Handout IRTG, May 2009

- Jan Reedijk. Leiden Institute of Chemistry
- Handout #2; metal-DNA/metal-anticancer
- Slide copies, stored as pdf; allowed for private use only.

10 pages; May 6, 2009

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Metal DNA & anticancer related to ligands

- Introduction cisplatin
- Mechanism of action Pt anticancer
- Other metal-anticancer compounds
- Multifunctional interactions

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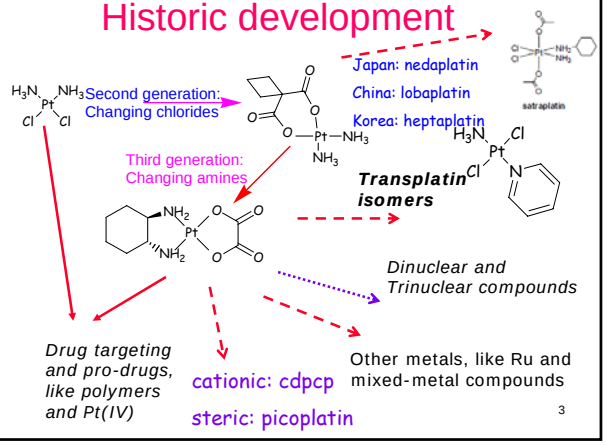
Historic development



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Statistics Pt use

- A patient will receive about 0.5 gram cisplatin, or up to 5 grams carboplatin.
- 20-30% of population will one day receive chemotherapy
- 5% of population will one day receive Pt therapy
- Average age 80; so each year 1/80 of polutaion to be considered;
- Germany 80 million; 5% of 1 million, 50000, each 1-2 gram: is 50-100 kg Pt each year
- (annual Pt production: 1200 ton/yr)

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Pt details

- 1200 ton/year world wide
- Costs: 20 euro gram
- Anti-cancer market 2007:
- All: 50 Geuro; Pt: 3 Geuro (75% is oxaliplatin);
- Drug development: 200 Meuro/drug
- P.J. Sadler, Chemistry & Industry 23 February 2009, page 18-20

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Hypothesis for the mechanism of cisplatin and related compounds

- Cisplatin binds at DNA on a very specific site: N7 positions of two guanine bases.
- The resulting distortion of the DNA is relatively small (a kink); replication (transcription) of platinated DNA in cells is blocked.
- The distortion is not recognized in certain (tumor) cells (and DNA is NOT repaired).
- In other (healthy, resistant) cells the damage is recognized (and repaired).

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Differences Organic and Inorganic Drugs

- Easy one-electron reductions possible with many metal ions;
- Easy changes in structure due to metal-ligand dissociation reactions:
  use as pro-drugs;
  kinetic differences are metal dependent.
- Metal ions as such are non-degradable

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Two major types of metal drugs

- Drugs with slow metals (like Ru, Pt) or slowly exchanging chelating ligands, like porphyrins;
- Drugs with rapid metals (fast ligand exchanges, on cellular/biological time scales).
- NB: in both cases the M-L bond strength is 80-150 kJ/mol

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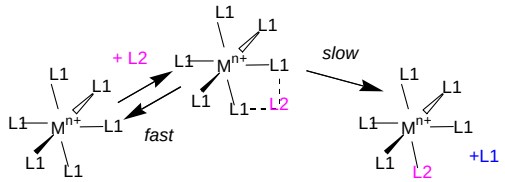
Metal-Ligand Exchange Kinetics



First step: diffusion controlled
Second step: relatively slow

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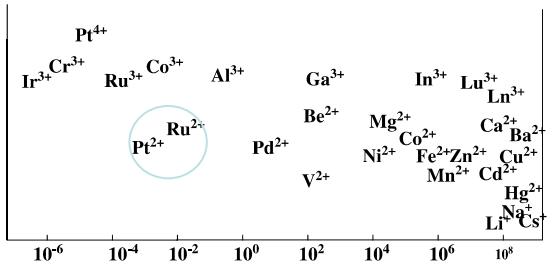

Ligand exchange rates (s^{-1}) for aqua complexes of metal ions

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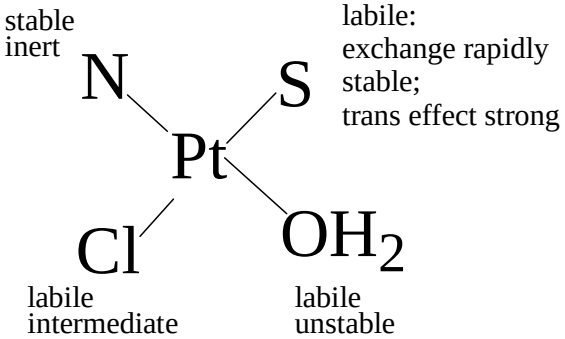

labile:
exchange rapidly
stable;
trans effect strong

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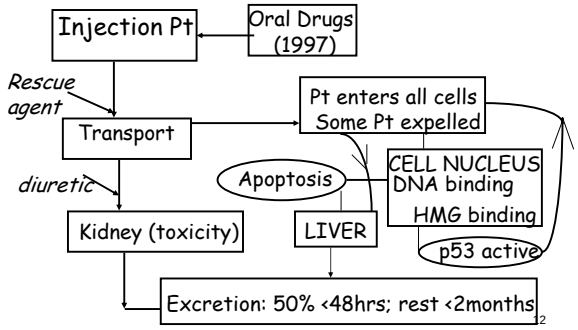
Transport of Pt in the body



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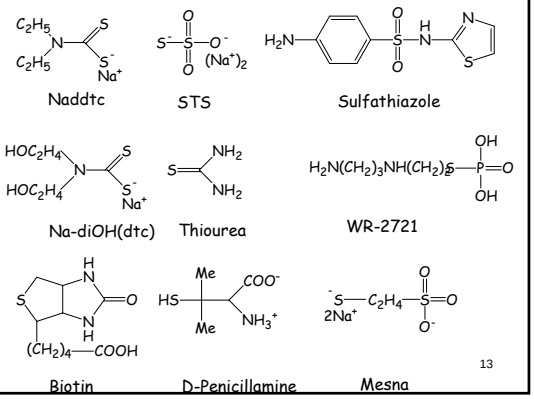
Rescue agents overview



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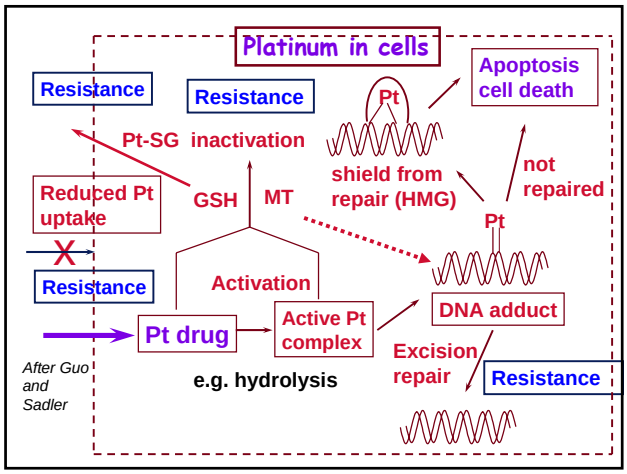

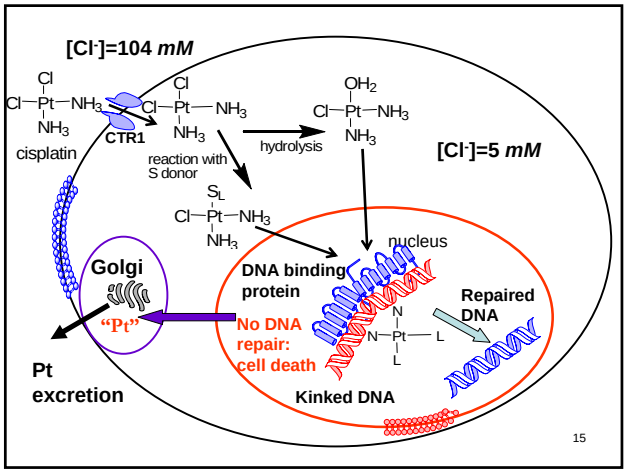

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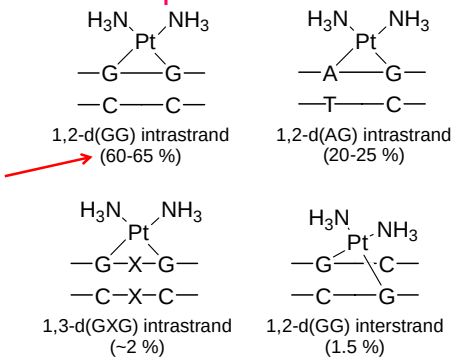
Most frequent Pt-DNA adducts



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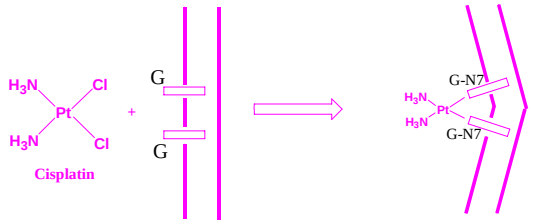
Most frequent chelation on DNA



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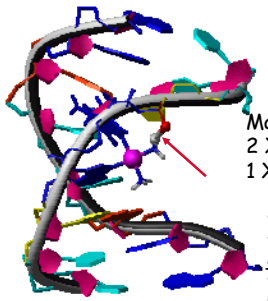
1,2 (GG) intrastrand structure



Many NMR structures
2 X-ray structures (high res)
1 X-ray complexed with HMG

- *Pt in major groove*
- *Duplex is bent toward major groove*
- *Duplex is unwound*
- *B-DNA preserved , A/B hybrid in the X-ray structure*
- *minor groove opens up*

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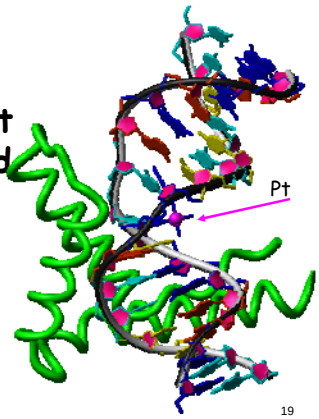
Structure of HMG protein at 1,2 intrastrand GG adduct



S.J. Lippard

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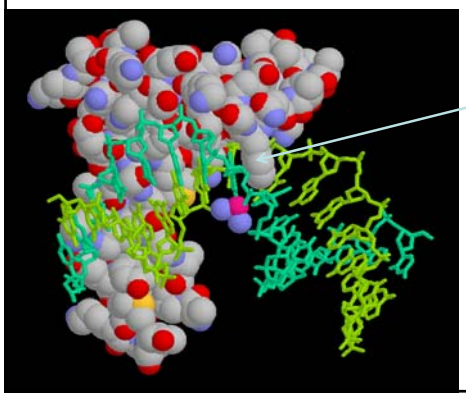
A protein maintains the kink



Phenylalanine sits in between the G's of the coordinated GG

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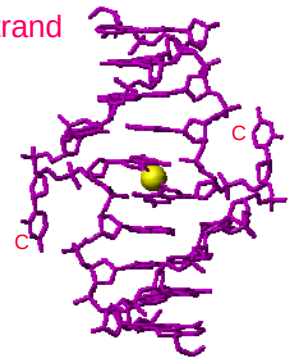
Cisplatin GG interstrand structure

TCTCGTCTCT
Pt
AGAGCAGAGA



Malinge, Leng: 1999

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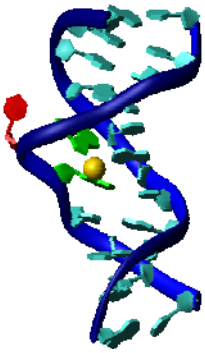
1,3 (GTG) intrastrand structure



- Pt in major groove
- ~30 bending toward major groove
- central thymine extruded
- minor groove widened

J.M. Teuben, PhD thesis, Leiden

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Why do we still need new drugs?

- Better water and saline solubility
- Avoid/reduce the toxic side effects
- Easy administration (e.g. oral)
- Circumvent resistance development
- Selectivity for tumors that are not responding to cisplatin

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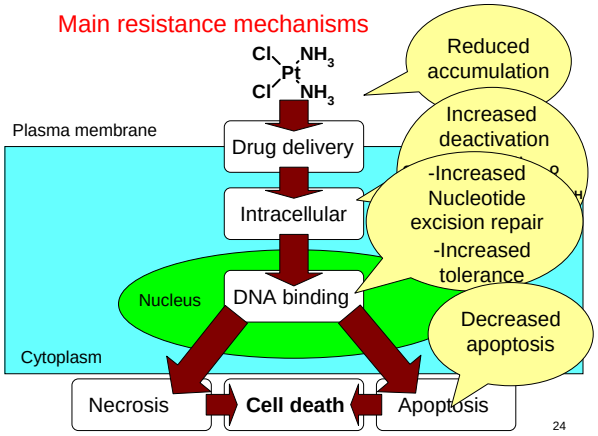
Main resistance mechanisms



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Chemical Requirements for new antitumor drugs

- Should be patentable
  Water soluble and water stable
- Survive blood protein attacks (pro-drug?)
  Pass (tumor) cell membranes
- Survive S-attack in cell; binds to DNA
- Give unique DNA binding (resistance)
  Active against cisplatin resistant cells

THIS CAN BE ANY METAL COMPOUND, provided it has proper ligand exchange kinetics

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Many approaches are followed worldwide

- **EXAMPLES from others and us:**
- Photoactivation of Pt compopunds
- Trans compounds
- Ru coordination compounds
- Organometallic Ru compounds
- Dinuclear and trinuclear compounds
- Recent: monofunctional Pt compound

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Newest data

- Introduction of bifunctionality
- Fluorescers attached
- Second metal (homonuclear): bis-Pt
- Second metal (heteronuclear):
  Pt-Ru and Pt-Cu

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Why interest in bi- and trifunctional (metal) binding to DNA?

- Recognition and action can be separated in space (and time);
- Monitor function (like fluorescer) can be added to an action function;
- Two (different) actions can be combined, perhaps even synergistically.
- Way to new drugs? Examples.

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Selection from our newest data

- Add bifunctionality to compounds:

- Fluorescers attached (for detection)
- Second metal (homonuclear): bis-Pt
- Second metal (heteronuclear): Pt-Ru and Pt-Cu
- Intercalators attached (they may be fluorescers at the same time)

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Following the Pt species in the cell to and from DNA

- Make a cisplatin analog with as a tail a fluorescing label (or a precursor);
- Incubate cells with this compound; label and Pt must stay together;
- Follow fluorescence in time and space, using digital analysing techniques;
- Visualise the process with a camera.

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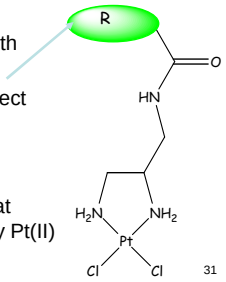
Powerful method to monitor cellular distribution; see JBIC, 9 (2004), 414

- Study Pt distribution using digital fluorescence microscopy
- Functionalize cisPt analogue with fluorescent tag, i.e.
  - Fluorescein derivative for direct detection in living cells
  - A smaller probe for indirect immunodetection
- 2 parallel techniques ensure that observed results are induced by Pt(II) metal



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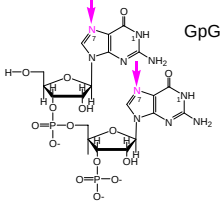
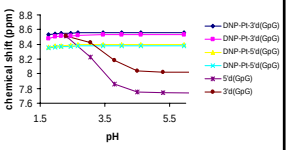
Reactions of CFDA-Pt and DNP-Pt

- CFDA-Pt and DNP-Pt obtained in acceptable yield (55-70%)
- Purity analyzed by ^{195}Pt and ^{1}H NMR
- DNA binding capacity tested by reacting with GpG , pH titration with NMR:
  ⇒ reacts like parent compound





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Main Conclusions Fluorolabeling

- Dynamic Imaging possible of Pt compounds in *living* cells.
- Experiments gave new insights in Pt(II) distribution:
  - rapid cellular uptake (<15 min) (Role of Cu and CTR1?)
  - Accumulation in the nucleus (<2 h)
  - After 6-8 h secretion of CFDA-Pt and accumulation in Golgi apparatus
  - After 24 h <1% of CFDA-Pt remains in the cell
  - **Now also applied for drugs which contain intercalating fluorescers (JBIC, 9 (2004), 414)**

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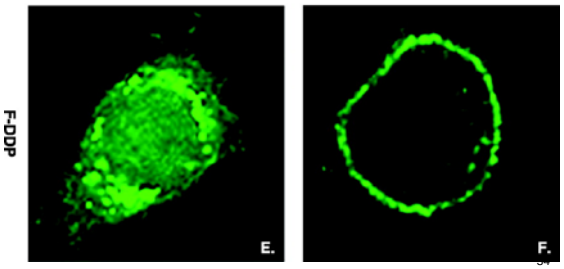
Left: normal cell; Right: resistant cell: no uptake of the fluorescent Pt



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Can we design new Pt compounds that precisely bind to DNA and that:

- 1) Give crosslinks,
- 2) Have similar H-bonds and kinetics (preferentially somewhat slower than cisplatin)
- 3) Do not distort the DNA very much
- YES:
  Dinuclear Pt compounds with a rigid bridge!

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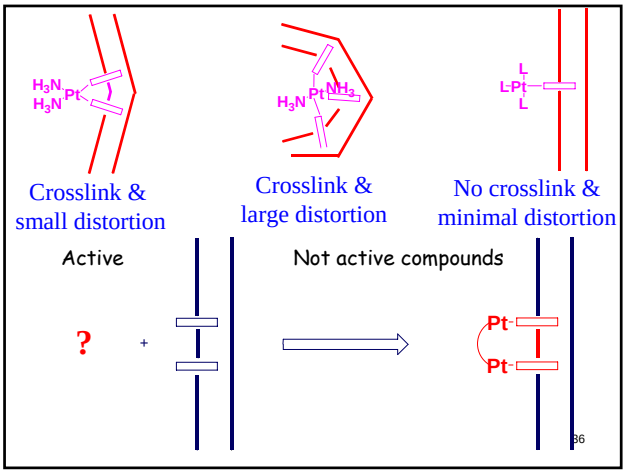

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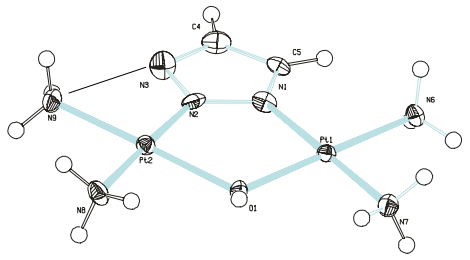
Crystal structure of a **cationic** triazolato bis-Pt complex



S. Komeda, PhD thesis, Leiden University

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Kinetics of DNA binding for the dinuclear Pt-azole bridged compounds:
It is in between cisplatin and carboplatin!

- Role of bridging azole: stabilisation (thermodynamically)
- Role of bridging hydroxide: stabilisation (kinetically)

S. Komeda et al. *J. Med. Chem.*, *46*, 1210-1219.

- Review:(J. Reedijk, EURJIC, 1303-1312)

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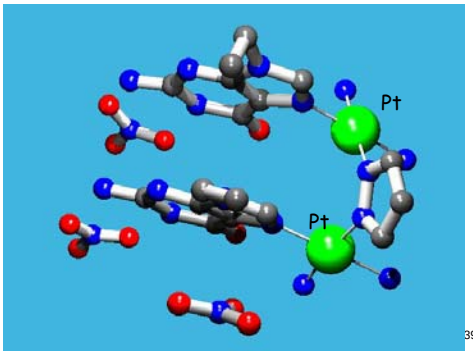
Reaction with model DNA base: Almost co-planar G bases



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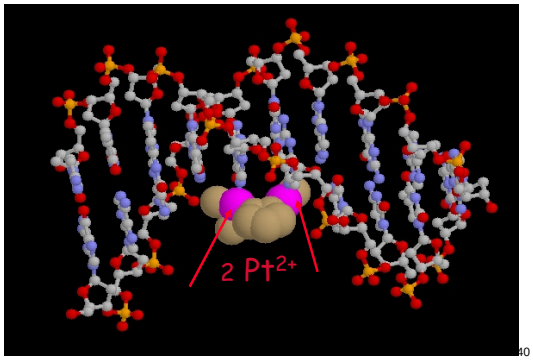
Two platinum atoms together can better bind to the DNA; Chem.Eur.J. 2006, *12*, 3741



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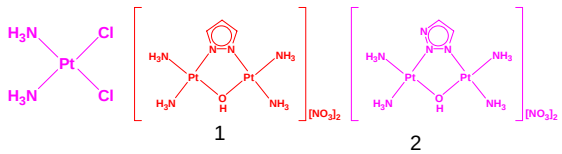

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IC_{50}(μM) values in 7 tumor cell lines

Compound	MCF7	EVSA-T	WIDR	IGROV	M19	A498	H226
Cisplatin	2.33	1.41	3.22	0.56	1.86	7.51	10.9
1	0.06	0.15	0.12	0.59	0.05	0.53	0.68
2	0.09	0.32	0.40	0.13	0.19	1.24	2.72

MCF7: breast cancer, EVSA-T: breast cancer, WIDR: colon cancer, IGROV: ovarian cancer; M19: melanoma, A498: renal cancer, H226: non-small cell lung cancer

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What next in Pt-based compounds?

- Addition of a second metal (Ru, Cu) Addition of a redox function
- Intercalator added
- Drugs with a built-in target (tumor cell) finder?
- Drugs with special functionalities to prevent side effects, to find the nucleus in the cell, to help excretion after cell killing, etc.

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How do Pt and Ru differ?

- Redox behaviour (1e, 2e); potentials
- Coordination Numbers (4 vs. 6)
- Ligand-exchange kinetics
- Preferred Oxidation States (2+ and 4+ for Pt; 2+ and 3+ for Ru)
- Similarities: Affinity for DNA bases; Ligand exchange kinetics
- Reviews: J. Reedijk, PNAS, 100, 3611-3616; Plat Met.Rev. 52, 2-11

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Some data on Pt-Ru compounds

- Dinuclear Pt-Ru compounds: Pt goes to N7 of DNA (major groove), while Ru can bind in minor groove:
- Synthesis, structure in solution, XRD and anticancer activity
- Oligonuclear Pt and Ru compounds

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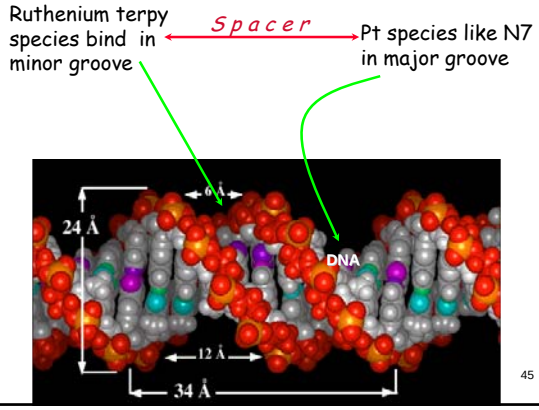

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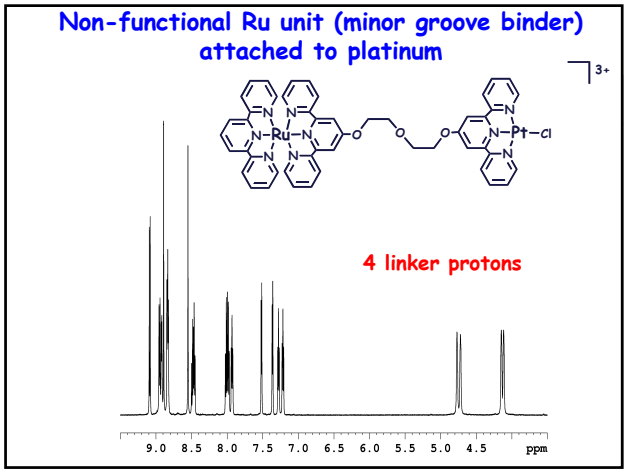

Binding of Ru species to DNA

1. $Ru(LL)_3^{n+}$ could bind as intercalator (with very large LL) or in groove (Barton, Norden)

2. $Ru(LL)_2(X)_2^{n+}$ could bind to a purine-N7 (preference for G) (Thorpe, Mestroni, Marzilli, Van Vliet)

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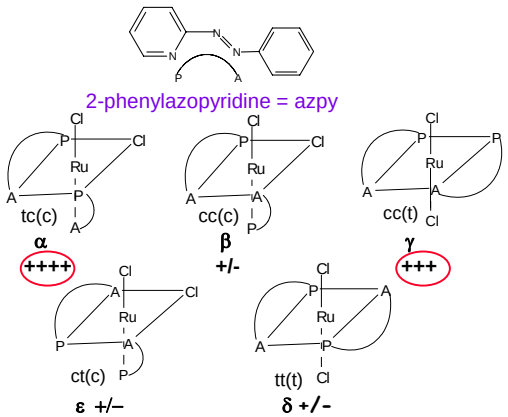
2 active of 5 Isomers of $[Ru(azpy)_2Cl_2]$



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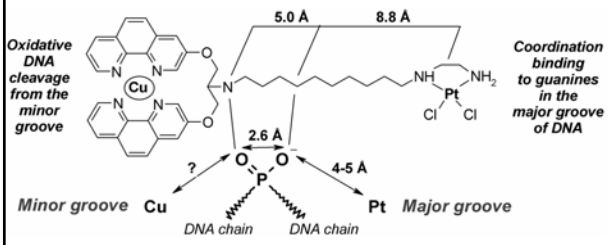
Copper and Platinum



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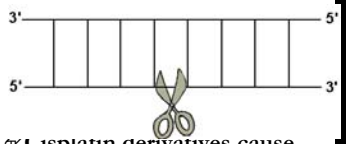
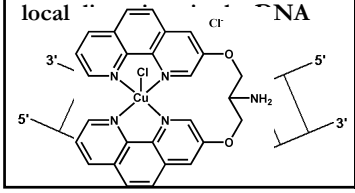
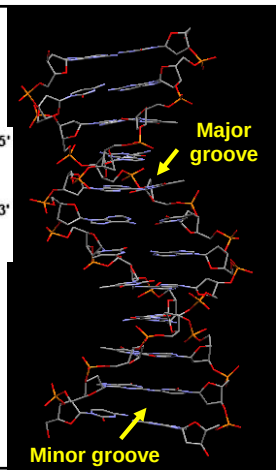
Aim of the project

Cu(3-Clip-Phen) is able to cleave DNA



Cisplatin derivatives cause local [illegible] DNA

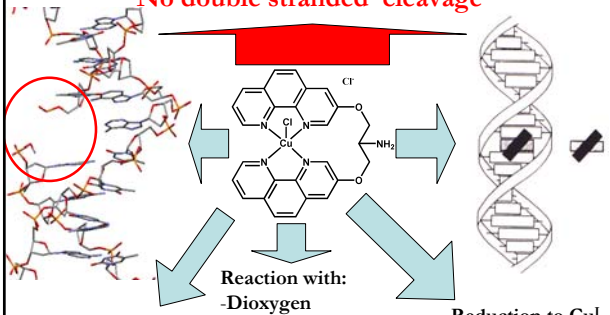




Mechanism of action of Cu(3-clip-phen)

Pitié, M *et al.* Advances in Inorganic Chemistry. **2006**, *58*, 77

No double stranded cleavage



H1', H4' or H5' abstraction

Reaction with:
-Dioxygen
-Dihydrogen peroxide
-Alkyl hydroperoxides

Reduction to Cu^{I}

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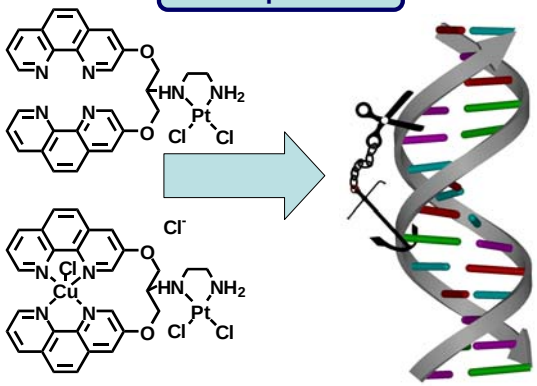
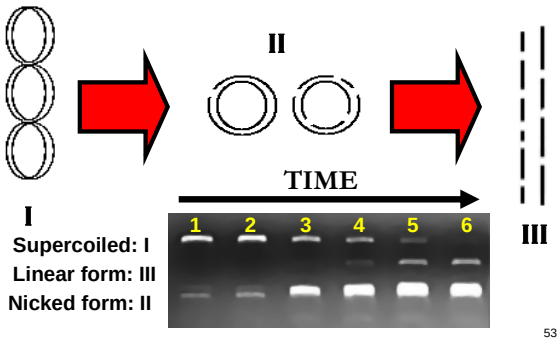
Complexes



Testing the DNA-cleaving activity on a plasmid



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First results on the Pt/Cu species

(P. de Hoog, et al., *J. Med. Chem., 50*, (2007), 3148-55; *J. Biol. Inorg. Chem.*, *13*, (2008), 575-86)

- Antitumor activity depending on length of the spacer: some much better than cisplatin; others hardly active.
- Double-strand cutting of plasmid DNA is possible in some cases; **depends on spacer and the length of the bridging chain**; significant differences between compounds have been observed.
- Sequence-dependent cutting by Cu-clip part, is induced by Pt binding at nearby GG sites.

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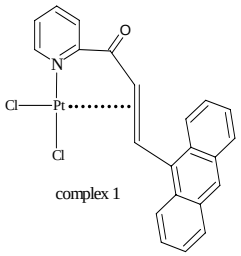
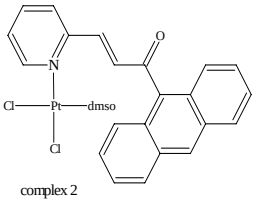
Acridine-based ligands and Pt complexes



complex 1



complex 2

No activity; high activity; also cbdca

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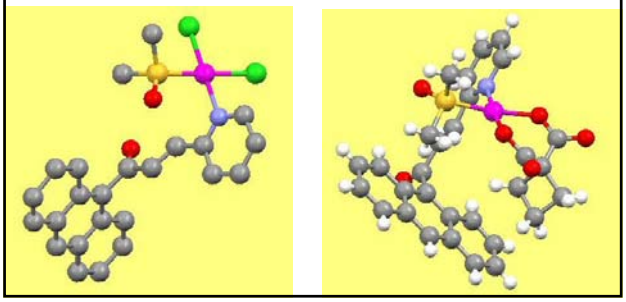
Newest compounds: Pt species with an acridine intercalator: highly active in several cell lines

IC_{50} (A_{2780}) = 2.4 µm, resp. 1.8 µm



Concluding Remarks(I)

- In metal-DNA binding the kinetics of the M-L binding are more important than the thermodynamic binding.
- The role of additional H-bonding interactions, both in the kinetics of the process, and in the stabilization of the adduct structure, is very important.

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Concluding Remarks(II)

- Cisplatin has many targets in the cells
- Much of the Pt species will never reach DNA
- Much of the Pt species will not stay long enough on the DNA
- A small fraction can reach the nuclear DNA and binds to guanine-N7
- Targeting of Pt is possible, e.g. to follow the pathway in the cell, or to add cell finder or, a DNA-cutting agent (Cu)

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Not (or poorly) understood matters

- Molecular basis rescue agents, side effects?
- Why cisplatin cures certain tumor cells only?
- How Pt enters the (tumor) cell membranes? How Pt reaches DNA with S ligands in cell? How is Pt removed from DNA (repair)?
- Is GG lesion most or only important lesion on the DNA? Any role for AG? For interstrand?
- How important is spacer length between metals
- How do "non-classical" Pt (Metal) antitumor drugs work? DNA binding, or other target?

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The future

- **Next stages antitumor drug design**:
- Development of dedicated drugs which comprise both the transport (through the membranes, or recognizing tumor cell surfaces), the survival in the cell, the binding to the DNA and - eventually - the excretion from the body, with minimum side effects.
- For this process, both (kinetically controlled) metal coordination and hydrogen bonding will be key factors on the molecular level.
- Reviews: J. Reedijk, PNAS, 100, 3611-3616; Plat Met.Rev. 52, 2-11; EURJIC, 2009, 1303-12

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