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Applications of *N*-Heterocyclic Carbene Complexes: From Catalytic Aldehyde Olefination to Anticancer Therapy

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Vollständiger Abdruck der von der Fakultät für Chemie der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

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Die Dissertation wurde am 07.12.2017 bei der Technischen Universität München eingereicht und durch die Fakultät für Chemie am 16.01.2018 angenommen.

„Wer immer tut, was er schon kann,

bleibt immer das, was er schon ist.“

Henry Ford

Die vorliegende Arbeit entstand im Zeitraum von Februar 2015 bis Dezember 2017 unter der Anleitung von Herrn Prof. Dr. Fritz E. Kühn, Professur für Molekulare Katalyse der Technischen Universität München, und Frau Prof. Dr. Angela Casini, Chair of Medicinal and Bioinorganic Chemistry der University of Cardiff.

Ich bedanke mich herzlich bei meinem Doktorvater

Herrn Prof. Dr. Fritz E. Kühn

für die freundliche Aufnahme in seinen Arbeitskreis, die vielfältigen und spannenden Forschungsmöglichkeiten, für die kreativen Freiheiten bei der Gestaltung meines Promotionsprojektes, für die großartige Unterstützung in den letzten drei Jahren sowie für die Ermöglichung einer fruchtbaren internationalen Kooperation.

Weiterhin gilt mein besonderer Dank meiner Zweitbetreuerin

Frau Prof. Dr. Angela Casini

für die stetige Unterstützung in zahllosen fachlichen Belangen und für die Ermöglichung von spannenden und lehrreichen Forschungsaufenthalten an der Cardiff University.

Diese Arbeit wurde unterstützt durch Fördergelder der TUM Graduate School und des ERASMUS-STA Programms.

Danksagung

Darüber hinaus, möchte ich mich an dieser Stelle bei einer Vielzahl weiterer Personen herzlich bedanken, ohne deren Wirken diese Arbeit in dieser Form nicht möglich gewesen wäre.

Ein besonderes Dankeschön gilt **Dr. Robert Reich** für die unermüdlichen Ratschläge in fachlichen und organisatorischen Angelegenheiten und die motivierenden Gespräche.

Dr. Markus Anneser danke ich für die großartige Hilfsbereitschaft und die unvergleichlichen theoretischen und praktischen Anregungen im Labor, die mir den Einstieg in die Eisenchemie grundlegend erleichtert haben.

Ein großes Dankeschön gilt auch **Dr. Jens Kück** für seine tatkräftige Unterstützung sowie wertvollen Tipps beim Verfassen meines ersten Manuskripts.

Dr. Oksana Storcheva für die tolle Zusammenarbeit und die erfreulichen Unterhaltungen während den letzten drei ACI-Praktika.

Dr. Alexander Pöthig für die Ausbildung von vorbildlichen Kristallographen wie **Philipp Altmann**, dem ich für sein starkes Engagement, meine herausfordernden Kristalle nicht aufzugeben, danke.

Den ehemaligen MedChem Gruppenmitgliedern **Eva Hahn**, **Sophie Jürgens** und insbesondere **Andrea Schmidt** für die konstruktiven Gespräche und die herrliche gemeinsame Zeit in Cardiff.

Lilli, **Jens** und **Bruno** für die tolle Atmosphäre und die gute Zusammenarbeit im Rahmen der MedChem Gruppe.

Weiterhin gilt mein besonderer Dank meinen Laborkollegen **Caro**, **Tommy**, **Felix** und **Anja** sowie **Ben**, **Pauline**, **Han**, **Andreas** und **Yang** für die besonders angenehme Arbeitsatmosphäre und die spaßigen Zeiten in den CRC Räumen 4006-4008.

Meinem Masterstudenten **Marco Bernd**, auf den ich mich während meines Forschungsaufenthalts in Cardiff stets verlassen konnte.

Allen weiteren Kollegen der Professur für Molekulare Katalyse danke ich ebenfalls für eine tolle Zeit während meiner Promotion.

Ebenso danke ich meinen Kollegen in Cardiff **Valeria Scalcon**, **Samuel Meier-Menches**, **Brech Aikman** und **Riccardo Bonsignore** sowie **Benjamin Woods**, **Darren Wragg**, **Margot Wenzel** und **Andreia Almeida** für die fruchtbare Zusammenarbeit und die unterhaltsamen Donnerstagabende im „Pen & Wig“.

Den freundlichen Sekretärinnen **Ulla Hifinger** und **Irmgrad Grötsch** für die großartige Hilfe bei zahlreichen organisatorischen Belangen.

Jürgen Kudermann und **Maria Weindl** für die ausgiebigen NMR-Kinetiken und die lehrreichen Gespräche während den Messungen.

Ulrike Ammari, **Bircan Dilki**, **Petri Ankenbauer**, **Rodica Dumitrescu** und **Pauline Fischer** für die Charakterisierung zahlreicher Verbindungen, sowie **Martin Schellerer** für die jahrelange Bestellung von Chemikalien und zügige Versendung von Proben.

Mein größter Dank gebührt meiner **Familie**, insbesondere meinen **Eltern**, meiner **Schwester** und nicht zuletzt **Andrej**, für die unermüdliche Unterstützung und den unvergleichlichen Rückhalt für alle meine Vorhaben.

Kurzzusammenfassung

Der erste Teil dieser Arbeit hat sich aus der steigenden Nachfrage nach Olefinen, welche u.a. für die Herstellung von Basis- und Feinchemikalien benötigt werden, entwickelt. Obwohl die Wittig-Reaktion eine etablierte Methode ist um C=C Doppelbindungen herzustellen, sind zahlreiche Nachteile dieser Reaktion bekannt. Mit der Entwicklung der katalytischen Olefinierung von Aldehyden gilt es diese Einschränkungen zu überwinden.

In diesem Rahmen werden Eisen(II) Komplexe, welche durch *N*-heterozyklische Carbene stabilisiert sind, auf ihre katalytische Aktivität geprüft. Der aktivste Komplex mit dem gemischten Ligandensystem, bis(NHC)-bis(Pyridin), wird für die Optimierung der Reaktionsbedingungen im Hinblick auf Verhältnisse der Ausgangsstoffe und unterschiedlichen Temperaturen verwendet. Es werden hohe Produktausbeuten mit durchgehend sehr guter *E*-Selektivität erreicht. Desweiteren werden ausführliche Untersuchungen zur katalytischen Wirkungsweise durchgeführt und diese mit literaturbekannten Mechanismen verglichen. Organische sowie eisenhaltige Schlüsselverbindungen werden mittels GC MS Analysen sowie ^{31}P NMR- und ^{1}H NMR-Studien charakterisiert. Dabei wird die katalytische Herstellung von Phosphor-Yliden beobachtet, welche analog zu einer Wittig-ähnlichen Reaktion mit Aldehyden weiterreagieren. Im Gegensatz zu anderen publizierten Mechanismen, wird hier vermutet, dass die Ylid-Bildung auf zwei unterschiedliche Wege, von denen einer nicht literaturbekannt ist, erfolgt.

Im zweiten Teil der Arbeit wird die synthetische Vielfalt von *N*-heterozyklischen Carbenen genutzt um hydrophile Gold(I) NHC Komplexe als potentielle Antikrebsmittel zu entwickeln. Während Gold(I) Komplexe für seine zytotoxische Wirkung bekannt sind, wird im Allgemeinen eine ausreichende Hydrophilie/Wasserlöslichkeit der Verbindungen für zuverlässige *in vitro* Untersuchungen sowie verbesserte biologische Verfügbarkeit unter physiologischen Bedingungen vorausgesetzt.

Es wurde hierzu eine Reihe von mono- und bimetallischen Gold(I) Komplexen unter Verwendung von sulfonierten bis(NHC) und hydroxylierten mono(NHC) Liganden synthetisiert und charakterisiert. Das Wachstum von Zellen des Ovarialkarzinoms 2008 wird besonders durch die mono(NHC) Au(I) Komplexe gehemmt. Stabilitätsstudien in wässriger/gepufferter Lösung geben Aufschluss über die Anfälligkeit der mono(NHC) Komplexe für Ligandenaustauschreaktionen mit möglichen Nukleophilen. Um das biologische Wirkprinzip dieser Gold(I) Verbindungen zu beleuchten, werden ihre Interaktionen mit dem zentralen Zielmolekül, dem Selenoenzym Thioredoxin Reduktase (TrxR), erforscht. Eine deutliche und selektive Bindung an die nukleophilen Aminosäurereste im aktiven Zentrum des Enzyms wird mittels biochemischer und massenspektrometrischer Analysen bestimmt. Studien basierend auf Fluoreszenz-Resonanzenergietransfer (FRET) hingegen deuten auf keine Interaktion mit der G -Quadruplex (G4)-

bildenden Telomersequenz der DNS hin. Letzteres stellt eine neue und vielversprechende Art von biologischer Zielstruktur für bestimmte Gold(I) NHC Komplexe dar.

Abstract

The first part of this work derives from the rising demand for the C=C motif for the production of a large number of fine and bulk chemicals. The Wittig reaction is a well-established method to generate carbon-carbon double bonds but it is also known for a series of limitations. In order to overcome those, the olefination of aldehydes in a catalytic fashion has emerged as a promising approach.

For this purpose, iron(II) complexes bearing *N*-heterocyclic carbene ligands are evaluated for their catalytic performances. The optimization of the reaction is achieved using the most active complex bearing a mixed bis(NHC)-bis(pyridine) ligand system upon variation of substrate ratios and temperature. As a result, high olefin yields with excellent *E*-selectivity are obtained. Moreover, in-depth mechanistic investigations are performed and compared to literature-known pathways. By means of GC-MS analysis, ^{31}P NMR and time-dependent ^{1}H NMR spectroscopic studies iron-based as well as organic key intermediates are revealed. Overall, a mechanism including the catalytic formation of the phosphorus ylide which subsequently reacts with aldehydes in a Wittig-like reaction is observed. In contrast to other literature-known mechanisms, the phosphorus ylide is assumed to be generated *via* two different pathways, of which one is proposed for the first time.

In the second part of the thesis the synthetic flexibility of *N*-heterocyclic carbenes is exploited for the development of hydrophilic gold(I) NHC complexes as potential anticancer drugs. While gold(I) complexes generally display promising cytotoxic properties, the sufficient hydrophilic/water-soluble character of a drug is essential for reliable *in vitro* investigations along with an enhanced bioavailability under physiological conditions.

Therefore, a series of mono- and dinuclear gold(I) complexes exhibiting sulfonated bis(NHC) ligands and hydroxylated mono(NHC) Au(I) compounds are synthesized and characterized by means of NMR spectroscopy, mass spectrometry and elemental analysis. In particular, all mono(NHC) complexes display high antiproliferative effects against ovarian carcinoma cell line 2008. In contrast to the bis(NHC) compounds, the mono(NHC) complexes are prone to ligand exchange reactions as demonstrated in stability studies in aqueous/buffered solution. In order to elucidate the mode of biological action, in-depth mechanistic studies are performed with the pivotal seleno-enzyme target thioredoxin reductase (TrxR). By means of biochemical assays and mass spectrometric approaches selective and distinct binding properties of the gold(I) compounds with both possible binding sites of TrxR is described. In contrast, fluorescence resonance energy transfer (FRET) assays showed no considerable interaction with telomeric G-quadruplex (G4) DNA sequences, which represent a novel biological target for certain gold(I) NHC complexes.

List of Abbreviations

ADMET	acyclic diene metathesis polymerization
ASK	apoptosis signal-regulating kinase
Asn	asparagine
AQP	aquaporin
BAX	Bcl-2-associated X protein
BIAM	biotin-conjugated iodoacetamide
bipy	bipyridine
BTX	benzene, toluene, xylene
Bz	benzyl
CLL	chronic lymphocytic leukemia
Cys	cysteine
DFT	density functional theory
DLC	delocalized lipophilic cations
DNA	deoxyribonucleic acid
dppe	1,2-bis(diphenylphosphino)ethane
EDA	ethyl diazoacetate
equiv.	equivalent
ERK	extracellular signal-regulated
ESI	electrospray ionization
FDA	US Food and Drug Administration
FRET	fluorescence resonance energy transfer
GC	gas chromatography
Gly	glycine
GR	glutathione reductase
GSH	glutathione
G4	G-quadruplex
h	hour
IC_{50}	half maximal inhibitory concentration
kt	kiloton

L	ligand
M	metal
MAP	mitogen-activated protein
MDO	methylrhenium dioxide
Me	methyl
Mes	2,4,6-trimethylphenyl
min	minute
MPT	membrane permeability transition
MS	mass spectrometry
MTO	methylrhenium trioxide
NADPH	nicotinamide adenine dinucleotide phosphate
n-Bu	*n*-butyl
NHC	*N*-heterocyclic carbene
NMR	nuclear magnetic resonance
(N)SCLC	(non-)small cell lung cancer
PARP	poly(adenosine diphosphate (ADP)-ribose) polymerase
PET	positron emission tomography
ppm	parts per million
Prx	peroxiredoxin
PTA	1,3,5-triaza-7-phosphaadamantane
RCM	ring-closing metathesis
ROMP	ring-opening metathesis polymerization
ROS	reactive oxygen species
Sec	selenocysteine
TPP	*meso*-tetraphenylporphyrin
Trx	thioredoxin
TrxR	thioredoxin reductase
TTP	*meso*-tetra(*p*-tolyl)porphyrin
Val	valine
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 N-Heterocyclic Carbenes: A Versatile Ligand

Carbenes are defined as neutral compounds possessing a divalent carbon atom with a six-electron valence shell. Due to their incomplete electron configuration and coordinative unsaturation they were traditionally regarded as highly reactive species merely present as reaction intermediates.[1,2] Indeed, prior to the challenging isolation of free carbenes, metal complexes bearing and stabilizing carbene moieties as ligands were introduced (Fig. 1).

It was E. O. Fischer who prepared and characterized the first metal carbene complex, methoxyphenylmethylene tungsten(0) pentacarbonyl, in 1964.[3] Ever since, so-called "Fischer carbene complexes" represent electrophilic complexes of late transition metals in low oxidation states which are coordinated to singlet carbenes adjacent to electronegative substituents.[4,5] The nucleophilic congener of Fischer-type carbene complexes were first prepared by R. R. Schrock in 1974 and are referred to as alkylidene or "Schrock carbene complexes".[6] The latter usually incorporate early transition metals of high oxidation states which are bound to triplet carbenes adjacent to hydrogen or alkyl groups.[4,5]

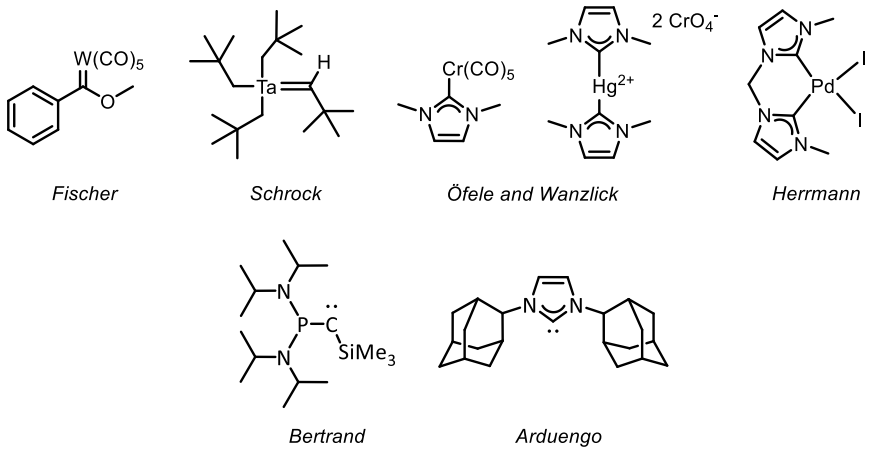


Fig. 1. Milestones in chemistry of carbenes. Above: Early transition metal carbene complexes by Fischer[3] and Schrock[6]. First isolated NHC complexes by Öfele[7] and Wanzlick[8] as well as Pd-based NHC complex[9] applied in homogeneous catalysis. Below: First stable carbene and *N*-heterocyclic carbene by Bertrand[10] and Arduengo[11], respectively.

One of the most prominent class of Fischer-type carbenes are *N*-heterocyclic carbenes (NHC) of which chromium and mercury-based complexes were, for the first time, independently prepared by Öfele[7] and Wanzlick[8] in 1968. A couple of decades later, a milestone in the quest for free carbenes was reached when Bertrand *et al.*[10] isolated a carbene compound stabilized by silicon and phosphorus atoms, followed by Arduengo *et al.*[11] who then described the first free and "bottleable" *N*-heterocyclic carbene, 1,3-di(adamantyl)imidazol-2-ylidene, in 1991. This was the triggering event for a plethora of structurally diverse *N*-heterocyclic carbenes reported to date.[1,2,12–15]

Initiated by Herrmann *et al.* who described the first catalytic application of palladium-based NHC complexes in 1995[9], *N*-heterocyclic carbenes have ever since become indispensable in homogeneous catalysis either serving as organocatalysts[16] or, to a much greater extent, acting as excellent ligands for different transition metals[14] including Fe[17], Pd[18], Au[19] and Ru[20]. Indeed, over the last years, they have superseded the common phosphine ligand for good reason. NHCs and phosphines behave similarly when binding to metals as both exhibit strong σ-donor and weak π-acceptor character. However, carbenes are typically more electron-donating leading to thermodynamically stronger metal-ligand bonds which are often persistent towards moisture, oxygen and heat. Furthermore, the spatial arrangement of phosphines is cone-like shaped whereas NHC ligands can be visualized as fans or umbrellas with the N-substituents ('wing tips') closer positioned towards the metal center. As a consequence, the choice of the nitrogen side chains can serve as a means to control the metal's reactivity and catalytic potential. Indeed, one of the most exceptional properties of NHCs is their synthetic flexibility giving rise to a series of tailor-made designs by facile structural and electronic modifications of the 'wing tips', the backbone and by the choice of heterocycle (Fig. 2).[1,5,13]

The unique characteristics of NHC ligands in metal complexes have soon been utilized in research fields beyond catalysis. Today, they are additionally largely applied as functional materials and have even demonstrated promising potential in the treatment of pathogenic microorganisms and cancer (Fig. 2).[1,21]

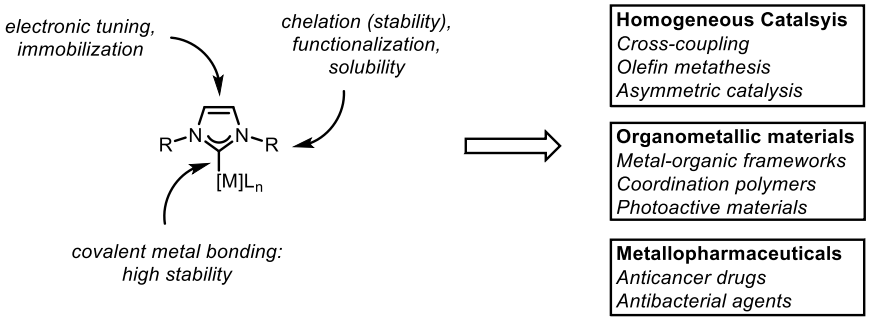


Fig. 2. General properties of organometallic NHC complexes offering a wide variety of application fields, mainly in homogeneous catalysis, as organometallic materials and metallopharmaceuticals.[1,21]

1.2 Olefins Today – Demand and Offer

1.2.1 Rising Quest for the C=C Motif

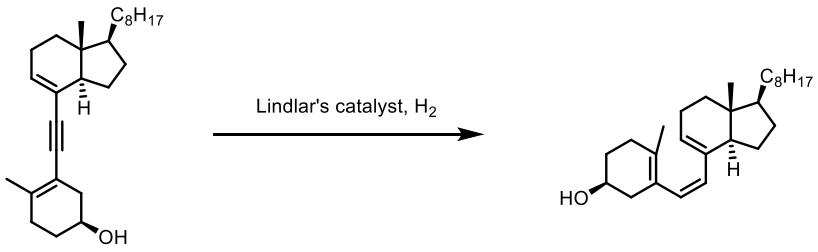
Next to ammonia and methanol, lower alkenes (ethene, propylene, butadiene) and aromatics ("BTX": benzene, toluene, xylene) belong to one of the limited number of base chemicals exploited as starting material for approximately 85% of chemical products such as agrochemicals, consumer chemicals, pharmaceuticals and polymers.[22] In particular, the increasing demand of the latter since the beginning of industrial polymer production in the 20th century has boosted the production scale of olefins deriving from steam cracking of naphtha (or ethane) and catalytic reforming processes.[22,23] In 2015, for example, the European petrochemicals industry produced 20.000 kt of ethylene, of which 62% was solely used for the synthesis of polyethylene. The residual amount of ethylene serves as feedstock for other essential organic products including ethyl chloride, ethylene oxide, ethyl benzene and ethanol.[24]

In fact, the rich chemical reactivity of olefins turns them into versatile synthons for a vast variety of organic transformations.[25,26] Due to the planar molecular geometry the exposed sites along the double bond exhibit high electron density and concurrently low steric hindrance which allows facile attacks by various electrophilic compounds. Therefore, olefins are typically prone to electrophilic addition paving the way for diverse reactions including halogenation, hydration and epoxidation. Nonetheless, olefins which are appropriately substituted with electron-withdrawing groups (*e.g.* α,β-unsaturated carbonyl compounds) can also undergo nucleophilic addition reactions such as the Michael reaction, a common method for the formation of novel carbon-carbon bonds. Today, olefins are utilized as feedstocks in a great variety of industrial processes for the production of *e.g.* alkyl nitriles (hydrocyanation), aldehydes (hydroformylation)[27] and carbon-carbon bonds[28] (olefin metathesis, polymerization).

1.2.2 Synthetic Routes to C-C Double Bonds

A considerable amount of olefins still derives from the petrochemical industry which, depending on the geographical conditions, offers different feedstocks including light saturated hydrocarbons (*e.g.* North America), naphtha (*e.g.* Europe) and light and heavy gas oils.[22,23] Next to other methods, steam cracking is the principal industrial process applied for the production of light alkenes, in particular of ethylene. During this process low-boiling alkenes such as pentene and butadiene as well as benzene-rich pyrolysis gasoline are obtained as important byproducts. In general, steam cracking operates at high temperatures (400 – 500 °C) in the presence of large amounts of steam. Due to finite fossil fuels the scientific community increasingly focuses on the replacement of petrochemicals by biomass resources such as fatty acids and carbohydrates.[29]

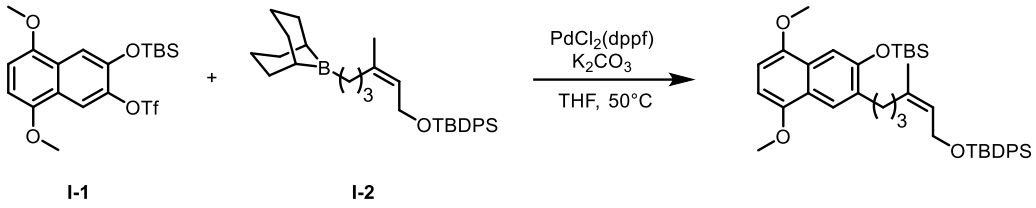
In a much smaller scale *cis*-alkenes can be selectively synthesized by the partial hydrogenation of alkynes in the presence of the commercially available heterogeneous Lindlar's catalyst. It consists of palladium and quinoline deposited on $CaCO_3$ and is poisoned with various forms of lead co-catalysts (lead acetate, lead oxide) in order to prevent total hydrogenation to alkanes.[30,31] The stereoselectivity is due to the nature of the heterogeneous catalysis where hydrogen can only be bound on the catalytic surface resulting in *cis*-alkenes exclusively. Although the Lindlar catalyst is widely used, particularly in the synthesis of natural products (Scheme 1) and pharmaceuticals[32,33], it suffers from low yields and low selectivity for the semihydrogenation triggering investigations on updated Pd-based reducing agents.[34]



Scheme 1. Application of the Lindlar's catalyst during the synthesis of Vitamin D.[33]

In the last quarter of the 20th century transition metal-catalyzed cross-coupling reactions have emerged as powerful tools in organic chemistry to generate new carbon-carbon bonds - a strategy for which Suzuki[35], Negishi[36] and Heck[37] were awarded with the Nobel prize in 2010. The catalytic cycle is believed to be initiated by the oxidative addition of the organic substrate bearing a potent leaving group (**I-1**, Scheme 2) to the catalytically active metal center (usually Pd or Ni) followed by the transmetalation step

with a metallated compound (**I-2**) leaving the catalytic metal center with both organic moieties. In the final reductive elimination step the organic moieties are coupled with the reformation of the catalyst. It should be noted that the mechanism is slightly different for the Mizoroki-Heck reaction.[37] Today, there is a series of cross-coupling reactions incorporating metallated substrates based on different metals including Sn, Zn, Mg and Si, and each bearing characteristic advantages and limitations. Notably, *N*-heterocyclic carbenes have increasingly been applied and gradually outweigh traditional phosphine ligands.[38]



I-1 **I-2**

Scheme 2. Example of Suzuki coupling reaction utilized during the total synthesis of halenaquinone.[39]

A further wide-spread synthetic route for alkenes is the catalytic olefin metathesis which is referred to as the bimolecular cleavage and reconstitution of carbon-carbon double bonds by the exchange of alkylidene groups. The reaction was first reported in the mid-1950s and has enjoyed great recognition not least due to its potential to synthesize functionalized polymers according to ROMP (ring-opening metathesis polymerization) and ADMET (acyclic diene metathesis polymerization) and to transform terminal alkenes into internal ones.[40] Moreover, RCM (ring-closing metathesis) is commonly used as a straightforward formation of ring motifs as exemplarily shown in Fig. 3.[41] After the determination of the mechanistic key intermediates by Chauvin, the design and thus the activity of the catalyst could be accordingly developed. In general, the catalyst complexes bear an alkylidene moiety which have been increasingly optimized over the last decades. The initially applied molybdenum-based Schrock carbenes (1990) were soon replaced by ruthenium-based catalysts, Grubbs' 1st generation (1992) and Grubbs' next generations bearing *N*-heterocyclic carbene ligands[20] (since 1999), respectively.

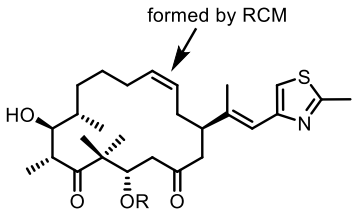
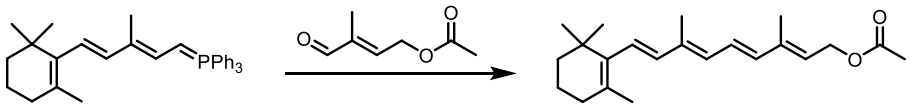


Fig. 3. Example of RCM (ring-closing metathesis) applied for the synthesis of an advanced intermediate of cytotoxic agent epothilone A.[41]

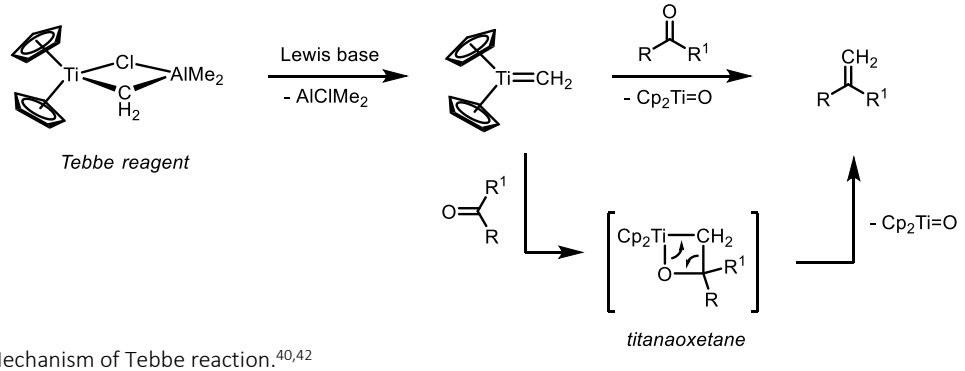
To date, the probably most prominent and common metal-free method to generate C-C double bonds is the Wittig reaction.[42,43] It describes the reaction of a phosphorus ylide (phosphorane, Wittig reagent) and a carbonyl compound (aldehyde and/or ketone) to a four-membered phosphaoxetane ring

intermediate which subsequently splits into alkene and phosphorus oxide as byproduct. The Wittig reaction is favored due to its high stereoselectivity which can be tuned by varying different factors including nature of ylide, carbonyl compound and type of solvent. In general, *cis*-selectivity is achieved from "non-stabilized" ylides (electron-donating group, *e.g.* alkyl) and bulky and/or aliphatic aldehydes whereas "stabilized ylides" (electron-withdrawing group, *e.g.* CN) and aromatic aldehydes favor *trans*-alkene formation. Although the Wittig reaction is widely applied in laboratories and industrial processes (Scheme 3)[44] it suffers from disadvantages such as limited conversion with less reactive carbonyl compounds, side reactions with base-sensitive substrates and hindered separation of the $Ph_3P=O$ byproduct. Ameliorated alternatives which can overcome some of the mentioned drawbacks are the Horner-Wadsworth-Emmons reaction and Julia reaction, respectively.[42]



Scheme 3. Industrial application of the Wittig reaction during one of the final BASF process steps for the total synthesis of vitamin A.[44]

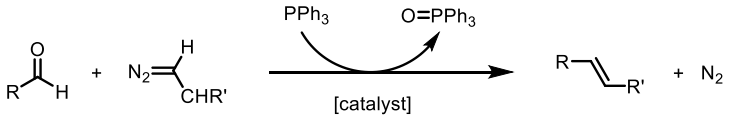
The organometallic equivalent to the Wittig reaction was introduced in the 1970s when R. R. Schrock demonstrated that niobium and tantalum complexes with neopentylidene ligands behave as "transition metal ylides" by transferring the neopentylidene ligand to carbonyl moieties including less reactive esters.[42] Triggered by this discovery, Fred Tebbe, a co-worker of Schrock during that time, observed a similar chemical behavior for a titanium-based alkylidene complex.[45] The latter is formed *in situ* in the presence of a *Lewis* base (*e.g.* 4-Dimethylaminopyridine) which then reacts with carbonyl compounds to terminal olefins *via* titanaoxetane intermediate (Scheme 4).[40,42] The range of transferable carbene moieties, which is restricted to methylene for the Tebbe reagent, was broadened with the introduction of a tungsten-based alkylidene complex. Hence, the transfer of carbenes bearing cyclic, aliphatic and aromatic moieties was possible.[46] However, since this reaction requires equimolar quantities of metal compounds, subsequent research focused on the design of organometallic complexes which can overcome this limitation in a catalytic fashion.



Scheme 4. Mechanism of Tebbe reaction.[40,42]

1.3 Catalytic Olefination of Aldehydes

Since the 1980s the catalytic olefination of aldehydes represents an elegant alternative in order to generate carbon-carbon double bonds. In contrast to the Tebbe reagent, the original carbene donor is most commonly a diazo compound (*e.g.* ethyl diazoacetate, EDA) which readily reacts with the formation of gaseous dinitrogen. Furthermore, similar to the Wittig reaction a tertiary phosphine compound such as triphenylphosphine is utilized as oxygen acceptor from the aldehyde (Scheme 5).



Scheme 5. Schematic aldehyde olefination using a diazo compound and triphenylphosphine in the presence of a metal-based catalyst.

Over the last years a series of catalysts based on various metals including Mo, Re, Ru and Fe bearing different types of ligands have been studied for their catalytic activity. As a result of this catalyst screening, three main mechanistic pathways have emerged which are displayed in Fig. 4. In the following section the presented modes of action will be discussed by means of selected metal complexes.

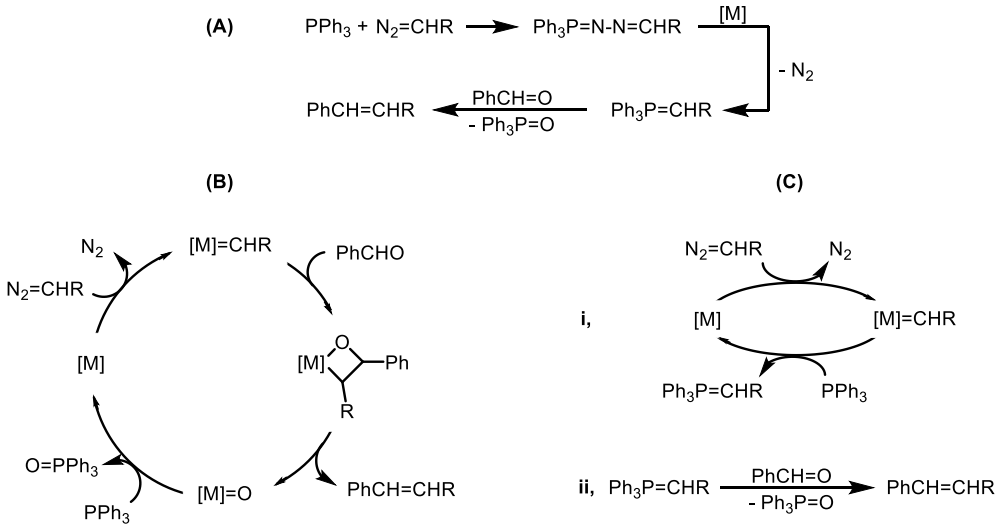
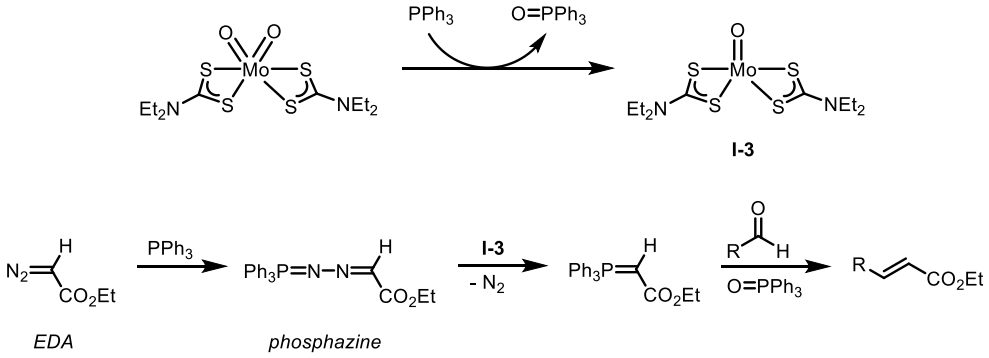


Fig. 4. Three major mechanistic pathways of the aldehyde olefination for a series of metal-based catalysts reported to date. (A) Catalytic ylide formation from phosphazine. (B) Olefination *via* metallacycle intermediate. (C) Catalytic carbene transfer from metal intermediate to phosphorus.

In 1986, Schwartz *et al.* demonstrated that a simple molybdenum complex, $MoO(S_2CNEt_2)_2$, reacts readily with diazoalkanes (N_2CHR) to give adducts, "metalloazines", which behave as carbonyl

equivalents interacting with phosphoranes according to the Wittig reaction. The reaction is accompanied by the release of dinitrogen at room temperature. Thus, depending on the substituents of the diazoalkane and the phosphorane, a variety of asymmetric olefins can be obtained.[47] Although free phosphine and molybdenum complex are regenerated in this reaction, the reagents were observed to continue to react resulting in mixtures of by-products which impede a clean catalytic reaction.

A few years later, Lu *et al.* reported on a similar molybdenum complex, $MoO_2(S_2CNEt_2)_2$, which, however, was catalytically applied (10 mol%) in the synthesis of olefins from various aldehydes, EDA and PPh_3. In general, high olefin yields (< 83%) with strong *E*-selectivity were obtained at 80 °C after few hours. In particular, aromatic aldehydes bearing an electron-donating group yielded higher olefin amounts than aliphatic aldehydes and aromatic congeners exhibiting electron-withdrawing substituents. As this order of activity is in contrast to the one observed in the Wittig reaction, a different mechanistic pathway was assumed. Investigations resulted in the development of two possible pathways, whereas a catalytic ylide formation from phosphazine (as demonstrated in Fig. 4A) was considered as the prevailing one (Scheme 6). The catalytically active molybdenum species **I-3** is initially formed *in situ* by reducing $MoO_2(S_2CNEt_2)_2$ with PPh_3. Subsequently, phosphazine, which is readily formed from ethyl diazoacetate and triphenylphosphine, releases dinitrogen in the presence of catalyst **I-3** resulting in the production of phosphorane. The latter gives rise to the olefinic coupling product with aldehydes according to a Wittig-like reaction. The reaction intermediates were each synthetically confirmed, *e.g.* isolated phosphazine was treated with **I-3** resulting in the Wittig reagent, $Ph_3P{=}CHCO_2Et$.[48]



Scheme 6. Proposed main pathway in the aldehyde olefination reaction catalyzed by molybdenum complex $MoO_2(S_2CNEt_2)_2$.[48] This pathway correlates with the general illustration in Fig. 4A.

Recently, Liu and Chang *et al.* reported on stereoselective aldehyde olefination using differently substituted Fe(IV)-corrole complexes as catalysts (Fig. 5, left).[49] Among these, the highest activity was observed for complex **I-4a** and was reduced with increasing number of electron-withdrawing corrole substituents ($-C_6F_5$). The reaction of benzaldehyde (1.0 equiv.), EDA (2.0 equiv.) and triphenylphosphine (1.1 equiv.) yielded 88% of an isomer mixture of olefin (*trans*/*cis* = 24:1) in the presence of 1 mol% of **I-4a** at 40 °C after 12h. Similar to the observations gained for the Mo-based complex described above,

olefin yields were lower for electron-deficient aryl aldehydes. By means of ^{31}P NMR spectroscopy it has been observed that the reaction of PPh_3, EDA and catalytic amounts of **I-4a** yielded large amounts of phosphazine which gradually was replaced by phosphorus ylide leading to the assumption of a catalytic ylide formation as shown in Fig. 5, right. In more detail, a side-on coordination of the phosphazine to the iron metal center which, based on electrostatic interaction, leads to a four-membered ring intermediate has been proposed. The latter releases dinitrogen and the Wittig reagent upon intramolecular rearrangement regenerating the free catalyst.

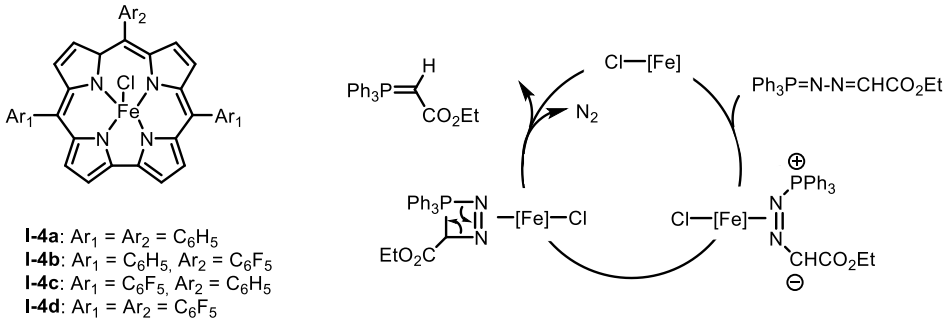


Fig 5. Left: Iron(IV)-corrole catalyst applied in the aldehyde olefination by Liu and Chang *et al.* Right: Catalytic ylide formation from Fe(IV)-corrole complex and phosphazine. [49]

To date, the olefination of aldehydes proceeding *via* metallacycle intermediates (Fig. 4B) is mainly limited to Re-based catalysts and has been supported by various studies.[50–58] In 1991, Herrmann *et al.* first demonstrated that methyltrioxorhenium(VII) (MTO) is highly active in catalyzing the olefination of aldehydes[50] next to numerous other catalytic reactions[57] including olefin metathesis[59] and epoxidation[60,61]. Stochiometric amounts of different aldehyde and diazoalkane species were treated with PPh_3 in the presence of MTO (1-10 mol%) and no significant change in the high product yield was observed when varying the reaction temperature (- 20 °C to + 80 °C). The catalysis with MTO distinguishes in several aspects from the one observed for the molybdenum-based complex reported by Lu *et al.* For example, higher olefin yields are obtained for aldehyde substrates bearing electron-withdrawing substituents. Consequently, Herrmann *et al.* assumed a novel possible mechanism for the Re-catalyzed aldehyde olefination, namely the one presented as pathway B in Fig. 4.[52,54,56]

The first step of the catalytic cycle is the *in situ* formation of the supposed active catalyst species, methyldioxorhenium (MDO), which was already known to be generated upon oxygen abstraction by phosphines from MTO (Fig. 6).[51] Then MDO forms a rhenium carbene species with the diazoalkane under the liberation of dinitrogen and release of the two coordinated phosphine compounds. The metal carbene subsequently reacts with the aldehyde to give a metallacycle intermediate which decomposes into the olefinic product upon regenerating MTO.

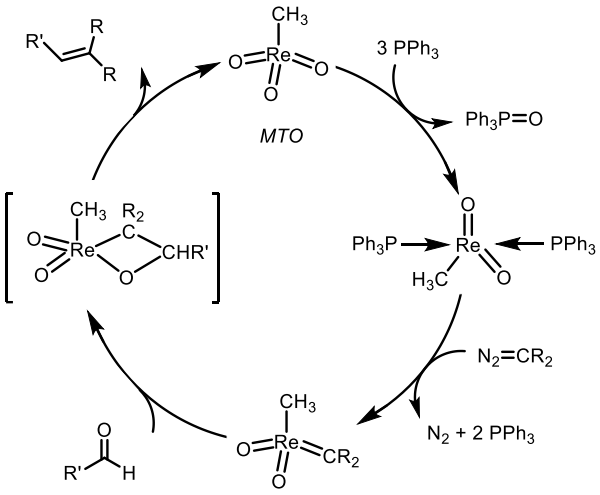


Fig. 6. Mechanism of aldehyde olefination catalyzed by MTO proposed by Herrmann *et al.*[50–52,54,56] This pathway correlates with the general illustration in Fig. 4B.

In contrast to the postulated metallacycle intermediate in Fig. 6, the existence of a metal carbene species during the reaction could be confirmed by follow-up studies. By means of ^{13}C NMR spectroscopy Romão and Kühn *et al.*[58] were able to detect a Re(V) carbene signal with the typical chemical shift in the far low field ($\delta(^{13}C)$ = 323.333 ppm). For this purpose, they prepared a mixture of PPh_3, EDA and $CH_3ReO_2[(C_6H_5)C \equiv C(C_6H_5)]$ (1:1:0.5), an adduct of MDO and diphenylacetylene, in deuterated chloroform and measured it at -30 °C. Further mechanistic investigations were performed for a plethora of $Re(VII)O_3$, $Re(V)O_2$ and Re(V)O complexes bearing C-, N- and P-based ligands, respectively. As a result, the most active Re-based catalysts are described as highly Lewis acidic metals coordinated to sterically unsaturated moieties (*e.g.* as in MTO) or easily removable ligands (*e.g.* PPh_3). Moreover, selected Re-complexes were shown to interact with phosphazine to give a Re carbene species instead of phosphorane which is in contrast to the molybdenum-based mechanism by Lu *et al.*[48]

Although Fujimura *et al.*[62] were the first working group to describe a catalytic cycle as shown in Fig. 4C in 1998, a detailed mechanistic examination on the ylide formation as a result of carbene transfer from the ruthenium carbene intermediate to the phosphorus moiety is missing. It was Woo *et al.* who performed in-depth investigations for an iron(II) porphyrin system (Fig. 7) which showed efficient and stereoselective olefination of aldehydes and ketones.[63,64] At ambient temperature olefin yields of up to 99% were reached in the presence of 0.1-2 mol% of Fe^{II} complexes bearing *meso*-tetra(*p*-tolyl)porphyrin (TTP)[63] and *meso*-tetraphenylporphyrin (TPP)[64] ligands, respectively. In order to exclude a potential metallaoxetane species (in this case oxoiron(IV)) indirect methods were performed. Since iron(II) complexes are known for their potential to epoxidize olefins *via* oxoiron(IV) species[65], reactions of benzaldehyde, EDA and styrene were set up in the presence of catalytic amounts of iron(II) porphyrin. Instead of epoxidation products cyclopropanation products were yielded indicating an iron carbene

intermediate.[66] Attempts to replace styrene by cyclohexene, which is less prone to cyclopropanation reactions, also failed as the products included ethyl maleate and fumarate deriving from iron carbene intermediates. Based on the typical reactivity profile known for metal carbenes (cyclopropanation, dimerization of carbene ligands) and the key control experiment that PPh_3, EDA and catalyst give phosphorane (as identified by means of ^{1}H and ^{31}P NMR spectroscopy) a mechanism demonstrated in Fig. 7 was assigned to the iron(II) complexes. Shortly after, a series of potential metalloporphyrins incorporating V, Cr, Mn, Co, Ni, Cu and Ru metal centers were introduced whereas catalytic activity was exclusively observed for Fe^{III}(TPP)Cl and Ru^{II}(TPP)CO and to a lesser extent for Co^{II}(TPP). With regard to the mechanism of the iron(III) complex Zhang *et al.* assumed the *in situ* reduction to iron(II) by EDA followed by the catalytic phosphorane formation similar to the mechanism proposed by Woo *et al.*[67,68]

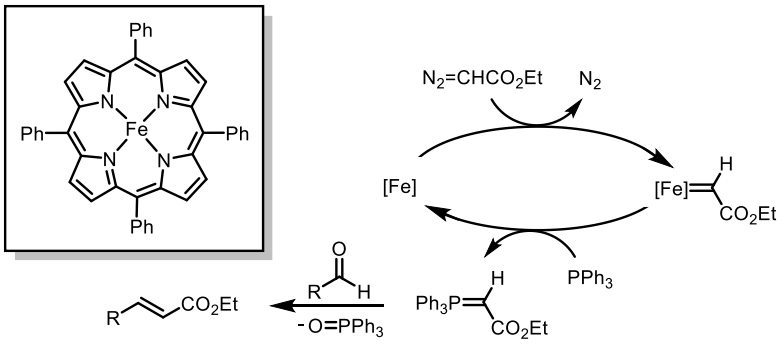


Fig. 7. Catalytic ylide formation during aldehyde olefination exemplified for the Fe(II) TPP system.[63,64] This pathway correlates with the general illustration in Fig. 4C.

Initiated by the pioneering work of Woo *et al.* the use of iron-based natural product catalysts for the aldehyde olefination was further spread throughout the scientific community.[69] In particular, Fasan *et al.* reported on the first engineered myoglobin variants as highly stereoselective and efficient biocatalysts reaching turnover numbers of 1,100-4,900 in the presence of $AsPh_3$ yielding olefins for a variety of aryl aldehydes and alkyl α-diazo acetates.[70]

Interestingly, in contrast to the majority of Re-based literature indicating a catalytic mode of action according to Herrmann *et al.* (Fig. 4B and Fig. 6) Chang *et al.* steered in another mechanistic direction for $[ReO_3(bipy)]^+[ReO_4]^-$.[71] By means of time-dependent ^{31}P NMR spectroscopy they observed a correlation between the decrease of phosphine and the increase of phosphorus ylide formation whereas the latter reduced according to the rise of phosphine oxide. Hence, they excluded the catalytic cycle proposed by Herrmann *et al.* where the phosphine is directly transformed to phosphine oxide and no other phosphorus species is present. Furthermore, DFT calculations and gas-phase reactions of mass-selected ions indicate the presence of rhenium carbene and rhenium phosphorane species analogously to the mechanism depicted in Fig. 7.

1.4 Gold NHC Complexes in Anticancer Therapy

1.4.1 Cancer – An Overview

According to the World Health Organization (WHO) noncommunicable diseases such as cardiovascular diseases, cancers and diabetes are related to the highest number of deaths worldwide.[72] Each year, noncommunicable diseases cause up to 38 million deaths (particularly in low- and middle-income countries) which is equal to 68% of global deaths.[73] Among these diseases cancer represents a growing burden for humanity which significantly increases annually. While in 2003 approximately 10 million people developed cancer and 6.2 million cancer-related deaths were reported, in 2014 cancer incidents rose up to 14.1 million with a morbidity rate of 8.2 million.[74] For the near future an upward trend of these numbers is expected due to the increasing life expectancy of modern population coupled with an unhealthy life-style. The most common risk factors include malnutrition, physical inactivity, drug abuse and urban air pollution. Furthermore, the use of tobacco causes approximately 20% of global cancer deaths in addition to viral infections (*e.g.* Hepatitis B and C viruses, Human papillomavirus) which are related to 20% of cancer-related deaths in low-middle income countries.[75] In 2012, the three most common sites of cancer diagnosed for men were lung, prostate and colon and for women breast, lung and colon, respectively.[73] Due to the progressive achievements in medicine the survival rates for patients of breast and prostate cancers have slightly increased over the last years, however substantial development in this area of research is strongly needed.

Cancer is a term for a collection of diseases based on malfunctioning cells which have lost the ability to grow and divide creating tissues of normal shape and function, but instead, proliferate in an uncontrolled fashion resulting in tumors which destroy adjacent tissues.[76] The general conception of tumorigenesis (the formation of tumors) describes a multistep process consisting of tumor initiation, progression and proliferation. The tumor initiation is a result of genetic and/or epigenetic alterations including mutations, chromosomal aberrations, methylation of DNA and histone modifications.[77] In most cancer instances, mutations cause the production of oncogenes and the inactivation of tumor suppressor as well as DNA repair genes.[78] The transformation of normal to cancerous cells can be induced and promoted by various physical, chemical and biological factors. For example, ultraviolet and ionizing radiation induce DNA double-strand breaks, asbestos leads to the formation of hazardous radicals and the human papillomavirus inhibits tumor suppressor proteins. During the progression phase of tumorigenesis, the expression of cell growth-regulating genes is accordingly altered offering the platform for the uncontrolled cell proliferation. As tumor cells grow and divide rapidly they provide

themselves with the required supplies of oxygen and other essential nutrients by growing blood vessels (angiogenesis) in adjacent tissues followed by the spread of the tumor (metastasis).[79]

Apart from radiation and surgery a further common method to treat cancer is chemotherapy. The treatment of diseases by systematically modified chemicals was first achieved by Paul Ehrlich (1845 – 1915) in 1908 for the anti-syphilitic agent arsphenamine (trade name: Salvarsan). Thus, Ehrlich's ground-breaking chemotherapeutic approach triggered the development of novel cytotoxic substances such as Paclitaxel (trade name: Taxol) or chlormethine (trade name: Mustargen).[79]

1.4.2 Development of Anticancer Metallodrugs

In the late 1960s Barnett Rosenberg *et al.* accidentally discovered the first metal-based anticancer drug, namely cisplatin (trade name: Platinol, Fig. 8). After successful *in vivo* tests showing antitumor activity on mice, the compound was taken for clinical testing and was approved by the FDA (US Food and Drug Administration) in 1978.[80] Today, cisplatin is applied worldwide mainly for the treatment of testicle, ovarian and bladder cancer.

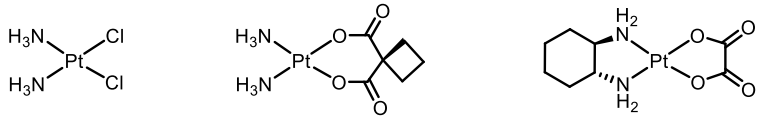


Fig. 8. Platinum complexes approved by the FDA for the worldwide treatment of cancer.

The first step of the established mode of biological action involves the activation of cisplatin $Pt[(NH_3)_2Cl_2]$ by intracellular aquation producing the species $Pt[(NH_3)_2(H_2O)_2]$. Then, DNA is platinated at the most nucleophilic binding sites, namely at the N7 positions of purine residues guanine and adenine. In general, one platinum center can bind two bases of the same DNA strand or on different strands resulting in intrastrand (major product) and interstrand (minor product) DNA cross-links, respectively. Thus, platination of the DNA generates substantially distorted DNA structures triggering cell cycle arrest and, to a greater extent, the inhibition of transcription resulting in the programmed cell death (apoptosis).[81]

Although cisplatin belongs to one of the most significant benchmark chemotherapeutic drugs it is notably toxic to the gastrointestinal tracts and kidneys (nephrotoxicity). Other common side effects are ototoxicity, nausea and vomiting. Moreover, during the course of chemotherapy many patients are faced with acquired drug resistance which may be due to (i) reduced intracellular accumulation, (ii) intracellular detoxification by binding to nucleophiles other than DNA (*e.g.* glutathione) and (iii) enhanced DNA-repair machineries.[80] With the purpose to overcome these limitations, further platinum-based metallodrugs (Fig. 8) were developed among which carboplatin (trade name: Paraplatin) and oxaliplatin (trade name: Eloxatin) were granted by the FDA in 1989 and 2002 for the worldwide treatment of ovarian and colorectal cancer, respectively.[82]

However, the intrinsic drawback of platinum complexes to bind DNA with limited selectivity for cancerous cells gave rise to synthetic approaches incorporating other biologically active metals. Over

the last years, a plethora of promising cytotoxic complexes bearing different metals were reported.[77,83,84] However, only a few accessed clinical trials (Table 1, Fig. 9).

Table 1. Selected metal complexes for therapeutic[a] and imaging[b] purposes currently in clinical trials for the treatment of cancer.

Drug name	*Incorporated Metal*	*Development Status*	*Indication*	*ClinicalTrials.gov Identifier*
TOOKAD®soluble	Pd	Phase III	prostate cancer[a]	NCT01875393
^{64}Cu-ATSM	Cu	Phase II	cervical cancer[b]	NCT00794339
(N)KP1339	Ru	Phase I	solid tumors[a]	NCT01415297
auranofin	Au	Phase II	CLL[a]	NCT01419691
		Phase I/II	NSCLC, SCLC[a,c]	NCT01737502

[c] combinational therapy with sirolimus. CLL = chronic lymphocytic leukemia. (N)SCLC = (non-)small cell lung cancer.

TOOKAD®soluble is a palladium-based photosensitizer suitable for patients with early prostate cancer. By irradiation with a specific wavelength it is believed to generate reactive oxygen species (ROS) which damage blood vessels leading to the deprivation of oxygen and nutrient to tumor cells.[85] The radiopharmaceutical agent ^{64}Cu-ATSM is used in combination with PET (positron emission tomography) imaging applications as a marker for tumor hypoxia enabling the localization of tumor sites.[86] Hypoxia is a condition where cells are deprived of oxygen which is an important determinant for tumors rapidly draining blood and thus oxygen supplies.[87] Besides, ruthenium(III) complexes such as (N)KP1339 which are attributed to lower toxicity and higher selectivity towards cancerous cells are currently in clinical trials. They were shown to target both the DNA and enzymes, while in particular protein kinases are regarded as their primary biological target.[84,88]

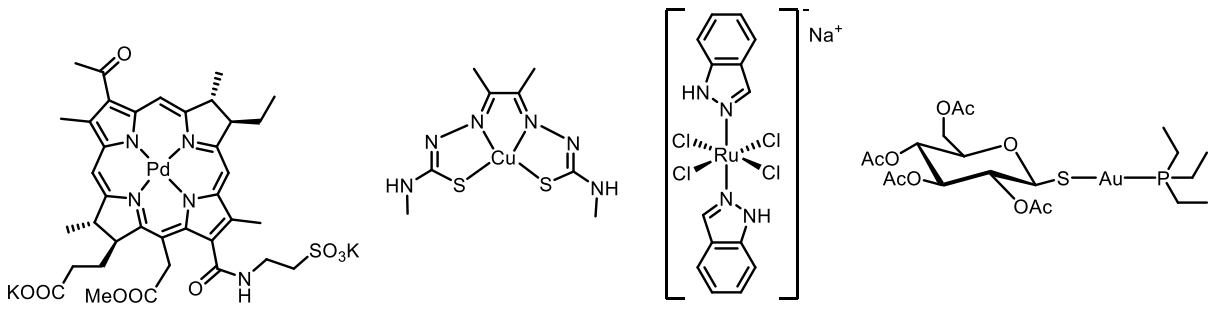


Fig. 9. Structures of metal-based anticancer agents currently in clinical trials.

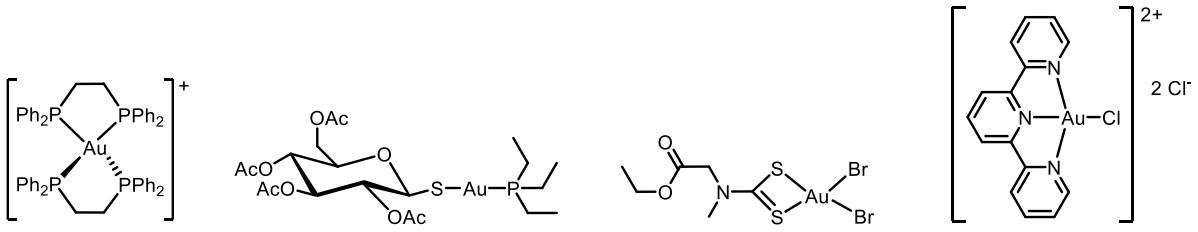
In particular, gold-based complex auranofin has gained increasing attention when the FDA-approved antirheumatic agent was repurposed for cancer treatment. Auranofin (trade name: Ridaura) currently

undergoes clinical trials for the treatment of CLL (chronic lymphocytic leukemia) and (Non-)Small Cell Lung Cancer, respectively. In CLL cells, auranofin activates the oxidative and endoplasmic reticulum (ER) stress responses which, under prolonged exposure, result in the programmed cell death (apoptosis).[89] Oral administration of the drug to *in vivo* mice models distinctly reduced the tumor expansion.[90,91] Notably, auranofin is almost completely eliminated from the body within two months and only traces of the administered dose is accumulated in the kidneys.[92] The damaging stress responses are attributed to the ability of auranofin to inhibit the seleno-enzyme thioredoxin reductase. In fact, this protein is referred to as the principal biological target for gold-based drugs.

1.4.3 Gold Complexes in Anticancer Therapy

The use of gold complexes for medicinal purposes dates back to over 2000 years ago.[93–95] Gold therapy for clinical purposes began in the early 20th century when the healing effect on joint pain associated with rheumatoid arthritis was observed.[96,97] Today, different antiarthritic gold(I) complexes such as auranofin, aurothiomalate (trade name: Myocrisin) and aurothiosulfate (trade name: Sanocrysin) are therapeutically applied, among which auranofin is unique for its oral administration. Gold-related side-effects were therefore mainly assessed from rheumatoid arthritis patients showing most commonly skin and mucous membrane hypersensitivity, diarrhea and to a lesser extent hematological abnormalities and nephrotoxicity.[98]

In 1979 pioneering work by Lorber *et al.* revealed cytotoxic properties of auranofin on HeLa cervical cancer cells.[99] Subsequent studies reporting its promising anticancer potential *in vivo*[100] and towards cisplatin-resistant cell lines[101] initiated the evaluation of structure-activity relationships for novel cytotoxic gold complexes bearing various types of ligands, including those are presented in Fig. 10.[102,103]



phosphines *[Au(dppe)$_2$]$^+$* | **thiolates** *auranofin* | **dithiocarbammates** | **N-donor ligands**

Fig. 10. Early reported cytotoxic gold complexes coordinated to different classes of ligand systems.[102] dppe = 1,2-bis(diphenylphosphino)ethane.

The common oxidation states of gold are +3, +1 and 0, among which Au(III) and, to a greater extent, Au(I) are predominantly used as therapeutic agents in the form of metal complexes. Nevertheless, it should be noted that over the last years gold nanoparticles have gained increasing interest as drug delivery systems, in photodynamic therapy and as therapeutic agent on its own.[104–106] Gold(III) ions are known to form square-planar complexes that are isostructural and isoelectronic (d^8) to Pt(II) complexes which explains the scientific interest for potential Au(III) anticancer drugs. However, the majority of studies focus on gold(I) complexes, partly due to their higher chemical stability in aqueous solution and the early discovery of the cytotoxic activity of auranofin. After the administration of coordination gold(I)

compounds to cells, they can readily undergo up to two step ligand exchange reactions with sulfur-containing biomolecules such as the tripeptide glutathione (GSH) and the drug-carrier protein albumin which transfers the metal into cells. The pharmacologically active species is therefore in many cases not the administered one.[107] For auranofin, the acetylthioglucose ligand is initially released, followed by the slower liberation of phosphine with the formation of $Ph_3P=O$.[108]

Although the exact mode of biological action is not completely understood, several studies point towards a completely different mechanism with respect to cisplatin and analogues. In fact, while Pt(II) complexes appear to be able to target nucleic acids as well as peptides and proteins, mechanistic studies on cytotoxic Au(I)/Au(III) complexes over the last years strongly suggest induction of apoptosis *via* targeting mitochondria in cancer cells. Mitochondria are not only the main energy producers for cells but also play a major role in initiating cell death *via* release of proapoptotic factors and production of ROS (reactive oxygen species).[109] Depending on the ligand system, Barnard and Berners-Price[110] reported two pathways for gold phosphine complexes to impair mitochondria:

(i) Cationic, tetrahedral gold(I) complexes such as the bis-chelated $[Au(dppe)_2]^+$ (Fig. 10) are less prone to ligand exchange reactions, and are well accumulated in cancer cells since they behave as delocalized lipophilic cations (DLCs). Thus, they can induce membrane permeability transition (MPT). The latter phenomenon is associated with loss of the mitochondrial membrane potential ($\Delta\psi_m$) resulting in the opening of mitochondrial permeability transition pores through which water and other small molecules can enter the organelle. This results in swelling of the mitochondrial matrix, bursting of the outer membrane and thus apoptosis.[111] In contrast to healthy cells, mitochondria in cancerous cells possess enhanced membrane potentials $\Delta\psi_m$ which is exploited as an approach to selectively target cancer by DLCs. In other words, the chemical nature of DLCs offer (i) the required lipophilic character to pass through the lipid bilayer and (ii) the positive charge in order to selectively accumulate in mitochondria of cancer cells instead of cytoplasm. Although $[Au(dppe)_2]^+$ showed promising *in vivo*[112] cytotoxicity, its great lipophilicity gives rise to toxic side-effects which is attributed to non-selective MPT also in healthy cells.[113] Consequently, the investigation on fine-tuning the lipophilic-hydrophilic balance of anticancer complexes was emphasized.

(ii) Neutral, linear gold(I) phosphines such as auranofin are more prone to thiol reactivity under physiological conditions and are often reported as potent inhibitors of the redox-active enzyme thioredoxin reductase (TrxR), which is known to be overexpressed in several types of cancer.[114] Indeed, the vast majority of published studies suggest TrxR as the principal biological target of cytotoxic gold(I) complexes.

The thioredoxin system consists of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH and regulates crucial cell functions including defense against oxidative stress and apoptosis and redox

signaling associated with many diseases.[114] For mammals three isoforms of TrxR are identified which are highly expressed in cytosol (TrxR1), mitochondria (TrxR2) and testis (TrxR3). At the N-terminal active site they possess a conserved -Cys-Val-Asn-Val-Gly-Cys- catalytic site which is similar to human glutathione reductase (GR). Although both TrxR and GR share similar structural and catalytic functions (both serve in the removal of hydrogen peroxide), the intracellular concentrations of GR and TrxR are in the millimolar and micromolar range, respectively. Moreover, thioredoxin reductases possess a highly accessible selenocysteine (Sec) residue on its flexible C-terminal arm which offers a unique binding site for the selective inhibition by potential anticancer agents. The thioredoxin system catalytically reduces many essential proteins including peroxiredoxin (Prx) which in turn transfers electrons to hydrogen peroxide generating water (Fig 11A). Biological assays demonstrate that auranofin and related compounds exhibit much higher affinity for TrxR than GR which is tentatively associated with the pronounced "soft" character of selenium compared to sulfur.[115]

This binding preference of Au(I) ions towards Sec was also substantiated by mass-spectrometry using a tetrapeptide that matches the C-terminal sequence of TrxR.[116] The studies indicate a preferential binding of gold to selenocysteine affording distinct Sec-Au adducts next to Sec-Au/Cys-Au dinuclear species (B1 and B2, Fig. 11). Notably, studies have shown that gold(I) complexes are usually more effective TrxR inhibitors than gold(III) compounds and that the cytosolic isoform of TrxR is more sensitive to gold species than the mitochondrial one.[109]

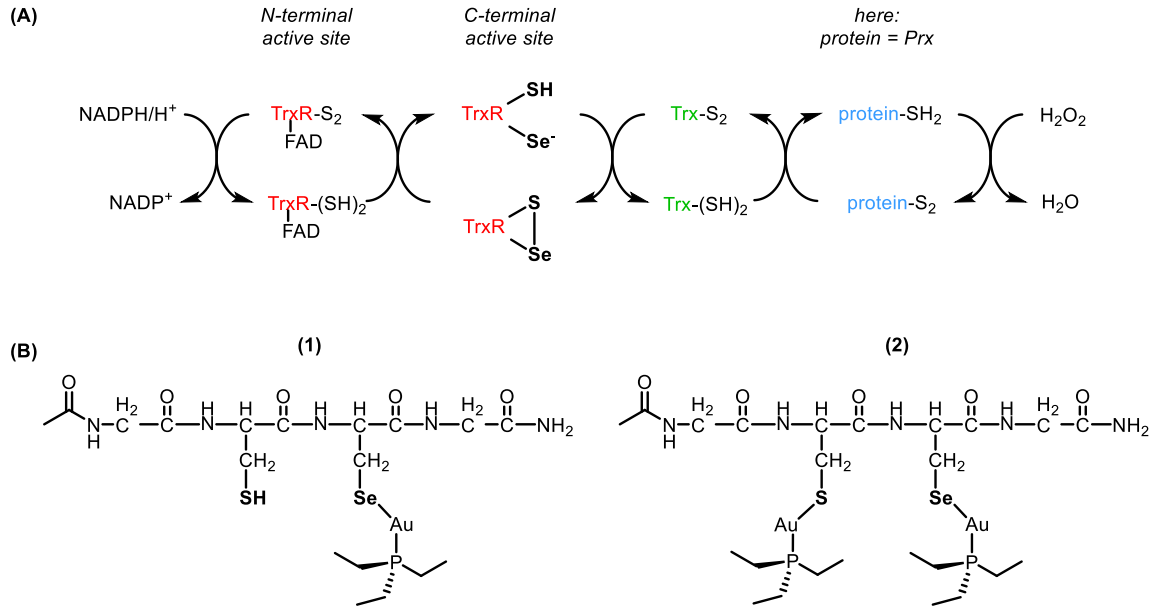


Fig. 11. (A) Thioredoxin system mediating the catalytic reduction of many proteins including peroxiredoxin (Prx) which in turn reduces hydrogen peroxide. (B) ESI-MS studies reveal that artificial C-terminal tetrapeptide sequence of TrxR treated with auranofin yields (a) monoadduct where gold selectively binds selenocysteine (Sec) and (b) bisadduct with both cysteine (Cys) and Sec, respectively.[116]

The crucial consequences of mitochondrial TrxR inhibition are release of proapoptotic factors and loss of the catalytic activity and ability to reduce intracellular H_2O_2 to water (Fig. 12). It should be noted that hydrogen peroxide is a ROS species and a natural metabolic product from the respiratory chain. Particularly in mitochondria, H_2O_2 concentrations are mainly regulated by TrxR as well as GR and a long-term imbalance of the cellular redox state by insufficient removal of ROS leads to cell death. Following inhibition of TrxR by gold compounds, resulting in enhanced H_2O_2 concentration, the oxidant crosses the mitochondrial membrane entering cytosol where it cannot be reduced by the likewise inhibited TrxR1 isoform and thus activates different apoptosis-stimulating signaling pathways (Fig. 12).[109]

Concerning Au(III) compounds, although TrxR is regarded as one of the main biological targets, recent studies demonstrate their reactivity with other proteins, in particular the water and glycerol membrane channel aquaporins, as well as the DNA repair protein PARP-1 (poly(adenosine diphosphate (ADP)-ribose) polymerase 1)[117]. Interestingly, in few cases gold compounds have been shown to also bind to DNA domains, as it will be discussed in the next section.[118,119]

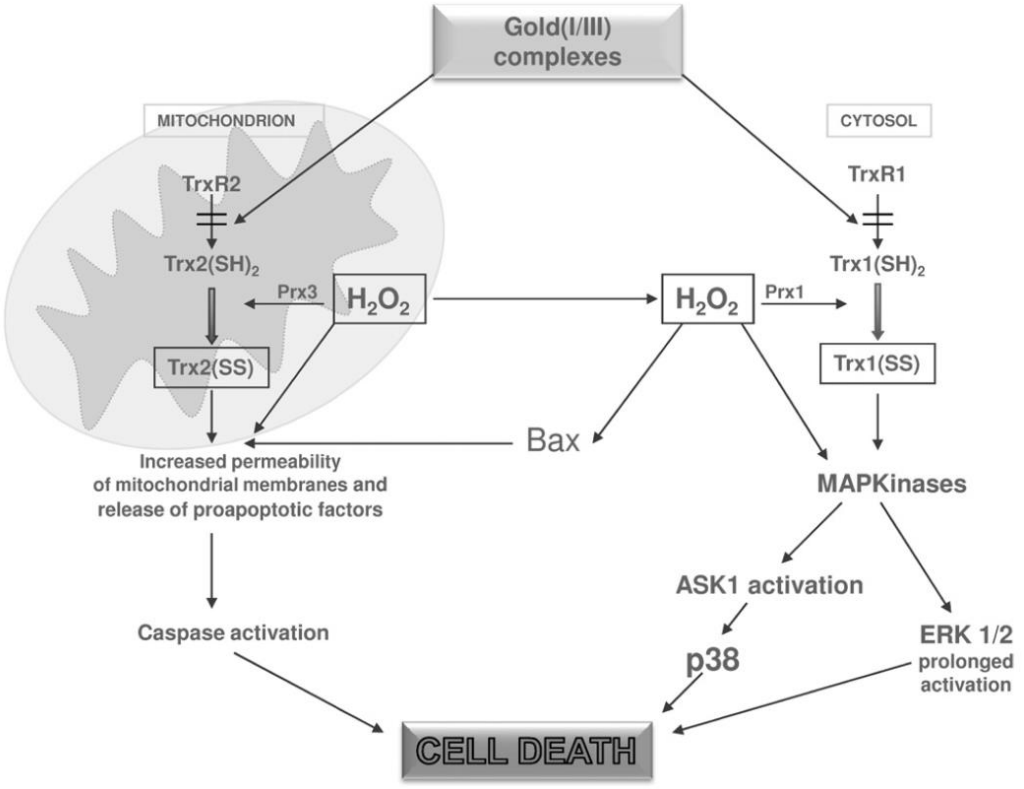


Fig. 12. Schematic illustration of cell death mechanism induced by gold(I, III) complexes. The inhibition of mitochondrial TrxR2 prevents the reduction of H_2O_2 by peroxiredoxin (Prx3) leading to the opening of mitochondrial permeability transition pores and release of proapoptotic factors. Hydrogen peroxide is released to cytosol where likewise inhibited TrxR1 cannot reduce it and stimulates the MAP (mitogen-activated protein) kinase signal transduction pathway causing apoptosis. ASK1 = Apoptosis signal-regulating kinase 1. ERK 1/2 = extracellular signal-regulated kinase 1/2. BAX = Bcl-2-associated X protein. *Reprinted from Coord. Chem. Rev., 253/11-12, Bindoli, A.; Rigobello, M. P.; Scutari, G.; Gabbiani, C.; Casini, A.; Messori, L., Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs, 1692-1707, Copyright 2009, with permission from Elsevier.*

Under physiological conditions, intracellular thiols such as glutathione (GSH) and albumin can readily reduce Au(III) ions to Au(I) and Au(0) which can give rise to enhanced toxicity of gold(III) as shown in mice models,[120] but also to extensive compound's deactivation in cells. In turn, naked Au(I) ions can undergo disproportionation reactions yielding Au(III) and Au(0) species. In order to sufficiently stabilize the therapeutic agents under physiological conditions, special emphasize has been put on organometallic gold complexes in recent years.

1.4.4 Rational Design of NHC Gold(I) Complexes

In order to achieve metallodrugs endowed with sufficient stability in physiological conditions, and still maintaining reactivity with pharmacological targets (e.g. via ligand exchange reactions), organometallic compounds have attracted increasing attention as promising anticancer agents. Specifically, organometallic gold complexes reported to display antiproliferative effects, can be roughly subdivided into three groups: (i) cyclometalated Au(III) complexes with di- or terdentate C,N donor ligand systems, (ii) Au(I) alkynyl complexes and (iii) gold complexes bearing *N*-heterocyclic carbene complexes (Fig. 13).[121] In particular, gold(I) NHC complexes have gained increasing interest as anticancer agents[122] as the ligand exhibits vast synthetic flexibility allowing the straightforward development of structure-activity relationships. In Fig. 13 a list of common characteristics of cytotoxic NHC gold(I) complexes studied to date are shown and will be illustrated by selected compounds (Fig. 14).

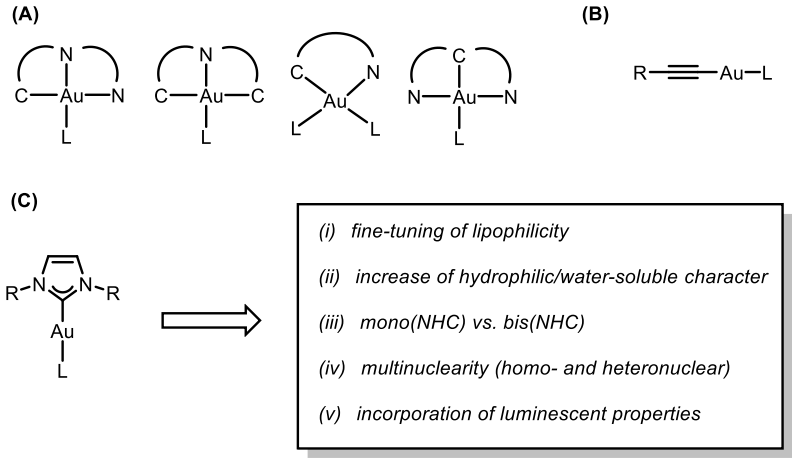


Fig. 13. General representation of organometallic gold(I,III) complexes bearing cyclometalated (A), alkynyl (B), *N*-heterocyclic carbene (C) ligands, respectively. The box includes common features of cytotoxic gold(I) NHC complexes studied to date.

For example, Berners-Price and Filipovska *et al.* demonstrated for three gold(I) NHC complexes **I-5** how the NHC ligand system can be readily fine-tuned in terms of lipophilicity which is necessary for balancing both cytotoxicity and selectivity towards cancerous cells.[123] At lower concentrations (< 25µM) all three complexes exhibit toxicity towards breast cancer cell lines (*e.g.* MDA-MB-231) but not towards healthy cells (HMEC). However, at higher concentrations the most lipophilic species **I-5c** loses its selectivity and becomes toxic towards normal cells whereas the least lipophilic compound **I-5a** remains selective but loses its general cytotoxicity. In contrast, the intermediate lipophilic compound **I-5b** retains the most optimal selectivity and cytotoxicity. Despite the required lipophilic character of a drug for membrane

permeability, a sufficient degree of hydrophilicity/water solubility is essential for *in vitro* investigations and efficient *in vivo* administrations associated with an enhanced bioavailability under physiological conditions. For this purpose, the NHC ligand is an ideal candidate as previously reported by Herrmann and Kühn *et al.*[124] For example, Dias and Santini *et al.* reported ester- and amide-functionalized gold(I) complexes **I-6a** and **I-6b** exhibiting moderate antiproliferative activity towards a series of cisplatin-sensitive (*e.g.* lung cancer A549) and -resistant (ovarian cancer C13*) cancer cell lines along with non-toxicity against healthy cells (human embryonic kidney cells HEK293).[125]

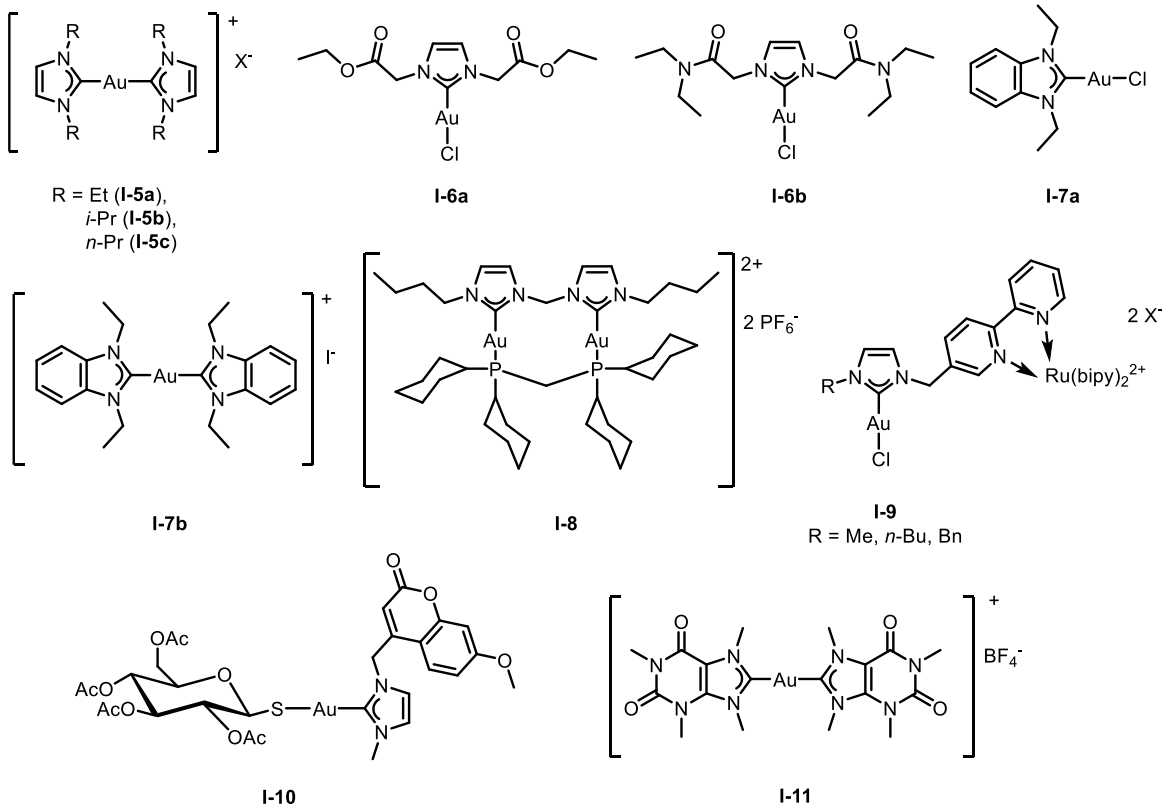


Fig. 14. Selected gold(I) NHC complexes exhibiting different (bio-)chemical and photophysical features depending on their ligand systems. X = PF_6^-/Cl^-/Br^-.

In addition, several research groups have investigated the effect of mono and bis(NHC) ligated gold(I) complexes on the anticancer efficacy.[126–129] Regarding the primary biological target, TrxR, a mono(NHC) gold(I) compound seems to possess a higher inhibitory activity due to the more facile ligand exchange reaction with Cys and/or Sec residues of the enzyme. For example, this trend was observed for the benzimidazolylidene gold(I) complexes **I-7a** and **I-7b**.[126] However, a more labile second ligand (*e.g.* halide) is also more reactive and thus more prone to deactivation due to reactions with other non-targeted cellular components.[130] Moreover, as TrxR is not the only biological target for anticancer therapy, a general trend correlating its inhibition and compounds' cytotoxicity is still treated with caution. For example, for a gold(I) complex structurally similar to **I-7b** additional DNA targeting character was attributed.[131]

Previous literature has also focused on the synthesis and biological evaluation of multinuclear gold(I) NHC complexes. For example, Che *et al.* demonstrated that the cytotoxicity of dinuclear gold(I) species **I-8** is less affected by pre-incubation with serum albumin than for mononuclear auranofin.[132] By means of ^{1}H NMR spectroscopy, a distinct 1:1 binding ratio between model TrxR peptide (Gly-Cys-Sec-Gly) and gold complex **I-8** was observed under liberation of the bis(dicyclohexylphosphine)methane ligand, indicating the inhibition of both binding sites, Cys and Sec. Furthermore, NHCs allow the incorporation of other cytotoxic metal moieties (*e.g.* Ru(II) arene[133]) and the formation of heterobimetallic complexes by appropriate functionalization of the wing-tip substituents. An elegant approach to combine both the cytotoxicity of gold(I) NHCs with the photophysical properties of $Ru(bipy)_3$ was shown by Hemmert and Gornitzka *et al.* for **I-9**.[134] The obtained luminescent properties of the mixed metal complex were therefore used to localize the compound in the cellular components and thus gain insight into the mode of biological action. Indeed, *N*-heterocyclic carbene ligands are ideal candidates for *in vitro* imaging purposes as they can be readily attached to typical fluorophores such as anthracenyl[135] and coumarin (*e.g.* compound **I-10**)[136] moieties, respectively.

Notably, caffeine-derived bis(NHC) gold(I) complex **I-11** was recently reported to be a selective binder of telomeric G-quadruplexes (G4) instead of duplex DNA[131] and that both caffeine ligands interact with guanine moieties of G4 by π-stacking.[137] G4s are guanosine-rich intrastrand secondary structures of DNA formed by G-quartets in a pseudoplanar arrangement (Fig. 15).[138] This type of DNA architecture is present in telomeric and promoter regions of the genome and are assumed to play important roles in key cellular events. Telomeric G4s are the guanine-rich repeats at the single-stranded ends of chromosomes and protect DNA from degradation. The enzyme telomerase is known to be overexpressed in many cancer cells and plays an important role in their immortalization. Stabilizing telomeric G4s and, thus, indirectly inhibiting the telomerase activity, is an elegant approach to selectively target cancer cells. Furthermore, the stabilization of G4s in promoter regions of certain oncogenes can also regulate their transcription.[139–141] In both cases, G4 stabilization can lead to either cytostatic or cytotoxic effects.

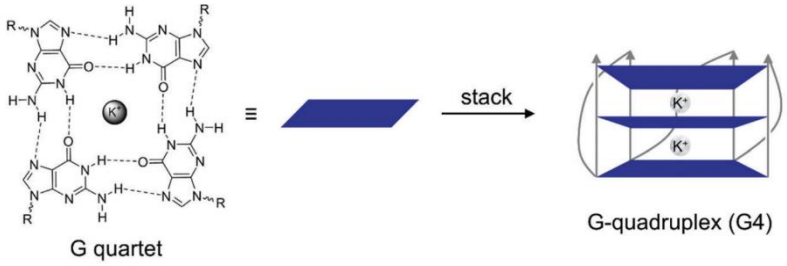


Fig. 15. Four guanine bases with central potassium cation in a Hoogsten hydrogen-bonding arrangement (G-quartet) forming G4 structures upon stacking.[138]

CHAPTER 2

OBJECTIVE

The first part of this work focuses on the olefination of aldehydes catalyzed by iron(II) complexes bearing *N*-heterocyclic carbene ligands. The dicationic complexes bearing bis(*N*-heterocyclic carbene)-bis(pyridine)[142] and cyclic tetra(*N*-heterocyclic carbene)[143] (**1** and **2**, Fig. 16) ligand systems were previously published by Herrmann and Kühn *et al.* and have proven to be efficient catalysts in a range of various oxidation reactions. Based on their distinct similarities with iron(II) porphyrin complexes (**3**) which are active in the aldehyde olefination (see section 1.3), compounds **1** and **2** display ideal candidates to be evaluated for their catalytic potentials. Notably, these Fe^{II}-based complexes form square-planar geometries and bear strong polydentate σ-donor ligands.

Furthermore, the proceeding mode of catalytic action is investigated compared with literature-known mechanisms as shown in Fig. 4. In doing so, the potential generation of organic and metal-based key intermediates including iron(IV)-carbene and oxo-iron(IV) species, respectively, is investigated. While the former intermediate is observed with similar iron(II) complexes such as **3**, the formation of oxo-iron(IV) species from **1** and **2** is assumed during catalytic epoxidation reactions.

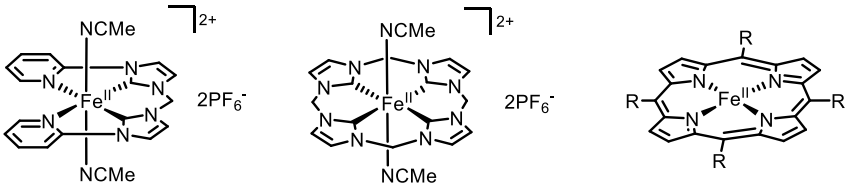


Fig. 16. Structures of iron(II) complexes bearing square-planar bis(*N*-heterocyclic carbene)-bis(pyridine) (**1**) and cyclic tetra(*N*-heterocyclic carbene) (**2**) and *meso*-tetraphenyl-porphyrin (**3**)[63,64] ligand systems utilized in the catalytic olefination of aldehydes.

The objective of the second part of this work is based on the development of hydrophilic gold(I) *N*-heterocyclic carbene complexes applied as potential anticancer agents which are investigated for their biological target. Next to an adequate lipophilicity in order to cross cell membranes, a promising anticancer drug ought to concurrently provide sufficient hydrophilic/water-soluble character. The latter is not only required for reliable *in vitro* investigations but it is also essential for an efficient drug administration along with an enhanced bioavailability under physiological conditions. For this purpose, especially the wing-tips of NHC ligands offer facile synthetic flexibility for an adequate functionalization.

The final gold(I) NHC complexes are studied for their mode of biological action, in particular for the compounds' inhibitory effect on the pivotal cellular target thioredoxin reductase (TrxR) in comparison with the inhibition of the structurally related glutathione reductase (GR). By means of biochemical assays and mass spectrometric approaches the molecular reactivity toward possible binding sites of TrxR is studied in-depth. The gold(I) compounds' overall cytotoxicity is evaluated using ovarian cancer

cell line 2008. Furthermore, beyond the anticancer drug target thioredoxin reductase (TrxR), G-quadruplex DNA is investigated as an additional potential target.

To date, a series of NHC-based organometallic complexes exhibiting anticancer properties have been reported to interact with several secondary DNA structures *via* different binding modes. In particular, non-coordinative binding of guanosine-rich quadruplex structures of DNA anticipates a promising strategy for the development of novel anticancer drugs exhibiting enhanced target selectivity along with reduced side effects.

CHAPTER 3

PUBLICATION SUMMARIES

3.1 Iron(II) N-heterocyclic Carbene Complexes in Catalytic One-Pot Wittig Reactions: Mechanistic Insights

Özden Karaca, Markus R. Anneser, Jens W. Kück, Anja C. Lindhorst, Mirza Cokoja, Fritz E. Kühn

Journal of Catalysis, 2016, *344*, 213-220

In this paper the catalytic performances of iron(II) *N*-heterocyclic carbene complexes **1** and **2** (Fig. 16) are investigated for the olefination reaction with aldehydes. The targeted olefin product, *E*-ethyl cinnamate, from benzaldehyde, ethyl diazoacetate (EDA) and PPh_3 was only obtained using complex **1**. In contrast, a distinct decomposition product from a possible oxidative addition reaction of complex **2** and EDA was identified. At optimal reaction conditions (70 °C, benzaldehyde/EDA/PPh_3/catalyst = 1/1.2/2/0.1) up to 90% of *E*-olefins are obtained after 2 h. Notably, the yield of *E*-ethyl cinnamate strongly depends on an excess of PPh_3.

In order to elucidate the proceeding mode of catalytic action, investigative approaches were conducted using **1**. Overall, as the key reaction the catalytic generation of the phosphorus ylide from a mixture of PPh_3, EDA and catalyst was demonstrated by different analytical techniques including GC-MS and NMR spectroscopy. The catalytically formed phosphorus ylide subsequently reacts with differently functionalized aldehydes according to an order of reactivity observed for the classic Wittig reaction: 4-nitrobenzaldehyde > benzaldehyde > 4-methoxybenzaldehyde. In fact, by means of time-dependent ^{1}H NMR spectroscopy, the gradual conversion of aldehyde and *in situ* generated phosphorus ylide to the target olefination product was observed at room temperature (Fig. 17).

Further studies were performed in order to rationalize the catalytic formation of the phosphorus ylide. In doing so, two possible pathways are assumed, namely (i) *via* an iron(IV) carbene intermediate (Fig. 4A) and (ii) *via* the transformation of phosphazine in the presence of uncoordinated PPh_3 (related to Fig. 4C), respectively. The first pathway is supported by the determination of products deriving from catalytic cyclopropanation of styrene and catalytic dimerization of EDA, respectively. These are typical reactions commonly applied as indirect methods for the detection of iron carbene intermediates, in this case of [Fe]=CH(CO_2Et). The second pathway is based on the detection of phosphorus ylide upon treating phosphazine, Ph_3P=N-N=CH(CO_2Et), with **1**. Notably, this reaction was exclusively observed in the presence of free PPh_3 which serves as nucleophile initiating the catalytic formation of the Wittig reagent from iron-phosphazine species **4** (Fig. 18).

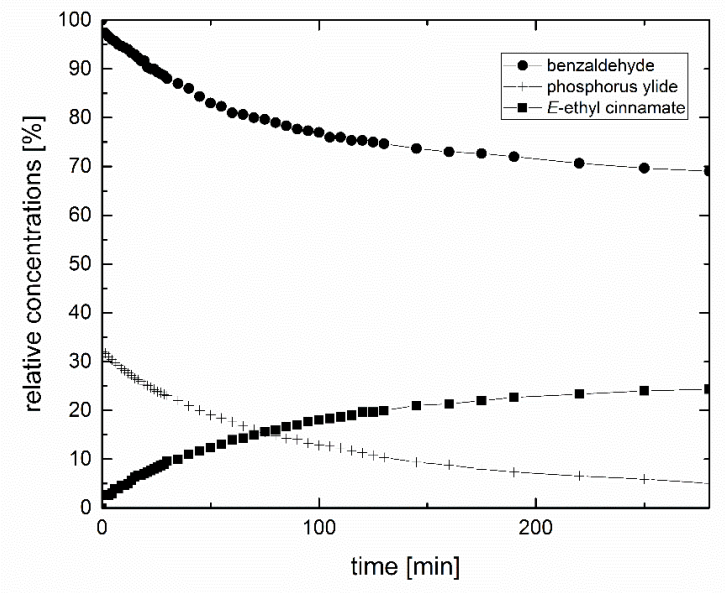


Fig. 17. Time-dependent product formation corresponding to conversion of aldehyde and *in situ* generated phosphorus ylide.

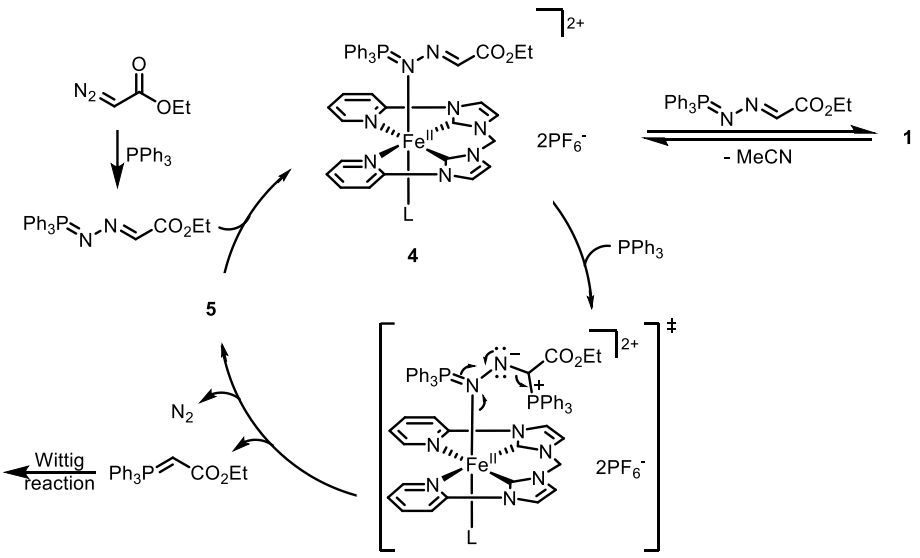


Fig. 18. One of two proposed modes of action for the catalytic formation of phosphorus ylide using **1** as catalyst. **5** is referred to as complex **1** with one axial acetonitrile ligand exchanged by PPh_3. L = acetonitrile/PPh_3.

3.2 Characterization of Hydrophilic Gold(I) N-Heterocyclic Carbene (NHC) Complexes as Potent TrxR Inhibitors Using Biochemical and Mass Spectrometric Approaches

Özden Karaca, Valeria Scalcon, Samuel M. Meier-Menches, Riccardo Bonsignore, Jurriaan M. J. L. Brouwer, Frederica Tonolo, Alessandra Folda, Maria Pia Rigobello, Fritz E. Kühn, Angela Casini

Inorganic Chemistry, 2017, *56*, 14237–14250

In this article, a series of hydroxylated mono(NHC) Au(I) as well as mono- and dinuclear gold(I) complexes bearing sulfonated bis(NHC) ligands are reported as potential anticancer agents (Fig. 19). The test compounds were synthesized and fully characterized by NMR spectroscopy, ESI-MS and elemental analysis. Furthermore, by means of mass spectrometric approaches their stability in (buffered) aqueous solution was studied demonstrating the potential of exclusively mono(NHC) gold(I) complexes to undergo ligand exchange reactions upon replacing the chloride moiety by potential nucleophiles.

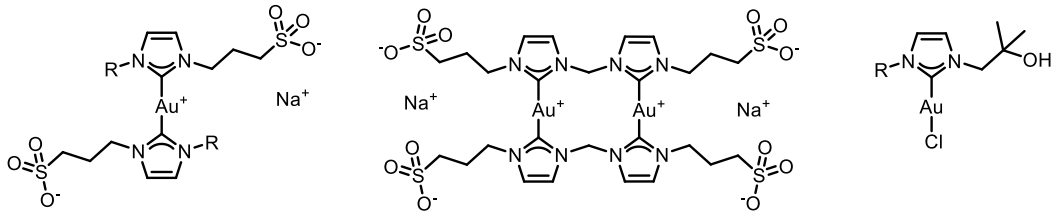


Fig. 19. Structures of hydrophilic NHC Au(I) complexes studied as potential anticancer agents targeting seleno-enzyme TrxR. Mes = 2,4,6-trimethylphenyl.

The antiproliferative effects of the gold complexes were investigated in ovarian carcinoma cell line 2008. In particular, compounds 10-12 display strong cytotoxicity with IC_{50} values in the low-micromolar range even after 1 h of incubation time (Fig. 20).

In vitro biochemical assays demonstrated for all mono(NHC) gold(I) compounds an effective inhibition of purified TrxR similar to auranofin. The same compounds were also strong inhibitors of total TrxR in 2008 cancer cell lysates which correlates with the observed cytotoxicity profile. Notably, a binding selectivity was observed for cytosolic TrxR (TrxR1) over mitochondrial TrxR (TrxR2) along with a distinct general preference for TrxR over GR. In addition, a pronounced formation of cellular ROS which is, as previously mentioned, correlated with an efficient TrxR inhibition was clearly observed for 10-12.

Regarding the molecular reactivity of the mono(NHC) Au(I) complexes with the assumed principal target TrxR, a strong inhibition of both binding sites, selenocysteine and cysteine, was confirmed by means of two independent techniques, namely BIAM (biotin-conjugated iodoacetamide) assays and mass spectrometric studies.

Moreover, both mono and bis(NHC) gold(I) complexes display selectivity towards protein targets with regard to nucleic acids, which was demonstrated by means of FRET (fluorescence resonance energy transfer) melting assays using telomeric G-quadruplex DNA.

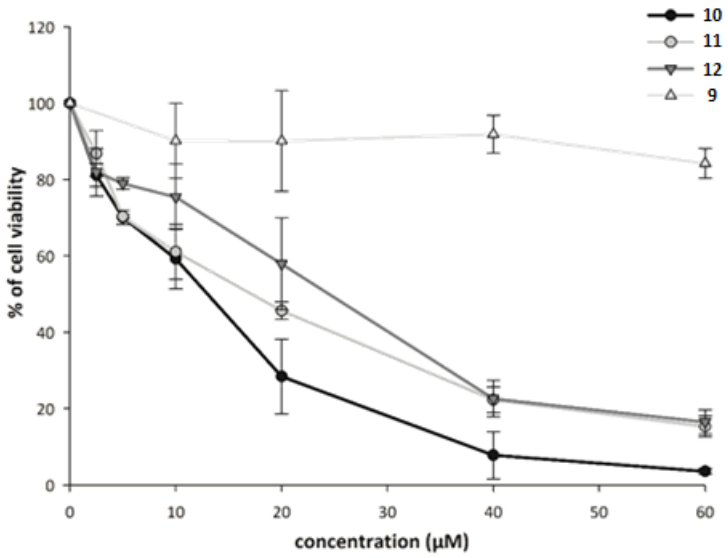


Fig. 20. Dose-dependent viability of ovarian carcinoma cells 2008 after 1 h incubation with selected NHC gold(I) complexes **9** and **10-12**.

3.3 On the binding modes of metal NHC complexes with DNA secondary structures: implications for therapy and imaging

Özden Karaca, Samuel M. Meier-Menches, Fritz E. Kühn, Angela Casini

Chemical Communications, **2017**, *53*, 8249-8260

This feature article provides an overview of anticancer metal NHC complexes that target DNA secondary structures *via* different binding modes. Organometallic complexes have gained increasing attention as therapeutic agents gradually entering clinical trials. With regard to cytotoxic NHC metal compounds, their modes of biological action have been predominantly related to interactions with crucial protein-based biomolecules. However, recently NHC metal complexes have broadened the scope in the development of novel anticancer drugs by introducing the interaction with distinct secondary structures of the DNA while the selective targeting of cancerous cells is anticipated.

Prominent motifs of the DNA which have been associated with cytotoxic implications are double stranded DNA including DNA mismatches and intrastrand DNA structures such as G-quadruplex (G4) DNA sequences, respectively (Fig. 21). Notably, the type of binding of the different motifs highly depends on the nature of the applied metallodrugs. A series of organometallic NHC complexes were shown to target double-stranded DNA by coordinative and intercalative binding as well as by inducing DNA cleavage, respectively. While many platinum-based NHC complexes are known for their direct coordination, complexes of various metals bearing planar aromatic moieties within their ligand systems favor non-coordinative stacking interactions (intercalation).

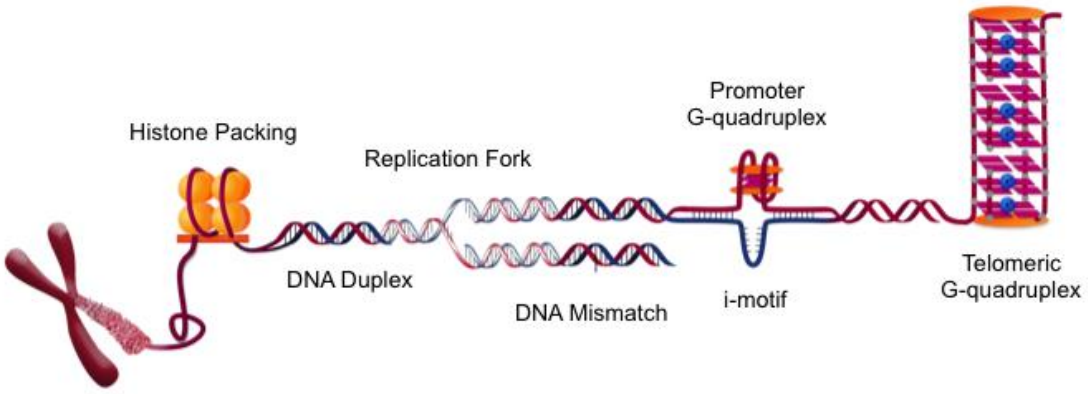


Fig. 21. Scheme of the DNA secondary structures as possible targets for organometallic NHC complexes.

In recent years, guanine-rich sequences of DNA have gained increasing attention as novel targets and have initiated the development of metallodrug designs which can selectively interact with quadruplex DNA. Among the rare number of organometallic NHC complexes reported to date, caffeine-derived gold(I) NHC complex $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ (**I-11**, Fig. 14) demonstrated promising selectivity in targeting telomeric G4 structures over duplex DNA, while showing selective cytotoxic effects on tumorigenic with respect to healthy cells. In this context, due to their pronounced stability and synthetic flexibility, NHC metal complexes display powerful tools in the development of novel anticancer drugs exhibiting tailor-made pharmacological properties.

CHAPTER 4

CONCLUSION AND OUTLOOK

The first part of this work presents the first investigation of dicationic NHC iron(II) complexes as environmentally benign catalysts in the olefination of aldehydes. Complex **1** (Fig. 16) provides a straightforward and selective Wittig-type olefination in a one-pot fashion without the need of preformation of the phosphorus ylide as known in a classic un-catalyzed Wittig reaction. At optimized catalytic conditions, in particular in the presence of excessive amounts of the substrate triphenylphosphine, high *E*-olefin yields along with minimized by-products were achieved.

Through in-depth investigations the catalytic mode of action of compound **1** was disclosed in comparison with literature-known pathways. Initial studies displaying the necessity of PPh_3 for the catalytic olefin formation excluded a possible procedure previously observed for Re-based catalysts including MTO (Fig. 4B and 6). Instead, the formation of phosphorus ylide was exclusively observed in the presence of catalytic amounts of iron(II) NHC complex **1**. The catalytically generated phosphorus ylide was demonstrated to react with aldehydes to olefins, while the reaction rate was enhanced using aldehydes exhibiting electron-withdrawing groups while those bearing electron-donating groups reduced the olefination rate; an order of reactivity typically observed for the classic Wittig reaction. The catalytic formation of the phosphorus ylide was proposed to occur *via* two possible pathways. On the one hand, an iron(IV) carbene intermediate which is formed from **1** and EDA transfers the carbene moiety to triphenylphosphine as previously reported by Woo *et al.* for an iron(II) porphyrin system. On the other hand, phosphazine, which is readily formed from EDA and PPh_3, reacts with the catalyst to phosphorus ylide by releasing dinitrogen. The latter suggested pathway has not been previously published but explains the necessity of excessive PPh_3 amounts for high olefin yields. Apart from its function as oxygen abstractor from the aldehyde, PPh_3 furthermore was proposed to serve as a nucleophile initiating the transformation of the intermediate iron-phosphazine adduct to phosphorus ylide under the reformation of the active catalyst species (Fig. 18).

Further investigations on this part of the dissertation should focus on a wider substrate screening, *e.g.* aliphatic aldehydes and ketones, along with targeting similar high olefin yields, however at lower reaction temperatures. The latter can be ideally achieved with an enhanced catalytic activity implying the assessment of thorough structure-activity relationships. It was already demonstrated that in contrast to compound **1** exhibiting the mixed bis(NHC)-bis(pyridine) ligand system the cyclic tetra(NHC) iron(II) complex **2** (Fig. 16) is completely inactive due to its assumed deactivation by EDA. In order to rationalize this crucial effect of the ligand system, the library of electronically and structurally diverse tetradentate iron(II) NHC complexes synthesized in our working group can be screened. In contrast, the variation of the axial ligands might have a lower effect on the overall catalytic behavior as they were demonstrated to readily undergo exchange reactions with PPh_3, which is present in excessive amounts in the olefination reaction.

The second part of this work focuses on the synthesis of hydrophilic gold(I) NHC complexes which were evaluated for their biological properties as potential anticancer agents. In order to incorporate the hydrophilic characteristic, the *N*-heterocyclic carbenes were functionalized at their wing-tips by hydroxyl and sulfonate substituents. The obtained hydroxylated mono(NHC) and sulfonated bis(NHC) gold(I) complexes exhibit slight and high solubility in water, respectively. The compounds' antiproliferative effects were evaluated towards ovarian carcinoma cell line 2008 which demonstrated a notably enhanced cytotoxicity of all three mono(NHC) gold(I) compounds.

A series of biochemical assays were furthermore conducted in order to rationalize a possible correlation between the observed antiproliferative effects and the inhibition of thioredoxin reductase, which is the central biological target for gold(I) complexes. All mono(NHC) gold(I) complexes demonstrated a strong and selective binding affinity of TrxR over GR (glutathione reductase), which is structurally similar but lacks the selenocysteine residue. Notably, the studies were performed on purified enzymes and on cell extracts *in vitro*. Additionally, the enhanced cellular production of ROS, which is correlated with the inhibition of redox-active TrxR and to the oxidative character of the Au(I) center, was determined. By means of biochemical and mass-spectrometric methods the binding of both cysteine and selenocysteine residues of TrxR by gold(I) complexes was revealed. Notably, in 2008 cells the tested mono(NHC) gold(I) complexes display a similar order of activity with regard to their cytotoxicity and TrxR inhibition which highlights the distinct role of this biomolecular target for the antiproliferative effects of the gold compounds. This mechanistic hypothesis needs to be further validated by future studies.

For this purpose, luminescent moieties can be attached to the NHC ligand which enables the tracking of the gold(I) compounds within the cellular compartments by means of imaging techniques such as fluorescence microscopy. Furthermore, mass spectrometry studies demonstrated the facile exchange of the chloride ligand in mono(NHC) complexes by possible nucleophiles. Although this reactivity is required for the coordinative binding and thus inhibition of TrxR, a series of potential nucleophiles are present in the biological environment and may inactivate the drug before it arrives to the anticipated biological target. In particular, cysteines were strongly bound by the tested gold(I) NHC complexes displaying a potential limitation for the envisaged drug distribution. Therefore, the highly reactive chloride ligand can be replaced by 1,3,5-Triaza-7-phosphaadamantane (PTA). The latter presents a suitable ligand due to its non-toxicity, hydrophilic character and the desired balance between stability and reactivity.

Furthermore, the tested gold(I) NHC complexes were shown to lack stacking interactions with telomeric G-quadruplex DNA, a nucleic acid-based target which is particularly expressed in certain cancerous cells. This target is scarcely investigated for NHC-based metal complexes but has gained increasing attention due to the discovery that bis-caffeine gold(I) NHC complex **I-11** displays binding selectivity for G4 over

duplex DNA along with a higher cytotoxicity for cancerous over healthy cells. This topic is currently under investigation in the working group of Prof. Kühn in cooperation with Prof. Casini. In doing so, the effect on the G4-binding is examined by exchanging the gold center *e.g.* by silver and varying the ancillary non-caffeine ligand.

CHAPTER 5

REPRINT PERMISSIONS

5.1 Elsevier Journal

"Iron(II) *N*-heterocyclic Carbene Complexes in Catalytic One-Pot Wittig Reactions: Mechanistic Insights"

Journal of Catalysis 2016, *344*, 213-220, DOI: 10.1016/j.jcat.2016.09.029

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JOURNAL OF CATALYSIS

Title: Iron(II) N-heterocyclic carbene complexes in catalytic one-pot Wittig reactions: Mechanistic insights

Author: Özden Karaca,Markus R. Anneser,Jens W. Kück,Anja C. Lindhorst,Mirza Cokoja,Fritz E. Kühn

Publication: Journal of Catalysis

Publisher: Elsevier

Date: December 2016

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Inorg. Chem. 2017, *56*, 14237-14250, DOI: 10.1021/acs.inorgchem.7b02345

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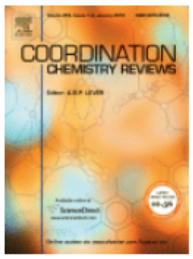

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Journal of Catalysis 2016, *344*, 213-220

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Direct link: DOI 10.1016/j.jcat.2016.09.029

Characterization of Hydrophilic Gold(I) N-Heterocyclic Carbene (NHC) Complexes as Potent TrxR Inhibitors Using Biochemical and Mass Spectrometric Approaches

Özden Karaca[a,b,‡], Valeria Scalcon[c,‡], Samuel M. Meier-Menches[b], Riccardo Bonsignore[b], Jurriaan M. J. L. Brouwer[c,d], Federica Tonolo[c], Alessandra Folda[c], Maria Pia Rigobello[c,*], Fritz E. Kühn[a,*], Angela Casini[b,d,e,*]

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On the binding modes of metal NHC complexes with DNA secondary structures: implications for therapy and imaging

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Direct link: DOI 10.1039/C7CC03074F

CHAPTER 7

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CHAPTER 8

COMPLETE LIST OF PUBLICATIONS

8.1 Journal Articles

[3] Characterization of Hydrophilic Gold(I) *N*-Heterocyclic Carbene (NHC) Complexes as Potent TrxR Inhibitors Using Biochemical and Mass Spectrometric Approaches

Özden Karaca[‡], Valeria Scalcon[‡], Samuel M. Meier-Menches, Riccardo Bonsignore, Jurriaan M. J. L. Brouwer, Frederica Tonolo, Alessandra Folda, Maria Pia Rigobello, Fritz E. Kühn, Angela Casini

Inorg. Chem. **2017**, *56*, 14237-14250.

[2] On the binding modes of metal NHC complexes with DNA secondary structures: implications for therapy and imaging

Özden Karaca, Samuel M. Meier-Menches, Angela Casini, Fritz E. Kühn

Chem. Comm. **2017**, *53*, 8249-8260

[1] Iron(II) *N*-Heterocyclic Carbene Complexes in Catalytic One-Pot Wittig Reactions: Mechanistic Insights

Özden Karaca, Markus R. Anneser, Jens W. Kück, Anja C. Lindhorst, Mirza Cokoja, Fritz E. Kühn

Journal of Catalysis **2016**, *344*, 213-220.

[‡]equally contributing co-authors

8.2 Conference Contributions

[2] Caffeine-derived transition metal *N*-heterocyclic carbenes: Insights onto their binding affinity towards telomeric G-quadruplexes and anticancer properties

Ö. Karaca, M. Bernd, S. M. Meier-Menches, R. Bonsignore, A. Casini, F. E. Kühn

Conference Poster, 18th International Conference on Biological Inorganic Chemistry (ICBIC18), Florianópolis, Brazil, July/August **2017**.

[1] Water-Soluble Gold(I) NHC Complexes: Synthesis and Biological Properties

Ö. Karaca, J. M. J. L. Brouwer, A. Citta, A. Folda, M. P. Rigobello, A. Casini, F. E. Kühn

Conference Poster, 42nd International Conference on Coordination Chemistry (ICCC16), Brest, France, July **2016**.

CHAPTER 9

APPENDIX