Synthesis, characterization and cytotoxicity studies of novel organo-metallic compounds

Esther Sirignano

UNIVERSITÀ DEGLI STUDI DI SALERNO

UNIVERSITÀ DEGLI STUDI DI SALERNO
Dipartimento di Farmacia

Dottorato di ricerca
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Coordinatore: Chiar.mo Prof. *Gianluca Sbardella*

SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY STUDIES OF NOVEL ORGANO-METALLIC COMPOUNDS

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Dottorando

Dott.
Esther Sirignano

Tutore

Chiar.mo Prof.
Carmela Saturnino

Contents

Abstract

Despite the discovery of cis-platin in the treatment of cancer there has been a considerable exploration on the antitumoral activity of other transition metal complexes. One of the main problems about the application of transition metal complexes for chemotherapy is their potential toxicity. For instance, recently the attention has been focused on titanium based complexes, which could have significant potential effect against solid tumor. The advantage of Ti(IV) complexes is their relative biological compatibility, which mostly leads to mild and revisable side effects. However, the hydrolytic instability of known Ti(IV) complexes and formation of various different species upon water addition makes their therapeutic application problematic, and raises a strong interest in the development of relatively stable Ti(IV) complexes with well defined hydrolytic behavior that demonstrate appreciable cytotoxic activity. Strong ligand binding is also of interest to avoid complete ligand stripping by transferrin, so that the ligand may be used as a target for structure–activity relationship investigations.

Titanocene dichloride (Cp_2TiCl_2) shows an average antiproliferative activity in vitro but promising results in vivo. Considerable work has been performed in developing therapeutic analogues of Cp_2TiCl_2 by varying the central metal, the labile ligands (Cl) and the bis-cyclopentadienyl moiety. In particular, small changes to the Cp ligand can strongly affect

the hydrolytic stability and water solubility properties of the metallocenes and have an impact on the cytotoxic activity.

For a better exploration of the parameters affecting activity and its mechanistic aspects, the synthesis and investigation of particularly designed complexes based on different strongly coordinating ligands has been our main purpose

We synthesized novel titanocene and half-titanocene derivatives, having substituted cyclopentadienyl ligands; all the complexes have been fully characterized by NMR, elemental analysis and MS. Additionally we studied the rate of hydrolysis of these complexes. Starting from the reflection that the different activities of the complexes could be related to their different stabilities, the hydrolysis stability represents a first possible indication on the achievable cytotoxic effects of synthesized compounds.

The synthesized compounds have been evaluated for their cytotoxic potential against cancer cell lines. Most of these compounds showed significant anti-proliferative effects compared to cisplatin

Chapter One

This chapter contains an overview on all the medical fields in which organo-metallic compounds are involved, with a special focus on organo-metallic antcancer drugs.

1 Introduction

Biomedical inorganic chemistry, known as elemental medicine, is an important new area of chemistry. It really offers the potential for the design of novel therapeutic and diagnostic agents and hence for the treatment and understanding of diseases which are now intractable[1-2] (Figure 1). Inorganic elements has a central roles in biological and biomedical processes, and it is evident that many organic compounds used in medicine do not have a merely organic mode of action; some of them are activated or biotransformed by metal ions including metallo-enzymes[4], others have a direct or indirect effect on metal ion metabolism. Elemental medicine offers real possibilities to pharmaceutical industries, which have traditionally been dominated by organic chemistry alone, for the discovery of novel drugs with new mechanisms of action. This field has been encouraged by the success of cisplatin, the worlds best selling anticancer drug, and platinum complexes with oral activity, reduced toxicity, and activity against resistant tumors are now on clinical trial. [3]

The organo-metallic complex titanocene dichloride has also been tested on patients, and Ru^{III} complexes have a promising metastatic activity.

The toxicity of Gd^{III} complexes can be controlled so that they can be injected in gram quantities as magnetic resonance imaging (MRI) contrast agents with no risk and ligand design allows paramagnetic ions to be targeted to specific organs.

Designed ligands also allow the targeting of radiodiagnostic (e.g. ^{99m}Tc) and radio- therapeutic isotopes (e.g. ^{186}Re).

There has been recent impreovment in understanding the coordination chemistry and biochemistry of older metallo-drugs such as gold antiarthritic and bismuth antiulcer drugs, and further work might lead to their effective use.

Current areas with exciting clinical potential include vanadium insulin mimics, manganese superoxide dismutase mimics, lanthanide-based photo-ensitizers, ruthenium nitric oxide scavengers, and metal-targeted organic agents.

The increasing knowledge of organo-metallic chemistry will provide an opportunity for the design of new drugs (both inorganic and organic) in many other areas too, for example neuropharmaceutical and antiinfective

agents. Progress in medicinal coordination chemistry is closely dependent on understanding not only the thermodynamics (equilibria and structures) but also the kinetics (and mechanisms) of reactions of metal complexes, especially under biologically relevant conditions.

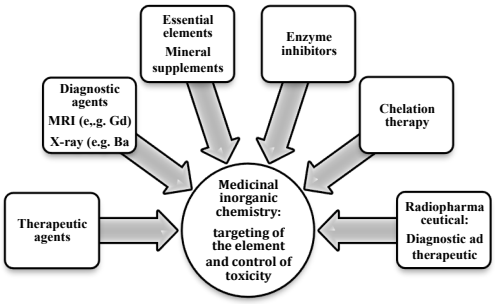

Figure 1 *Some of the key areas of medicinal inorganic chemistry*

1.1 MRI Contrast Agents

Magnetic resonance imaging is now a strong instrument in clinical diagnosis.[5] Diseases can be detected from differences in ^1H NMR resonances (mainly of H_2O) between normal and abnormal tissues by injections of external paramagnetic agents, known as contrast agents. Most contrast agents contain Fe^{III} Gd^{III}, or Mn^{II} ions which have a large number of unpaired electrons (7, 5, and 5, respectively, high spin) and long electron spin relaxation times.[6, 7]

Four Gd^{III} complexes have been approved for clinical use, and are nowadays widely used, for example, for the detection of abnormalities of the blood-brain barrier.[14] Complexes containing DTPA (1, Magnevist) and DOTA ligands (2, Dotarem) are ionic, whereas those of BMA-DTPA (3, Omniscan) and HP-DOTA (4, Prohance) are neutral; their low osmolarity decreases the pain of the injections. All these agents are extracellular, and they diffuse rapidly into the interstitial space. The Gd^{III} center is nine-coordinate in each complex and contains one bound H_2O

ligand.Water exchange on Gd^{III} is dissociative and steric hyndrance at the H_2O site increases the exchange rate.

Complex 1 does not enter cells and is excreted almost exclusively by the kidney.

The insertion of a benzyloxymethyl substituent on the a-C atom of a terminal acetate of DTPA as in BOPTA gives a Gd^{III} complex (5, Gadobenate), which enters hepatocytes and is excreted in bile.[8] The coordination sphere of Gd^{III} in complex 5 is almost identical to that in complex1 (distorted tricapped trigonal prism, nine-coordinate), and both complexes show similar stabilities and relaxivities (see Figure 3).[8]

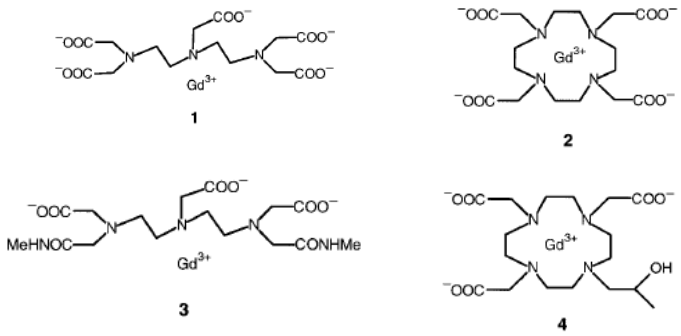

Figure 2 *Complexes used as MRI agents*

The distorted octahedral complex 6 (Teslascan is the mangafodipir trisodium salt),[9] is currently in clinical use for increasing contrast in the liver (detection of hepatocellular carcinomas).[10] The relaxivity of 6 is about 35 % greater than that of Mn complexes of DTPA and DOTA, which also do not contain directly coordinated water.[11]

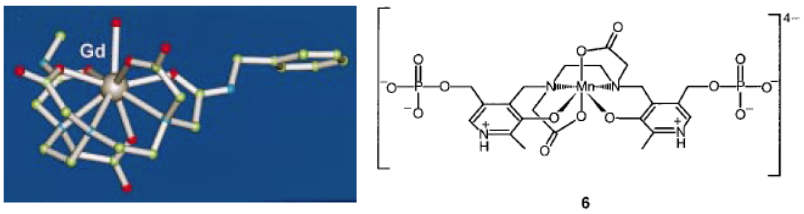

Figure 3 *Crystal structure of 5 (on the left) and Teslacan (on the right).*

1.2 Radiopharmaceuticals

Clinical interest in radionuclides centers not only on highintensity γ -ray emitters, especially ^{111}In, ^{67}Ga, ^{51}Co ^{99m}Tc and ^{201}Tl, , ^{51}Cr, and ^{169}Yb for diagnostic imaging, but also on β- emitters, for example ^{186}Re, ^{89}Sr, and ^{153}Sm for therapy.[1] Some ^{99m}Tc-based radiopharmaceuticals and several other radionuclides are currently utilized in clinical diagnosis. Complex 7 ([^{99m}Tc$_V$(dl-hm-pao)], Ceretec) is an approved perfusion imaging agent for diagnosis and evaluation of cerebral stroke. It is absorbed by the brain and is transformed into a more hydrophilic drug which is retained in the brain. Complex 8 ([99mTcI(sestamibi)], Cardiolite), instead, is used for myocardial perfusion imaging. It was projected on the idea that cationic lipophilic complexes behave as potassium mimics and are taken up by the heart.[13] The sequential metabolism of the six equivalent methoxy groups of 8 to hydroxyl groups in the liver leads to formation of ^{99m}Tc complexes with stronger hydrophilicity which are not retained in myocardial tissues.[14] Monoclonal antibodies (mAbs) combined with radionuclides such as ^{111}Insatumomab pendetide (which contains the murine mAb B72.3, which is directed to TAG-72, an antigen expressed by many adenocarcinomas) are currently used for diagnosis of ovarian and colorectal cancer.[15] Several other murine mAbs linked to ^{111}In and ^{99m}Tc are now in clinical trials.[16] Substantial progress has been performed recently in the development of ^{99m}Tc-based receptor-specific

radiopharmaceuticals.[17] Encapsulation in fullerenes may also give a novel method for the delivery of radionuclides to target sites.[18, 19]

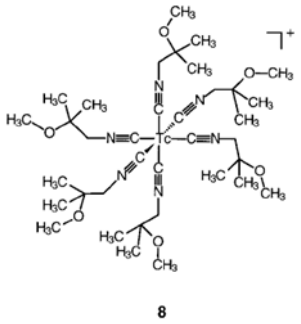

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Figure 4 *Structure of Cardiolite.*

1.3 Antiinfective Agents

Historically silver compounds have widely been used as antimicrobial agents in medicine. Silver has a low toxicity and is active at low concentrations. The practice of instilling the eyes of babies with 1% $AgNO_3$ solution immediately after delivery is still common in some countries for prevention of ophthalmia neonatorum.[20] Silver sulfadiazine 9, made from Ag^I and sulfadiazine A, is currently used as an antifungal and antimicrobial agent. It is a polymeric insoluble drug which releases Ag^I ions slowly, and is applied topically as a cream to prevent bacterial infections in cases of acute burns. The slow release of antimicrobial Ag^I ions from inorganic or organic polymer matrices[21] is important for pharmaceutical industries.

The mode of action of AgI is unknown. Cell-wall damage may be irelevant, and it has been shown that Cys150 in phosphomannose isomerase, an essential enzyme for the biosynthesis of cell walls of Candida albicans, is a Ag^I target in this organism.[22] Silver-resistant bacteria are well known,[20] and improvement in understanding the mechanism of resistance is now being made.

Antimony has been used for medical purposes for many centuries. Complexes of Sb^{III} are conventionally more toxic than those of Sb^V. Two Sb^V drugs, sodium stibogluconate (Pentostam) and N-methylglucamine

antimonate (Glucantime), are currently used for the treatment of leishmaniasis, an infection caused by intracellular parasites.[23] The carbohydrates contained in the drugs may sbe useful for delivering Sb^V to macrophages. The Sb^V complexes may be considered prodrugs because the more toxic Sb^{III} forms at or near the site of action. There is current interest in improving the solubility, stability, and efficacy of Sb drugs, and Sb^{III} and Sb^V complexes with yeast mannan are reported to be promising.[24, 25]

The iron chelating complex, named desferrioxamine 10 is clinically approved for the treatment of malaria disease. Its activity may results from the interfernce of Fe^{III} metabolism inside the digestive vacuole of malaria parasites.[26]

Several antisense oligonucleotides are strong inhibitors of HIV-1 integrase,[27] and are currently on clinical trial. These have key sequences such as 5'-d(GTGGTGGGTGGGTGGGT) (11, T30175), and are only formed of thymidine and deoxyguanosine. They fold up in the presence of K^+ ions to give four-stranded structures dominated by two stacked guanine-quartet motifs.[28]

This structure is very stable under physiological conditions (e.g., K-11 is resistant to serum nucleases with a half-life of 5 h) and is probably critical for biological properties. Macrocyclic bicyclam ligands such as 12 (JM3100) are certainly amongst the strongest inhibitors of HIV ever described, being active at nanomolar concentration.[29] Since they are safe at these levels, they have a high selectivity index (ca. 10^5).

The zinc complex of ligand 12 is also active.[30] The bicyclams appear to obstacle the virus entry and membrane fusion through interaction with the CXCR4 coreceptor during the early stages of the retrovirus replicative cycle.[31]

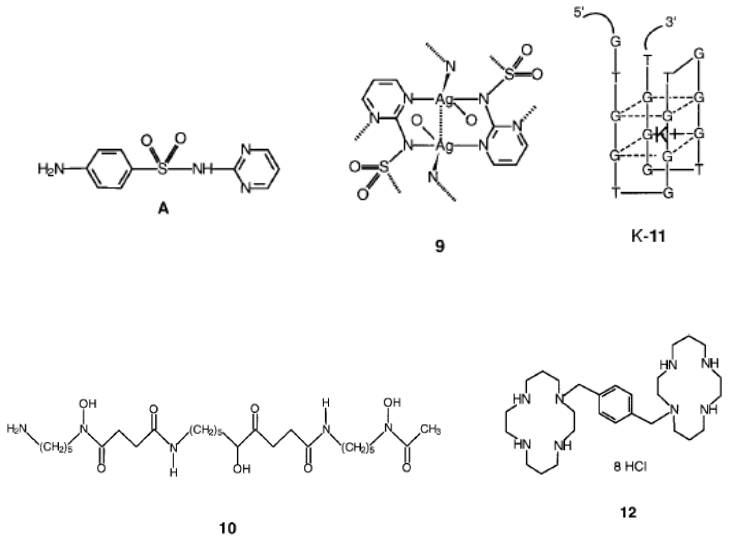

Figure 5 *Structure of complexes used as antinfective agents*

1.4 Superoxide Dismutase Mimics

The free radical superoxide, O_2^-, is a product of endothelial cells and activated leukocytes, and has been postulated to mediate the ischemia-reperfusion injury as well as inflammatory and vascular diseases. It can react with NO forming damaging peroxynitrite, ONO^{2-}. The metalloenzyme superoxide dismutase, known as SOD can destroy O_2^-: Cu,Zn-SOD in the cytoplasm of eukaryotic cells, Mn-SOD in mitochondria [see Eqs. (1) and (2) below].

$$M^{n+1} + O_2^- \longrightarrow M^{n+} + O_2 \quad (1)$$

$$M^{n+} + O_2^- + 2H^+ \longrightarrow M^{n+1} + H_2O_2 \quad (2)$$

However, the use of SOD in therapy is restricted by itsbrief plasma half-life (clearance by the kidney) and inability to penetrate cell membranes giving only an extracellular activity. Low molecular mass mimics of SOD

are, for this reason,of much potential medical interest.[32] For example, a variety of Fe-based and Mn^{-} porphyrins and macrocyclic complexes show SOD mimic activity.[33-36] Notably, Mn^{II} and Mn^{III} macrocycles appear to be promising.[37, 38] For example, complex 13 (SC-52608) is able to scavenge superoxide and therefore effectively protect the ischemic and reperfused myocardium from injury.[39] Another example is manganese(III) 5,10,15,20-tetrakis(4-benzoic acid)- porphyrin (MnTBAP), which can protect against neurodegeneration and is for this reason of potential interest for the treatment of brain diseases such as Alzheimer's and Parkinson's diseases.[40]

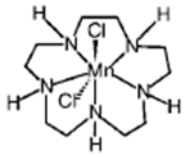

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Figure 6 *Structure of SC-52608*

1.5 Cardiovascular System

The Fe^{II} complex sodium nitroprusside 14 is the only clinically used among the metal-nitrosyl complex.[41] It is often used to rapidly decrease the blood pressure in humans. Its hypotensive effect is evident within seconds after infjection, and the required blood pressure is usually obtained within one to two minutes. It is also useful in cases of heart attacks, emergency hypertension, or surgery.[42] The therapeutic effects of 14 is based on the quick release of nitric oxide, which relaxes vascular smooth muscle. Activation in vivo may involve reduction to $[Fe(CN)_5(NO)]^{3-}$, which then releases cyanide to give $[Fe(CN)_4(NO)]^{2-}$ and then nitric oxide.[43, 44]

Ruthenium complexes such as K[Ru(Hedta)Cl] K-15 (JM1226) have been proposed as nitric oxide scavengers for the control of NO levels under conditions of clinical interest.

In water complex 15 is in equilibrium with the aqua species 16 (JM6245; the pKa of the dangling arm carboxyl group is 2.4).[45] Both complexes bind to NO very quickly (rate constant>$10^8 m^{-1} s^{-1}$ at body temperature 310 K) and tightly (K>$10^8 m^{-1}$), giving the linear nitrosonium Ru^{II}-NO^+ adduct 17.[45] Complex 16 has been demonstrated to reverse the poor response of the artery to vasoconstrictor drugs,[46] which is one of the major clinical problem in the treatment of patients with septic shock (caused by very high levels of circulating bacteria in the body). The excessive production of NO not only appears to be a main contributory factor in septic shock, but also in arthritis, inflammation, diabetes and epilepsy.

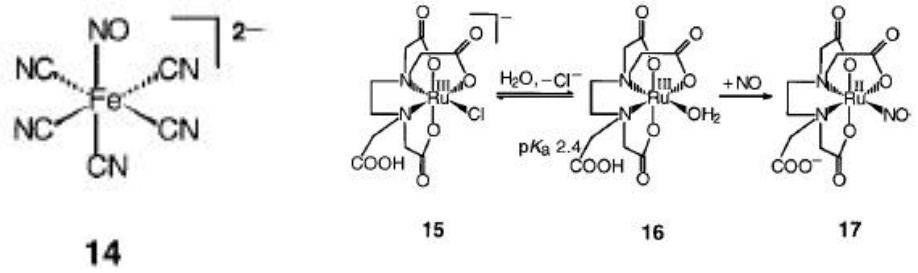


Figure 7 *Structures of complexes used in the cardiovascular system*

1.6 Insulin Mimetics

Nearly 20 years ago It was founded that V^V as vanadate and V^{IV} as vanadyl can mimic some of the effects of insulin (stimulate glucose uptake and oxidation as well as glycoge synthesis).[47, 48] Vanadium complexes having organic ligands are often less toxic and can show improved aqueous solubility and lipophilicity. The orally active complex bis-(maltolato) oxovanadium(IV) (18,BMOV)[49] is about three times more effective in vivo as an insulin-mimetic agent than $VOSO_4$[50] Complex 18 has, in the solid-state, a five-coordinate square pyramidal geometry with the oxo ligand in the axial position and trans maltolato ligands.[51] In aerobic aqueous solutions, the complex is quickly oxidized to the dioxovanadium(V) species.[52]

Low molecular weight chromium-binding substance (LMWCr), a naturally occuring oligopeptide (ca. 1500 Da, consisting of Cr^{III}, Asp, Glu, Gly, and Cys in a 4:2:4:2:2 ratio), has been demostrated to activate

the insulin-dependent tyrosine kinase activity of the insulin receptor protein, with the activity being proportional to the Cr content of the oligopeptide (maximium activity with four Cr^{III} per oligopeptide) .[53] The trinuclear cation 19 can stimulate the tyrosine kinase activity of the insulin receptor in a manner quite identical to that of LMWCr.[54] Interestingly when acetate groups replaces propionate in 19, the complex does not activate but rather inhibits both the kinase and membrane phosphatase activity. Its potential for diabetes treatment has yet to be discovered.

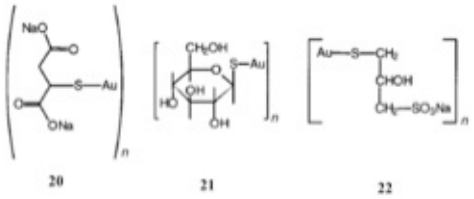
18 19

Figure 8 *Structures of complexes used as insulin mimics.*

1.7 Gold Antiarthritic Drugs

Some injectable 1:1 AuI thiolato complexes are used clinically for the treatment of severe cases of rheumatoid arthritis, including sodium aurothiomalate (20, Myocrisin), aurothioglucose (21, Solganol), and sodium aurothiopropanol sulfonate (22, Allochrysine).[55] Most of the gold thiolates are polymeric complexes (e.g. chain or ring forms) with thiolate S-bridging and linear AuI ions and have a gold-to-ligand ratio close to 1:1, as shown by EXAFS[56] and WAXS data.[57] Crystal structures of the gold thiolate drugs themselves have been ambiguous, the only example is given by the crystallization of 20 by Bau[58] using techniques for macromolecules crystallization. The linear S-Au-S units are disposed into polymeric double-helical chains. Hexameric Au6S6 rings of the type previously predicted [59] have also been demostrated to exist in crystals with 2,4,6-tri(isopropyl)thiophenol as the thiolato ligand.[60] The formulated gold thiolate drugs often contain thiols in small

molar excess (e.g. 10%) over AuI, and readily undergo thiolate exchange and thiolate addition reactions.[61]



20 21 22

Figure 9 *Structures of gold antiarthritic drugs*

The only oral gold antiarthritic compound currently in use is the Auranofin, a phosphane complex. It is also highly cytotoxic to cancer cells *in vitro*, and is also reported to be active against psoriasis if used topically.[62]

A common metabolite found in the plasma and urineof patients treated with gold drugs is $[Au(CN)_2]^-$,[63] an ion which promptly enters cells and is capable of inhibiting the oxidative burst of white blood cells. Therfore it could therefore be an active metabolite of gold drugs. It also shows anti- HIV and anticancer activity. The elevated Au content in the red blood cells of smokers receiving gold therapy has been realted to the inhalation of HCN through smoke.[64] Simulating the red blood cell concentrations, Elder et al.[65] have observed that $[Au(CN)_2]^-$ reacts with GSH to form $[Au(SG)2]^-$, which is very stable. In all probability $[Au(SG)_2]^-$ is to be formed inside the cells both from auranofin and gold thiolate drugs, and may be responsible for the inhibition of many enzymes such as the Se-enzyme glutathione peroxidase.[66] Compound 20 is a strong inhibitor of neutrophil collagenase, a zinc enzyme which includes Cys ligands in the metal-binding site.[67, 68] These interactions may be useful in joint tissue. $Gold^I$ shows a srtrong affinity for thiolate S, but binds only weakly to N and O ligands. Hence the proteins containing cysteine thiolate groups (mainly those with low pKa values) are targets for AuI, but binding of AuI to DNA is poor. Moreover, the thiolate exchange reactions are usually very rapid. During therapy, Au levels in the blood typically reach 20mm, and Au is transported by albumin bound to Cys34.

The rate of gold binding is regulated by the rate of opening of the cleft containing Cys34,[69, 70] and the Au binding seems to induce a "flip-out" of this residue.[71] $Gold^{I}$ drugs can strongly bind to thiol groups in DNA-binding proteins such as the transcription factors Jun-Fos and Jun-Jun, allowing the possibility that gold can regulate the activity of transcription factor.[72] The oxidation of Au^{I} to Au^{III} *in vivo* could be responsible for some of the side effects of gold drugs.[73,74] In inflammatory conditions, strong oxidants such as H_2O_2 and ClO^- are potentially available in vivo and can oxidize the metal centers in auranofin, 20 and 21 to Au^{III}.[75] $Gold^{III}$ has the unusual capability to deprotonate peptide amide groups even at low pH values (e.g. pH 2)[76] and may modify peptide recognition by T cells. $Gold^{III}$ is able tooxidize disulfide bridges in insulin and albumin[77] and methionine residues of ribonuclease.[78]

1.8 Bismute antiulcer Drugs

Some bismuth complexes have been used for treating gastrointestinal disease for at least two centuries.[79] These include alicylate salts, nitrate, sand colloidal bismuth subcitrate, all Bi^{III} compounds. $Bismuth^{V}$ is usually a powerful oxidant agent. The structures of Bi drugs are widely unknown. The coordination number of Bi^{III} is highly variable (3-10), and often the coordination geometry is irregular.[80] Bi^{III} is strongly acidic in water (first pKa ca. 1.5) and has an high tendency to form stable oxo-bridged and hydroxo clusters.

The $[Bi_6O_4(OH)_4](NO_3)_6 \cdot 4H_2O$ complex, known as "magisterium bismuti" since the 17th century was used as a beauty care product, and crystallizes at pH values under 1.2.[81] The main understood in structural terms are the citrate complexes, for which several X-ray structures have been performed,[82] although none has absolutely the same composition as the drugs themselves. The dominant aspect is the dimeric $[(cit)BiBi(cit)]^{2-}$ unit (H_4cit:citric acid), which contains bridging citrate anions. The Bi-O(alkoxide) bond is reallystrong and short (2.2 Å), and a stereochemical role for the $6s_2$ lone pair is evident in the structure. These dimers aggregate into chains and sheets in the crystal through a network of hydrogen bonds involving citrate, counter ions, and water to give polymers. Such polymerscould be deposited on the surface of ulcers.

Bismuth^{III} citrate complexes seem to be stable in solution in a pH range of about 3.5 to 7.5. [Bi^{III}(Hcit)] itself is solubilized by a variety of amines,[83] and the adduct with the organic histamine antagonist ranitidine, ranitidine bismuth citrate,[84, 85] has recently been approved as a new drug. The antibacterical activity of Bi^{III} against Helicobacter pylori seems to be important for its antiulcer activity.[86] This organism is also implicated in other diseases such as even cancer.

The biological activities of Bi^{III} are probably mainly due to binding to enzymes and proteins. The binding of Bi^{III} to DNA is unknown, so far. There is a poweful correlation between the strength of binding of Bi^{III} to a variety of ligands and that of Fe^{III}. Although Bi^{III} (ionic radius 1.03 Å) is bigger than Fe^{III} (ionic radius 0.64 Å high spin) it also strongly binds to transferrin, the serum Fe^{III} transport protein.[87] The strength of binding of transferrin to Bi^{III} can be related with the high acidity of Bi^{III}. Correlated proteins (periplasmic iron-binding proteins) are implicated in Fe uptake by some virulent bacteria.

Bismuth^{III} can promptly displace Zn^{II} from the metallothionein, Cys-rich protein, and bismuth metallothionein is stable even under deeply acidic conditions (pH 2).[88] Cysteine and glutathione may have a crucial role in the transport of Bi^{III} in cells and biofluids. These thiols can avoid the precipitation of colloidal bismuth subcitrate (CBS) at pH 2.0, and animal studies have shown that simultaneous oral administration of thiols and bismuth salts gives a significant increase in the bismuth concentration in blood plasma.[89, 90] The complex [Bi(SG)$_3$] is very stable (lgK.29.6) in a wide pH range (2-10) with bindingonly through the S atom.[91] Exchange of GSH between bound and free forms is quite rapid at biological pH (ca. 1500 s^{-1}).

1.9 Anticancer Agents: Platinum

1.9.1 Clinical Platinum Complexes

Platinum complexes are currently amongst the most world-widely used drugs for the treatment of cancer. Four injectable Pt^{II} complexes have been approved for clinical use and many other cis-diam(m)ine complexes are on clinical trials, including an oral Pt^{IV} complex. Today cisplatin (23) is one of the most largely used anticancer drugs, along with the second generation drug carboplatin [$Pt(NH_3)_2$(CBDCA-*O,O′*)] (24). The glycolato complex 25 (nedaplatin, 254-S) and oxalato complex 26 (oxaliplatin, *l*-OHP, which contains R,R-1,2- diaminocyclohexane, DACH) have also been approved for clinical use in Japan and in all the countries, respectively. These drugs are particularly effective in "cocktail" or combination chemotherapy for treatment of advanced colorectal, lung, and ovarian cancers.[92, 93]

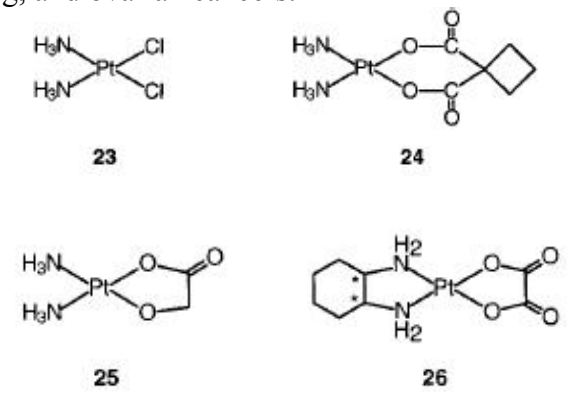
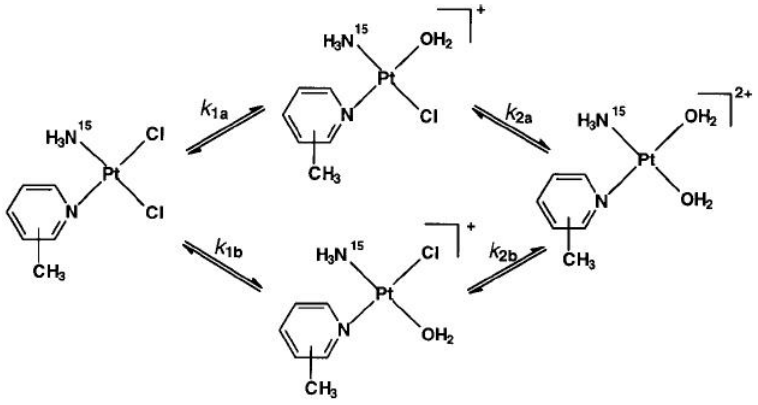


Figure 10 *Stuctures of Platinum complexes*

The sterically-hindered complex 27 (ZD0473) is active (by oral administration and injection) against an acquired cisplatin-resistant cell line of a human ovarian carcinoma xenograph,[94] and entered clinical trials in 1997. It is certainly less reactive than cisplatin, for example binding to plasma proteins and inducing DNA interstrand crosslinks in cells less quickly. The hydrolysis rate of 27 are at least two to three times slower than those of cisplatin (table 1).

Table. 1 *Rate constants k (Scheme 1) for hydrolysis of the platinum- picoline anticancer complex 27 and the 3-picoline analogue at 310 K (0.1m $NaClO_4$), in comparison with cisplatin (308 K, 0.32m KNO_3)*

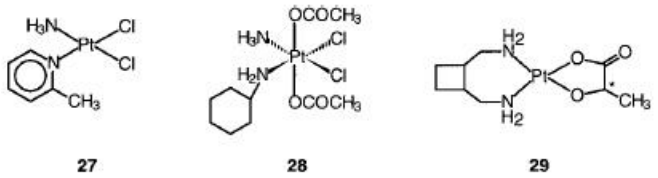
Compound	k [$\times 10^{-6}$ s^{-1}]	Compound	k [$\times 10^{-6}$ s^{-1}]
2-picoline – Pt complex **27**	k_{1a}: 31.9 ± 1.5	3-picoline – Pt complex	k_{1a}: 44.7 ± 1.9
	k_{1b}: 22.1 ± 1.4		k_{1b}: 103 ± 4
	k_{2a}: 73 ± 14		k_{2a}: 35.0 ± 1.7
	k_{2b}: 3.5 ± 2.5		k_{2b}: 78 ± 60
cisplatin (**23**)	k_1: 75.9		



Scheme. 1 *Reaction step in the hydrolysis of a(m)mineplatinum complexes*

The orally active complex 28 (JM216) is demonstrated to have stronger *in vitro* and *in vivo* activity compared to cisplatin against small cell lung, human cervical, and ovarian carcinoma cell lines.[96] Incubating 28 with human plasma, it is transformed into at least six biotransformation products, which include the dichloroplatinum(II) complex cis-[$PtCl_2(NH_3)$(cyclohexylamine)] and the monoand dihydroxo Pt^{IV} complexesas the major metabolite.[97,98]

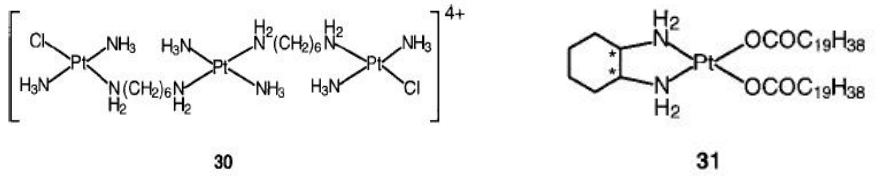
Complex 29 (Lobaplatin, D-19466) was approved into clinical trials in 1992.



27 28 29

Figure 11 *Structure of Platinum complexes*

The central Pt unit in the trinuclear complex 30 (BBR3464, counteranion = $NO3^-$), is capable only of hydrogen-bonding interactions with DNA and is reported to be up to 100-fold more potent than cisplatin against human tumor cell lines resistant to cisplatin .[99-101] The general charge of 4 greatly enhances DNA affinity, characterized by long-range interstrand cross-linking up to six base pairs apart with significant efficient, unwinding and irreversible conversion of B- to Z-DNA. The adducts are capable of terminating the DNA synthesis *in vitro*. The cytotoxic effect of the complex is insensitive to the p53 status of cisplatin-resistant cells (effective against tumors showing a mutant p53).
cis-Bis(nonadecanoato)(trans-R,R-1,2-diaminocyclohexane)- platinum^{II} 31 (N-DDP) is a ipophilic liposome-incorporated cisplatin analogue that has demonstrated promising *in vivo* activity against tumors resistant to cisplatin and liver metastases.[102]



30 31

Figure 12 *Structure of Platinum complexes*

1.9.2 Platination of DNA

The guanine N7 is the most electron-rich site on DNA (most easily oxidized), and the major adducts of platinum drugs with DNA are 1,2-GpG and 1,2-ApG intrastrand crosslinks. The features of these adducts have been widely characterized and reviewed.[103] The NMR solution structure of cis-[{$Pt(NH_3)_2$}$^{2+}${d(CCTG*G*TCC)·d(GGACCAGG)}] (where * denotes Ptbound G) indicates that the B-DNA backbone conformation is mainly altered to accommodate the platinated lesion (**Figure *13***).[104]

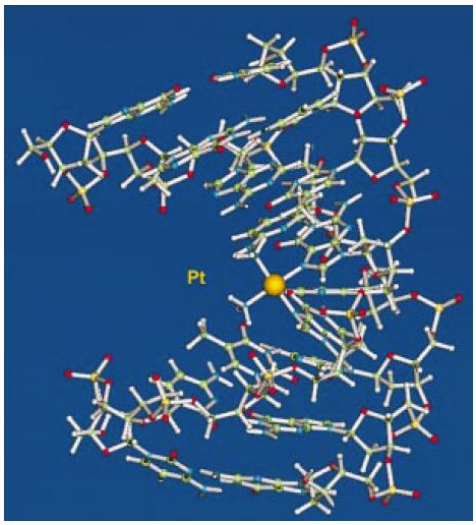


Figure 13 *NMR solution structure of cis-[{Pt(NH3)2}2+{d(CCTG*G*TCC) ·d(GGACCAGG)}]*

A novel X-ray crystal structure of the cisplatin adduct of the duplex d(CCTCTG*G*TCTCC)· d(GGAGACCAGAGG) (Figure 4) shows that cisplatin bends DNA by 35-40° in the direction of the major groove with a dihedral angle of 268 between the two guanine rings.[105,106] The duplex acquires a juxtaposition of A-like and B-like helical DNA segments. It is notable that the conformation surrounding the GG platination site in the solid-state X-ray crystal structure [106] is strongly similar to that in solution.[104,107] The Pt ion is removed from the planes of the coordinated

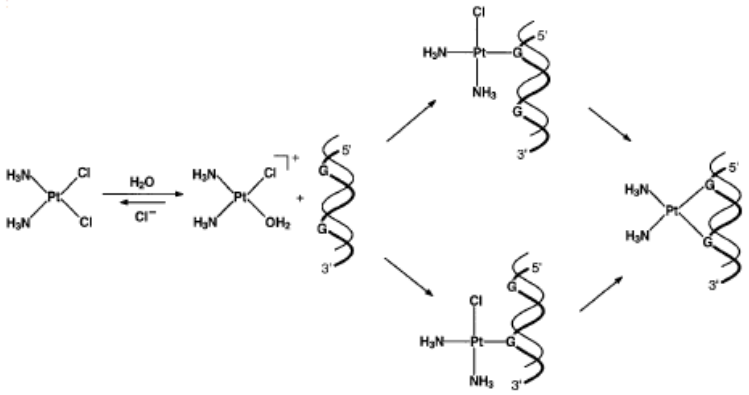
G-bases by about 0.8 **Å**, suggesting the presence of critical strain in this lesion. The properties and nature of minor cisplatin -DNA lesions which account for about 10%of the adducts, including 1,3-d(GpNpG) interstrand and intrastrand cross-links and monofunctional adducts, are less well know. Interstrand cross-linking of DNA occurs mainly between two guanine residues on opposite strands, and requires a distance of approximately 3 **Å** between the two N7 atoms. The most conventional interstrand cross-linking sequences are 5'-CG and 3'-CG.[108] In these sequences the two guanine residues are separated by at least 7-9 **Å**; therefore a large distortion of double-helical B-DNA is need to achieve the cross-linking.

The solution NMR structure (see **Figure *14*** on the left) of *cis*-[{$Pt(NH_3)_2$}$^{2+}${$d(CATAGCTATG)_2$}] shows that the duplex endures a strong rearrangement at the lesion site, locating the platinum atom in the minor groove.[109] The deoxyribose of the platinated G residue is inverted so that O4' is pointing in the opposite direction respect to the remaining nucleotides. Moreover, the C residue which was initially base-paired to the platinated G is extruded and is transformed in extra-helical

(see **Figure *14*** on the right). In the solution NMR structure of the interstrand cross-linked [{$Pt(NH_{3)2}$}$^{2+}$}-{d(CCTCG*CTCTC) ´d(GAGAG*CGAGG)}], the cis-{Pt- $(NH_3)_2$}$^{2+}$ moiety is also placed in the minor groove.[110] The stacking of the two cross-linked guanine residues with the surrounding bases forms a bend of 40° towards the minor groove. A very similar bend occurs in the X-ray crystal structure of this interstrand cross-link. The two cytosin residues are extruded from the double helix, and the platinum residue is embedded in a cage of nine water molecules. The general mechanism of formation of GG cross-links on DNA by cisplatin is evident in Scheme 2. Cisplatin is hydrolyzed to give monoaqua and diaqua species with a half-life of 1.7 h at 310 K. The aqua complexes are considered to be the active species towards DNA.

The finding that the 5'-G monofunctional adduct formed during the reaction of cisplatin with the 14-mer DNA duplex d(ATACATGGTACATA) • d(TATGTACCATGTAT) is very long lived with a half-life of 80 h at 298 K was unexpected.[111,112] The half-life of the two monofunctional G adducts of the GG single strand are similar, proposing that the three-dimensional structure of DNA has a role in stabilizing the 5'-G adduct either by constraining the position of the

incoming 3'-G N7 ligand or by shielding the Cl ligand from hydrolysis. Molecular modeling studies prove that hydrogen-bonding between the carbonyl groups on DNA and the NH_3 ligands plays a main role in determining the orientation of the Pt-Cl bonds and their accessibility. Molecular mechanics calculations demonstrate that although the chloride ligand in the monofunctional adduct faces outward, away from the helix, the aqua ligand which replaces it after hydrolysis faces inwards on account of its strong hydrogenbonding properties.[112] Modeling of transition states is now necessary.



Scheme. 2 *Mechanism of reaction of cisplatin with DNA*

Kinetic analyses based on HPLC results provide the accurate determination of the rates of both platination and chelation steps for reactions of cisplatin diaqua with oligonucleotides.[113,114] For the double-stranded oligonucleotide d(TTGGCCAA)$_2$ the formation of the 5'-G monoaqua adduct is faster than that of the 3'-G monoadduct, and macrochelate ring closure of the 5'-G monoaqua to give the bifunctional adduct (half-life of 3.2 h at 293 K) is much slower than that of the 3'-G monoadduct. The biological importance of long-lived monofunctional adducts on DNA remains to be detected, but these alone may be sufficient to kill cells if they are not repaired, which seems to be the case for complex 33, the active trans iminoether (see Section 1.9.3).[115] Long-lived monofunctional adducts may also contribute to the formation of DNA- protein cross-links.

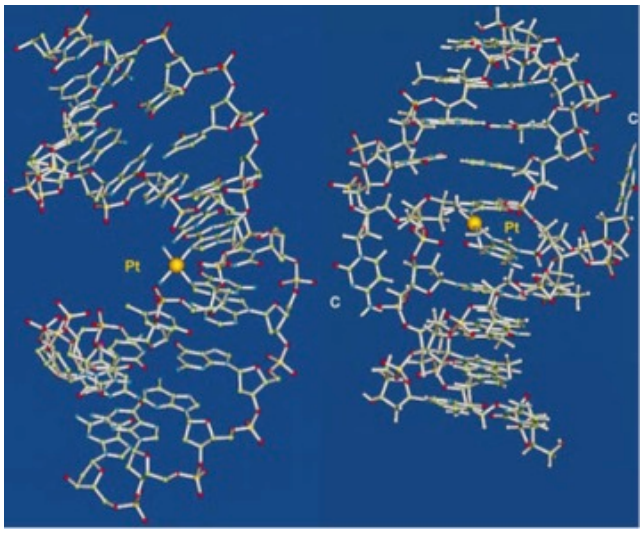
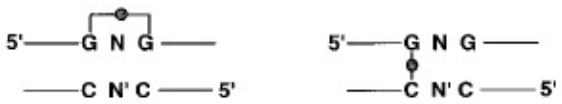


Figure 14 *Crystal structure of* $[\{Pt(NH_3)_2\}^{2+}$*-{d(CCTCG*CTCTC)´ d(GAGAG*CGAGG)}] (on the left) and of cis-*$[\{Pt(NH_3)_2\}^{2+}$*{d(CATAGCTATG)*$_2$*}] (on the right)*

The stability of the Pt-N7G bond is demonstrated to be very high, and it can be broken only by a very strong nucleophiles such as thiourea or cyanide. However, recent studies have found that this bond can be labile in certain DNA adducts.

The adduct trans-$\{Pt(NH_3)_2\}^{2+}${d(TCTACG*CG*TTCT)}] (1,3-GG cross-link) is at neutral pH unstable and rearranges to form the linkage isomer trans-[$\{Pt(NH_3)_2\}^{2+}${d(TCTAC*GCG*TTCT)}] (1,4-CG cross-link).[116] It was indicated subsequently that intra- and interstrand transplatin -DNA adducts undergo isomerization in both double- and single-stranded and DNA.[117-119] For instance interstrand cross-links between a platinated 5'-G and the complementary C residue can be formed.[117]



Scheme. 3 *Rearrangement of a trans-[{Pt(NH*$_3$*)*$_2$*}-DNA] intrastrand cross-link (left) to an interstrand cross-link (right)*

The isomerization of the intrastrand d(CCTG*G*TCC) · d(GGACCAGG) cross-link to a d(CCTG*GTCC) · d(GGACCAGG*) interstrand cross-link proves that even Pt-N7 bonds in Pt - 1,2-GpG cross-links can be destabilized.[104] This process seems to be assisted by Cl^-.

1.9.3 Protein Recognition

In tumor cells the repair of platinated DNA lesions may be protected by a class of proteins named HMG high mobility group proteins.[120, 121]
The protein HMG1 sows three structural domains, two of them, known as domains A and B, are positively charged, and the third domain comprises a 30-amino-acid stretch of acidic residues at the C terminus site. Several NMR structures of HMG domains have been performed.[122,123] Domain B of HMG1 consists of three α-helical regions joined by small loops, and folded into an L-shaped structure. The two domains, the positively charged domain A and the central domain B bind to DNA, while the acidic C-terminal domain interacts with histones only.
The binding affinity of HMG1 towards a series of cisplatin modified 15-mer DNA duplexes d(CCTCTCN1G*G*- N2TCTTC) · d(GAAGAN3CCN4GAGAGG) is strongly dependent on the bases adjacent to the Pt lesion.[124] The affinity for HMG1 domain A decreases by at least two orders of magnitude in the order N2=dA>T>dC. When N1=N2=dA, Pt-DNA binds 100-fold stronger to HMG1 domain A (Kd.1.6nM) than to HMG1 domain B (Kd.134nM). The HMG binding succed ind increasing the bending of platinated DNA to as much as 90°.[125] NMR studies of a GG 14-mer platinated with cisplatin show that the kinked duplex binds in the elbow region of HMG1 domain A,[126] and recent X-ray studies of HMG1 domain A bound to a 16-base-pair DNA fragment containing a cisplatin-DNA 1,2-d(GpG) intrastrand crosslink suggest that the protein inserts a Phe side chain into the platinum site.
The linker histone H1, a nuclear protein, also binds much more strongly to DNA modified by cisplatin than to transplatin or unmodified DNA.[127] Competitive binding of a cisplatin modified 123-base-pair DNA fragment suggest that histone H1 binding is stronger than that of HMG1. Also the TATA box-binding protein (TBP)/TFIID, a promoter recognition factor binds selectively to and is sequestered by cisplatin-damaged DNA. This may lead TBP/TFIID away from its normal promoter sequence explaining

the inhibition of RNA synthesis.[128] Therefore these proteins may also have an important roles in the mechanism of action of platinum complexes drugs.

1.9.4 Active *trans* Complexes

Most of the work on the design of platinum anticancer complexes has focused on cis-diam(m)ine complexes, since it was demonstrated, years ago, that trans-$[PtCl_2(NH_3)_2]$ is inactive. However, the discovery that trans-$[PtCl_2(py)_2]$ (py = pyridine) is strongly cytotoxic has stimulated renewed interest in trans complexes.[129] The trans Pt^{IV} complex 32 is active against both human and murine subcutaneous tumor models,[130] and efficiently promotes single-strand breaks and DNA interstrand cross-links.[131] These properties may explain its unusual cytotoxicity property against cisplatin-resistant tumors.[132] Strikingly the corresponding trans Pt^{II} complexes,without the axial hydroxo groups, are inactive. Direct reactions of the Pt^{IV} complexes with DNA may be crucial, but in vivo reduction could give reactive intermediates.

Trans-Imino ether platinum complexes such as 33 are more cytotoxic than the cis analogues (Ome and Pt are cis with respect to C=N in the Z isomer, and trans in the E isomer).[133,134] The mechanism of action of these agents seems to be different from that of cisplatin and may be correlated to the properties of the imino ether ligands.[135] Although these trans complexes react with DNA more slowly than cisplatin, they give the same level of DNA binding after 24 hrs. The trans E,E complex is the m strongest complex in inhibiting DNA synthesis and cell proliferation, but does not induce large local DNA conformational changes.[136] It especially forms monofunctional adducts at guanine residues in double-helical DNA even after long incubation times (48 hrs at 310 K).[137a] The reactivity of the second chloride ligand in the monofunctional adduct is strongly reduced. The trans E,E monofunctional adducts are not recognized by HMG proteins.[137b,c]

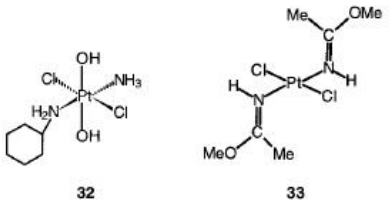


Figure 15 *Structures of trans Pt complexes*

1.9.5 Biotransformation

l-Methionine (L-MetH) may have an important role in the metabolism of platinum anticancer drugs. The Pt^{II}- L-Met complexes form quickly in plasma after injection of cisplatin into rats.[138] The complex [Pt(Met-S,N)$_2$] has been detected in the urine of patients[139] and exists mainly as the cis isomer (cis:trans=87:13) in solution (**Figure *16***).[140]

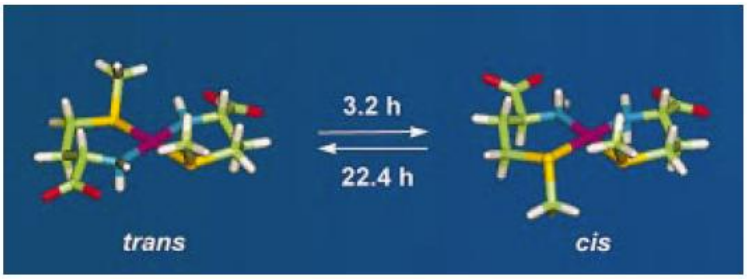


Figure 16 *Isomerization of R,R-trans- and S,R-cis-[Pt(Met)$_2$], a metabolit of cisplatin*

HAS, the human serum albumin is a single-chain 66-kDa protein which has 17 disulfide bridges and one free thiol group at Cys34, as well as six Met residues: M87, M123, M298, M329, M446, and M548. Reaction between HAS and cisplatin is believed to be the main route for platinum binding in human blood plasma.[141] Recent data suggest that cisplatin reacts strongly with methionine residues of albumin, forming a Met- S,N macrochelate together with minor adducts with Cys34 and monodentate Met-S residues.[142]

The reaction of carboplatin 24 with l-Met gives way to to a surprisingly stable ring-opened intermediate with a half-life of 28 hrs at 310 K.[143] Similar ring-opened complexes seems to be present in urine after

carboplatin administration.[144] Therefore it can be possible that the reaction of carboplatin with Met or its derivatives could give an activation pathway for the drug. The binding of the methionine sulfur atom is reversible, and it can be displaced by guanine N7.
Intracellular thiols such as glutathione (the tripeptide g- Glu-Cys-Gly, GSH), often present at concentrations of 3-10mM, are capable of inactivating platinum drugs. The main product from reaction between GSH and cisplatin is a high molecular mass polymer with a Pt:GSH ratio of 1:2.[145] The formation of these polymers may be responsible for depletion of platinum from the circulation. The active export of cisplatin from cells is one of the main mechanisms of resistance, and a GS-X pump, an ATP-dependent export pump for GS-conjugates, is able to pump out GS-Pt complexes from cancer cells.[146] Reaction between cisplatin and the cysteine-rich protein metallothionein (MT) leads to displacement of the ammine ligands and gives rise to $Pt_{7-10}MT$ containing PtS_4 clusters.[147] This gives way to another pathway for the inactivation of Pt drugs.

1.9.6 Photoactivation

Both Pt^{II} and Pt^{IV} complexes have long been considered to be photoreactive, and there are many field for the application of photodynamic reactions in pharmacology and biochemistry. A new approach to DNA platination involves the use of iodoplatinum(IV) complexes which are activated by visible light.[148] The toxicity of trans,cis-$[Pt(en)(OAc)_2I_2]$ against human bladder cancer cells is enhanced (by 35%) when the treated cells are irradiated with light of λ >375 nm. It has been shown that visible light can give way to the aquation of this complex followed by photoreductive platination of guanosine monophosphate, in contrast to the dihydroxo analogue.[149] Reactions of iodoplatinum(IV) ethylenediamine complexes with glutathione in the absence of light involve the initial attack of thiol on an iodide ligand of Pt^{IV} to give a reactive chelate ringopened intermediates.[150] Electron transfer driven labilization of trans ligands provides a new concept in drug design. UV light induced cleavage of both inter and intrastrand cross-links involving Pt-G bonds has been observed. Irradiation of DNA modified by cisplatin with UV light (λ>300 nm) can produce specific

cross-links to the protein HMG1, thought to involve Lys6 in domain B with labilization of a - purine bond.[151, 152]

1.9.7 Octahedral Platinum(IV) Complexes

Octahedral Pt^{IV} complexes show a tendency towards ligands substitution by a dissociative mechanism respect to an associative mechanism for Pt^{II}, with the net result that Pt^{IV} compounds are moderately more substitutionally inert. This is desirable for lower toxicity and oral bioavailability but it is not desirable for DNA intercalation. Despite that, most Pt^{IV} complexes are showing considerable activity in initial trials, with functionality thought to depend on the in vivo reduction of Pt^{IV} to Pt^{II}, producing reactive intermediates capable of interacting with DNA. In order to obtain oral bioavailability and beat cellular resistance, reserchears have tried multiple strategies to improve cisplatin properties. The reduction of Pt^{IV} to Pt^{II} compounds by biological agents is crucial to exert their antitumor activity. The reduction potential of Pt^{IV} complexes depends on the type of equatorial and axial ligands [153-160]. Choi et al. in another study, proved that the reduction rates which correlate with the reduction potentials depend on the electron withdrawing influence of the axial ligand [157]. In relation to the studied ethylenediamine-based Pt^{IV} complexes the fastest reduction rate ($OH < OCOCH_3 < Cl < OCOCF_3$) corresponds to the most electron withdrawing ligand and correspond to the highest observed cytotoxicity. Kelland et al. reported the synthesis of about 25 trans-Pt^{IV} complexes and their trans-Pt^{II} counterparts, and then evaluated and compared *in vitro* as well as *in vivo* anticancer activity of these complexes. The described compounds had the following general formula: trans- [(amine)(ammine)Cl_2Y_2] Pt^{IV} (Y =OH or Cl). Two amino groups atoms together with two chloride lie in the same plane, in equatorial positions. In opposition to them two hydroxide groups or two chloride atoms (Y ligands) are the axial ligands,. The anticancer activity studies gave very promising results. The complexes were tested in vitro against a series of human ovarian carcinoma cell lines, which contained tumor cells possessing either acquired (41McisR, CH1cisR and A2780cisR) or intrinsic (HX/62 and SKOV-3) resistance to cisplatin. Many of the complexes had a comparable anticancer activity respect to cisplatin and also did not show acquired cisplatin resistance. Notably, 14

complexes showed significant anticancer activity *in vivo* in the subcutaneous murine ADJ/PC6 plasmacytoma model. Among these, 13 complexes had axial hydroxido ligands and one had axial ethylcarbamate ligands, whereas all the platinum^{II} and tetrachloroplatinum^{IV} complexes were inactive [161].

1.9.8 Polynuclear Platinum Complexes

By the structural point of view, polynuclear platinum complexes are a different group. These innovative complexes have two or more platinum centers linked by various types of ligand (aromatic, aminoalkane, etc.). Thus, polynuclear complexes contravene many structure–activity rules for platinum complexes. Different SARs rules were created for polynuclear complexes based on their *in vitro* cytotoxicity results. One of the conditions to obtain good antitcancer activity of the complexes is the location of the leaving group, preferably chlorido, in trans position to the bridging linker. In the meanwhile, many trans and cis polynuclear complexes were synthesized, mainly by Farrell et al. [162-164]. Binuclear platinum^{II} complexes with bifunctional spermine, thiourea and modified tetraamine linkers have been synthesized and studied. The secondary, protonated, uncoordinating amines in these compounds may give additional interaction with DNA by electrostatic interaction and hydrogen bonding and thus provide a stronger activity over the parent dinuclear agents such as BBR3005 [{trans-PtCl $(NH_3)_2\}_2$-μ-[{trans-$Pt(NH_3)_2(H_2N(CH_2)_6NH_2)_2\}](NO_3)_2$ (Fig. 17.-complex 1) [165,166].
Trinuclear and tetranuclear platimun complexes have also been eavaluated [167,168]. The trinuclear complex BBR3464 (Fig. 17.- complex 2) is the first example of multinuclear compound which entered clinical trials in late 1997. Its preclinical antitumor profile was accentuated by remarkable potency, therapeutic doses approximately ten-folds lower that cisplatin, and activity in a wide set of solid human cancer [169,170]. Currently, BBR3464 is being entered in phase II clinical trials under the auspices of Novuspharma SpA. The investigations of phase I trials demonstrated that neutropenia and diarrhea were present as dose-limiting toxicities. The efficacy of this complex was not limited by neurotoxicity nephrotoxicity, or pulmonary toxicity [171]. The complex interacts with DNA with a different mechanism respect to cisplatin or other

mononuclear platinum complexes [172]. The tetranuclear platinum complex (Fig. 17.- complex 3), had low cytotoxic activity perhaps due to problems during trasport across the cell membranes [168].

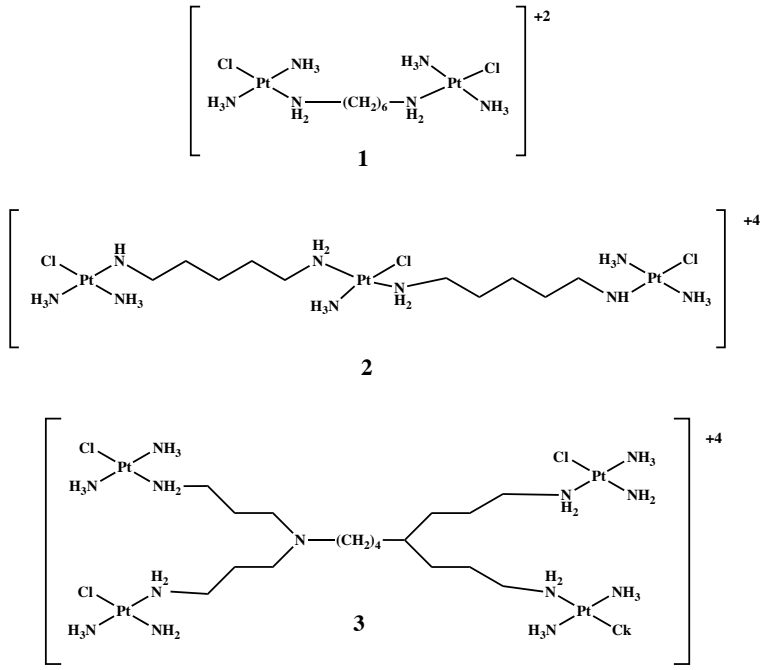


Figure 17 *Structure of polynuclear platinum complexes*

1.10 Palladium Complexes as Alternative Potential Antitumor Agents

The strong analogy between the coordination chemistry of Pt^{II} and Pd^{II} compounds has encouraged studies of Pd^{II} complexs as anticancer compounds[173,174]. A crucial factor that might explain is the ligand-exchange kinetics. The hydrolysis of leaving ligands in palladium complexes is too rapid, 105 times faster than their corresponding platinum complexes. They dissociate promptly in solution giving very reactive species that are unable to reach their pharmacological targets. In addition, some of them acquire an inactive trans-conformation. This

considerably stronger activity of palladium complexes suggests that in order to develop an antitumor palladium drug it must be stabilized by a strongly coordinated nitrogen ligand and a suitable leaving group. If this group is acceptably non labile, the drug can maintain its structural integrity *in vivo* for an enough long time. Many simple Pd^{II} compounds with interesting cytotoxic features have been previously reported such as [cis-$(NH_3)_2PdCl_2$] (Fig. 18- complex 1), [trans-$(NH_3)_2PdCl_2$] (Fig. 18- complex 2), [(1,5-COD)$PdCl_2$], [(Π-C_3H_5)$PdCl_2$]$_2$ and [(cyclopentyl)$_2PdCl_2$] Recent progresses in this field have also been based on Pd^{II} compounds having bidendate ligands as a way to prevent any possible cis-trans isomerism [175-177] .

1 **2**

Figure 18 *Two palladium (II) compounds with biological properties*

1.10.1 Trans-Palladium(II) Complexes Containing Bulky Monodentate Ligands

Many trans-Pd complexes with interesting activity against cancer cells have been prepared. Generally, the strategies that have been used to design these compounds were on the window of reactivity usually employed for the potential platinum anticancer drugs. A comparative study on anticancer activity was carried out between the diethyl-2-quinolmethylphosphonate (2-dmqmp) and dihalide Pd^{II} complexes of monoethyl-2-quinolmethylphosphonate (2-Hmqmp)[178]. The diester ligand has two potential donors, the the O from phosphoryl giving the complex [trans-(2-dqmp)$_2PdCl_2$] and N from quinoline and (Fig. 19- complex 1). The complexes of the diester 2-dmqmp were found to be more active than those of the monoester-based ligand (2-Hmqmp). This may partly be due to the greater leaving activity of the halogen ligands in the complex having the 2-dmqmp ligand and to their greater solubility or lipophilicity.

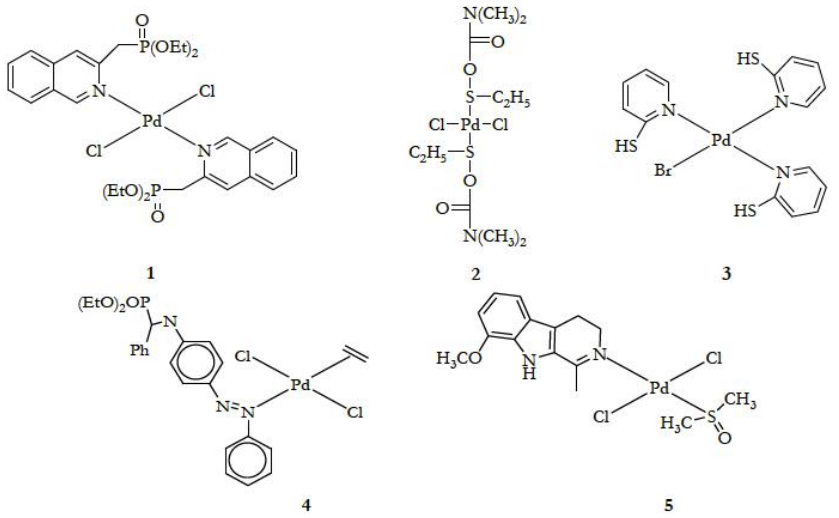


Figure 19 *Trans-palladium (II) complexes containing bulky monodentate ligands*

Fulrani et al., reported the synthesis and cytotoxic studies of some trans-$[(L)_2Pd(X)_2]$ complexes (**Figure *19*** complex 2) (L= N,N-dimethyl-O-ethylthiocarbamate: DMTC or N-methyl-O-ethylthiocarbamate: MTC, X= Cl, Br). Other palladium complexes based on 2-Mercaptopyridines (MP) were also synthesized. The $[(MP)_3Pd(Br)]Br$ (Fig. 19- complex 3) is of potential clinical use since it shows lower IC50 values on LoVo cell lines than cisplatin and about the same as its Pt^{II} analogue [179].

Palladium^{II} complexes containing alkyl phosphonates derived from quinoline and aniline were reported. Most of the aniline compounds (e.g. (Fig.19, complex 4)) had cyctotoxic properties *in vitro* against animal and human tumor cell lines. Complexes with naturally occurring compounds have also been used. The palladium complex which contains the bulky nitrogen ligand harmine (7-methoxy-1-methyl- 911-pyrido[3,4-b]indole, trans-[Pd(harmine) $(DMSO)Cl_2$] (**Figure *19*** complex 5) shows a greater cytotoxic activity against K562 and L1210 cell lines than cisplatin [180].

Abu-Surrah et al., recently, reported the synthesis and molecular structure of a new chiral, enantiometrically pure, trans-palladium(II) complex, trans-$[2\ \{(R)\text{-}(+)\text{- bornyl-amine}\}_2Cl_2]$ (Fig. 10) that has the bulky amine ligand R-(+)-bornyl-amine [181]. The complex had similar anticancer

activity against HeLa cells when compared to the activity of the standard drugs, cisplatin, carboplatin and oxaliplatin.

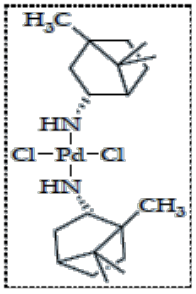


Figure 20 *Structure of the palladium (II) complex*

1.10.2 Palladium^{II} Complexes with Bidentate N∩N Ligands

Dichloro palladium^{II} complexes with spermine and spermidine were reported by Navarro-Ranninger et al. [182]. This type of chelating ligands have been utilized because of their relevant biological activity; they are involved in differentiation and proliferation of cells in membrane stabilization and DNA replication. Complexes of spermidine (Fig. 21- complex 1) show values of IC50 similar to cisplatin, whereas those of spermine (Fig. 21- complex 2) have low anticancer activity. Ethylendiamine palladium^{II} complexes with pyridine or its derivatives were also reported[183]. The increase of the electron donor properties of the substituents mainly lead to an increase of the donor strength of the coordinated pyridines, which directly lead to the increase of the cytotoxic activity of the palladium compounds.

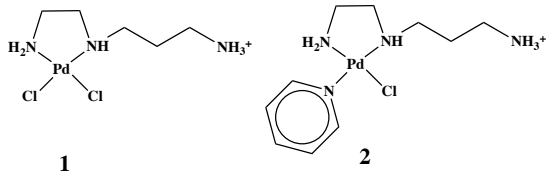


Figure 21 *Palladium(II) complexes with bidentate N∩N Ligands*

Abu-Surrah et al., recently, used an alternative method to prepare the enantiometrically pure DACH-based palladium^{II} complexes [184,185]. In this procedure, the desired organic bidentate ligand was allowed to react

with [cis-Pd(PhNC)$_2$Cl$_2$], a palladium^{II} starting material that is soluble at 25°C in most organic solvents, in CH_2Cl_2. Following this method, the nucleophilic substitution reaction of the complex [cis-Pd(PhNC)$_2$Cl$_2$] with (1R,2R)-(-)-1,2-diaminecyclohexane afforded the square planar Pd(II) complex [(1R,2R)-(-)-(DACH)PdCl$_2$] (Fig. 22- complex 1) with an high yield. The corresponding cationic, aqua complex, [(DACH)Pd(H$_2$O)$_2$](NO$_3$)$_2$ (Fig. 22- complex 2) and the oxalate complex [(DACH)Pd(C$_2$O$_4$)] (Fig. 22- complex 3) have also been synthesized and characterized [186]. A series of other oxaliplatin like complexes such as [(DACH)Pd(O-O)] has also been synthesized by Khokhar et al. [187] (O-O: malonate, methylmalonate, phenylmalonate, xylate). Unluckly, the influence of the different dicarboxylate ligands could not be focused since the complexes lack the anticancer activity. This could be related to the low stability and solubility of the above complexes in solution.

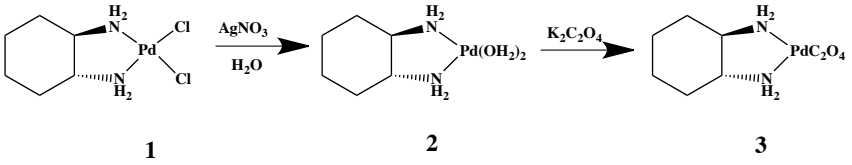


Figure 22 *DACH-based palladium (II) complexes*

In order to increase the stability of the palladium^{II} complexes, two chelates forming two rings around the central atom were synthesized and studied. Modified amino acids such as py-CH$_2$-accys (Fig. 23- complex **1**) (accys: N-acetyl-S-methylene-2-(2-pyridine)-L-cysteine) have been used [188]. The S,N-chelation mode of these ligands is important, since only the side chain of the aminoacid is involved in metal coordination, whereas the aminoacid function remains uncoordinated, leaving this functional group accessible for the attachment of other aminoacid or peptides. It has been demonstrated that the reactivity of these palladium complexes compete with some platinum^{II} complexes. Another study investigated compounds having NO$_3$ as a chelate in addition to a bidentate nitrogen ligand [189]. A comparison among [(bipy)Pd(NO$_3$)$_2$], [(AMP)Pd(NO$_3$)$_2$], [(AEP)Pd(NO$_3$)$_2$], [(DACH)Pd(Meorot)] (bipy = 2,2′-bipyridyl, AMP = 2-aminomethylpyridine, AEP = 2-aminoethylpyridine, Meorot = 3-methylorotate) proved that only [(DACH)Pd(Meorot)] (Fig. 23- complex 2) was active, showing an high activity against sarcoma 180 but a low one

against P388 leukemia. Similarly, [(DACH)Pd(5-fluroorot)] (Fig. 23- complex 3) later reported [190], showed a significant anticancer activity. These strong chelating ligands, replacing nitro or chloro ligands, induce a reduction in the percentage of hydrolysis.

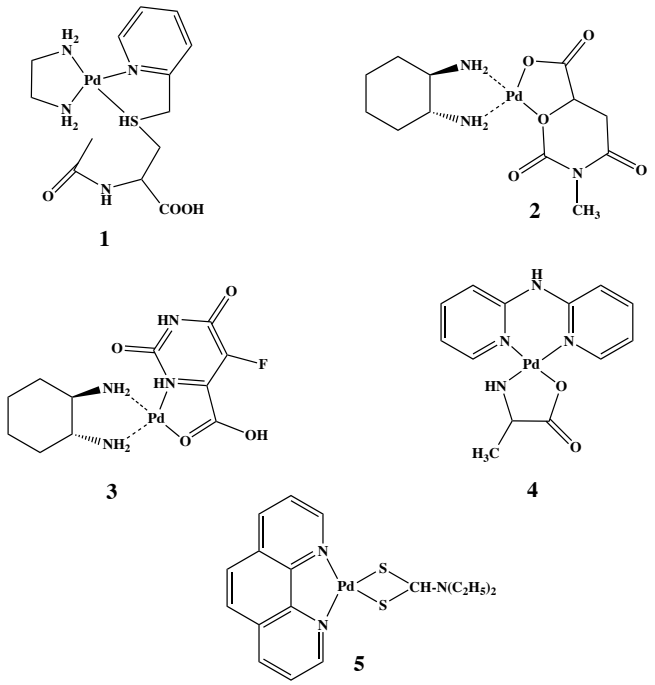


Figure 23 *Palladium ^{II} complexes bearing two bidentate chelates or tetradentate nitrogen ligands*

2-2'-bipyridylamine-based palladium^{II}complexes having L-alanine or glycine have been reported and evaluated [191]. The alanine based complex (Fig. 23- complex 4) dysplaied stronger cytotoxic activity against P388 lymphocytic leukemia cells than the glycine based one. Other aromatic ligands such as 1,10-phenanthroline, which is one of the most widely used ligands in coordination chemistry, has been utilized in the field of antitcancer-transition metal chemistry. Its planar nature allows its participation as a DNA intercalator. Several derivatives of it were synthesized and used as tetradentate ligands. The activities of [N,N-dialkyl-1,10- phenanthroline-2,9-dimethamine)Pd(II)] (Fig. 23- complex 5) (alkyl: Me, Ethyl, propyl, cyclohexyl) are significantly dependent on

the nature of the alkyl substituents. The complexe having the bulkiest groups showed lower IC50 values than cisplatin [192].

1.10.3 Palladium complexes with Biphosphine P ∩ P Ligands

Many of the prepared palladium^{II} complexes showed a moderate anticancer activity *in vitro* compared to the platinum-based drugs because of their very high lability in biological fluids [193]. Therefore, it has been suggested that the organometallic biphosphine-based cyclopalladated complexes which are more stable and less toxic could have *in vivo* a more specific anticancer activity[194]. Many cyclopalladate complexes based on biphosphine ligands (Fig. 24) have been synthesized and evaluated for their anticancer properties in a murine syngeneic B16F10 melanoma model. The ionic complex killed 100% tumor cells even at very low concentration (<1.25 μM). Other Pd^{II} complexes containing bidentate phosphine ligands of the general formula $[L_2PdXm]n+nX$ (L= Ph_2P-A-PPh_2, A= $(CH_2)_2$, $(CH_2)_3$, X= Cl, Br, NO_3) were synthesized and studied for *in vitro* cytotoxicity, anticancer activity in murine tumor model and mechanism of action. The mode of action of these complexes seems tp be different from that of cisplatin in relation to the effects on DNA.

Figure 24 *Palladium (II) complexes with biphosphine P ∩ P Ligands*

1.10.4 Palladium(II) complexes with N ∩ S Mixed Donor Ligand

Khan et al. reported some palladium^{II} complexes with mixed sulfur-nitrogen ligands such as substituted pyrimidines (mercapto or amino) and methionine [195]. Methionine coordinates to Pd^{II} through sulfur and amino nitrogen, thus leaving a carboxylic group free. It has been demonstrated

that the complex [(methionine)Pd(2-merpy)Cl]Cl (Fig. 25) has IC_{50} value lower than 10 μg/ml, *in vitro*, so it could be a potential anticancer agent.

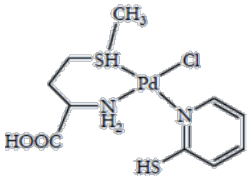


Figure 25 *Palladium(II) complexes with N ∩ S Mixed Donor Ligand*

1.10.5 Dinuclear Palladium(II) Complexes

In the field of dinuclear palladium complexes chemistry, the use of strongly coordinated dinitrogen ligands is preferred. The synthesis of putrescine and spermine-based dinuclear complexes: $[PdCl_4(Put)_2]$ and $[PdCl_4(Sperm)_2]$ has been reported by Navarro-Raninger.The complex (Fig. 26- complex 1) is a coordination complex with a dimeric nature. In the 4 amino groups of the spermine coordinate to two cis-Pd-centers (Fig. 26- complex 2). The cytotoxicity results showed that the putrescine complex is much more reactive respect to the spermidine one [182]. Zhao et al. evaluated dinuclear palladium complexes having two functional $[Pd(en)(pyridine)Cl]^+$ units bridged by Se or S.[196]. The complexes are soluble in water. The Se-Bridged Pd^{II} dimmer (Fig. 26- complex 3) has a lower IC50 than the S analogue or *cisplatin* against the HCT8 cancer cells Dinuclear cyclopalladated organometallic complexes having biphosphine ligands were also studied by Rodrigues et al. [197]. The dimeric Pd^{II} complex showed to be the most active *in vivo* compared to the corresponding mononuclear complexes. It reduces tumor growth and increases animal survival.

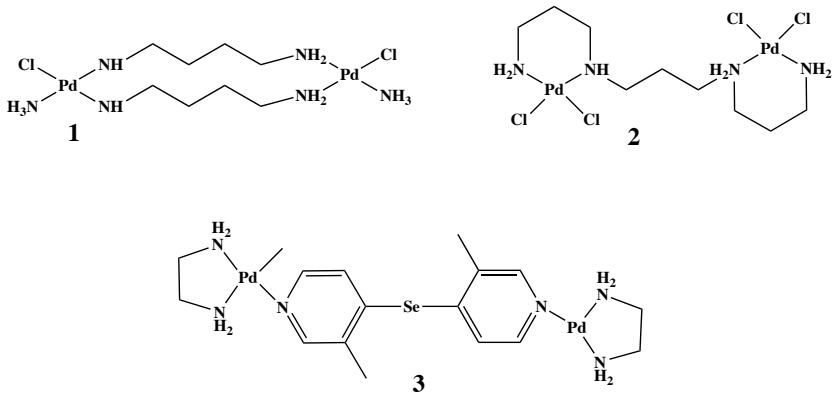


Figure 26 *Structure of multinuclear Palladium complexes*

1.11 Ruthenium (II & III) Complexes as Antitumor Agents

Recently it was demonstrated that ruthenium has several favorable chemical properties, suggesting that it may be a strong candidate to substitude platinum and to give a basis for rational anticancer drug design. Ruthenium is less toxic than platinum and it is believed that the remarkable antitumor activity of ruthenium is related to its ability to mimic iron in binding to several biomolecules, including albumin and serum transferrin. Two Ru^{III} complexes, named *trans*-$[RuCl_4(Im)(DMSO)]ImH$ (NAMI-A) and *trans*-$[RuCl_4(Ind)_2]IndH$ (KP1019) are undergoing clinical trials. Whilst KP1019 has cytotoxic activity against cancer cells, NAMI-A has anti metastatic activity being relatively non-toxic[198-203]. It has been proposed that the activity of Ru^{III} complexes, which are usually relatively inert towards ligand substitution, is dependent on *in vivo* reduction to more labile Ru^{II} analogues. Thus, the activity of Ru^{II} complexes is currently being evaluated. In particular, the potential of Ru^{II}–(h6-arene) complexes as anticancer agents is under investigation since arenes are known to stabilize ruthenium in its 2^+ oxidation state[203-205]. Half-sandwich (h6-arene) Ru^{II} complexes with sulfoxide, phosphane, imidazole, chelating amino acidato, and diamine or diimine ligands have also been studied for cytotoxic activity. Both the

lability of the Ru–Cl bond and the size of the arene and have a crucial role in determining the cytotoxicity of ruthenium^{II} complexes such as [(h6-arene)RuCl(LL′)](PF6) with bidentate ligands LL′. Compounds having extended polycyclic arenes (e.g. tetrahydroanthracene) and LL′ = ethylenediamine (en) are mainly active towards A2780 human ovarian cancer cells, whereas those with polar substituents on the arene such as $COOCH_3$ (an electronwithdrawing group) or 1,10- phenanthroline (phen), or with aromatic diimine ligands such as 2,20-bipyridine (bpy) have either poor or no activity.

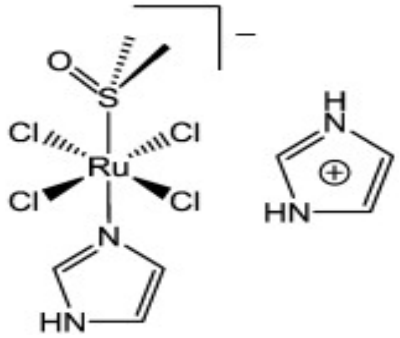


Figure 27 *Structure of the anticancer drug NAMI-A*

Further increases in the size of the polypyridyl ligand (pp) lead, however, to a decisive reversal of the latter trend and the *in vitro* cytotoxicities of the complexes $[(\eta^6\text{-}C_6Me_6)RuCl(pp)](CF_3SO_3)$ (Fig. 28) towards the human cell lines HT-29 (colon cancer) and MCF-7 (breast cancer) are mainly dependent on the surface area of the aromatic system. For example, the IC50 values decreases from 11.1 over 2.12 to 0.13 μM for MCF-7 cells as the size of the polypyridyl ligand increases in the order dpq < dppz < dppn (dpq = dipyrido- [3,2-f:2′,3′- h]quinoxaline; dppz = dipyrido[3,2-a:2′,3′-c]phenazine; dppn = benzo[i]dipyrido[3,2-a:2′,3′-c]phenazine). These value are related with the cellular uptake efficiency, which increases from 1.1 over 146.6 to 906.7 ng(Ru)/mg(protein) within the series. The kinetically inert complexes $[(\eta^6\text{-}C_6Me_6)Ru\{(NH_2)_2CS\}(pp)](CF_3SO_3)_2$ (pp = dppz, dppn) (Fig. 29) are also cytotoxic and this suggests that specific properties of the large polypyridyl ligands (e.g. DNA cleavage and/or intercalation) may be responsible for their biological activity. DNA binding studies show that (h6- C6Me6)Ru^{II}

compounds containing dpq or particularly dppz ligands are good metallointercalators but that the dppn ligand is too large to support stable intercalation between the base pairs of the double helix [206-214]. The in vitro and in vivo assessment of a series of h6-arene ruthenium complexes containing a pta ligand (pta = 1,3,5-triaza-7- phosphaadamantane) were studied as anticancer agents [215]. In addition to the toluene, para-cymene benzene and hexamethylbenzene derivatives, three systems with functionalised arene ligands were evaluated.

All pta complexes were demonstrated to give a pH-dependent DNA damage, with the DNA damage at the typical pH of hypoxic tumor cells, whereas little or no damage was observed at characteristic pH values of healthy cells. This behavior was correlated to the pta ligand, which can be protonated at low pH, and the protonated form was considered to be the active agent. Therefore, the introduction of functionalised pendant arms on the arene ligand, such as in the below complexes (Fig. 28), did not have any significant improvement in the cytotoxicity of the compounds (IC50 (TS/A cells) = 66 μM,; 103μM,; 159 μM,) if compared to the h6-aromatic hydrocarbon systems.

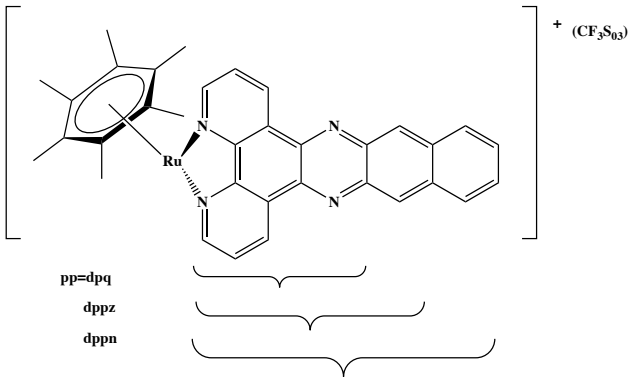


Figure 28 *Structure of [(η^6-C_6Me_6)RuCl(pp)](CF_3SO_3)*

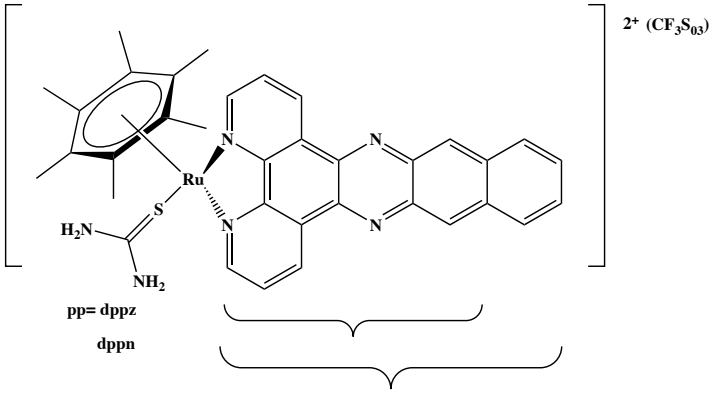


Figure 29 *Structure of $[(\eta^6\text{-}C_6Me_6)Ru\{(NH_2)_2CS\}\text{- }(pp)](CF_3SO_3)_2$*

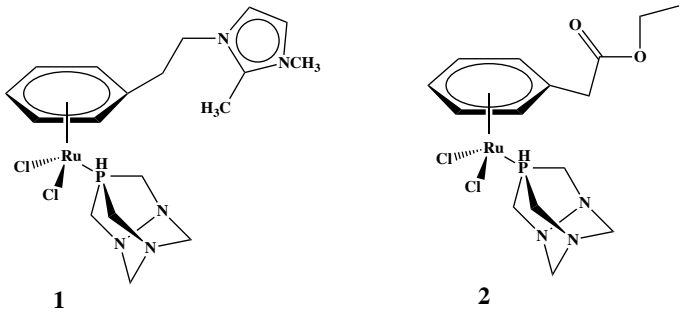


Figure 30 *Chemical structure of a series of η^6- arene ruthenium complexes contraining a pta ligand*

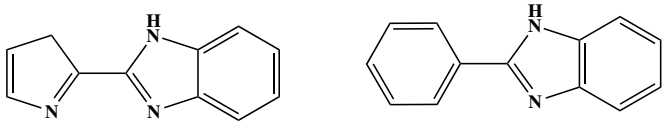
1.12 Copper Complexes as Antitumor Agents

The anticancer activities of a set of simple copper complexes having different types of nitrogen donor ligands such as thiosemicarbazone, imidazole, purine, benzohydroxamic acid and amino acid ligands have been evaluated[216-220]. Some mixed chelate copper-based drugs showed greater antineoplastic properties than cisplatin *in vitro* and *in vivo* studies [221,222].

Copper complexes seem to have a mechanism of action certainly different respect to the clinically used drug cisplatin. For example, the complex

[Cu(malonate)(phen)$_2$] was demonstrated to induce apoptosis in cultured mammalian cells and is able to mediate significant cellular oxidative stress, interfere with mitochondria respiratory activity in fungal cells and promote membrane lipid peroxidation [223,224]. Similar copper chelates of phen were studied and also had high antineoplastic activity by inhibiting ATP synthesis and respiration[225]. Moreover, complexes of general formula $[Cu(phen)]^{2+}$ are known to bind to DNA both intercalating and non-intercalating, and are known as potent oxidative nucleases but the exact structure of $[Cu(phen)]^{2+}$ when bound to DNA has not been poperly characterized [226].

Recently Devereux et al. have been studing alternative chelating ligands such as TBZH and 2-PyBZIMH (Fig. 21). The anti-cancer activity of TBZH is mainly increasedif it is bound to a copper centre [227,228] but none of the reported complexes have exhibited activity close to that of cisplatin so far. Reports of the biological activity of 2-PyBZIMH are scarce with no papers published describing the anti-tumor activity of its copper complexes. Its use as a ligand in the synthesis of novel Pt^{II} and Pd^{II} anti-tumor agents has been reported [219]. In this study thee reserachers did not test the free 2-PyBZIMH ligand but found that [Pt(2- PyBZIMH)Cl_2] was the most efficient anti-cancer agent against eight brain tumour cell lines, but the activity was significantly lower than that of cisplatin.



TBZH **2-PyBZIMH**

Figure 31 *Structure of TBZH and 2-PyrBZIMH ligands*

Recently, Sathisha et al. prepared a complex of Cu^{II} with bis(3 acetylcoumarin) thiocarbohydrazone (Fig. 32). This octahedral distorted complex has demonstrated promising cytotoxic activity when screened using the *in vitro* method for certain types of cell lines.

Bravo-Gómez et al. selected a mixed chelate copper^{II} complex of [Cu(N–N)(acac)]NO_3 and [Cu(N– N)(gly)]NO_3 with several substituents on the diimine ligand to obtain a quantitative structure–activity relationship (QSAR) study. Moreover, Chen et al. have synthesized copper^{II}

complexes of ethyl 2-[bis(2-pyridylmethyl)amino]propionate ligand (ETDPA). These complexes are stable in air with the formula of [(ETDPA)CuCl2] and [(ETDPA)Cu(phen)]$(ClO_4)_2$.
The DNA-binding capability of the two Cu complexes with DNA was different. The binding mode of [(ETDPA)CuCl2] is not the classical intercalation binding, and the binding mechanism of [(ETDPA)Cu(phen)]$(ClO_4)_2$ with DNA is intercalation. The cytotoxic assay shows that the [(ETDPA)Cu(phen)]$(ClO_4)_2$ is more active on the proliferation of cancer cells than the [(ETDPA)$CuCl_2$].

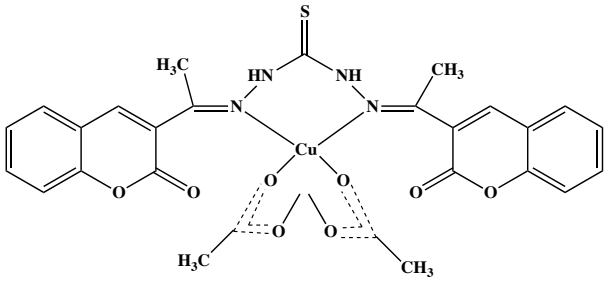


Figure 32 *Proposed structure of the copper complex synthesized by Sathisha et al.*

1.12.1 Other Metal Anticancer Agents

Tetrahedral bis(diphosphanyl)gold^{I} complexes (see figure 33) are much less reactive, than linear AuI antiarthritic complexes for example towards thiols. This complex (lactate salt) shows goood activity in a range of cancer models. It has a different mechanism of action respect to cisplatin, and is targeted to mitochondria, where it destroys membrane potentials. It showed to be too cardiotoxic for clinical use, but the introduction of pyridylaryl substituents on P increases the hydrophilicity of diphosphane bischelated AuI complexes, and produce strong and selective activity towards cisplatin resistant and sensitive ovarian tumor cells.[229] It should be evaluated whether the cardiotoxicity is reduced.
Gallium salts are known to exhibit anticancer activity, and Ga^{III} is probably delivered to cancer cells through the transferrin, a serum protein. Recent interest in gallium salts derives from their synergistic effect with cisplatin in the treatment of carcinoma of the urothelium and lung cancer.[230] Gallium^{III} maltolate has entered clinical trials for conditions related to the treatment of bone disease.[231]

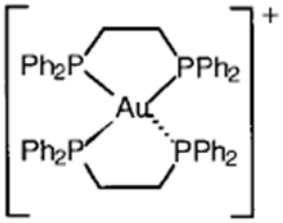


Figure 33 *Structure of Tetrahedral bis(diphosphanyl)gold(I) complex*

1.13 Anticancer Agents: Titanium Complexes

Among all the evaluated metals– which comprise much of the periodic table – complexes of titanium have shown particular promise due to high activity against tumors that are resistant to cisplatin combined with low toxicity[232-240]. In particular, the titanium complexes bis(cyclopentadienide)titanium dichloride (Cp_2TiCl_2, titanocene dichloride, 1) and cis-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV) [(bzac)2- $Ti(OEt)_2$, budotitane, 2] (Fig 34) have been the first metal-based chemotherapeutics to reach Phase I clinical trials since the development of cisplatin. Although both complexes showed promising properties in these preliminary studies, 1 has not progressed beyond Phase II due to its low efficacy respect to toxicity ratio,[241,242] and 2 has not progressed past Phase I due to formulation problems.[243] These difficulties have increased the development of titanium complexes that show higher potency and hydrolytic stability.

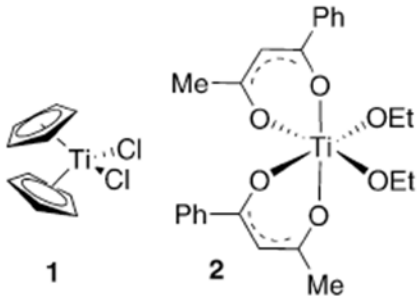


Figure 34 *Structure of TDC (1) and Budotitane (2)*

The strong cytotoxic activity of TDC against implanted Ehrlich ascites tumors (EAT) in mice was reported in 1979 where cure rates greater than 80% were observed with little of the heavy-metal toxicity observed for cisplatin.[244] In following studies the authors reported the key observation that while 1 and vanadocene dichloride showed similar activities against EAT in-vivo, the cytotoxicity of TDC was ca. 100 times less than that for vanadocene dichloride *in-vitro*.[245] The reason for the reduced activity was hypothesized to be due to the hydrolytic instability of TDC, which rapidly forms insoluble aggregates in aqueous solution with eventual formation of TiO_2, a feature that often occurs with many Ti^{IV} complexes because of their oxophilic and electron-poor nature. A detailed study of the hydrolytic behavior of TDC in neat water at various pH ranges showed that at pH 5, following a rapid loss of the first chloride ion, complete hydrolysis of both chloro groups occurs with a $t_{1/2}$ of ca. 50 min, while hydrolysis of the Cp ligands is slower ($t_{1/2}$ ca. 54 h).[246] On the other hand, vanadocene dichloride was stable toward hydrolysis at pH 7, which certainly explains the difference between the *in-vitro* and *in-vivo* results for these two complexes. The in-vitro cytotoxicity could be resolved by using a galenic formulation,[229] and a subsequent patent reported a water-soluble formulation of 1, MKT-4, which allowed the intravenous administration during clinical trials.[247]

In 1982 the anticancer properties of Budotitane (2) were reported, for the first time.[248] It shows cytotoxic activity towards animal cancer such as EAT and colon tumors.[233,243] During the hydrolysis tests, the addition of 2 to neat water gave a suspension in which the complex remained completely undecomposed.[233] However, if water was added to a solution

of 2 in methanol or acetonitrile, rapid hydrolysis ensued. The half-life for hydrolysis of the ethoxy groups in water was calculated as $t_{1/2}$ = 20 s, while hydrolysis of the diketonato ligand in acetonitrile/water solutions of **2** takes places after 2.5 h. The use of smaller diketonato ligands was demonstrated to give even a more rapid ligand hydrolysis. NMR studies show that **2** exists as the cis isomer.[233] MM3 studies suggest that the predominance of the cis compound is primarily due to steric factors. In an interesting demonstration of the additional structural complexities that can accompany the use of transition metals as drugs, the unsymmetrical ligands of 2 can potentially give rise to three sets of enantiomeric cis complexes. In $CDCl_3$ solution at 23 °C, 2 exists as a 4:1 mixture of two cis isomers. The relative cytotoxicity of these two compounds has not been investigated, although the crystal structure of one of them has been determined.[249]

1.13.1 Structure–Activity Relationships of Titanocene Dichloride and Related Compounds

Considerable work has been performed in developing therapeutic analogues of 1 by varying the central metal (M), the labile ligands (Cl) and the bis-cyclopentadienyl moiety.

The study of cytotoxic metallocenes other than titanium has been described in detail elsewhere.[250] Substitution of the chloro leaving ligands has been evaluated in order to obtain analogues with improved water solubility, but in general has not been shown to have a deep impact on cytotoxicity. For example, titanocenes of the general formula Cp_2TiX_2 (X = Cl, Br, NCS, maleate, O_2CCl_3) all achieved cure rates of 100% in mice with fluid EAT, I agreemement with the hypothesis that the cytotoxic Ti^{IV} compounds is generated from displacement of the labile halide ligands in 1.[251,252] In contrast, small changes to the Cp ligand can strongly impact the hydrolytic stability and water solubility features of the metallocenes and thereby affect the cytotoxic activity. Moreover, it has been suggested that substitution can also impact the capability of the ligand to intercalate with DNA, although further work are needed to establish thismechanism.

Substitution of one of the hydrogen atoms of the Cp ligand by R [R = Et (3), R = $Si(CH_3)_3$ (4)] (figure 35) showed to reduce cure rates of mice

with fluid EAT to 60– 80%, while substitution of both Cp ligands by R reduced cure rates drastically to 10–30%.[232]

Pentamethyl-substituted titanocenes were reported to be completely devoid of cytotoxic activity. The extent to which this lowered activity represents the reduced water solubility of these compounds is not sure. Studies on the hydrolytic behavior of the methylated metallocene complex $(C_5H_4Me)_2TiCl_2$ (5; C_5H_4Me = 1-methylcyclopentadienide) revealed that the CpMe ligands were less promptly hydrolyzed than TDC at physiological pH. Furthermore, when the chloride leaving ligands were replaced with glycine in this system 6 (figure 35), water-soluble complexes with high hydrolytic stability were obtained at physiological pH. The compounds were observed to generate stable complexes with nucleotides at pH 7, although further biological studies were not reported.[253]

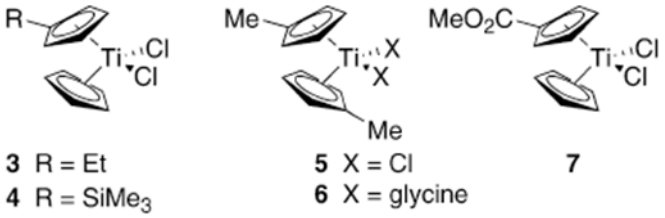


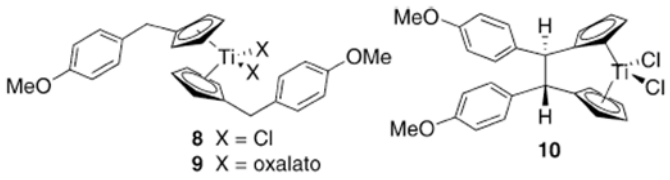
Figure 35

Substitution of the Cp ring with electron-poor substituents (R = CO_2Me 7) (Figure 35) was reported to increase cytotoxicity in a human small-cell lung-cancer cell line but revealed lower cytotoxic properties towards a number of other studied cancer cells.[254] This could be justified by reduced hydrolytic stability due to the lower ligand-to-metal electron donation.

Many titanocene analogues containing aromatic groups appended to the Cp ligand have also been prepared.[240] Although generalizations regarding structure–activity relationships are not yet clear, these complexes have shown promising *in-vitro* activity against the LLC-PK cell line.

When tested against a panel of 36 human tumor-cell lines, the most promising candidate, titanocene Y (8) (figure 36), was found to have

good activity against renal cell cancer. Interestingly, the oxalato complex 9 (figure 26), possessing a substantially less labile chelating ligand in replacement of the chloro groups, was reported to have a 13- fold increase in activity relative to 8 during *in-vitro* studies against the LLC-PK cell line,[255] yet *in-vivo* studies showed quite identical activity if compared to to 8.[256] Although the hydrolysis behavior of 8 and 9 was not studied in detail, it is possible that differences in the IC50 values of 8 and 9 obtained *in vitro* may in fact reflect the greater hydrolytic stability of 9. In general, lower activity was found in analogues where the two Cp units were bound by a two-carbon bridge, obtained as mixtures of stereoisomers (10, figure 36), although other ansa-metallocene complexes studied recently revealed promising activity.[257,258]



8 X = Cl
9 X = oxalato

10

Figure 36

Several attempts have been performed to improve the aqueous solubility of 1 through appendage of polar side chains to the Cp ligands. For instance, titanocenes with alkylammonium hydrochloride moieties gave improved aqueous solubility.

Compound 11 (figure 37) showed good activity against both A2780 and the cisplatin-resistant A2780/CP cell lines.[259] Interestingly, an analogue of 11 with a bulkier Cp ligand (12, figure 37) possessed similar cytotoxicity.[260] Concurrently, in a parallel study, a significant number of similar alkylammonium complexes were prepared and their *in-vitro* cytotoxic properties evaluated against H209, A549, and A2780 cell lines in direct comparison with the water soluble synthesis of TDC, MKT-4.[261] In initial studies an intersting difference in cytotoxicity was found between bis(alkylammonium)cyclopentadienide complexes and those containing a single alkylammonium Cp ligand.

However later studies on related compounds, have found that this "bis(alkylammonium)" effect is not necessarily general.[262]

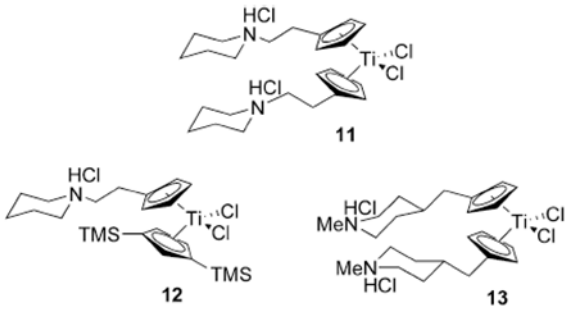


Figure 37

A number of water-soluble and cationic derivatives of TDC have been synthesized through incorporation of secondary amide or an ester on to the Cp ligand.[263,264] By using an acylation reaction, a diverse array of amide and ester-containing titanocenes were prepared. *In-vitro* studies of complexes with the general structure 14 (Figure 38) demonstrated good activity against BJAB (leukemia) cell lines.[265] Notably, complex 15 (Figure 38) possesses a fluorescent aminopyrene unit which may be of potential application in studies in order to understand the biological activity mechanism.

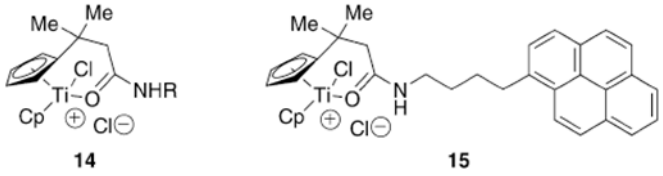


Figure 38

1.13.2 Structure–Activity Relationships of Budotitane and Related Compounds

The structure–activity information collected from the preparation of 200 derivatives of budotitane with the general formula D_2MX_2 (D = 1,3 dicarbonyl;M = metal; X = labile ligand) and their activity against the sarcoma 180 ascitic tumor model were reported.[233] Among the studied metals, the cytotoxic activity decreased in the order Ti>Zr >Hf >Mo >Sn >Ge.

No significant difference in cytotoxicity was found between compounds incorporating the leaving groups X (X = F, Br, Cl, OEt), although the OEt group was eventually preferred due to its stronger hydrolytic stability.

Among the 1,3-dicarbonyl groups reportes, acetylacetonato (acac) compounds possessed very low activity, while optimal antitumor activity was found for the benzoylacetonato (bzac) ligand. Incorporation of donating (OMe) or electron-withdrawing (Cl, NO_2) groups on the aromatic ring had a marginally deleterious effect upon the observed cytotoxic activity, as did the replacement of phenyl for tertbutyl, although considerable cytotoxicity was preserved.

The cytotoxic activity of structurally related mononuclear and polynuclear titanium^{IV} 4-acyl-5-pyrazolonato (16 Figure 39) species which are structurally related to 2 were studied.[234,266] The cytotoxic acrivity of a tetranuclear species 17 (Figure 39) was encapsulated in a liposome to improve its insufficient water solubility and found to display cytotoxicity against TA-3 and HEP-2 cell lines in-vitro. Synthetic studies found that selectivity for mononuclear compounds could be obtained by rigorous absence of moisture during their synthesis. Notably, addition of trace water to a solution of the monomeric compound 18 (Figure 39) in anhydrous [d_6]benzene formed a highly stable species that was hypothesized to be hydrolyzed oxo-bridged oligomers retaining the chelating ligand within 15 mins, unlike the observations with budotitane.[233]

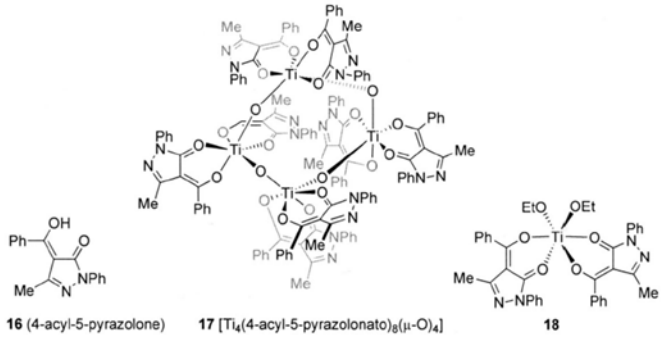


Figure 39

1.13.3 Comparison of the Biological Profiles of Titanocene Dichloride and Budotitane

In general, the cytotoxic activity profiles of TDC and Budotitane show many congruencies. Comparing the *in-vivo* studies, 1 and 2 have good activity against slow-growing tumors of the liver, colon, and kidney, and relatively low activity against faster growing cancers such as leukemia. Notably, emesis and other symptoms associated with the toxicity of cisplatin were not present during clinical trials of 1 and 2. Both complexes hydrolyze quickly, and require galenic preparations with water-soluble ligands in order to improve water solubility and stability. Additionally, both compounds contain a set of aromatic ligands that undergo hydrolysis under physiological conditions within several hours. The observation that 1 and 2 do not exert their maximal cytotoxic effects until >24 h after administration – in hard contrast to cisplatin – certainly leads to the question of how this relates to the half life of ligand hydrolysis.[267] In addition, the observation that 1 shows stronger cytotoxicity following aging in certain organic co-solvents[268] raises questions regarding the peculiar lability required of the active compounds.

While a number of detailed biological studies have been performed on 1, analogous biological studies for 2 are largely absent. Although it is plausible to hypothesize that 1 and 2 share a similar mechanism of action, too little is currently known to draw definite conclusions. The following

section will attempt to summarize what is currently known about the mechanism of action of these cytotoxic titanium compounds.

1.13.4 Mechanistic Aspects of Biological Activity

Early hypotheses that 1 share a mechanism of action with cisplatin[239] have been challenged by the efficacy of 1 against cisplatin-resistant cell lines, as well as by the differnt chemical behavior of "soft", water soluble $Cl_2Pt(NH_3)_2$ with "hard", hydrolytically unstable Cp_2TiCl_2 observed in aqueous solution under physiological conditions.[241] Nucleic acid metabolism appears to be disturbed by titanocene compounds. Analysis of the intracellular localization of titanium after treatment of TDC found titanium accumulation in cellular regions rich in nucleic acids.[269] Interestingly, while cisplatin promptly gives cessation of DNA-metabolic activity, TDC does not affect cell transit through the S phase but cells are irreversibly unable to perform mitosis.[270] It has been concluded that the three main phenomena observed in cells after treatment with TDC – specifically, the activation of endogenous viruses, the formation of giant cells, and the development of cellular necrosis – are all consistent with an interaction of 1 with intracellular DNA.[271] Adducts of TDC (Cp_2Ti–DNA and CpTi–DNA) with calf thymus DNA has been detected through spectroscopical studies,[272] as was also supported by model studies with particular nucleotides.[273] The specific interaction of the active titanium species with DNA remains not clear yet, although an interaction with the phosphate backbone has been proposed.[274]

As far as budotitane is involved, the contribution of the aromatic diketonato ligand toward higher cytotoxic activity has led to hypotheses concerning DNA intercalation.[233,266]

However, because of the considerable activity retained when C_6H_5 is replaced by tert-butyl, the uncertain nature of the active species, and the lack of biological studies on 2, this proposal remains speculative.

Another study described the interaction of TDC with topoisomerase II, an enzyme catalyzing the opening and rejoining of the DNA phosphate backbone during the cell cycle. TDC was reported to be a strong inhibitor of topoisomerase II, which may also lead to antiproliferative effects.[275]

Much recent work has focused on the mechanism thorugh which anticancer titanium complexes are transported into cells. Mainly, the

question of whether ligands remain attached to the titanium during transport into the cell or whether the ligands merely serve to protect the titanium center from hydrolysis in the intercellular medium remains a critical not answered question. Discovering the mechanism of titanium transport would have important implications for the design of more effective and potent cytotoxic titanium complexes.

Observing the increased number of transferrin receptors on the surface of tumor cells and the implication of transferrin in transport of metal ions such as Ru^{III} and Ga^{III} to cancer cells,[276, 279] it has been proposed that transferrin (Tr) is responsible for uptake of titanium from the intercellular medium.[279-281] Transferrin, the 80 kDa protein responsible for iron transport into cells, is present in the intercellular medium at a concentration of ca. 35 μM and contains two metal binding domains that are about 30% saturated with Fe^{III} in blood plasma.

Supplementary studies have shown that Ti^{IV} binds more strongly to human serum transferrin than does Fe^{III}.[282,283] UV/Vis and ^1H NMR studies support the conclusion that TDC as $[Cp_2Ti(citrate)_2]$ binds to transferrin with complete hydrolysis of the Cp ligands.

After reaction with transferrin for 30 mins at pH 7, ^1H NMR signals attributed to bound Cp in TDC completely disappeared, replaced by resonance signals characteristic of free cyclopentadiene. Interestingly, at low pH (<5.5) titanium was released from Ti_2Tr to bind to ATP. In this mechanism, TDC, or its analogues,[284,285] could possibly act as a pro-drug, with the ligands protecting the titanium center from hydrolysis into titanium dioxide in the intercellular medium. Although TDC is completely hydrolyzed by transferrin in the presence of added citrate, the binding of transferrin to the potentially oxo-bridged hydrolysis products of TDC may occur with different selectivities and/or rates.

Notably, however, no such observations were reported for Budotitane or its analogous. Moreover, the water-stable tetranuclear titanium complex 20 based on the diketonato analogue maltol ligand 19 (Figure 40) does not donate Ti^{IV} to transferrin, even if is active against HT-29 cell lines in-vitro with values comparable to that of TDC, unlike other titanocene derivatives evaluated in comparison.[286]

Thus, it would appear that further work is certainly necessary in establishing transferrin as the exclusive agent of active transport for cytotoxic titanium complexes.

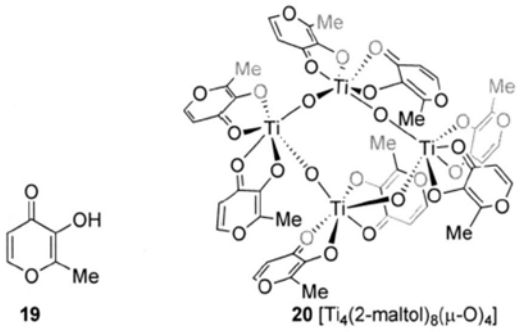


Figure 40

A recent paper proposes a different theory for the titanium transport into the cells through the action of serum albumin (HSA).[287] HSA, which is present in high concentrations in the intercellular medium (ca. 700 μM), has a variety of metal-binding sites and has been demonstrated to bind intact metal complexes such as contrast agents and photosensitizers.
NMR studies proved that, in contrast to albumin-free controls, solutions of TDC in the presence of HSA showed no clear signals for either free Cp_2TiCl_2 or cyclopentadiene after many days. Instead, two proton signals were formes, and proposed to represent Ti^{IV}-bound Cp protons. These results certianly suggest that HSA stabilizes the Cp2Ti moiety at physiological pH, and that the extent to which albumin binding stabilizes complexes may be important for biological activity.
This was even supported by studies on other metallocene analogues.[288]
Otherwise, binding studies with the dichloro analogue of Budotitane showed less than TDC equiv. of Ti bound to the protein and the presence of free ligand in the NMR spectrum.
It was also reported that while transferrin outcompeted HSA for titanium citrate, exchange of Ti from a preformed HSA-Ti complex to transferrin was quite slow and incomplete.
The important feature of this model is that the ligands of the cytotoxic titanium complex remain bound, allowing for a biological mechanism implicating the ligands in a putative DNA-binding event within the cell.
An important unanswered question in this model is whether there is a mechanism by which the HSA-Ti complex is transported into the cell, or whether HSA serves as a protecting group for the active titanium complex

until it is transported by passive diffusion into the cell. The discovery that serum albumin can stabilize otherwise the hydrolytically unstable titanium compoundshas, therefore, added a new dimension to the study of these compounds.

It is certainly clear that more studied are needed to improve what is known about Ti^{IV} complexes as cytotoxic agents. Despite the many years since the initial studies regarding the involvement of Ti^{IV} metal in biological interactions of a therapeutic potential, very little is known about the actual important biological interactions involved in the cytotoxicity mechanism, as well as about the exact requirements of a suitable complex and its attendant ligands. Consequently, only complexes of Cp, diketonato, or related ligands have been explored, although some recent derivatives demonstrate strongly improved properties. Nevertheless, the enormous potential of the Ti^{IV} center due to its notably low toxicity requires a reinvestigation of its diverse complexes.

1.13.5 Phase II trial of titanocene dichloride in advanced renal-cell carcinoma

1.13.5.1 Patients and methods

From May 1995 to June 1996, 14 patients with advanced renal-cell cancer (RCC) were recruited into phase II trial. Patients were eligible if they had histologically confirmed RCC, bidimensionally measurable meta- static or locally recurrent unresectable disease, no prior medical antineoplastic cure, a life expectancy of at least 3 months along with a Karnofsky performance status of above 70%, and adequate renal, hepatic, and bone marrow function. Informed consent was obtained from each patient. The patients' characteristics are reported in Table 2.

Before to study entry, patients had a complete workup, including a physical, history examination, blood chemistry, and blood count chest, X-ray, abdominal ultrasonography. Additional imaging procedures were performed as indicated by symptoms or history.

All patients were treated with titanocene dichloride (provided by Medac, Hamburg, Germany) given at 270 mg/m^2 on day 1 by i.v. injection. Treatment cycles were repeated every 21 days. Patients who achieved partial or complete responses or stable disease after two cycles were continued on titanocene dichloride until either progression or stabilization

of their disease for two cycles. The response to treatment was assessed after two cycles of therapy and every 3 months thereafter according to WHO response criteria [289].

Table. 2 *Patients' characteristics*

Characteristics	Number of patients (%)
Eligible patients	14
Sex:	
Male	11 (79%)
Female	3 (21%)
Age (years):	
Median (range)	60 (33–72)
Karnofsky index (%):	
80	1 (7%)
90	9 (64%)
100	4 (29%)
Nephrectomy	10 (71%)
Sites of metastatic disease:	
Lung	3 (21%)
Lung and others	4 (29%)
Bone	4 (29%)
Lymph nodes	1 (7%)
Local recurrence	2 (14%)

1.13.5.2 Response

A total of 11 patients received 2 cycles of titanocene dichloride. One patient who presented with stable disease continued the treatment for a further two cycles and one patient died due to tumor progression within 4 weeks. Because of side effects, one patient dropped out of the treatment schedule after one cycle. There was no complete or partial response. Two patients had metastases in the lung; one of them had been nephrectomized. The other patient had bone metastases only. Three patients (21%) had stable disease (SD) for 1, 3, and 3 months, respectively. All patients with SD had a good performance status (Karnofsky index 100%). The remaining ten patients (79%) had shown evidence of progression while under treatment with titanocene dichloride. The median survival time of all patients amounted to 50 weeks, and the overall survival was 43% at 1 year.

1.13.5.3 Toxicity

Mild upper abdominal discomfort with loss of appetite was universally experienced for 1-2 days after the administration of titanocene dichloride by all patients entered into this study. In eight patients (57%) symptoms of vomiting, nausea, and weight loss occurred; three of them suffered from grade 3 WHO toxicity and two patients, from grade 2 toxicity. Subsequently, the dose of titanocene dichloride was reduced to 75% of the original dose in two patients. Although the treatment level had been reduced, one patient chose to withdraw from further participation in the study. There was no other organ toxicity (see Table 3). Four patients (29%) developed an increase in serum creatinine concentration corresponding to WHO grade 2 toxicity (maximal 2.6 mg/dl) and three patients (21%) developed grade 1 toxicity. The treatment-related increase in serum creatinine levels resolved after completion of the therapy. With regard to hematologic parameters and chemistry values of liver function, no significant difference was detectable between baseline levels and control values during the treatment.

Table. 3 *WHO toxicity of tratment with titanocene dichloride (n=14)*

Side effects	WHO grade			Total (%)
	I	II	III	
Loss of appetite	14	0	0	14 (100%)
Nausea/vomiting and weight loss	3	2	3	8 (57%)
Increase in creatinine	3	4	0	7 (50%)

In conclusion TDC produced responses in proportions too small to justify its use in single-agent therapy.

1.13.6 Methoxy alkyl substituted titanocenes

Recently, some of us have reported the synthesis and the cytotoxic activity of some titanocene derivatives obtained replacing the aryl-methoxylic group on cyclopentadienyl of titanocene Y with the ethenyl-methoxy group, in order to have a stronger electron donor effect on the cationic species responsible for the cytotoxic activity (fig.41).

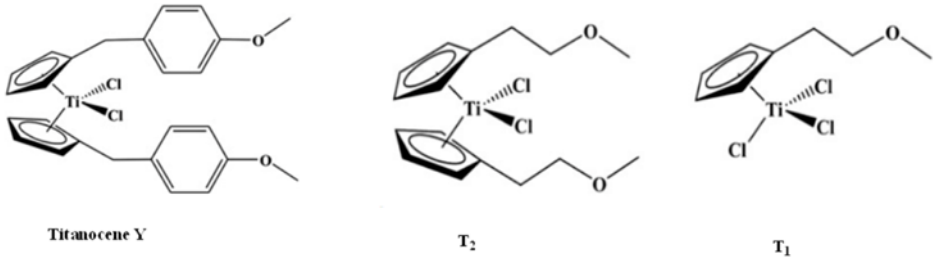


Figure 41 *Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium dichloride (titanocene Y); bis-cyclopentadienyl-ethenylmethoxyl-titanium dichloride (T_2) and cyclopentadienyl-ethenylmethoxyltitanium trichloride (T_1)*

We also verified the influence of leaving ligands on the activity by substituting chlorine atoms with dimethylamide, oxalate or aminoacid groups.[290]

It is worth noting that in ref. 290, the highest cytotoxic activity was reported for half-titanocene complex T_1, compared to titanocene T_2, titanocene Y and cis-platinum. Some of the synthesized compounds showed a good cytotoxicity, in particular the complexes bis-cyclopentadienyl-ethenylmethoxyl-titanium dichloride T_2 and cyclopentadienyl-ethenylmethoxyl-titanium trichloride T_1 gave values very similar to cis-platin on MCF-7 cell lines, with the IC_{50} comparable to the ones reported for titanocene Y. Moreover, the half-titanocene complex (T_1) also showed a good cytotoxic activity, comparable to that of cis-platin, on HEK-293 cells. The results of the hydrolysis of our titanocenes showed unequivocally that the leaving groups (Cl, $N(CH_3)_2$, C_2O_4 or glycine) significantly affect even the hydrolysis rate of cyclopentadienyl groups, being chloride and oxalate more stable.[290]

Thus, the presence of substituents, aryl methoxy group on cyclopentadienyl ring in titanocene Y or ethenyl-methoxy group in titanocene T_2 or in the half-titanocene T_1, produces compounds having interesting cytotoxic activity. Although generalizations regarding structure-activity relationships are not yet clear, we could hypothesize that the neutral nucleophilic substituents of cyclopentadienyl (aryl methoxy or ethenyl-methoxy group) could intramolecularly coordinate to the titanium cation, thus preventing decomposition reactions. On the other hand, this hypothesis was suggested for analogous complexes able to give polymerization of propene or styrene having microstructures strongly

influenced by the possible coordination of neutral substituent of cyclopentadienyl at the metal center.[291-293]

As mentioned above, in the literature several examples of titanocene-complexes showing cytotoxic activity were reported, but, to the best of our knowledge, the cyclopentadienyl-ethenylmethoxyl-titanium trichloride represents the first example of half-titanocene complex with interesting cytotoxic activity.

Chapter Two

This chapter introduces the aims of this project and provides an extensive discussion on the obtained results.

2 Aim of the project

One of the crucial consideration about the application of transition metal complexes for chemotherapy is their potential toxicity. The advantage of Ti^{IV} compounds is their relative biological compatibility, which mostly leads to moderate and reversible side effects. However, the hydrolytic instability of known Ti^{IV} complexes and formation of various different species upon water addition makes their clinial use debatable, and promote a marked interest in the development of relatively stable Ti^{IV} complexes with well defined hydrolytic behavior that show considerable cytotoxic activity. Strong ligand binding is also of interest to avoid complete ligand stripping by transferrin, so that the ligand may be useful as a target for structure–activity relationship studies.

For a worthier evaluation of the parameters affecting the cytotoxic activity and the hydrolytic behaviour, the synthesis and investigation of specifically designed complexes based on different strongly coordinating ligands has been our main purpose; our main ambition is the synthesis of complexes with imroved hydrolytic stability and welldefined hydrolytic behavior.

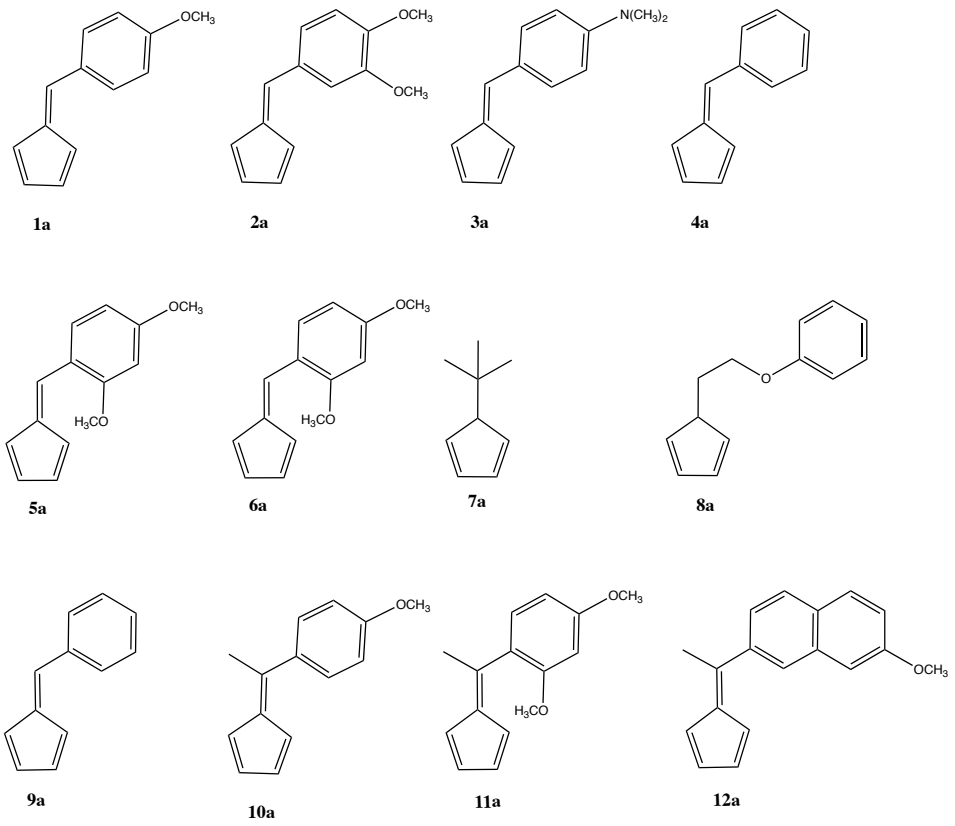
Therefore, the aim of this study was the synthesis and the characterization of some titanocene and half-titanocenes compounds by nuclear magnetic resonance (NMR), mass spectroscopy and elemental analysis. All these complexes contain different substituents on the Cp ligands, able to stabilize the titanium cation by intramolecular or intermolecular coordination. Preliminary cytotoxic studies of these titanium-based compounds have been carried out, as well.

Moreover we have evaluated the cytotoxic activity of our pro-ligands, fulvene.

2.1 Ligand and Complex Design

As the hydrolytic instability of Ti^{IV} complexes is one of the main difficulty that needs to be considered, our ligands have been projected to enhance stable binding to Ti^{IV} and enforce hydrolytic stability. The considered parameters are the following:

(a) neutral complexes for granting cell penetration by passive diffusion and the most stable oxidation state for the Ti^{IV} center ;
(b) inert ligand that will hopefully remain bound to the metal center during the biological interactions and allow for activity in a controlled manner (see cheme 4)
(c) the preferred geometry for the Ti^{IV} center is tetrahedral;
(d) complexes of the general structure $LTiX_3$ in order to investigate the citotoxic properties of half-titanocene (see scheme 5)
(e) complexes of the general structure L_2TiX_2 (see scheme 6)
(f) two leaving ligands, to enable potential chelate binding to the biological target of interest, as happens for cisplatin and as could be proposed for titanocene dichloride, both having this characteristic.[233]

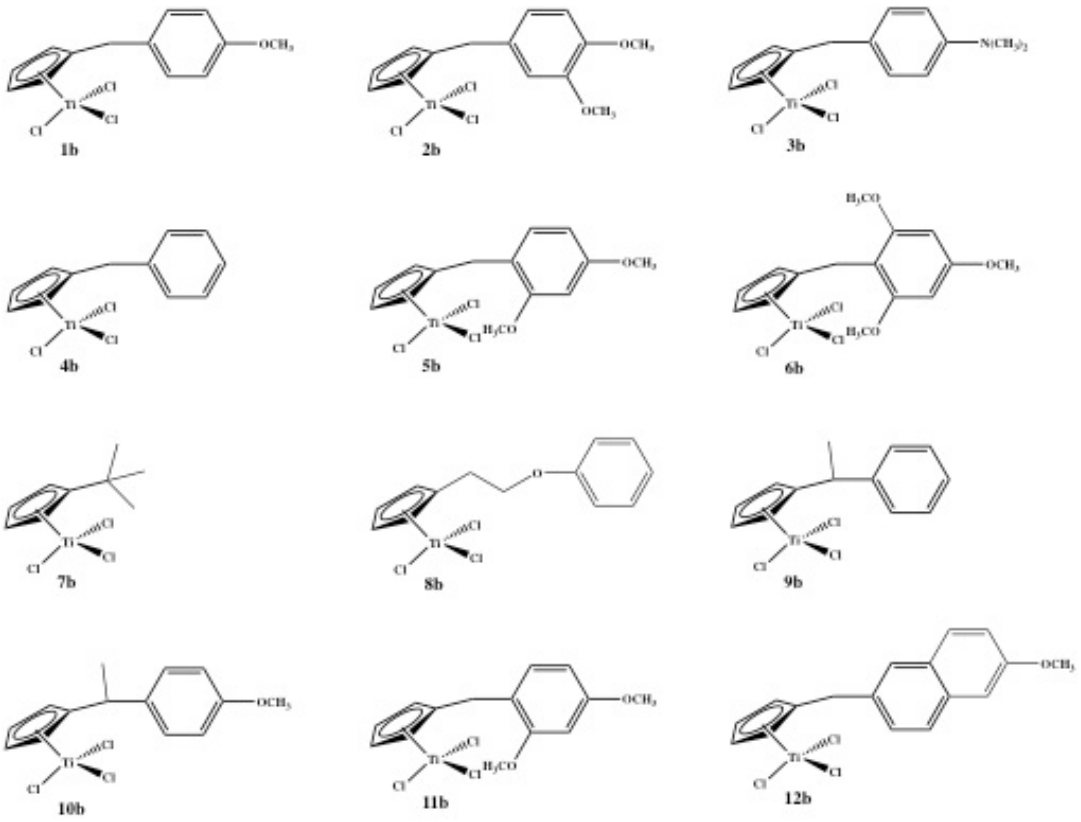


1a **2a** **3a** **4a**

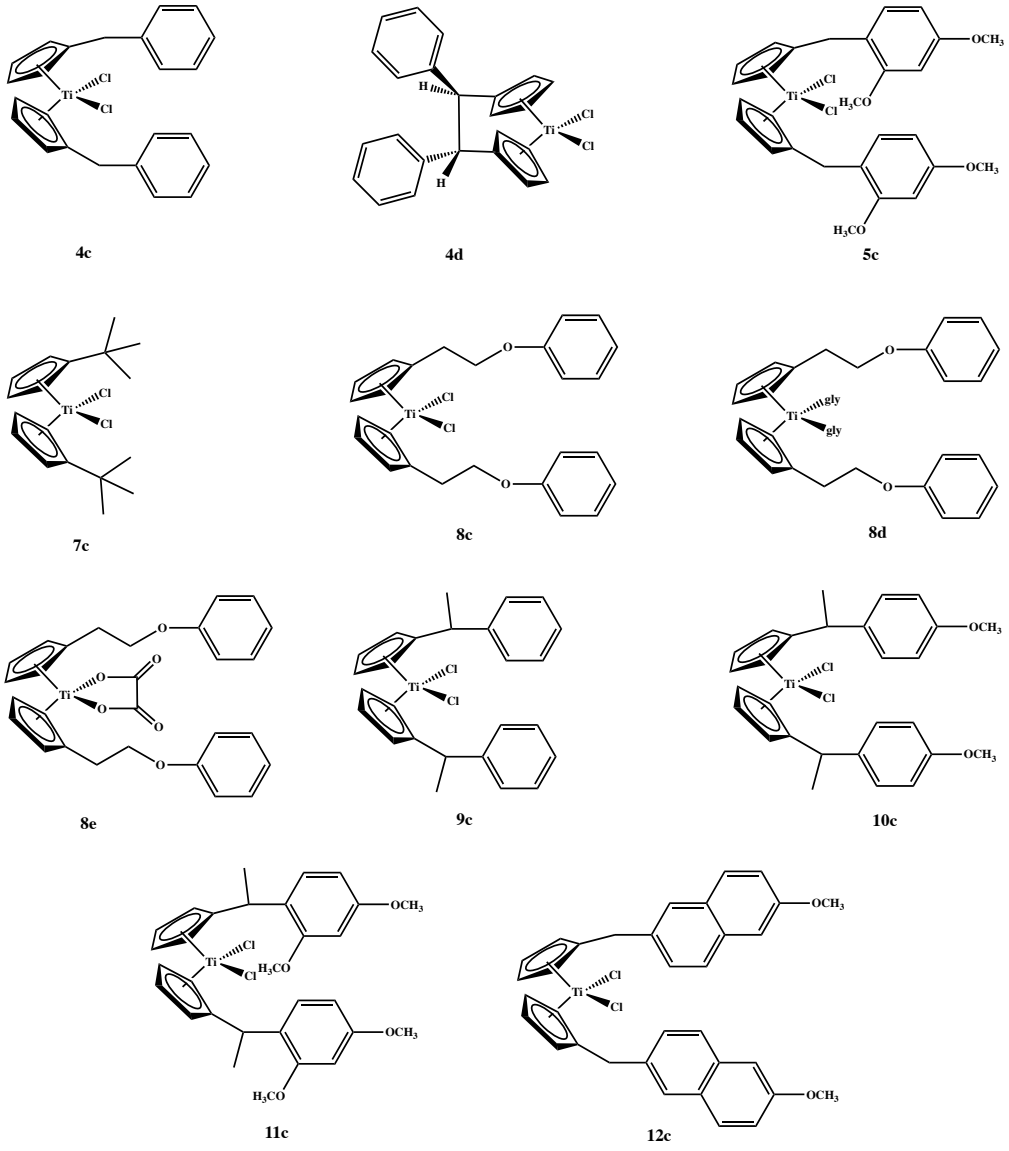
5a **6a** **7a** **8a**

9a **10a** **11a** **12a**

Scheme. 4 *Structure of synthesized ligands*



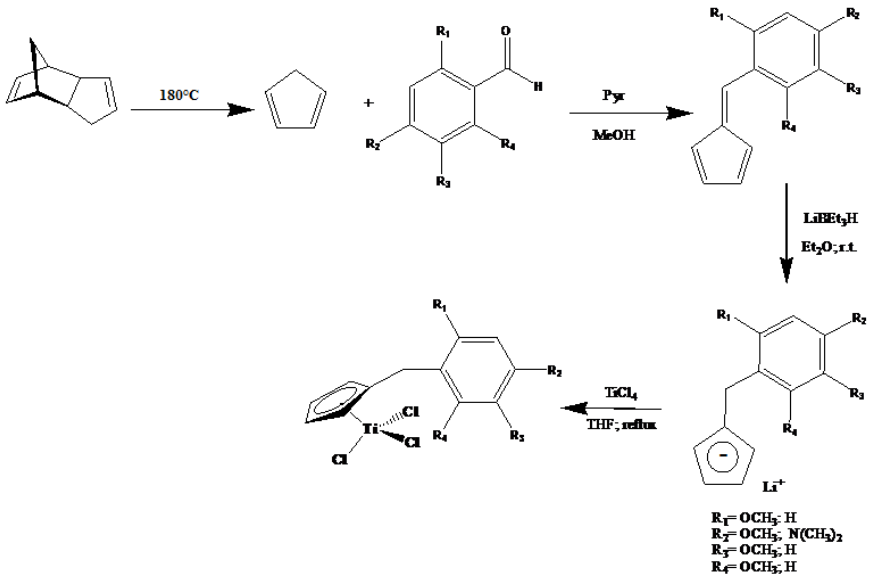
Scheme. 5 *Structure of synthesized half-titanocene complexes*



Scheme. 6 *Structure of synthesized titanocene complexes*

2.2 Synthesis and characterization of aryl-substituted half-titanocene

The half-titanocene 1b-6b have been synthesized and fully characterized through ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and elemental analisys.
The aim of these syntheses has been to investigate the cytotoxic activity of these compounds, being the cyclopentadienyl-ethenylmethoxyl-titanium trichloride ($T_{1,}$) the first example of half-titanocene complex with interesting cytotoxic activity in leterature.[290]
1b was synthesized in order to verify if the activity was higher for half-titanocene Y than titanocene Y, as it was for the bis-cyclopentadienyl-ethenylmethoxyl-titanium dichloride T_2 and cyclopentadienyl-ethenylmethoxyl-titanium trichloride T_1.
2b, 5b and 6b have other methoxyl groups, which may make ligands much more coordinating, apart from the methoxyl in position 4.
3b has a dimethylamino-group in position 4 of the aryl which has strong capabilities to bond metal-cation.
4b has no substituents on the aryl, but the phenyl is of course able to coordinate to titanium.
The synthesis of complexes was carried out according to scheme 7. The synthesis of pre-ligands fulvene 1a-6a were carried out as outlined in references,[294-297] starting from the suitable and purified following general procedures.
The lithium salt of the ligand was obtained by reacting the suitable fulvene with Super Hydride ($LiBEt_3H$) in diethyl ether dry. Then, it was isolated and subsequently reacted with one equivalent of $TiCl_4 \cdot 2THF$ in dry THF. The reaction product was purified following common procedures and isolated in high yield (see Scheme 5).
Elemental analysis (C, H, N) agreed with the proposed formulation. ^{1}H COSY experiments allowed the assignment of all the proton resonances of the ^{1}H NMR spectrum, whereas DEPT experiments were useful for the attribution of ^{13}C NMR signals (see experimental part). The synthesized half-titanocenes were also characterized by mass spectrometry. The mass spectra show the molecular ion and the fragmentation of the complexes (*i.e.:* ligand, [ligandTi]). The set of these data allowed us to have an unambiguous structural determination, as reported in scheme 5.



Scheme. *7 Synthetic route for the preparation of complexes 1b-6b*

In Fig. 42 the ^{1}H and ^{13}C NMR spectra of complex 4b are reported as an example.

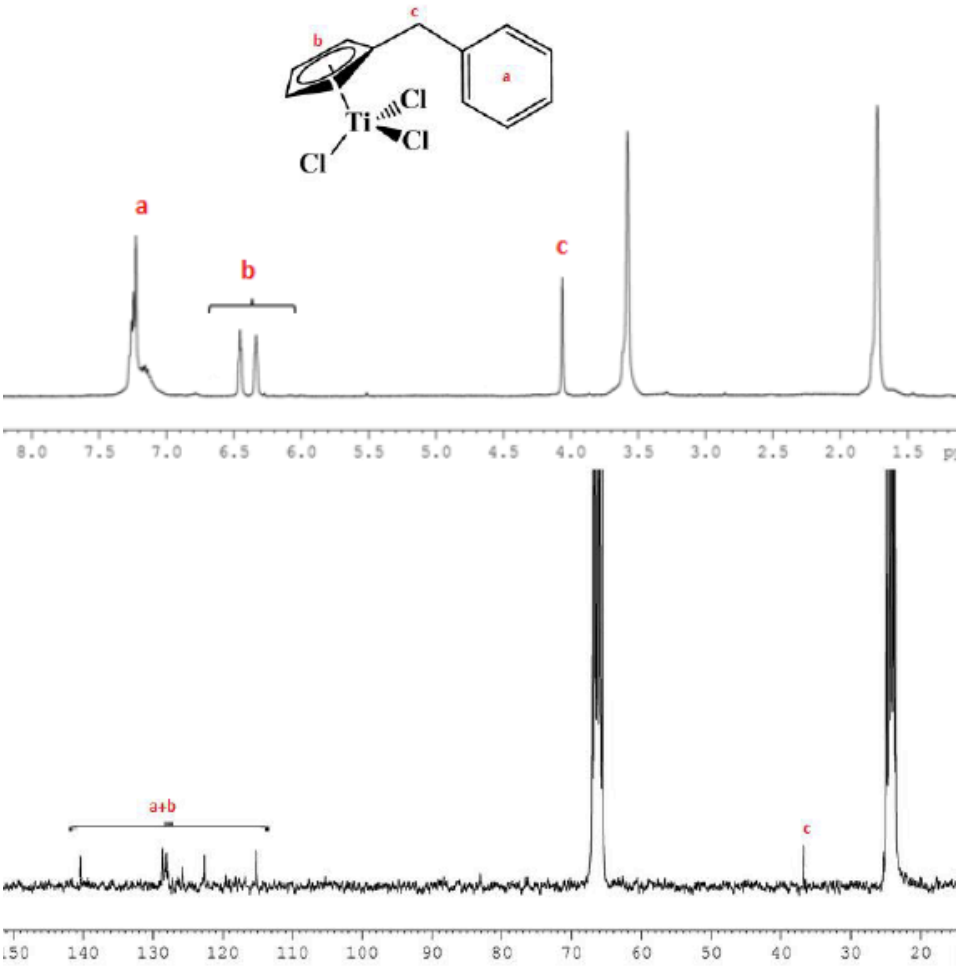


Figure 42. *^{1}H and ^{13}C NMR spectra of complex 4b*

2.2.1 Hydrolysis test of complexes 1b-11b

Hydrolysis stability of the half-titanocene complexes (1b-6b) has been determined in aqueous solution, 90% DMSO by ^{1}H NMR spectroscopy, in order to correlate the chemical stability and coordination chemistry of these complexes with their observed cytotoxic activity. Since we can expect that rapid hydrolysis of the leaving group (-Cl) and cyclopentadienyl ligands could give way to biologically inactive species, active species could be generated if the Cp rings remain metal bound.
Hydrolysis of aromatic rings of 1b-6b was evaluated by integrating the two signals of protons of cyclopentadienyl bonded to metal, as to newly formed multiplet of substituted cyclopentadiene. Table 4 reports the results of our hydrolysis tests and Fig. 43 shows, as an example, the evolution in time of unsaturated region of ^{1}H NMR spectra of 4b.

Table. 4 *Hydrolysis results of 1b-6b complexes in DMSO/D_2O solution at rt followed by ^{1}HNMR*

Complex	% Cp rings hydrolysis			
	5 mins	4 hrs	8 hrs	24 hrs
1b	<1	<1	<1	<5
2b	<1	<1	<1	<5
3b	<1	<1	<1	<5
4b	9	24	40	41
5b	<1	<1	<1	<5
6b	<1	<1	<1	<5

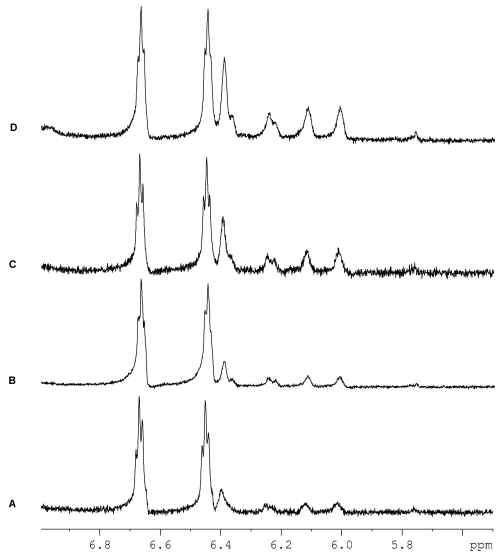


Figure 43 *^{1}H NMR spectra of 4b at rt in 90% DMSO (A) 5 mins afterthe dissolution, (B) 4.5 hrs after the dissolution, (C) 8.5 hrs after the dissolution and (D) 24 hrs after the dissolution.*

All the complexes show an high hydrolytic stability. In particular, the cyclopentadienyl rings of all the complexes are hydrolyzed only for less than 5% after 24 hrs, whereas complexes 4b is hydrolyzed for 41%. (Table 4).
These data provide sufficient evidence that the presence of coordinating groups on the aryl substituent of the cyclopentadienyl are effective for the stabilization of the complexes. Therefore, these coordinating groups might be fundamental to increase, if active, their biological effectiveness.

2.2.2 Cytotoxic tests

In order to investigate the effects on cancer cell proliferation of the novel compounds synthesized, we treated for 5 days MCF7 and SkBr3 breast cancer cells with each compound. Cells were also exposed to cisplatin in order to compare the anticancer effects of the complexes to this well-known chemotherapeutic.

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers.[298] Of the two mastectomies she received, the first revealed the removed tissue to be benign. Five years later, a second operation revealed malignant adenocarcinoma in a pleural effusion from which was taken cells for MCF-7. The woman was treated for breast cancer with radiotheraphy and hormonotherapy.

Prior to MCF-7, it was not possible for cancer researchers to obtain a mammary cell line that was capable of living longer than a few months

MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen, in the form of estradiol, via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line.

In addition to retaining their estrogen sensitivity, MCF-7 cells are also sensitive to cytokeratin. They are unreceptive to desmin, endothelin, GAP, and vimentin. When grown in vitro, the cell line is capable of forming domes and the epithelial like cells grow in monolayers. Growth can be inhibited using tumor necrosis factor alpha (TNF alpha), and treatment of MCF-7 cancer cells with anti-estrogens can modulate insulin-like growth factor finding protein's, which ultimately have the effect of a reduction in cell growth.

Genetically, the MCF-7 line has not been maintained exactly. Originally, it was described as having a karyotype containing 85 chromosomes, which has since been reduced by 16 chromosomes. Today's cell line has a karyotype containing 69 chromosomes. Furthermore, there are genetic discrepancies between the MCF-7 cell line from the Michigan Cancer Foundation and the ATCC cell line. This indicates that the ATCC cell line is of a different source than the other MCF-7 cell lines.

SkBr3 is a cell line isolated by the Memorial Sloan-Kettering Cancer Cells in 1970. It was derived from a pleural effusion due to an adenocarcinoma originating in the breast of a 43 year old, caucasian female. The cell line over-expresses the HER2 gene product.

The SkBr3 line is synonymous (derived from the same patient) with the AU565 cell line.

2.2.2.1 Cytotoxicity results

The effects of each compound on cell viability were determined with the MTT assay, which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme.

Cells were seeded in quadruplicate in 96-well plates in regular growth medium and grown until 70-80% confluence. Cells were washed once they had attached and then treated with 10 μM each compound for indicated time (for one day up to 5 days).

Relative cell viability was determined by MTT assay according to the manufacturer's protocol (Sigma-Aldrich, Milan, Italy). Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted vs drug concentration.

It should be noted that by using the compounds mentioned above, SkBr3 cells resulted to be more responsive to the treatment compared to MCF7 cells.

Among all tested compounds, 4b, 5b and 6b elicited repressive effects on the proliferation of both cell lines (Fig. 44). In particular, 4b strongly decreased the viability of MCF7 cells after 3 days of treatment, whereas 5b showed the highest antitumor activity on SkBr3 cells after 2 days, being the most active compound on this cell line. In particular 4b, 5b and 6b showed a strongest cytotoxic effect on MCF7 than cisplatin. Moreover 5b showed a similar cytotoxic activity on SkBr3 compared to cisplatin.

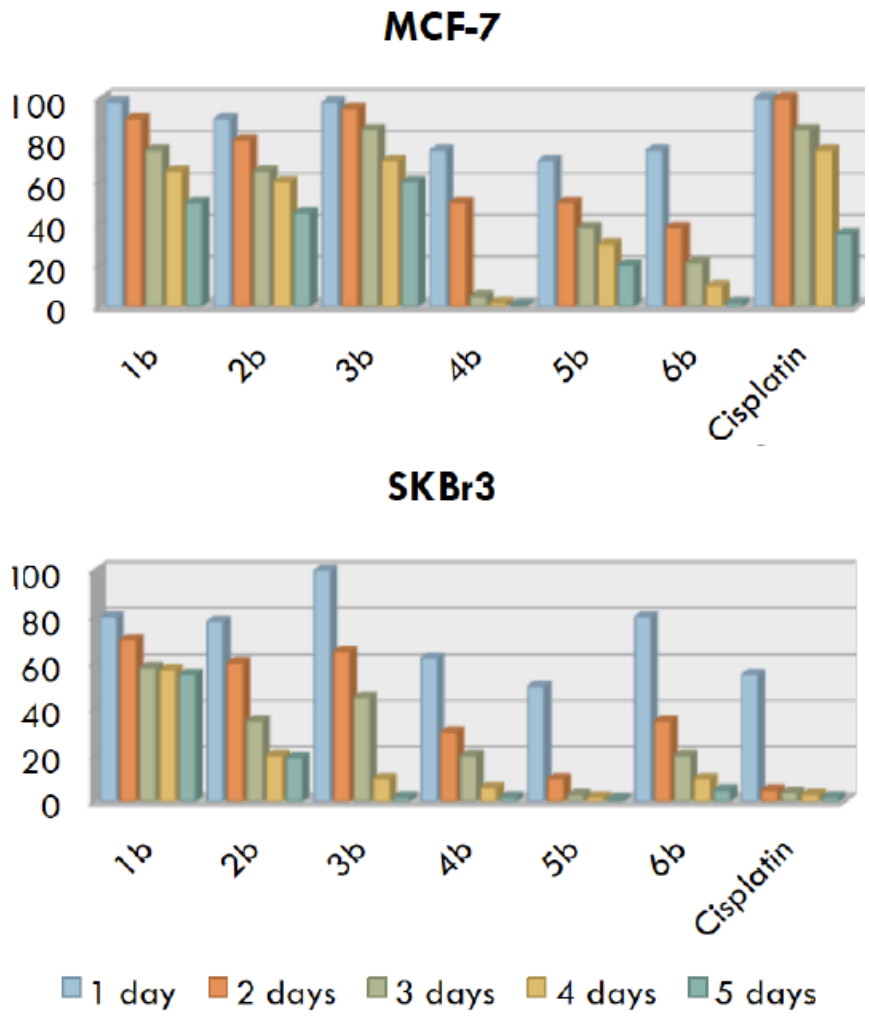


Figure 44 *Evaluation of growth responses to 10 μM of 1b-6b in MCF7 and SkBr3 breast cancer cells, as determined by using the MTT assay. Cell viability was expressed as the percentage of cells treated with the different compounds compared to cells treated with vehicle. Cells (5–8·10^4 mL^{-1}) were treated for one day up to 5 days, as indicated*

The cytotoxic activity of complexes 4b and 5b has been tested even against NCI-H1299.

Human non-small-cell lung carcinoma NCI-H1299 clones were grown in RPMI1640 medium and they were maintained at 37°C in a humified

atmosphere of 5% (v/v) CO_2 in air. In order to test cell viability, we use MTS assay (Promega, Italy), so cells were plated in 96 wells plates at 32000 cells/mL and they were allowed to grow for 24 hours. After this period, cells were treated for 72 hours with different concentrations of the drugs, that were diluted in medium just before their use. Cisplatin was removed after 2 hours from the treatment, in order to avoid that some of its derivates could damage cells. The data of the survival curves and histograms were plotted as percentages of untreated controls. Three independent experiments consisted of at least three replicates for each point, were conducted.

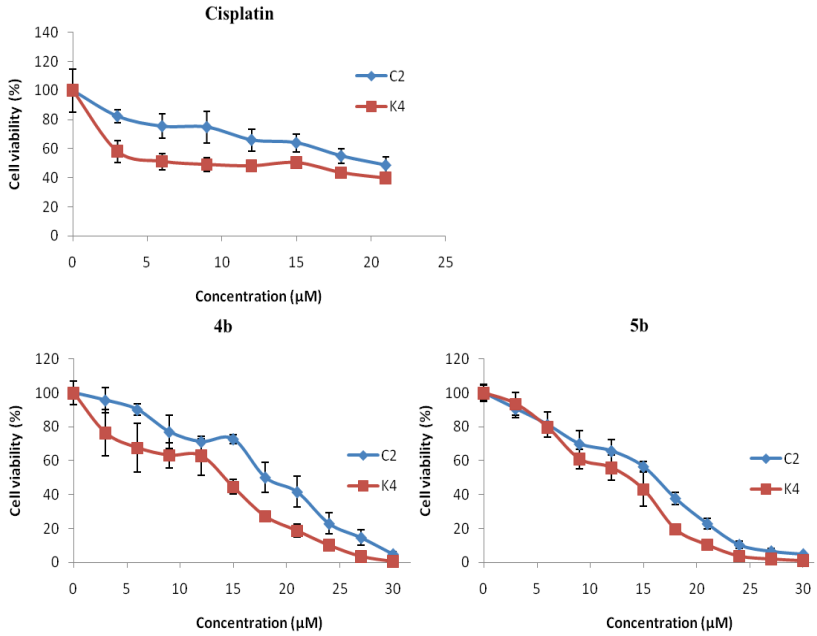


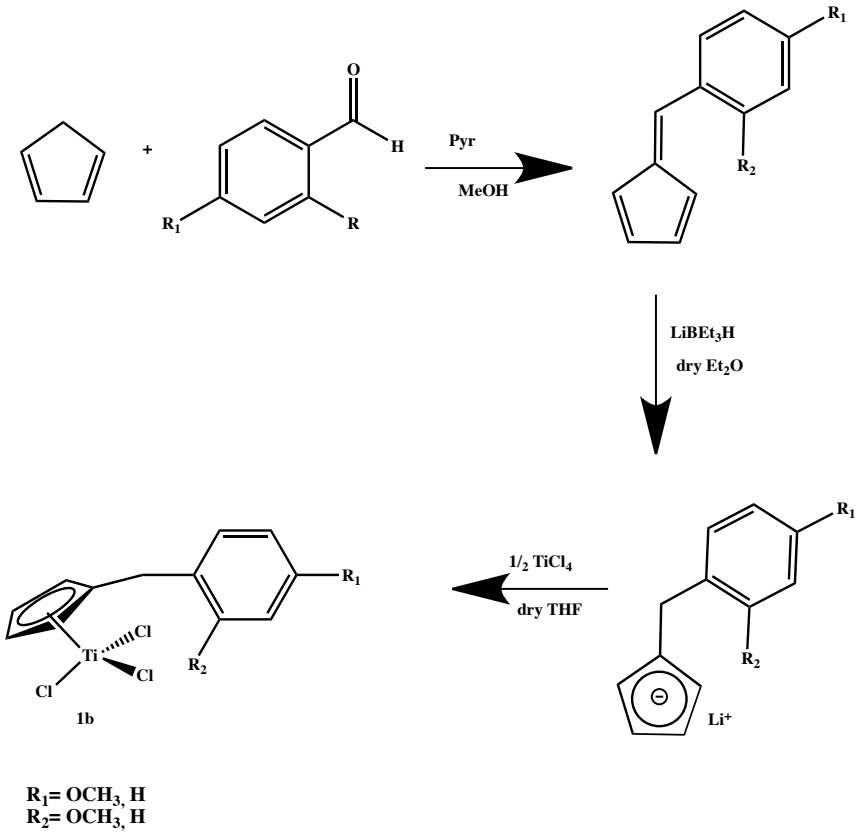
Figure 45 *Curves dose-response of two Human non-small-cell lung carcinoma NCI-H1299 clones treated with different concentration of 4b, 5b and Cisplatin*

As it can be seen in figure 45, complex 4b showed a stronger antiproliferative effect on WT KRAS clone compared with the effect on G12C KRAS clone. Moreover, 4b displayed a similar activity to cisplatin, even if its cytotoxic activity is stronger at higher concentrations. Complex 5b displayed a very similar activity to the one of 4b, because the

antiproliferative effect is stronger on WT KRAS clone and it is comparable to the one of cisplatin.

2.3 Synthesis and characterization of titanocene derivatives complexes

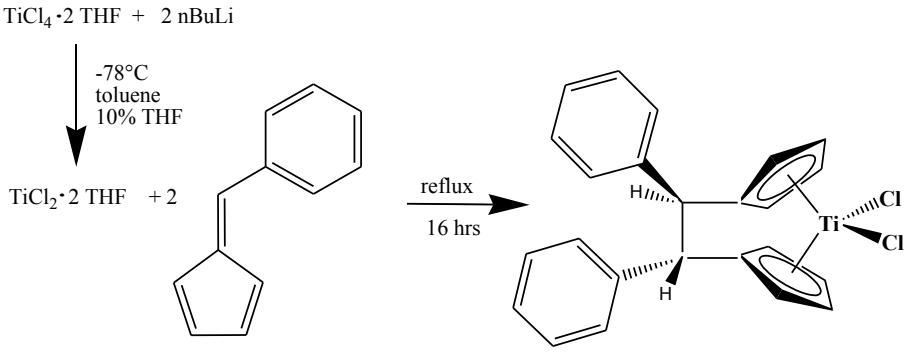
In order to verify the cytotoxic activity of half-titanocene, compared to the analogous titanocene complexes, we synthesized the titanocene derivatives having the same ligands of the more cytotoxic half titanocene complexes previously reported (4b and 5b).
In particular complex 4c was synthesized to test if the ether group, which is present on the titanocene Y, on T_1 and T_2, is necessary to have a significant higher cytotoxic activity or if a more labile ligand, having greater ability of π–donation, can produce a molecule with likewise interesting cytotoxic properties; otherwise its half-titanocene derivatives had shown very interesting cytotoxic properties when compared to the half-titanocene of the well-known Titanocene Y.
The complexes have been synthesized starting from fulvcne 4a and 5a.
The synthesis of the fulvene was carried out as previously outlined for their half-titanocene complexes 4b and 5b; the lithium salt was obtained by reaction with Super Hydride ($LiBEt_3H$) in dry diethyl ether. Then it was isolated and subsequently reacted with half-equivalent of $TiCl_4 \cdot 2THF$ in dry THF (see scheme 8). The reaction product was purified following common procedures and isolated in high yield.



Scheme. 8 *Synthetic route for the preparation of complexes 4c and 5c*

Complex 4d (1,2-diphenyl-1,2-dicyclopentadienyl-ethane)-titanium-dichloride was synthesized in order to have a complex very similar to 4c, but with a chelating ligand, which would prevent the intramolecular coordination of phenyl groups to metal, and consequently produce a less stable complex than 4c.

Complex 4d was synthesized according to scheme 9, following a literature method.



Scheme. 9 *Synthetic route for the preparation of complex **4d***

Elemental analysis (C, H, N) agreed with the proposed formulation. ^{1}H COSY experiments allowed the assignment of all the proton resonances of the ^{1}H NMR spectrum, whereas DEPT experiments were useful for the attribution of ^{13}C NMR signals (see experimental part). The synthesized half-titanocenes were also characterized by mass spectrometry. The mass spectra show the molecular ion and the fragmentation of the complexes (*i.e.:* ligand, [ligandTi]).
In Fig. 46 the ^{1}H and ^{13}C NMR spectra of complex 4c are reported as an example.

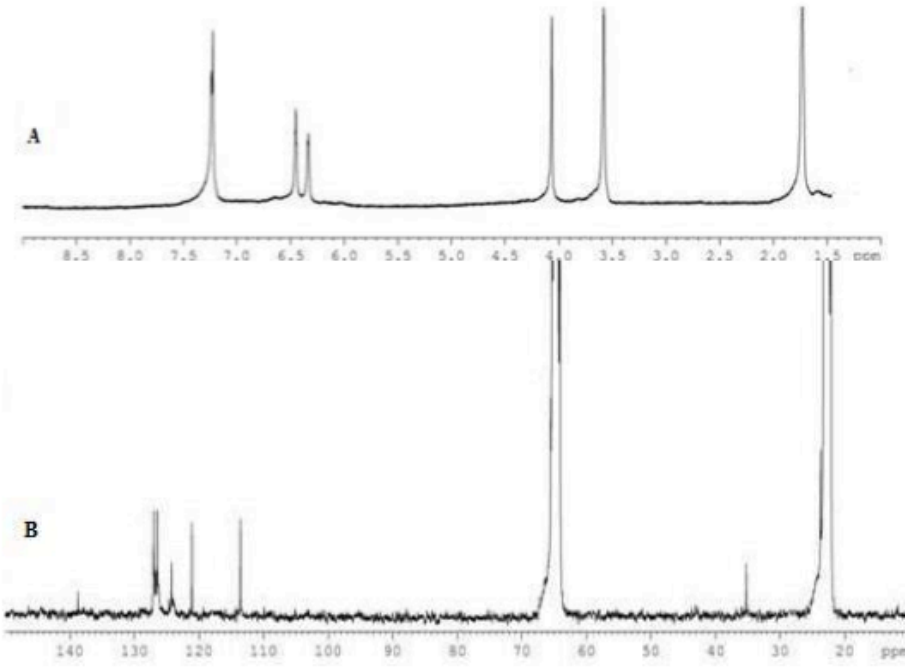


Figure 46 *^{1}H (A) and ^{13}C NMR (B) spectra of 4c in THF*

2.3.1 Hydrolysis test of titanocene compounds

Hydrolytic stability of the titanocene complexes has been determined in aqueous solution, 90% DMSO by ^{1}H NMR spectroscopy, in order to possibly correlate the chemical stability of these complexes with their observed cytotoxic activity.

Table 5 reports the results of our hydrolysis tests and Fig. 47 shows, as an example, the evolution in time of unsaturated region of ^{1}H NMR spectra of 4c.

Table. 5 *Hydrolysis results of complexes DMSO/D_2O solution at rt followed by ^1H NMR*

Complex	% Cp rings hydrolysis			
	5 mins	4 hrs	8 hrs	24 hrs
4c	<1	<1	13	40
4d	25	40	48	60
5c	<1	<1	<1	<5

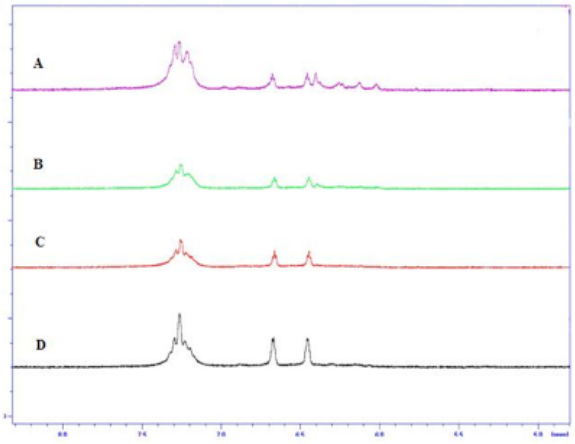


Figure 47 *^1H NMR spectra of unsaturated region of 4c at RT in 90% DMSO (A) 5 mins after dissolution, (B) 4 hrs, (C) 8 hrs and (D) 24 hr*

These data confirm the previous ones, being complex 4c the less stable of the unbridged aryl titanocene, missing an electron donor group on the phenyl ring capable of coordinating to the metal centre; it is worth noting that complex 5c, the ansa derivatives, is muh less stable of its unbridged related compound 4c, being already hydrolyzed for 25% after 5 minutes. It can be justified considering that the bridge between the two benzilic carbons prevents the coordination of the phenyl ring on the metal centre, making it less stable.

2.3.2 Cytotoxic activity

In order to investigate the effects on cancer cell growth of the compounds synthesized, MCF7 and SkBr3 breast cancer cells were used as model systems. Cells were treated for 5 days with each compound and were also exposed to *cisplatin*, in order to compare the anticancer effects of the chemicals used to this well-known chemotherapeutic. In dose-response and time course experiments, most of the tested compounds showed the capability to inhibit, in a dose-dependent manner, the proliferation of both cell lines, just after a 24 hour treatment).

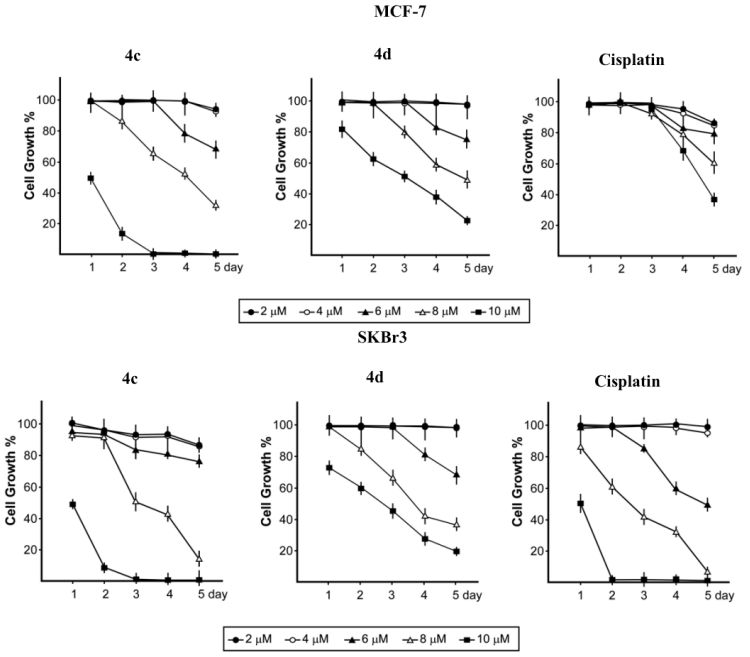


Figure 48 . *Evaluation of cell growth treating MCF7 and SKBr3 breast cancer cells with tested compounds, as determined by using the MTT assay. Cells (5–8·10^4 mL^{-1}) were treated for one day up to 5 days with increasing concentrations of tested compounds, as indicated. Cell viability was expressed as the percentage of cells treated with the different compounds compared to cells treated with vehicle. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test).*

Among all tested titanocene derivatives, complexes 4c elicited strong repressive effects on the proliferation of both cell lines (Table 6). In particular, complex 4 showed quite similar patterns of response across the two cancer cell models (with IC_{50} values of 10 μM in both cell lines after 24 h treatment). Moreover 4c inhibited the proliferation of MCF7 cells more strongly compared to cisplatin.
The low activity of complex 4d can be justified considering that the bridge between the two benzilic carbons prevents the coordination of the phenyl ring on the metal centre, making it less stable, and therefore less active.
Complexes with an alkyl group on the linker between Cp ring and phenyl group show a lower activity.

Table. 6 *Cytotoxic activity of tested compounds on MCF7 and SkBr3 breast cancer cells after one 24hrs treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test).*

Compound	Cell lines (IC50 values)	
	MCF-7	SKBr3
4c	10 (±3)	10 (±1)
4d	27 (±6)	25 (±5)
5c	n.d	n.d
Cisplatin	29 (±8)	10 (±2)

Complex 4c has been then tested against NCI-H1299 with the previously reported method.
Human non-small-cell lung carcinoma NCI-H1299 clones were grown in RPMI1640 medium and they were maintained at 37°C in a humified atmosphere of 5% (v/v) CO_2 in air. In order to test cell viability, we use MTS assay (Promega, Italy), so cells were plated in 96 wells plates at 32000 cells/mL and they were allowed to grow for 24 hours. After this period, cells were treated for 72 hours with different concentrations of the drugs, that were diluted in medium just before their use. Cisplatin was removed after 2 hours from the treatment, in order to avoid that some of its derivates could damage cells. The data of the survival curves and histograms were plotted as percentages of untreated controls. Three

independent experiments consisted of at least three replicates for each point, were conducted.

In Figure 49, we can see that 4c showed a stronger antiproliferative activity both on WT KRAS clone and on G12C KRAS clone compared to cisplatin. However, the effect is higher on WT KRAS clone.

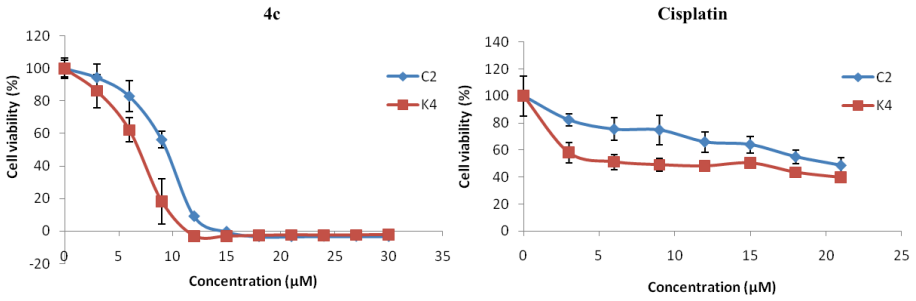


Figure 49 *Curves dose-response of two Human non-small-cell lung carcinoma NCI-H1299 clones treated with different concentration of 4c and Cisplatin*

The cytotoxic activity of complex 4c was evaluated even against others cell lines with the following method.

A375 (melanoma cell line), A549 (human lung alveolar basal epithelial cell) and HEK-293 (3.5 10^4 cells/well) were plated on 96-well microtiter plates and allowed to adhere at 37°C in a 5% CO_2 atmosphere for 2 h. Thereafter, the medium was replaced with fresh one (50 µL) and a 75 µL of 1:4 serial dilution of each tested compound was added, and then the cells incubated for further 72 h. Cis-Platinum was used as reference drug.

Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of MTT to formazan and cells viability was assessed accordingly to the method of Mosman. Briefly 25 mL of MTT (5 mg/mL) was added and the cellswere incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilised with 100 mL of a solution containing 50% (v:v) N,N-dimethylformamide, 20% (w:v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. The viability of each cell line in response to treatment with tested compounds and 6-mercaptopurine was calculated as: % dead cells =100-(OD treated/OD control) ×100

Table. 7 *The cytotoxic activity of compounds was evaluated as IC50 (μM): the concentration of compound that affords cell growth by 50% as compared to control on the following cell lines: A375 (melanoma cell line), A549 (human lung alveolar basal epithelial cell) and HEK-293 human embryonic kidney cells, The cis-platin was used as standard drug*

	Cell line (IC50, μM)		
Complex	**A375**	**A549**	**HEK-293**
4c	12 (±4)	70 (±8)	>100
TDC	>100	>100	>100
Cisplatin	22 (±8)	12 (±2)	67(±5)

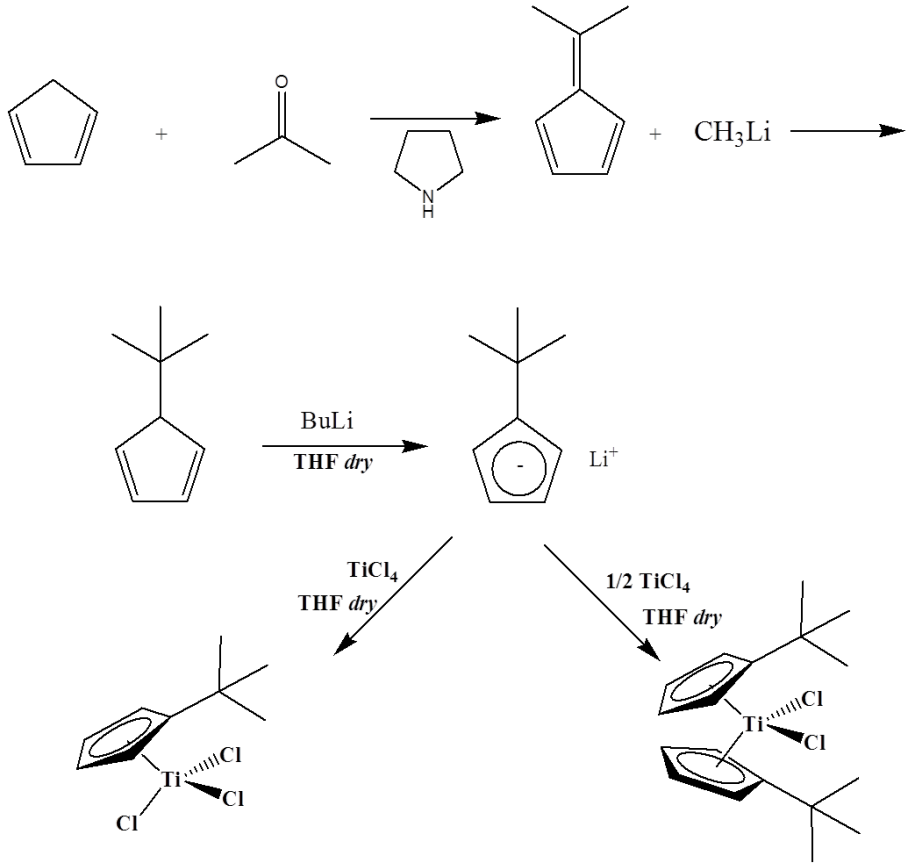
2.4 Synthesis and characterization of alkyl-substituted titanocene

In order to enforce our hypothesis about the efficacy of a coordinating group to stabilize the metal center, we decided to synthesize complexes 8b and 8c. These compounds have a strong electron-donor group on the Cp ring (a tert-butyl group) able to strengthen the sigma bond (σ bond) between Cp ring and the metal center, but no capability of coordination to the Ti centre

Complexes 7b and 7c have been synthesized and fully characterized through ^{1}H NMR, ^{13}C NMR and elemental anlisys.

The synthesis of 7b and 7c were carried out by reaction of the lithium salt of the ligand (7a) with one equivalent of $TiCl_4$ to obtain 8b, or half equivalent of $TiCl_4$ to obtain 8c (see scheme 9)

The reaction of 6,6-dimethylfulvene with acetone in dry diethyl ether at low temperature produced tert-butylcyclopentadiene (7a). Then, the ligand reacted with BuLi to form the correspondinh lithium salt which was isolated and then reactes with one equivalent or half quivalent of $TiCl_4$ in dry THF. The complexes were purified following common procedures and isolated in good yield.



Scheme 9. *Synthetic route for the preparation of complex 7b.7c*

2.4.1 Hydrolysis test

Hydrolysis stability of the complexes (7b and 7c) has been determined in aqueous solution, 90% DMSO by ^{1}H NMR spectroscopy, as well. Table 8 reports the results of our hydrolysis test.

Table. 8 *Hydrolysis results of 8b-8ccomplexes in DMSO/D_2O solutionat rt followed by ^{1}HNMR*

Complex	% Cp rings hydrolysis			
	5 mins	4 hrs	8 hrs	24 hrs
7b	<1	58	76	>99
7c	<1	52	59	72

As can be easily visualized through the table 5, complex 7b showed a moderate instability after 24 hrs, which almost total hydrolysis, whereas complex 7c proved to be more stable.
These data enforce our hypothesis that a coordinating groups on the cyclopentadienyl ring is necessary for the stabilization of the complexes, especially for half titanocene complexes, whose metal centre is more electron poor compared to titanocene derivative.

2.4.2 Cytotoxic activity

As for the previous complexes, these compunds have been tested on the same cell lines (MCF-7 ans SKBr3), but their observed citotoxicity was quite low if compared to Cisplatin (see table 9).

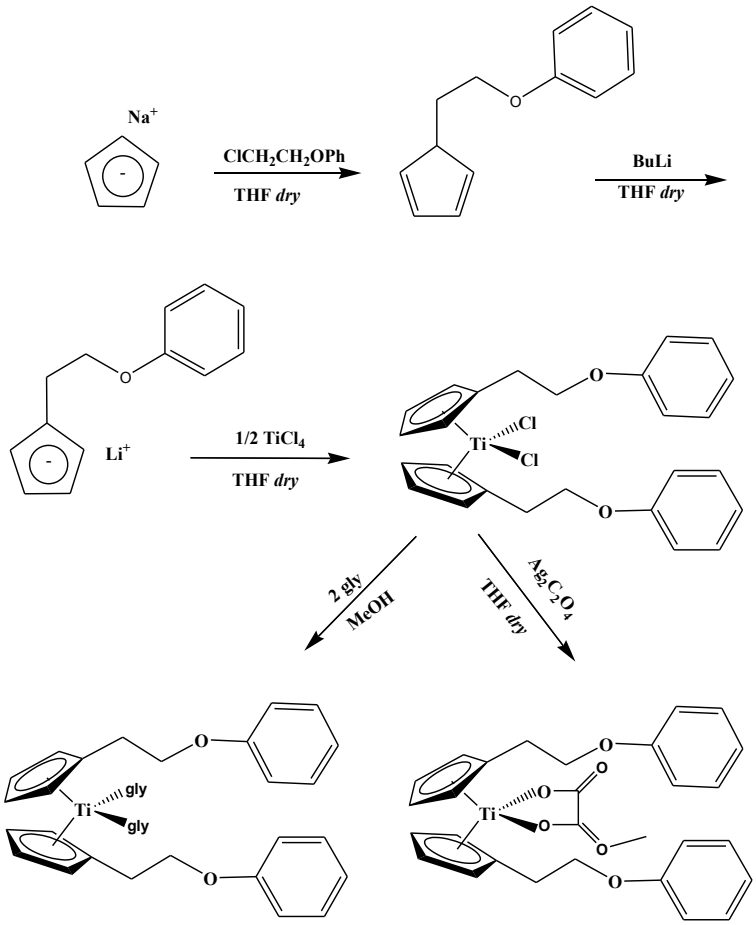
Table. 9 *Cytotoxic activity of tested compounds on MCF7 and SkBr3 breast cancer cells after one 24h treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test)*

	Cell lines	
Compound	MCF-7	SKBr3
7b	>100	>100
7c	>100	>100
Cisplatin	29 (±8)	10 (±2)

2.5 Synthesis and characterization of ethoxybenzyl-cyclopentadienyl titanocene

As indicated by our results, the presence of a coordinating group, such as phenyl or ether one, is necessary to stabilize the active species and generate a cytotoxic complex. This conclusion is supported by the lower activity of complex 4d (three times less cytotoxic than the unbridged complex 4c), in which the bridge beetwen the Cp ligands prevent the coordination of phenyl group on the metal centre, and of the lack of activity of complex 7c,with no coordinating group in its structure.
Moreover, the position of the phenyl ring and of the ether group may influence the activity, as can been easily seen through the half-titanocene 2b and 5b; these two complexes have two methoxylic groups, but in different positions and this lead to different cytotoxic activity, being complex 5b more cytotoxic than 2b, perhaps because of a stronger coordinating capability of the methoxy group in position 2, compared to the one in position 3.
In order to investigate an alternative arrangement of the phenyl group and of the ether group we decided to synthesized complexes 8b-8c, in which the phenyl ring and the ether group can more easily coordinate the metal centre, being the whole complex much more flebible.
As can be easily verified, this ligand may stabilize the titanium cation either with oxygen atom or with phenyl ring.
Moreover, complexes 8b-c allowed to test the effect of phenoxy group, instead of methoxy one present in T1 and T2, on the effectiveness of this titanocene and half-titanocene.
Complexes 8d and 8e were synthesized so as to have different leaving groups on titanocene, compared to chloride, and this could improve the activity of the complexes, by means of favorable pharmacokinetic properties.
The synthesis of 8b and 8c were carried out in good yields by reaction of the lithium salt of the ligand Li[C_5H_4-CH_2CH_2OPh] with one equivalent of $TiCl_4$ or half an equivalent respectively.
The synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-bis-glycine [C_5H_4-CH_2CH_2OPh]$_2$Ti(gly)$_2$ (8d) was carried out by the reaction of 8c with two equivalents of glycine in methanol containing 1% of water

(see scheme 10).
Instead, the synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-oxalate $[C_5H_4CH_2CH_2OPh]_2Ti(C_2O_4)$ (8e) was performed by reaction of 8c with silver-oxalate in dry THF (see Scheme 10).
All the synthesized complexes were purified following common procedures and isolated in good yield. Elemental analysis (C, H, N) were in agreement with the proposed formulae. ^{1}H COSY experiments allowed the assignment of all the proton resonances of the ^{1}H NMR spectrum, whereas DEPT experiments were useful for the attribution of ^{13}C NMR signals. The synthesized titanocenes were also characterized by mass spectrometry.



Scheme. 10 *Synthetic route for the preparation of complexes 8b-8e*

2.5.1 Hydrolysis test

Even for these complexes, hydrolytic stability has been determined in aqueous solution, 90% DMSO by ^{1}H NMR spectroscopy. Table 10 reports the results of our hydrolysis tests with**.** All the complexes show an high hydrolytic stability. In particular, the cyclopentadienyl rings of complexes are hydrolyzed only for less than 5% after 24 hrs

Table. 10 *Hydrolysis results of **8b-8c**complexes in DMSO/D_2O solutionat rt followed by ^{1}HNMR*

Complex	% Cp rings hydrolysis			
	5 mins	4 hrs	8 hrs	24 hrs
8b	<1	<1	<1	<5
8c	<1	<1	<1	<5
8d	<1	<1	<1	<5
8e	<1	<1	<1	<5

2.5.2 Cytotoxic activity

The synthesized compounds have been evaluated for their cytotoxic potential against two human breast cancer cell lines. Most of these compounds showed significant anti-proliferative effects compared to *cisplatin* when tested against both MCF7 and SkBr3 cells in MTT assays. Cells were treated for 5 days with each compound and were also exposed to *cisplatin*, in order to compare the anticancer effects of the chemicals used to this well-known chemotherapeutic. In dose-response and time course experiments, most of the tested compounds showed the capability to inhibit, in a dose-dependent manner, the proliferation of both cell lines, just after a 24 hour treatment.

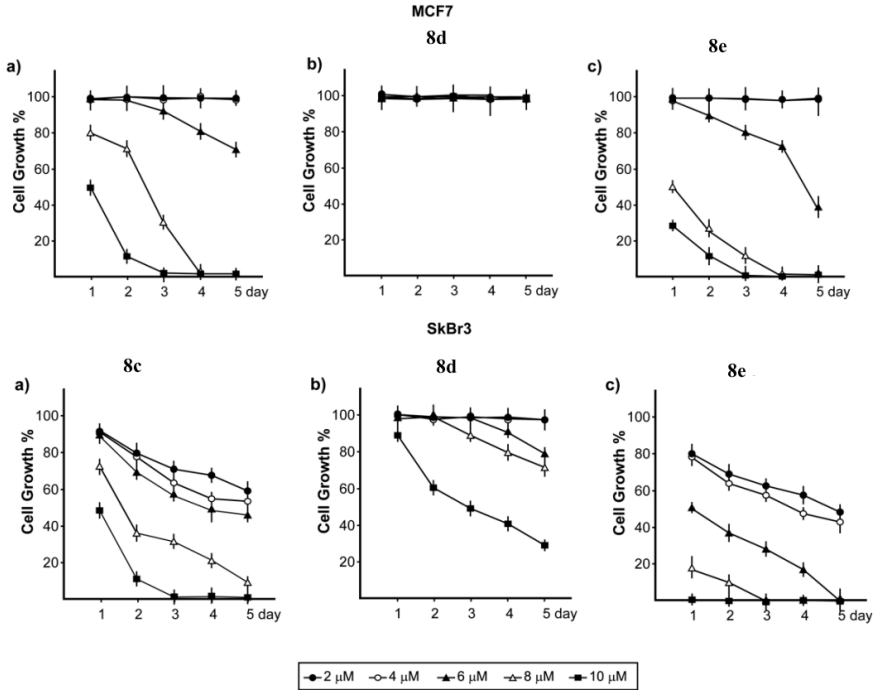


Figure 50 *Evaluation of cell growth treating MCF7 and SKBr3 breast cancer cells with tested compounds, as determined by using the MTT assay. Cells (5–8·10^4 mL^{-1}) were treated for one day up to 5 days with increasing concentrations of tested compounds, as indicated. Cell viability was expressed as the percentage of cells treated with the different compounds compared to cells treated with vehicle. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test).*

Among all tested titanocene derivatives, complexes 8c and 8e elicited strong repressive effects on the proliferation of both cell lines (Table 11). Complex 8e exhibited stronger antiproliferative effects in SkBr3 cells with respect to those elicited in MCF7 cells (IC_{50} values of 9 μM in MCF7 and 6 μM in SkBr3 cells) after a one-day-treatment (Table 11). Remarkably, a 24-hour-treatment with complex 8e (10 μM) was able to fully prevent SkBr3 cell growth (Fig. 50). Finally, it should be pointed out that complexes 8c and 8e inhibited the proliferation of MCF7 cells more strongly compared to *cisplatin* (Fig. 50).
Complex 8d showed a good cytotoxic activity only against SkBr3, whereas the half-titanocene 8b was active only at very high concentration (>100 μM).

Table. 11 *Cytotoxic activity of tested compounds on MCF7 and SkBr3 breast cancer cells after one 24h treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test).*

Complex	Cell line (IC50, µM)		
	MCF-7	**SKBr3**	**HEK-293**
8b	>100	>100	>100
8c	10 (±2)	10 (±1)	>100
8d	>100	38 (±5)	>100
8e	9 (±1)	6 (±2)	>100
TDC	>100	>100	>100
Tit. Y	72 (±9)	>100	>100
Cisplatin	29 (±8)	10 (±2)	67(±5)

Moreover, complexes 8c and 8e were tested again NCI-H1299.
Human non-small-cell lung carcinoma NCI-H1299 clones were grown in RPMI1640 medium and they were maintained at 37°C in a humified atmosphere of 5% (v/v) CO_2 in air. In order to test cell viability, we use MTS assay (Promega, Italy), so cells were plated in 96 wells plates at 32000 cells/mL and they were allowed to grow for 24 hours. After this period, cells were treated for 72 hours with different concentrations of the drugs, that were diluted in medium just before their use. Cisplatin was removed after 2 hours from the treatment, in order to avoid that some of its derivates could damage cells. The data of the survival curves and histograms were plotted as percentages of untreated controls. Three independent experiments consisted of at least three replicates for each point, were conducted

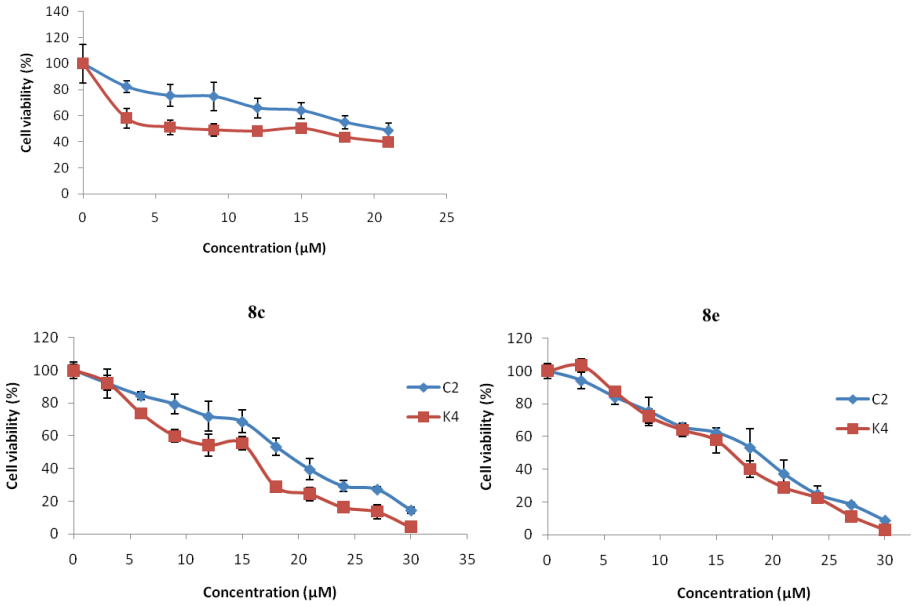
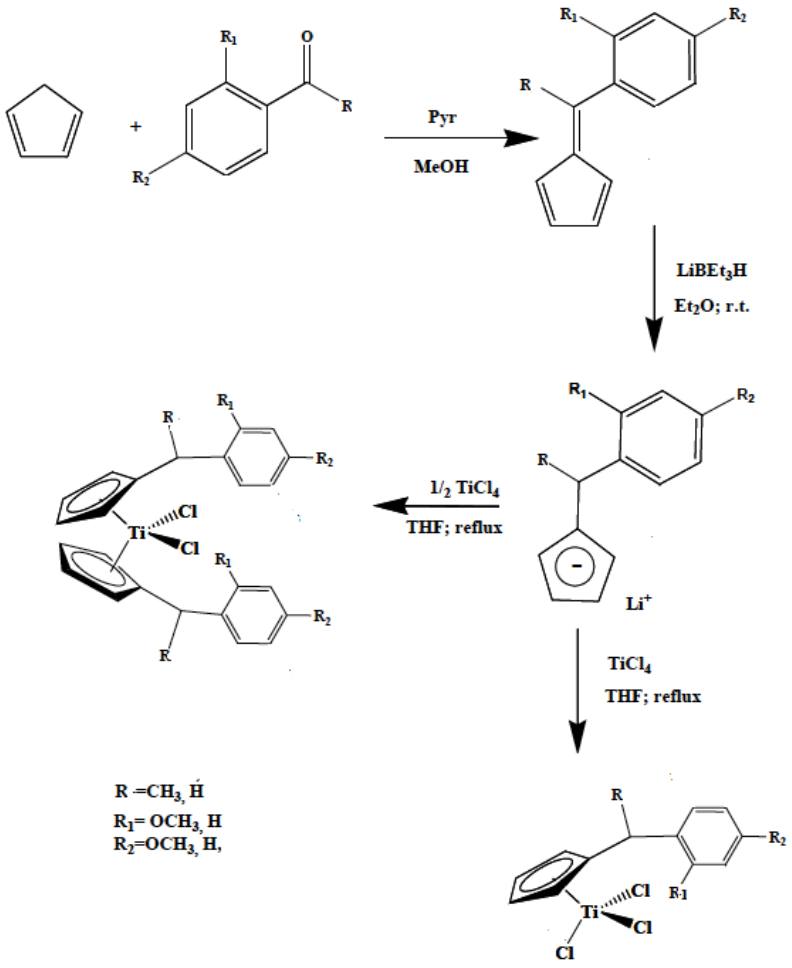


Figure 51. *Curves dose-response of two Human non-small-cell lung carcinoma NCI-H1299 clones treated with different concentration of 8c, 8e and Cisplatin*

In Figure 51, the dose-response curve of 8c showed again a similar activity to cisplatin and a stronger effect of the drug on WT KRAS clone. Morevoer complex 8e is the only titanocene complex that showed a similar activity on WT KRAS clone and G12C KRAS clone.

2.6 Synthesis and characterization of titanocene and half-titanocene derivatives having a methyl group on the linker between Cp ring and phenyl group

In order to investigate the importance of the linker between the Cp ring and the phenyl ring, we synthesized and fully characterized complexes 9b-9c, 10b-10c and 11b-11c.



Scheme. 11 *Synthetic route for the preparation of complexes 9b-11b and 9c-11c*

These complexes have the same ligand of the most active compounds previously reported, and in addition a methyl group on the linker between Cp rings and phenyl group in order to obtain even a more lipophilic complex.

The synthesis has been carried out according the same method used for complexes 1b-6b, starting from the suitable acetophenone, as shown in scheme 11.

All the synthesized complexes were purified following common procedures and isolated in good yield. Elemental analysis (C, H, N) were in agreement with the proposed formulae. ^{1}H COSY experiments allowed the assignment of all the proton resonances of the ^{1}H NMR spectrum, whereas DEPT experiments were useful for the attribution of ^{13}C NMR signals. The synthesized titanocenes were also characterized by mass spectrometry.

2.6.1 Hydrolysis test

Even for these complexes, hydrolytic stability has been determined in aqueous solution, 90% DMSO by ^{1}H NMR spectroscopy.
Table 12 reports the results of our hydrolysis tests**.** All the complexes show an hydrolytic stability, whereas complexes 9b and 9c are the less stable ones. These data agree with those previously reported.

Table. 12 *Hydrolysis results of complexes in DMSO/D_2O solution at rt followed by ^{1}H NMR.*

Complex	% Cp rings hydrolysis			
	5 mins	4 hrs	8 hrs	24 hrs
9b	15	30	55	55
9c	14	35	52	55
10b	<1	<1	<1	<5
10c	<1	<1	<1	<5
12	<1	<1	<1	<5
11c	<1	<1	<1	<5

2.6.2 Cytotoxic activity

The synthesized compounds have been evaluated for their cytotoxic potential against the same human breast cancer cell lines.

Table. 13 *Cytotoxic activity of tested compounds on MCF7 and SkBr3 breast cancer cells after one 24h treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test).*

Complex	Cell line (IC50, µM)	
	MCF-7	SKBr3
9b	>50	>50
9c	>50	>50
10b	>50	>50
10c	>50	>50
11b	>50	>50
11c	>50	>50
TDC	>100	>100
Tit. Y	72 (±9)	>100
Cisplatin	29 (±8)	10 (±2)

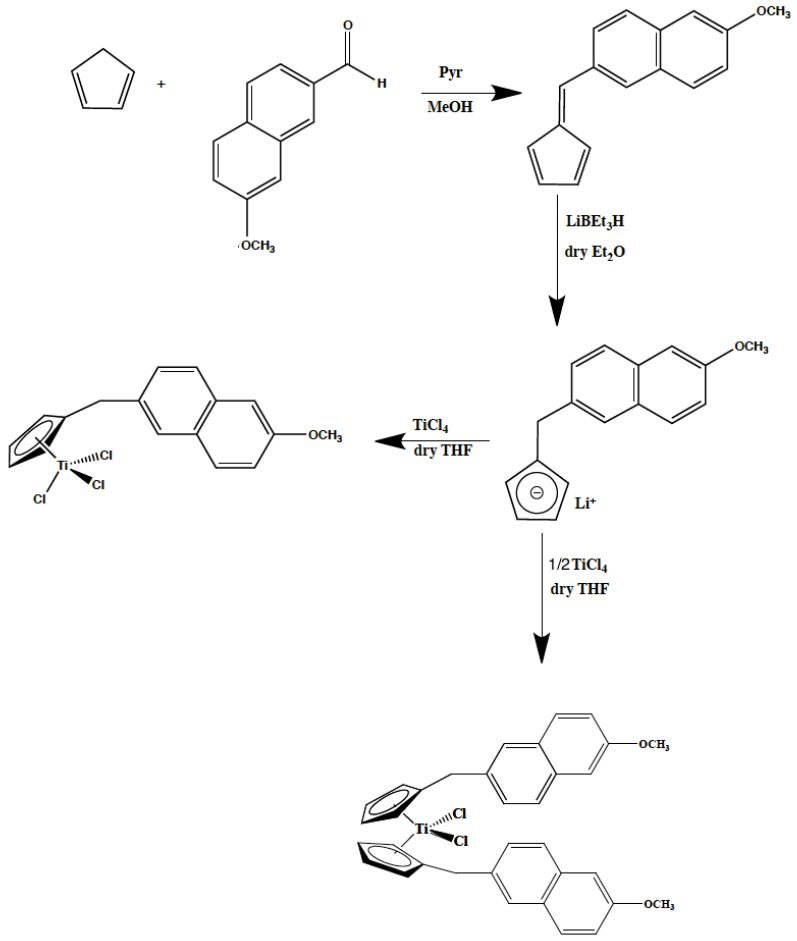
Cells were treated for 5 days with each compound and were also exposed to *cisplatin*, in order to compare the anticancer effects of the chemicals used to this well-known chemotherapeutic. In dose-response and time course experiments, most of the tested compounds showed the capability to inhibit, in a dose-dependent manner, the proliferation of both cell lines, just after a 24-hour treatment.
As snown in table 13, complexes 9b-11b and 9c-11c show no significant activity when tested against the same cell lines.

2.7 Synthesis and characterization of naphtyl-substituted complexes

Complexes 12b and 12c have the naphtyl ring instead of the phenyl ring and the methoxy group in the same position of Titanocene Y; the naphtyl

and phenyl groups have similar coordination properties but very different steric hindrance.

The synthesis was carried out starting from the suitable benzaldehide, as shown in scheme 12.



Scheme. 12. *Synthetic route for the preparation of complexes 12b and 12c*

2.7.1 Hydrolysis test

Even for these complexes, hydrolytic stability has been determined in aqueous solution, 90% DMSO by ^{1}H NMR spectroscopy.
Table 14 reports the results of our hydrolysis tests with**.** All the complexes show an hydrolytic stability even after 24 hours.

Table. 14 *Hydrolysis results of complexes in DMSO/D_2O solution at rt followed by ^{1}H NMR.*

Complex	% Cp ring hydrolysis			
	5 mins	4hrs	8 hrs	24 hrs
12b	<1	<1	<1	<5
12c	<1	<1	<1	<5

2.7.2 Cytotoxic activity

The synthesized compounds have been evaluated for their cytotoxic potential against the same human breast cancer cell lines but their cytotoxicity is lower if compared to cisplatin.

Table. 15 *Cytotoxic activity of tested compounds on MCF7 and SkBr3 breast cancer cells after one 24h treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ test).*

Complex	Cell line (IC50, µM)	
	MCF-7	SKBr3
12b	>50	>50
12c	>50	>50
TDC	>100	>100
Titanocene Y	72 (±9)	>100
Cisplatin	29 (±8)	10 (±2)

2.8 Cytotoxic activity of fulvene

All the synthesized fulvene have been tested for their cytotoxic acitivity against five cancer cell lines: MCF-7, SkBr3, Ishikawa, LnCap and A549. In addition to the well known MCF7 and SkBr3 cell lines, we tested them against:

- *Ishikawa* cell line: the cell line Ishikawa was established from an endometrial adenocarcinoma from a 39 year old woman. The cells induced well differentiated adenocarcinoma in athymic nude mice. Estrogen and progesterone receptors were demonstrated both in cell culture and in induced tumours. Ishikawa has been reported to produce corticotropin-releasing hormone, placental alkaline phosphatase and chorionic gonadotrophin, and responded to steroid hormones.
- *LnCap* cell line: LNCaP cells are androgen-sensitive human prostate adenocarcinoma cells derived from the left supraclavicular lymph node metastasis from a 50-year-old caucasian male in 1977. They are adherent epithelial cells growing in aggregates and as single cells. High-affinity specific androgen and estrogen receptors are present in the cytosol and nuclear fractions
- *A549* cell line: A549 cells are adenocarcinomic human alveolar basal epithelial cells. The A549 cell line was first developed in 1972 by D. J. Giard, et al. through the removal and culturing of cancerous lung tissue in the explanted tumor a of 58-year-old caucasian male. In nature, these cells are squamous and responsible for the diffusion of some substances, such as water and electrolytes, across the alveoli of lungs. They are able to synthesize lecithin and contain high level of desaturated fatty acids, which are important to maintain the membrane phospholipids in cells

With the aim of investigating the effects on cancer cell growth of the newly synthesized compounds, we evaluated by MTT tests their cytotoxic potential against a panel of f the ive human tumor cell lines.
MCF7 breast and A549 lung cancer cells were maintained in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 100 mg/ml

penicillin/streptomycin and 2 mM L-glutamine (Life Technologies, Milan, Italy). LNCaP prostate and SkBr3 breast cancer cells were cultured inRPMI-1640 medium with and without phenol red respectively, supplemented with 10% FBS, 100 mg/ml penicillin/streptomycin and 2 mM L-glutamine (Life Technologies, Milan, Italy). Ishikawa endometrial cancer cells were maintained in MEM supplemented with 10% FBS, 100 mg/ml penicillin/streptomycin, 2 mM L-glutamine and 1% Non-Essential Amino Acids Solution (Life Technologies, Milan, Italy).

The effects of each compound on cell viability were determined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme.[27] Cells were seeded in quadruplicate in 96-well plates in regular growth medium and grown until 70–80% confluence. Cells were washed once they had attached and then treated with increasing concentrations each compound for 1day in regular medium supplemented with 1% FBS. Relative cell viability was determined by MTT assay according to the manufacturer's protocol (Sigma–Aldrich, Milan, Italy). Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted versus drug concentration. IC_{50} values represent the drug concentrations that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.

Cells were treated for 24 hrs with each compound and were also exposed to *cis*-diamminedichloroplatinum(II) (*cis*platin), in order to compare the anticancer effects of the chemicals synthesized to this widely used anti-cancer chemotherapy drug. As *cis*platin is used to treat among others testicular, ovarian, bladder, head and neck, esophageal, small and non-small cell lung, breast, cervical, stomach and prostate cancers, we employed breast MCF7 and SkBr3, endometrial Ishikawa, prostate LnCaP and lung A549 cancer cells as model systems. The *in vitro* cytotoxic activity measurements indicated that most of the compounds studied are able to inhibit the proliferation of the five cell lines.

As reported in table x. compounds 1a-6a and 12a show a significant cytotoxic activity on all the cell lines, being the Ishikawa the most sensitive and the LnCap the less one.

In particular, among the investigated compounds, 5a exhibited the most powerful antiproliferative activity against all the human tumor cell lines

evaluated. Interestingly, the aforementioned compound showed higher inhibitory effects on cell growth than to *cis*platin.
It should be noted that 3a did not exhibit any antiproliferative potential, whereas 11a just blocked the proliferation of MCF7 breast cancer cells, confirming the importance of both two methoxy groups in 2' and 4' position. The inability of this compound to block the proliferation of SkBr3, Ishikawa, LnCaP and A549 cells might be related to the presence of the methyl group in position 6.

Table. 16 *Cytotoxic activity of tested compounds on breast MCF7 and SkBr3, endometrial Ishikawa, prostate LnCaP and lung A549 cancer cells, after 24 h treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P<0.05$, $\chi 2$ tst).*

Compound	**IC_{50} (µM)**				
	MCF7	**SkBr3**	**Ishikawa**	**LnCaP**	**A549**
1a	14(±2)	13(±4)	15(±2)	10(±4)	12(±4)
2a	10(±1)	13(±2)	9(±3)	11(±2)	13(±5)
3a	>50	>50	>50	>50	>50
4a	15(±3)	4(±1)	11(±3)	7(±1)	15(±4)
5a	8 (±2)	9(±2)	3(±1)	5(±2)	5(±1)
6a	11(±3)	10(±2)	9(±3)	5(±2)	25(±4)
9a	15(±2)	35(±6)	10(±2)	>50	>50
10a	6(±1)	15(±2)	6(±2)	>50	22(±4)
11a	10(±1)	>50	>50	>50	>50
12 a	30(±5)	>50	9(±1)	8(±1)	13(±2)
1a hydro	10(±2)	>50	10(±3)	12(±2)	>50
Cisplatin	22 (±6)	12 (±1)	14(±3)	10(±1)	12(±2)

Chapter Three

This chapter contains a summary of the performed work together with the main conclusions.

3 Conclusions

We have synthesized and characterized several ligands and titanium complexes. All the complexes have been tested in order to value their hydrolytic behavior.

The synthesized compounds have been evaluated for their cytotoxic potential against cancer cell lines. Most of these compounds showed significant anti-proliferative effects compared to cisplatin

As indicated by our results :

- *the presence of a coordinating group, such as phenyl or ether one, is necessary to stabilize the active species, delay hydrolysis and generate a cytotoxic complex*
- *the ether groups are important to improve the hydrolysis stability but are not necessary to have a significant cytotoxic activity*
- *a more labile ligand, as the benzyl group, having greater ability of π–donation, produce complexes with interesting cytotoxic properties;*
- *lower activity was found in the analogues where the two Cp units were bound by a two-carbon bridge (4d)*
- *half-titanocene showed lower cytotoxic activity compared to their titanocene analogues*
- *the labile ligands (Cl) do not have a strong impact on cytotoxic activity*

- *a methyl group on the linker between Cp ring and the phenyl group decrease the cytotoxic activity*
- *small steric hyndrance alla round the complex is favored*
- *the proligands show interesting cytotoxic activity but are not related to complexes cytotoxicity*
- *further study are needed to understand the exact mechanism through which these compounds exercise their action.*

We strongly believe that the real merit of Ti^{IV} complexes for clinical applications is yet to be discovered and, certainly, appreciated.

Chapter Four

This chapter contains all the experimental data relative to the synthesis, and thecharacterizations of all the ligands and complexes.

4 Experimental section

4.1 General procedure

All manipulations were carried out under oxygen- and moisture-free atmosphere in an MBraun MB 200 glove-box. All the solvents were thoroughly deoxygenated and dehydrated under argon by refluxing over suitable drying agents, while NMR deuterated solvents (Euriso-Top products) were kept in the dark over molecular sieves. $TiCl_4$, Titanium^{IV} chloride tetrahydrofuran complex and all chemicals were obtained from Aldrich chemical Co. and used without further purification. Cyclopentadiene was obtained by freshly cracked dicyclopentadiene. Silver oxalate was prepared by following the reported procedure.[255]
The elemental analyses for C and H were recorded on a ThermoFinnigan Flash EA 1112 series and performed according to standard microanalytical procedures. ^1H NMR, homodecoupled ^1H NMR, ^1H COSY and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance 300 Spectrometer operating at 300 MHz (^1H) and 75 MHz (^{13}C) and referred to internal tetramethylsilane. Molecular weights were determined by ESI mass spectrometry. ESI-MS analysis in positive and negative ion mode, were made using a Finnigan LCQ ion trap instrument, manufactured by Thermo Finnigan (San Jose, CA, USA), equipped with the Excalibur software for processing the data acquired. The sample was dissolved in acetonitrile and injected directly into the electrospray source, using a syringe pump, which maintains constant flow at 5 µl /min. The temperature of the capillary was set at 220°C. GC analyses were carried out with a GC-MS 7890A/5975C spectrometer (Agilent Technologies) equipped with an OPTIMA 17MS column and a mass-selective detector.

4.2 Synthesis of ligands

4.2.1 Synthesis of 6-p-(methoxyphenyl) fulvene (1a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of p-methoxy-benzaldehyde (2.4 ml, 20 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution immediately turned from colourless to red-orange. Large amounts of an orange solid precipitated out the solution. When TLC analysisi showed only one product band after 15 min, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 3.6 g of an orange product were obtained (98%yield)
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.70, 6.64, 6.45, 6.30 (C_5H_4, 4H m); 7.53, 7.50, 6.71, 6.88, 6.85 (C_6H_4, 4H m); 3.75 ($(OCH_3)_2$, 6H s); 7.10 (Ph-C*H*-Cp, 1H s)

4.2.2 Synthesis of 6-(3',4'- dimethoxyphenyl)fulvene (2a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of 3,4-dimetoxy-benzaldehyde (2.0 g, 10 mmol) and Cyclopentadiene (5 ml, 75.0 mmol) in 30 ml of methanol. After addition, the colour of the solution slowly turned from colourless to red-orange. After 20 hours no solid precipitated out the solution; acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 2.05 g of a red product were obtained (99% yield).

^{1}H NMR (δ ppm $CDCl_3$, 250 MHz 6.73, 6.69, 6.50, 6.34 (C_5H_4, 4*H* m); 6.93,6.90.6.79 (C_6H_3, 3H m); 3.94 ((OCH_3)$_2$, 6H s); 7.19 (Ph-C*H*-Cp, 1H s)

4.2.3 Synthsis of 6-(p-N, N-Dimethylanilinyl) fulvene (3a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of p-(N, N-dimethylamino)benzaldehyde (3.0 g, 30 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution immediately turned from colourless to red-orange. Large amounts of an orange solid precipitated out the solution. When TLC analysisi showed only one product band after 15 min, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 3.6 g of an orange product were obtained (90% yield)
^{1}H NMR (*δ* ppm $CDCl_3$, 250 MHz): 6.79, 6.63, 6.43, 6.31 (C_5H_4, 4H m); 7.58, 7.55, 6.71, 6.88 (C_6H_4, 4H m); 3.03 (N(CH_3)$_2$, 6H s); 7.13 (Ph-*CH*-Cp, 1H s)

4.2.4 Synthesis of 6-phenylfulvene (4a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of benzaldehyde (2.03 ml, 20 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution slowly turned from colourless to red-orange. After 20 hours no solid precipitated out the solution; acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 3.17 g of a red oil were obtained (97% yield).

^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.70, 6.52, 6.36, 6.33 (C_5H_4, 4H m); 7.61, 7.59, 7.43,7.40, 7.36 (C_6H_5, 5H m); 7.24 (Ph-C*H*-Cp, 1H s)

4.2.5 Synthesis of 6-(2',4'- dimethoxyphenyl)fulvene (5a)

The synthesis was carried out under nitrogen. Pyrrolidine (1.3 ml, 15mmol) was added to as olution of 2,4-dimethoxy-benzaldehyde (2.0 g, 10 mmol) and Cyclopentadiene (2.1 ml, 26.0 mmol) in 30 ml of methanol. After addition, the colour of the solution slowly turned from colourless to red-orange. After 4.5 hours no solid precipitated out the solution; acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 2.05 g of a red product were obtained (96% yield).
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.58, 6.55, 6.48, 6.37 (C_5H_4, 4*H* m); 7.63, 7.61, 7.52 (C_6H_3, 3H m); 3.87 ($(OCH_3)_2$, 6H s); 6.65(Ph-C*H*-Cp, 1H s)

4.2.6 Synthesis of 6 (2',4',6'-trimethoxyphenyl)fulvene (6a)

The synthesis was carried out under nitrogen. Pyrrolidine (1.3 ml, 15 mmol) was added to as olution of 2,4,6-trimethoxy-benzaldehyde (1.7 g, 10 mmol) and Cyclopentadiene (2.1 ml, 26.0 mmol) in 30 ml of methanol. After addition, the colour of the solution slowly turned from colourless to red-orange. After 4.5 hours no solid precipitated out the solution; acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 2.31 g of a red product were obtained (95% yield).
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.45, 6.43, 6.38, 6.32 (C_5H_4, 4*H* m); 6.15 (C_6H_2, 2H m); 3.86, 3.81 ($OCH_3)_3$, 9H s); 7.18(Ph-C*H*-Cp, 1H s)

4.2.7 Synthesis of (tert-butyl) cyclopentadiene (7a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to a solution of acetone (0.45 ml, 6.0 mmol) and Cyclopentadiene (1.2 ml, 15.0 mmol) in 20 ml of methanol. When TLC analysisi showed only one product band after 12 min, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure 1.3 g of an orange-red product (6,6-dimethyl-fulvene) were obtained (81% yield).
1g of 6,6-dimethyl-fulvene (9.4 mmol), were dissolved in 50 ml of dry diethyl ether to give a red solution. 7 ml of methyllithium solution (1.6 M in diethyl ether, 11 mmol) were added drop-wise at -78° C, giving a brown solution. The solution was warmed up to room temperature and left stirred overnight to give a white precipitate and a yellow solution. Afterwards the mixture was quenched with cold water. The organic product was extracted by 3x80 ml ether fraction.
The solution was dried over magnesium sulphate and the solvent was removed at reduced pressure to yield 1.14 g of a red orange oil (yield 99%).
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 1.22 [C_5H_5-*C(CH₃)₃*, 9H, m]; 2.97 [C_4H_4-*CH*-$C(CH_3)_3$, 1H, s]; 6.01-6.66 [*C₅H₅*-$C(CH_3)_3$, 5H, m].
^{13}C NMR (δ ppm $CDCl_3$, 62.89 MHz): 29.6[C_5H_5-*C(CH₃)₃*]; 30.8 [C_5H_5-*C*$(CH_3)_3$]; 39.9 [(C_4H_4-*CH*)$C(CH_3)_3$]; 122.2-133.4 [*C₅H₅*-$C(CH_3)_3$].

4.2.8 Synthesis of 1-[(2-(cyclopentadienyl)ethoxy]benzene (8a)

5.23 ml of 1-(2-chloroethoxy)benzene (38.45 mmol), previously distilled on CaH_2, were dissolved in 50 ml of dry THF to give a colourless solution. 25 ml of a 2M solution in THF of sodium cyclopentadienide were added drop-wise at -78° C. The solution was warmed up to room temperature and left stirred overnight to give a white precipitate and a dark pink solution. Afterwards the mixture was quenched with methanol and cold water. The organic product was extracted by 3x50 ml ether fraction. The solution was dried over magnesium sukphate and the solvent was removed at reduced pressure to yield a brown oil (yield 89%)

^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 2.88 [C_5H_5-(CH_2*CH_2*OC_6H_5), 2H,t]; 4.14 [C_5H_5-(CH_2*CH_2*OC_6H_5), 2H,t]; 3.01[C_4H_4-*CH*-($CH_2CH_2OC_6H_5$),1H, m]; 6.17-6.52- [C_4H_4-CH-($CH_2CH_2OC_6H_5$), 4H , m]; 6.92-7.32 [C_5H_5 -($CH_2CH_2OC_6H_5$), 5H,m].
^{13}C NMR (δ ppm C_6D_6, 62.89 MHz): 30.1 [C_5H_5-(*CH_2*CH_2OPh)]; 67.8 [C_5H_5-(CH_2*CH_2*OPh)]; 44.1[C_4H_4-*CH*-(CH_2CH_2OPh)] ; 114.8-120.9-128.1-129.7-134.4-134.9-159.1 [*C_4H_4* –CH-(CH_2CH_2O*Ph*)]
GC-MS: 186.1 [M^+]

4.2.9 Synthesis of 6-methyl-6-phenyl-fulvene (9a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of acetophenone (2.40 g, 20 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution turned from colourless to red-orange. After 20 hours, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. The crude product was purified by column cromatography over silica gel anda mixture of n-hexane/ethyl acetate (9:1) as the eluent, yielding 1.05 g (31% yield).
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.65, 6.63, 6.53, 6.47 (C_5*H_4*, 4H m); 7.39 (C_6*H_5*, 5H m); 2.54 (C*H_3,* 3H s)

4.2.10 Synthesis of 6-methyl-6-(4'-methoxyphenyl)fulvene (10a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of 4'-methoxyacetophenone (3.00 g, 20 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution turned from colourless to red-orange. After 20 hours, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic

solution was dried over magnesium sulfate. The crude product was purified by column cromatography over silica gel anda mixture of n-hexane/ethyl acetate (9:1) as the eluent, yielding 0.71 g (20% yield).
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.63, 6.54, 6.45, 6.23 (C_5H_4, 4H m); 7.38, 7.31, 6.95, 6.91 (C_6H_4, 4H m); 2.52 (CH_3, 3H s) 3.83 ((OCH_3) 3H s)

4.2.11 Synthesis of 6-methyl-6-(2',4'-dimethoxyphenyl)fulvene (11a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of 2',4'-methoxyacetophenone (3.60 g, 20 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution turned from colourless to red-orange. After 20 hours, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. The crude product was purified by column cromatography over silica gel anda mixture of petroleum spirit/ethyl acetate (8:2) as the eluent, yielding 1.01 g (22% yield).
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.47, 6.43, 6.40, 6.06 (C_5H_4, 4H m); 7.04, 6.64, 6.56(C_6H_3, 3H m); 2.50 (CH_3, 3H s) 3.85, 3.73 ((OCH_3)$_2$ 6H s);

4.2.12 Syhthesis of 6-(4'-methoxynaphtalyl)fulvene (12a)

The synthesis was carried out under nitrogen. Pyrrolidine (2.5 ml, 30.0 mmol) was added to as olution of 6-methoxy-2naphtaldehyde (2.4 g, 20 mmol) and Cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution immediately turned from colourless to red-orange. Large amounts of an orange solid precipitated out the solution and after 16 hours acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl eher and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3 x 20 ml). The combined organic extracts were washed with a satured aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When

the solvent was removed under reduced pressure, 0.69 g of an orange product were obtained (18% yield)
^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 6.36, 6.38, 6.53, 6.69 (C_5H_4, 4*H* m); 7.97, 7.79, 7.75, 7.18, 7.15, 7.14 ($C_{10}H_6$, 4H m); 3.95 ((OCH_3) 3H s); 7.34 (Ph-C*H*-Cp, 1H s)

4.3 Synthesis of half-titanocene complexes

4.3.1 Synthesis of [(4-methoxybenzyl)cyclopentadienyl]-titanium (IV)trichloride (1b)

$LiBEt_3H$ (7 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1.13g, 6.1 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
0.350 g (1.83 mmol) of the lithium cyclopentadienide intermediate were dissolved in 20 ml of dry THF to give a colourless solution. 0.19 ml(18.3 mmol) of $TiCl_4$ were added at 0°C to give a dark red solution. The solution was refluxed for 19 hours and then cooled. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a black solid.
^{1}H NMR (δ ppm, CD_2Cl_2, 300 MHz): 3.78 [3H, C_5H_4-CH_2-C_6H_4-OCH_3], 4.01 [2H, C_5H_4-CH_2-C_6H_4-OCH_3], 6.8 [4H,C_5H_4-CH_2-C_6H_4-OCH_3], 6.83-7.01 [4H, C_5H_4-CH_2-C_6H_4-OCH_3].
^{13}C NMR (δ ppm, CD_2Cl_2, 75 MHz): 55.9 [C_5H_4-CH_2-C_6H_4-OCH_3], 45.0 [C_5H_4-CH_2-C_6H_4-OCH_3], 114.0- 128.7-130.0-132.0-135.9-147.8-158.6 [C_5H_4-CH_2-C_6H_4-OCH_3].

Elemental analysis : Calcd. for $C_{14}H_{16}Cl_3OTi$ (%): C 47.43, H 4.55. Found (%): C 47.81, H 4.54.
Mass (E.I., 70 eV., m/z): 273 $[L\text{-}TiCl\text{-}Li]^{\cdot+}$, 186 $[L]^{\cdot+}$.

4.3.2 Synthesis of [(3,4-dimethoxybenzyl)-cyclopentadienyl]-titanium-trichloride (2b)

$LiBEt_3H$ (7 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1g, 4.7 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4$ (0.24 ml, 2.26 mmol) was added to 20 ml of dry THF . The solution turned immediately from colourless to pale yellow. 0.5 g (2.26 mmol) of the lithium cyclopenadienide intermediate were dissolved in 20 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled. The colour of the solution became finally brown.The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a brown solid.
^1H NMR (δ ppm, THF-d8 300 MHz): 3.73 [6H,C_5H_4-CH_2-C_6H_3-*$(OCH_3)_2$*], 3.98 [2H, C_5H_4-*CH_2*-C_6H_3-$(OCH_3)_2$], 6.29-6.42 [4H,*C_5H_4*-CH_2-C_6H_3-$(OCH_3)_2$], 6.71-6.84 [3H, *C_6H_3*-$(OCH_3)_2$].
^{13}C NMR (δ ppm, CD_2Cl_2, 75 MHz): 55.5 [C_5H_4-CH_2-C_6H_3-*$(OCH_3)_2$*], 36.8 [C_5H_4-*CH_2*-C_6H_3-$(OCH_3)_2$], 112.2, 113,3 115.4 120.8 122.5 132.8 137.1 149.2 150.3 [*C_5H_4*-CH_2-*C_6H_3*-$(OCH_3)_2$].
Elemental analysis : Calcd. for $C_{15}H_{18}Cl_3O_2Ti$ (%): C 46.85, H 4.72. Found (%): C 46.91, H 4.74

Mass (E.I., 70 eV., m/z): 286 $[L\text{-}Ti\text{-}Na]^{\cdot+}$.

4.3.3 Synthesis of [(4-(N,N-dimethylbenzanilido)-cyclopentadienyl]-titanium-trichloride (3b)

$LiBEt_3H$ (6.2 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1.15g, 5.8 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4 \cdot 2$ THF (0.564 g) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.35 g of the lithium cyclopenadienide intermediate were dissolved in 20 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to red during addition. After this addition, the mixture was refluxed overnight and then cooled. The solvent was removed under reduced pression. The remaining residue was extracted with toluene (30 ml) and filtered twice through celite to remove the LiCl. The dark red filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a red solid.
^1H NMR (δ ppm, THF-d8,, 300 MHz): 2.98 [6H, $C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$], 3.89 [2H, $C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$], 6.45-6.67 [4H,$C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$], 7.10-7.31 [4H, $C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$].
^{13}C NMR (δ ppm, THF-d8, 75 MHz): 41.7 [$C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$], 33.1 [$C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$], 115.9-117.6-119.2-128.7-130.7-136.0-137.1 [$C_5H_4\text{-}CH_2\text{-}C_6H_4\text{-}N(CH_3)_2$].
Elemental analysis : Calcd. for $C_{15}H_{19}Cl_3NTi$ (%): C 49.02, H 5.21, N 3.81. Found (%): C 49.12, H 5.17, N 3.79.
Mass (E.I., 70 eV., m/z): 371 $[L\text{-}TiCl_3\text{-}Na]^{\cdot+}$

4.3.4 Synthesis of [(benzyl)-cyclopentadienyl]-titanium-trichloride (4b)

$LiBEt_3H$ (9.7 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1.g, 6.5 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4 \cdot$ 2 THF (0.617g, 1.86 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.300 g (1.86 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to red during addition. After this addition, the mixture was refluxed overnight and then cooled. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark red solid.
^{1}H NMR (δ ppm, THF-d8 300 MHz): 4.06 [2H, C_5H_4-CH_2-C_6H_5], 6.33-6.45 [4H, C_5H_4-CH_2-C_6H_5], 7.22-7.24 [9H, C_5H_4-CH_2-C_6H_5].
^{13}C NMR (δ ppm, THF-d8 75 MHz): 36.7 [C_5H_4-CH_2-C_6H_5], 110.9-115.1-122.6-128.0-128.6-140.6 [C_5H_4-CH_2-C_6H_5].
Elemental analysis : Calcd. for $C_{13}H_{14}Cl_3Ti$ (%): C 48.12, H 4.35. Found (%): C 48.17, H 4.32.
Mass (E.I., 70 eV., m/z): 242 $[L\text{-}Ti\text{-}K]^{\cdot +}$, 163 $[L\text{-}Li]^{\cdot +}$

4.3.5 Synthesis of [(2,4-di-methoxybenzyl)-cyclopentadienyl]-titanium-trichloride (5b)

$LiBEt_3H$ (7 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1g, 4.7 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4 \cdot 2$ THF (0.75g, 2.26 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.5 g (2.26 mmol) of the lithium cyclopenadienide intermediate were dissolved in 20 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled. The colour of the solution became finally brown. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a brown solid.
^{1}H NMR (δ ppm, THF-d8 300 MHz): 3.72-3.76 [6H,C_5H_4-CH_2-C_6H_3-*(*OCH_3*)*$_2$], 3.90 [2H, C_5H_4-*CH_2*-C_6H_3-$(OCH_3)_2$], 6.28-6.29 [4H,*C_5H_4*-CH_2-C_6H_3-$(OCH_3)_2$], 6.40-6.46-7.01 [3H,C_5H_4-CH_2-*C_6H_3*-$(OCH_3)_2$]
^{13}C NMR (δ ppm, CD_2Cl_2, 75 MHz): 55.4-55.5[C_5H_4-CH_2-C_6H_3-*(*OCH_3*)*$_2$], 31.8 [C_5H_4-*CH_2*-C_6H_3-$(OCH_3)_2$], 99.1-104.8-116.6-121.9-123.3-131.4-137.6-159.2-161.0 [*C_5H_4*-CH_2-*C_6H_3*-$(OCH_3)_2$].
Elemental analysis : Calcd. for $C_{15}H_{18}Cl_3O_2Ti$ (%): C 46.85, H 4.72. Found (%): C 46.91, H 4.74.
Mass (E.I., 70 eV., m/z): 286 $[L\text{-}Ti\text{-}Na]^{\cdot +}$.

4.3.6 Synthesis of [(2,4,6-tri-methoxybenzyl)-cyclopentadienyl]-titanium-trichloride (6b)

$LiBEt_3H$ (6 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1g, 4.1 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4 \cdot 2$ THF (0.504 g, 1.52 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.380 g(1.52 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a black solid.
^{1}H NMR (δ ppm, THF-d8 300 MHz): 3.72-3.77 [9H,C_5H_4-CH_2-C_6H_2-*$(OCH_3)_3$*], 3.90 [2H, C_5H_4-*CH_2*-C_6H_2-$(OCH_3)_3$], 6.30-6.34 [4H,*C_5H_4*-CH_2-C_6H_2-$(OCH_3)_3$], 6.47 [2H, C_5H_4-CH_2-*C_6H_2*-$(OCH_3)_3$].
^{13}C NMR (δ ppm, THF-d8 75 MHz): 54.7 [C_5H_4-CH_2-C_6H_2-*$(OCH_3)_3$*], 31.05 [C_5H_4-*CH_2*-C_6H_2-$(OCH_3)_3$], 98.4-104.0-115.8-122.5-130.6-137.7-158.3-160.3[*C_5H_4*-CH_2-*C_6H_2*-$(OCH_3)_3$].
Elemental analysis : Calcd. for $C_{16}H_{20}Cl_3O_3Ti$ (%): C 46.36, H 4.86. Found (%): C 46.39, H 4.84.
Mass (E.I., 70 eV., m/z): 317 $[L\text{-}Ti\text{-}Na]^{\cdot +}$.

4.3.7 Synthesis of (tert-butyl-cyclopentadienyl)-titanium (IV) trichloride (7b)

To a solution of neutral ligand 14a (0.500 g, 4.1 mmol) in dry n-hexane (30 mL), 1,4 ml of n-BuLi (2.5 M solution in hexane, 2.35 mmol) was slowly added at 0° C. The solution was warmed up to room temperature and left stirred overnight, obtaining a yellow lithium intermediate which was washed twice with hexane, dried under reduced pressure and isolated in good yield. Afterward to a solution of the lithium intermediate (0.110g, 0.85 mmol) in 30 ml of dry at -78° C 0.08 mL (0.85 mmol) of $TiCl_4$ were added, The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled.The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a brown solid (0.070 g, 30% yield)

^{1}H NMR (δ ppm toluol, 250 MHz): 1.26 [C_5H_4-$C(CH_3)_3$, 9H , s]; 5.80-6.08 [C_5H_4-$C(CH_3)_3$, 4H , s]

^{13}C NMR (δ ppm toluol, 62.89 MHz): 31.4 [C_5H_4-$C(CH_3)_3$]; 32 [C_5H_4-$C(CH_3)_3$];116-121-149 [C_5H_4-$C(CH_3)_3$].

4.3.8 Synthesis of (2-cyclopentadienyl-ethoxy-benzene)-titanium-(IV) trichloride (8b)

To a solution of neutral ligand (1.0 g, 5.37 mmol) in dry THF (40 mL), a stoichiometic amount of n-BuLi (2.5 M solution in hexane, 2.149 mL) was slowly added at 0°C. The solution was warmed up to room temperature and left stirred overnight, obtaining a yellow lithium intermediate. Afterward the solution was treated at -78 °C with 0.59 mL (5.37 mmol) of $TiCl_4$ and stirred overnight. The solvent was then removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pressure to give a black solid (yield 65%)

^{1}H NMR (δ ppm $CDCl_3$, 250 MHz): 3.23 [C_5H_4-(CH_2CH_2OPh), 2H,t]; 4.21 [C_5H_4-(CH_2CH_2OPh), 2H,t]; 6.44 [C_5H_4-(CH_2CH_2OPh), 4H , s]; 6.87-7.1 [C_5H_4 -(CH_2CH_2OPh), 6H,m].

^{13}C NMR (δ ppm, C_6D_6, 62.89 MHz): 25.7 [C_5H_4-(CH_2CH_2OPh)]; 71.9 [C_5H_4-(CH_2CH_2OPh)];114.7- 115.1- 115.9- 121.3- 123.7- 130.0- 130.2- 159.7 [C_5H_4 -(CH_2CH_2OPh)].
Elemental analysis: Calcd. for $C_{13}H_{13}Cl_3OTi$ (%): C 46.00, H 3.86. Found (%): C 46.21, H 3.84.
Mass data (E.I., 70 eV., m/z): 233 [L-Ti]$^{\cdot+}$.310 [L-$TiCl_2$-Li]$^{\cdot+}$.

4.3.9 Synthesis of 3-(phenylethyl)cyclopentadienyl-titanium(IV) trichloride (9b)

$LiBEt_3H$ (10 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (0.84g, 5 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4$·2 THF (0.13g, 0.4 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.070g (0.4 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark brown solid.
^{1}H NMR (δ ppm THF-d8, 300 MHz): 1.58 [3H, C_5H_4-CH*(CH_3)*-$C_6H_4(OCH_3)$], 4,45 [1H, C_5H_4-*CH*(CH_3)-C_6H_5] 6.35, 6.43 [4H, *C_5H_4*-$CH(CH_3)$-C_6H_5)], 7.17, 7.18, 7.21, 7.23 [5H, C_5H_4-$CH(CH_3)$-*C_6H_5*].
^{13}C NMR (δ ppm, THF-d8, 75 MHz): 23.9 [C_5H_4-CH*(CH_3)*-$C_6H_4(OCH_3)$], 43.6 [C_5H_4-*CH*(CH_3)-C_6H_5] 109.2, 114.8, 120.1, 127.5, 127.8, 138.8 [*C_5H_4*-$CH(CH_3)$-*C_6H_5*].

Elemental analysis: Calcd. for $C_{13}H_{12}Cl_3Ti$ (%): C 48.42, H 3.75. Found (%): C 48.21, H 3.74.
Mass (E.I., 70 eV., m/z): 286 $[L\text{-}Ti\text{-}Cl_2]^{\cdot+}$

4.3.10 Synthesis of [3-(4-methoxyphenylethyl)cyclopentadienyl]titanium(IV) trichloride (10b)

$LiBEt_3H$ (7.2 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (0.71g, 3.6 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4 \cdot 2$ THF (0.149 g, 0.45 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.090g (0.45 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark brown solid (yield 44.2%)
^{1}H NMR (δ ppm, C_6D_6, 300 MHz): 3.30 [3H, C_5H_4-CH(CH_3)-C_6H_4*(OCH$_3$)*], 1.58 [3H, C_5H_4-CH*(CH$_3$)*-C_6H_4(OCH_3)], 4,63 [1H, C_5H_4-*CH*(CH_3)-C_6H_4(OCH_3)] 5.45, 5.75 [4H, *C_5H_4*-CH(CH_3)-C_6H_4(OCH_3)], 6.37, 6.46, 6.74, 7.04 [4H, C_5H_4-CH(CH_3)-*C_6H_4*(OCH_3)].
^{13}C NMR (δ ppm, C_6D_6, 75 MHz): 55.0 [C_5H_4-CH(CH_3)-C_6H_4*(OCH$_3$)*], 23.2 [C_5H_4-CH*(CH$_3$)*-C_6H_4(OCH_3)], 40.6 [C_5H_4-*CH*(CH_3)-C_6H_4(OCH_3)],

114.2-114.5-120.0-120.8-122-123.3-129.5-138.8-159.1 [C_5H_4-CH(CH_3)-C_6H_4(OCH_3)].
Elemental analysis: Calcd. for $C_{14}H_{14}Cl_3OTi$ (%): C 47.70, H 4.00. Found (%): C 47.81, H 3.94.
Mass (E.I., 70 eV., m/z): 317 [L-Ti-Cl_2]$^{\cdot+}$

4.3.11 Synthesis of [3-(2,4-dimethoxyphenylethyl)cyclopentadienyl]titanium(IV) trichloride (11b)

$LiBEt_3H$ (8.8 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (1g, 4.4 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4$·2 THF (0.28 g, 0.85 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.200 g (0.85 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled.The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a brown solid (yield 40.875%).
^{1}H NMR (δ ppm, C_6D_6, 300 MHz): 3.22 3.23 [6H, C_5H_4-CH(CH_3)-C_6H_3*(OCH_3)$_2$*], 1.7 [3H, C_5H_4-CH*(CH_3)*-C_6H_3(OCH_3)$_2$], 5.06 [1H, C_5H_4-*CH*(CH_3)-C_6H_3(OCH_3)$_2$] 5.92, 6.33, 6.43, 6.54, 6.61, 6.91 [7H, C_5H_4-CH(CH_3)-C_6H_3(OCH_3)$_2$].

^{13}C NMR (δ ppm, C_6D_6, 75 MHz): 54.3 55.2 [C_5H_4-CH(CH_3)-C_6H_3*(OCH_3)$_2$*], 23.7 [C_5H_4-CH*(CH_3)*-$C_6H_3(OCH_3)_2$], 30.5 [C_5H_4-*CH*(CH_3)-$C_6H_3(OCH_3)_2$], 98.6-103.7-114.5-120.8-121.4-131.4-136.4-150.2-160.1 [*C_5H_4*-CH(CH_3)-*C_6H_3*(OCH_3)$_2$].
Elemental analysis: Calcd. for $C_{15}H_{16}Cl_3O_2Ti$ (%): C 47.10, H 4.22. Found (%): C 47.11, H 4.19.
Mass (E.I., 70 eV., m/z): 347 $[L\text{-}Ti\text{-}Cl_2]^{\cdot+}$

4.3.12 Synthesis of [(4-methoxynaphtalyl)cyclopentadienyl] titanium (IV) trichloride (12b)

$LiBEt_3H$ (8.1 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 1a (0.69g, 2.9 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4 \cdot 2$ THF (0.27 g, 0.82 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.200 g (0.82 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a black solid .
^1H NMR (δ ppm THF-d8, 300 MHz): 6.38, 6.46 (C_5H_4, 4*H* m); 7.66, 7.64, 7.60, 7.34, 7.16. 7.07 (C_{10}*H_6*, 6H m); 3.85 ((OCH_3) 3H s); 4.19 (Ph-C*H_2*-Cp, 2H s)

^{13}C NMR (δ ppm CD_2Cl_2, 75 MHz): 104.9, 124.1, 125.4, 128.1, 128.7, 129.3, 132.5, 147.0, 155.9 (C_5H_4 and $C_{10}H_6$); 55.9 ((OCH_3) 3H s); 35.1 (Ph-CH_2-Cp, 2H s)
Elemental analysis: Calcd. for $C_{17}H_{14}Cl_3OTi$ (%): C 52.55, H 3.63. Found (%): C 52.78, H 3.65.
Mass (E.I., 70 eV., m/z): 353 [L-Ti-Cl_2]$^{\cdot+}$

4.4 Synthesis of titanocene derivatives

4.4.1 Synthesis of bis-[(benzyl)-cyclopentadienyl]-titanium-dichloride (4c)

$LiBEt_3H$ (9.8 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 4a (1g, 6.5 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under nitrogen.
$TiCl_4$·2 THF (0.308g, 0.93 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.300 g (1.86 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added *via cannula* to the solution containing the $TiCl_4$.The solution turned from yellow to red during addition. After this addition, the mixture was refluxed overnight and then cooled. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark red solid (yield 55%).
^{1}H NMR (δ ppm, THF-d8, 250 MHz): 4.06 [C_5H_4-CH_2-C_6H_5, 2H, s], 6.33-6.44 [C_5H_4-CH_2-C_6H_5, 4H, m], 7.22- 7.24 [C_5H_4-CH_2-C_6H_5, 5H, m].

^{13}C NMR (δ ppm, THF-d8, 62.89 MHz): 36.7 [C_5H_4-*CH_2*-C_6H_5], 110.9-115.1-122.6-128.0-128.6-140.6[*C_5H_4*-CH_2-*C_6H_5*].
Elemental analysis: Calcd. for $C_{24}H_{20}Cl_2Ti$ (%): C 67.48, H 4.72. Found (%): C 67.39, H 4.77.
Mass data (E.I., 70 eV., m/z): 426 [L_2-$TiCl_2$-Li]$^{\cdot+}$; 357 [L_2-Ti]$^{\cdot+}$.

4.4.2 Synthesis of [1,2-Di(cyclopentadienyl)-1,2-bis1,2-di(phenyl)ethanediyl]titanium-dichloride) (4d)

$TiCl_4 \cdot 2$ THF (1.076g, 3,24 mmol) was added to 20 ml of dry toluene containing 10% dry THF. The solution turned immediately from colourless to pale yellow. The mixture was stirred and cooled down to -78° C, followed by drop wise addition of *n*-butyl lithium (4 ml, 6,48 mmol). The solution turned from yellow to brown during addition. After this addition the mixture was allowed to warm up slowly to room temperature. The colour of the solution became finally black. After 20 h, stirring at r.t, a solution of phenyl-fulvene (6.48 mmol, 1g) in 10 ml of dry toluene was added to the $TiCl_2 \cdot 2$ THF solution at r.t under nitrogen. Then it was stirred under reflux for another 20 hours. 10 ml of the solvent were removed and then the remaining mixture was filtered through celite. The solvent was then removed under vacuum and washed with dichlorometane and finally twice with hexane to give a dark brown solid (yield 49%).
^{1}H NMR (δ ppm, $CDCl_3$, 250 MHz): 5.54 [*trans*-C_5H_4-*CH_2*-C_6H_5, 2H, s], 4.85[*cis*-C_5H_4-*CH_2*-C_6H_5 2H, s], 6.20-6.37 [*C_5H_4*-CH_2-C_6H_5,4H, m], 6.89-7.29 [C_5H_4-CH_2-*C_6H_5*, 5H, m].
^{13}C NMR (δ ppm, $CDCl_3$, 62.89 MHz): 54.2 [*trans*-C_5H_4-*CH_2*-C_6H_5], 51.0 [*cis*-C_5H_4-*CH_2*-C_6H_5], 110.0-113.4-117.1-127.5-128.1-128.3-128.6-133.8-138.3[*C_5H_4*-CH_2-*C_6H_5*].
Mass (E.I., 70 eV, m/z): 433.04 [L_2-$TiCl_2$-Li]$^{\cdot+}$.
Elemental analysis: Calcd. for $C_{24}H_{20}Cl_2Ti$ (%): C 67.48, H 4.72. Found (%): C 67.39, H 4.77.

4.4.3 Synthesis of bis-[(2,4 dimethoxybenzyl)-cyclopentadienyl] titanium (IV) dichloride (5c)

$LiBEt_3H$ (7 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 60° C under a vacuum of 10^{-2} mbar for 40 minutes and to 90° C for other 20 minutes. The concentrated reagent was dissolved in diethyl ether (50 ml) and was transferred to a solution of 4a (1g, 4.7 mmol) in diethyl ether (60 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate precipitated from the solution and the colour of the solution changed from red to yellow. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. The white lithium salt was then collected on a frit and washed with diethyl ether (25 ml), dried briefly in vacuo and transferred to a Schlenk flask under argon.
$TiCl_4 \cdot 2$ THF (0.308 g, 0.93 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.300 g (1.35 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added *via cannula* to the solution containing the $TiCl_4$.The solution turned from yellow to red during addition. After this addition, the mixture was refluxed overnight and then cooled. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark red solid (yield 55%).
^{1}H NMR (δ ppm, C_6D_6 300 MHz): 3.32-3.38 [6H,C_5H_4-CH_2-C_6H_3-*$(OCH_3)_2$*], 4.25 [2H, C_5H_4-*CH_2*-C_6H_3-$(OCH_3)_2$], 6.19-6.29 [4H,*C_5H_4*-CH_2-C_6H_3-$(OCH_3)_2$], 6.38-6.46-7.11 [3H,C_5H_4-CH_2-*C_6H_3*-$(OCH_3)_2$].
^{13}C NMR (δ ppm, CD_2Cl_2, 300 MHz): 55.1-55.2[C_5H_4-CH_2-C_6H_3-*$(OCH_3)_2$*], 31.8 [C_5H_4-*CH_2*-C_6H_3-$(OCH_3)_2$], 99.4-104.6-115.7-121.7-123.0-131.7-137.2-159.0-160.7 [*C_5H_4*-CH_2-*C_6H_3*-$(OCH_3)_2$].
Elemental analysis : Calcd. for $C_{28}H_{28}Cl_2O_4Ti$ (%): C 61.45, H 5.16. Found (%): C 61.91, H 5.17.
Mass (E.I., 70 eV., m/z): 499 $[L\text{-}Ti\text{-}Na]^{\cdot +}$.

4.4.4 Synthesis of bis(tert-butyl-cyclopentadienyl) titanium (IV) dichloride (7c)

To a solution of neutral ligand 14a (0.518 g, 4.2 mmol) in dry THF (50 mL), 2.6 ml of n-BuLi (2.5 M solution in hexane, 2.35 mmol) was slowly added at -78° C. The solution was warmed up to room temperature and left stirred overnight, obtaining a yellow lithium intermediate. Afterward the solution was treated at -78° C with 0.23 mL (2.1 mmol) of $TiCl_4$ and stirred overnight. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a brown solid. (0.060g, 36% yield)

^{1}H NMR (δ ppm toluol, 250 MHz): 1.27 [C_5H_4-$C(CH_3)_3$, 9H , s]; 5.81-6.09 [C_5H_4 -$C(CH_3)_3$,4H ,s].

^{13}C NMR (δ ppm toluol, 62,89 MHz): 30.89 [C_5H_4-$C(CH_3)_3$]; 31.4 [C_5H_4-$C(CH_3)_3$]; 117.7-119.4-149.1 [C_5H_4-$C(CH_3)_3$].

Elemental analysis : Calcd. for $C_{19}H_{24}Cl_2Ti$ (%): C 61.45, H 5.16. Found (%): C 61.91, H 5.17.

Mass (E.I., 70 eV., m/z): 288 $[L\text{-}Ti\text{-}Cl]^{\cdot+}$.

4.4.5 Synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium (IV) dichloride (8c)

To a solution of neutral ligand (0.45 g, 2.41 mmol) in dry THF (40 mL), a stoichiometic amount of *n*-BuLi (2.5 M solution in hexane, 1.5 mL) was slowly added at 0 °C. The solution was warmed up to room temperature and left stirred overnight, obtaining a yellow lithium intermediate. Afterward the solution was treated at -78 °C with 0.13 mL (1.21 mmol) of $TiCl_4$ and stirred overnight. The solvent was then removed under reduced pressure. The remaining residue was extracted with dichloromethane (30 mL) and filtered through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 mL) and then dried under reduced pressure to give a black solid (yield 37%).

^{1}H NMR (δ ppm THF-d8 300 MHz,) δ 3.58 [t, 4H, C_5H_4-($CH_2$$CH_2OC_6H_5$)], 4.68 [t, 4H, C_5H_4-(CH_2CH_2O C_6H_5)], 6.75-6.85 [m,

8H, C_5H_4-($CH_2CH_2OC_6H_5$),], 6.90-7.10 [m, 10H, C_5H_4-($CH_2CH_2OC_6H_5$)].
^{13}C NMR (δ ppm 75 MHz, C_6D_6) δ 25.1 [C_5H_4-($CH_2CH_2OC_6H_5$)], 67.5 [C_5H_4-($CH_2CH_2OC_6H_5$)], 114.0, 114.9, 128.9 [C_5H_4-($CH_2CH_2OC_6H_5$)], 115.2, 118.6, 120.2, 157.8, 159.4 [C_5H_4-($CH_2CH_2OC_6H_5$)].
Elemental analysis Calcd for $C_{26}H_{26}Cl_2O_2Ti$ (%): C, 63.83; H, 5.36. Found (%): C, 64.02; H 5.32.
Mass (E.I., 70 eV, m/z): 496 [L_2-$TiCl_2$-Li]$^{\cdot+}$.

4.4.6 Synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-bis-glycine (8d)

In a round-bottom flask 50 mg of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride (0.16 mmol) was dissolved in 30 mL of methanol (containing 1% of water). To this brown solution 17 mg of glycine (0.23 mmol) was added at room temperature and the mixture was stirred for 4 h. The solvent was removed in vacuum, obtaining a red/orange solid, the yield was quantitative.
^{1}H NMR (δ ppm 300 MHz, DMSO-d_6) δ 3.16 [t, 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 4.11 [t 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 6.70-7.20 [m, 18H, C_5H_4-($CH_2CH_2OC_6H_5$)]; 3.30 [s, 4H, Ti-OCO-CH_2-NH_2]
^{13}C NMR (δ ppm 75 MHz, THF-d8) δ 29.7 [C_5H_4-($CH_2CH_2OC_6H_5$)], 68.3 [C_5H_4-($CH_2CH_2OC_6H_5$)], 112.5, 114.3, 120.5, 129.1, 130.0, 159.5, 160.2 [C_5H_4-($CH_2CH_2OC_6H_5$)], 185.6 [Ti-OCO-CH_2-NH_2], 47.1 [Ti-OCO-CH_2-NH_2].
Elemental analysis Calcd for $C_{30}H_{34}N_2O_6Ti$ (%): C, 63.61; H 6.05. Found (%): C, 63.45; H 6.09.

4.4.7 Synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-oxalate (8e)

Oxalic acid dihydrate (0.80 g, 6.33 mmol) was dissolved in ethanol (30 mL) to give a colourless solution. To this solution, freshly distilled triethylamine (0.90 mL, 12.64 mmol) was added, which remained colourless. Silver nitrate (1.16 g, 6.80 mmol) was dissolved in acetonitrile (5 mL) and ethanol (30 mL) to give a colourless solution. The two solutions were added and shielded from light exposure and stirred for two hours. When the stirring was stopped, the white precipitate was allowed

to stand for one more hour. The solution was filtered, and the white solid was dried in vacuo whilst being shielded from thelight

Silver oxalate (0.04 g, 0.12 mmol) and (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride (0.04 g, 0.08 mmol) were dissolved in THF (30 mL) in a round-bottom flask, shielded from the light. The solution was left stirring for 24 h at room temperature. The suspension was gravity filtered to give a red-orange colored filtrate. The solvent was removed in vacuum and a red-orange solid was obtained (yield 59.4%).

^{1}H NMR (δ ppm 300 MHz, THF-d_8) δ 3.16 [t, 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 4.07 [t, 4H, C_5H_4-($CH_2CH_2O\ C_6H_5$)], 6.73-7.16 [m 18H, C_5H_4-($CH_2CH_2OC_6H_5$)].

^{13}C NMR (δ ppm 75 MHz, C_6D_6) δ 30.8 [C_5H_4-($CH_2CH_2OC_6H_5$)], 68.3 [C_5H_4-($CH_2CH_2OC_6H_5$)], 115.4, 116.2, 119.7, 121.2, 130.0, 130.2, 134.0, 159.1 [C_5H_4-($CH_2CH_2OC_6H_5$)], 160.2 [Ti-C_2O_4].

Anal. Calcd for $C_{28}H_{26}O_6Ti$ (%): C, 66.41; H 5.18. Found (%): C, 66.21; H 5.21.

4.4.8 Synthesis of bis-[3-(phenylethyl)cyclopentadienyl]-titanium(IV) dichloride (9c)

The lithium intermediate was obtained by the first step of the synthesis of 9b

$TiCl_4 \cdot 2$ THF (0.094g, 0.28 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.100g (0.56 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark red solid

^{1}H NMR (δ ppm, THF-d8, 300 MHz): 1.58 [3H, C_5H_4-CH(CH_3)-$C_6H_4(OCH_3)$], 4,44 [1H, C_5H_4-$CH(CH_3)$-C_6H_5] 6.35, 6.40 [4H, C_5H_4-$CH(CH_3)$-C_6H_5)], 7.16, 7.18, 7.20, 7.23 [5H, C_5H_4-$CH(CH_3)$-C_6H_5].

^{13}C NMR (δ ppm, THF-d8, 75 MHz): 22.9 [C_5H_4-CH*(CH_3)*-$C_6H_4(OCH_3)$], 43.4 [C_5H_4-*CH*(CH_3)-C_6H_5] 110.2, 115.1, 120.4, 127.0, 127.8, 139.1 [*C_5H_4*-CH(CH_3)-*C_6H_5*].
Elemental analysis Calcd for $C_{26}H_{24}Cl_2Ti$ (%): C, 68.60; H, 5.31. Found (%): C, 68.62; H 5.32.
Mass (E.I., 70 eV, m/z): 384 $[L_2\text{-}Ti]^{\cdot+}$.

4.4.9 Synthesis of bis-[3-(4-methoxyphenylethyl)cyclopentadienyl]titanium(IV) dichloride (10c)

The lithium intermediate was obtained by the first step of the synthesis of 10b
$TiCl_4 \cdot 2$ THF (0.074g, 0.23 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.093g (0.46 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark red solid (yield 38.2%)
^{1}H NMR (δ ppm, C_6D_6, 300 MHz): 3.28 [6H, C_5H_4-CH(CH_3)-C_6H_4*(OCH_3)*], 1.58 [6H, C_5H_4-CH*(CH_3)*-$C_6H_4(OCH_3)$], 4.6 [2H, C_5H_4-*CH*(CH_3)-$C_6H_4(OCH_3)$] 5.44, 5.81 [8H, *C_5H_4*-CH(CH_3)-$C_6H_4(OCH_3)$], 6.37, 6.45, 6.75, 7.04 [8H, C_5H_4-CH(CH_3)-*C_6H_4*(OCH_3)].
^{13}C NMR (δ ppm, C_6D_6, 75 MHz): 54.6 [C_5H_4-CH(CH_3)-C_6H_4*(OCH_3)*], 21.9 [C_5H_4-CH*(CH_3)*-$C_6H_4(OCH_3)$], 40.2 [C_5H_4-*CH*(CH_3)-$C_6H_4(OCH_3)$], 113.8-114.2-115.7-119.6-120.3-129.1-138.3-142.7-158.7 [*C_5H_4*-CH(CH_3)-*C_6H_4*(OCH_3)].
Elemental analysis Calcd for $C_{28}H_{28}Cl_2O2Ti$ (%): C, 65.26; H, 5.48. Found (%): C, 65.32; H 5.42.
Mass (E.I., 70 eV, m/z): 479 $[L_2\text{-}Ti\text{-}Cl]^{\cdot+}$.

4.4.10 Synthesis of bis-[3-(2,4-dimethoxyphenylethyl)cyclopentadienyl]titanium(IV) dichloride (11c)

The lithium intermediate was obtained by the first step of the synthesis of 11b

$TiCl_4 \cdot 2$ THF (0.141g, 0.42 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.200g (0.84 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a dark red solid (yield 37.3%).

^{1}H NMR (δ ppm, C_6D_6, 300 MHz): 3.20 3.32 [12H, C_5H_4-CH(CH_3)-C_6H_3*(OCH_3)$_2$*], 1.7 [6H, C_5H_4-CH*(CH_3)*-$C_6H_3(OCH_3)_2$], 5.07 [2H, C_5H_4-*CH*(CH_3)-$C_6H_3(OCH_3)_2$] 5.70, 5.92 [8H, *C_5H_4*-CH(CH_3)-$C_6H_3(OCH_3)_2$], 6.28, 6.30, 6.88 [6H, C_5H_4-CH(CH_3)-*C_6H_3*$(OCH_3)_2$].

^{13}C NMR (δ ppm, C_6D_6, 75 MHz): 52.1 53.2 [C_5H_4-CH(CH_3)-C_6H_3*(OCH_3)$_2$*], 23.3 [C_5H_4-CH*(CH_3)*-$C_6H_3(OCH_3)_2$], 38.8 [C_5H_4-*CH*(CH_3)-$C_6H_3(OCH_3)_2$], 98.4-103.5-115.8-121.3-122-136.3-135.5-153.0-159.6 [*C_5H_4*-CH(CH_3)-*C_6H_3*$(OCH_3)_2$].

Elemental analysis Calcd for $C_{30}H_{32}Cl_2O_4Ti$ (%): C, 62.63; H, 5.61. Found (%): C, 62.59; H 5.59.

Mass (E.I., 70 eV, m/z): 504 $[L_2\text{-}Ti]^{\cdot +}$.

4.4.11 Synthesis of bis-[(4-methoxynaphtalyl)cyclopentadienyl] titanium (IV) dichloride (12c)

The lithium intermediate was obtained by the first step of the synthesis of 12b

$TiCl_4 \cdot 2$ THF (0.13g, 0.41 mmol) was added to 20 ml of dry THF. The solution turned immediately from colourless to pale yellow. 0.200 g(0.82 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 ml of dry THF and added dropwise to the solution containing the $TiCl_4$.The solution turned from yellow to dark red during addition. After this addition, the mixture was refluxed overnight and then cooled..The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (30 ml) and filtered twice through celite to remove the LiCl. The black filtrate was washed twice with hexane (20 ml) and then dried under reduced pression to give a black solid

^{1}H NMR (δ ppm CD_2Cl_2, 300 MHz): 6.38, 6.35 (C_5H_4, 4*H* m); 7.68, 7.67, 7.59, 7.34, 7.17, 7.01 ($C_{10}H_6$, 6H m); 3.88 ((OCH_3) 3H s); 3.90 (Ph-CH_2-Cp, 2H s)

^{13}C NMR (δ ppm CD_2Cl_2, 75 MHz): 105.4, 124.3,125.2, 128.0, 128.8, 129.4, 132.8, 146.8, 156.1 (C_5H_4 and $C_{10}H_6$); 55.2 ((OCH_3) 3H s); 34 (Ph-CH_2-Cp, 2H s)

Elemental analysis Calcd for $C_{34}H_{28}Cl_2O_2Ti$ (%): C, 69.53; H, 4.80. Found (%): C, 69.58; H 4.79.

Mass (E.I., 70 eV, m/z): 516 $[L_2\text{-}Ti]^{\cdot +}$.

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