Structure-activity relationships of titanocene complexes with antitumor properties

by

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- My parents and grandmother – Moral support
- All my friends and fellow students – Moral support

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Candidate: Susanna Brink
Promotor: Prof Simon Lotz
Department: Chemistry
Degree: Philosophiae Doctor

Summary

This study involves the synthesis and characterization of new organometallic complexes of Ti(IV). Such complexes were modelled on titanocene dichloride and designed to act as antitumor agents incorporating ligands with specific functions. As target for biological activity, the DNA double helix was identified. The desired complexes displayed (i) a labile halogen ligand for covalent bond formation with DNA, (ii) a planar condensed 3-membered heteroaromatic ring ligand that could act as intercalator in the major groove of DNA and (iii) two bulky, non-labile stopper ligands which will control the degree of intercalation. The complexes prepared in this study were representative of two groups, namely those where the transition metal was bonded directly to a carbon of the planar ring ligand and those where a sulfur atom was located between the metal and the ring ligand. The sulfur atom acts as a spacer and causes the ring unit to lie out of the plane of the metal. Dinuclear metal complexes were also investigated and two classes of complexes were prepared. The complexes where two [$TiCp_2$] fragments were linked and connected to two Cp rings and complexes where a [Ti] and a [Pt] fragment were connected through a Cp and ethylene diamine ligand, respectively.

New complexes [$TiCp_2(R)Cl$], [$TiCp_2(R)_2$], [$TiCp_2(SR)Cl$] and [$TiCp_2(SR)_2$] (RH = thianthrene, dibenzodioxin, dibenzofuran, benzo[b]furan, dibenzothiophene and benzo[b]thiophene) were prepared by adding the lithiated heteroaromatic precursors or the metallated thiolates to titanocene dichloride. The composition of the new complexes was determined by using NMR studies, mass spectrometry and micro analysis. The structures of [$TiCp_2(Dbz)Cl$], [$TiCp_2(SDbt)Cl$] and [$TiCp_2(Dbf)_2$] were confirmed by single crystal X-ray diffraction studies. Reaction pathways for the synthesis of [$\{\mu\text{-}\eta^5,\eta^5\text{-}C_{14}H_{16}\}Ti_2Cp_2X_2Cl_2$] (X = Cl, SDbf) and [$\{\mu\text{-}\eta^5,\eta^5\text{-}C_{14}H_{16}\}Ti_2Cp_2XCl_3$] (X = Dbf, SDbf) were established.

Complexes were selected for *in vitro* tests against HeLa and CoLo cells. The results were correlated with the geometry of the complexes, which made it possible to determine the antitumor structure-activity relationships. It was found that the greatest inhibition of tumor cell growth was achieved when a complex had a thiolate ligand (i.e. SDbf) for intercalation as well as a labile chloro ligand for substitution. These results supported the initial objectives and assumptions of the study. Also studied was the intercalative behaviour of selected complexes and their ligand substitution in aqueous medium. Using flow cytometry it was possible to show that the complexes did not intercalate and the high activity could probably be ascribed covalent bond formation of the complexes. Many other factors that could play a role such as the rate of substitution of ligands in aqueous medium were investigated.

Abbreviations

Nucleobases

5´-dAMP	5'-Deoxynucleoside adenosine monophosphate
5'-dCMP	5'-Deoxynucleoside thiamine monophosphate
AMP	Adenine monophosphate
dGMP	Deoxynucleoside guanine monophosphate
dTMP	Deoxynucleoside thiamine monophosphate
UMP	Uracil monophosphate
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
U	Uracil

Heteroaromatics

BfH/Bf	Benzo[b]furan/ Deprotonated benzo[b]furan
BtH/Bt	Benzo[b]thiophene/ Deprotonated benzo[b]thiophene
BuSDbz	1-Butylsulfanyl dibenzodioxin
DbfH/Dbf	Dibenzofuran/ Deprotonated dibenzofuran
DbfH-Me/Dbf-Me	4-Methyl dibenzofuran/ Deprotonated 4-methyl dibenzofuran
DbtH/Dbt	Dibenzothiophene/ Deprotonated dibenzothiophene
DbzH/Dbz	Dibenzo[1,4]dioxin/ Deprotonated dibenzo[1,4]dioxin
$DpeH_2$/Dpe	Diphenyl ether/ Deprotonated diphenyl ether
HSBf/SBf	Benzo[b]furan-2-thiol/ Benzo[b]furan-2-thiolate
HSBt/SBt	Benzo[b]thiophene-2-thiol/ Benzo[b]thiophene-2-thiolate
HSDbf/SDbf	Dibenzofuran-4-thiol/ Dibenzofuran-4-thiolate
HSDbf-Me/SDbf-Me	6-Methyl dibenzofuran-4-thiol/ 6-Methyl dibenzofuran-4-thiolate
HSDbt/SDbt	Dibenzothiophene-4-thiol/ Dibenzothiophene-4-thiolate
HSDbz/SDbz	Dibenzodioxin-1-thiol/ Dibenzodioxin-1-thiolate
HSThr/SThr	Thianthrene-1-thiol/ Thianthrene-1-thiolate
ThrH/Thr	Thianthrene/ Deprotonated thianthrene

Nuclear magnetic resonance spectroscopy

COSY	Correlation spectroscopy
HETCOR	Heteronuclear correlation spectroscopy
NMR	Nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon nuclear magnetic resonance
d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
m	Multiplet
s	Singlet
t	Triplet
J	Coupling constant
δ	Chemical shift

Other techniques and instruments

Anal. Calc.	Micro analysis calculated values
EELS	Electron energy loss spectroscopy
ICP	Inductive coupled plasma
[M^{+}]	Molecular ion
Mp	Melting point
UV	Ultraviolet

Substituents

Bu	Butyl
Cp	Cyclopentadienyl
R	Heteroaromatic ligand
X	Labile ligand

Solvents and bases

THF	Tetrahydrofuran
TMEDA	Tetrametylethylenediamine

Tumor cell lines

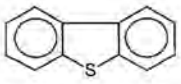
CoLo	Colorectal carcinoma
EhAT	Ehrlich ascites tumour
HeLa	Human cervix epitherioid carcinoma

Other

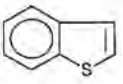
FS	Forward scatter
SS	Side scatter
L	Ligand
M	Transition metal
ppm	Parts per million
Å	Angstrom (10^{-8}m)
μ	Indicates a bridged ligand
d^n	Superscript indicates the number of valence electrons in the d-orbital
η^n	Superscript indicates the number of coordinated carbons

List of Compounds

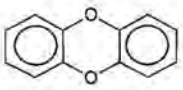
Organic Starting Compounds



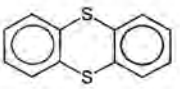
Dibenzothiophene [DbtH] **L2-01**



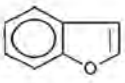
Benzo[b]thiophene [BtH] **L2-02**



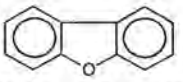
Dibenzo[1,4]dioxin [DbzH] **L2-03**



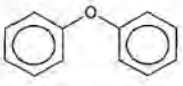
Thianthrene [ThrH] **L2-04**



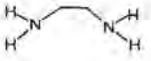
Benzo[b]furan [BfH] **L2-05**



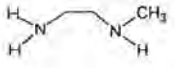
Dibenzofuran [DbfH] **L2-06**



Diphenylether [$DpeH_2$] **L2-08**



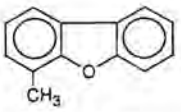
Ethylene diamine **L4-01**



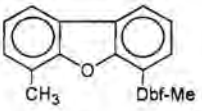
N-methyl ethylene diamine **L4-02**

N,N'-dimethyl ethylene diamine **L4-03**

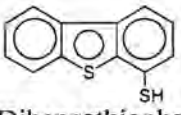
Organic Compounds Synthesized



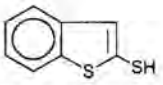
4-Methyl dibenzofuran [DbfH-Me] **L2-07**



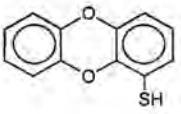
4,4′-Dimethyl-6,6′-bi(dibenzofuran)
[Me-Dbf-Dbf-Me] **L2-07b**



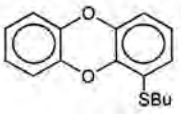
Dibenzothiophene-4-thiol [HSDbt] **L3-01**



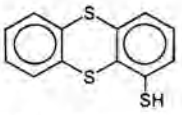
Benzo[b]thiophene-2-thiol [HSBt] **L3-02**



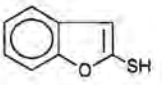
Dibenzodioxin-1-thiol [HSDbz] **L3-03**



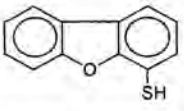
1-Butylsulfanyl dibenzodioxin [BuSDbz] **L3-03b**



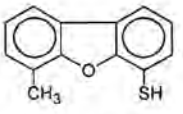
Thianthrene-1-thiol [HSThr] **L3-04**



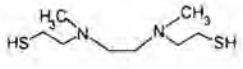
Benzo[b]furan-2-thiol [HSBf] **L3-05**

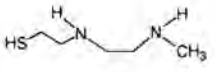


Dibenzofuran-4-thiol [HSDbf] **L3-06**



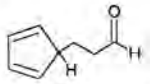
6-Methyl dibenzofuran-4-thiol [HSDbf-Me] **L3-07**



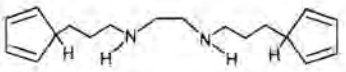


2-({2-[(2-mercaptoethyl)methylamino]ethyl}methylamino)ethanethiol [$C_8H_{20}N_2S$] **L4-04**

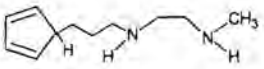
2-(2-methylamino ethylamino)ethanethiol [$C_5H_{14}N_2S$] **L4-05**



3-cyclopentadienyl propionaldehyde **L4-06**

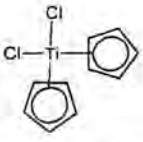


N,N´-bis-(3-cyclopentadienyl propyl) ethylene diamine **L4-07**

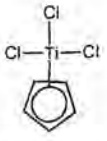


N-(3-cyclopentadienyl propyl)-N´-methyl ethylene diamine **L4-08**

Organometallic Starting Complexes

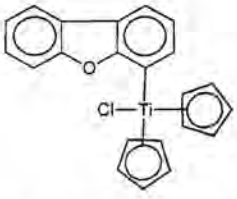


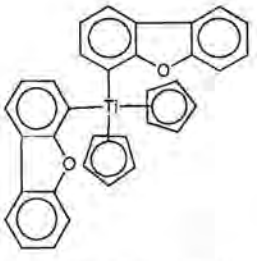
Titanocene dichloride [$TiCp_2Cl_2$] **S-01**



Trichloro cyclopentadienyl titanium (IV) [$TiCpCl_3$]

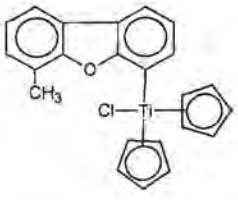
Organometallic Complexes Synthesized

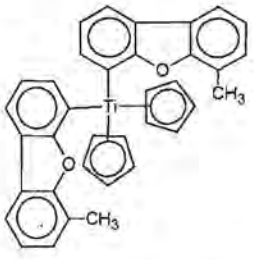




Chlorobis(cyclopentadienyl)(dibenzofuran-4-yl)titanium(IV) [$TiCp_2(Dbf)Cl$] **2-01**

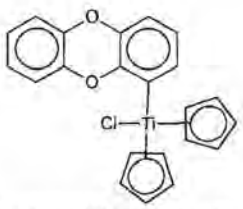
Bis(cyclopentadienyl)bis(dibenzofuran-4-yl)titanium(IV) [$TiCp_2(Dbf)_2$] **2-02**

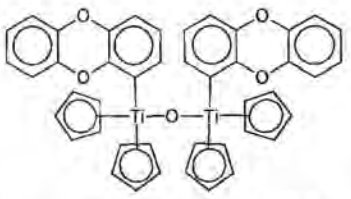




Chlorobis(cyclopentadienyl)(6-methyl dibenzofuran-4-yl)titanium(IV) [$TiCp_2(Dbf\text{-}Me)Cl$] **2-03**

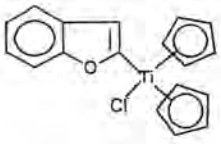
Bis(cyclopentadienyl)bis(6-methyl dibenzofuran-4-yl)titanium(IV) [$TiCp_2(Dbf\text{-}Me)_2$] **2-04**

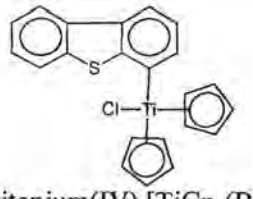




Chlorobis(cyclopentadienyl)(dibenzodioxin-1-yl)titanium(IV) [$TiCp_2(Dbz)Cl$] **2-05**

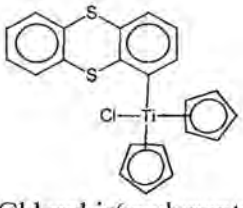
(μ-Oxo)bis[{bis(cyclopentadienyl)}(dibenzodioxin-1-yl)titanium(IV)] [$(\mu\text{-O})\{TiCp_2(Dbz)\}_2$] **2-06**

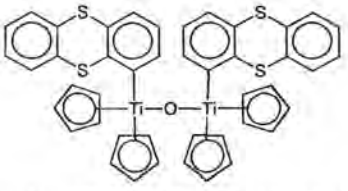




(Benzofuran-2-yl)chlorobis(cyclopentadienyl)titanium(IV) [$TiCp_2(Bf)Cl$] **2-07**

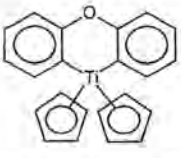
Chloro{bis(cyclopentadienyl)}(dibenzothiophen-4-yl)titanium(IV) [$TiCp_2(Dbt)Cl$] **2-08**



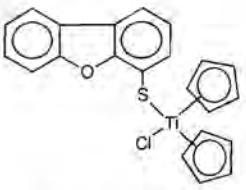


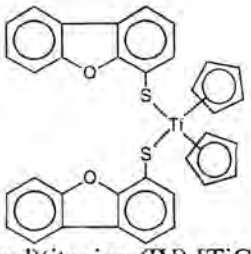
Chlorobis(cyclopentadienyl)(thianthren-1-yl)titanium(IV) [$TiCp_2(Thr)Cl$] **2-09**

(μ-Oxo)bis[bis(cyclopentadienyl)(thianthren-1-yl)titanium(IV)] [$(\mu\text{-O})\{TiCp_2(Thr)\}_2$] **2-10**



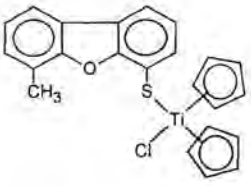
Bis(cyclopentadienyl)(diphen-2,2´-yl ether)titanium(IV) [$TiCp_2(Dpe)$] **2-11**

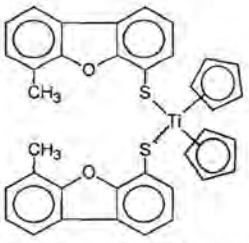




Chlorobis(cyclopentadienyl)(dibenzofuran-4-ylsulfanyl)titanium(IV) [$TiCp_2(SDbf)Cl$] **3-01**

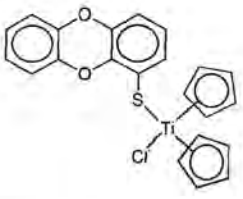
Bis(cyclopentadienyl)bis(dibenzofuran-4-ylsulfanyl)titanium(IV) [$TiCp_2(SDbf)_2$] **3-02**

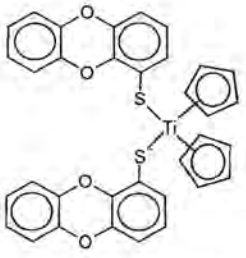




Chlorobis(cyclopentadienyl)(6-methyl dibenzofuran-4-ylsulfanyl)titanium(IV) [$TiCp_2(SDbf\text{-}Me)Cl$] **3-03**

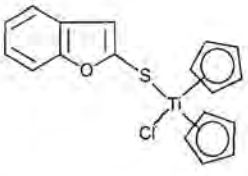
Bis(cyclopentadienyl)bis(6-methyl dibenzofuran-4-ylsulfanyl)titanium(IV) [$TiCp_2(SDbf\text{-}Me)_2$] **3-04**

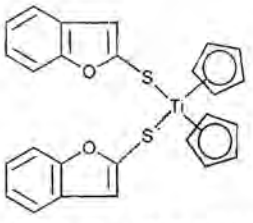




Chlorobis(cyclopentadienyl)(dibenzodioxin-1-ylsulfanyl)titanium(IV) [$TiCp_2(SDbz)Cl$]**3-05**

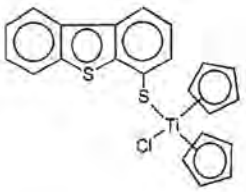
Bis(cyclopentadienyl)bis(dibenzodioxin-1-ylsulfanyl)titanium(IV) [$TiCp_2(SDbz)_2$] **3-06**

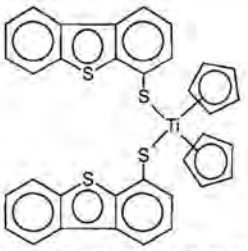




(Benzofuran-2-ylsulfanyl)chlorobis(cyclopentadienyl)titanium(IV) [$TiCp_2(SBf)Cl$] **3-07**

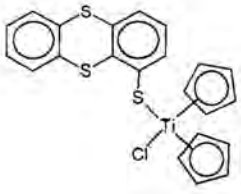
Bis(cyclopentadienyl)bis(benzofuran-2-ylsulfanyl)titanium(IV) [$TiCp_2(SBf)_2$] **3-08**

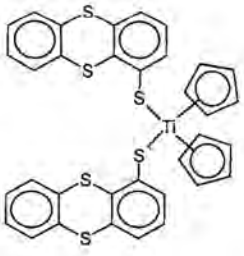




Chlorobis(cyclopentadienyl)(dibenzothien-4-ylsulfanyl)titanium(IV) [$TiCp_2(SDbt)Cl$] **3-09**

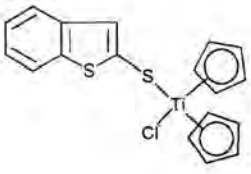
Bis(cyclopentadienyl)bis(dibenzothien-4-ylsulfanyl)titanium(IV) [$TiCp_2(SDbt)_2$] **3-10**

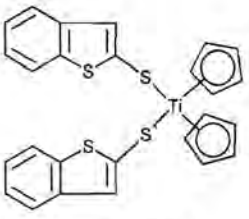




Chlorobis(cyclopentadienyl)(thianthren-1-ylsulfanyl)titanium(IV) [$TiCp_2(SThr)Cl$] **3-11**

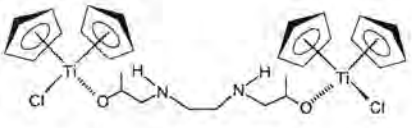
Bis(cyclopentadienyl)bis(thianthren-1-ylsulfanyl)titanium(IV) [$TiCp_2(SThr)_2$] **3-12**



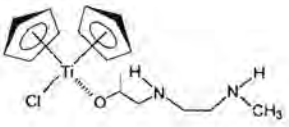


(Benzothien-2-ylsulfanyl)chlorobis(cyclopentadienyl)titanium(IV) [$TiCp_2(SBf)Cl$] **3-13**

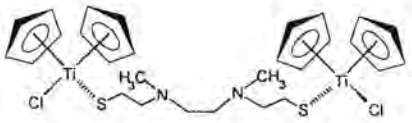
Bis(cyclopentadienyl)bis(benzothien-2-ylsulfanyl)titanium(IV) [$TiCp_2(SBf)_2$] **3-14**



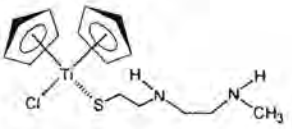
[$\{\mu\text{-}C_8H_{18}N_2O_2\}Ti_2Cp_4Cl_2$] **4-01**



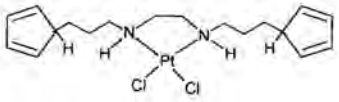
[$TiCp_2(OC_6H_{15}N_2)Cl$] **4-02**



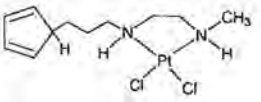
[{μ-$C_8H_{18}N_2S_2$}$Ti_2Cp_4Cl_2$] **4-03**



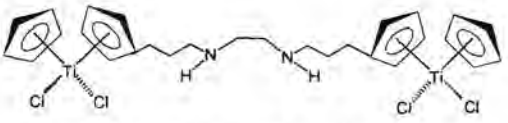
[$TiCp_2(SC_6H_{15}N_2)Cl$] **4-04**



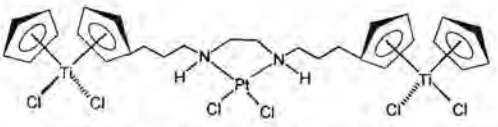
[$Pt(\mu\text{-}N,N'C_{18}H_{28}N_2)Cl_2$] **4-05**



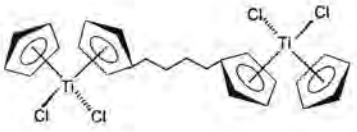
[$Pt(\mu\text{-}N,N'C_{11}H_{20}N_2)Cl_2$] **4-06**



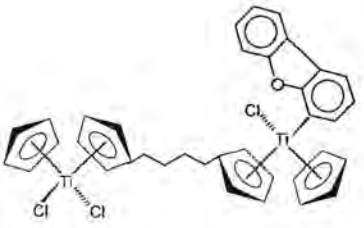
[{μ-η^5,η^5-$C_{18}H_{26}N_2$}$Ti_2Cp_2Cl_4$] **4-07**



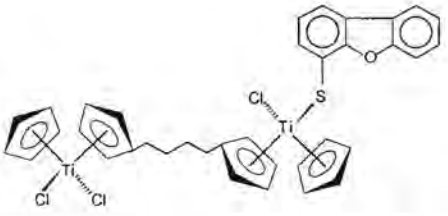
[Ti_2{μ-η^5,η^5-$(Pt(N,N'\text{-}C_{18}H_{26}N_2)Cl_2)$}$Cp_2Cl_4$] **4-08**



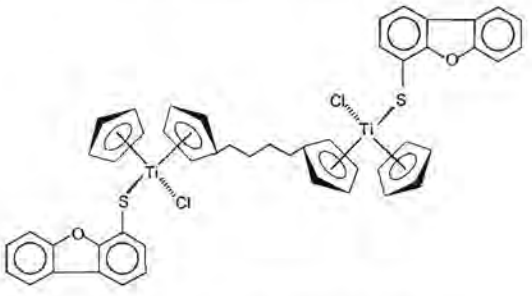
[{μ-η^5,η^5-$C_{14}H_{16}$}$Ti_2Cp_2Cl_4$] **4-09**



[{μ-η^5,η^5-$C_{14}H_{16}$}Ti_2(Dbf)Cp_2Cl_3] **4-10**



[{μ-η^5,η^5-$C_{14}H_{16}$}Ti_2(Dbf-S)Cp_2Cl_3] **4-11**



[{μ-η^5,η^5-$C_{14}H_{16}$}{Ti(Dbf-S)CpCl}$_2$] **4-12**