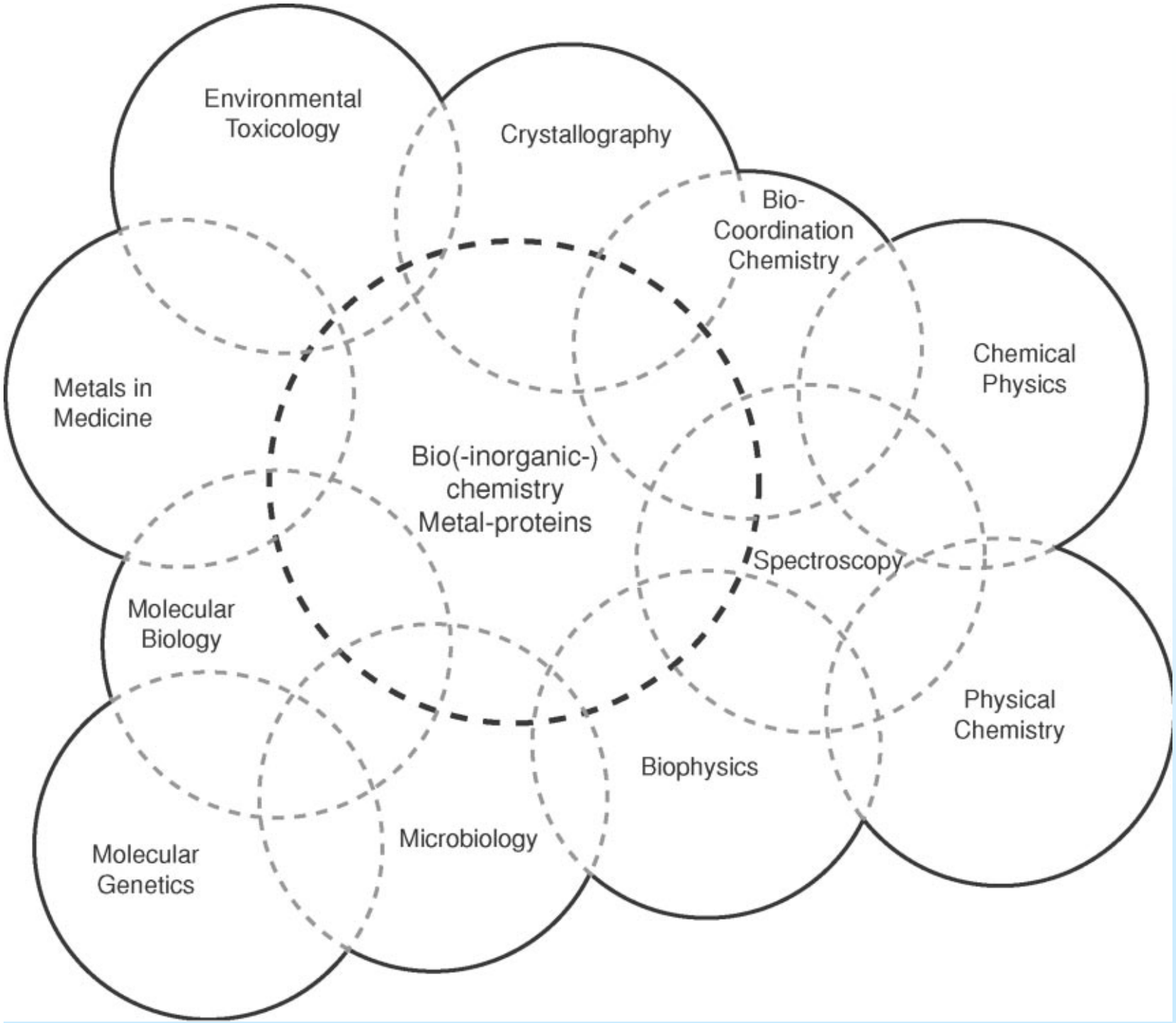
ΜΕΤΑΛΛΟ ΘΕΡΑΠΕΥΤΙΚΑ - Ή ΑΝΟΡΓΑΝΑ ΦΑΡΜΑΚΑ . Η ΧΡΗΣΗ ΤΩΝ ΜΕΤΑΛΛΩΝ ΣΤΗΝ ΙΑΤΡΙΚΗ

Δρ. Σ. Κ. Χατζηκακού

Καθηγητής


Environmental Toxicology
Crystallography
Bio-Coordination Chemistry
Metals in Medicine
Chemical Physics
Bio(-inorganic-) chemistry Metal-proteins
Spectroscopy
Molecular Biology
Physical Chemistry
Biophysics
Microbiology
Molecular Genetics

1. Metal ions in disease. The use of chelating agents

2. Metalloproteins as drug targets

3. Organelles as targets. The mitochondrion

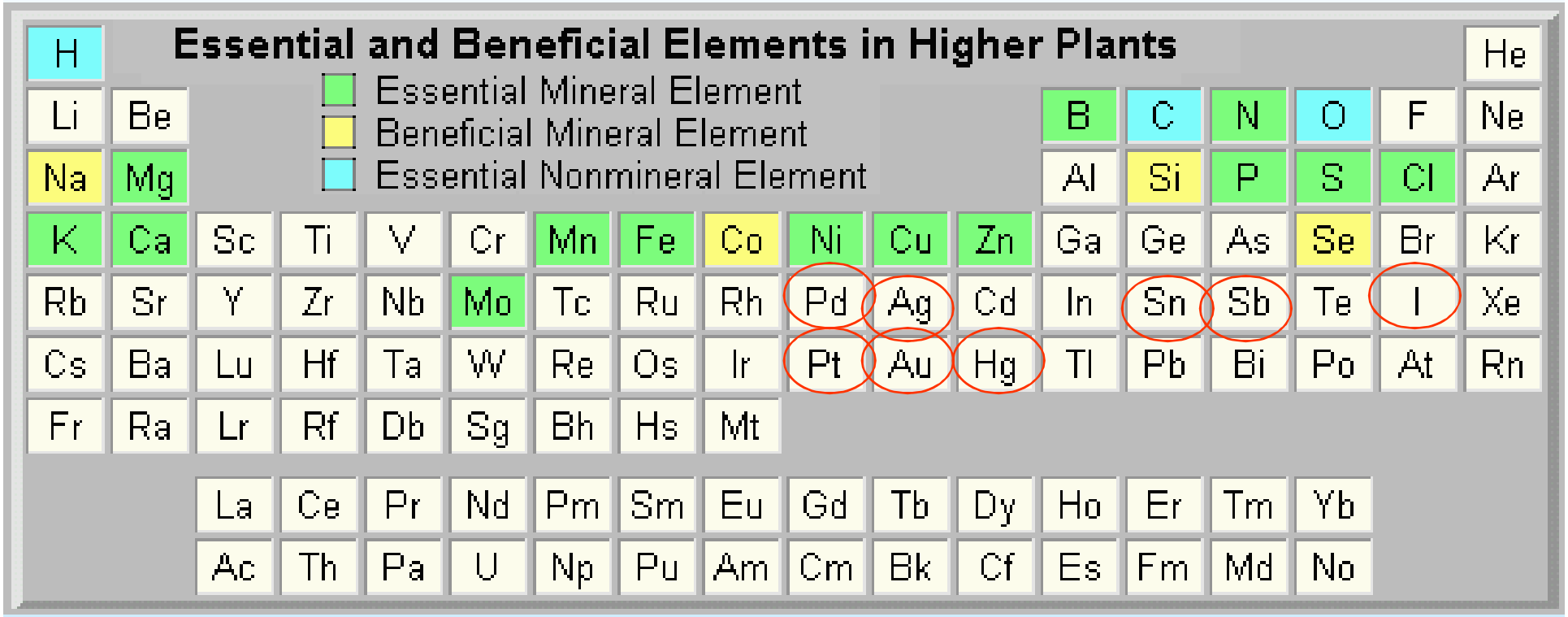
4. Metal/drug interaction

5. Metal-based chemotherapeutic drugs

6. Radioisotopes in medicine

ΕΙΣΑΓΩΓΗ.

Η υγεία των θηλαστικών βασίζεται στα βιο-δραστικά ιχνοστοιχεία τα οποία είναι απαραίτητα για την λειτουργία τουλάχιστον του ενός τρίτου των πρωτεϊνών και των ενζύμων τους. Πολλά διατροφικά συμπληρώματα περιέχουν βιο-ενεργά ιχνοστοιχεία



Το πρώτο σύγχρονο μέταλλο-θεραπευτικό φάρμακο είναι το SALVARSAN που ανακαλύφθηκε από τον Paul Ehrlich. Το μέταλλο-θεραπευτικό φάρμακο αυτό βασίζεται στο αρσενικό As και χρησιμοποιήθηκε για τη θεραπεία της σύφιλης.

Το αρσενικό αν και είναι ένα ισχυρό δηλητήριο όμως το οξείδιο του αρσενικού As_2O_3 χρησιμοποιήθηκε στη θεραπεία της λευχαιμίας.

Υπάρχει μια ισορροπία ανάμεσα στα πλεονεκτήματα από την δράση των μετάλλων και τη τοξικότητα τους.

Η τοξική δράση των μετάλλων μπορεί να περιοριστεί από τη χηλική θεραπεία όπως για παράδειγμα στην περίπτωση της ασθένειας Wilson (δηλητηρίαση από το χαλκό) όπου γίνεται θεραπεία με ψευδάργυρο.

Ασθένειες που θεραπεύονται με μέταλλο-θεραπευτικά ή ανόργανα φάρμακα:

(α) αναιμία (Fe),
(β) άσθμα (Au, Mg)
(γ) διαταραχή ηλεκτρολυτών (Li),
(δ) Διαβήτης (V)
(ε) ρευματοειδής αρθρίτιδα (Au)
(ζ) καρδιακή προσβολή (Mg)
(η) τροπικές ασθένειες (Sb)
(θ) έλκος (Bi)

Το πιο διαδεδομένο μέταλλο-θεραπευτικό φάρμακο για την θεραπεία του καρκίνου σε κλινική χρήση εδώ και τρις δεκαετίες είναι το cis-platin. Η τεράστια επιτυχία του φαρμάκου αυτού κατηύθυνε την έρευνα στην μελέτη και άλλων ανόργανων ενώσεων που περιέχουν Pt ή και άλλα μέταλλα όπως Pd, Sn κα.

Αντικατάσταση ενός ατόμου ενός οργανικού φαρμάκου με ένα μέταλλο έχει σαν αποτέλεσμα την αύξηση της δράσης του φαρμάκου κατά πολλές τάξης μεγέθους.

Τα μέταλλα μπορεί να δρουν είτε ως μεταφορείς του οργανικού φαρμάκου στο στόχο παρακάμπτοντας τον κανονικό μεταβολισμό του φαρμάκου είτε δρουν αυτά κάθε αυτά έχουν άμεση θεραπευτική δράση όπως πχ ο Pt δρά στο DNA.

Αυτή τη στιγμή τα μέταλλα μελετώνται για τη χρήση τους
[1] Ανάπτυξη δεύτερης Γενιάς Μέταλλο-θεραπευτικών Φαρμάκων για την αντιμετώπιση του καρκίνου
[2] Διαγνωστικά φάρμακα Magnetic Resonce Imaging MRI agents σύμπλοκα με παραμαγνητικά μέταλλα π.χ. γαδολίνιο Ga(III) έχει 7 μονήρη ηλεκτρόνια
[3] ραδιοθεραπευτικά.

Στόχος της έρευνας αυτή τη στιγμή είναι να αναπτυχθούν νέα σύμπλοκα άλατα με **καλύτερη δράση**, **μικρότερη τοξικότητα** και **λιγότερες παρενέργειες**.

Αυτή τη στιγμή (2006) ο οργανισμός φαρμάκων FDA στις ΗΠΑ, έχει χορηγήσει άδεια για κλινική χρήση ενός συμπλόκου του Sn με πορφυρίνη για τη θεραπεία της γεροντικής ανίας (εξασθένηση της οξυδέρκειας).


Medicinal Inorganic
Chemistry
and the
Treatment of Disease

Medicinal Inorganic Chemistry

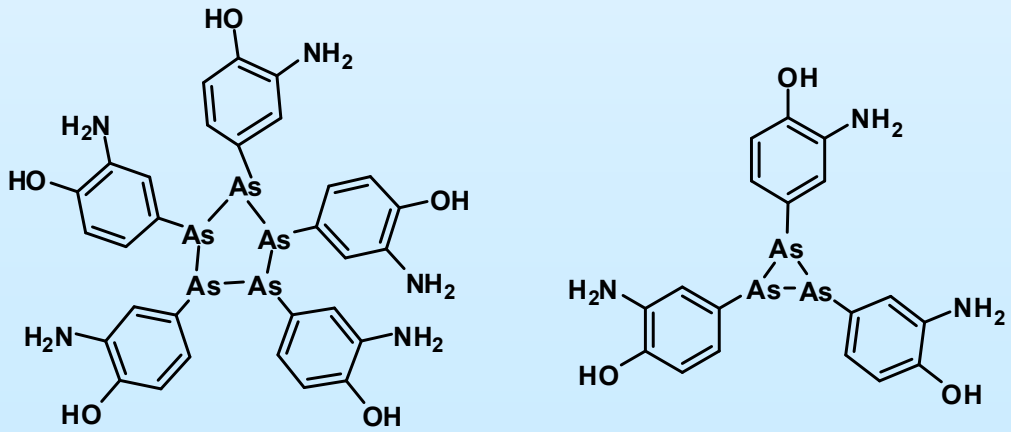
3000 BC : Egyptians used **Cu** to sterilize water

2500 BC : Chinese empire uses **Au** in a variety of medicine

400 BC : Hippocrates used **Hg**

1600s : Paracelsus pioneered the use of minerals in medicine using **Sb**, **As**, **Mg** salt

Early 1900s : Metals started making an impact on modern medicine
K[Au(CN)$_2$] used for tuberculosis
Salvarsan for the treatment of syphilis



Jaouen, G. *Bioorganometallics*, **2006,** 1st Ed. pp. 1-32
Orvig, C. Abrams, M.J. *Chem. Rev.* **1999**, 99, 2201

Outline

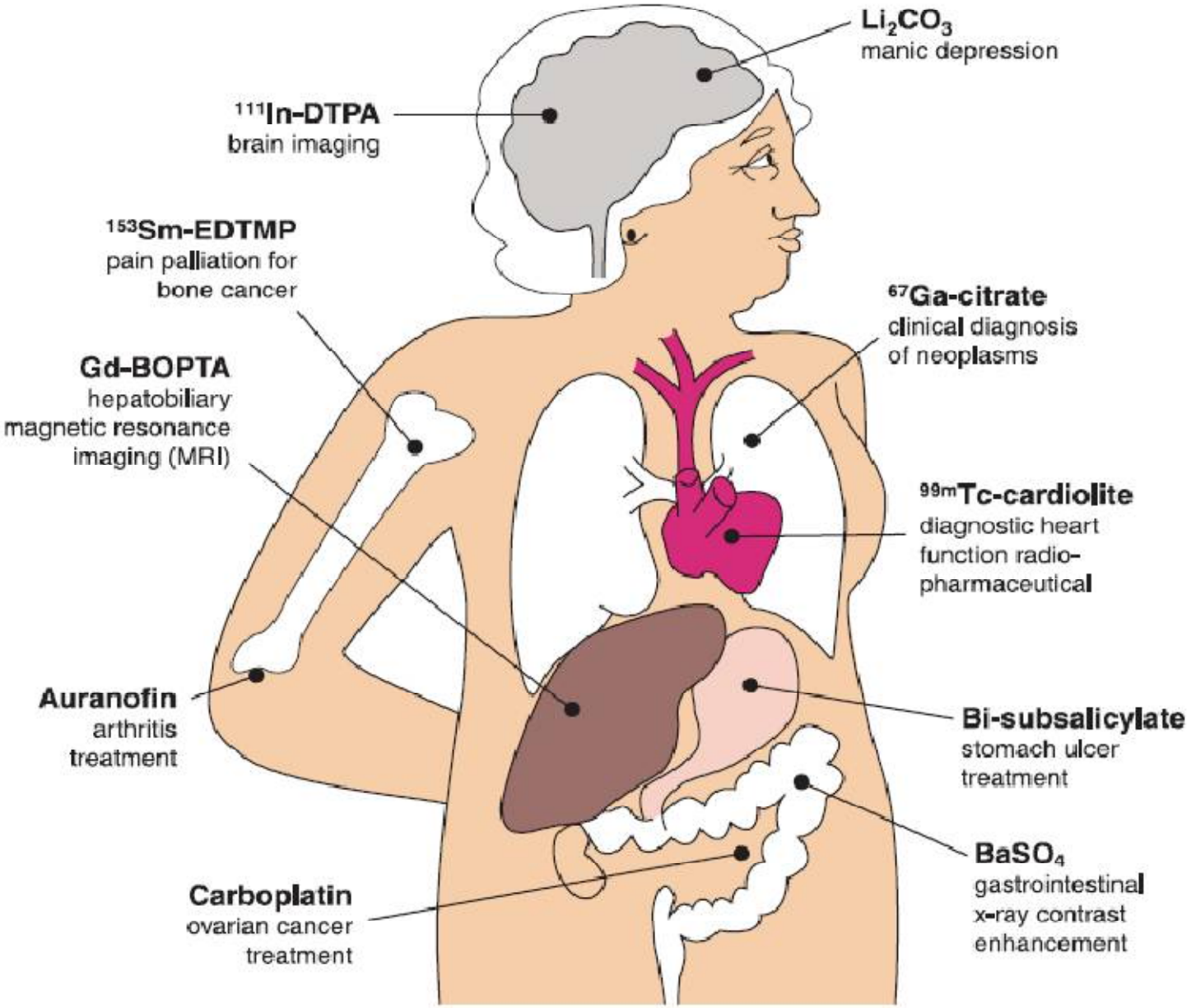
1. Traditional applications of inorganic compounds:

   - Chelation
   - Imaging properties

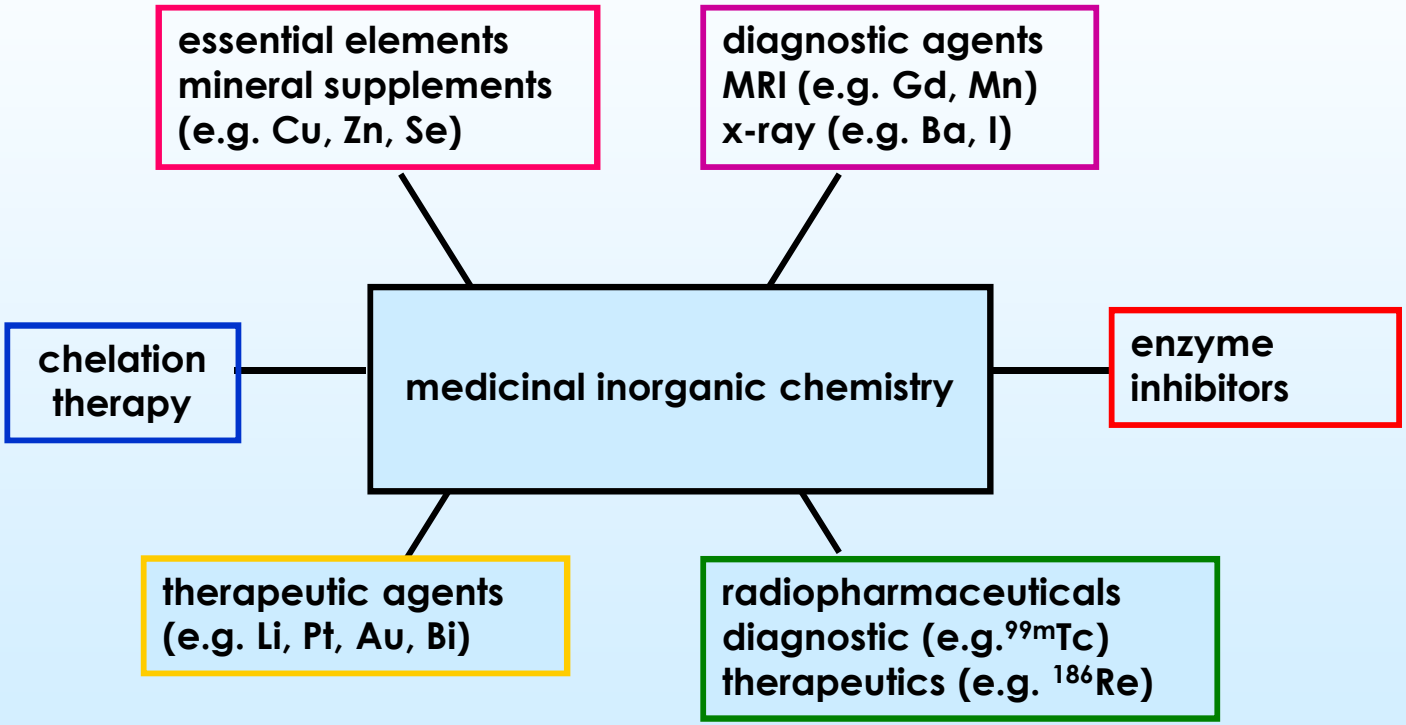
2. Inorganic compounds that utilize reactivity of metals

3. Inorganic compound that utilizes both the structure of metal and their reactivity in biological system

4. Inorganic compounds that utilize the unique structural opportunities of metals



Thompson, K.H, Orvig, C.; *Science*, **2003**, *300*, 936



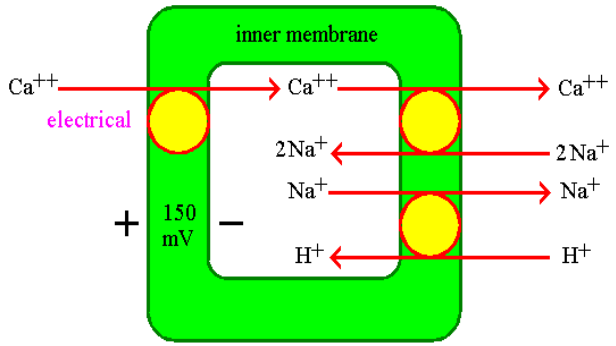
Guo, Z. Sadler, P.J. *Angew. Chem. Int. Ed*. **1999**, *38,* 1512
Orvig, C. Abrams, M.J. *Chemical Reviews*, **1999**, 99, 2201

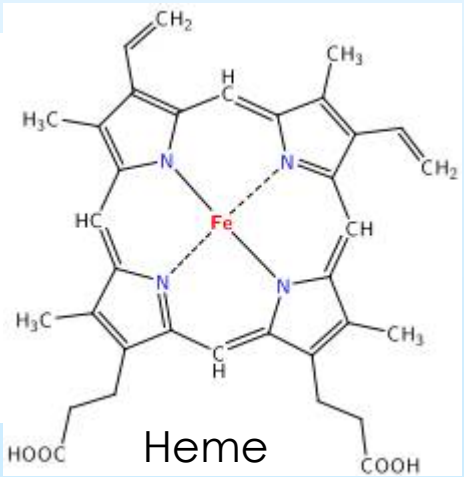
Medicinal Inorganic chemistry: Essential Elements

"Organic" elements: **C, H, N, O**

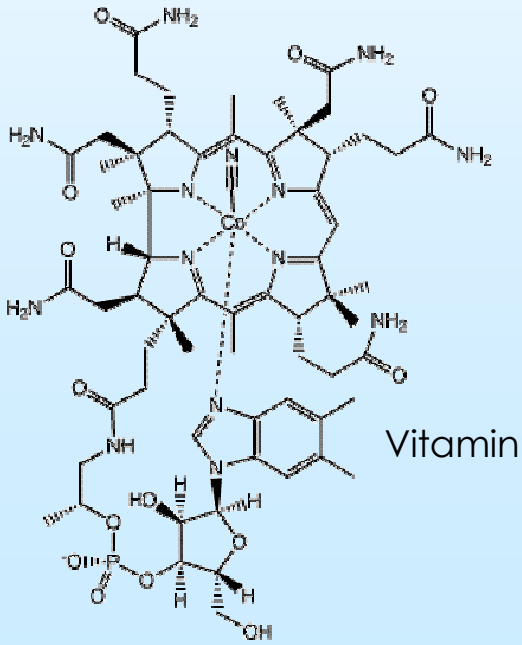
Macronutrients: Na, K, Mg, Ca, S, P, Cl, Si, Fe

Micronutrients: **V, Cr, Mn, Co, Ni, Cu, Zn, Mo, W, Se, F, I**





Heme

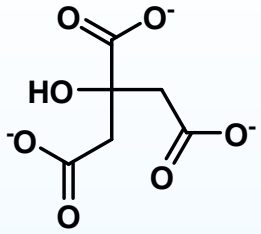

Vitamin B12

http://fr.wikipedia.org
Cotton,F.A.; Wilkinson, G.; Gaus, P.L.; Basic Inorganic Chemistry, 3rd Ed. (1995), pp. 729-753
http://www.daviddarling.info/encyclopedia/V/vitamin_B12.html

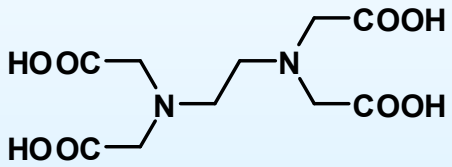
Medicinal Inorganic chemistry: Chelation Therapy

Used for metal intoxication

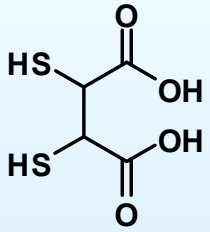
1941: Citrate is used for acute lead intoxication



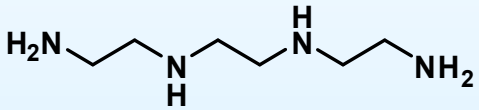
Since then, other chelating agents have come into clinical use:



EDTA



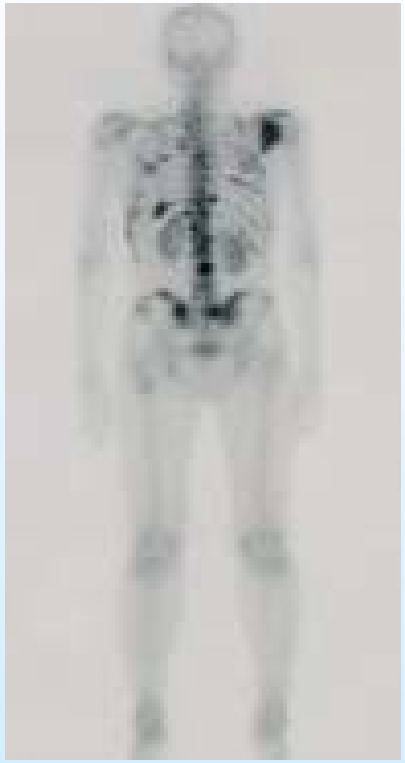
DMSA

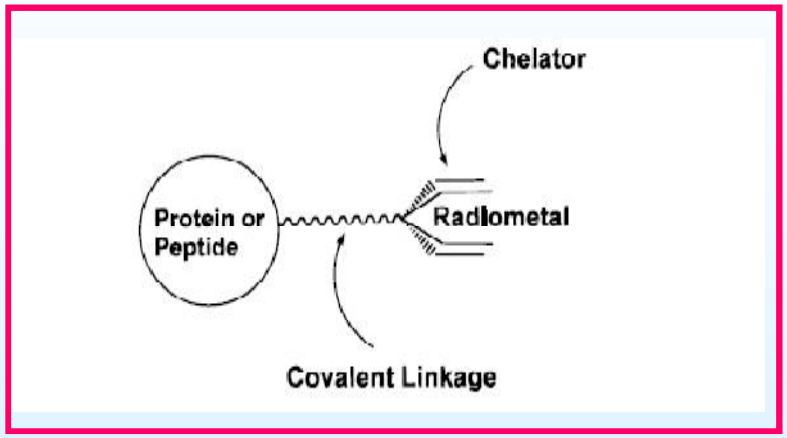


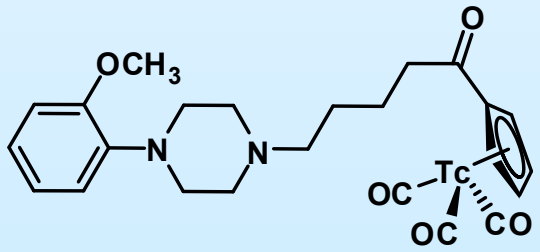
TETA

Andersen, O. *Chem. Rev.* **1999**, *99*, 2683

Medicinal Inorganic chemistry: Radiopharmaceuticals







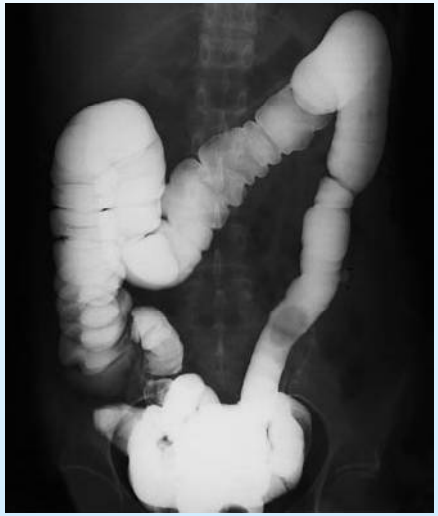
Anderson, C.J.; Welch, M.J. *Chem. Rev.* **1999,** *99,* 2219
Wang et al. *Bioconjugate Chem.* **1996,** *7,* 56
http://www.doemedicalsciences.org/
Jaouen, G. *Bioorganometallics*, **2006,** 1st Ed. pp. 1-32

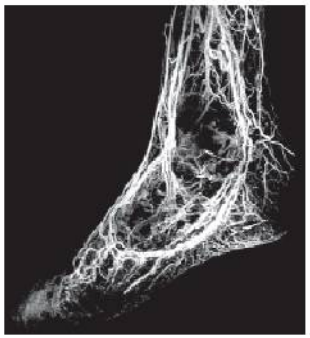
Medicinal Inorganic chemistry: Diagnostic Agents

Contrast agents:

- X-Ray:

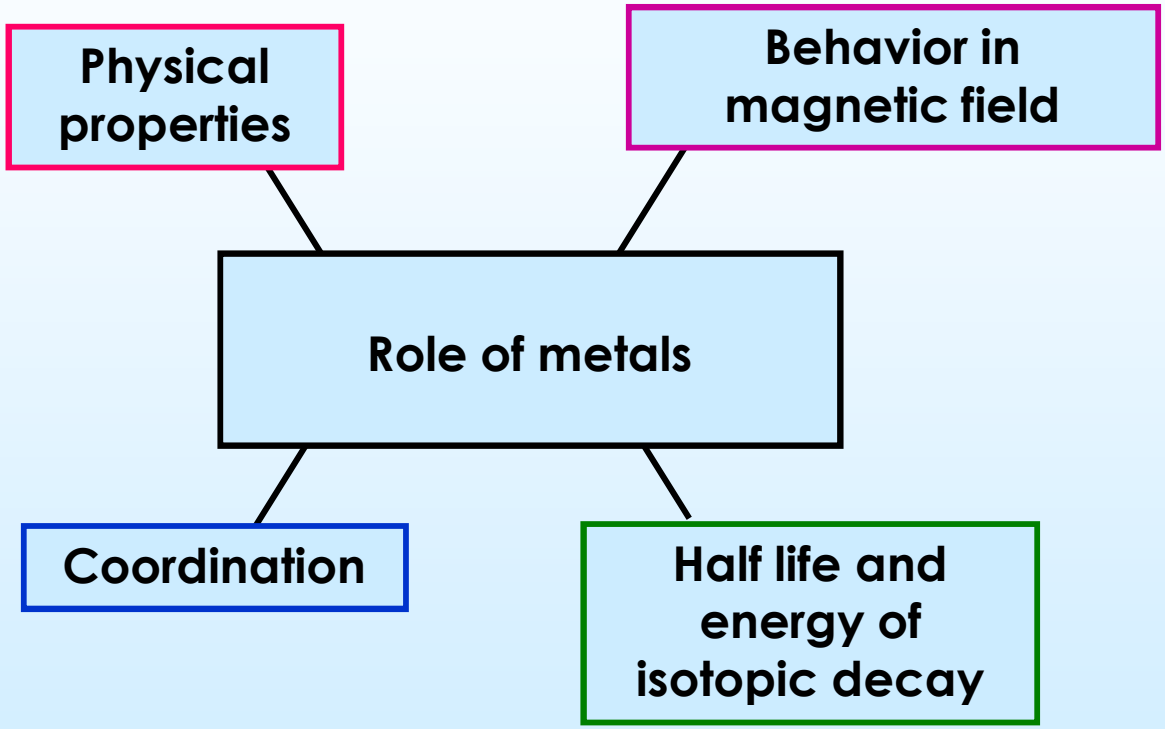
I, Ba, $BaSO_4$



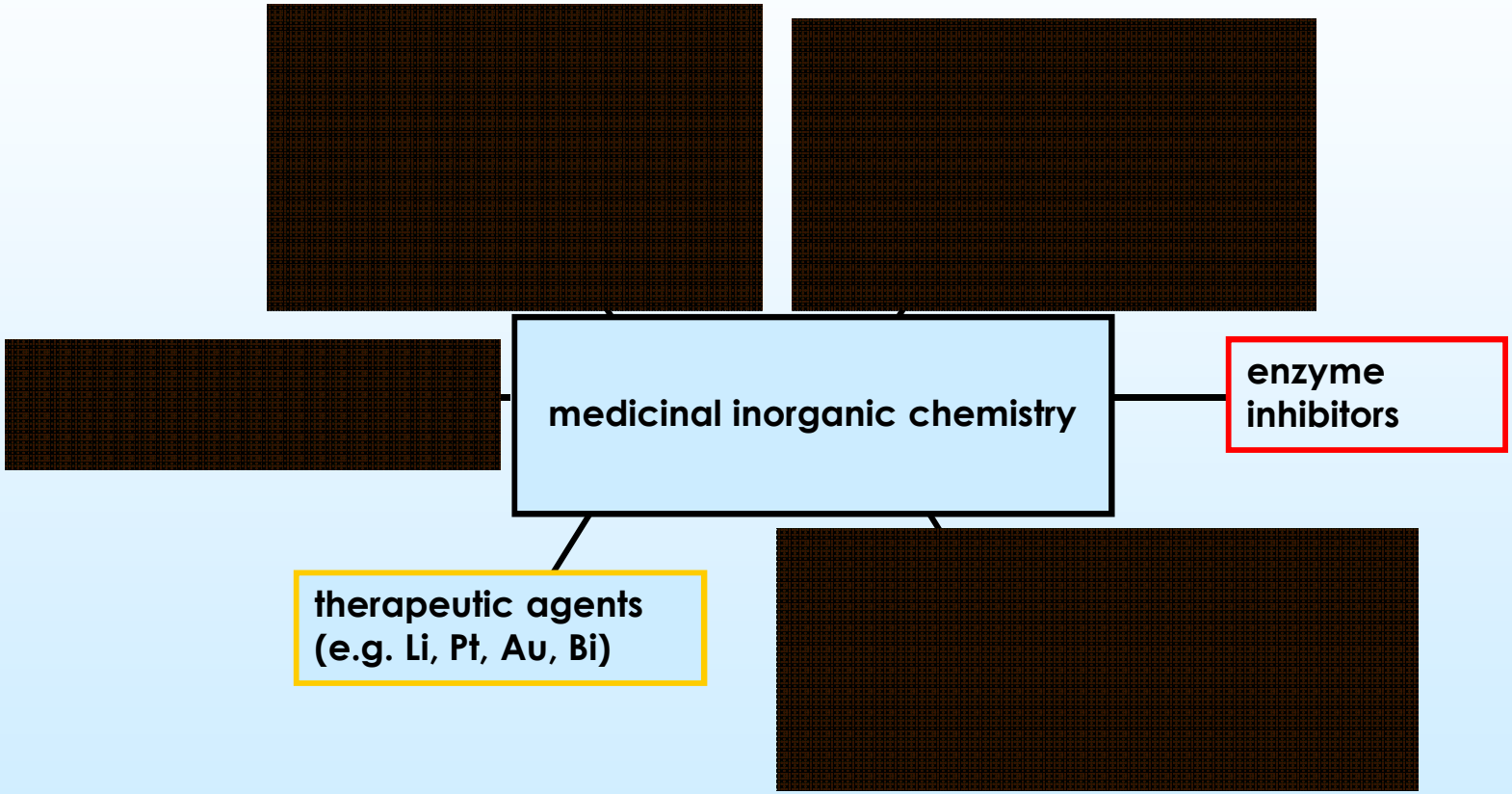


MRI:

Guo, Z. Sadler, P.J. *Angew. Chem. Int. Ed*. **1999**, *38*, 1512
www.asrt.org/content/ThePublic/AboutRadiologicProcedures/ContrastAgents.aspx
Thompson, K.H, Orvig, C.; *Science*, **2003**, *300*, 936



Guo, Z. Sadler, P.J. *Angew. Chem. Int. Ed*. **1999**, *38,* 1512
Orvig, C. Abrams, M.J. *Chem. Rev.* **1999**, 99, 2201



Guo, Z. Sadler, P.J. *Angew. Chem. Int. Ed*. **1999**, *38,* 1512
Orvig, C. Abrams, M.J. *Chemical Reviews*, **1999**, 99, 2201

- **Bioactivity is at the metal center**

  Cisplatin

- **Bioactivity is related to reaction caused by the metal center**

  Tamoxifen

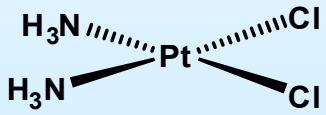
- **Metal is the structural scaffold**

  Pyridocarbazole ruthenium complexes

Therapeutic Agents

- Pharmaceutical industry usually dominated by organic drugs
- Certain Inorganic drugs have proven their utility: **Li, Bi**

Most important inorganic pharmaceuticals on the market:



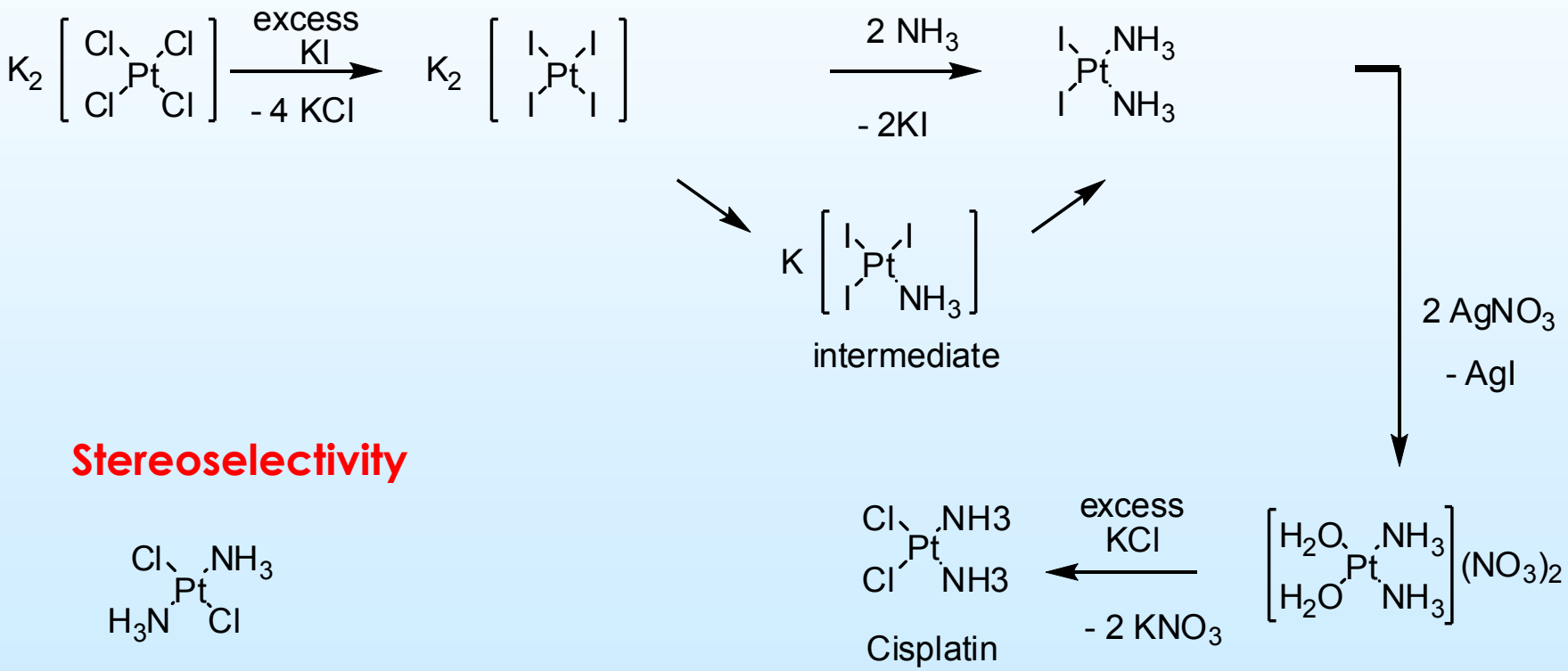
Cisplatin

- Discovered by chance by Rosenberg
- Used in the treatment of various cancers (testicular and ovarian)
- Approved for Clinical use in 1978
- World wide sales are around 2 billion U.S $

Guo, Z. Sadler, P. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 1512
Fricker, S.P. *Dalton Trans.*, 2007, 4903–4917
Alderden et al. *Journal of Chemical Education* **2006**, 83

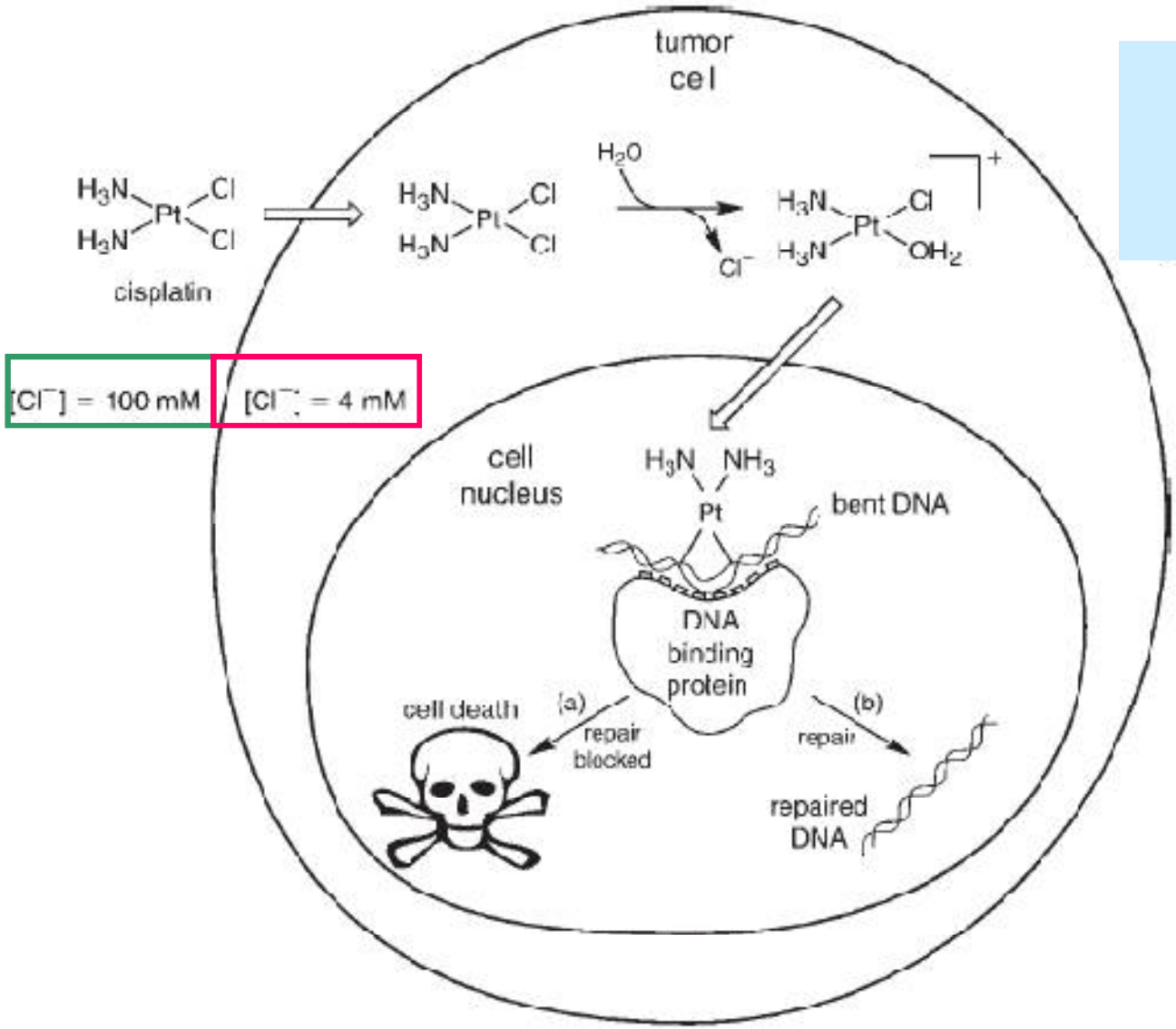
Cisplatin

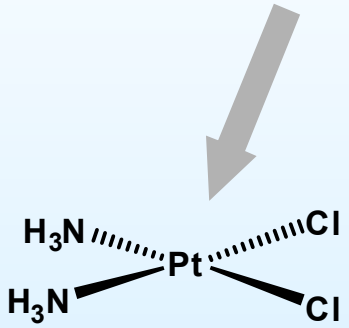
- Classic synthesis in inorganic chemistry; pioneered by Dhara in 1970



Stereoselectivity

Guo, Z. Sadler, P. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 1512
Fricker, S.P *Dalton Trans.*, 2007, 4903–4917
Alderden et al. *J. of Chem. Educ.* **2006**, 83





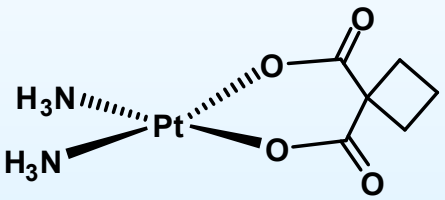
Platinum is the reactive adduct for cisplatin (coordination chemistry)

Guo, Z. Sadler, P. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 1512
Fricker, S.P. *Dalton Trans.*, 2007, 4903–4917
Alderden et al. *J. Chem. Educ.* **2006**, 83

The Search Continues

Cisplatin : Severe side effects (toxicity to kidneys and nervous system)

Resistance

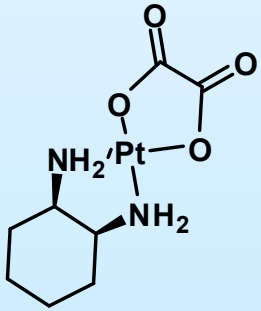


Carboplatin

Widespread clinical use

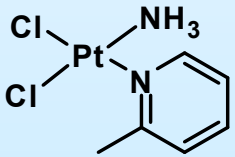
Less toxic and fewer side effects

Bidentate ligand is more stable; slower reaction in the body



Oxaliplatin

Colon cancer

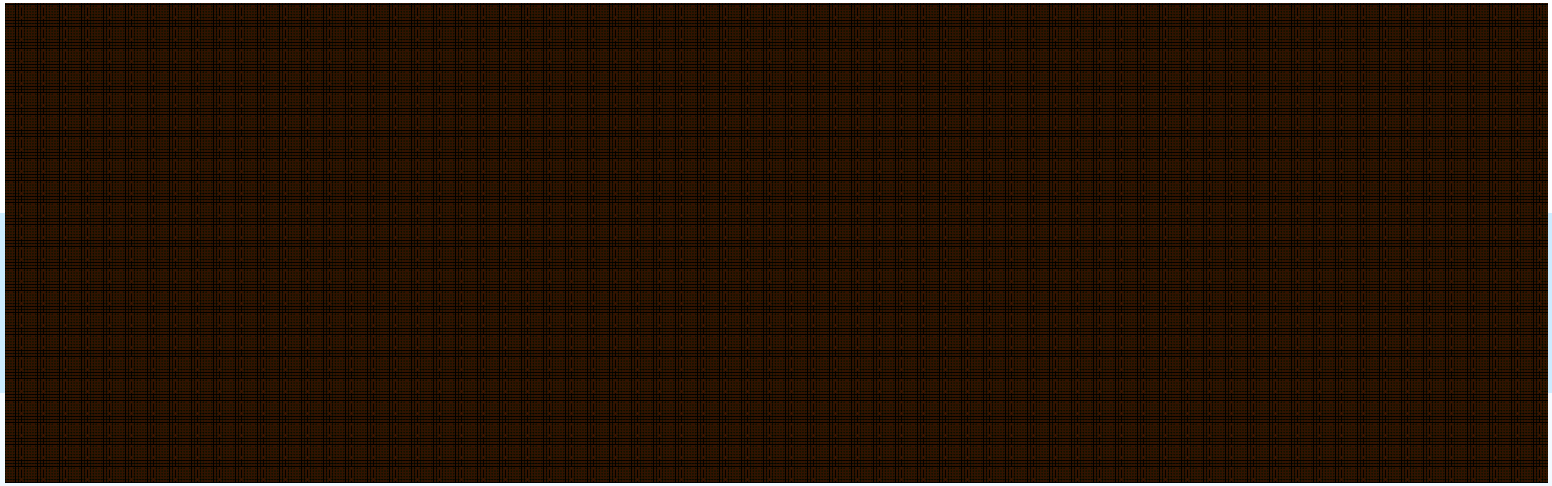


AMD473

Overcome resistance

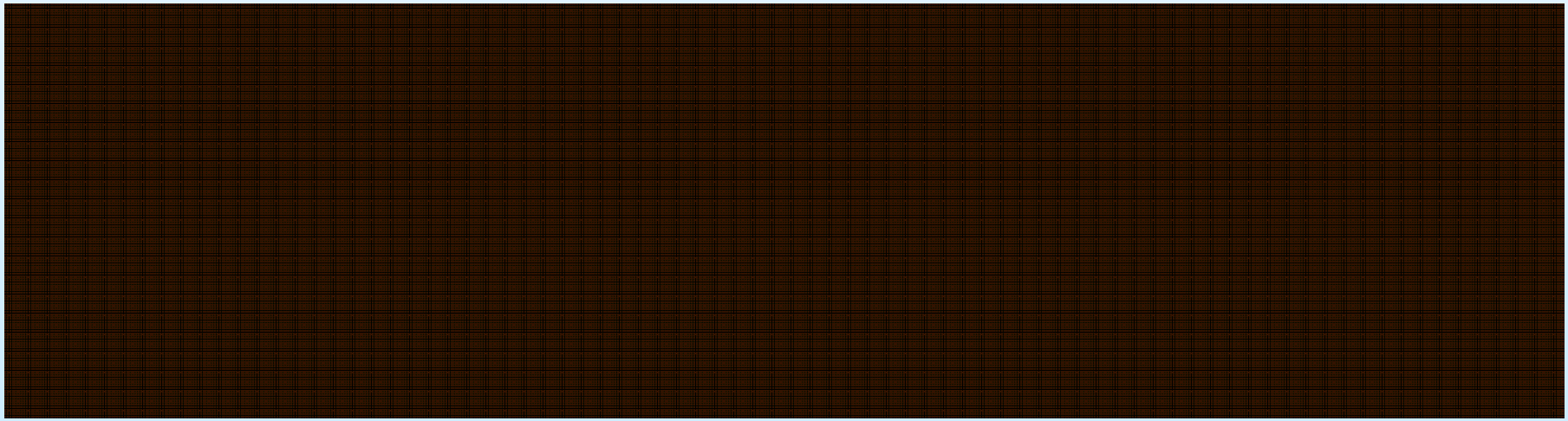
Sterics govern activity

Alderden et al. *J. Chem. Educ.* **2006**, 83



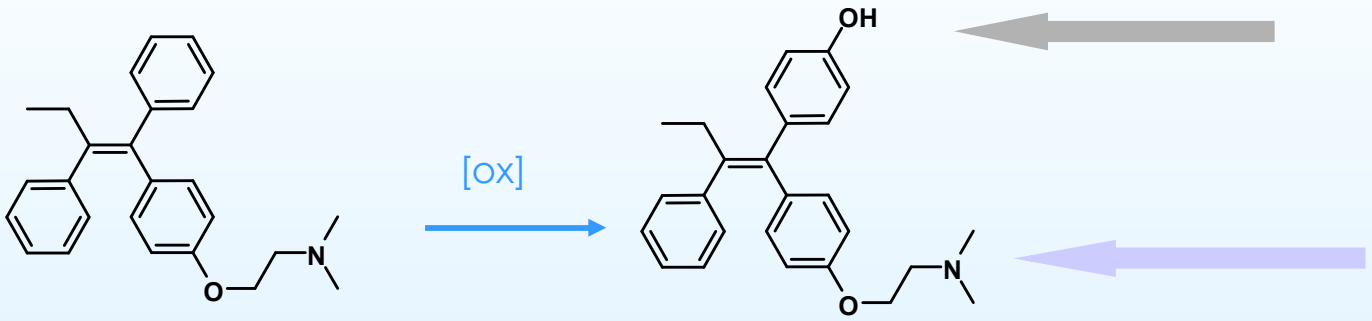
- **Bioactivity is related to reaction caused by the metal center**

  Tamoxifen

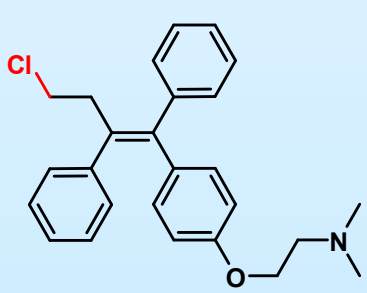


Tamoxifen

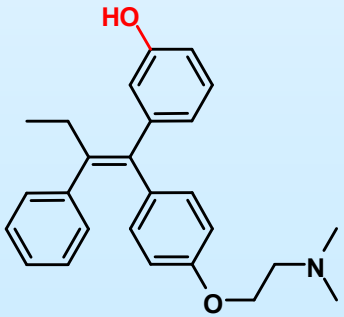
- Selective estrogen receptor modulator (SERM)
- The estrogen receptor plays a key role in the proliferation of hormone-dependent tumours



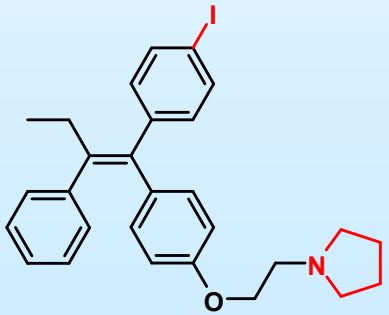
- Successful drugs but only active against ER+ tumors (60 %) and has developed resistance



Toremifene



Droloxifene

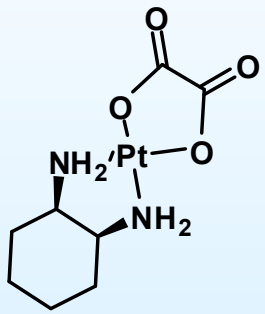


Iodoxifene

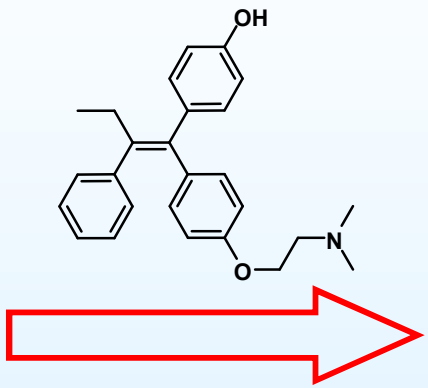
S. Top et al. *J. Organometal. Chem.* **2001**, *637*, 500
S. Top et al. *Chem. Eur. J.* **2003**, *9*, 5223

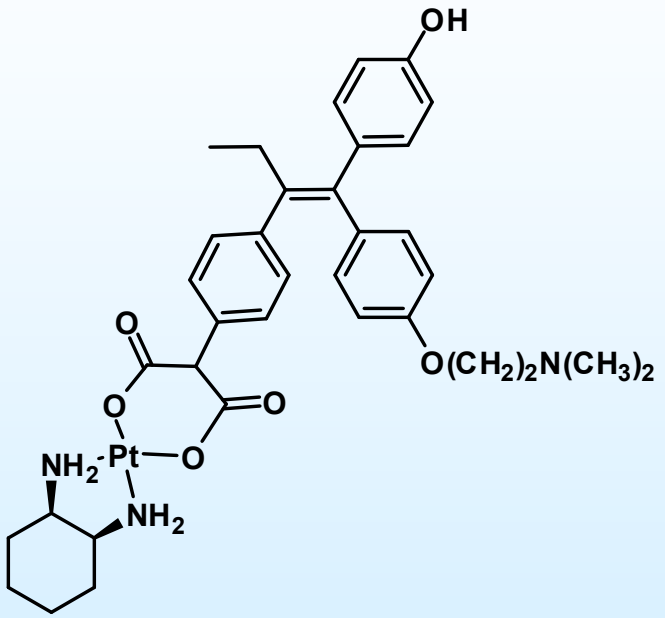
Metal Based Approach

Jaouen and coworker:



Oxaliplatin





Pt-N coordination bonds are too weak

- Hydrolyses too quickly

What other organometallic groups can be used?

S. Top et al. *J. Organometal. Chem.* **2001**, *637*, 500

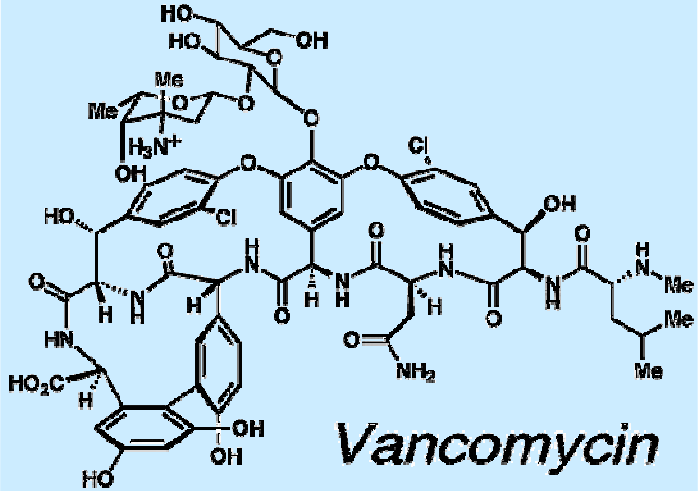
- **Metal as a structural scaffold**

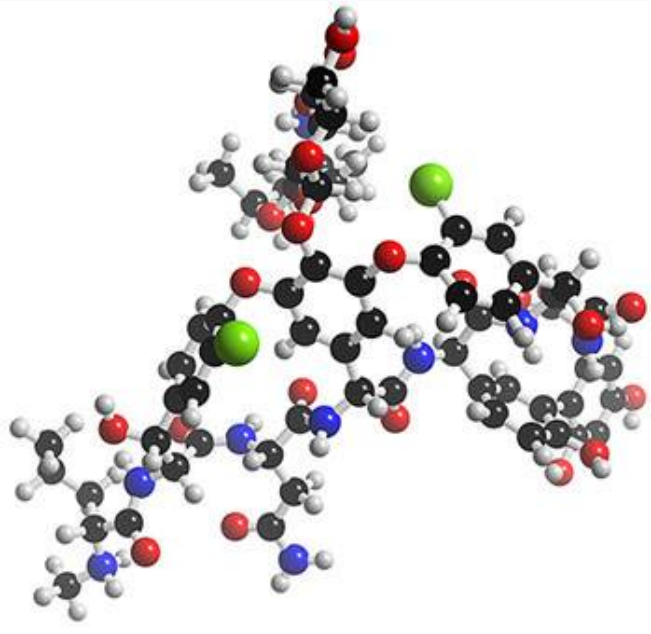
  Pyridocarbazole ruthenium complexes

Structural Diversity

Natural products display a high diversity of molecular skeletons:

- distinctive 3-D conformations





- Defined structures are important for their unique biological properties

Important challenge

Bregman, H.; Caroll, P.J.; Meggers, E. *J. Am. Chem. Soc.* **2006**, *128*, 877

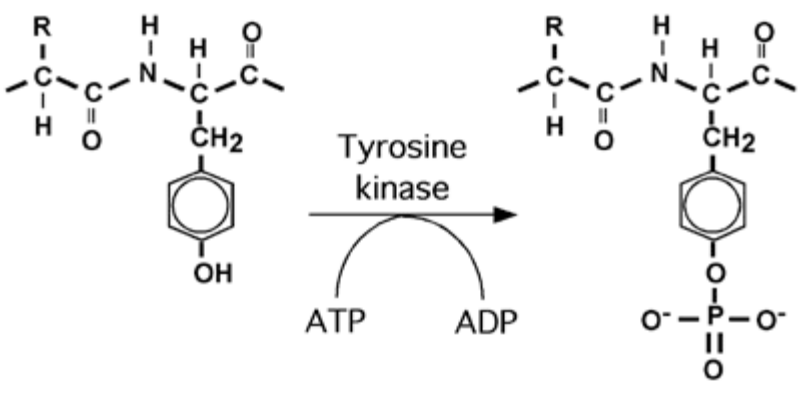
Outline

1. Target : Kinase; ATP binding site
2. Known inhibitor: Staurosporine
3. Metal scaffold
4. Synthetic approaches and development
5. Diversity oriented synthesis

Protein Kinases

Protein Kinases:

- Phosphorylation of proteins : turn them on or off

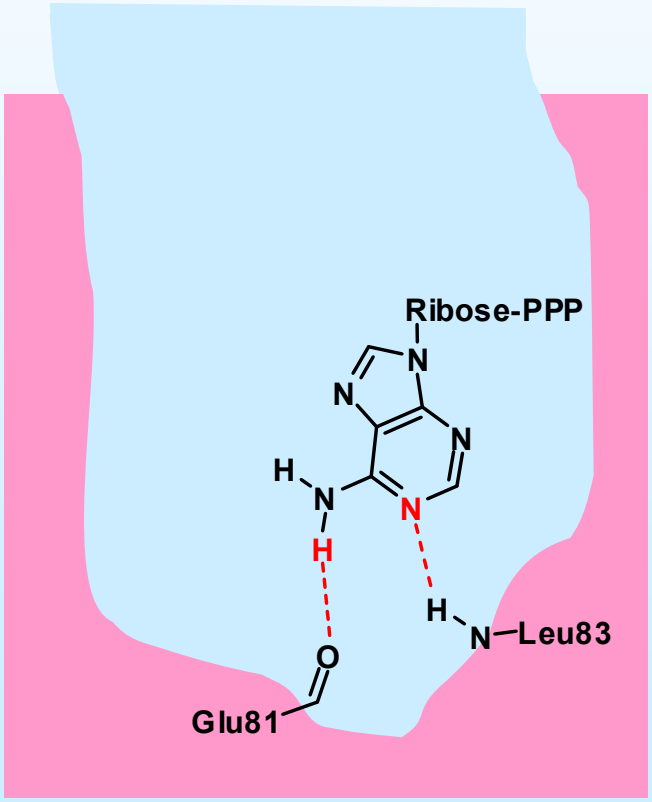


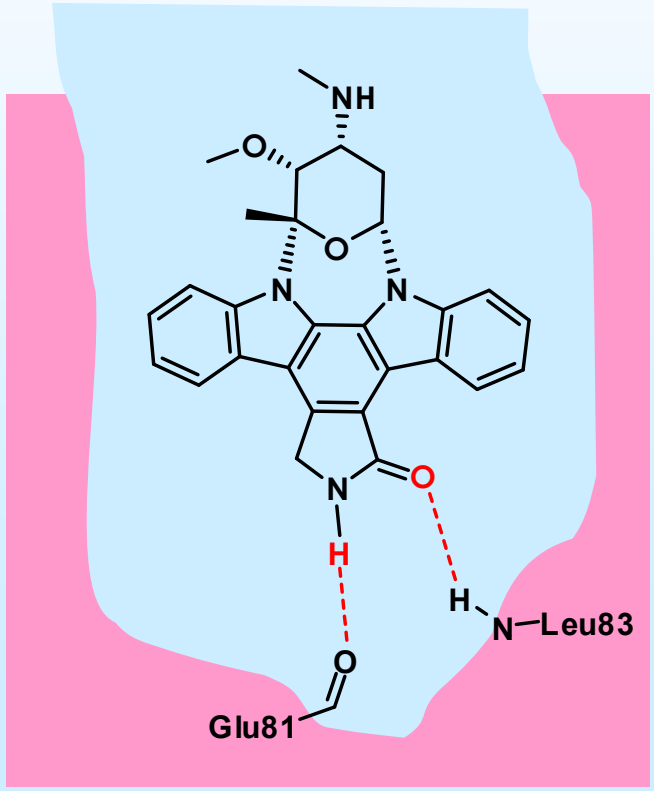
- Due to their involvement in various forms of cancers, PTKs have become prominent targets for therapeutics
- Regulate the majority of cellular pathways e.g DNA replication, cell growth
- Most kinases contain a 250-300 amino acid domain with a conserved core structure, compromising a binding pocket for ATP
- **These domains are more or less homologous**

Blume-Jensen. P.; Hunter, T. *Nature*, **2002**, *411*, 355
Fischer, P.M. *Curr. Med. Chem.* **2004**, *11*, 1583

ATP Binding

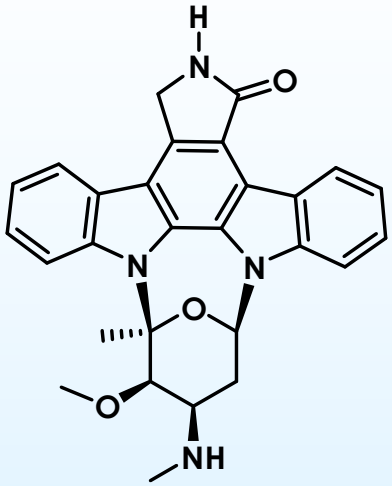
- ATP-binding site is an ubiquitous "receptor" in nature
- Most kinase inhibitors mimic mainly the adenine portion of ATP
- Approach is limited in terms of selectivity





Fischer, P.M. *Curr. Med. Chem.* **2004,** *11,* 1583

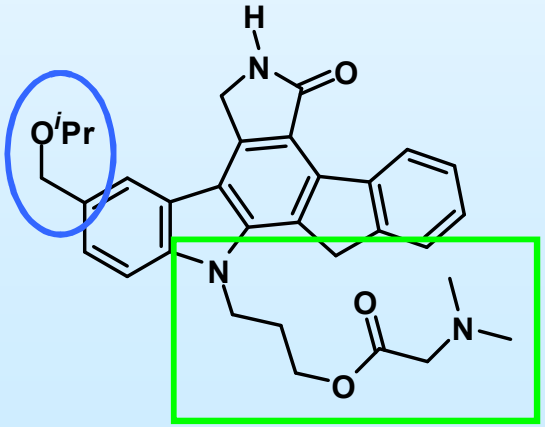
Bioorganometallic Chemistry: Staurosporine



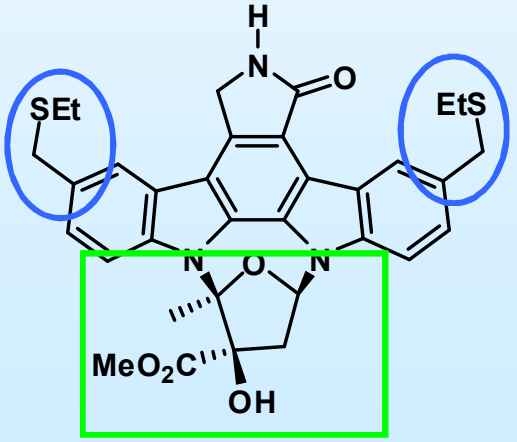
- discovered in 1977 while screening for microbials
- has gained great interest since it was reported to be potent against protein kinases
- Relatively potent; IC_{50} in the nanomolar range

Down side: Lacks specificity

Derivatives with modulated specificities are in preclinical trials as anticancer drugs



CEP-7055

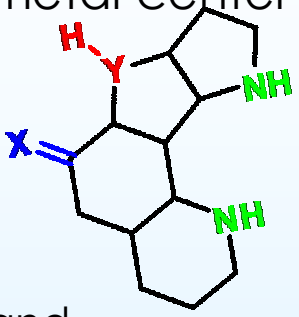


CEP-1347

Omura, S. et al. *J. Antibiotics*, **1994**, *48*, 535
M. Yang et al. Bioorg. Med. Chem. Lett. **2007**, 17, 326

Organometallic Chemistry

Meggers and coworkers: coordinate a known bioligand (staurosporine) to an inert metal center



Bioligand

Structural

OCH_2CH_3

C≡N

Specificity

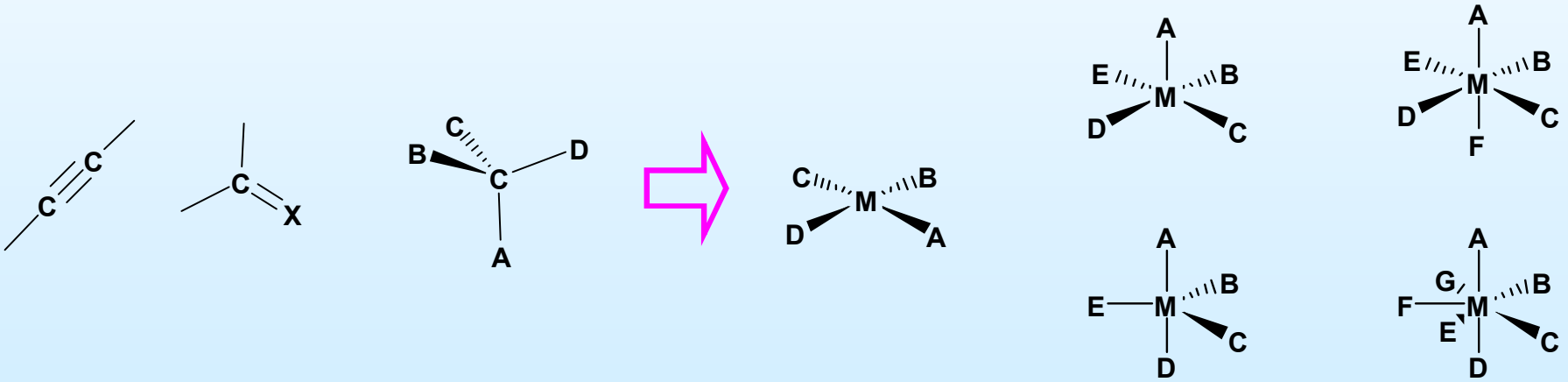
Inorganic compounds as structural scaffolds for the design of specific enzyme inhibitors

A Metal for Structure

Metals can be envisioned as hypervalent carbons

- new specificity can be achieved
- remove the limits imposed by the organic framework

Transition metals provide an expanded set of coordination geometries for the generation of molecular diversity



Octahedral with 6 different substituents can form 30 different stereoisomers

Meggers, E. *Curr. Opin. Chem. Biol*. **2007**, *11*, 287

Στο κεφάλαιο αυτό κάτω από τον τίτλο ΜΕΤΑΛΛΟΘΕΡΑΠΕΥΤΙΚΑ Ή ΑΝΟΡΓΑΝΑ ΦΑΡΜΑΚΑ θα μελετήσουμε τις εξελίξεις στη σύγχρονη έρευνα, στο αντικείμενο των εφαρμογών των συμπλόκων ενώσεων με Pt, Pd, Sn, Au, Sb, Ag, I και Se , στην Ιατρική.

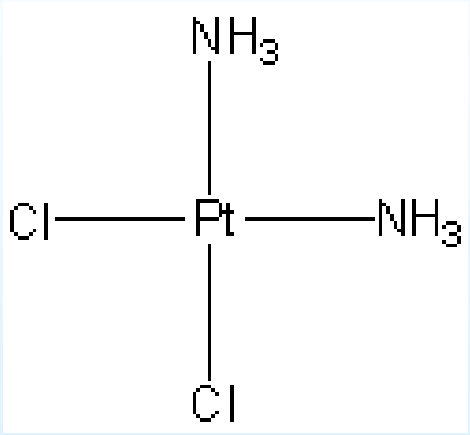
Στο ερευνητικό εργαστήριο βιοανόργανης χημείας με την επίβλεψη του Δρ Σ Χατζηκακού ασχολούμαστε με την

[1] σύνθεση νέων συμπλόκων ενώσεων των Sn, Au, Ag, I και Sb με αμίδια, σελενοαμίδια και αμίδια , και τη μελέτη της αντικαρκινικής δραστικότητας τους σε μια προσπάθεια ερμηνείας του μηχανισμού δράσης τους και βελτίωσης της αποτελεσματικότητας τους.

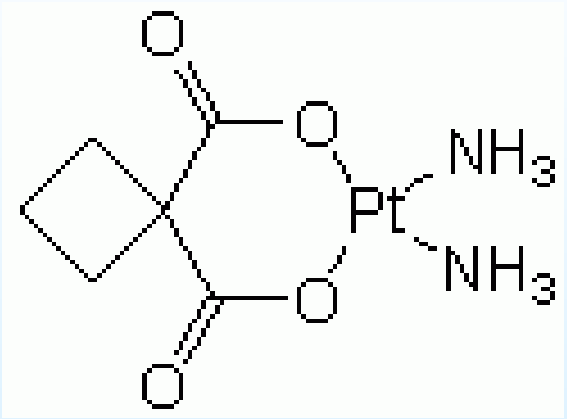
[2] μελέτη του μηχανισμού δράσης των θειοαμιδίων ως αντιθυροειδικών φαρμάκων σε σχέση με τη χημική τους συμπεριφορά ως προς το ιώδιο με στόχο τη βελτίωση των αντι-θυρεοειδικών φαρμάκων και

[3] μελέτη των θειοαμιδίων ως αντίδοτων σε δηλητηριάσεις από βαρεία μέταλλα όπως ο Hg

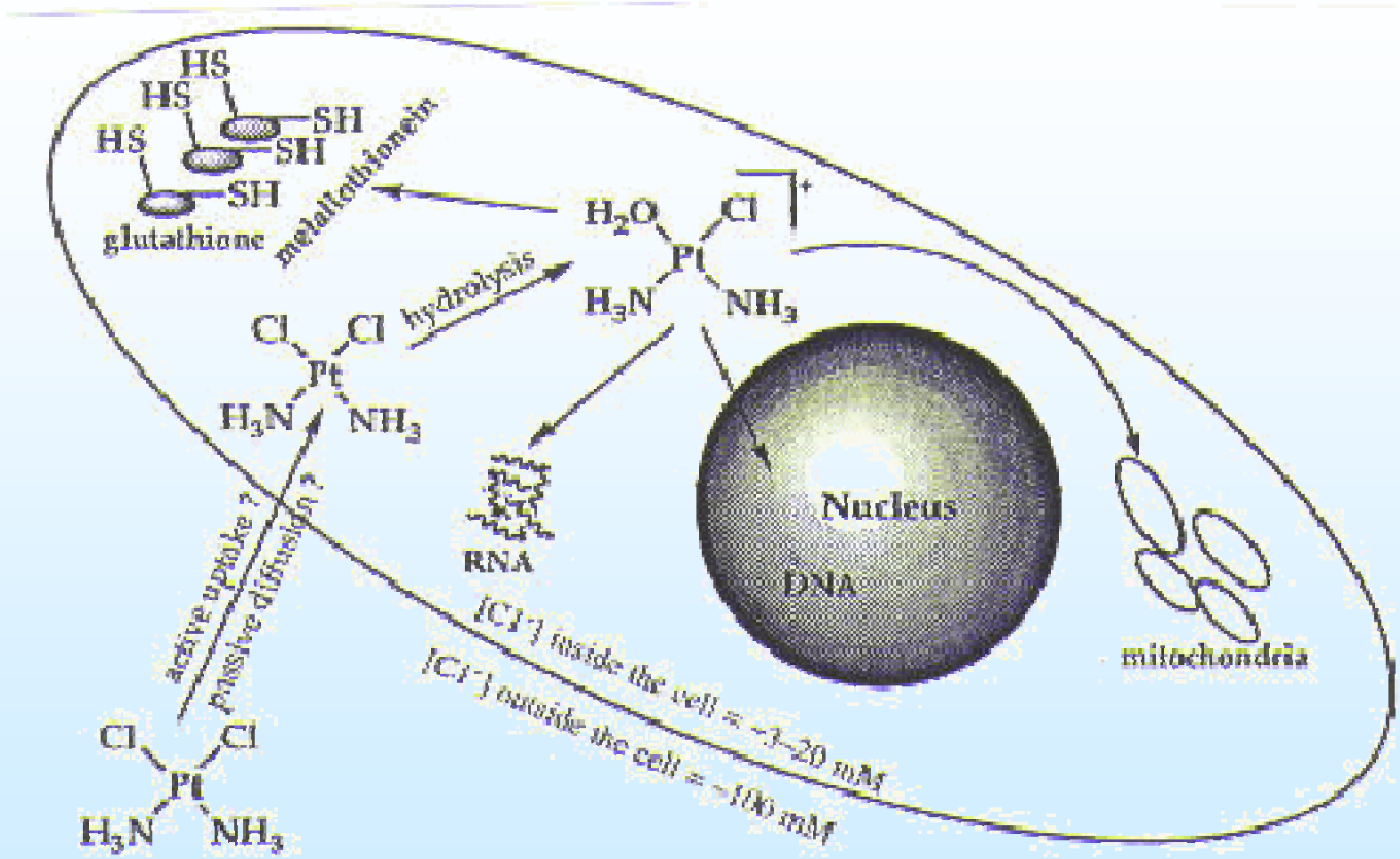
Cisplatin and carboplatin have a wide range of applications in cancer chemotherapy. **Since the discovery of the anti-proliferative properties of cisplatin, many platinum compounds have been synthesized, characterized and screened as anticancer agents**.

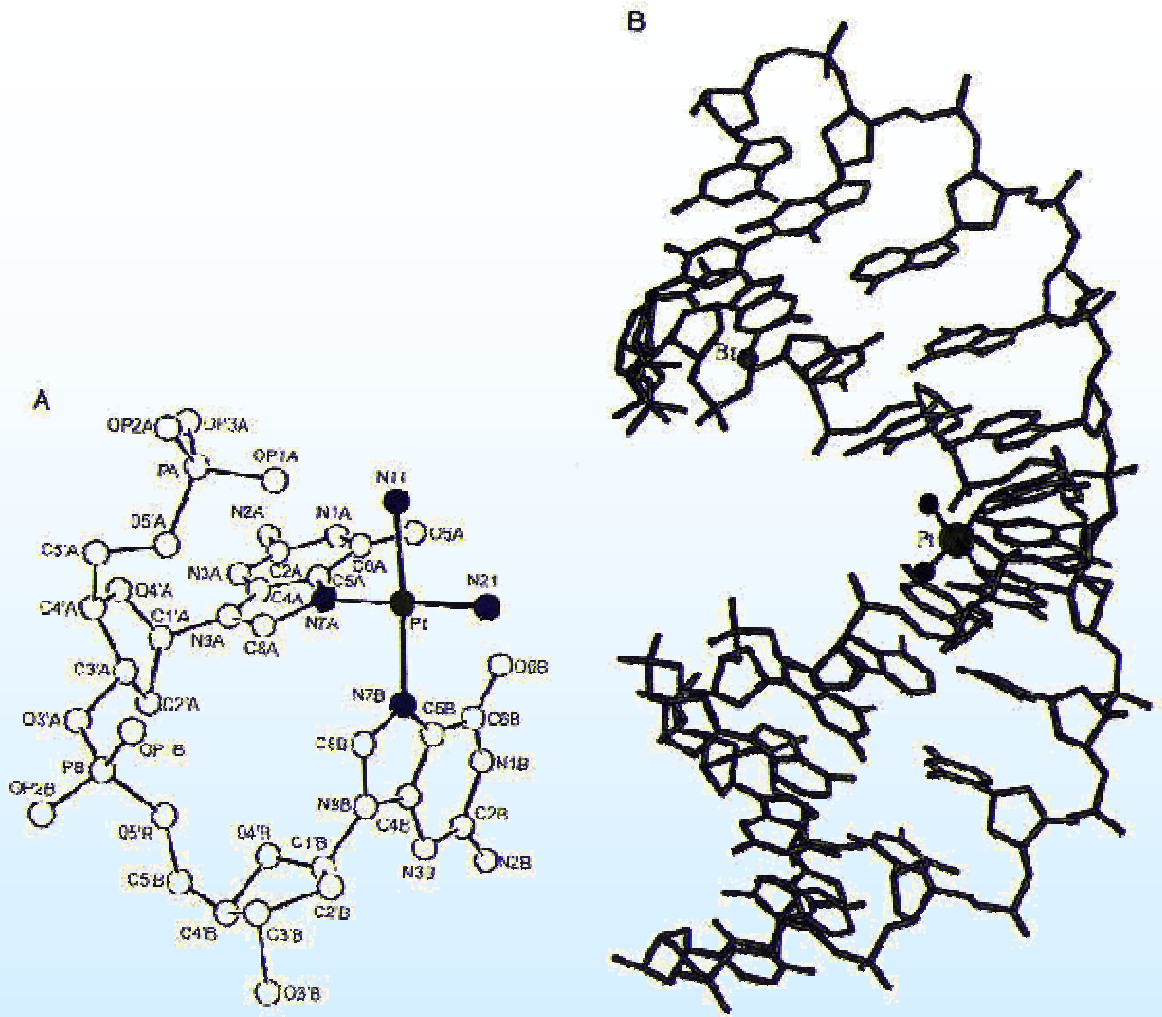


***cis*-platin**

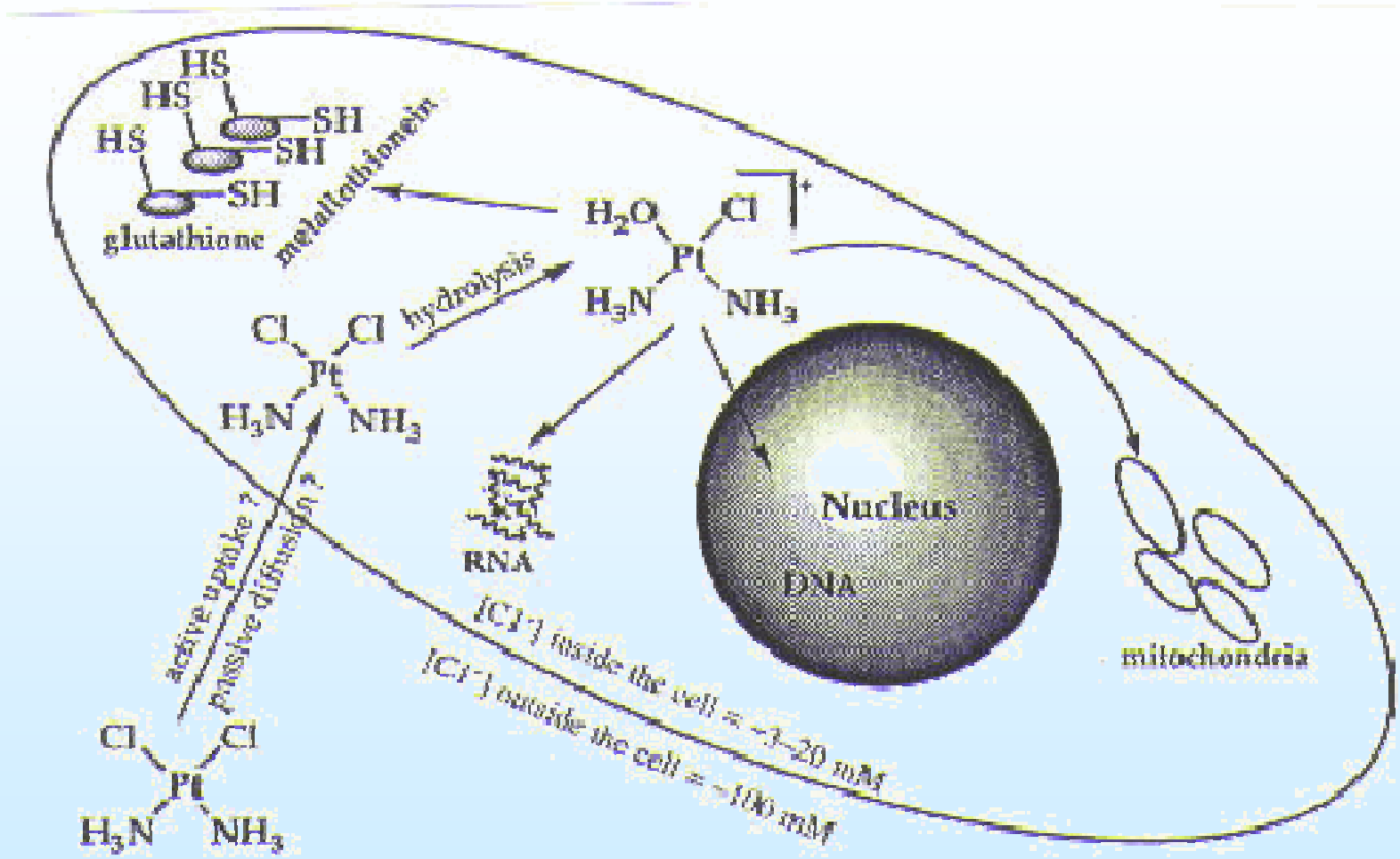


carboplatin


HS
HS
SH
HS
SH
SH
glutathione
metallothionein
H_2O
Cl
Pt
H_3N
NH_3
hydrolysis
Cl
Cl
Pt
H_3N
NH_3
RNA
Nucleus
DNA
mitochondria
active uptake ?
passive diffusion ?
Cl
Cl
Pt
H_3N
NH_3
[Cl⁻] inside the cell ≈ ~3–20 mM
[Cl⁻] outside the cell ≈ ~100 mM



The DNA base pairs. Cisplatin coordinates to the N7 atoms of the purine (guanine and adenine) bases.


HS
HS
SH
HS
SH
SH
glutathione
metallothionein
H_2O
Cl
Pt
H_3N
NH_3
hydrolysis
Cl
Cl
Pt
H_3N
NH_3
RNA
Nucleus
DNA
mitochondria
active uptake ?
passive diffusion ?
Cl
Cl
Pt
H_3N
NH_3
[Cl⁻] inside the cell ≈ ~3–20 mM
[Cl⁻] outside the cell ≈ ~100 mM

Compounds of other metal ions like organotin(IV), silver(I), gold(I/III), antimony(III) etc, are already known to possess antitumor activity. Our study aims to contribute to the elucidation of the antitumor action of organotins, silver, gold and antimony compounds and to establish a structure activity relationship (SAR).

for example see:

- ***Metallotherapeutic Drugs & Metal-Based Diagnostic Agents: The Use of Metals in Medicine*** **by M. Gielen and E.R.T. Tiekink, Eds., John Wiley & Sons, Chichester, UK, 2005**,
- **Zijian Guo, Peter J. Sadler**, ***Metals in Medicine***, ***Angew. Chem. Int. Ed.,*** **1999,** ***38***, **1512.**
- **E.R.T. Tiekink,** ***Critical Reviews in Oncology /Hematology,*** **2002,** ***42***, **217–224.**

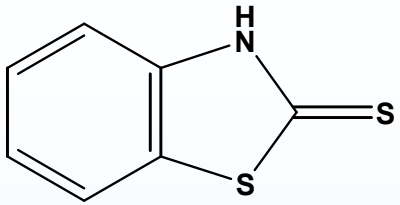
Organotin(IV) compounds

The anti-tumor activity of organotin(IV) complexes is well documented. Correlation efforts between structure and biological activity of organotin(IV) complexes are still a matter of research interest.

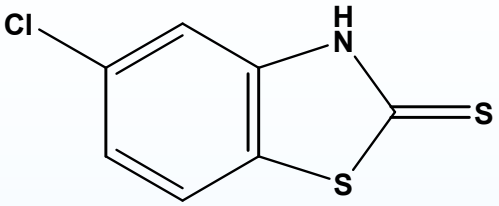
With the aim to synthesize new anti-tumor agents based on organotin(IV) compounds we studied the interaction of R_3SnCl and R_2SnCl_2, (R= Phenyl-, *n*-Butyl-, methyl-) with the thioamides and carboxylic acids.

[1] M.N. Xanthopoulou et.al., Bioinorganic Chemistry and Applications 2003, 1, 227
[2] M.N. Xanthopoulou et.al., J. Inorg. Biochem. 96 (2003) 425
[3] C.T. Chasapis et.al., Bioinorganic Chemistry and Applications , 2004, 2 , 43
[4] S.K. Hadjikakou, et.al., J. Organomet. Chem., 691 (2006) 1637–1642
[5] M.N. Xanthopoulou et.al., J. Organomet. Chem. 691 (2006) 1780
[6] M.N. Xanthopoulou et.al., Inorg. Chem. 2007, 46, 1187-1195
[7] M.N. Xanthopoulou et.al., Russian Chemical Bulletin, Intern. Edition, 2007, 56, 767
[8] M.N. Xanthopoulou et.al., Eur J Med Chem 43 (2008) 327-335
[9] S.K. Hadjikakou, et.al., J. Inorg. Biochem. 102 (2008) 1007–1015
[10] V.I. Balas, et.al., Bioinorganic Chemistry and Applications 2008, Article ID 654137.
[11] M.N. Xanthopoulou et.al., Polyhedron, 27 (2008) 3318–3324
[12] S.K. Hadjikakou, et.al.,Coord Chem Rev 253 (2009) 235–249
[13] M.A. Abdellah, et.al., Bioinorganic Chemistry and Applications 2009, Article ID 542979
[14] I.I. Verginadis, et.al., Eur J. Pharmaceutical Sciences, 42, (2011), 253-261
[15] V.I. Balas, et.al., Eur J Med Chem , 46, (2011), 2835-2844

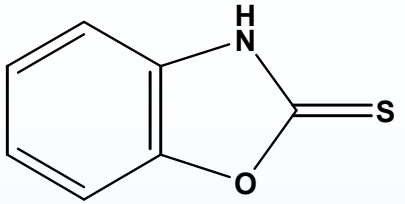
Ligands used for organotin complexes



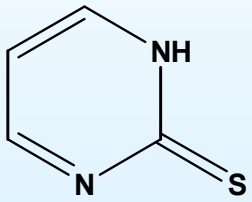
2-mercaptobenzothiazole
(mbztH)



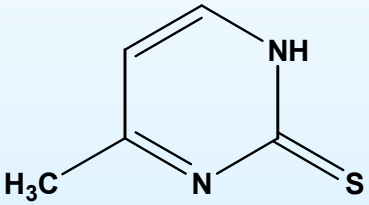
5-chloro-2-mercaptobenzimidazole
(ClmbztH)



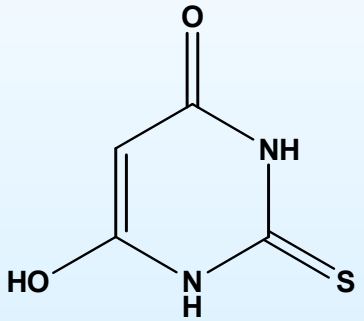
2-mercaptobazoxazole
(mbzoH)



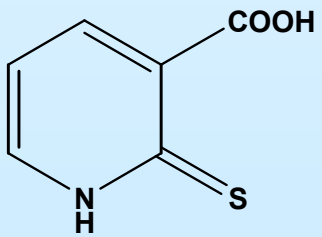
2-mercaptopyrimidine
(mpmH)



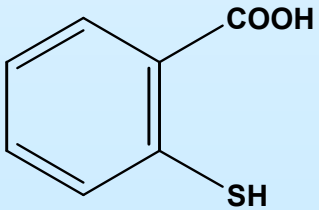
6-methyl-2-mercapto-pyimdine
(MempmH)



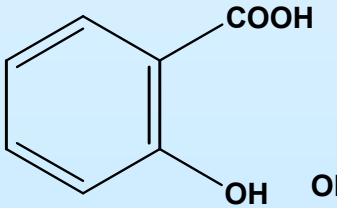
6-hydroxy-2-Thiouracil (tbaH)



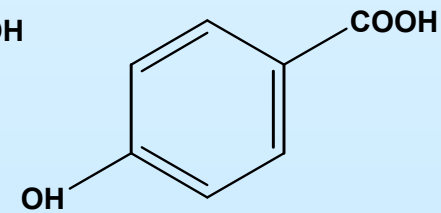
2-mercaptonicotinic acid
($mnaH_2$)



2-mercaptobenzoic acid
($mbaH_2$)



o-hydroxybenzoic acid
(o-OHbzaH)

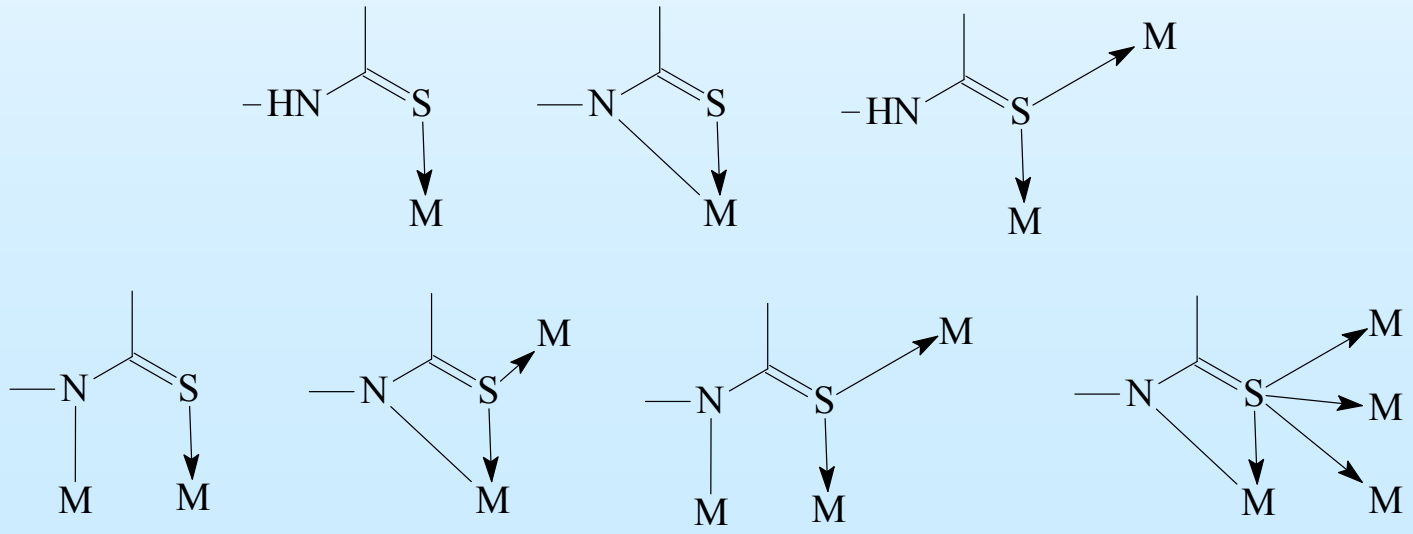


p-hydroxybenzoic acid
(p-OHbzaH)

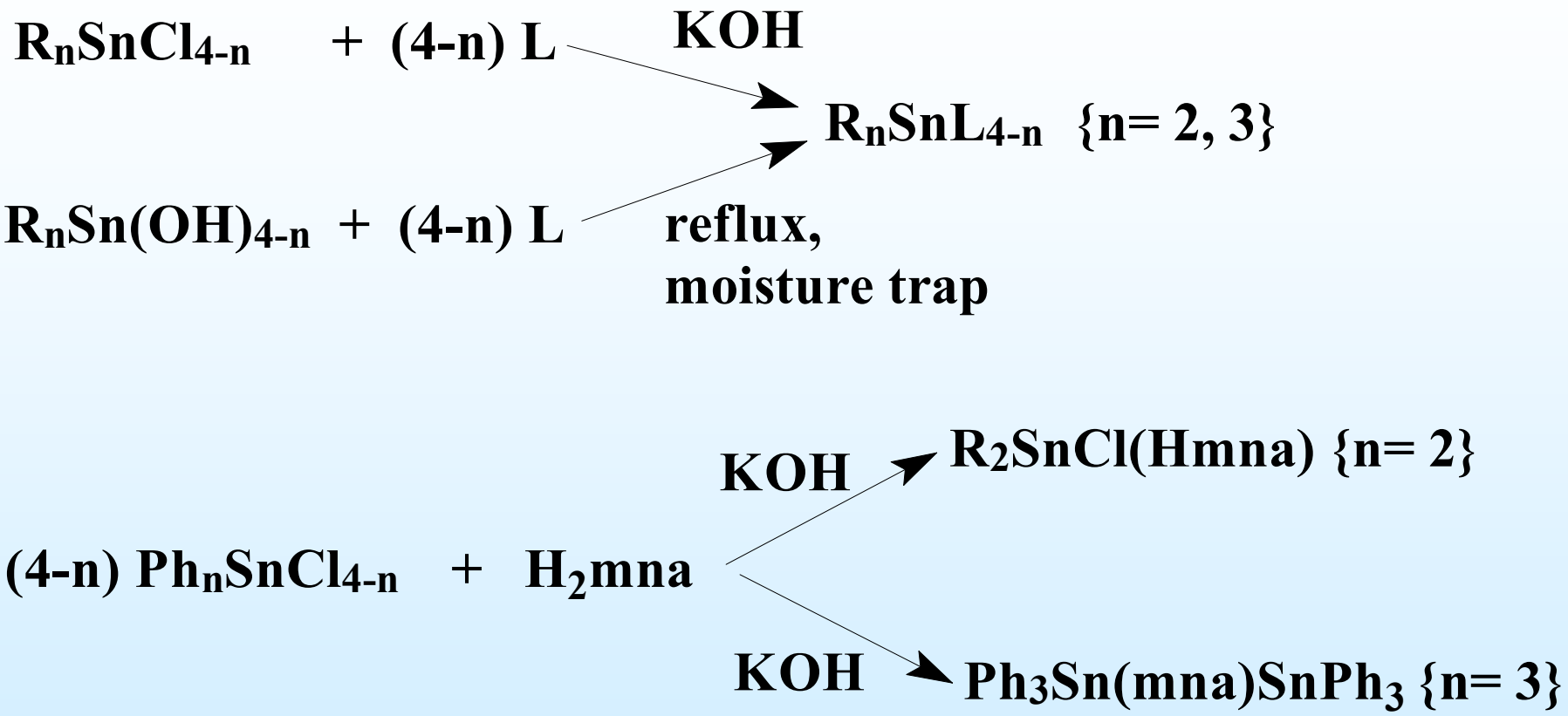
Thioamides such as 6-mercapto-purine, show anti-tumor activity in their own right. More platinum-pyridine thione complex has been patented for clinical use in cancer treatment.

Thioamides, also, may coordinate to a metal ion with a variety of coordination modes including: (i) *mono*-dentate (ii) chelading (iii) *bi*-dentate (iv) bridging (v) *tri*-dentate and (vi) poly-dentate. Thus, a variety of structural motifs can be obtained by the use of thioamide ligands.

[1] K.G. Van Scoik, C.A. Johnson, W.R. Porter, Drug Metab. Rev. 16 (1985) 157.
[2] J. Dehand, J. Jordanov and J.P. Beck, *Chem-Biol. Interactions*, 11 (1975) 605.
[3] Japanese Patent: JP8061522 (800509).



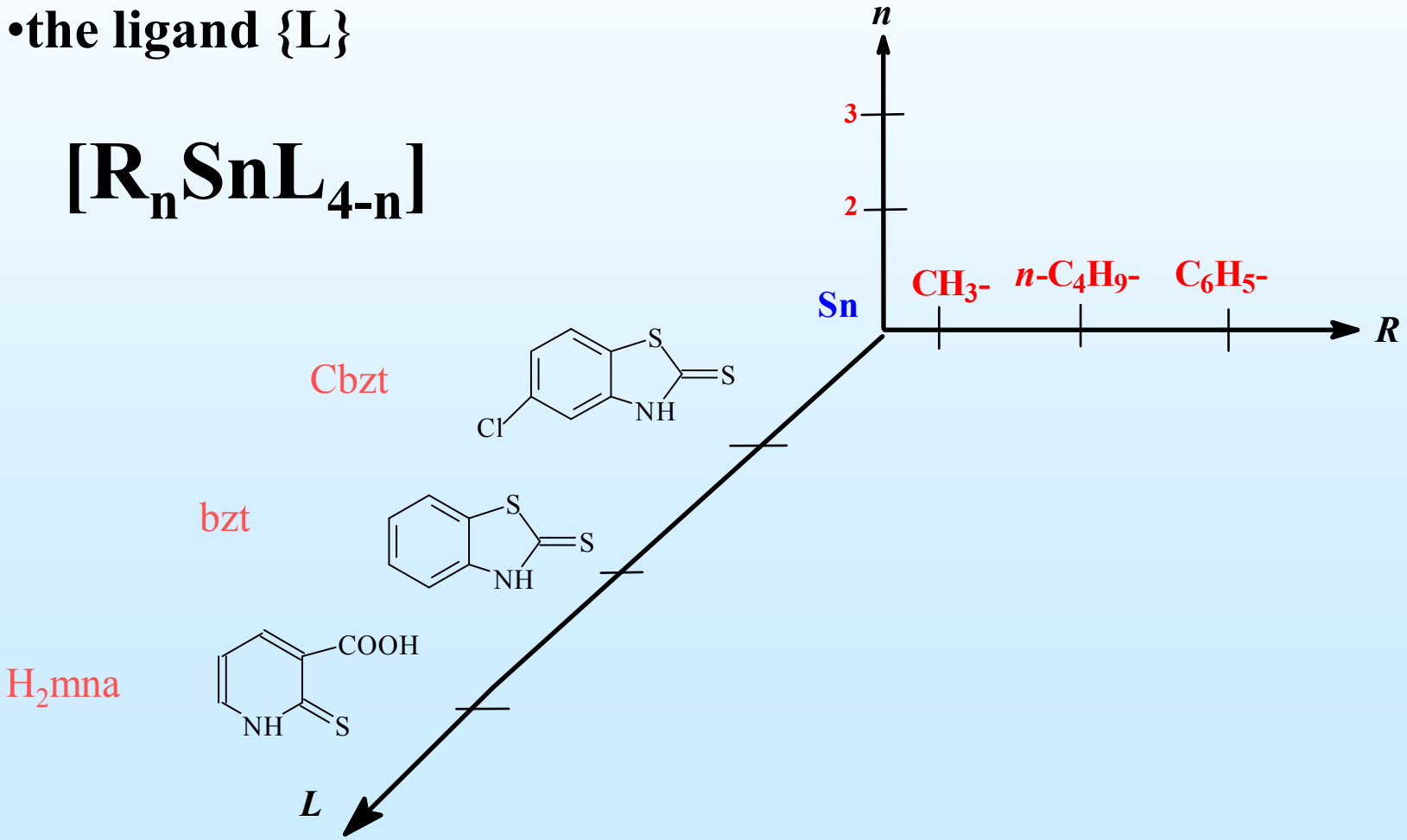
Synthesis of the complexes



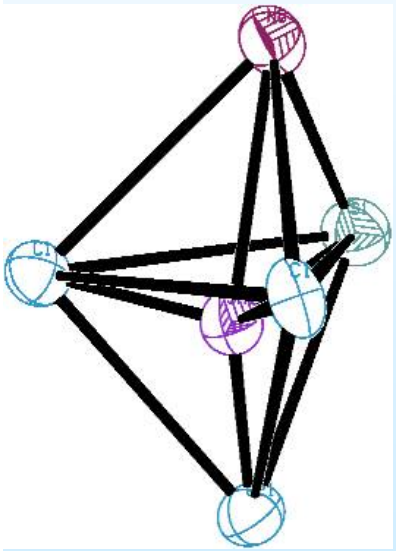
Structure - activity relationship studies on this type of complexes were based on the variation of:

- **the number of tin(IV) atoms in the complexes**
- **the alkyl group {R-},**
- **the number of alkyl groups bonded to Sn atom {n} and**
- **the ligand {L}**

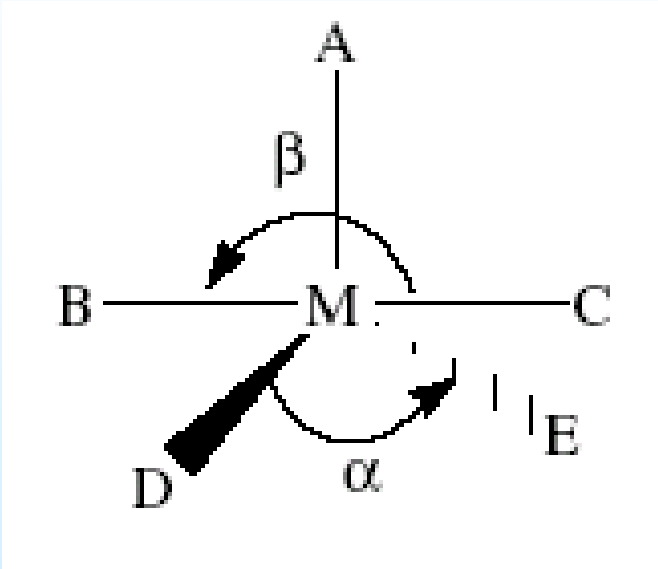
$$[R_nSnL_{4-n}]$$



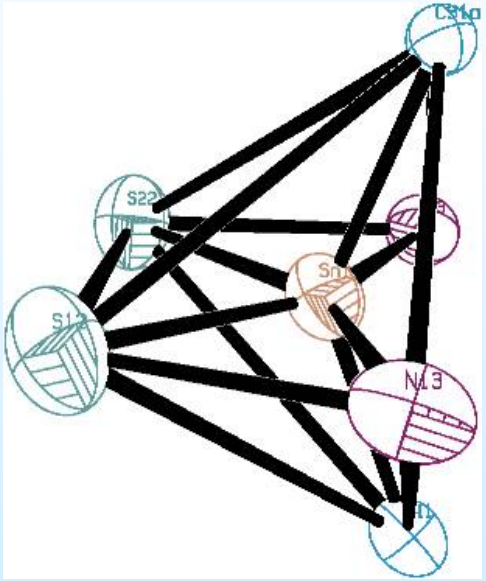
Tri-organotin compounds are mainly five or four coordinated with either trigonal bi-pyrmaidal or tetrahedral geometry, while **Di-organotin** compounds are mainly six or four coordinated with disorder octahedral, or tetrahedron geometry, around the metal center



Trigonal bi-pyramidal geometry

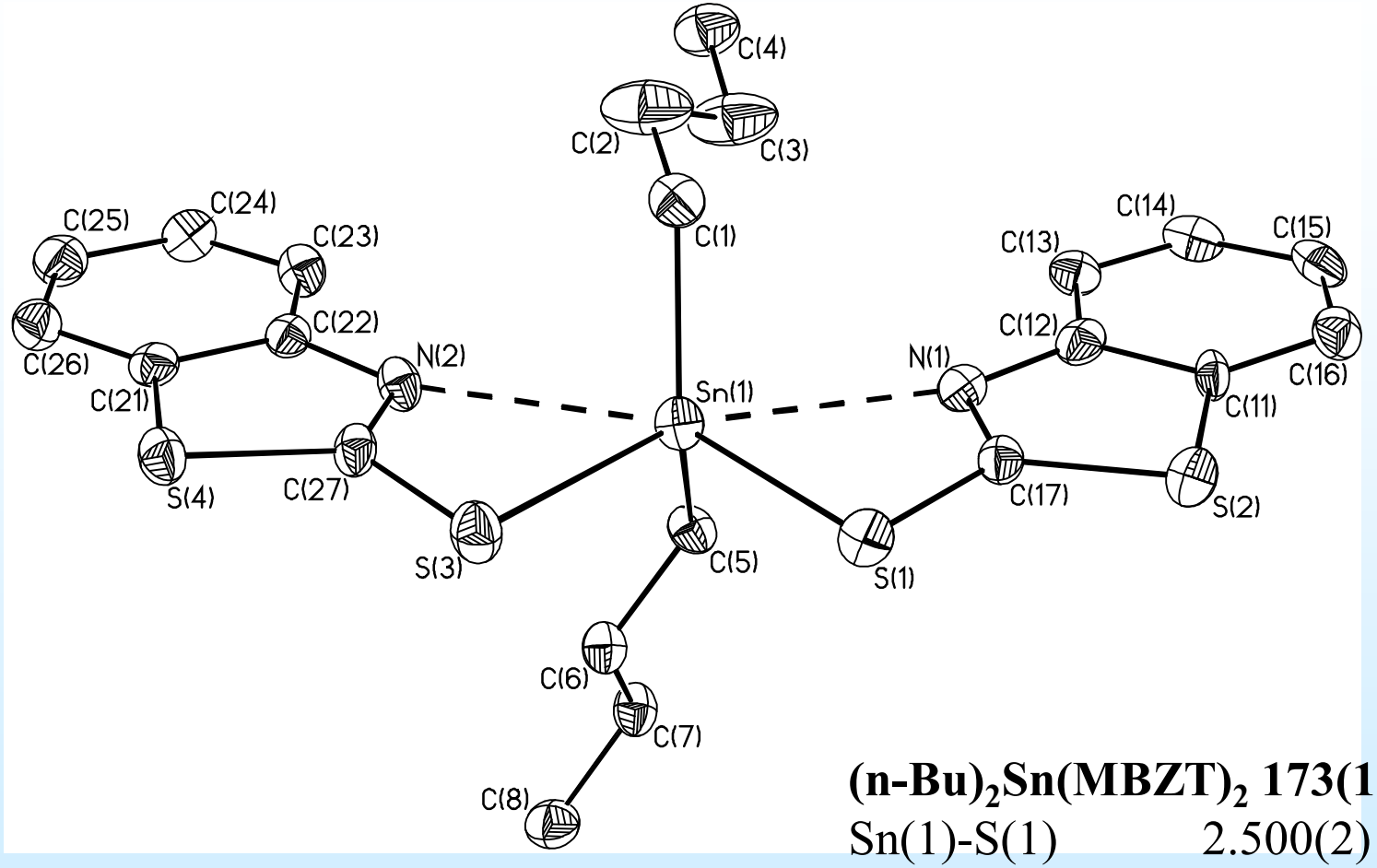


According to Reedijk
when the parameter $\tau = (b - a)/60$
equals zero a tetragonal
pyramidal structure is obtained, while in
trigonal bipyramidal
structure equals to unity



Distorted octahedral geometry

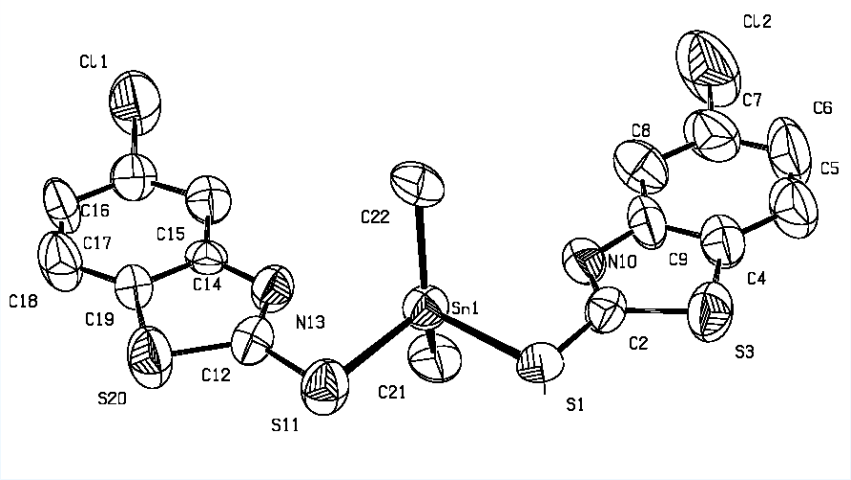
Six coordinated organotin complexes



$(n\text{-}Bu)_2Sn(MBZT)_2$ 173(1) K

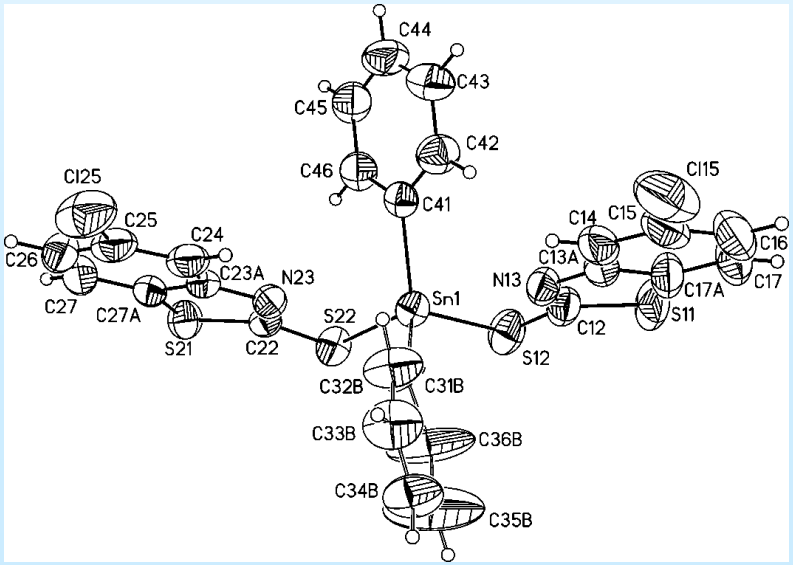
Sn(1)-S(1)	2.500(2)
Sn(1)-S(3)	2.5092(19)
Sn(1)-N(1)	2.730(5)
Sn(1)-N(2)	2.780(6)

Six coordinated organotin complexes



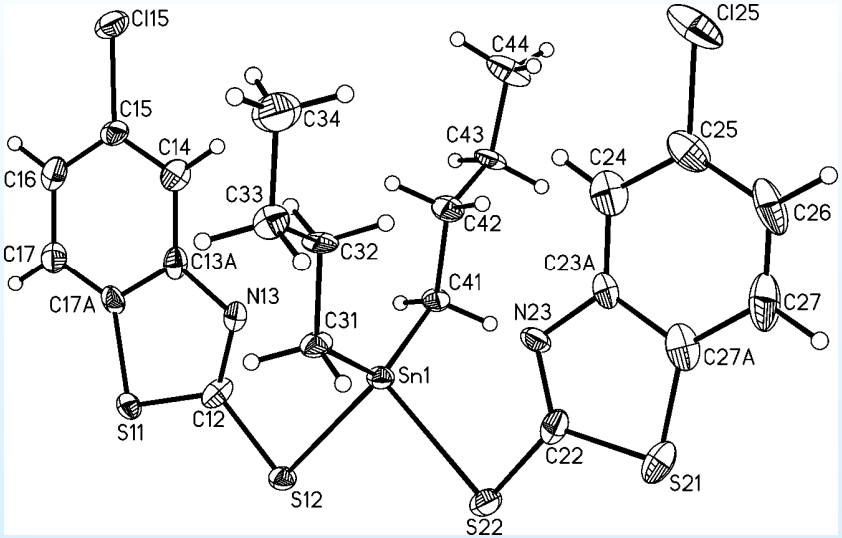
$Me_2Sn(CMBZT)_2$ 293(1) K

Sn(1)-S(11) 2.494(4)
Sn(1)-S(1) 2.509(4)
Sn(1)–N(10) 2.802,
Sn(1)–N(2) 2.635



$(n\text{-}Bu)_2Sn(CMBZT)_2$ 293(2) and 100(1) K

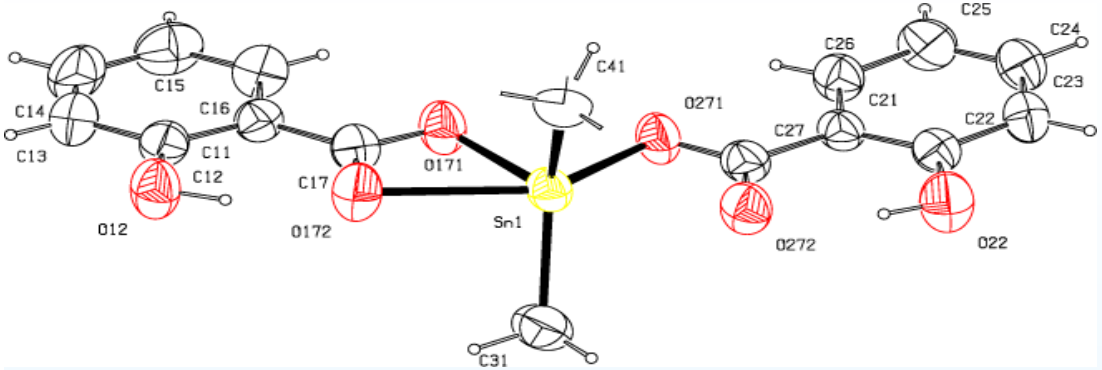
293(2) K		100(1)	
Sn1-S2	2.4885(16)	Sn1-S12	2.5042(19)
Sn1-S3	2.515(2)	Sn1-S22	2.5286(19)
Sn1-N1	2.783(9)	Sn1-N13	2.748(7)
Sn1-N2	2.789(10)	Sn1-N23	2.766(7)

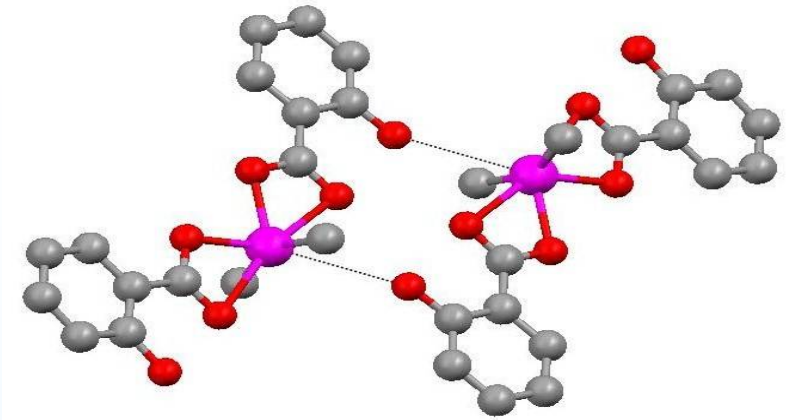


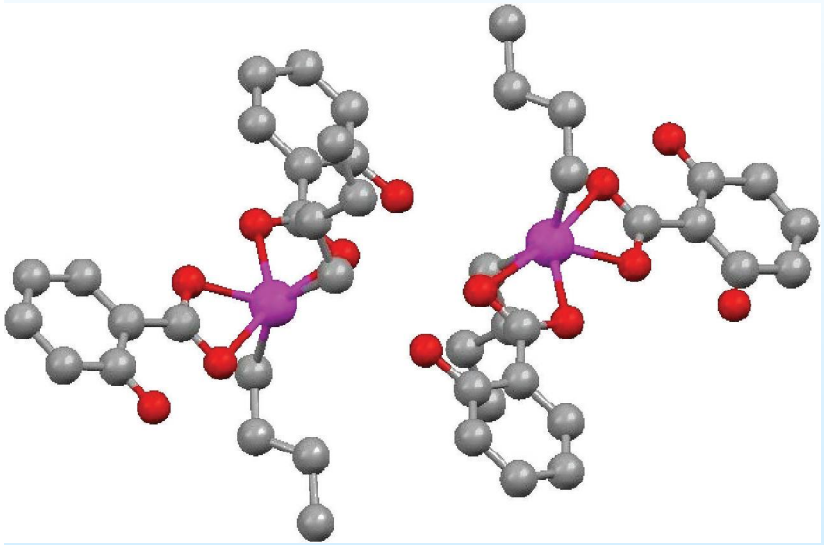
$Ph_2Sn(CMBZT)_2$ 293(1) K **D4**

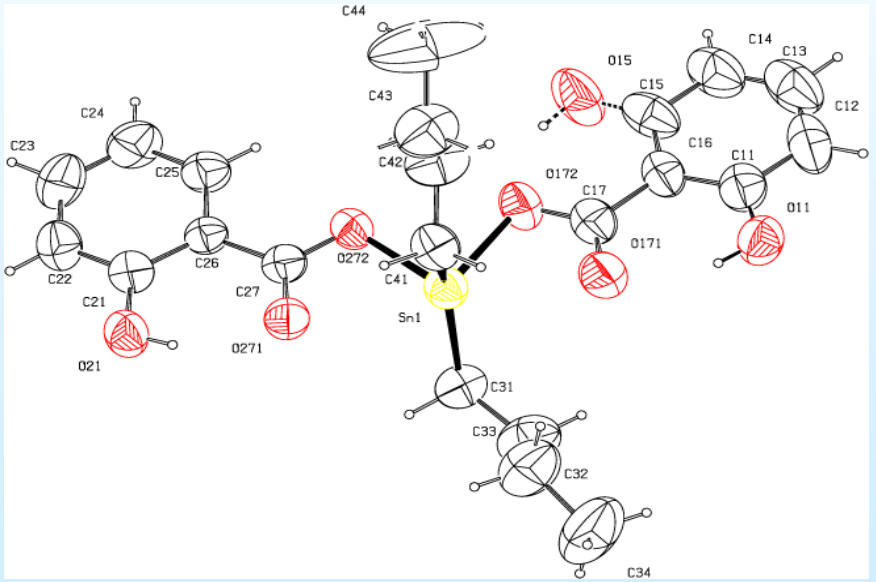
Isomer A	Isomer B
Sn(1)-S(12) 2.4947(7)	Sn(1)-S(12) 2.4947(7)
Sn(1)-S(22) 2.5020(7)	Sn(1)-S(22) 2.5020(7)
Sn(1)-N(13) 2.653(2)	Sn(1)-N(13) 2.653(2)
Sn(1)-N(23) 2.7718(18)	Sn(1)-N(23) 2.7718(18)

Six coordinated organotin complexes



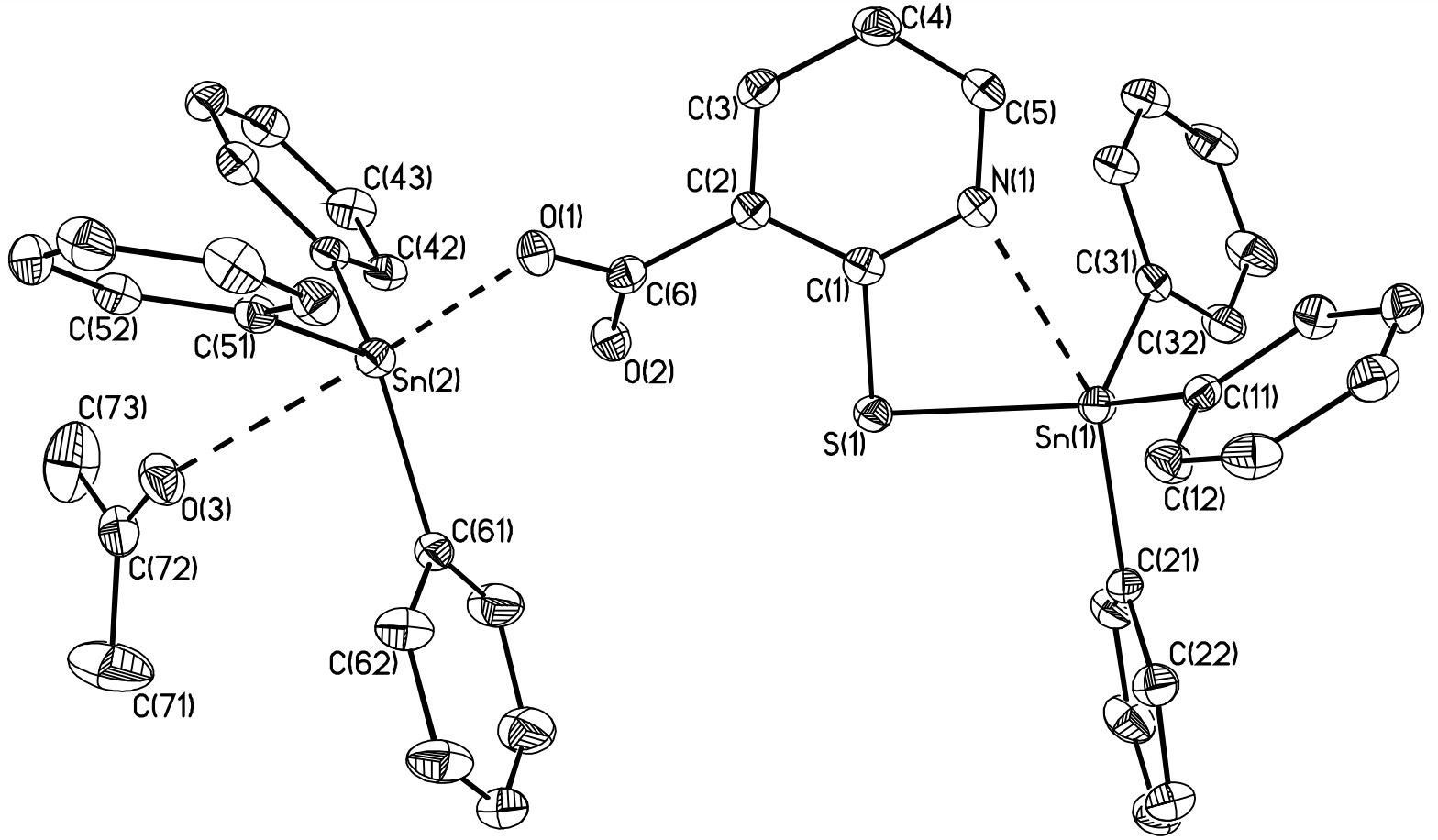






The Sn-O bond distances are 2.1060(18), 2.5147(15), 2.1079(15) and 2.577 Å in **H1** and 2.104(4), 2.564, 2.121(4) and 2.632 Å in **H2** showing an asymmetric bidentate coordination of the ligand to the Sn atom, leading into distorted octahedral geometry around the metal ion.

Five coordinated organotin complexes

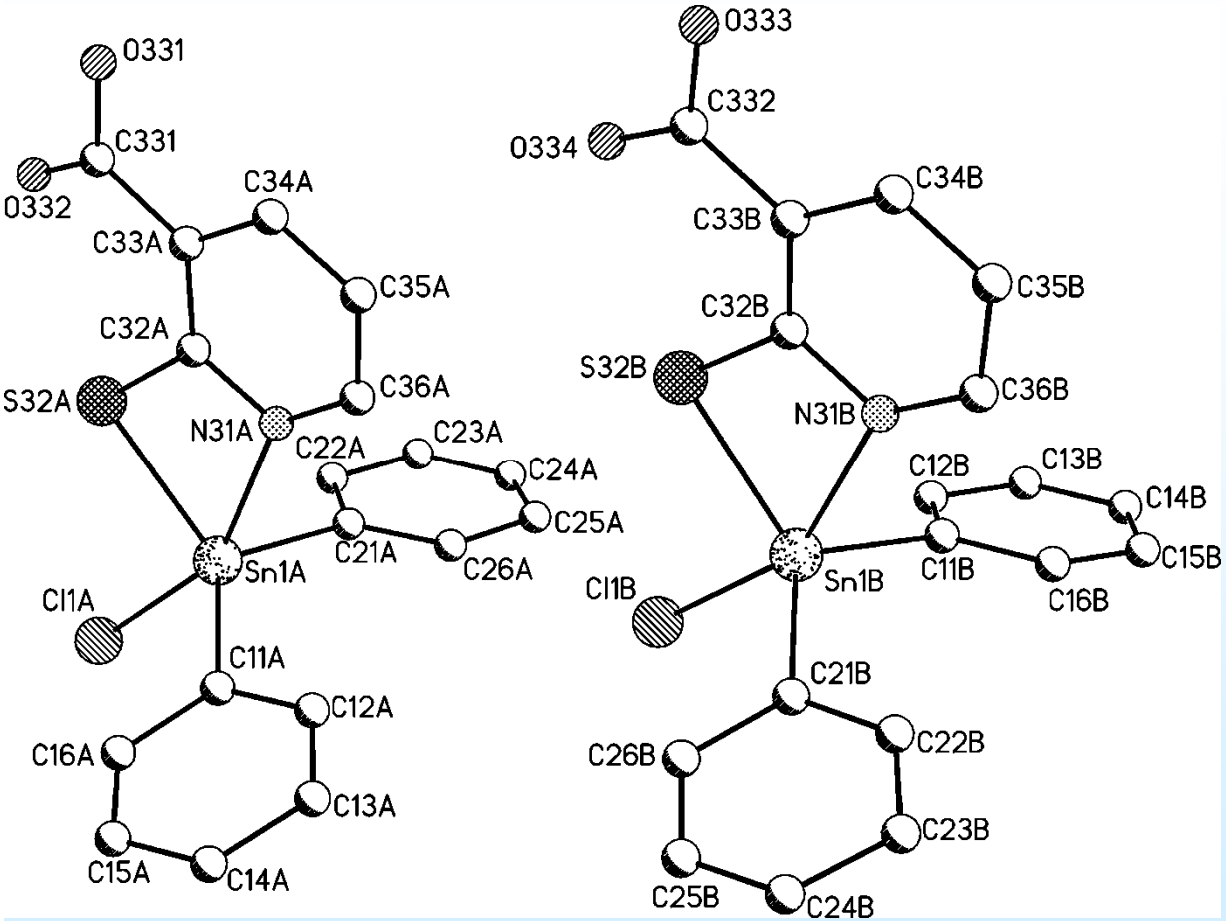


$Ph_3Sn(MNA)SnPh_3 \cdot Me_2CO$ 173(1) K B3

τ= 0.73

Sn(1)-S(1)	2.4450(7)	Sn(2)-O(3)	2.7763(18)
Sn(1)-N(1)	2.711(2)	Sn(2)-O(2)	2.7779(16)
Sn(2)-O(1)	2.1149(15)		

Five coordinated organotin complexes



$Ph_2SnCl(HMNA)$ C1

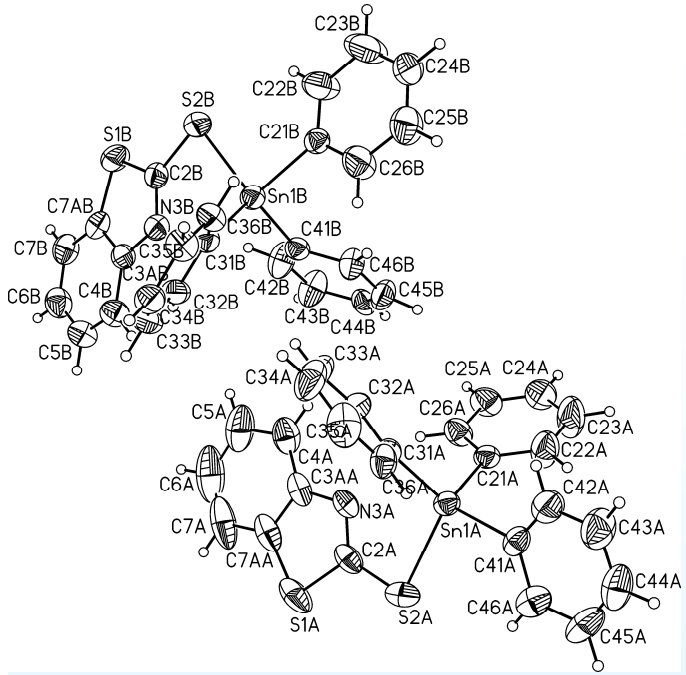
τ= 0.53(A); 0.59 (B)

Isomer A

Sn(1A)-N(31A)	2.435(5)
Sn(1A)-S(32A)	2.4383(17)

Isomer B

Sn(1B)-S(32B)	2.4396(18)
Sn(1B)-N(31B)	2.441(5)



$Ph_3Sn(MBZT)$ 295(1) K D1

τ= 0.71(A); 0.65 (B)

Isomer A

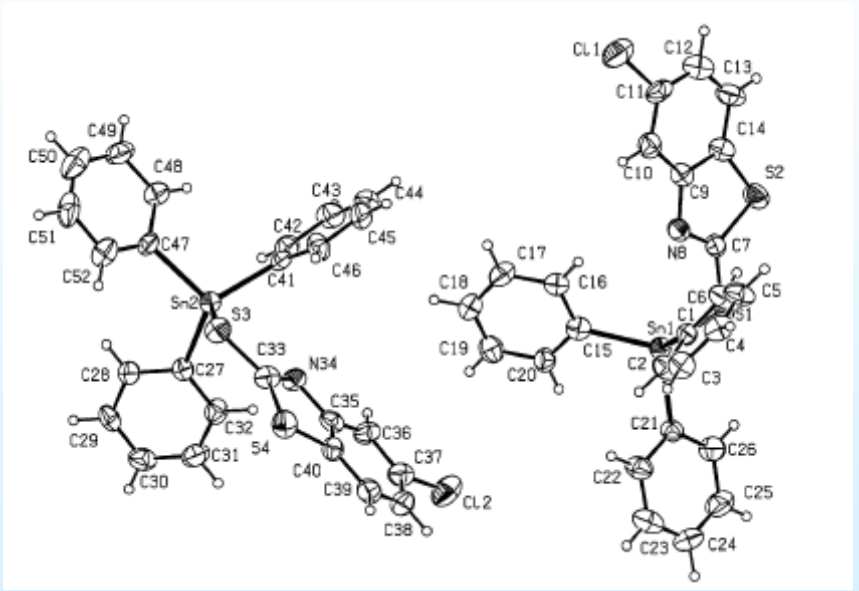
Sn(1A)-S(2A) 2.4540(15)

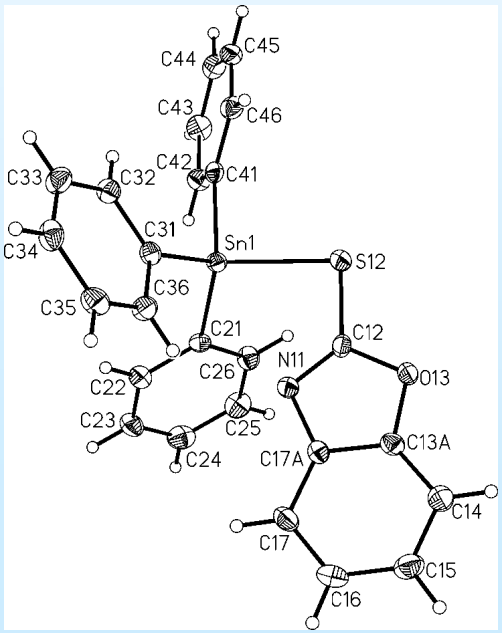
Sn(1A)-N(3A) 2.945(4)

Isomer B

Sn(1B)-S(2B) 2.4625(14)

Sn(1B)-N(3B) 2.898(4)





$Ph_3Sn(MBZO)$ 295(1), 100(1) K

τ= 0.62 (295 K); 0.61 (100 K),

295(1)K

Sn1-S12 2.4661(13)

Sn1-N11 3.078(4) weak interaction

100(1)K

Sn1-S12 2.4699(9)

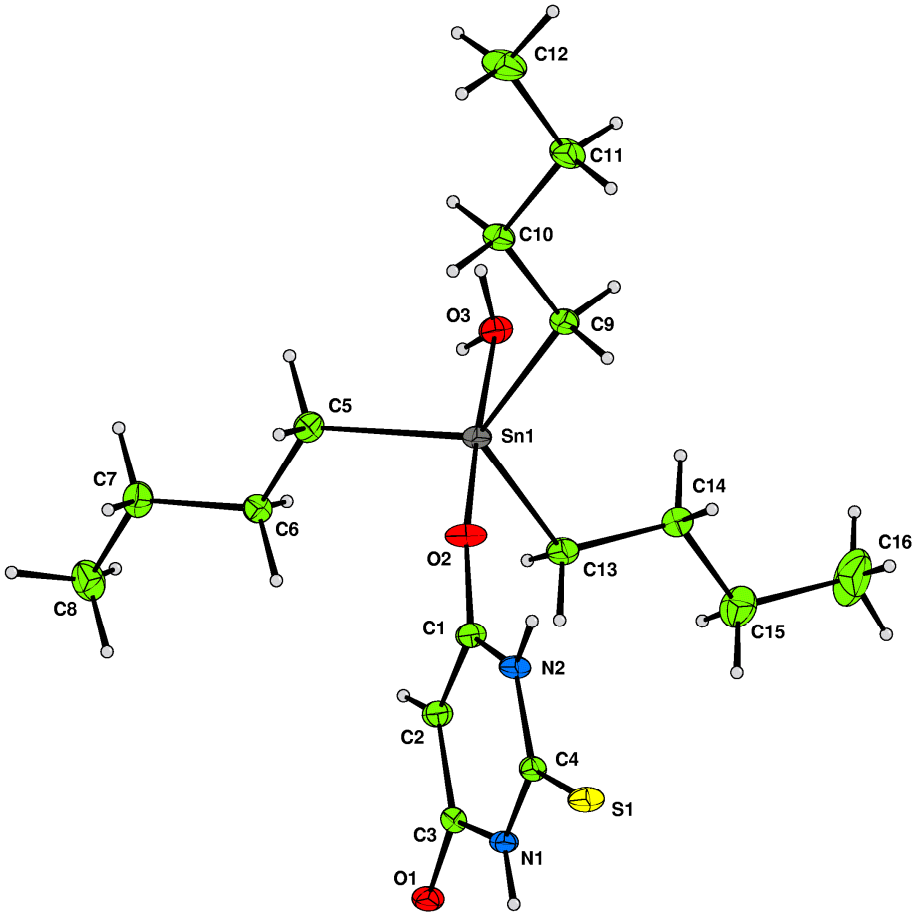
Sn1-N11 3.067(3) weak interaction

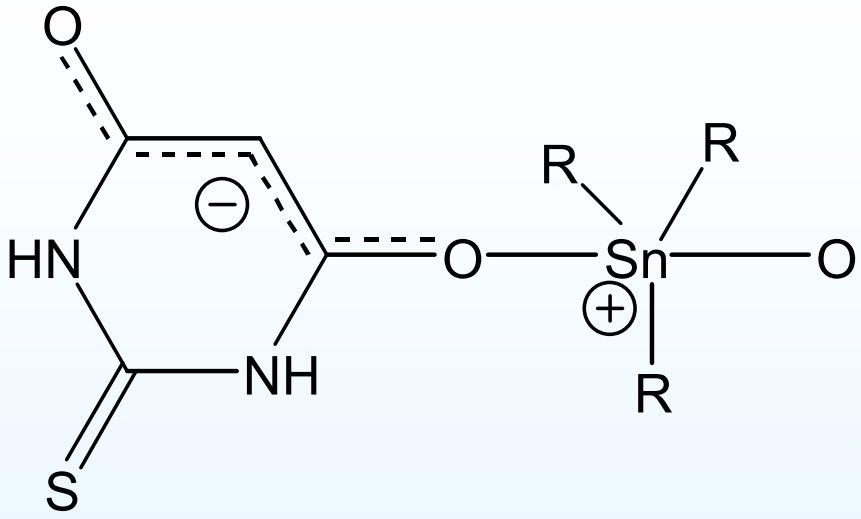
$Ph_3Sn(ClMBZT)$ 293(1) K

τ= 0.63(A); 0.58 (B)

Isomer A		Isomer B	
Sn(1)-S(1)	2.458(3)	Sn(2)-S(3)	2.456(3)
Sn(1)-N(8)	3.007(4)	Sn(1)-N(34)	3.010(3)

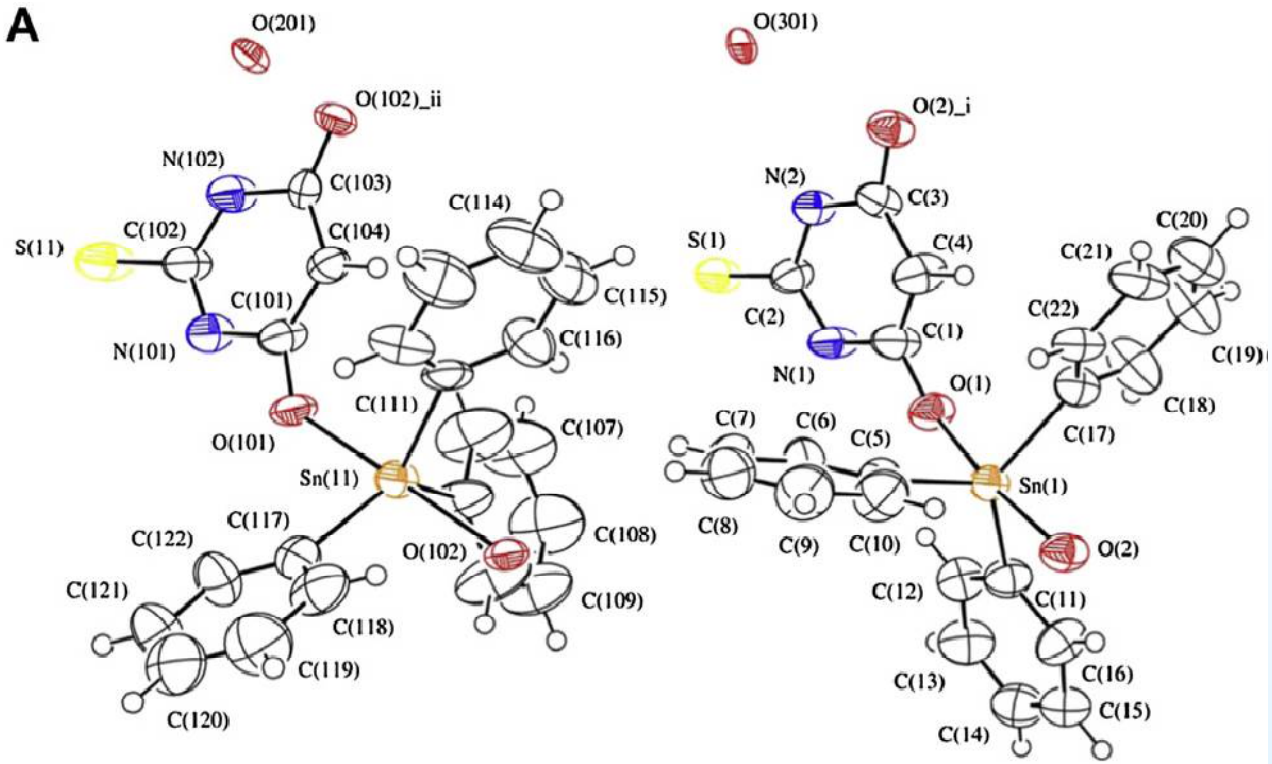
Five coordinated organotin complexes

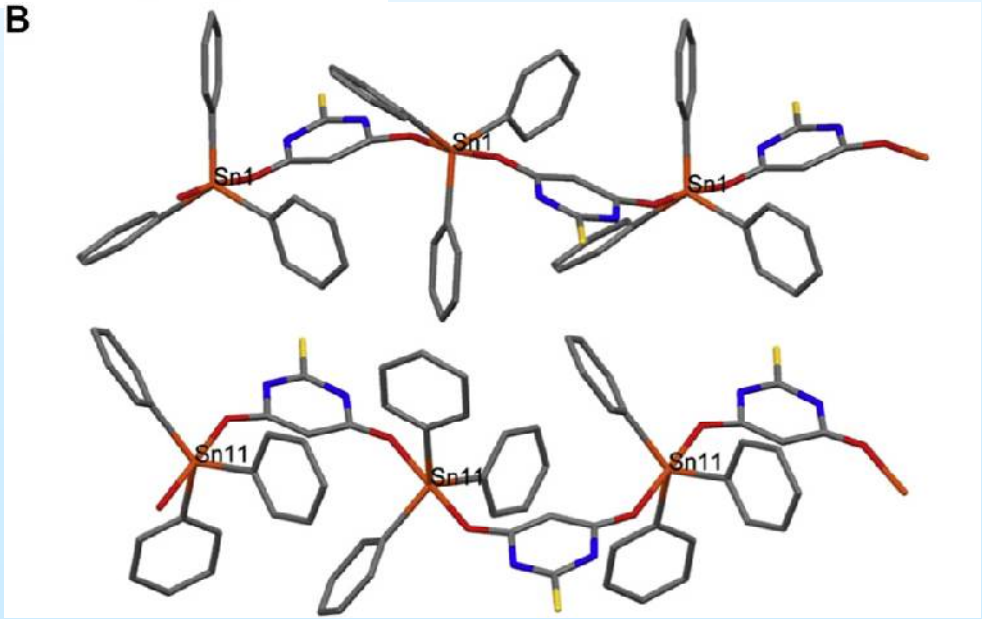




Two almost equal **C–O** bond distances (C1–O2 = 1.274(2), and C3–O1 = 1.261(2)) and two **C-C** bond lengths also equal (C1–C2 and C2–C3 of 1.386(3) and 1.394(3)), leads to a zwitterionic form of the compound.

$Bu_3Sn(TBA)(H_2O)$ J1
τ= 0.94
Sn1–O2 2.2287(14)





$\{[Ph_3Sn(O\text{-}HTBA)]\}_n$

τ= 0.83

O(1)-Sn(1) 2.214(16) O(101)-Sn(11) 2.225(16)

O(2)-Sn(1) 2.249(15) O(102)-Sn(11) 2.279(15)

Compound	IC_{50} cell activity
$[(CH_3)_2Sn(o\text{-}HBZA)_2]$	>2000 nM
$[(n\text{-}C_4H_9)_2Sn(o\text{-}HBZA)_2]$	150 nM
$[(n\text{-}C_4H_9)_3Sn(o\text{-}HBZA)]$	150 nM
$[(C_6H_5)_3Sn(o\text{-}HBZA)]$	**5-10 nM**
$[(n\text{-}C_4H_9)_3Sn(p\text{-}HBZA)]$	30-40 nM
$[(C_6H_5)_3Sn(p\text{-}HBZA)]$	25-35 nM
$[(n\text{-}Bu)_3Sn(TBA)\cdot H_2O]$	**125 nM**
$\{[Ph_3Sn(O\text{-}HTBA)]\}_n$	**133 nM**
$[(C_6H_5)_3Sn(MNA)Sn(C_6H_5)_3](acetone)]$ (B3)	**5 nM**
$[(C_6H_5)_3Sn(MBZT)]$	1500–3000 nM
$[(C_6H_5)_3Sn(MBZO)]$	1300–3000 nM
$[(C_6H_5)_3Sn(CMBZT)]$	500–800 nM
$[(C_6H_5)_2\ Sn(CMBZT)_2]$	**300–500 nM**
$[(n\text{-}C_4H_9)_2Sn(CMBZT)_2]$	600–800 nM
$[(CH_3)_2\ Sn(CMBZT)_2]$	5000–7500 nM
$[(CH_3)_2Sn(PMT)_2]$	20000–60000 nM
$[(n\text{-}C_4H_9)_2Sn(PMT)_2]$	700 nM
$[(C_6H_5)_2Sn(PMT)_2]$	1000-2000 nM
$[(C_6H_5)_3Sn(PMT)]$	**100 nM**

IC_{50} values of organotin(IV) complexes against Leimiosarcoma (LMS) cells.

Cisplatin IC_{50}= 4-5 μM

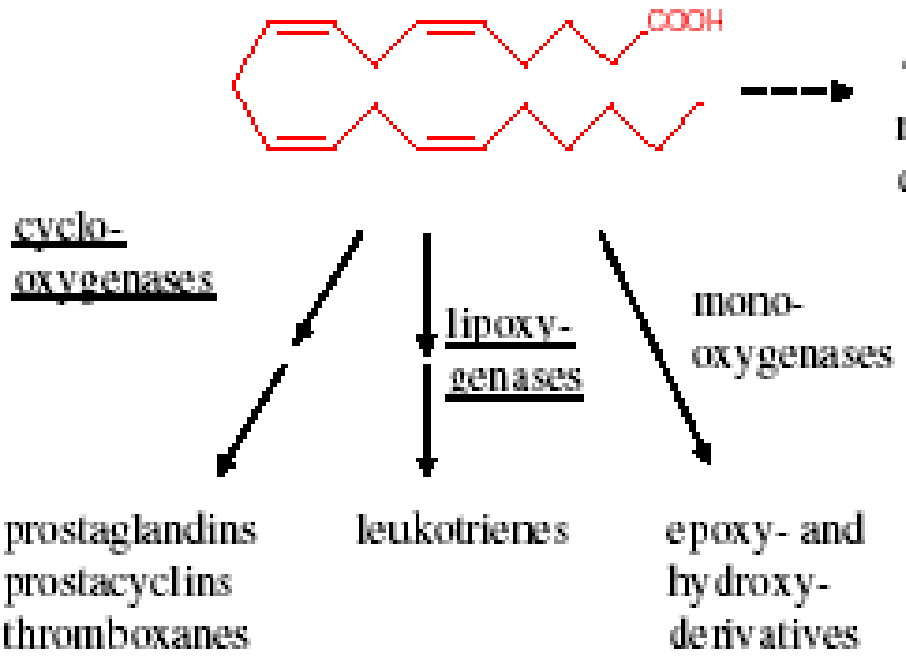
It is well known that many drugs which inhibit the growth of tumor cells act either by interfering with the the double helix of DNA or with the metalloenzymes that are necessary for the rapid growth of malignant cells.

No evidence for the $Et_2Sn(IV)$-nucleotide interaction was found at intermediate pH values (4.0-9.5), [M. Gielen, N. Hadjiliadis, et.al, Eur. J. Inorg. Chem. (2000) 513].

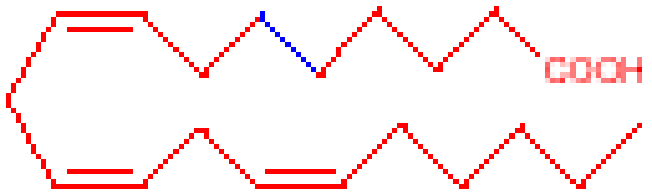
These results may indicate that organotin's anti-cancer activity may involve not only DNA –tin complexes interaction at physiological conditions but metalloenzymes-tin complexes as well.

Lipoxygenase (LOX) – organotin complexes interaction

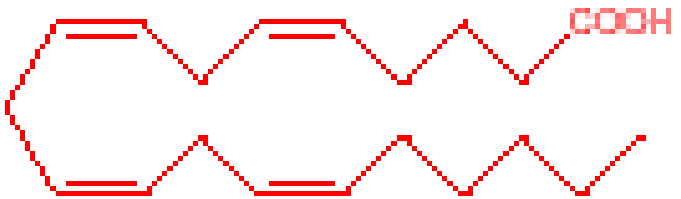
Enzymatic Peroxidation of poly-unsaturated fatty acids, such as **Linoleic acid** and **Arachidonic acid by the enzymes cycloxygenase (COX) or Lipoxygenase (LOX)**



Linoleic acid



Arachidonic acid



Poly-unsaturated fatty acids, such as Linoleic acid and Arachidonic acid are converted to prostaglandins (PG) or leukotrienes by **LOX** in an esential mechanism for the cell life. **Prostaglandines** contribute to tumorigenesis acting as angiogenesis factors [A.M. De Marzo et.al., *Cancer Letters*, 215, (2004), 1- 20]. **Linoleic acid**, discovered in beef and dairy products, was proven to be a potential mutagen inhibitor [M.W. Parka, *J. Agric. Food Chem.* 1989, 37, 75-81]

IC_{50} values for LOX inhibition activity and cell cytotoxic activity of organotin(IV) complexes

Compound	IC_{50} for LOX inhibition	IC_{50} cell activity
$[(C_6H_5)_3Sn(MNA)Sn(C_6H_5)_3](acetone)]$	**14 µM**	**5-20 nM**
$[(C_6H_5)_2Sn(HMNA)Cl]$	26 µM	
$[(C_6H_5)_3Sn(MBZT)]$	19 µM	1500–3000 nM
$[(C_6H_5)_3Sn(MBZO)]$	16 µM	1300–3000 nM
$[(C_6H_5)_3Sn(CMBZT)]$	21 µM	500–800 nM
$[(C_6H_5)_2 Sn(CMBZT)_2]$	**10 µM**	**300–500 nM**
$[(n\text{-}C_4H_9)_2Sn(CMBZT)_2]$	13 µM	600–800 nM
$[(CH_3)_2 Sn(CMBZT)_2]$	14 µM	5000–7500 nM
$[(CH_3)_2Sn(PMT)_2]$	61 µM	20000–60000 nM
$[(n\text{-}C_4H_9)_2Sn(PMT)_2]$	26 µM	700 nM
$[(C_6H_5)_2Sn(PMT)_2]$	21 µM	1000-2000 nM
$[(C_6H_5)_3Sn(PMT)]$	**17 µM**	**100 nM**

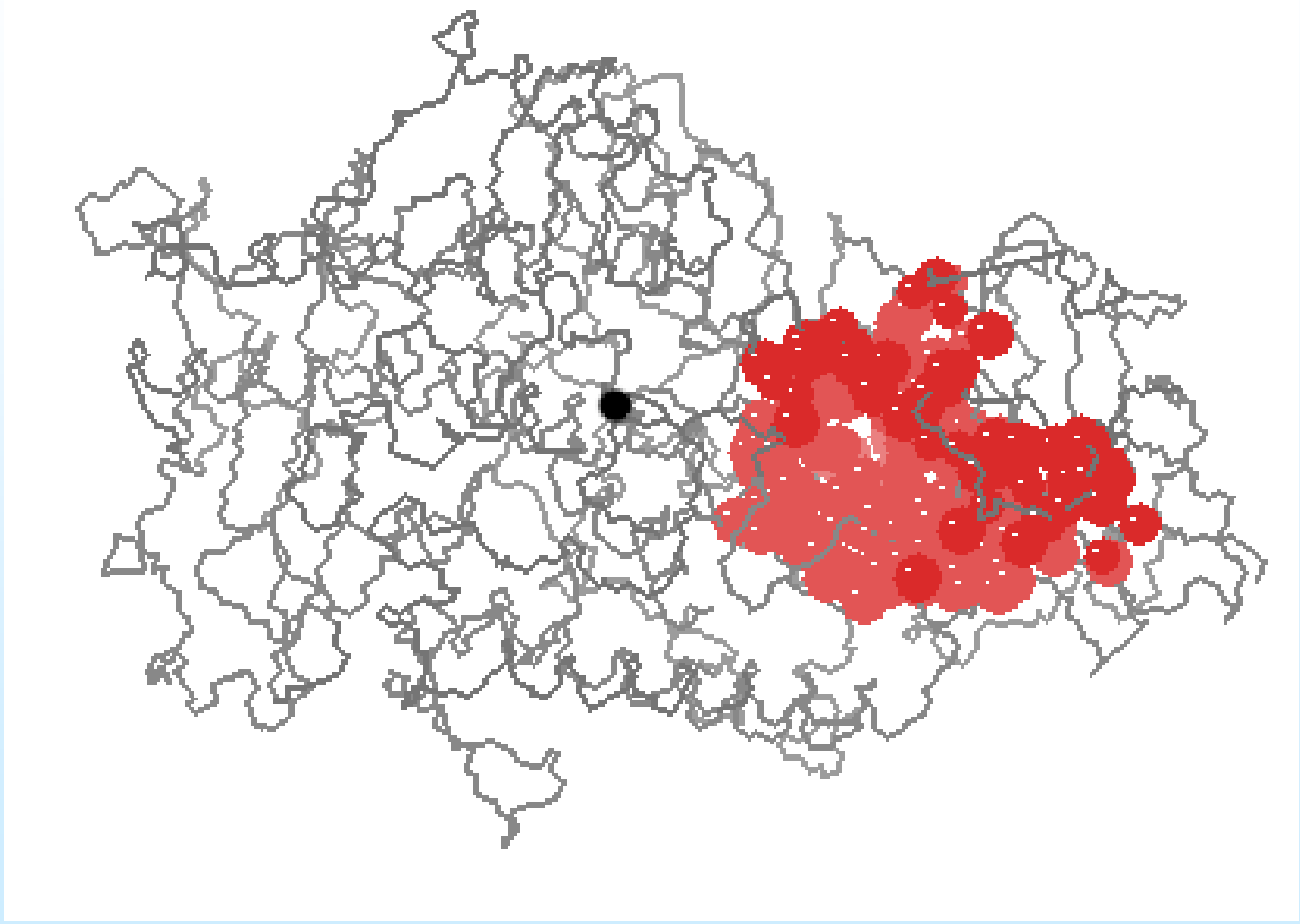
The inhibition type caused by the complexes, was studied by steady state kinetics.

The reversible type of inhibition was firstly tested. The incubating time showed no influence on enzyme activity in the presence of constant complex concentration, suggesting a reversible type of inhibition.

The kinetic parameters (Km and Vmax) of complexes studied were evaluated by performing a series of experiments with various substrate concentrations in the presence of complexes in constant concentrations.

Thus, the compounds studied, inhibit the enzyme with a reversible type, **mixed inhibition mechanism**. In this mechanism both the **EI** (enzyme–inhibitor) and **ESI** (enzyme–substrate–inhibitor) complexes are formed.

Binding sites of the inhibitors (**1C**) - (**5C**), (2A) studied.



Conclusions

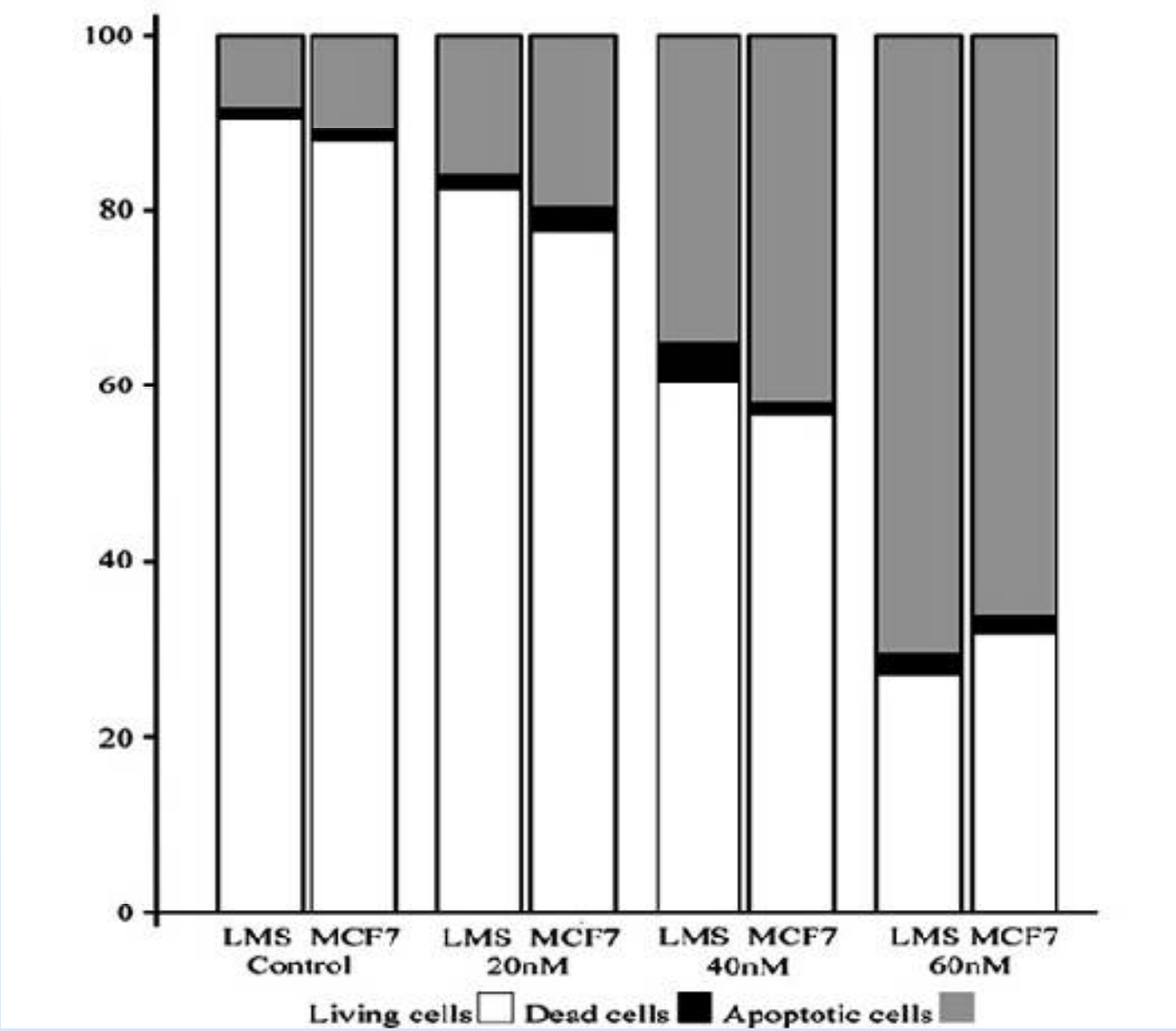
According to Huber et.al [*Coord. Chem. Rev.*; 95, (1989), 109-123] the structures of all organotins anti-tumor active compounds are characterized by (i) **the availability of coordination positions at Sn** and (ii) the occurrence of relatively **stable ligand-Sn bonds**, e.g. Sn-N and Sn-S and their slow hydrolytic decomposition.

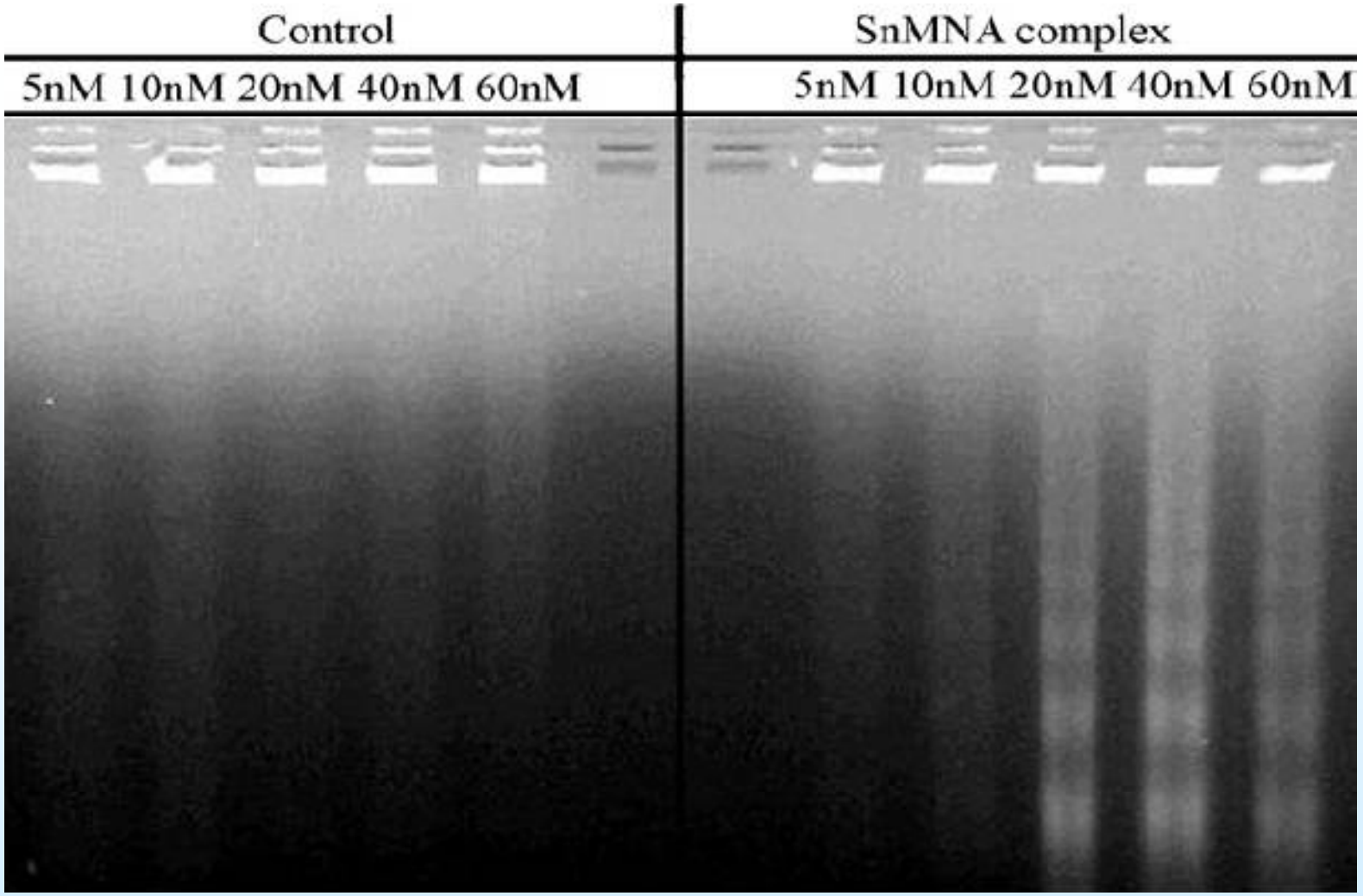
These general conditions are followed by organotin-thioamide complexes studied.

However, in case of complexes **D1-D5** and **B2** the highest cytotoxic activity is shown by the six coordinated complex **D4** which has no free coordination position while its Sn-S and Sn-N bond distances are found to be shorter than those found in tri-organotin complexes. Therefore, the geometrical feature of this type of complexes seems to play a less important role.

Anti-proliferative activity of the complexes studied here, follows the same rate found for the LOX activity inhibition.

The mechanism of action of the $Ph_3Sn(MNA)SnPh_3 \cdot Me_2CO$ complex was also studied by flow cytometry and DNA fragmentation assay





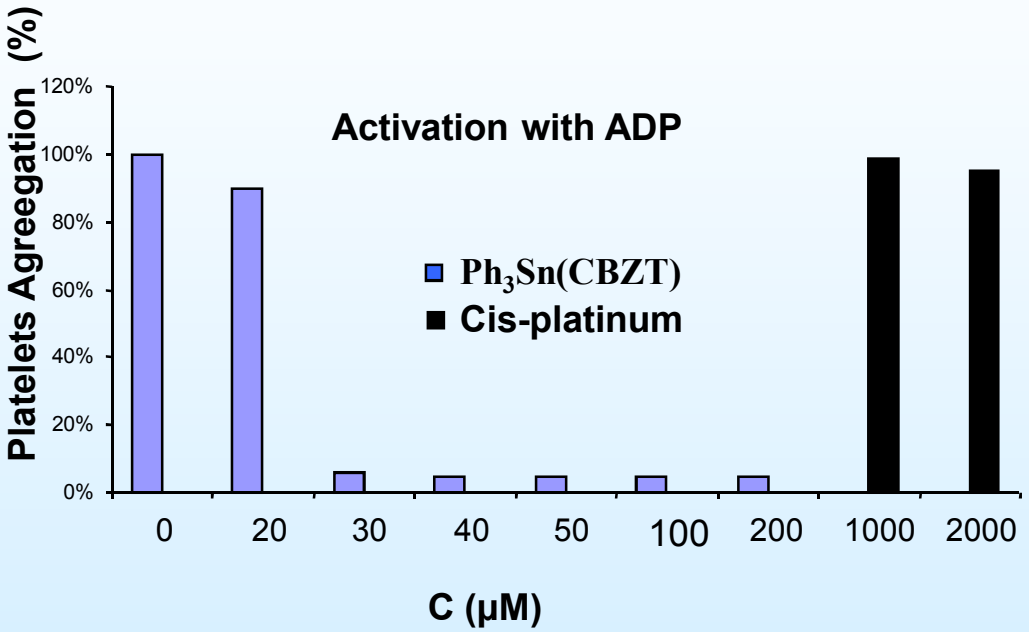
LMS cells were treated with increasing concentrations of the SnMNA complex from 5 nM to 60 nM.DNA laddering assay shows the typical pattern of fragmented or compacted nuclei. Untreated LMS cells showed viability and impact nuclei. SnMNA complex was able to caused apoptosis over 20nM

Antitumor activity of SnMNA given i.p. as repeated doses against tumor bearing Wistar rats.

Dose (mg/kg BW)	MST (days)	Mean tumor weight (g)	MTGR
Control (CG)	23.9 ± 3.21	68.1 ± 6.70	2.85 ± 0.20
4×5.4 (EG)	21.3 ± 2.87[a]	N/A	N/A
	48.0 ± 14.93[b,*]	30.7 ± 4.60[b,*]	0.78 ± 0.12[*]

N/A: not available–melted and regressed tumor. MST, mean survival time; MTGR, mean tumor growth rate; BW, body weight; CG, control group; EG, experimental group; SD, standard deviation.
a 40% of the animals with melted and regressed tumors.
b 30% of the animals with palpable tumors.
It is noteworthy that the tumors of 3 animals (30%) from the EG were totally regressed and the animals were cured and still alive
* Statistically different from the CG, $p < 0.05$. Data are presented as mean±SD.

Platelets aggregation which are enhanced with the metastatic potential of cancer cells



$Ph_3Sn(CBZT)$ complex inhibit the platelets aggregation

Antitumor activity of CBZT-5 given i.p. as repeated doses against tumor bearing Wistar rats.

Ομάδες	MST (days) MT +/-SD	BW (gr)	Mean Toumor Weight (gr)	MTGR (g/day) MT+/- SD
Control (CG)	23±3.3	190.0±8.9	68.1±5.3	2.96±0.4
Group 1 (EG) 4 x 0.5 mg/Kgr	33.8±2.7**	327.2±34.6**	111.4±32.2*	3.30±0.9
Group 2 (EG) 3 x 0.7 mg/Kgr	39.2±6.8**	338.4±74.3**	128.5±45.4*	3.26±0.9

CG, control group; EG, experimental group; MST, mean survival time; MTGR, mean tumor growth rate; BW, body weight; SD, standard deviation.
**Statistically different from the CG, $p < 0.05$. Data are presented as mean±SD.

Animals with metastasis after treatment with CBZT-5.

	Number of Animals	**Animals with metastasis**	**%**
Control (CG)	10	4	40%
Group 1 (EG)	15	0	0%
Group 2 (EG)	15	0	0%

IC_{50} values for cell viability found for complexes **1** and **2** against HeLa (cervical), OAW-42 (ovarian), MCF-7 (breast, ER positive), MDA-MB-231 (breast, ER negative), A549 (lung), Caki-1 (renal) and additionally, the normal human lung cell line MRC-5 (normal human fetal lung fibroblast cells) and normal immortalized human mammary gland epithelial cell line (MTSV17) cell lines

IC_{50} values (μM)*								
Compounds	**Cell lines**							
	MRC-5	**MTSV17**	**MCF-7**	**OAW-42**	**HeLa**	**A-549**	**MDA-MB-231**	**Caki-1**
1	0.130	0.092	0.103	0.200	0.105	0.235	0.203	0.120
2	0.108	0.070	0.068	0.072	0.065	0.240	0.106	0.406
cisplatin	6.3	24.7	18.5	3.1	5.0	16.4	50.0	12.5

*IC_{50} values were derived from the corresponding dose-effect curves drawn from sextuplicate determinations with CV lower than 5%.

Flow cytometry assay results, showed that, compounds 1 and 2 mediate a strong cytotoxic response to normal and cancer cell lines tested through apoptosis and induce cell cycle arrest in S phase of the cell cycle.

Apoptotic cell death requires interaction with DNA (direct or indirect). Since there is no evidence for a direct organotin(IV) - nucleotide interaction at intermediate pH values (4.0-9.5) the anti-cancer activity of organotin's may not involve direct- interaction with DNA at physiological conditions.

In contrast, complexes 1 and 2 were found to interfere with lipoxygenase, by preventing oxidation of polyunsaturated fatty acids (such as linoleic acid) to their peroxo derivatives by the enzyme and by thus to interact with DNA indirect. Therefore, it could be proposed that organotin complexes 1 and 2 may be interfere with metalloenzymes that induce apoptosis to malignant cells. Thus, although, complexes 1 and 2 were found to interact somehow with DNA the exact mechanism of this interaction still remains unknown.

AKNOWLEGMENTS

Group Students:

Dr. A Metsios, Dr. Marianna Xanthopoulou., Dr Ibrahim.I. Ozturk, Dr Kostantinos Antoniadis, Mr Panagiotis Zachariadis M.Sc. Mr Sotiris Zartilas M.Sc., Mr Vasilis Balas M.Sc. Mr Kostantinos Kouroulis, M.Sc., Mr Loukas Kyros, M.Sc

Prof. M Kubicki, *Department of Chemistry, A. Mickiewicz, University, Poznan, Poland.*

Prof. S. Skoulika, *Department of Chemistry, University of Ioannina, Greece.*

Prof. A. Tasiopoulos, *Department of Chemistry, University of Cyprus Cyprus*

Prof. K Charalabopoulos, **Prof. S. Karkabounas**, **Prof. N Kourkoumelis,** Medical School, University of Ioannina, Greece.

Prof. Jan Balzarini, Katholieke Universiteit Leuven, Rega Institute For Medical Research

Prof. E.R. Milaeva, *Chemistry Department, M.V. Lomonosov Moscow State University, Moscow, Russia.*

Prof. I.S. Butler, Department of Chemistry, McGill University, Montreal, Quebec, Canada