12600) sebagai bakteria Gram positif dan *Escherichia coli* (ATCC 25922) sebagai bakteria Gram negatif. Kesemua kompleks Ag(I)-NHC, secara umumnya, menunjukkan aktiviti antibakteria yang baik dengan nilai dalam lingkungan 10.0±0.5-32.9±2 mm untuk zon perencatan tumbuhan bakteria dan nilai MIC untuk kompleks adalah dalam

lingkungan 6.25-100 μg/ mL. Walau bagaimanapun, garam berkaitan adalah tidak aktif pada dasarnya bagi kedua-dua stren bakteria. Adalah diperhatikan bahawa terbitan kompleks Ag(I)-NHC dengan rantaian akil yang lebih panjang bersifat lebih bioaktif. Seterusnya keputusan juga menyokong bahawa kompleks Ag(I)-NHC dinuklear mempunyai potensi antibakteria yang lebih baik daripada seiring mononuklear mereka. Untuk memperolehi pandangan tempoh awal pada mod tindakan sebenar mereka, plasmid pekeliling pTS414 DNA/RNA telah didedahkan pada gel elektroforesis dan didapati Ag(I)-NHC mononuklear (**31, 34, 37,** dan **38**) dan Ag(I)-NHC dinuklear (**41, 44, 45, 46** dan **48**) adalah sangat efisien dalam menggalakkan rekahan/degradasi RNA dan DNA tanpa kehadiran zat tindakbalas iaitu dalam ketidakhadiran H_2O_2 dan reduktan tambahan.

Sintesis CNC bersepadu kompleks Pd(II)-NHC telah dicapaikan melalui tindakbalas pentranslogaman (pemindahan NHC) antara Pd(cod)Cl_2 dengan kompleks Ag(I)-NHC terbitan garam bis-[benz]imidazolium yang berkenaan. Kajian sinaran-X menunjukkan struktur mononuklear untuk tiga kompleks (**52, 54** dan **55**) dalam keadaan pepejal. Kompleks Pd(II)-NHC yang disediakan telah diuji dengan E. coli dan S. aureus. Kompleks yang diuji menunjukkan aktiviti pada bakteria yang disebut, tetapi lebih rendah dibandingkan dengan kompleks Ag(I)-NHC. Keputusan daripada aktiviti nuklease tempoh awal menunjukkan kompleks Pd(II)-NHC (**51**, **52**, **54** and **55**) adalah efisien dalam rekahan asid nukleik melalui mekanisme tak teroksidasi.

SYNTHESIS, STRUCTURE AND ANTIMICROBIAL PROPERTIES OF NEW SILVER(I)- AND PALLADIUM(II)-*N*-HETEROCYCLIC CARBENE COMPLEXES DERIVED FROM [BENZ]IMIDAZOL-2-YLIDENES

ABSTRACT

This thesis reports the synthesis, structure and antimicrobial properties of various silver(I)- and palladium(II)-NHC complexes bearing [benz]imidazolin-2-ylidene ligands. The findings of the research are presented in four chapters. Two types of [benz]imidazolium salts with different substituents were prepared. The first type is mono-[benz]imidazolium salts (**1**-**15**) with ether, nitrile and allyl functionalities. They are conveniently tuned, using different alkyl/alkyl aryl substituents at the 3-position of the [benz]imidazolium ring. While the second type is bis-[benz]imidazolium salts (**16**-**25**) derived from propyl, pyridine and para-xylene linked systems. The complexes were prepared by *in situ* reaction of Ag_2O with the corresponding [benz]imidazolium salts by varying the metal to salt molar ratio, resulting in the isolation of twenty five new Ag(I)-NHC complexes (**26**-**50**). X-ray crystal structures for fifteen of these complexes were determined showing a variation in the structural motifs across the series with mononuclear and binuclear species being generated. The salts and their complexes were characterized by spectroscopic methods (FTIR, ^{1}H and ^{13}C NMR), elemental analysis (CHN) and single crystal X-ray diffraction techniques. The effect of substitutions on antibacterial activity of these compounds has been investigated. Compounds were screened for their antibacterial efficacy against *Staphylococcus aureus* (ATCC 12600) as a Gram positive bacterium and *Escherichia coli* (ATCC 25922) as a Gram negative bacterium. All the Ag(I)-NHC complexes, in general, showed good antibacterial activities in the range 10.0±0.5-32.9±2 mm for the zone of bacterial growth inhibition and the MIC values of the complexes are in the range of 6.25-100 μg/ mL.

However, their corresponding salts were essentially inactive against both strains of bacteria. It was observed that the derivatives of the Ag(I)-NHC complexes with longer alkyl chain were more bioactive. Furthermore the results also suggest that binuclear Ag(I)-NHC complexes have relatively better antibacterial potential compared with their mononuclear counterparts. In order to gain preliminary insights into their actual mode of action(s), circular plasmid pTS414 DNA/RNA was exposed to gel electrophoresis and it was found that the mononuclear Ag(I)-NHC (**31, 34, 37** and **38**) and binuclear Ag(I)-NHC (**41**, **44**, **45**, **46** and **48**) are extremely efficient in promoting the cleavage/degradation of RNA and DNA in the absence of co-reactants i.e., in the absence of H_2O_2 and added reductant.

The synthesis of CNC pincer Pd(II)-NHC complexes has been achieved by the transmetallation (NHC transfer) reaction between $Pd(cod)Cl_2$ and the corresponding Ag(I)-NHC complexes derived from bis-[benz]imidazolium salts. X-ray studies revealed mononuclear structures for three of the complexes (**52**, **54** and **55**) in solid state. The prepared Pd(II)-NHC complexes were tested against the *E. coli* and *S. aureus*. The examined complexes showed an activity against the mentioned bacteria, but much lower than that of the Ag(I)-NHC complexes. The results of the preliminary nuclease activities demonstrate that Pd(II)-NHC (**51**, **52**, **54** and **55**) complexes are efficient in the cleavage of nucleic acids *via* non-oxidative mechanism.

CHAPTER ONE

INTRODUCTION

1.1 *N*-Heterocyclic Carbenes (NHCs)

NHCs are electron rich σ-donor ligands with zero formal charge on the carbene carbon.[1] Generally, NHCs possess the carbene carbon located at the 2-position between the two nitrogen substituents (Figure 1.1 a). They are singlet carbenes which are electronically and sterically stabilized. It is the inductive (-I) and the mesomeric (+M) push-pull effect that provides most of the stabilizing energy.[2,3,4] However, by changing the type of azole ring electronic properties can be adapted with the following order of electron donating power benzimidazole < imidazole < imidazoline (Figure 1.1 b).[5]

NHCs have been found as efficient substitutes to phosphine ligands due to many advantages as ancillary ligands.[6] The availability of sterically impeded functional group attached to the nitrogen atoms promotes reductive elimination of the product from the metal. The influence of the metal-carbene bond of the NHC complex facilitates ligand dissociation and they are also better σ-donors than phosphine ligands. Lastly the carbene carbon containing the unfilled orbitals is normally part of the heterocycle (azole ring system), therefore the properties of the NHCs can be finely tuned by the addition of electron donating substituents on the nitrogen atoms, affording them exceptional coordinating power and superior multidentate ligands.[7,8]

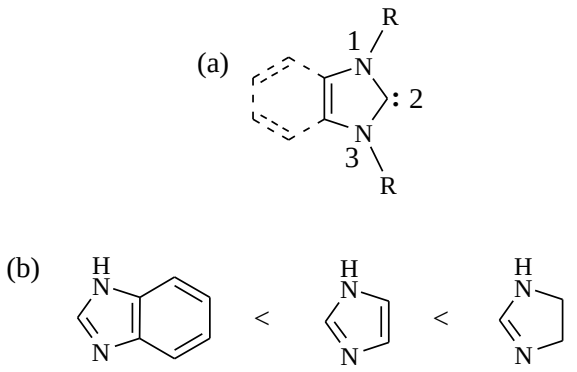
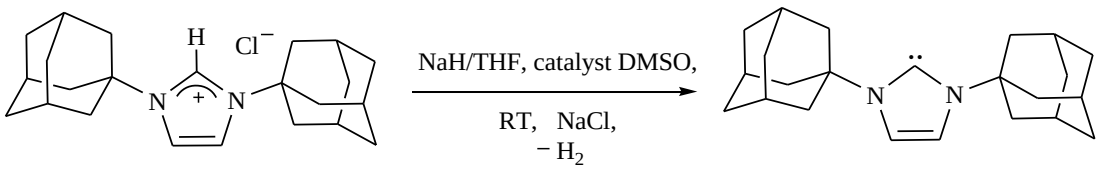


Figure 1.1: (a) Position of the carbene carbon (b) Order of electron donating power.

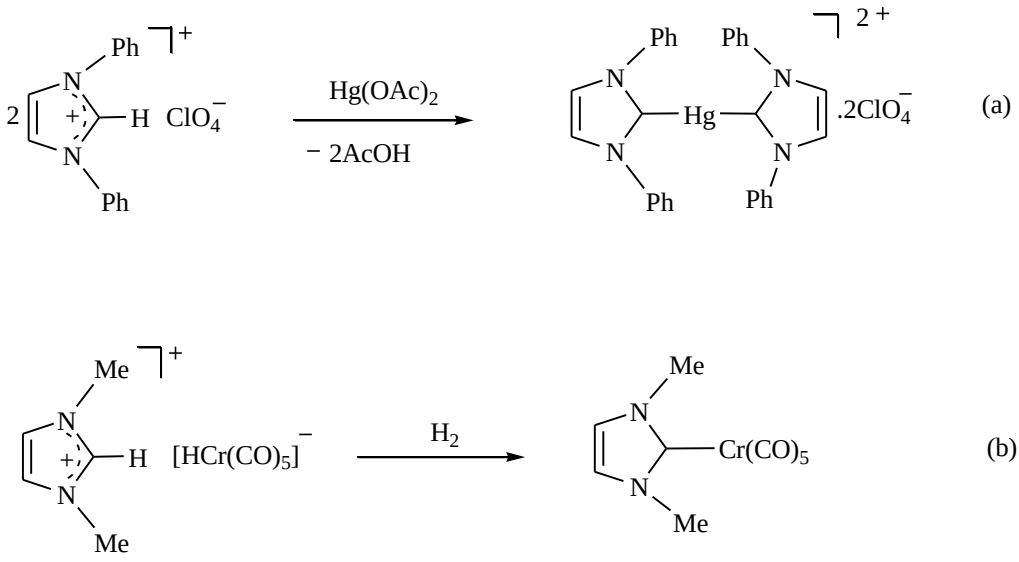
1.2 Background

Carbenes have been known as an exclusive type of intermediate, as far back as 1954 according to the earlier work by Doering.[9] After the introduction of carbenes by Doering to organic chemistry[9] and ten years later by Fischer and Maasbol to organometallic chemistry,[10] they have attracted the interest of synthetic[11] and organometallic[12] chemists exceedingly. However it was not until Arduengo isolated and characterised the first stable NHC in 1991 (Scheme 1.1)[13] that the interest of the scientific community was revived.[14-16] This discovery led to the use of NHC in the development of carbene system. After this, different methods for the isolation of heterocyclic carbenes have been reported by a number of research groups.[17,18,19,20]



Scheme 1.1: Preparation of the first isolated NHC.

NHCs have been known due to earlier work done in 1968 by Wanzlick and Ofele who were able to use them to form mercury-salt and chromate carbene complexes.[21,22] The mercury complex was formed from the metal acetate, while Ofele used carbonyl metalate for the chromate complex (Scheme 1.2). The carbenes intermediate were claimed to have been prepared by *in situ* technique from corresponding imidazolium salts.[22]

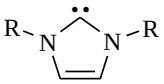


Scheme 1.2: Formation of NHC-metal complexes by (a) Wanzlick and (b) Öfele.[22]

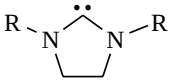
1.3 Structural diversities and stability

NHCs possess several variations in their basic structures (Figure 1.2). Examples of these are imidazol-2-ylidene **I**, imidazolidin-2-ylidene **II**, a five-membered 1, 2, 4-triazolin-(3, 5)-ylidene **IV** with the presence of carbenes at the 3 and 5-positions, thereby enhancing the possibility of binding to one or more metal centres.[23] Furthermore there is six membered tetrahydropyrimid-2-ylidine **V**[24] and several other heterocyclic carbenes have already been reported, such as four-

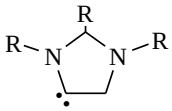
membered rings,[25] 1, 3-thiazole[26], cyclic alkyl(amino)carbenes,[27,28] P-heterocyclic carbenes (PHCs).[29,30] However five membered NHCs are the most broadly studied[31].



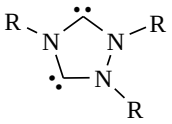
imidazol-2-ylidenes
I



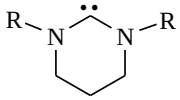
imidazolidin-2-ylidenes
II



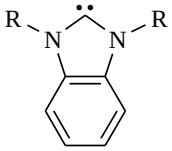
imidazol-5-ylidenes
III



1, 2, 4-trazolidin-3, 5-ylidenes
IV



tetrahydropyrimid-2-ylides
V



benzimidazol-2-ylidene
VI

Figure 1.2: Types of NHC ligands.

The contributions of the 6 π-electrons in unsaturated NHCs to the stability of the carbene centre have been a disputable contention.[28,32] NHCs of this type are proposed to stabilize the singlet state when there are unshared electrons paired in the same σ or π orbitals as a result of the inductive (-I) and the mesomeric (+M) push-pull effect (Figure 1.3).[32] Thus the negative inductive effect of the nitrogen on the carbene carbon decreases the energy of σ non-bonding orbital by increasing it s-character and leaving the Pπ orbital unaltered, thereby favouring the singlet state.[16] The mesomeric effect occurs when the carbon orbitals (s, pπ or px, py) interact with the p or π orbitals of the nitrogen substituents. The substituents lone pair improves the Pπ character of the singlet carbenes *via* electron transfer to the vacant Pπ orbitals of the carbenes.[16]

Dixon and Arduengo formerly stated that the stability of NHCs was mainly as a result of the inductive charge transfer from the carbene carbon to the nitrogen substituents, and that the mesomeric effect is quite insignificant.[28,33] It was also proposed that the double bond unsaturation would facilitate a delocalisation of

electron density around the ring, thereby providing a small but valid contribution to the overall stability of the NHC.[16,35-37]

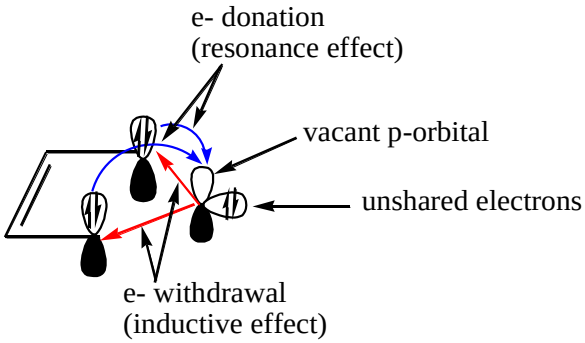


Figure 1.3: Electronic stabilisation of NHCs.

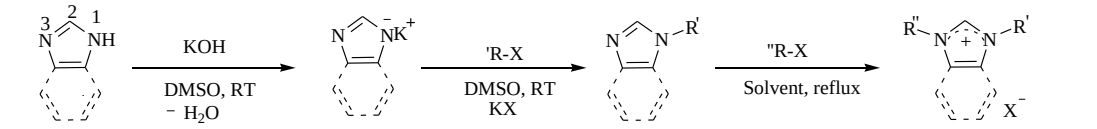
This was considered to have justified Arduengo's isolation of a free carbene with an unsaturated backbone, while Wanzlick's earlier work on saturated systems produced dimers.[38] However in 1995, NHC bearing saturated backbone was isolated and it became clear that the contribution of delocalisation could only give little stabilisation.[18] and that mesomeric π-electron donation from the nitrogen atoms to the carbene centre is adequate for the stabilisation of the free carbene.[35]

1.4 Synthesis of azolium salts: the NHC precursors

NHC precursors are prepared by two common pathways:[39]

1.4.1 Nucleophilic substitution

This route involves step-by-step alkylation of azole (imidazole, benzimidazole, triazole etc) in the presence of strong base such as KOH and NaOH. The potassium or sodium azolide formed is then treated with one equivalent of alkyl or aryl halide in a suitable solvent to obtain *N*-alkyl/aryl substituted azole.[40] The *N*-substituted azole is subsequently reacted with one equivalent of appropriate alkyl/aryl halide to yield disubstituted derivatives of imidazolium salts (Scheme 1.3).

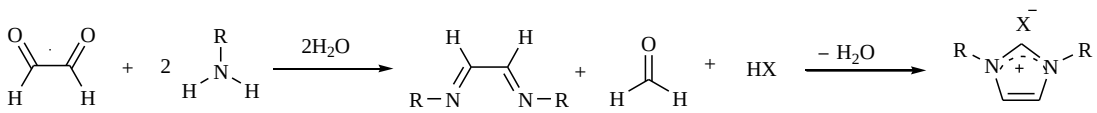


X = Halide

Scheme 1.3: Synthesis of [benz]imidazolium salts by nucleophilic substitution.[40]

1.4.2 Multi components one step reaction

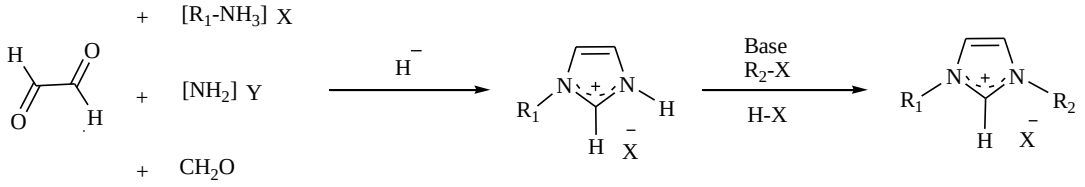
This route involves one pot synthesis, starting from glyoxal, primary amine and formaldehyde leading to the desired products. The reaction proceeds through a coupling between amine and glyoxal and forms the corresponding Schiff base. Condensation with formaldehyde leads to the imidazolium salt. The multicomponent reaction of primary amines, glyoxal, and formaldehyde in the presence of a Brønsted acid has been developed for the introduction of reactive *N, N′*-substituents (Scheme 1.4).[41]



X = Halide

Scheme 1.4: Synthesis of imidazolium salt by multi-components reaction.[41]

This flexible reaction gives access to a multitude of symmetrically *N, N'*-substituted imidazolium salts. Unsymmetrical substituted azolium salts can also be prepared by the combination of a multicomponent cyclization with an *N*-alkylation reaction. The initial cyclization gives an *N*-alkyl imidazolium salt, which is then alkylated at the second nitrogen atom to obtain the asymmetrically substituted derivative (Scheme 1.5).[42]



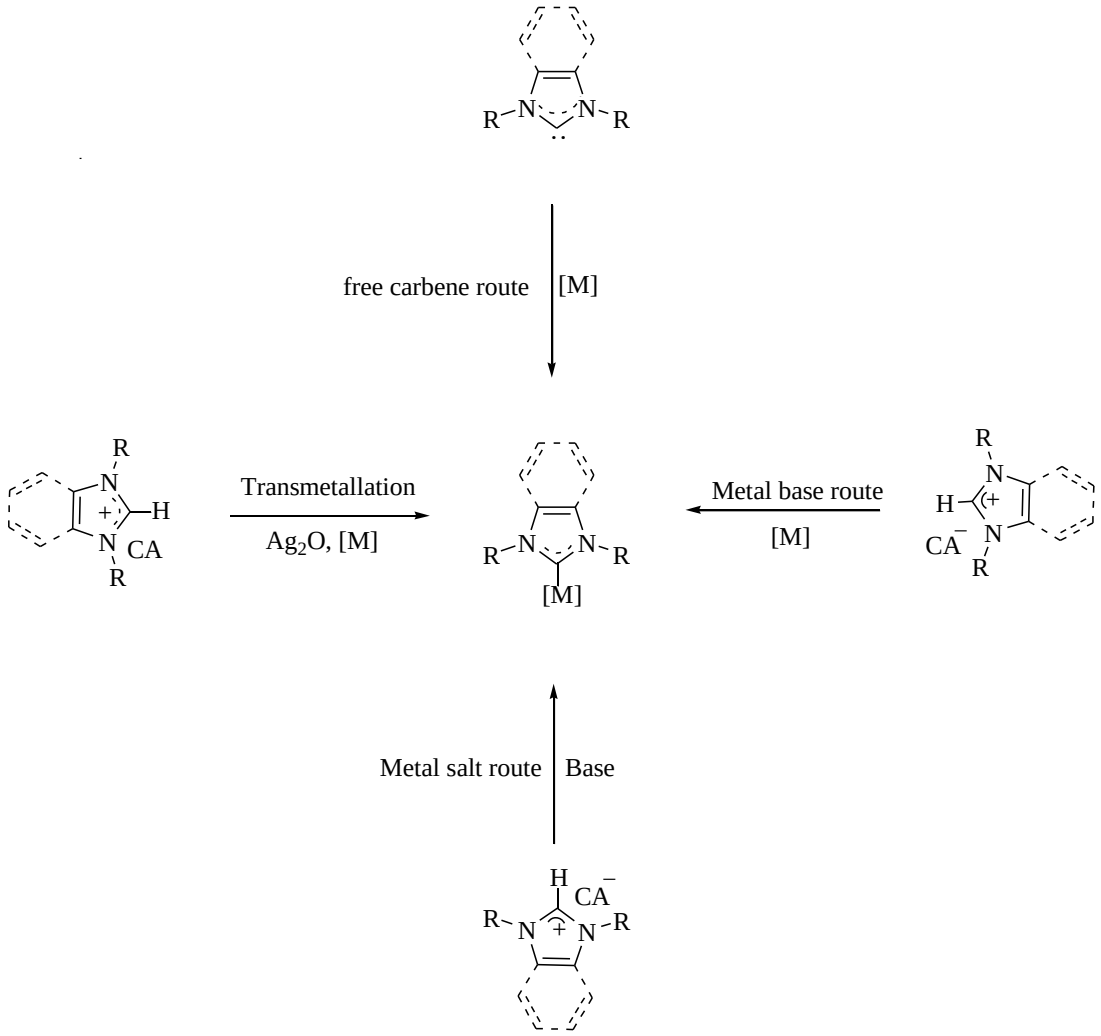
Scheme 1.5: Multi-component cyclization and *N*-alkylation.[42]

1.5 Preparation of NHC complexes

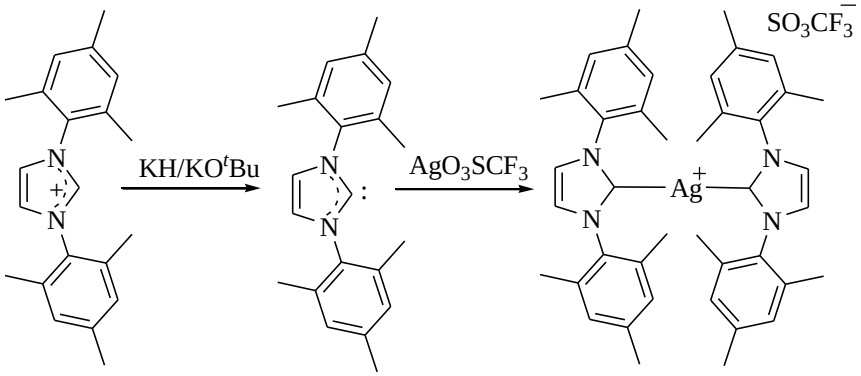
NHCs have been extensively used due to their favourable properties over phosphine and its strong coordination with main group and transition metals. Recently, researchers have adopted a number of synthetic procedures to prepare NHC complexes of d-block and main group metals.[43,44] The outline of the main synthetic methods is presented in Scheme 1.6.

(a) Free Carbene Route

This method was achieved by the reaction of suitable metal compounds with free NHCs. The deprotonation of the azolium salt results in the free NHC generation and the subsequent reaction with an appropriate metal compound to yield the transition metal carbene complex (Scheme 1.7).[45,46,47] Free carbene route has been adopted in the preparation of vast array of metal complexes but the choice of this procedure is constrained due to the conditions needed to afford the free carbene. Strong bases such as KH or KO^{t}Bu are often used and usually they can result in the deprotonation of some other acidic protons in the ligand. Therefore this method is limited to imidazolidin-2-ylidenes and benzimidazolin-2-ylidenes because the deprotonation can give rise to decomposition, especially in ligands where the methylene groups are in the α position of the nitrogen atoms of the NHC.[48-50]



Scheme 1.6: Primary synthetic routes towards formation of NHC complexes.[43]



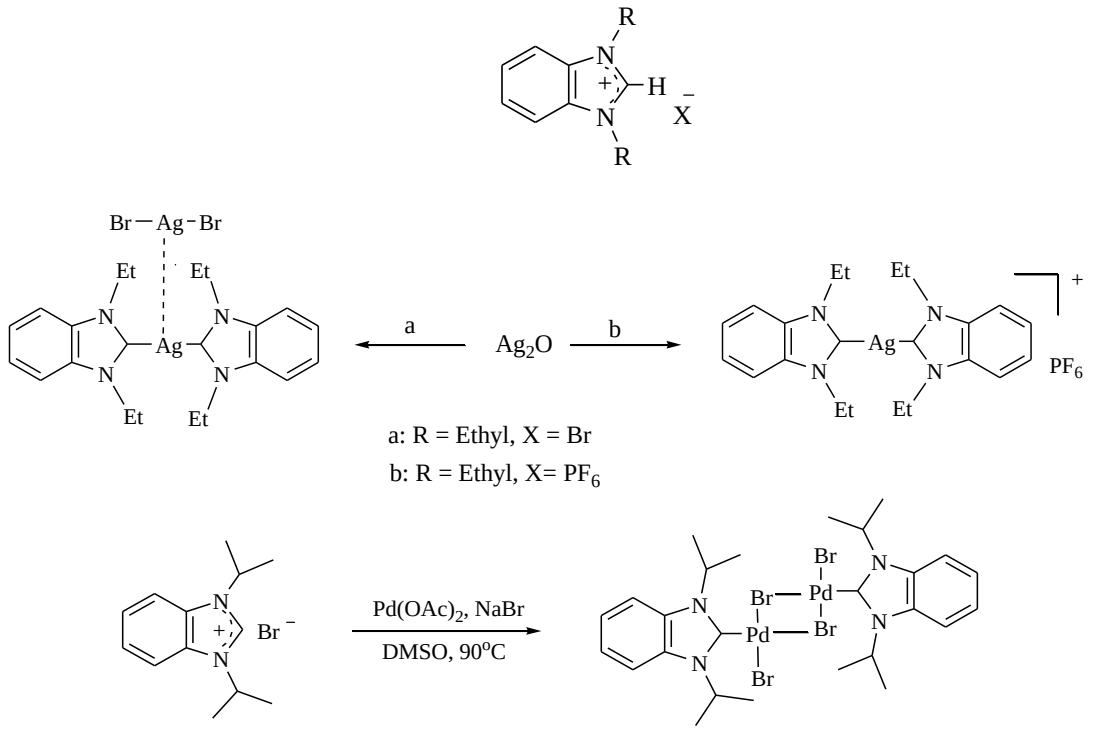
Scheme 1.7: Synthesis of Ag(I)-NHC *via* free carbene route.[45]

(b) *In situ* deprotonation of azolium salts

This method is based on the deprotonation of azolium salts by the use of external bases such as sodium acetate,[52] triethylamine,[53] NaH, *t*-BuOLi or *t*-BuOk,[14,51] and other bases have also been reported, such as silver oxide (Scheme 1.8)[54-57] and palladium acetate (Scheme 1.8).[58] The first NHC complexes reported by Wanzlick and Öfele have been prepared by this method.[21,53] This method is remarkably favourable, considering the fact that air and moisture sensitive free carbenes can be by-passed. Most of the complexes of NHCs discussed in this work were prepared using this method.

(c) Transmetallation (NHC transfer reaction):

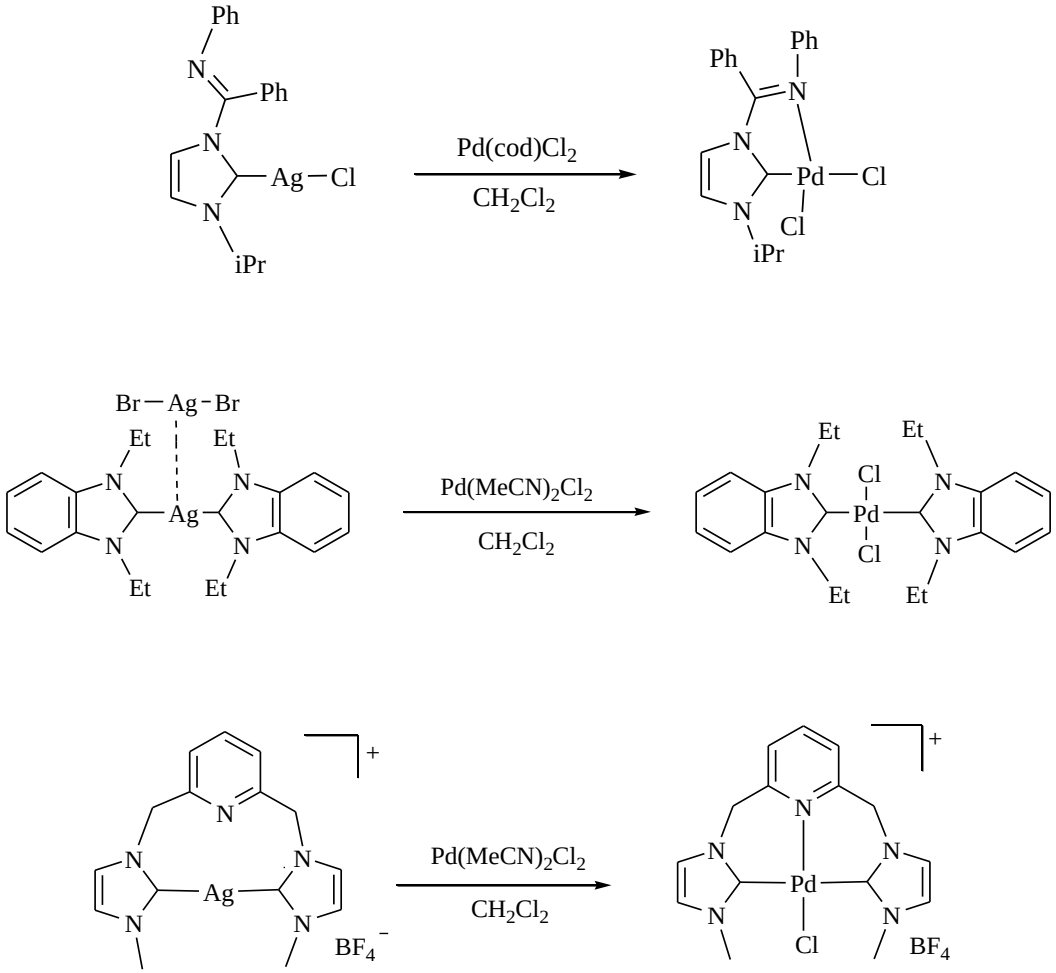
Lin and co-workers were the first to report this method in 1998.[54] In this method, Ag_2O was used as the basic metal source for the deprotonation of the azolium salts to give Ag-NHC complexes. This method has been successfully used with a variety of metals such as Rh, Ir, Pd, Pt, Ru, Cu and Au.[59,60]As a result of their lability, Ag(I)-NHC complexes are easily used as NHC transfer agents in the preparation of Pd (II) (Scheme 1.9), Au (I), Rh(I) and Ir(II) NHC complexes. This procedure has been extensively used due to the moderate reaction conditions, leading to the formation of more stable products. The absence of ^{107,109}Ag-^{13}C coupling in the ^{13}C NMR and the labile nature of the Ag-NHC complexes was suggested to be one the reasons for the easy NHC transfer.[54] The procedure is such that, a suitable azolium salt was treated with Ag_2O, $AgCO_3$ and AgOAc to obtain Ag(I)-NHC complexes. Thereafter, the obtained Ag(I)-NHC complexes were made to react with the other metal sources to give the proposed metal-NHC complexes.[61]



Scheme 1.8: Preparation of metal-NHC complexes by *in-situ* deprotonation method.[58]

The synthesis of Pd(II)–NHCs other than transmetallation method, such as direct metalation of azolium salt with $Pd(OAc)_2$ or with Pd(II)-precursor in the presence of external base were also investigated. The work by Gade and co-workers,[62] Tilset and co-workers,[63] Cavell and co-workers[64] and Shreev and co-workers[65] pointed out the inefficacy of direct metalation to prepare Pd(II)–NHCs. Consequently, transmetallation by Ag(I)–NHCs became a general interest. However, there are some short comings that were reported in using the transmetallation method. Hahn and co-workers,[66] Bildstein and co-workers,[67] Magil and co-workers[68] reported the low yield and non-success of transmetallation. Mata and co-workers also reported unsuccessful formation of a chelate complex with the intended metal-NHC complex.[69] These failures were proposed to have been caused by the presence of a

strong $C_{carbene}$-Ag bond. The Pd(II)-NHC complexes discussed in present work were obtained by this route.



Scheme 1.9: NHC transfer from Ag(I) centre to Pd(II) centre.[54]

1.6 Functionalized NHC complexes

Research on functionalised NHC complexes has developed rapidly over the last few years.[70-74] The formation of NHC complexes by reacting NHC precursor with a number of donors in the ligand framework gives innovative coordination chemistry.[74] Functionalized NHCs with excellent donor groups of nitrogen and oxygen are exclusively diverse in number. In this work, the synthesis and structural characterisation of NHC complexes functionalized with oxygen and nitrogen donor groups are highlighted as important part of the present study.

1.6.1 Functionalized NHCs and metal complexes containing nitrogen donors

The modelling of functionalized NHCs with nitrogen donor groups is given a considerable attention much more than phosphine donor groups. Especially, the inclusion of pyridine functionality into polydentate ligand framework of NHCs is of great significance. This high interest in pyridine functionality could be partially due to the simple synthetic procedure associated with the ligand precursors and the interesting rigidity transmitted to the metal complexes from the chelate rings and addition to the exceptionally thermal stability.[72] Pincer-type ligands based on pyridine–dicarbene have gained considerable attention as a result of the increased stability and a strengthened rigidity (Figure 1.4). Furthermore, NHC with a central imidazole ring and two flanking pyridine side-arms have been studied by several research groups.[68,75–78] The ligand precursor (**VII**) forms Pd (II) complex (**VIII**) due to the small bite angle resulting in the bidentate coordination of the two ligands (Scheme 1.10).[75] Complex (**VIII**) shows a fluxional behaviour in solution because of the hemilabile characteristic of the ligand with the coordinated and free pyridine arms.[75]

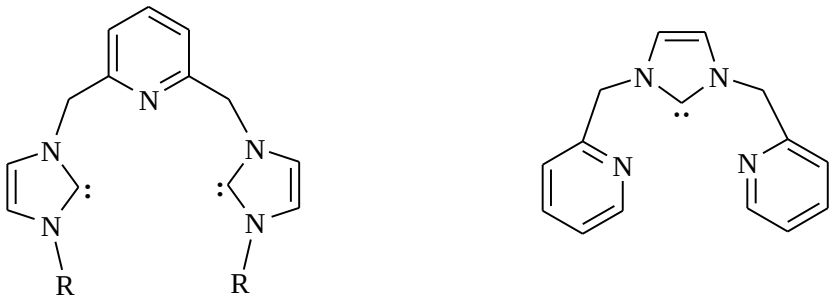
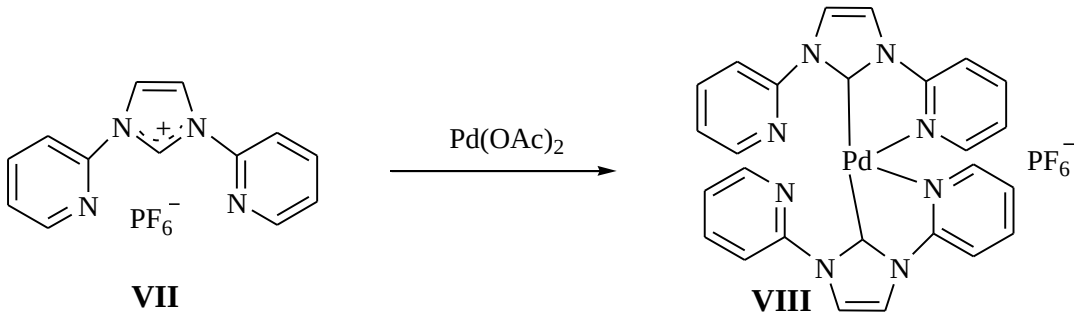


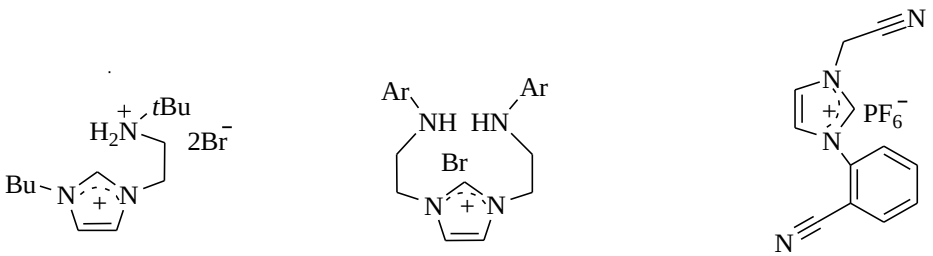
Figure 1.4: Pyridine NHC ligands.



VII **VIII**

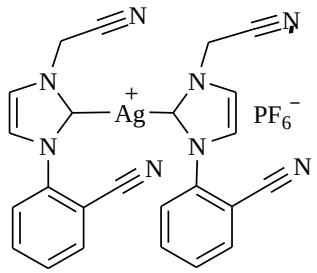
Scheme 1.10: Formation of Pyridine functionalized Pd(II)-NHC complex.[75]

Apart from pyridine functionality, other varieties of functionalized NHCs with nitrogen donor groups, like imine, amide, nitrile, oxazoline, pyrazole, and amine are also well-known.[79,80] The first amide functionalised NHC (**IX)** was reported by Arnold et al. and was prepared in good yield by the alkylation of *tert*-butyl imidazole with *t*-BuNHCH$_2$CH$_2$Br.HBr**.**[81] Fryzuk et al. was able to establish the first models of tridentate bis-amine NHC precursor (**X)**. In 2009 Wylie et al. reported the synthesis of unsymmetrical nitrile functionalized NHC precursor (**XI)** from 2-cyanophenylimidazole (Figure 1.5). The reactions of these novel imidazolium salt with Ag_2O yielded novel Ag(I)-NHC complex (**XII)**.[82] Although an analogous nitrile-functionalized NHC complex of Pd(II) (**XIII)** was reported earlier by Dyson and co-workers.[83] See Figure 1.6 below.

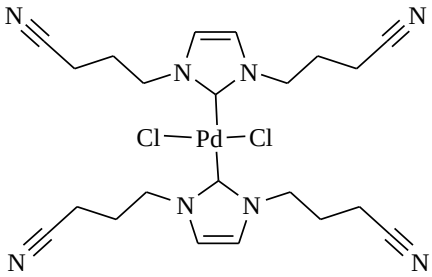


IX **X** **XI**

Figure 1.5: Examples of N-functionalized NHC precursors.



XII

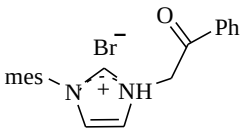


XIII

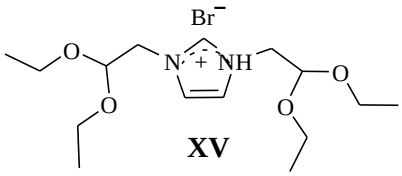
Figure 1.6: Examples of N-functionalized Ag(I)-NHC and Pd(II)-NHC complexes.

1.6.2 Functionalized NHCs and metal complexes containing oxygen donors

Functionalized NHCs bearing oxygen donors, like ester,[84,85] ether,[73,86,87] hydroxyalkyl[88, 89] and ketone components[85,90] have been reported. In most cases for the complexes, the oxygen functionalities of these NHC precursors are not coordinated[84,86,89,90](Figure 1.7) but complexes with the chelated oxygen functionalities are also known.[90,91] Früstner et al. showed that ester functionality can also be connected with the NHC moiety.[84] Takacs et al. also reported the preparation of acylfunctionalized NHC precursor[88] and was found as an effecient Pd-catalyzed Suzuki-Miyaura coupling reactions. According to cited literatures above, it is worthy of note to mention that most of the research on functionalized complexes have been directed towards catalysis. So far research involving functionalized NHC complexes for biological studies is quite rare.



XIV



XV

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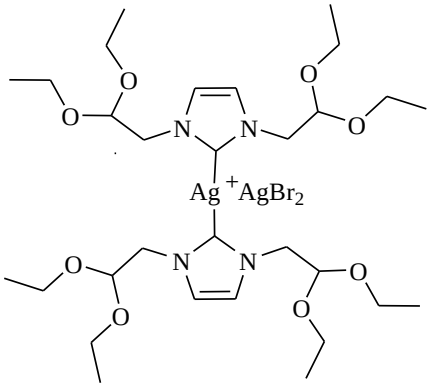
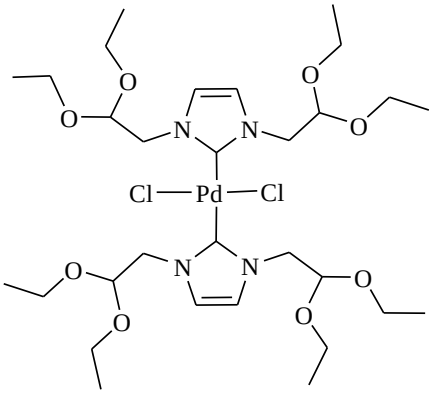


XVI



XVII

Figure 1.7: Examples of O-functionalised Ag(I)-NHC and Pd(II)-NHC complexes.

1.7 Antibiotics

Antibacterial agents, or antibiotics, are a class of a larger group of compounds called antimicrobial agents. Earlier, antibiotics were referred to as only naturally occurring molecules produced by a variety of microorganisms.[92] However, the term has transformed to include man-made synthetic compounds.[93] Antibiotics can be categorised into four distinct classes based upon their mechanism of action. Those four classes are (1) the agents that inhibit bacterial cell wall biosynthesis, (2) the agents that inhibit protein biosynthesis, (3) the agents that inhibit deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) synthesis, and (4) the agents that inhibit folate synthesis.[93]

Inhibitors of Bacterial Cell Wall Biosynthesis:

β-lactam antibiotics act on diverse targets within the biosynthetic cascade of cell wall formation. This family of compounds is comprised of four groups of

molecules: (1) the penicillins, (2) the cephalosporins, (3) the carbapenems, and (4) the monobactams. They all act *via* the same mechanism of action, and that is by binding and inhibiting the penicillin binding proteins (PBPs); more specifically they inhibit peptidoglycan transpeptidase causing lysis and cell death.[93]

Inhibitors of Protein Biosynthesis:

This class of compounds exert their antibacterial action by blocking one or more of the steps in protein biosynthesis that occur on either the 30S or 50S subunits of the bacterial ribosome. There are a number of families of molecules that make up this family including the macrolides, lincosamides, streptogramins, oxazolidinones, aminoglycosides, and tetracyclines.[93] However, the major families that compose this family are the aminoglycosides, macrolides, and tetracylines. These antibiotics show potent activity against gram-negative bacteria, but lack potent activity against most gram-positive bacteria. However, they are used in combination with β-lactams to treat some enterococcal infections.[93]

Inhibitors of DNA and RNA Synthesis:

This class of antibiotics works on one of three targets: DNA gyrase, topoisomerase IV, or DNA-directed RNA polymerase. There are two families of antibiotics included within this class, and those are the quinolones and the rifamycins. The activity of the quinolones is derived from their inhibition of DNA gyrase and topoisomerase IV.[92] While DNA gyrase and topoisomerase IV have similar mechanisms of action, the difference is the fact that DNA gyrase is more important for gram-negative organisms and topoisomerase IV is more important for gram-positive organisms.[94] The quinolones work by binding to DNA gyrase or topoisomerase IV and preventing the supercoiling of DNA, thereby inhibiting synthesis of DNA.[93] The quinolones were not widely used until the discovery of the

fluoroquinolones in the 1980s.[95] There are presently four generations of quinolones that have been developed and are in clinical use. The rifamycin family of antibiotics works on DNA-directed RNA polymerase. These agents bind to the β-subunit of DNA-directed RNA polymerase and inhibit the initiation of chain formation in RNA synthesis.[93] Rifamycin is a natural product with several natural analogs, but three semisynthetic analogs have since been synthesized. Those agents are rifampin, rifabutin, and rifapentine. These agents are front-line agents in the treatment of tuberculosis.[93]

Inhibitors of Folate Synthesis:

Folate is very important to the survival of all living organisms.[96] It plays a considerable role in DNA synthesis by serving in the transfer of methyl, formyl, and other single-carbon fragments in the biosynthesis of purine nucleotides.[97] Humans and bacteria obtain folate via different routes. Humans obtain folate through their diet, while bacteria synthesize folate through the folate biosynthetic pathway. This difference makes the folate biosynthetic pathway a very attractive drug target.[96] There are two enzymes in this pathway that are currently targeted by antibacterial agents: dihydropteroate synthesis (DHPS) and dihydrofolate reductase (DHFR). The family of agents that act on DHPS is the sulfonamides. DHFR is acted upon by a single agent, and that is trimethoprim.[92] The sulfonamides work by inhibiting DHPS, which catalyses the conversion of *p*-aminobenzoic acid (PABA) to dihydroteroate.[98,99] As a result of resistance issues, most of the originally developed sulfonamide drugs are no longer in use as a monotherapy. However, some are still in use for acne and urinary tract infections. The most commonly used drug in this class is sulfamethoxazole. This agent is often used in combination with trimethoprim,

which expands it antibacterial spectrum and reduces the potential for the development of drug resistance.[94]

1.8 Biological applications of Ag(I) and Pd(II)-NHC complexes

1.8.1 Background

Penicillin resistant *staphylococcus aureus* (*S. aureus*) strains were first reported in the 1940s. However, research on anti-microbial is considered to have started in 1928 with the accidental discovery of penicillin by *Alexander Fleming*.[100] Anti-bacterial drug discovery reached its apex in the 1950's and of the majority of these compound classes are still in use today.[101] In 1959 methicillin was introduced but again *S. aureus* quickly developed resistance which was later referred to as Methicillin-resistant *S. aureus* (MRSA).[101,102] Resistance by MRSAs were also reported in the earlier years of the 1980's against such antibiotics like erythromycin, neomycin, gentamycin and ciprofloxacin in 1990's.[103] After the introduction of streptogramins and quinolones earlier in the 1960's, no novel class of anti-bacterial agent was introduced into clinical practice until linezolid was launched in 2000. Considering the diversity of bacterial structures, drug discovery in this area requires agents with specific biomolecular targets or routes. Bacterial microorganisms have utilized anti-bacterial xenobiotics to develop a complex arsenal of defences to protect themselves from attack. Consequently, some researchers suggest the superior role of natural products, owing to evolutionary conditions, in producing an array of antibiotic producing microorganism, compared with synthetic libraries.[102] However, these antibiotics function by targeting specific biomolecular pathways and as a result have a propensity for the development of resistance to prophylactic treatment.

Later in 2002, it was reported that Vancomycin-resistant *S. aureus* (VRSA) was identified and isolated.[104,105] Bacteria can develop resistance surprisingly fast,

arising through either an alteration of the target site or enzyme, inhibition of the antibiotics to gain access and destruction or deactivation of the antibiotics.[106] Therefore highly resistant pathogenic bacteria causing life threatening infectious diseases are appearing, especially, strains which are no more responding to treatment by any possible antibiotics. A number of studies have been carried out on the effect of bacterial infections, one example of such is the effect of *S. aureus* peritoneal infection on a mouse model, the studied revealed the presence of intraperitoneal abscesses in the vicinity of inoculation sites, a disease that occurs frequently in humans undergoing continuous ambulatory peritoneal dialysis for end-stage renal disease (Figure 1.8).[107]

During the last two decades, numerous anti-bacterial agents have been developed and offered into the market, however their effectiveness has over the years deteriorated and failed to solve the problem of multidrug resistant bacteria.[108-110] Therefore, there is a need to develop novel anti-microbial compounds with an entirely different mechanism in order to curb the fatality of multidrug-resistant bacteria. In the light of this, organometallic derivatization was proposed as an attractive approach to subdue the resistance problem. This approach could possibly provide a metal mode of action which is specific and non-existence for the purely organic parent drug molecule.[111-114] In certain conditions, it was suggested that the effectiveness of a metal-based-drug could be as a result of the original activity and the inherent toxicity of the inorganic metal.[114] It has already been established that the potentiality of bacteria to cultivate resistance against silver-based anti-microbials is constrained, therefore affording such antibiotics to possess a long shelf life.[115,116] The development of metal-containing anti-cancer and anti-malarial compounds has shown a lot of advancement.[117-122] However, very little awareness has been shown

for the development of organometallic anti-bacterial drugs. Therefore, research on metal-based anti-bacterial compounds is very appropriate and desirable.

To date a large number of studies have been carried out on Ag(I) complexes as potential anti-microbials, anti-cancer and their carbene transfer ability.[123-126] Also Ag(I)-complexes have shown interesting DNA binding ability,[127-129] however there is paucity of data on their ability to cleave the nucleic acids (DNA/RNA). On the other hand, the interaction of Ag(I)–NHC complexes with nucleic acids is a significant field of research to be explored to a greater extent due to the utility of these carbene complexes in the development of spectroscopic probes, diagnostic agents and site-specific nucleic acid cleavers, among others.[130] It is worthy of mentioning that apart from DNA there are some other biological targets including RNA, enzyme or protein. Therefore it is considered worthwhile to investigate the interaction of these synthesized compounds with DNA and/or RNA as an initial experiment to study the likely mode of action(s) of the reported compounds.

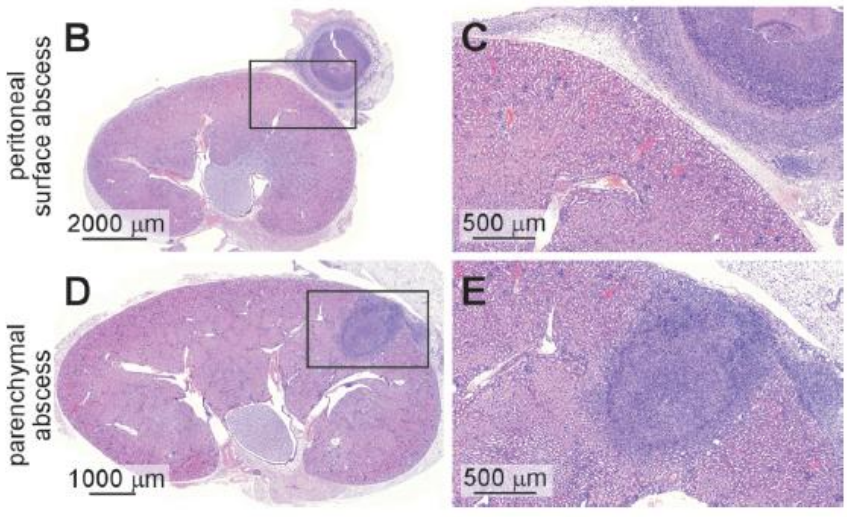
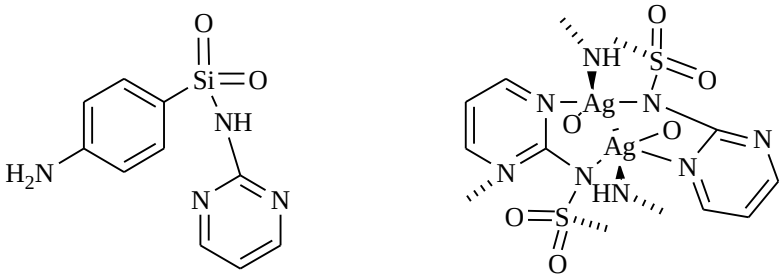


Figure 1.8: Staphylococcal lesions associated with the kidney.[107]

1.8.2 Biological applications of Ag(I)-NHC complexes

Biological studies of Ag(I)–NHC complexes are established on the knowledge that elemental silver and its salts have long been recognized for their potential in the protection of the eyes of newborns from infection, as well as their anti-microbial properties, essentially against chronic ulcers, extensive burns, and wounds.[131,132] This astounding activity of silver or Ag(I)–NHC complexes against bacteria, fungi, and yeast is due to slow delivery of silver cations across the cell membrane, which mostly interferes with the electron transport system of the cell and also interacts with the thiol groups of the vital enzymes of bacteria.[133] Ag(I)-NHC complexes offers encouraging results to overcome the problems of drug resistance of some pathogenic bacteria, which has so far eluded the effectiveness of sulphonamides. Recently, Ag(I)–NHC/coordination complexes have been found to display potential anti-microbial activity, particularly for the potential treatment of cystic fibrosis and chronic lung infections,[134,135] which makes them appropriate entities for drug development.[136] The enhanced stability of the complexes is anticipated to be useful for anti-microbial activity, because silver is released gradually as compared to ionic silver complexes such as $AgNO_3$ which is spontaneous in action but lose their effectiveness quickly resulting in reinfection.[137,138] Youngs research group was the first to report Ag-NHC in 2004 for anti-microbial studies.[139] One example of such series of compound (**XXII**) is shown in Figure 1.11 below. In this work Youngs recorded a highly promising results from this caffeine derived Ag-NHC which showed excellent anti-bacterial activity against drug resistant pathogenic bacteria.[140,141] The search for better biologically active Ag(I)-NHC complexes led to the synthesis of modified caffeine silver acetate complex.[134] Gosh and co-workers also synthesized Ag(I)-NHC complexes of 1-

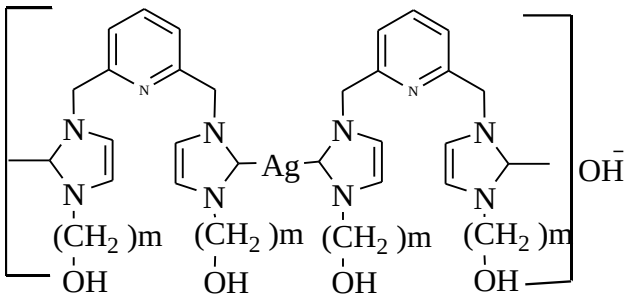
benzyl-3-tert-butylimidazolium chloride and tested on analytically important microorganism.[142] The Ag(I)-NHC complexes derived from 4,5-dichloroimidazole have been synthesized, having water stability for up to three days and this was attributed to the presence of electron withdrawing substituents present on the 4- and 5-positions of the imidazole ring.[141] In recent years, varieties of functionalized NHC complexes with bidentate chelating NHCs, tripodal NHCs, pincer type NHCs have also been investigated,[71,143-147] especially in catalysis while their anti-microbial properties are less explored. The effectiveness of the anti-microbial properties of Ag(I) complexes may be influenced by the type of ligands that bind to Ag(I) ion.[148] It was found that the type of substituents and chain length of alkyl chain have a significant effect on their anti-microbial properties.[149,150] Modification of NHCs can be easily achieved by introducing functional groups at the nitrogen atoms of the imidazole ring,[16,146,151] thereby fine-tuning the characteristic features like lipophilicity, charge, solubility of the spacers (bridging groups) and substituents. This has stimulated the design of complexes in favour of reaching a compromise between biological activity and toxic effects.[151] Examples of metal based antibiotics are shown in Figure 1.9 below.



XVIII **XIX**

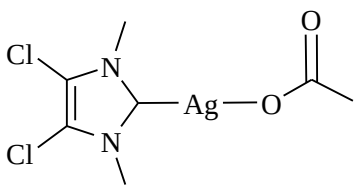
Figure 1.9: Sulfadiazine (**XVIII**) and Silver sulfadiazine complex (**XIX**).

A very fascinating characteristic of NHC chemistry is the simplicity with which a number of complexes with similar structures and different lipophilicity (Figure 1.10 & 1.11) can be synthesized by changing the substituents on the imidazolium salts.[14,16] The lipophilicity of a substance cannot be overlooked as one of the influential parameters in its biological activity.[134,141,143] The enhanced lipophilicity characters of some compounds have been found to have positive correlation with their anti-bacterial activity.[152,153]

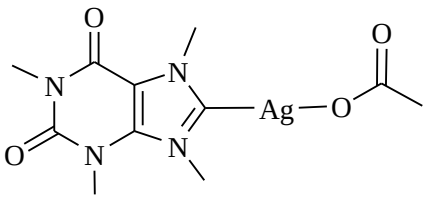


XX: m = 2 (**XX, XXI**)
XXI: m = 3

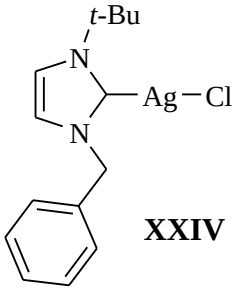
Figure 1.10: The first Ag(I)-NHC complexes used as anti-microbial agents.



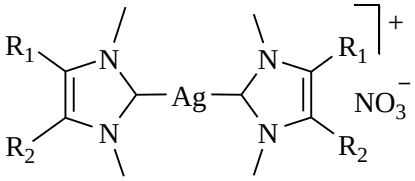
XXII



XXIII



XXIV



XXV R_1 = H
R_2 = Cl

Figure 1.11: Examples of Ag(I)-NHC complexes exhibiting significant anti-microbial activity.

Both functionalized and nonfunctionalized imidazole based NHCs, as well as their metal derivatives of wide structural diversity, have been studied as therapeutic agents for cancer treatment. Specifically, functionalized NHC ligands have attracted special interest from the point of view of tuning the coordination environment around the core metal, because of their flexible chelating nature. In principle, the lipophilicities and reactivities of Ag(I)–NHC complexes can be tuned systematically by changing the attached NHCs or substituents. Therefore in the present work, numerous varieties of Ag(I)–NHC complexes have been synthesized to determine the possible involvement of functional and non-functional substituents present at the N atom(s) of the [benz]imidazolium core.

1.8.3 Biological application of Pd(II)-NHC complexes

A number of complexes based on Pd(II) have been synthesized and their different biological activities have been documented.[154,155,156] The effect of various palladium complexes on the growth and metabolism of different groups of microbes has been investigated. In 2009 Garoufis et al. reviewed diverse scientific papers on anti-viral, anti-bacterial and antifungal activity of Pd-(II) complexes with various types of ligands (sulfur and nitrogen donor ligands, Schiff base ligands and drugs as ligands).[157] Over the past few years, literature has shown different activities of Pd-(II) complexes against several species of bacteria and fungi.[158-166] Although the biological activity of Pd-based drugs bearing a variety of ancillary ligands has been under intense investigation, only two reports of Pd-(II)-NHC complexes with anti-bacterial activity is found in recent literature (Figure 1.12).[167,168] Ghosh was the first to explore the cytotoxic capacity of two Pd complexes, **XXVI** and **XXVII** against three human tumor cell lines.[168] To date, numerous Pd(II)–NHC complexes have been reported for various activities. However, this field of chemistry has been