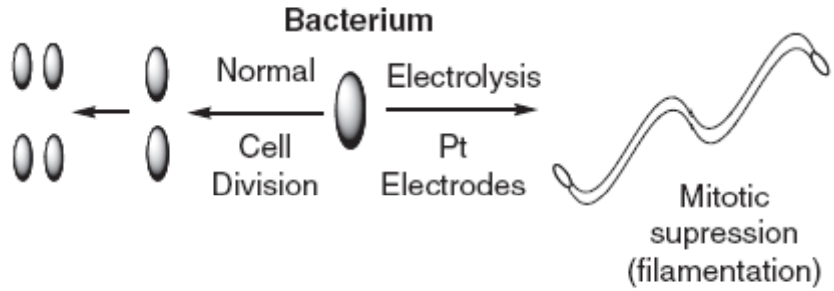
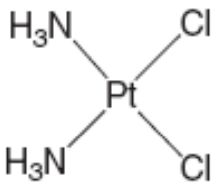
Cisplatin-based Anti-Cancer Drugs…

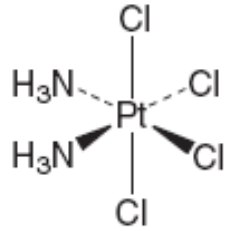
1964: *Michigan State Uni.*, Barnett Rosenberg found that Pt(II) inhibits cell division



A representation of mitotic suppression leading to filamentation



cis-DDP
or
cisplatin



cis- $[Pt(NH_3)_2Cl_4]$

cis-platin produced filamentation and *in the absence* of the electric field

cis-platin: prepared in 1845 by Peyrone…
in 1893 Werner predicted its correct formula kai geometry (sq. planar) !!!

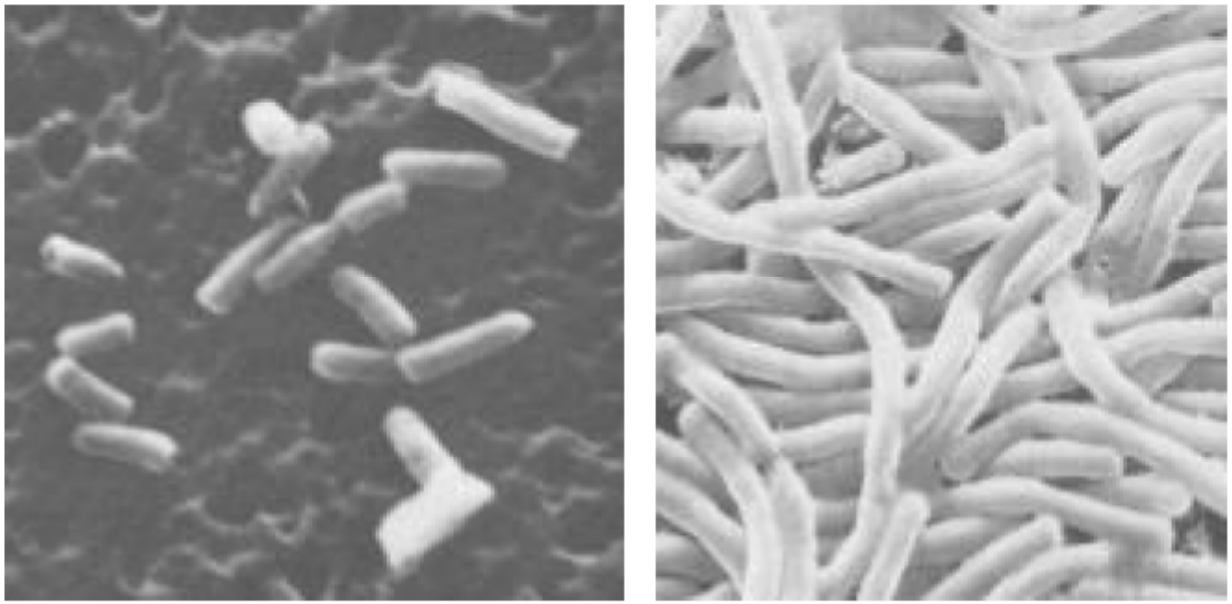


Figure 2.2. (A) *E. coli* bacteria, and **(B)** the filamentous growth of the same bacteria in the presence of cisplatin.

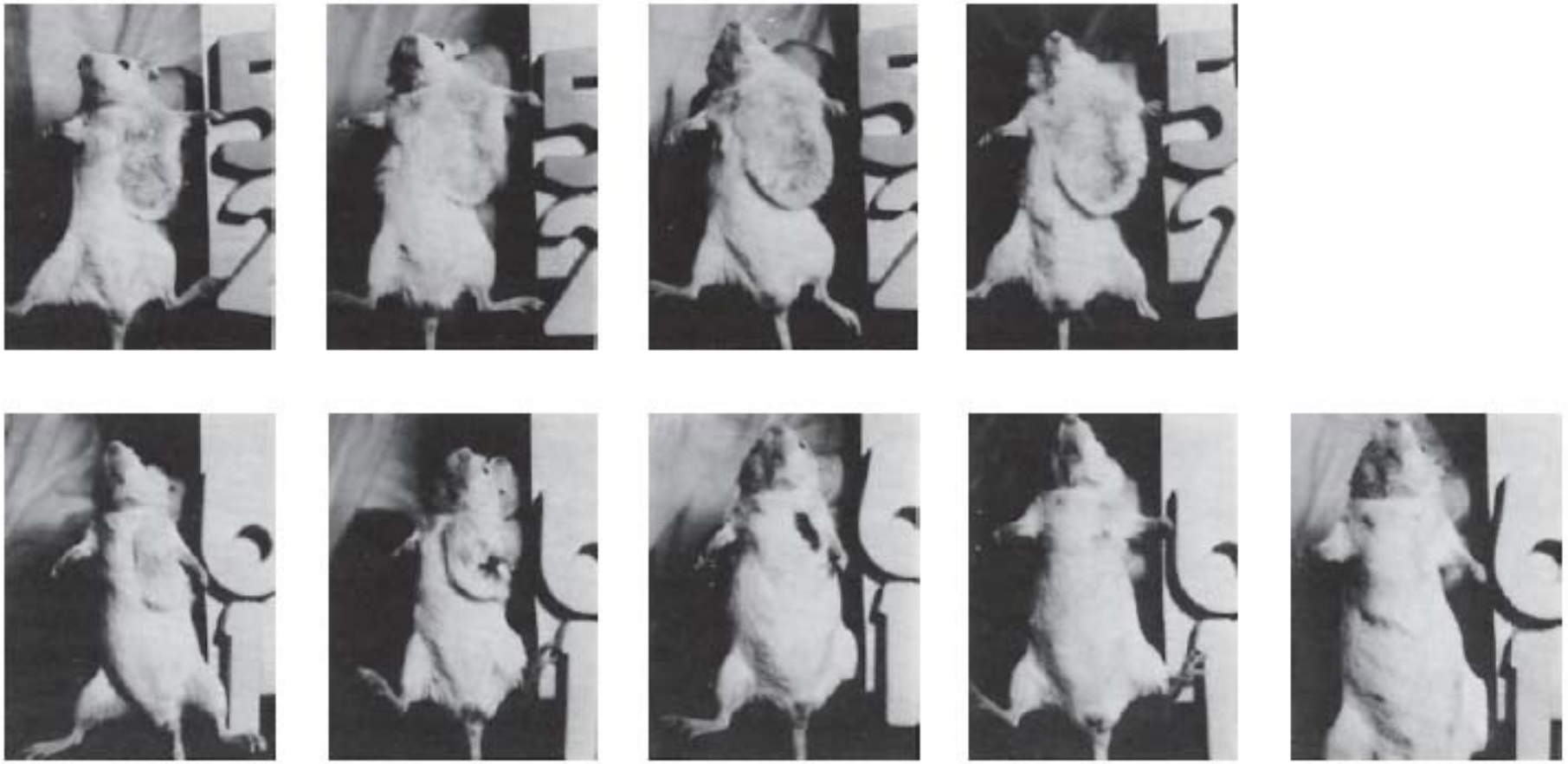


Fig. 3. *Time sequence photographs of two mice with solid Sarcoma-180 tumors*. The mouse at the top was an untreated negative control. She died on day 21 when the tumor weighed about 3 g. The bottom mouse was in the group treated on day 8 with an intraperitoneal injection of *cis*-dichlorodiammineplatinum(II). Her tumor was completely regressed six days after treatment, and she died of age-related causes almost 3 years later.

cis-platin

National Cancer Institute and MSU: mice with Sarcoma 180 tumors showed tumor regression after being injected with cis-platin. Control exps.: mice died after ~ 20 days when not treated with cis-platin.

Therapeutic Index: $LD_{50}/ID_{50} = 8$

1971: got into **Phase I**
1978: FDA approved it for **testicular** and **ovarian cancer**

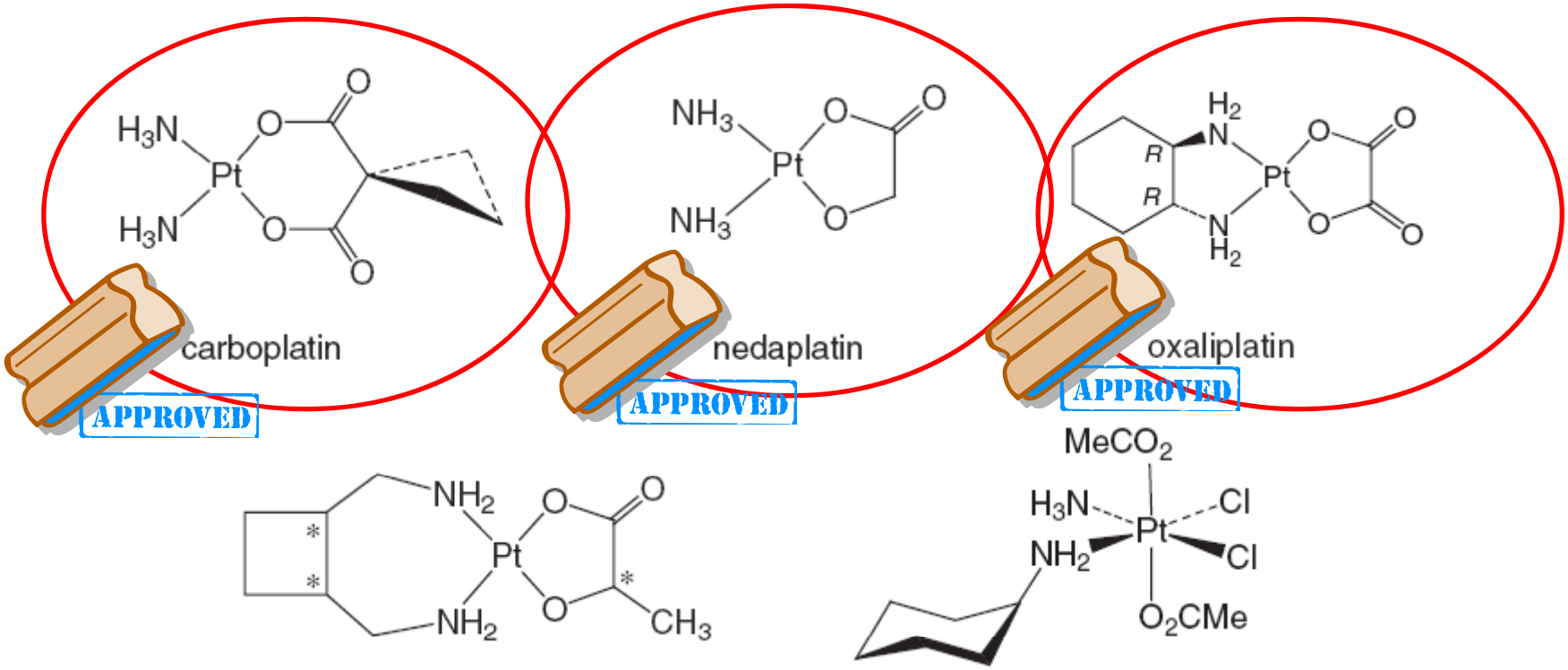
By **1983: the most famous a/c drug** for type **A** and **B carcinomas.**

Weekly doses of 5mg kg^{-1} over a month

Major limitation: kidney toxicity. Needs plenty of H_2O intravenously and diuretic agents to flash it out of the kidney. Nausea, vomiting, bone marrow suppression.

Over 3000 Pt cmpds. to impove cis-platin…1% has entered clinical tries.

Structure and Reactivity…2nd generation drugs



Lobaplatin

Phase II

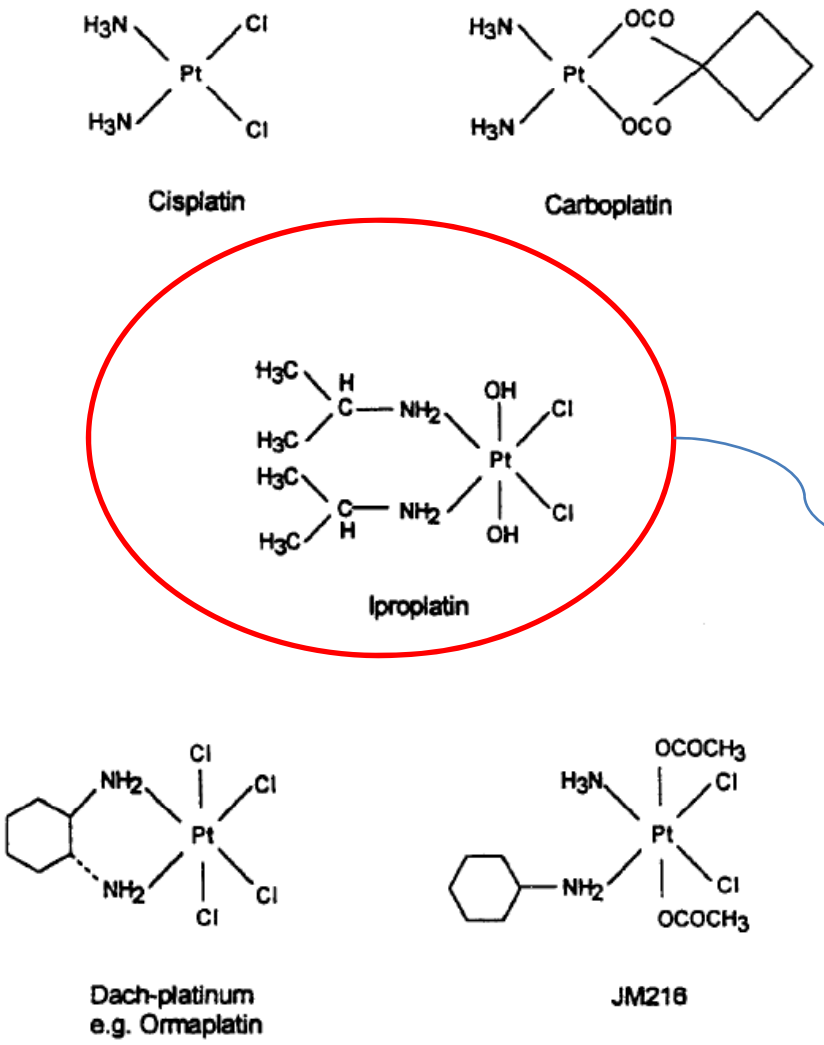
JM216

Oral,
Phase II

Cisplatin is unstable in water (half life 2.5 h at 310 K) and, for administration as an anticancer drug, it is formulated in saline solution to prevent **aquation**

Table 1 *Comparative clinical properties of cisplatin and carboplatin*

	Cisplatin	*Carboplatin*
Typical dose/schedule		
	100 mg m^{-2}; i.v. q 3–4 weeks or 20 mg m^{-2} daily for 5 days, q 3–4 weeks	400 mg m^{-2}; i.v. q 3–4 weeks
Major toxicities		
	Nephrotoxicity Severe nausea and vomiting Neurotoxicity (peripheral neuropathy) Ototoxicity (tinnitus/hearing loss)	Myelosuppression (mainly thrombocytopaenia)
Pharmacokinetics[22]		
(A) Total platinum		
$t_{1/2}\,\alpha$	20–40 min	1–3 h
$t_{1/2}\,\beta$	44–190 h	6.7– > 24 h
(B) Free (ultrafilterable) platinum		
$t_{1/2}\,\alpha$	22–78 min	87 min
$t_{1/2}\,\beta$	not seen	354 min
24 h Urinary platinum excretion (% of dose)[22]		
	16–35	65



Structures of platinum drugs that have undergone extensive clinical trials (cisplatin, carboplatin and iproplatin) or are new drugs currently in early clinical trials

Favourable efficacy in **preclinical** studies, *i.e.* Less nephrotoxicity but antitumour activity comparable to that of cisplatin!

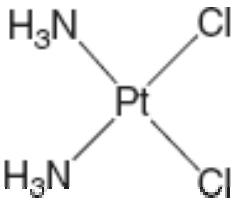
Iproplatin was the first quadrivalent platinum(IV) complex possessing an octahedral configuration, rather than the square-planar configuration of cisplatin and carboplatin, that entered clinical trials. To date, it is the only platinum drug besides cisplatin and carboplatin to have undergone extensive (including phase III) clinical trials....

But, **clinical trials** showed:

1) Toxicity profile similar to carboplatin,
2) Less active,
3) More toxic than carboplatin and significantly less active!

cis-platin

Well…how does cis-platin it work?...general Pt chemistry first



Platinum exists in two main oxidation states, Pt(II) and Pt(IV), respectively. In Pt(II) complexes such as cisplatin and carboplatin, the platinum atom has four bonds directed to the corners of a square plane at which the four ligand atoms are located.

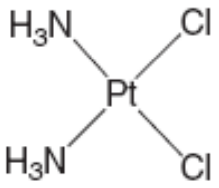
In contrast, in Pt(IV) complexes (such as iproplatin) there are six bonds and ligands: four in a square-planar configuration, and two located axially, directly above and below the platinum, thus producing an octahedral configuration.

The **stereospecificity** of the bonds is also of importance, as exemplified by the contrasting biological properties of the two isomers, cis-platin and trans-platin (which is inactive).

The chemical properties of Pt coordination complexes are largely **dependent on the relative displacement reactions of the various ligands**. While some platinum bonds *(e.g. those to N or S) are essentially irreversible* under physiological conditions, the stability of bonds to halogens and especially to **H_20 is much lower**. **Thus, cisplatin reacts primarily by stepwise exchange of its two labile Cl (leaving ligands) for water or hydroxyl ions. The final positively charged, highly reactive diaquo species (which is the same for cisplatin and carboplatin) is then capable of reacting with nucleophilic sites on DNA, RNA, or proteins**

Few important early facts…for in vivo mice cancer

1) *cis*-[$Pt(NH_3)_2Cl_4$] also works
2) *trans* **don't** work
3) Pd analogues **don't** work
4) Pt analogues with pyridine ligands **don't** work
5) When NH_3 is replaced by PR_3 or SR_2 **don't** work
6) 2 leaving groups in ***cis*** position
7) Non-leaving groups with weak trans-effect, e.g. NH_3
8) **At least one** NH group bound to Pt



cis-DDP
or
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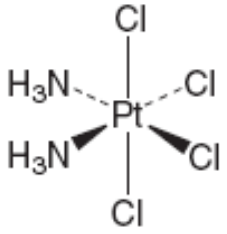
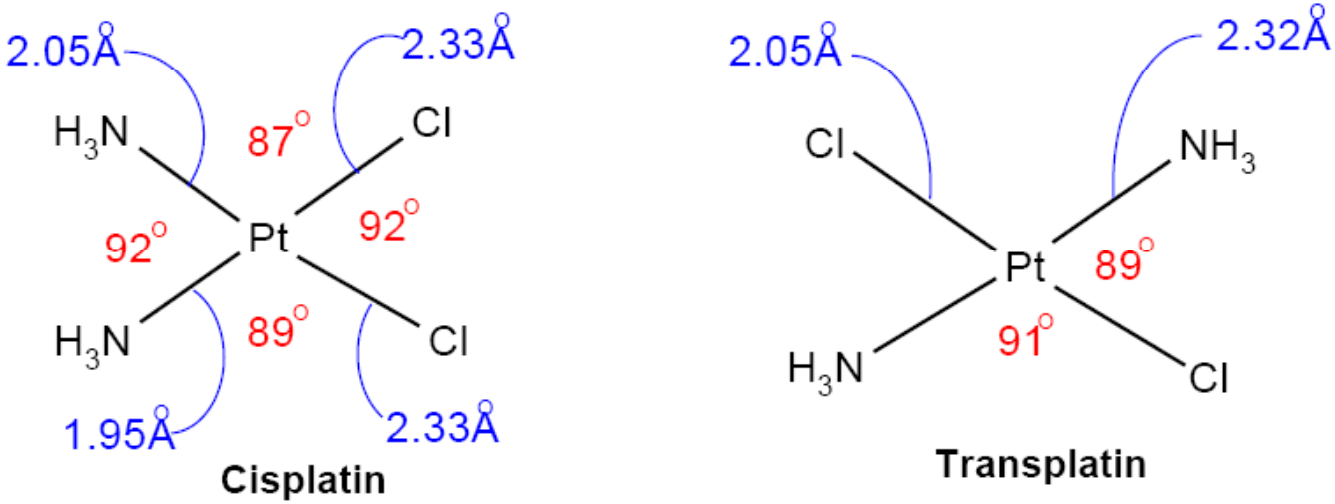


cis- [$Pt(NH_3)_2Cl_4$]



Structural differences between Cisplatin and Transplatin.

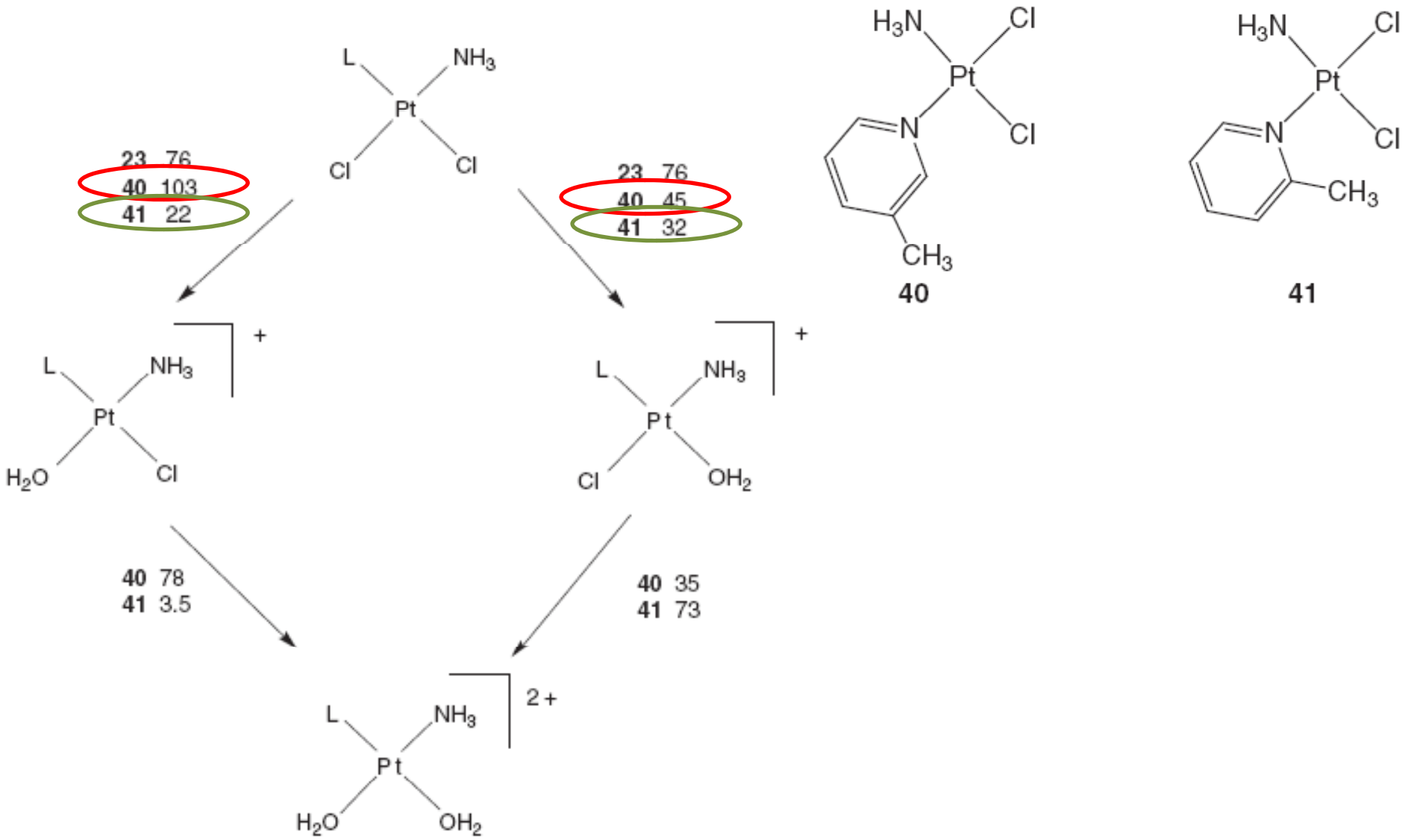
Metal nature….Pt vs. Pd

M(II) both d^8, square planar, similar ionic radii

1) **Pt(II) more kinetically inert than Pd(II)**
2) **Pd hydrolyzes rapidly compared to Pt**

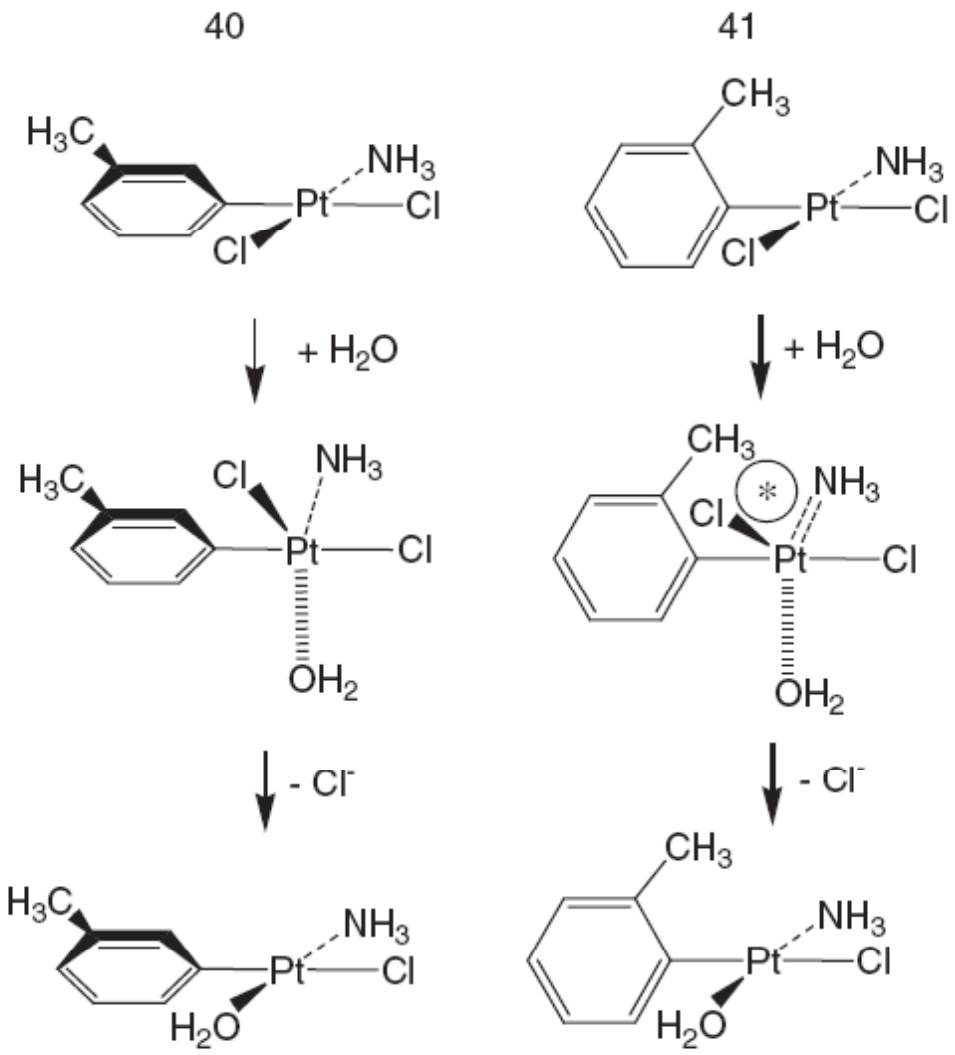
Amine ligands nature

1) **Solubility (goes up in fatty lipid environment when adding hydrocarbon to N**
2) ***trans*-effect to the trans ionic ligands**
3) **Size and shape affect the reactivity…solvent and other molecules can/can't come close)**



Rates of hydrolysis reaction x 10^{-6} s^{-1}

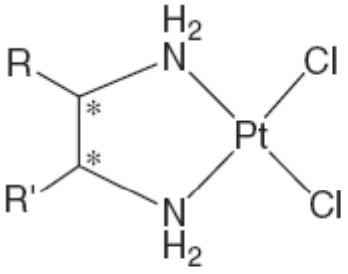
23 L = NH_3
40 L = NC_5H_4-3-CH_3
41 L = NC_5H_4-2-CH_3



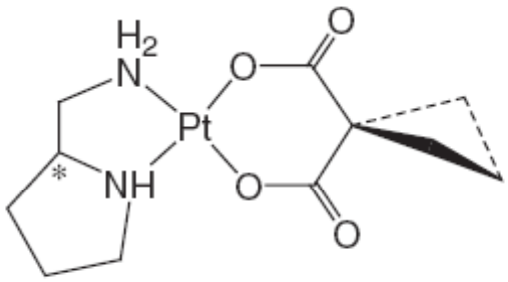
*An illustration of how the -Cl/CH_3- interaction in **41** can slow its aquation rate compared to **40** through a steric effect arising from the difference in ligand structure*

Chelate effects….changes lability of the complexes

Chirality…



42 R = CH_3, R' = H
43 R = R' = CH_3
44 R = R' = C_6H_4OH-3
45 R = R' = C_6H_4OH-4



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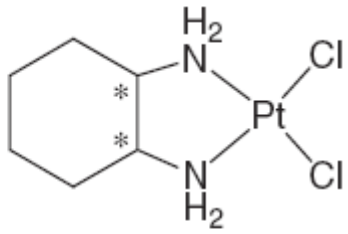

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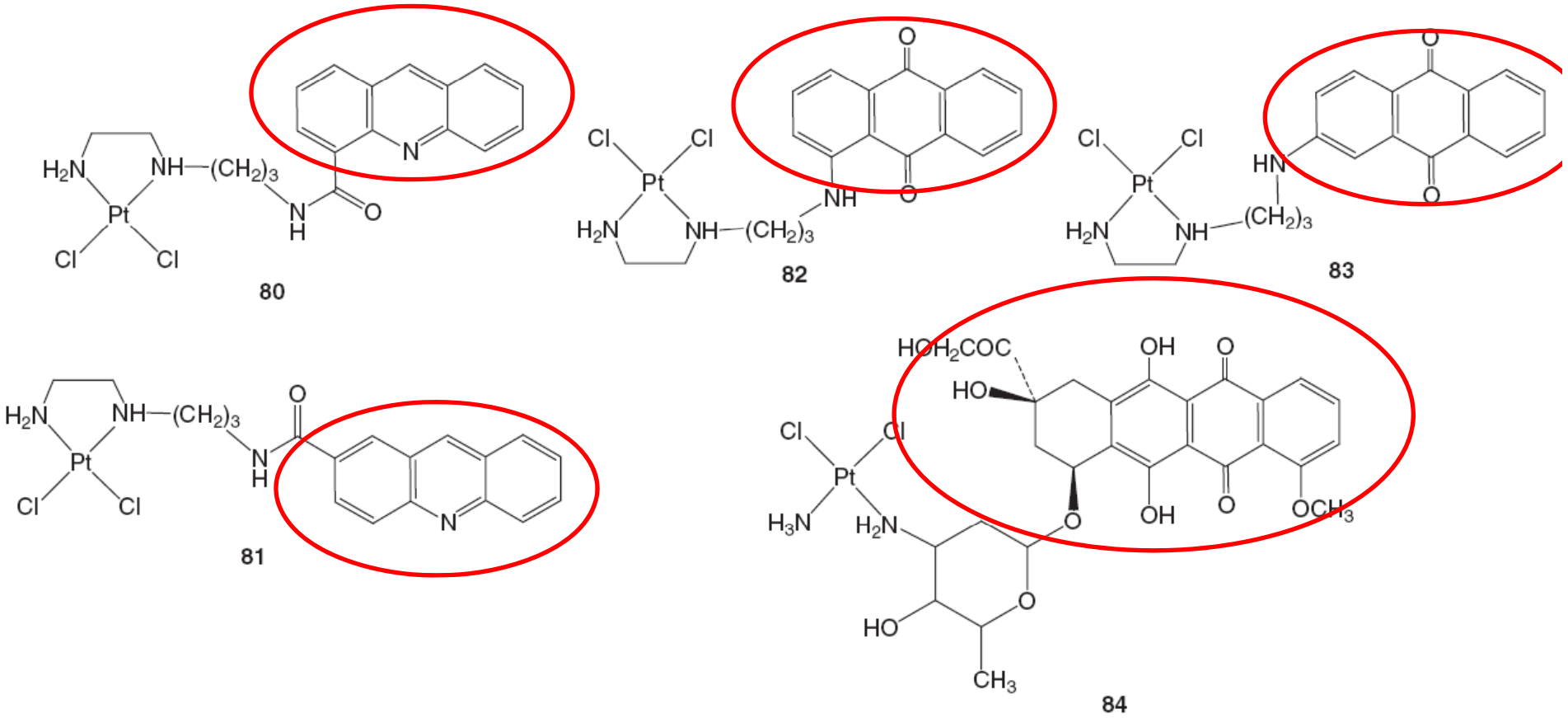
Not clear yet how chirality affects reactivity…

Ditopic agents….complexes with 2 biologically active parts

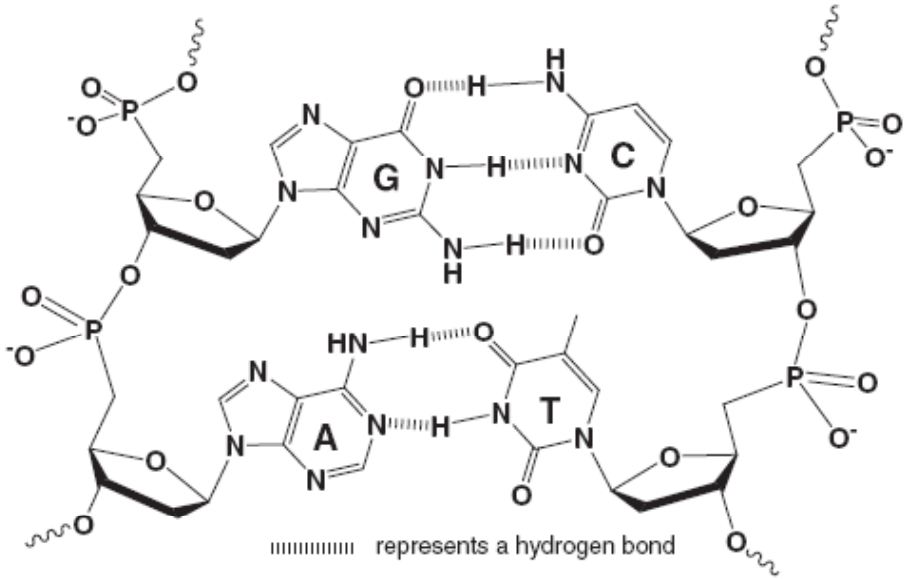


DNA intercalators…anthraquinone, acridine, etc…

…the position of the link!!!!!!

Possible Mechanisms of *cis*-platin…

(a) The Structure of DNA

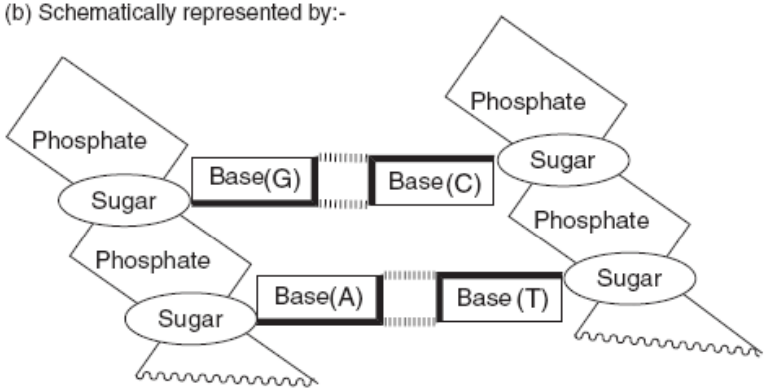


Complementary bases form pairs
G goes with C
A goes with T

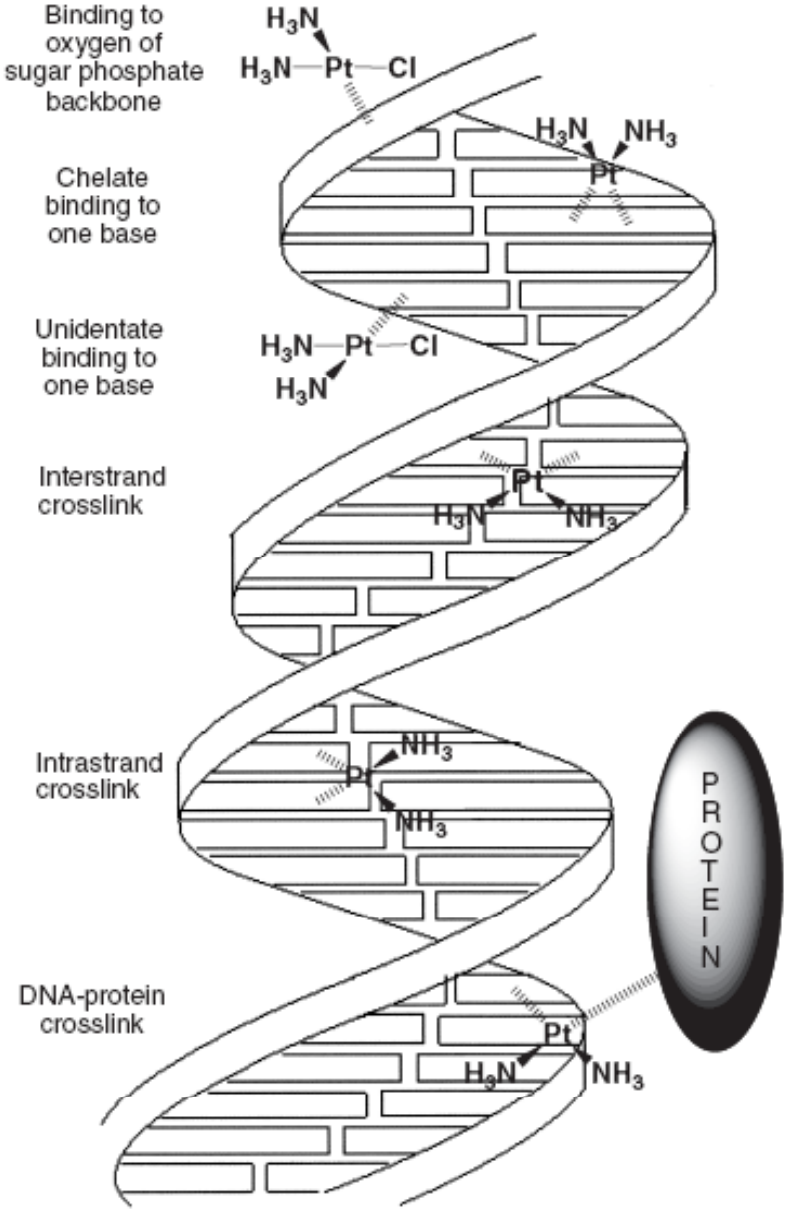
G = Guanine A = Adenine C = Cytosine T = Thymine

Purines Pyrimidines

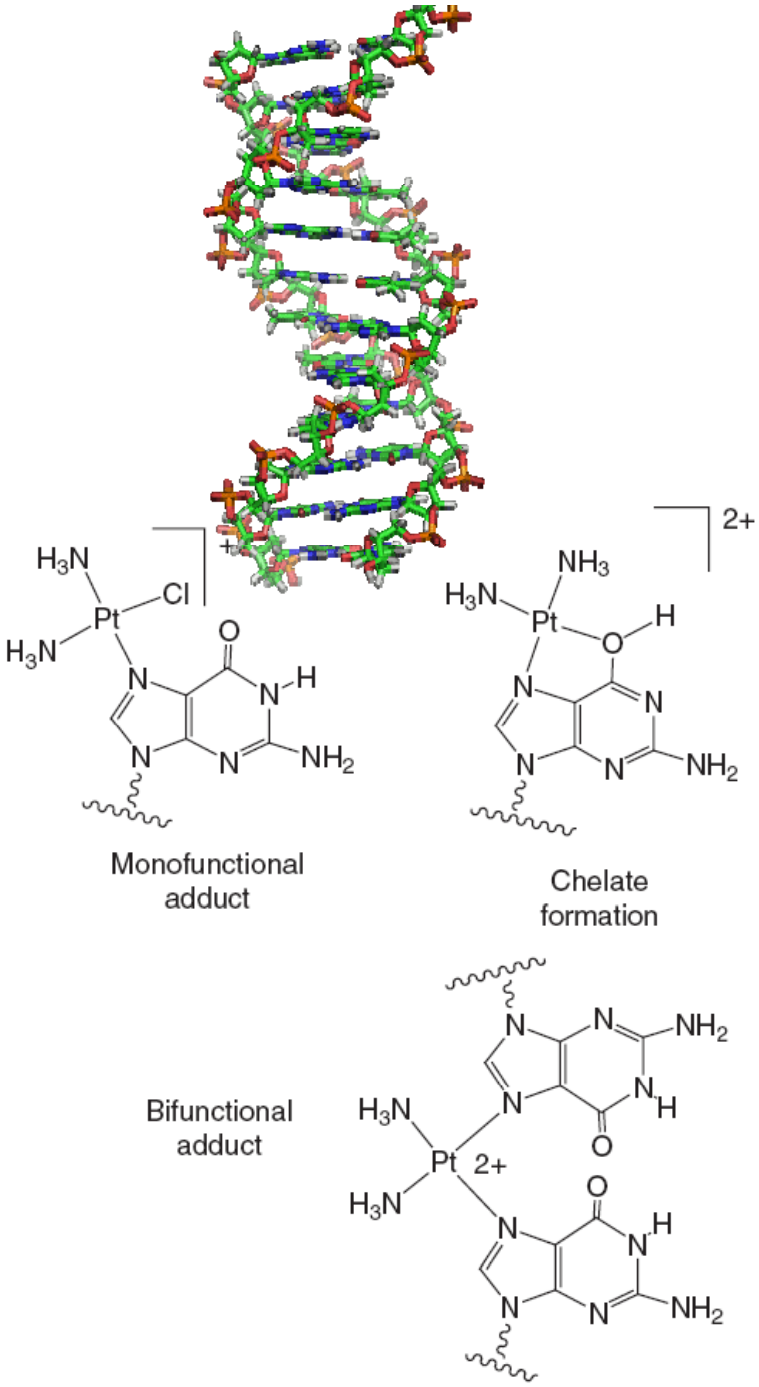
(b) Schematically represented by:-



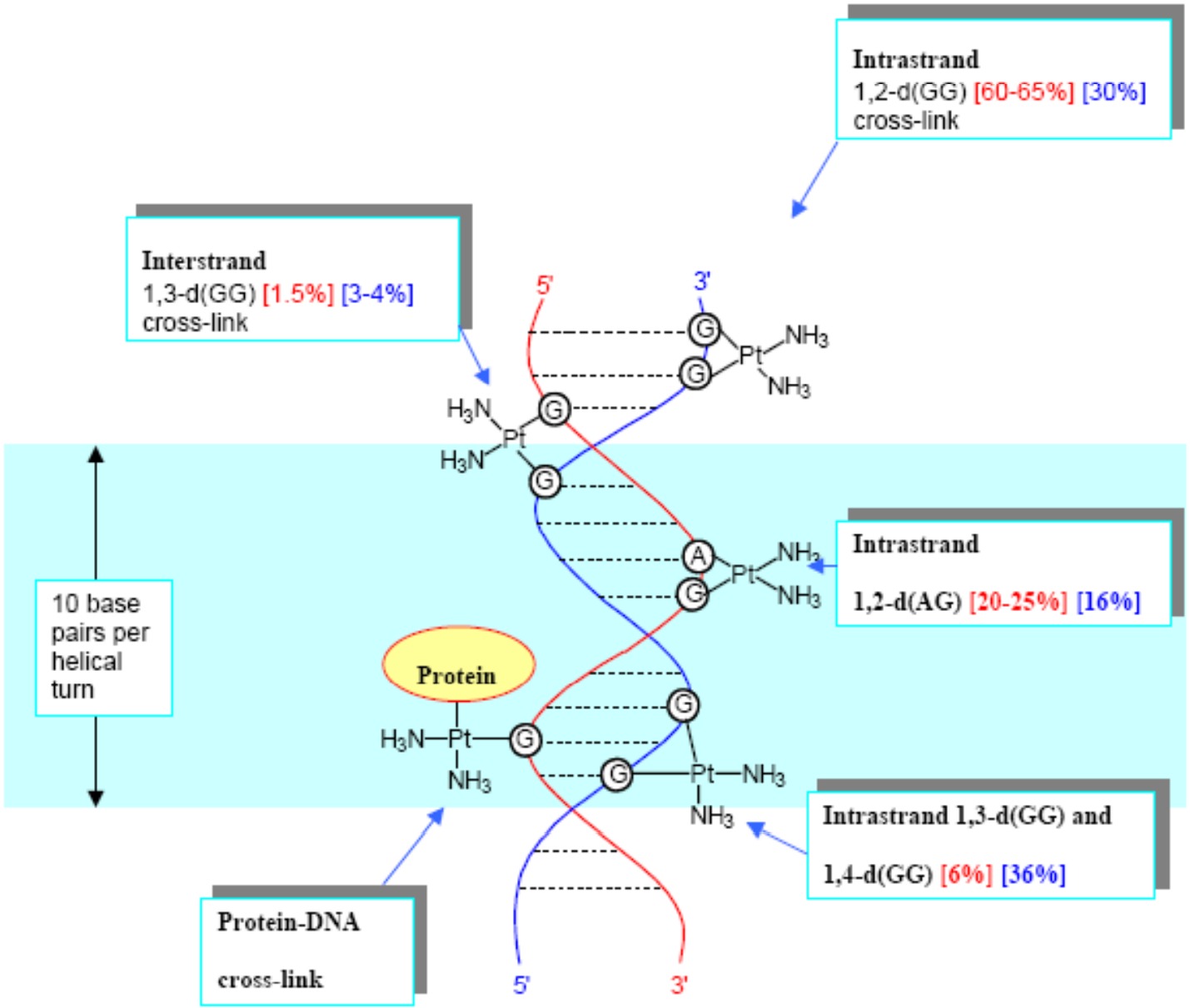
The structure of DNA showing (a) the complementary base pairing of the bases, Adenine with Thymine and Guanine with Cytosine and (b) a schematic representation of the 'double strand' structure



Some possible cis-DDP-DNA binding modes



Some possible binding modes of cis-[$PtCl_2(NH_3)_2$] to a guanosyl base in DNA



Platinum adducts with DNA. Typical populations after reaction with cisplatin are shown in red and for carboplatin in blue.

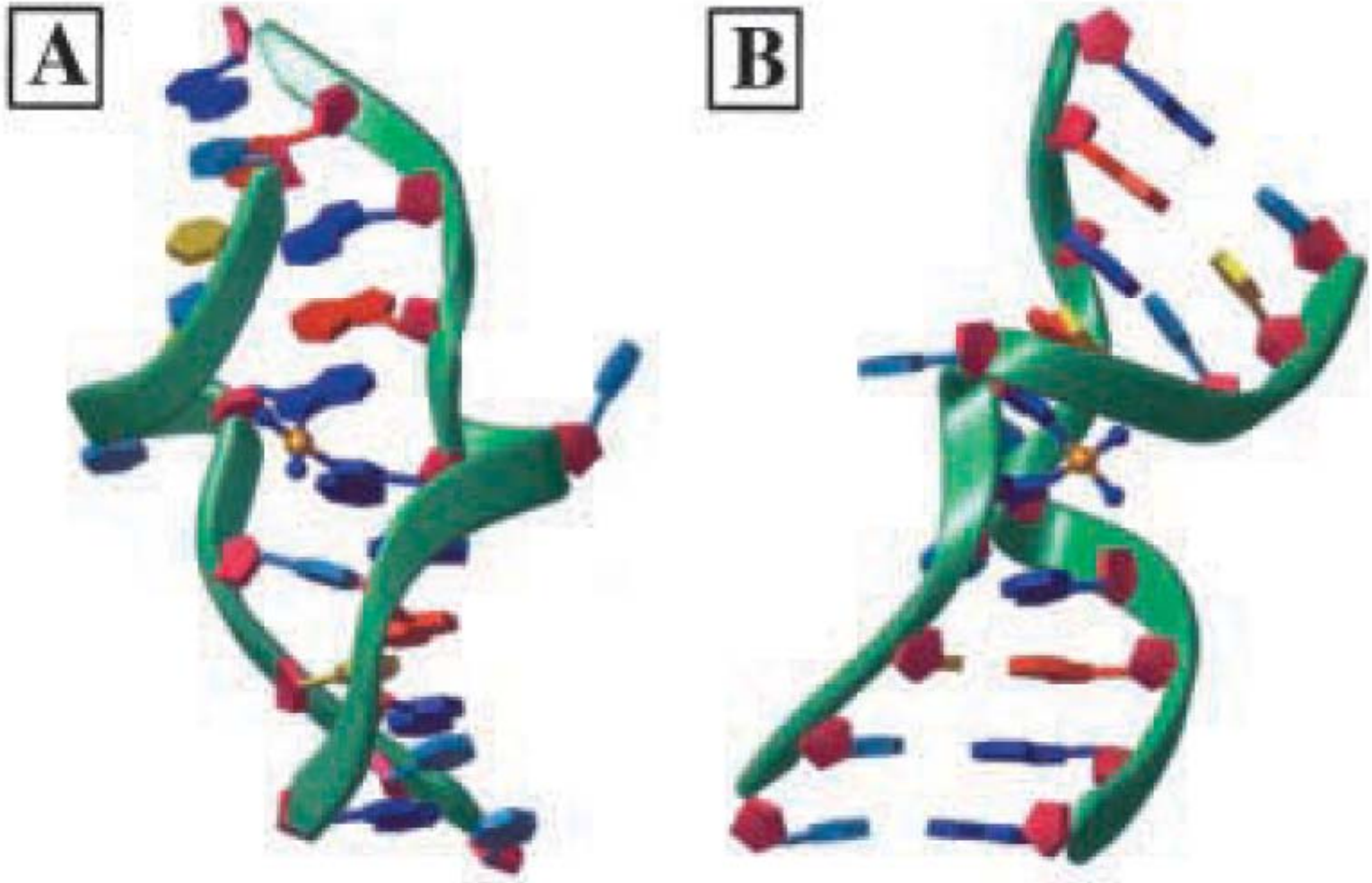
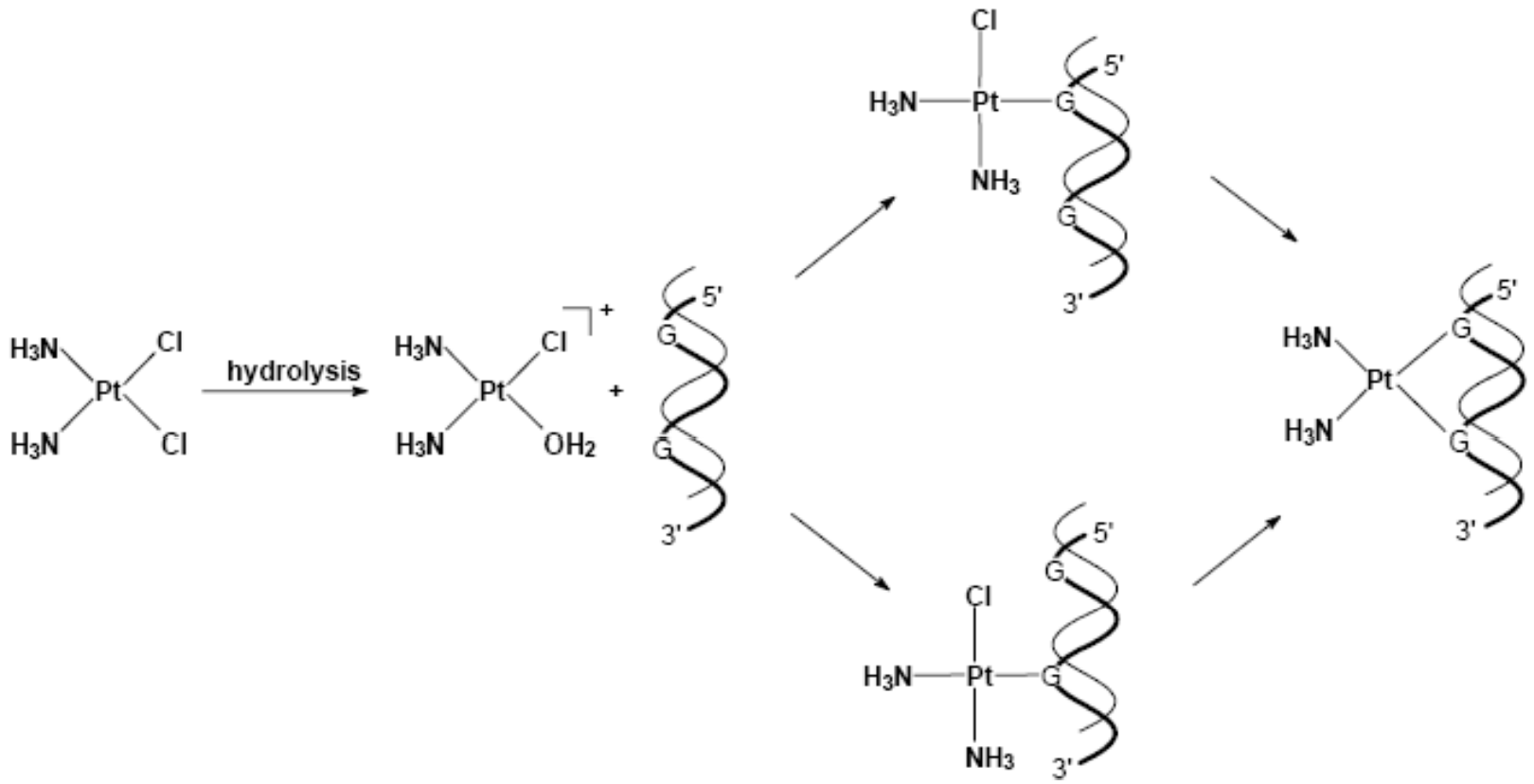


Fig. 1. *Two ribbon representations of the crystal structure of the DNA decamer d(CCTCG*-CTCTC/GAGAG*CGAGG) containing a unique cisplatin interstrand cross-link at d(GpC)·d(GpC) site* (asterisks indicate the chelated bases in the adduct). A front view (*A*) allows to see the structure with the lesion in the minor groove. A side view (*B*) shows the chicane of the backbone with the helix-sense reversal. Pt^{II} atom, yellow; ammine groups, navy blue; sugars, pink; guanines, navy blue; adenines, red; thymines, yellow; cytosines, light blue; phosphodiester backbone, green.



The presence of high chloride ion concentrations in extracellular fluid approximately 100 mM) is considered to suppress the aquation reactions and allow the uncharged complex to penetrate cell membranes. However, upon entering the cell, where the cytoplasmic chloride concentration is much lower (as low as 4 mM), the chloride ligands begin to exchange.

Why is trans-platin inactive????

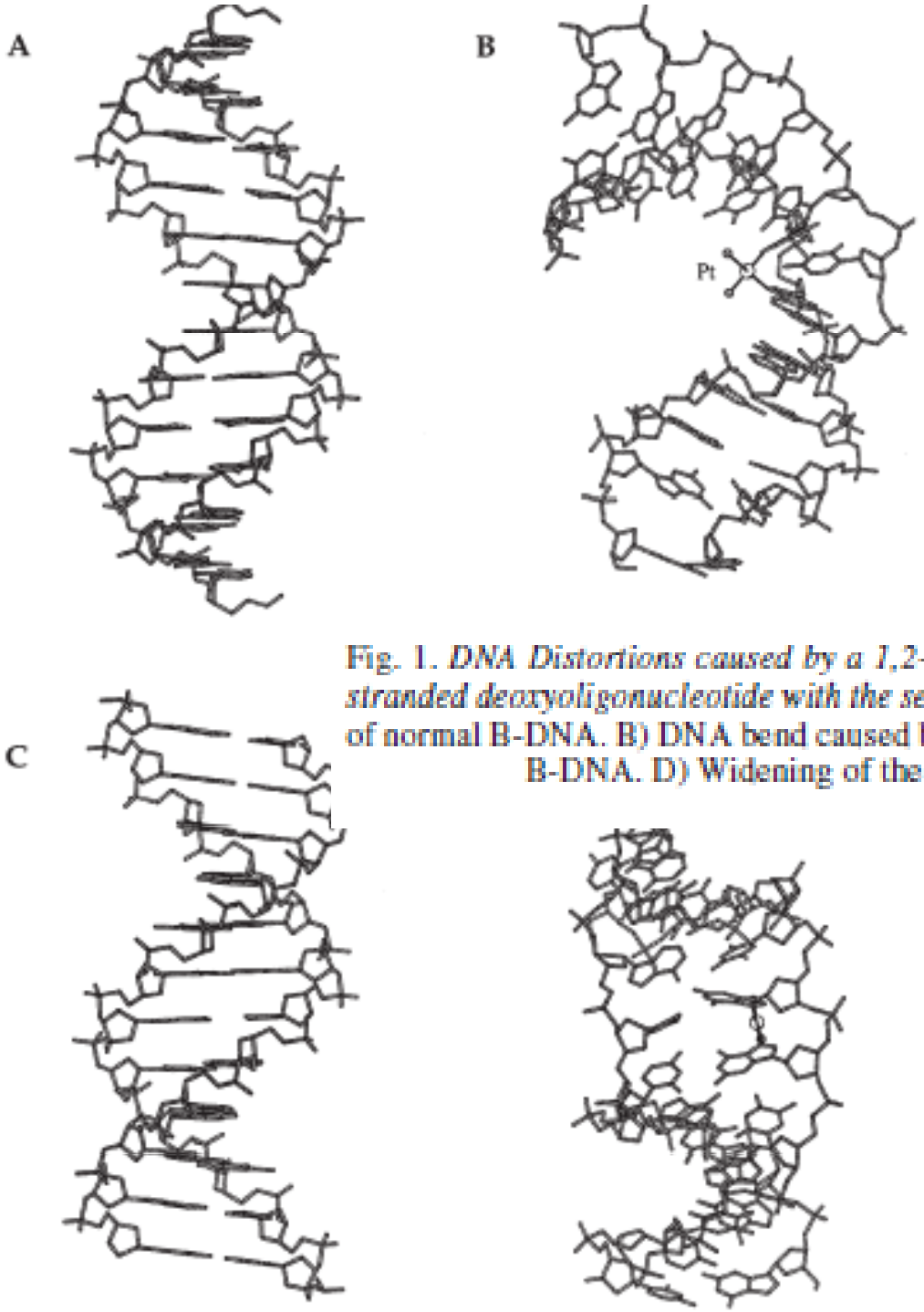


Fig. 1. *DNA Distortions caused by a 1,2-d(GpG)-cisplatin intrastrand adduct in a double-stranded deoxyoligonucleotide with the sequence d(CCTCTG*G*TCTCC).* A) Major groove of normal B-DNA. B) DNA bend caused by the cisplatin adduct. C) Minor groove of normal B-DNA. D) Widening of the minor groove by the cisplatin adduct.

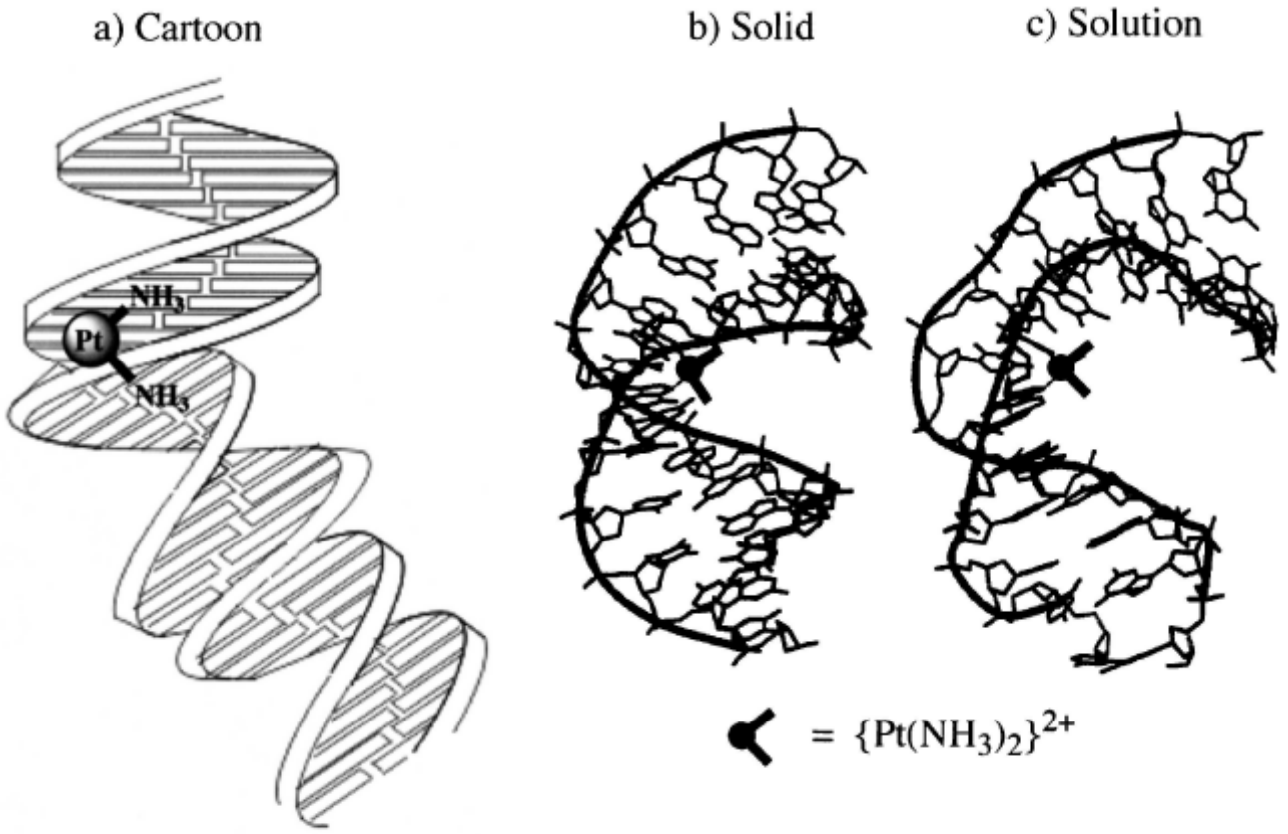
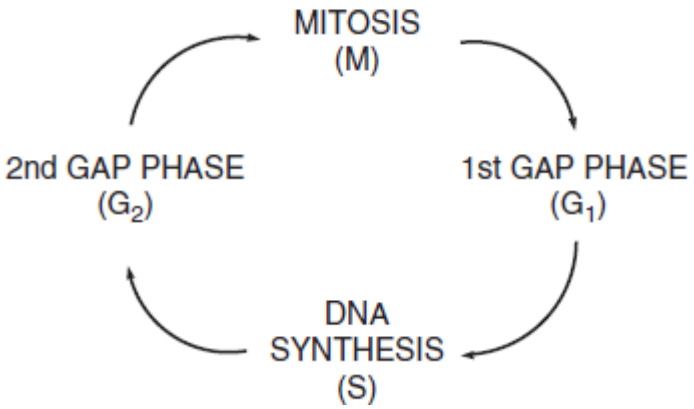


Figure 8 *Views of the effect of intra-strand cis-$\{Pt(NH_3)_2\}^{2+}$ crosslinking on DNA structure (a) a cartoon representation of DNA bending; (b) the solid state X-raydiffraction structure of the cis-$\{Pt(NH_3)_2\}^{2+}$ adduct of d(CCTCTG*G*TCCC).d(GGAGACCAGAGG) (G* denote Pt binding sites); (c) the solution NMR structure of the cis-$\{Pt(NH_3)_2\}^{2+}$ adduct of d(CCTCTG*G*TCCC).d(GGAGACCAGAGG)*

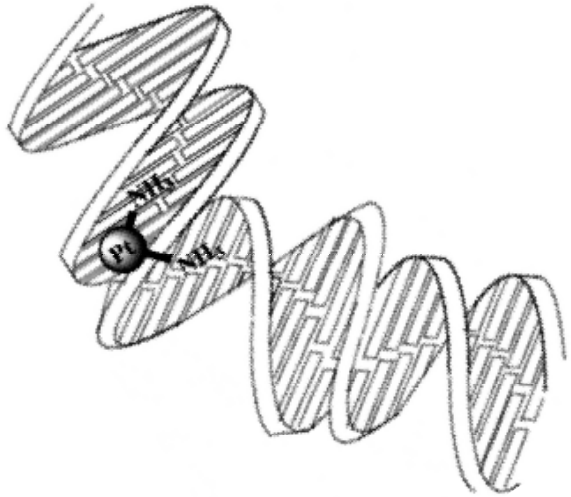
Consequences of DNA Platination…

Assuming that DNA **is the primary target molecule** associated with the antitumor activity of cisplatin, and having seen **structural** and chemical evidence that DNA does indeed form complexes with platinum compounds, the next question must **relate to how the platination of DNA might lead to antitumor activity**…

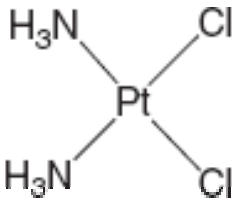


The **normal life cycle for a cell** involves its formation from another cell by **mitosis**, a **first gap phase (G1)** follows during which protein synthesis is occurring; next here is a **DNA synthesis phase (S)** in which new copies of DNA are produced, a **second gap phase (G2)** with continued protein synthesis and finally a **mitosis phase (M)** in which another new cell is formed. This cycle continues repeatedly until the cell dies

In the **DNA synthesis phase** it is necessary for a duplicate copy of the nuclear DNA to be produced. This involves **unravelling the DNA, separating the duplex into two strands, then using the strands as templates to synthesise new DNA for incorporation into the new cell**. If synthesis of the new DNA strands by DNA polymerases were blocked by platination, cell replication might be inhibited.



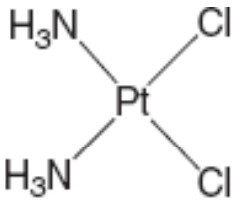
Another process necessary for cell division is **protein synthesis**. **If the transcription from DNA of the genetic information necessary for the synthesis of proteins were blocked, this too could prevent cell replication. The binding of proteins to DNA is another important process occurring in the cell, and the inhibition or promotion of protein binding could have a role to play in the activity of cisplatin.**



A further possibility is that platination of DNA leads to early cell death, apoptosis, **through binding to telomeric regions of DNA at the ends of chromosomes**. These telomeric regions protect the ends of chromosomes from degradation and are **guanosine rich** consisting of lengths of repeating 5'-TTAGGG-3' groups in humans.

Part of the telomeric region is lost with each successive cell division and eventually, when the telomeric region become sufficiently short, the cell dies. **However, there is a ribonucleoprotein, telomerase**, which can add sections to the telomeric regions thereby prolonging cell life. **If cisplatin binding to telomeric regions results in degradation of the telomeres, or the inhibition of telomerase activity, premature cell death would result**.

Measurements of telomere loss in HeLa cells have shown that cisplatin can degrade and shorten telomeric regions. **Cisplatin has also been shown to inhibit telomerase activity** when other agents which damage DNA, including trans-DDP, did not show this effect. It would seem possible therefore that cisplatin can cause premature cell death through interactions affecting telomeric DNA.

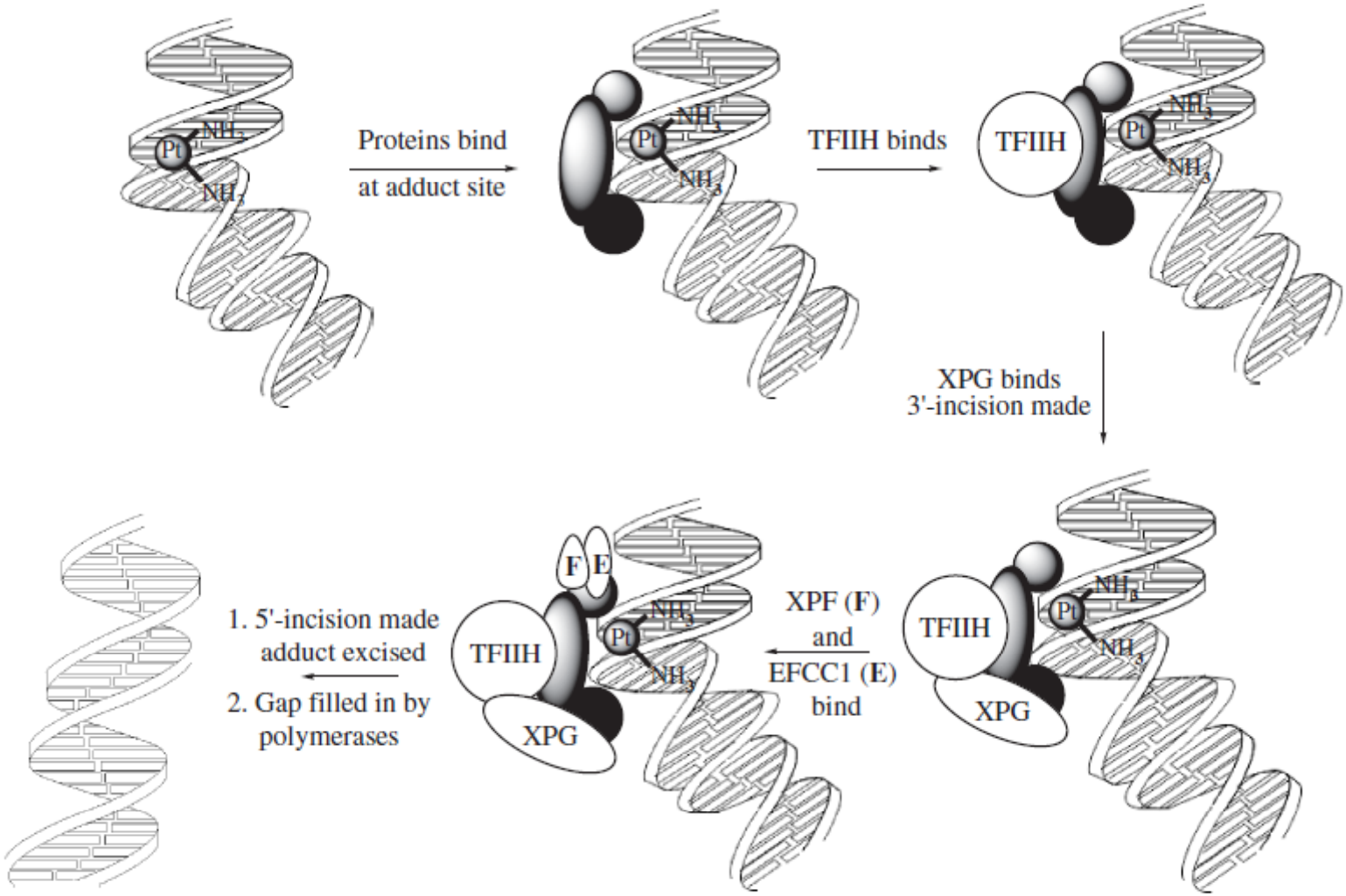


Finally it is important to note that, in addition to nuclear or genomic DNA (gDNA), there is also DNA in the nucleosomes of mitochondria. Mitochondrial DNA (mDNA) is not subject to natural DNA repair processes in the same way as gDNA, so platination of mDNA may have **a greater effect because its platinum adducts persist whereas in gDNA adducts may be removed by repair processes.**

Experiments have shown a 4- to 6-fold higher proportion of platinum adducts in mDNA than in gDNA after treatment with cisplatin. **This could be attributed to more extensive initial binding to mDNA or to greater retention of platinum adducts as a result of deficient repair**. It is not yet clear whether platination of mDNA is more or less important than platination of gDNA in the activity of cisplatin.

However, cells deficient in DNA repair have been shown to be more sensitive to cisplatin than those proficient in DNA repair indicating that **repair mechanisms may be important in moderating cisplatin activity.**

DNA repair processes…



Among the natural cellular processes for repairing damaged sequences of DNA, nucleotide excision repair (NER) appears to be important for the removal of platinum adducts.

Several proteins are implicated in the initial recognition of DNA damage caused by platinum binding. After these '**damage recognition' proteins** have bound to the platinated site the protein TFIIH binds to them. The presence of TFIIH allows binding of the protein XPG **which makes an incision** in the DNA strand **on the 3'-side** of the platination site. Thereafter the XPF-ERCC1 protein complex binds and makes **a second incision on the 5'-side** of the platination site. **The process cuts out a section of the DNA strand approximately 30 nucleobases in length and the gap left as the proteins dissociate is refilled by DNA polymerases to produce the repaired DNA duplex**.

Where repair mechanisms of this sort are effective in removing platinum from DNA they will limit the antitumor effect of the platinum compound....

Some of the proteins involved in NER appear to bind specifically to sites containing cis-$\{Pt(NH_3)_2\}^{2+}$ adducts and so NER may be an important factor limiting the effectiveness of cisplatin.

Resistance to cis-platin…major problem…

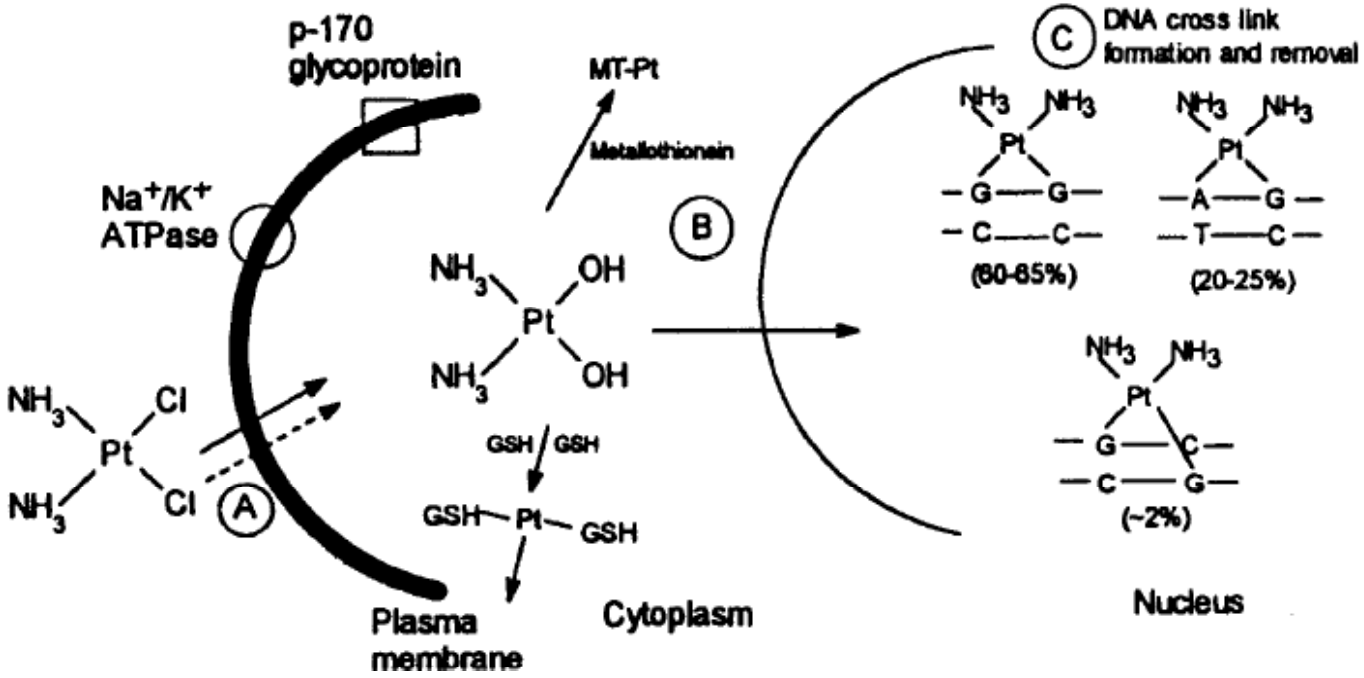
Some cells may be intrinsically resistant to cisplatin while others may develop resistance during treatment.

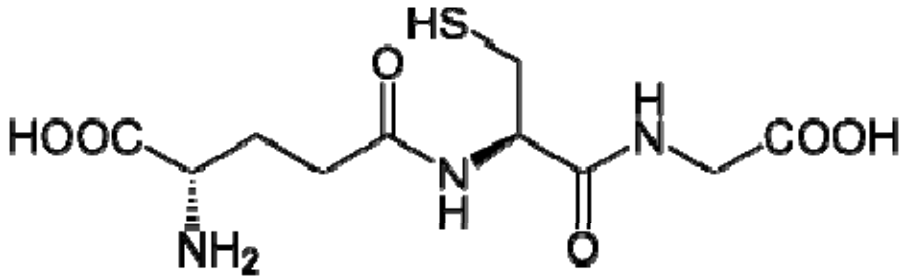
Several possible causes of cisplatin resistance have been proposed and any or all may play some part in the development of resistance.

1) The intracellular accumulation of cisplatin may decline, possibly due to decreasing uptake of the drug coupled with release rates which do not change significantly.

2) Stimulation by cisplatin of the production of complexing agents such as thiols which can bind to the cisplatin and **compete with DNA binding**. In particular **GSH** can be present in cells at concentrations of 0.5–10 mmol dm^3 and may bind to platinum.

3) Stimulation by cisplatin of enhanced DNA repair activity resulting in the excision of platinated regions of DNA. A number of experiments on different cell lines, including human ovarian cell lines, have shown enhanced repair activity to be common among cisplatin resistant cells although, there is not a complete correlation between repair activity and cisplatin resistance.



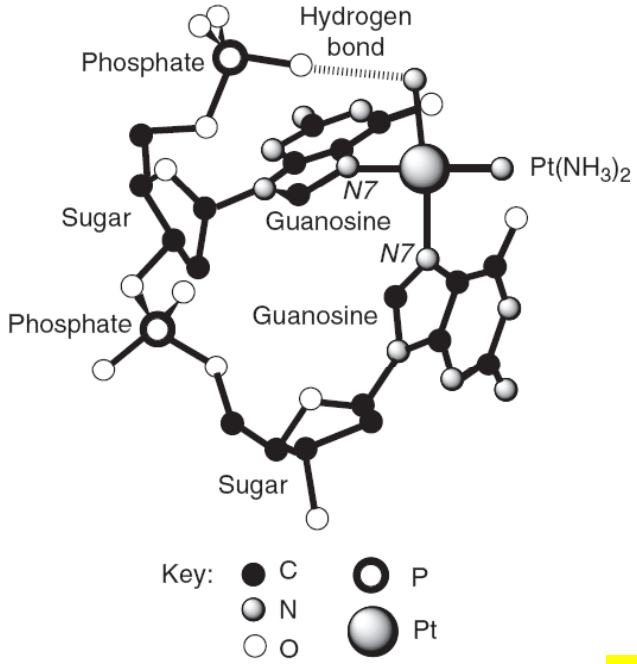


GSH: Glutathione

Concluding remarks…

Why is cis-[$Pt(NH_3)_2Cl_2$] retained until it finds the DNA?

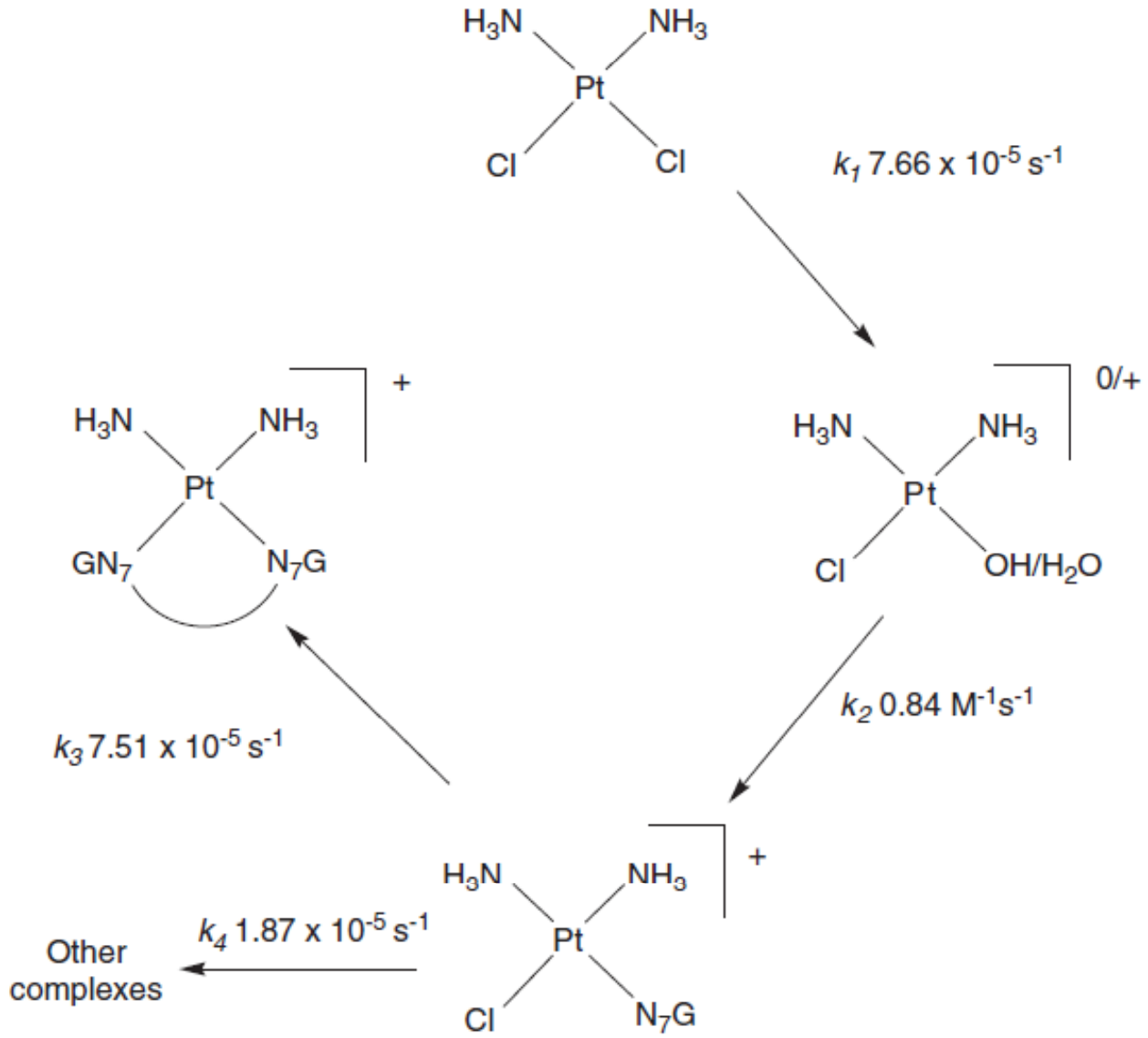
…binds at 2 guanosine N7s…intra-strand crosslink…

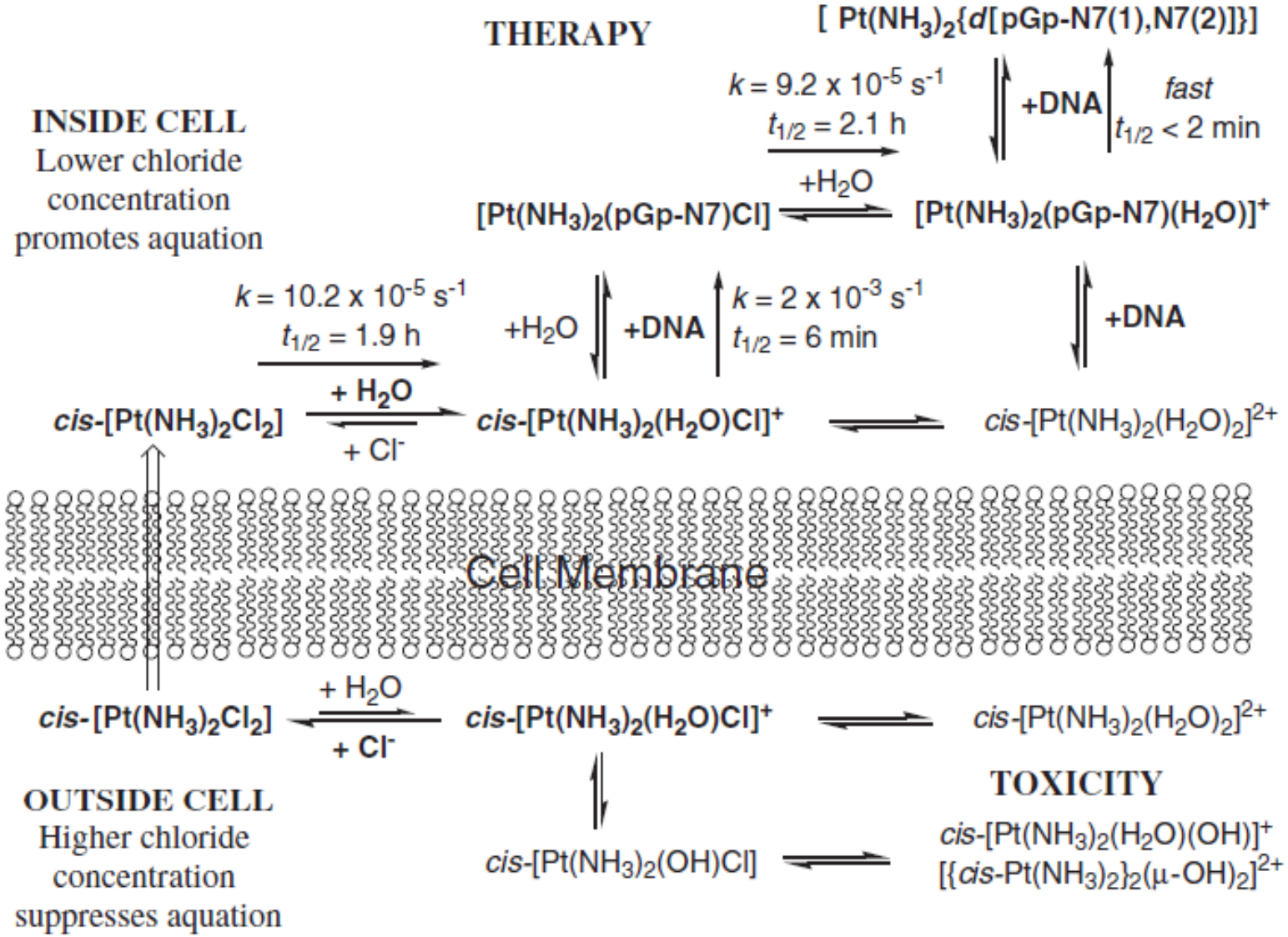


cis-[$Pt(NH_3)_2(GG)$]$^{2+}$ => DNA to bend…

1) Inhibition of DNA polymerase
2) Inhibition of transcription
3) Apoptosis
4) Selective binding of some proteins
5) Blocking of binding of some proteins

Tumor cells are among the most active in the body with respect to cell division and DNA processing, so they are more sensitive to the effects of cisplatin, a factor leading to its antitumor activity.


H3N
NH3
Pt
Cl
Cl
k1 7.66 x 10-5 s-1
0/+
H3N
NH3
Pt
Cl
OH/H2O
k2 0.84 M-1s-1
+
H3N
NH3
Pt
Cl
N7G
k3 7.51 x 10-5 s-1
+
H3N
NH3
Pt
GN7
N7G
k4 1.87 x 10-5 s-1
Other
complexes


THERAPY
[Pt(NH3)2{d[pGp-N7(1),N7(2)]}]
k = 9.2 x 10-5 s-1
t1/2 = 2.1 h
+DNA
fast
t1/2 < 2 min
+H2O
[Pt(NH3)2(pGp-N7)Cl]
[Pt(NH3)2(pGp-N7)(H2O)]+
INSIDE CELL
Lower chloride concentration promotes aquation
k = 10.2 x 10-5 s-1
t1/2 = 1.9 h
+H2O
+DNA
k = 2 x 10-3 s-1
t1/2 = 6 min
+DNA
+ H2O
cis-[Pt(NH3)2Cl2]
cis-[Pt(NH3)2(H2O)Cl]+
cis-[Pt(NH3)2(H2O)2]2+
+ Cl-
Cell Membrane
+ H2O
cis-[Pt(NH3)2Cl2]
cis-[Pt(NH3)2(H2O)Cl]+
cis-[Pt(NH3)2(H2O)2]2+
+ Cl-
OUTSIDE CELL
Higher chloride concentration suppresses aquation
TOXICITY
cis-[Pt(NH3)2(OH)Cl]
cis-[Pt(NH3)2(H2O)(OH)]+
[{cis-Pt(NH3)2}2(μ-OH)2]2+

How Much Cisplatin Does It Take to Kill a Cell?

Roberts measured the amount of DNA platination in many cell lines and compared this to the degree of cytotoxicity.

…cytotoxicity occurs when there are **around** **2–10 nmoles of Pt/g DNA**, **which reflects about**

1 Pt/100,000–500,000 nucleotides

2nd study: **1Pt/250,000 nucleotides**

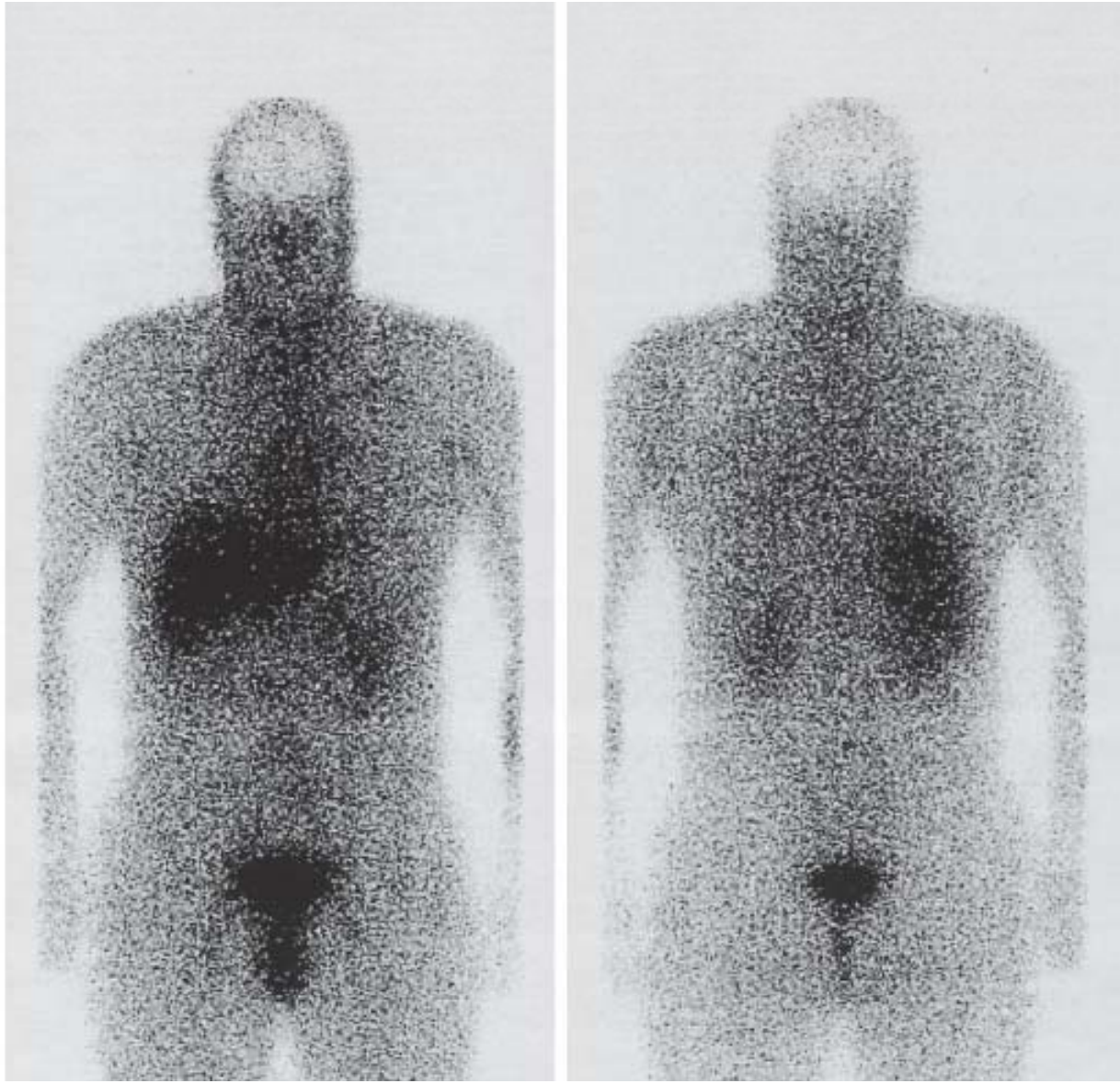
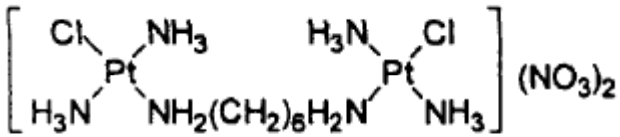
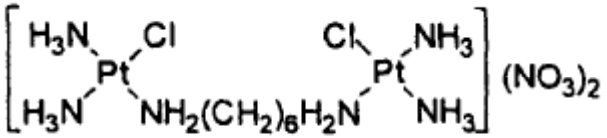


Fig. 7. *Whole-body image* (anterior to the left, posterior to the right) *of a testis cancer patient 1 h after the end of a [^{191}Pt]cisplatin infusion*. The platinum was still clearly detectable after 65 h but only weakly visible 7 days after the infusion. Chemotherapy was performed following surgery, so no macroscopic tumor tissue remained in the testis. In a different patient, however, platinum was detected in a metastatic tumor of the neck. Reprinted with permission from the Scandinavian University Press from [237].

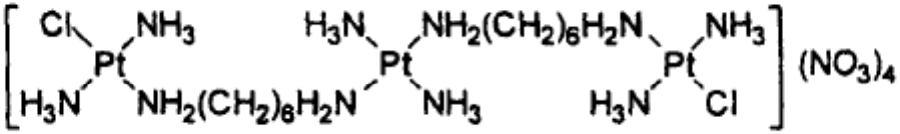
Other Pt-based Anti-Cancer Drugs…



1,1/t,t



1,1/c,c



BBR 3464 (1,0,1/t,t,t)

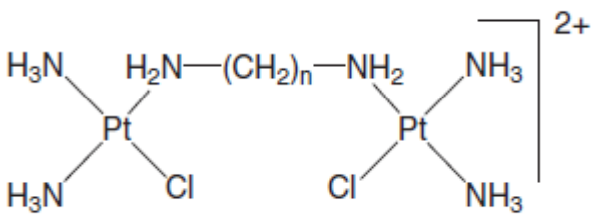
In early 1998 a novel trinuclear platinum compound, BBR3464, entered Phase I clinical trials, the first genuinely new platinum agent not based on the 'classical' cisplatin structure to do so.

Table 1 *Comparision at maximum tolerated dose of BBR3464* (0.2–0.4 mg kg^{-1}) *and cisplatin* (3–6 mg kg^{-1}) *after i.v. repeated treatment on staged tumors*

Clinical parameter[a]	*BBR 3464*	*Cisplatin*
Resistance, TWI < 50%	0	9 (4 NSCLC, 2 ovarian, 2 gastric, 1 prostatic)
Relative resistance, TWI 50–70%	3 (1 NSCLC, 1 gastric, 1 prostatic)	7 (2 SCLC, 2 NSCLC, 2 ovarian, 1 bladder)
Sensitivity, TWI > 70%	15 (3 SCLC, 5 NSCLC, 5 ovarian, 1 gastric,1 bladder)	2 (1 ovarian, 1 SCLC)

[a]See reference 7. TWI% is Tumor Weight Inhibition compared with controls. SCLC: small cell lung cancer; NSCLC: non-small cell lung cancer. The clinical parameter refers to the fact that clinical resistance, relative resistance and sensitivity are most likely to be seen at these TWI levels. Thus, BBR3464 is significantly more potent than cisplatin – good tumor sensitivity was observed in 15/18 cases for BBR3464.

BBR3464 clearly represents a significantly promising antitumor agent and a distinct departure from all previous mononuclear cisplatin agents. This is also true in comparison with other DNA-DNA cross-linking agents.



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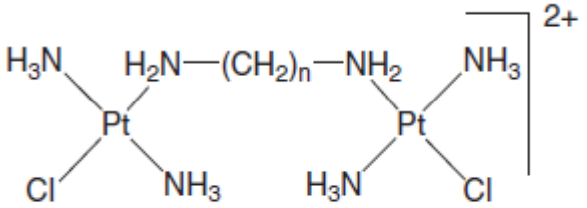

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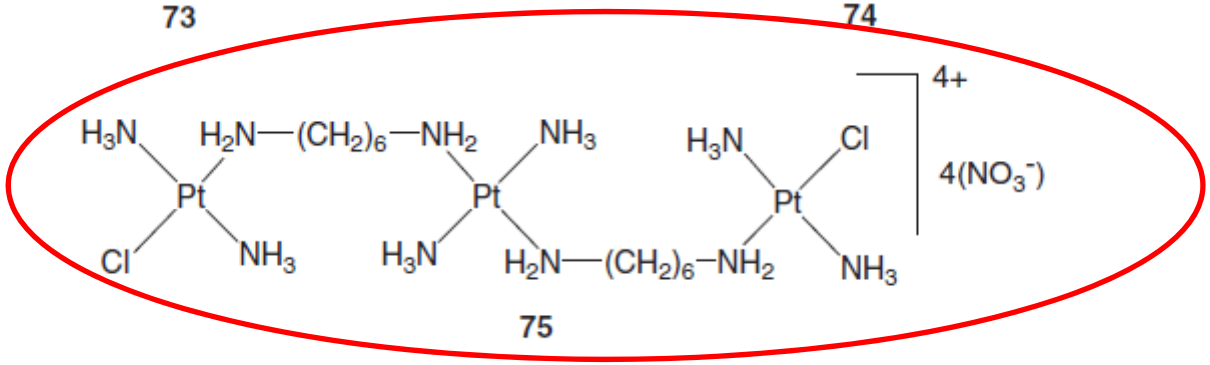

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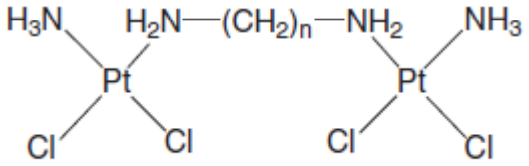

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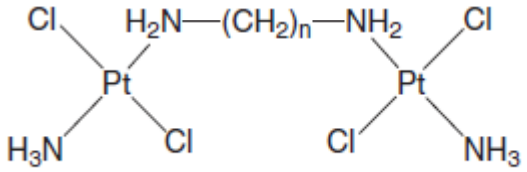

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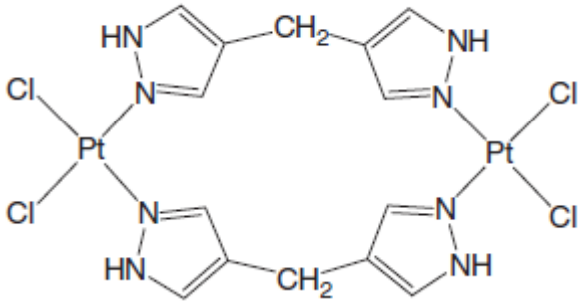

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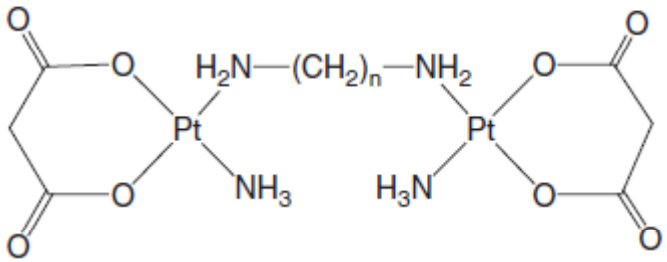

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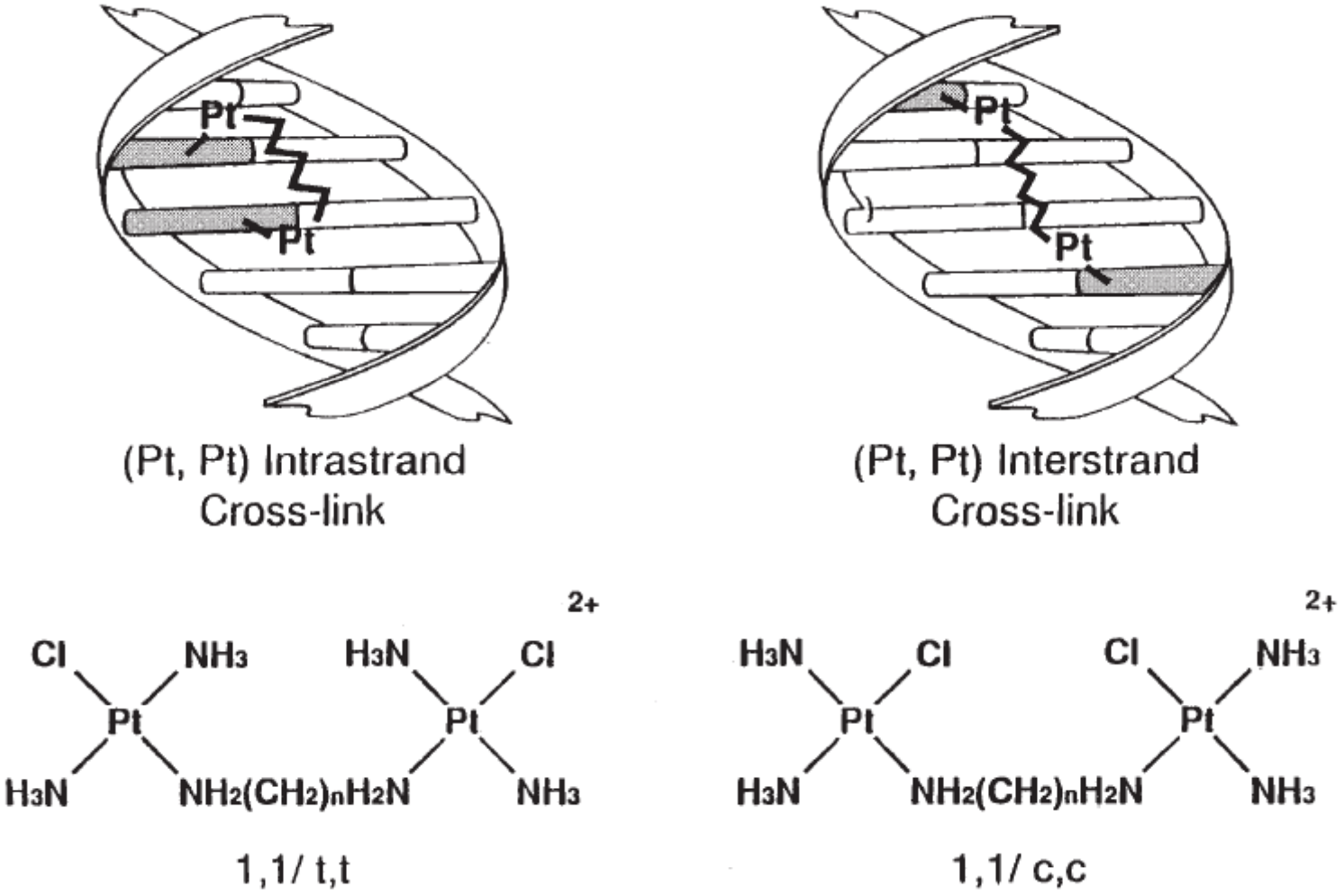
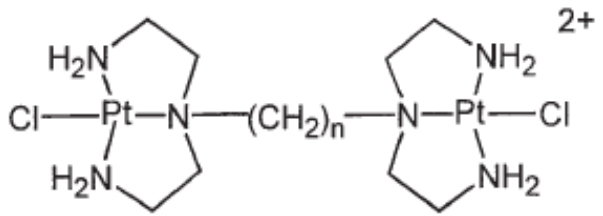
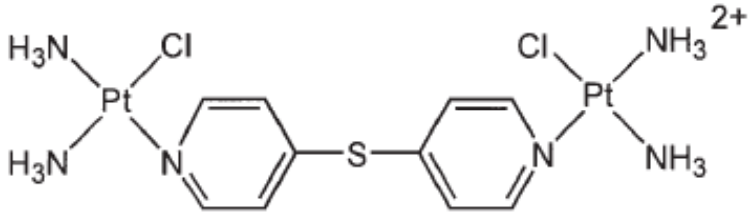


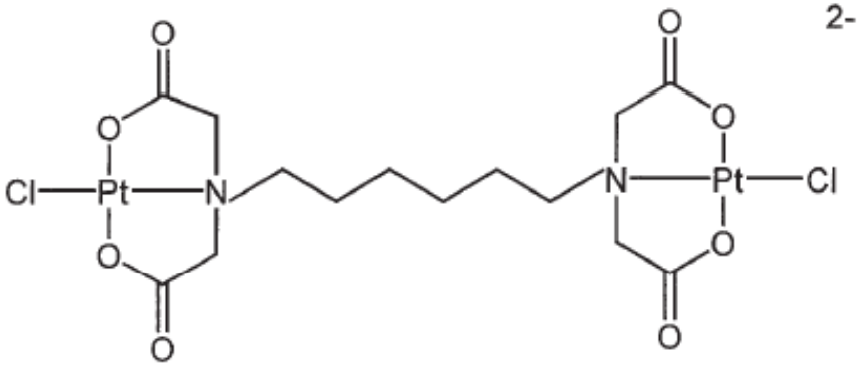
Fig. 2. *Schematic limiting binding modes for a di- or trinuclear bifunctional DNA-binding compound.* The 1,1/*t*,*t* geometry forms both types of adduct; the 1,1/*c*,*c* ($n = 4, 6$) forms only interstrand cross-links. Long-range intrastrand cross-links can, however, occur for a species such as BBR 3464.



(a)



(b)



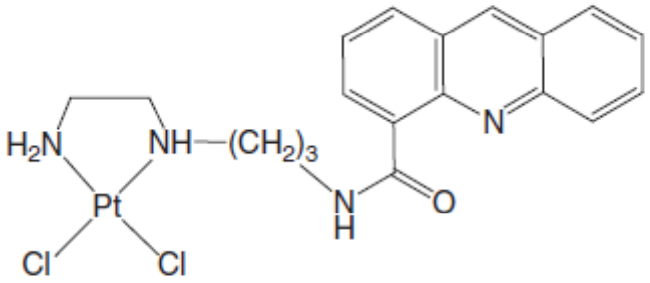
(c)

Miscellaneous bifunctional DNA-binding dinuclear compounds reported for their potential antitumor activity

Pt ditopic complexes…

A platinum centre linked to another quite different functional group designed to interact with a different biological target, or with the same target but in a different way

One logical approach to improving the efficacy of platinum anticancer drugs is to attach a platinum complex known to have antitumor properties to a bioactive group, which might improve its transport to the target site, or increase the likelihood of platinum exerting its effect at the target site



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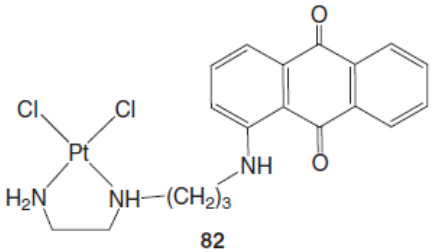

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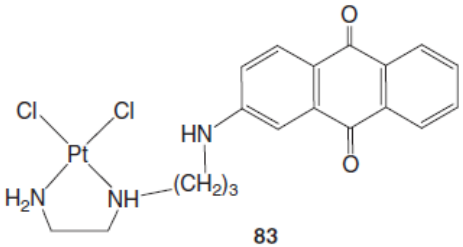
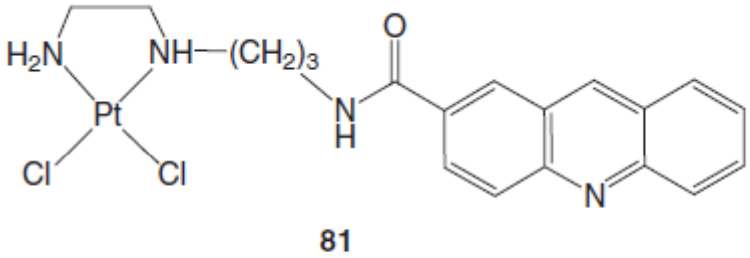


83

DNA intercalators



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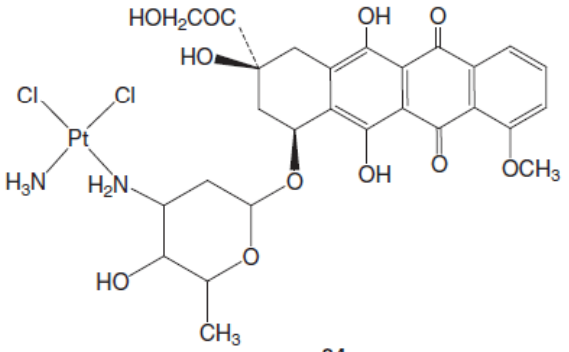
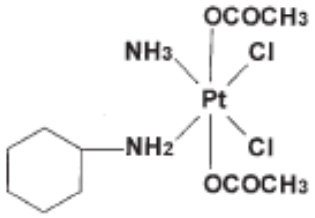


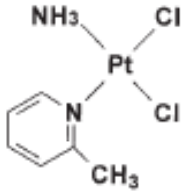
84

The Development of Orally Active Platinum Drugs



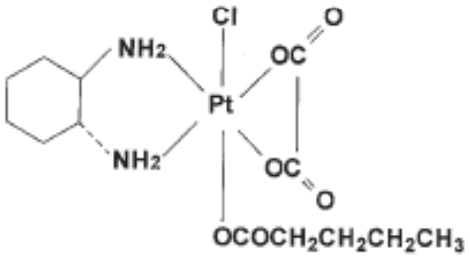
JM216

Phase I 1992



AMD473

Phase I 1997



C5-OHP-C1

preclinical development

Fig. 1. *Structures of published orally active platinum drugs*

over the past 30 years, many hundreds, perhaps thousands of analogues have been synthesized: why? …**cisplatin one of the most toxic drugs used in man** and second, many tumors exhibit resistance

The ability to deliver the drug orally …**much greater flexibility in dosing** and **increase the potential for the use of Pt drugs, especially in palliative care**.

Table. *Intraperitoneal* vs. *Oral Antitumor Activity in Mice Bearing the ADJ/PC6 Plasmacytoma for Selected Platinum Agents*[a])

Compound	i.p.		*TI*	oral		*TI*
	LD_{50} mg/kg	ED_{90} mg/kg		LD_{50} mg/kg	ED_{90} mg/kg	
Cisplatin	11.3	0.6	18.8	140	24	5.8
Carboplatin	180	14.5	12.4	235	99	2.4
JM149	17.4	0.4	44	118	18	7
JM216	30	5.7	5.3	330	5.8	56.9
AMD473	43	3	14.3	560	6.2	90.3

[a]) LD_{50}, 50% lethal dose; ED_{90}, dose required to reduce tumor mass by 90%; *TI*, therapeutic index, LD_{50}/ED_{90}. JM149 = *cis*-[$PtCl_2(OH_2)(NH_3)$(c-$C_6H_{11}NH_2$)]; JM216 = *cis*-[$PtCl_2(OCOCH_3)_2(NH_3)$(c-$C_6H_{11}NH_2$)]; AMD473 = *cis*-[$PtCl_2(NH_3)$(2-picoline)].

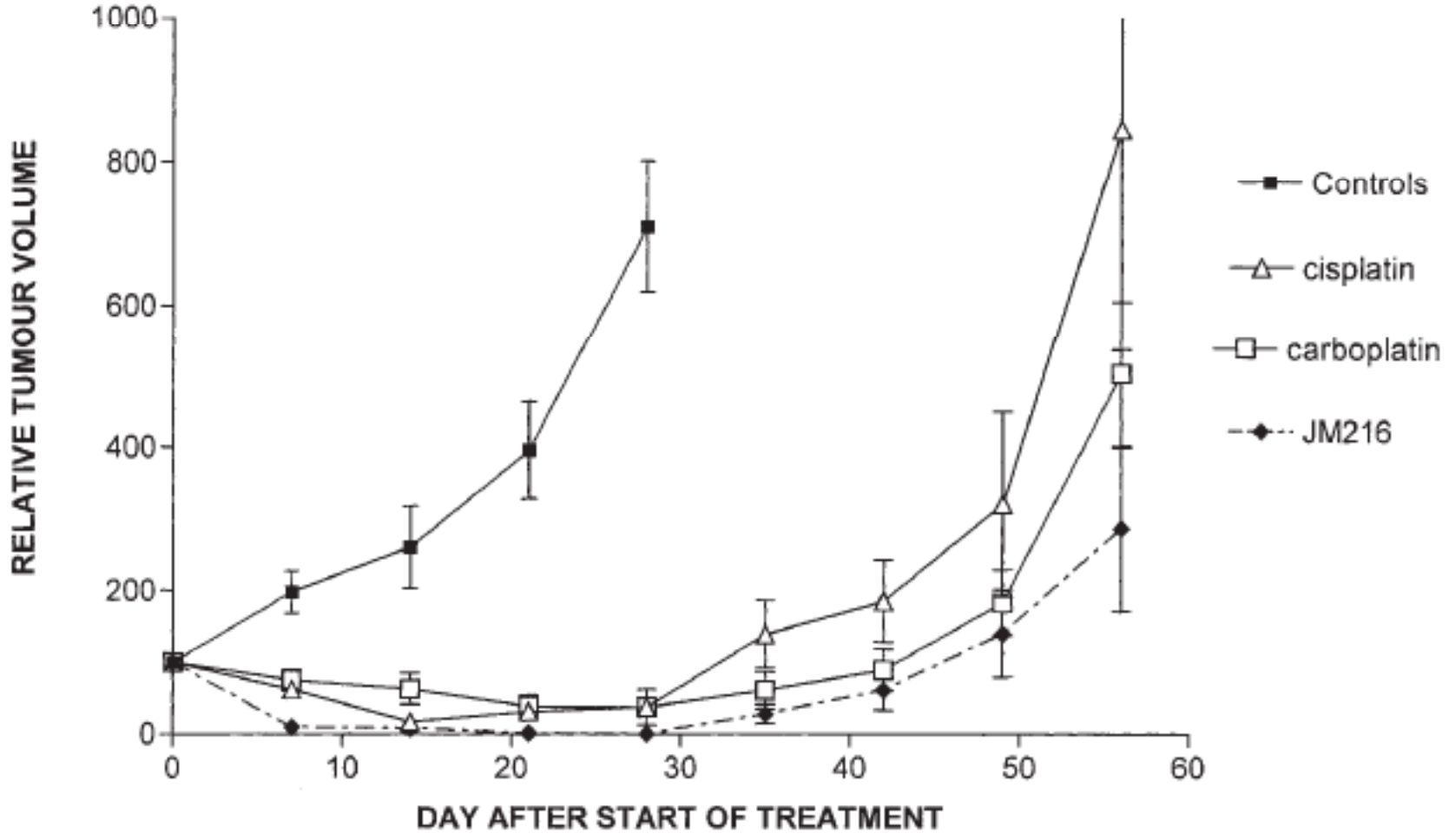


Fig. 2. *Comparative antitumor activity of orally administered JM216, 135 mg/kg q7d×4* (♦), *i.v. administered cisplatin, 3 mg/kg q 7d×4* (△), *and i.v. administered carboplatin, 90 mg/kg q7d×4* (□) *to mice bearing the CH1 human ovarian carcinoma xenograft* (controls, (■))

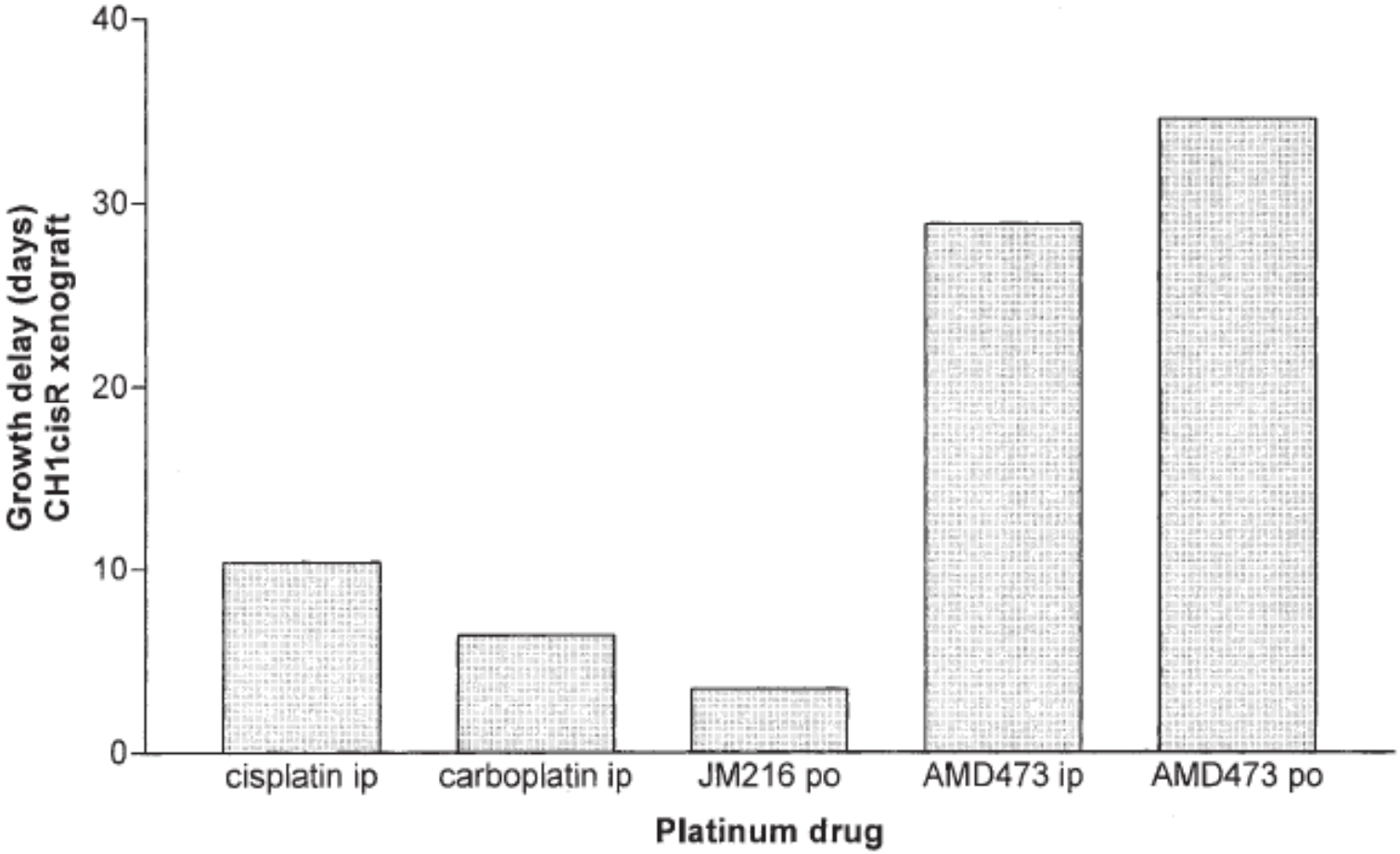
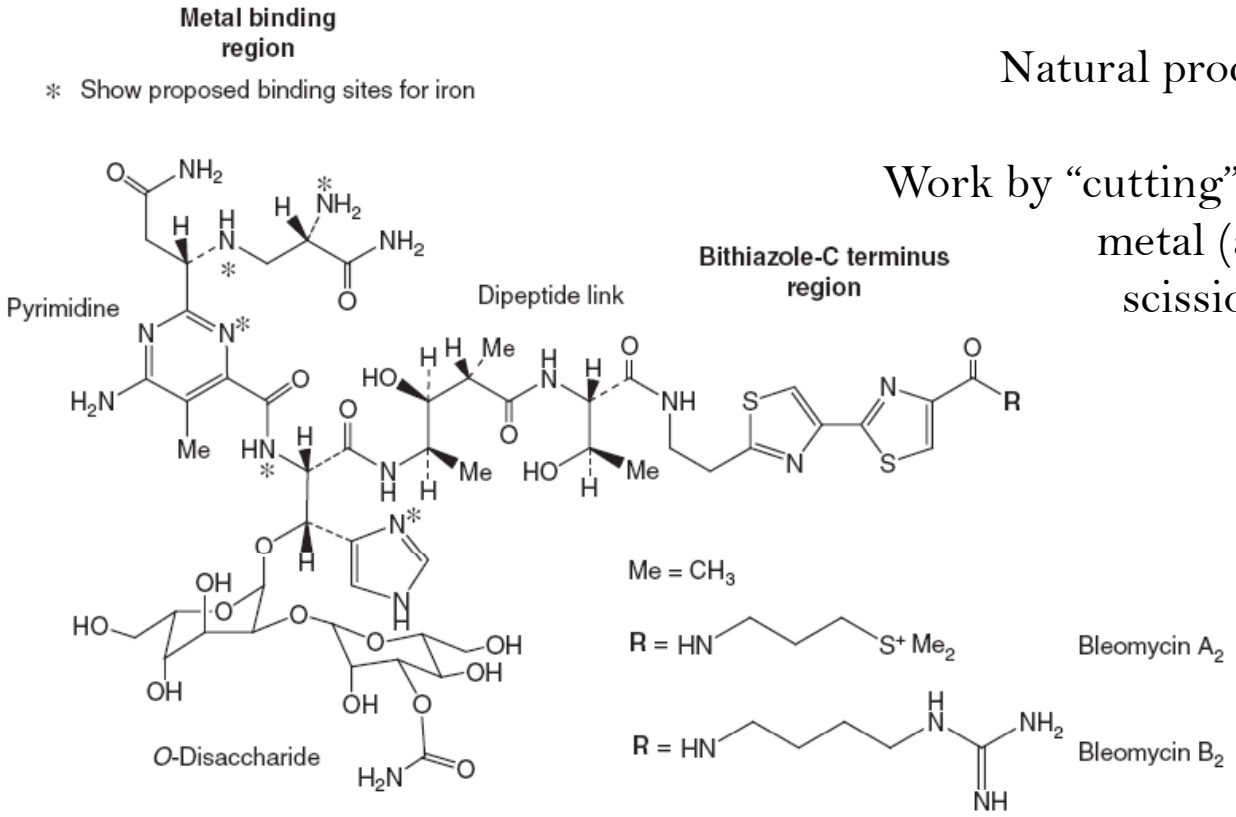


Fig. 4. *Antitumor activity in growth delay* (time taken in days for treated *vs*. control tumors to reach twice the volume at the start of drug treatment) *for i.p. administered cisplatin* (4 mg/kg q7d×4), *carboplatin* (80 mg/kg q7d×4), *oral JM216* (90 mg/kg q7d×4), *i.p. administered AMD473* (35 mg/kg q7d×4) *and oral AMD473* (400 mg/kg q7d×4) *in mice bearing the CH1*cis*R acquired cisplatin-resistant human ovarian advanced-stage s.c. xenograft*

Other non-Pt anticancer agents…

Bleomycins…



Natural products from glycopeptides.

Work by "cutting" DNA when activated by redox metal (and O_2 is present)…
scission of DNA strands

Testicular carcinomas, ovarian cancer, non-Hodgkin's lymphomas

Iron bleomycin is a remarkable multifunctional agent. At one end the molecule contains a DNA intercalator in the form of the bithiazole group which may serve as a sort of 'tether'. At the other end of the molecule is a metal binding region which not only binds and activates a metal centre towards reaction with dioxygen, it also contributes to the selectivity of DNA binding through recognising 5'-GC or 5'-GT regions in the sequence of bases.

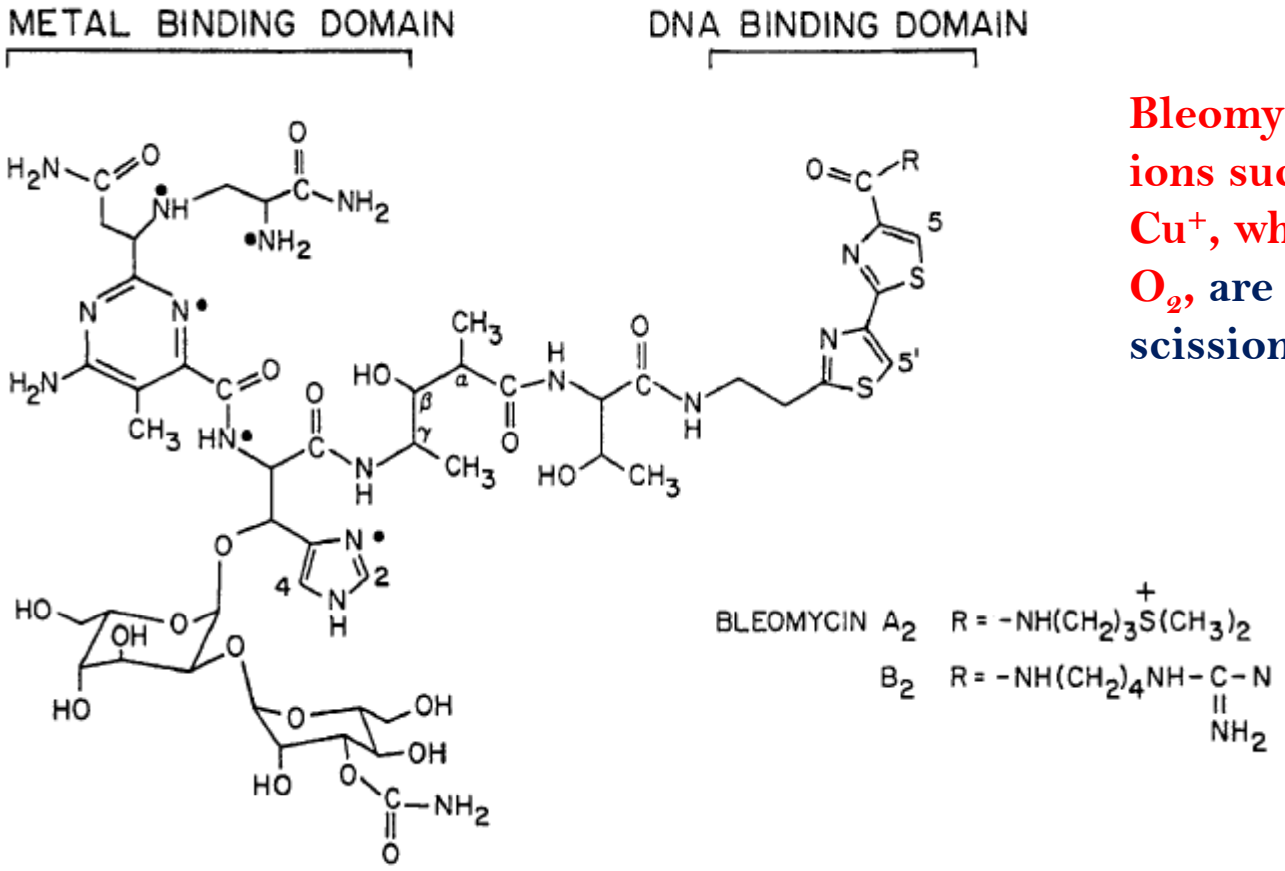
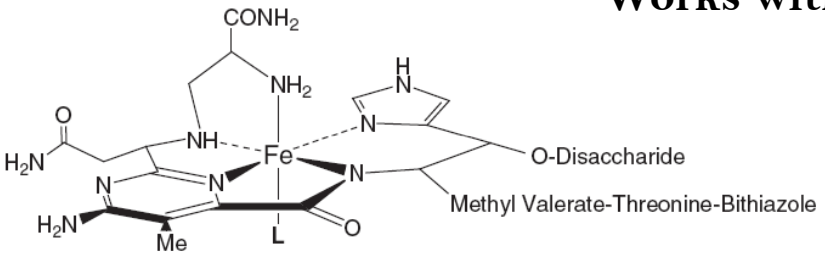


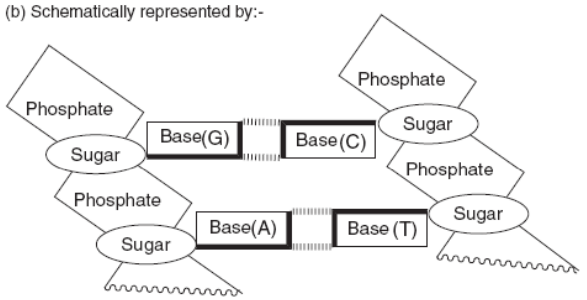
Figure 1 *Structure of bleomycin*

Bleomycin complexes of metal ions such as Fe^{2+}, Co^{2+} and Cu^{+}, which can be oxidised by O_2, are able to cause DNA scission when O_2 is present

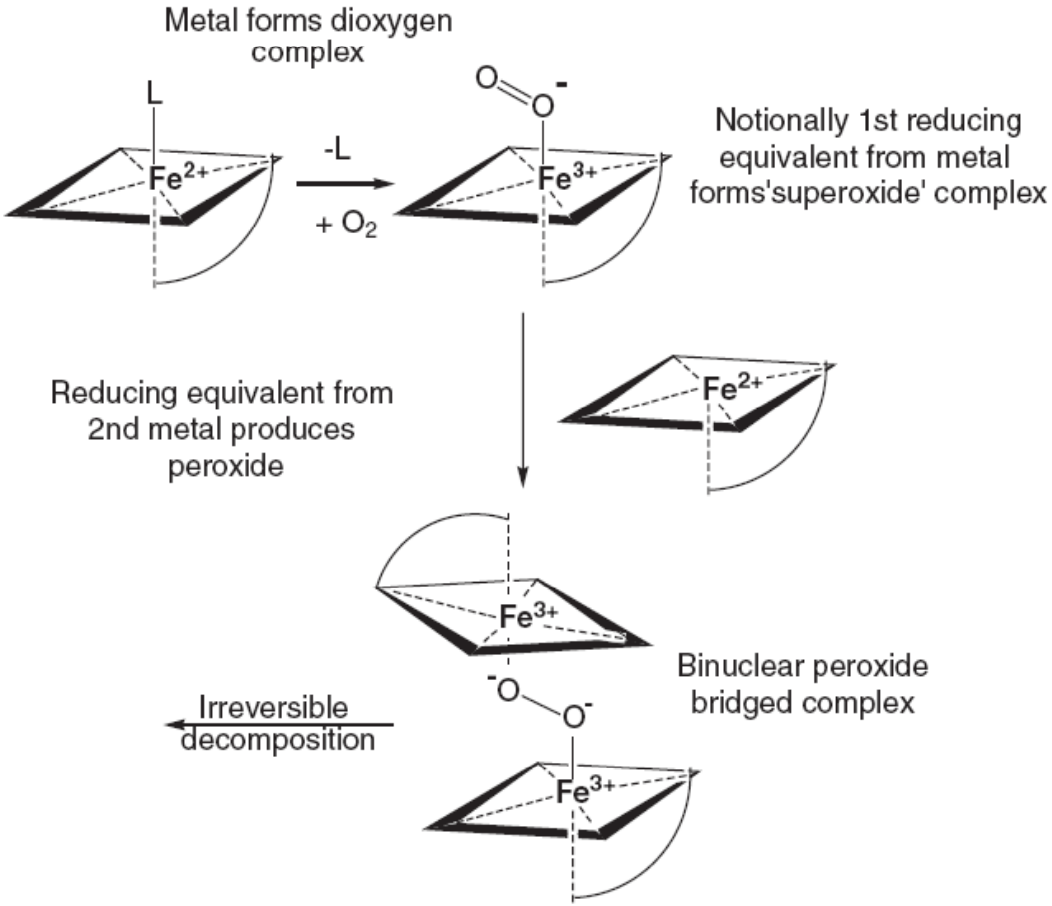
Works with Cu^{II} also!!!

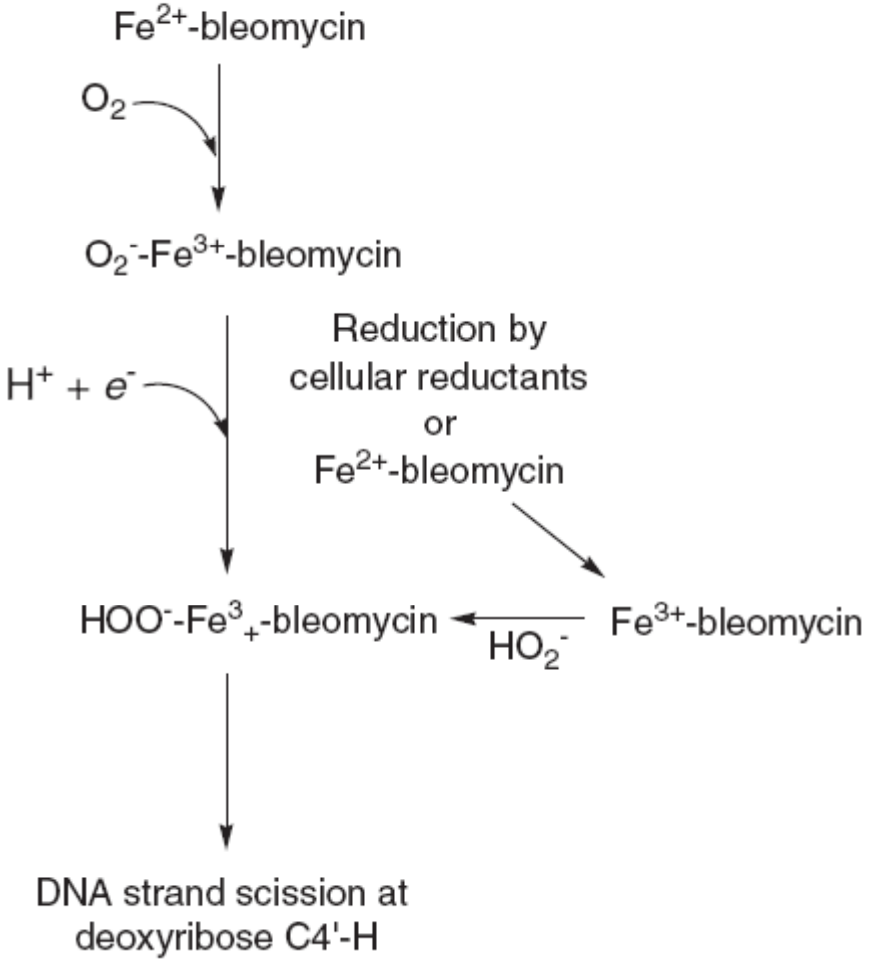


L = dioxygen or labile carbamoyl group from bleomycin



The structure of DNA showing (a) the complementary base pairing of the bases, Adenine with Thymine and Guanine with Cytosine and (b) a schematic representation of the 'double strand' structure





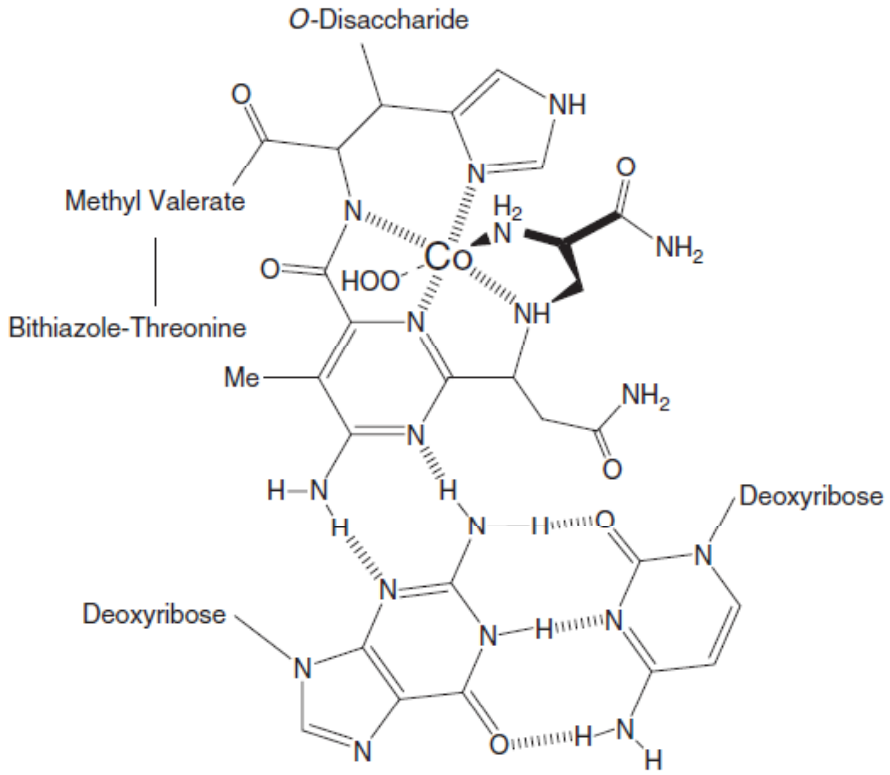
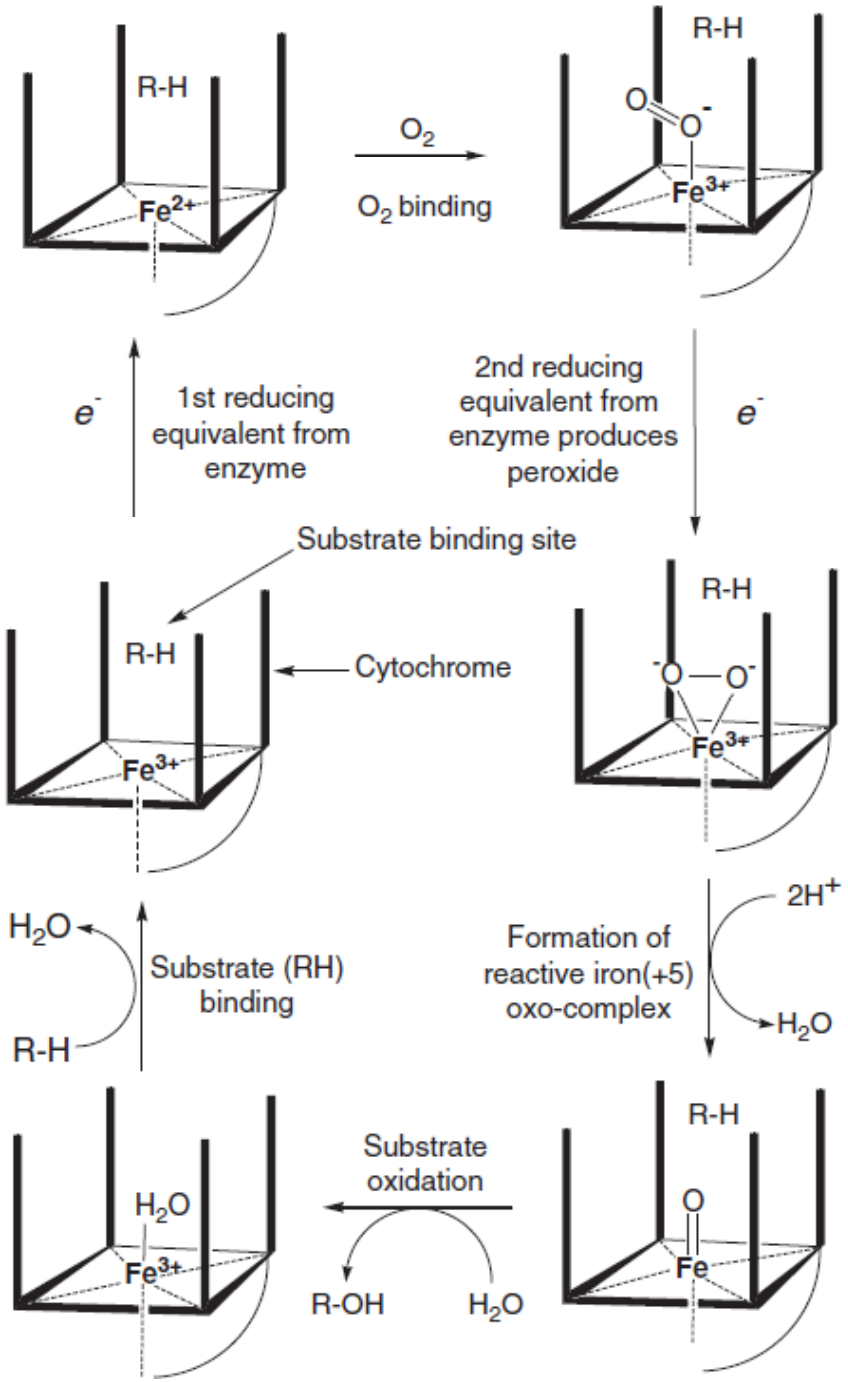


Figure 14 *A proposed interaction mode between the metal binding region of* Co^{3+}*-bleomycin and DNA*

Metallobleomycins preferentially bind to 5'-GC and 5'-GT sequences in DNA. This selectivity is dependent upon the nature of the metal binding region of the bleomycin but is unaffected by changes in the C-terminus. The presence of a bithiazole group in the structure of bleomycin is an important feature and sequence selectivity for 5'-GC and 5'-GT was lost when either thiazole ring was absent. The bithiazole group in bleomycin is known to be a DNA minor groove intercalator.

In bleomycin, the metal binding region is the major determinant of the sequence selectivity in DNA binding



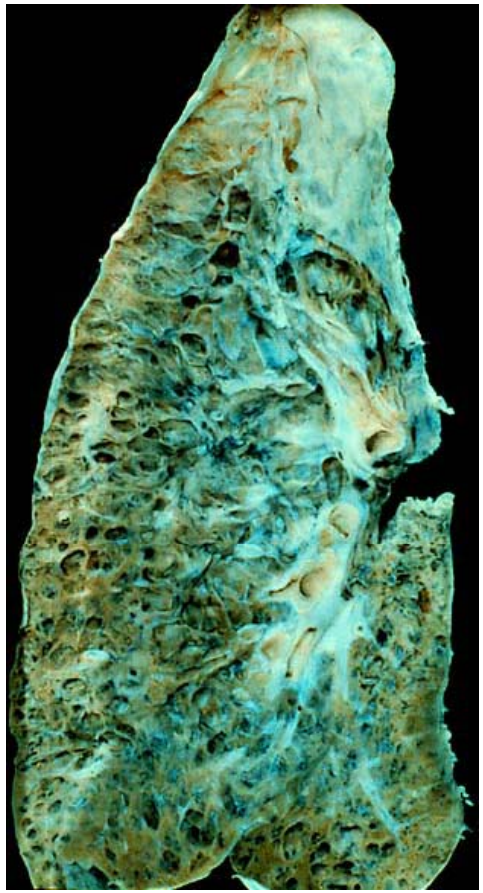
If the metal ion is structurally protected by the ligands so that a second reducing metal ion cannot approach the bound O_2 molecule, different behaviour results.

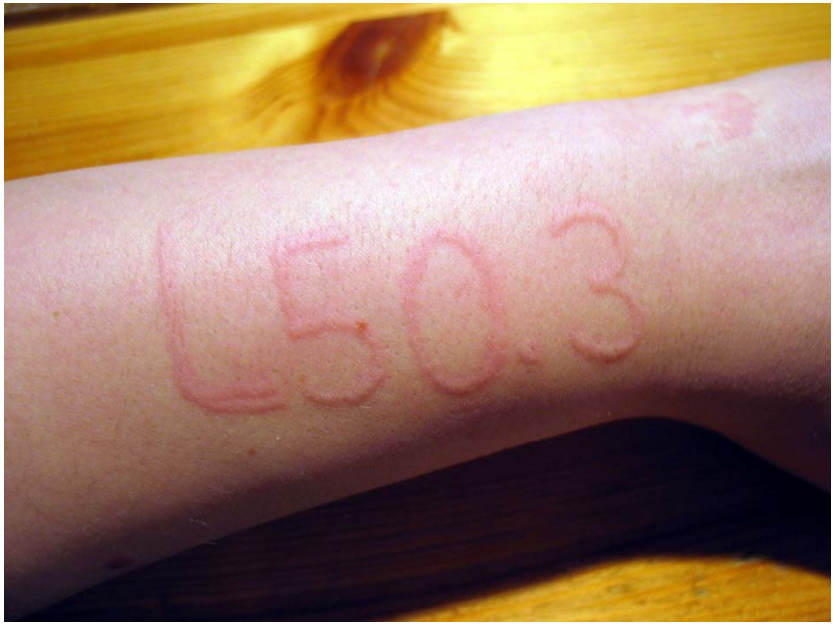
In the case of hemoglobin further reduction of the iron-dioxygen group is not possible and reversible dioxygen binding is observed.

In the case of cyt P_{450} a second reduction by the enzyme of the iron-dioxygen group produces a peroxide ligand bound to a single metal centre. This can form a reactive iron-oxo species which can effect oxidations such as converting a C-H group to C-OH.

Side effects:

1) Pulmonary fibrosis= deceases affecting the tissue and space around the air sacs of the lungs
2) Impaired lung function
3) Lung injury
4) Fever
5) Rash
6) Alopecia
7) Dermatographism



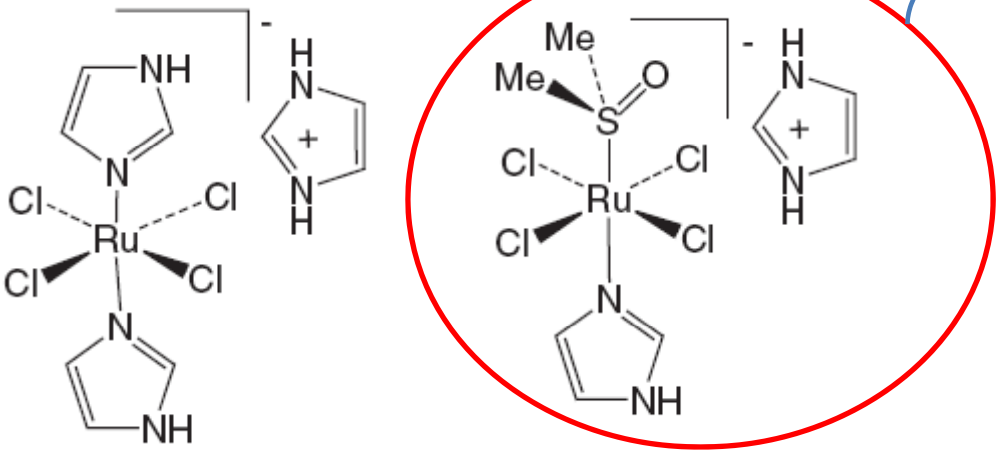


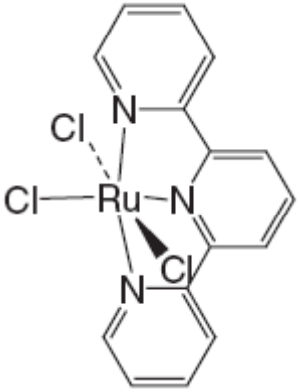
Ru-based anti-cancer drugs…

21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn
39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd
57 La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg

Ru: $5s^1\ 4d^7$
Ru^{II}: $4d^6$ low-spin
Ru^{III}: $4d^5$ low-spin

$[Ru_3O_2(NH_3)_{14}]^{6+}$ has shown anticancer reactivity
$[RuCl_3(NH_3)_3]$ …anticancer reactivity only for the fac isomer





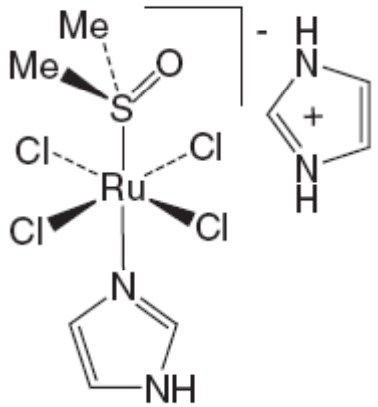
Activity against cancer metastases!!!
Ru-DMSO complexes:
Low toxicity…but high doses for therapeutic effects…

Ru(3+) to Ru(2+). ..propably inter- and not intra-strand DNA crossing.

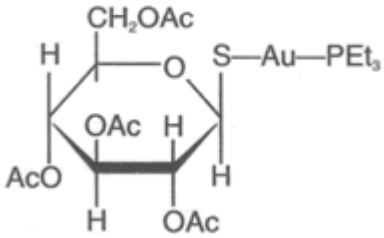
In summary ruthenium chloro-ammine, and related complexes, do show antitumor properties.

Transport of ruthenium to the tumor by transferrin seems likely and reduction of Ru^{3+} to Ru^{2+} complexes may be important in the release and antitumor action of the ruthenium.

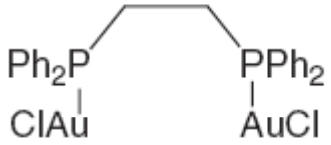
Furthermore, there is a possibility that ruthenium-oxo species might be formed in highly oxidising regions with the prospect of chemistry like that seen for iron in P_{450} or bleomycin occurring. As with cisplatin, binding to G sites in DNA seems favoured where DNA interactions occur but octahedral ruthenium complexes are not well suited to forming intrastrand crosslinks. Rather there is evidence of inter-strand crosslinks through purine binding to trans sites in the ruthenium complex.

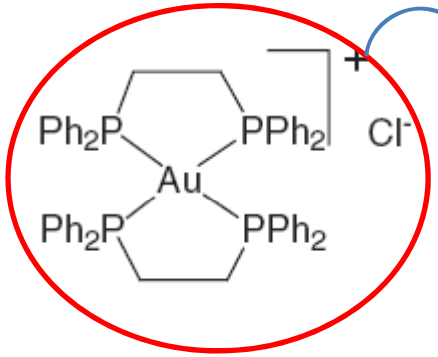


Au-based anti-cancer drugs…



Auranofin: promising in vitro results…but not that good in vivo.



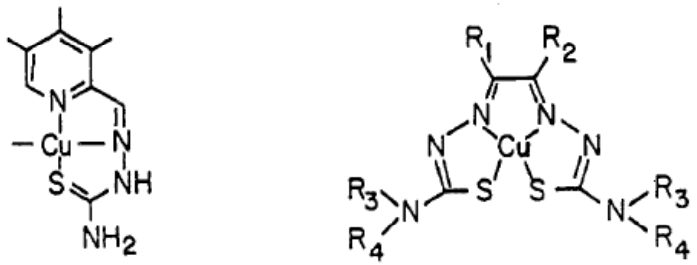


Active only when applied directly on the tumor…

bdppe: bis-diphenylphosphinoethane…Very good antitumor activity even without metal!

Inhibit protein synthesis (rather than DNA or RNA synthesis)
Forms DNA-protein crosslink and breaks DNA strands
Major dis.: Cardiovascular toxicity…

Cu-based anti-cancer drugs…



	R_1	R_2	R_3, R_4
CuKTS	$CH_3CH_2OCHCH_3$	H	H
$CuKTSM_2$	$CH_3CH_2OCHCH_3$	H	CH_3

Structures of thiosemicarbazonato Cu(II) complexes: (left) monothiosemicarbazones; (right) bis(thiosemicarbazones) CuKTS and $CuKTSM_2$

$$Cu(II)KTS + 2RSH \longrightarrow Cu(I)SR + \tfrac{1}{2}\,RSSR + H_2KTS$$

$$Cu(I)SR + O_2 \rightarrow Cu(II)SR + O_2^{-}\cdot$$

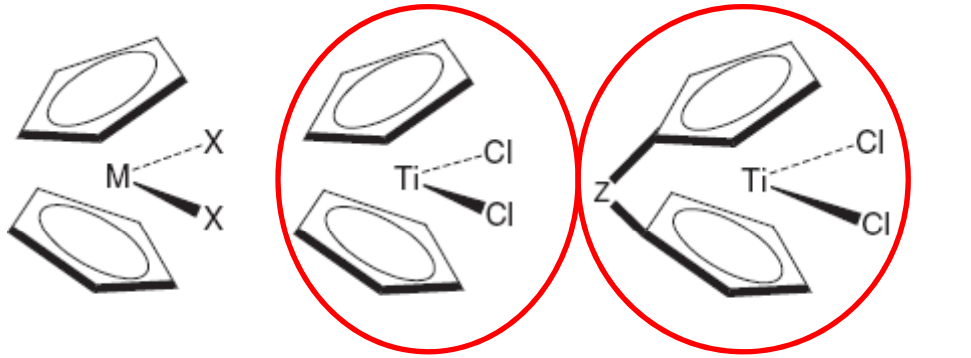
$$Cu(II)SR \rightarrow Cu(I) + \tfrac{1}{2}RSSR$$

$$2O_2^{-}\cdot + 2H^+ \rightarrow O_2 + H_2O_2$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + OH\cdot + OH^-$$

Organometallics-based anti-cancer agents…

Metallocenes: $[M(\eta^5\text{-}C_5H_5)_2X_2]$
M: Ti, V, Nb, Mo…significant activity
M: Ta, W, Zr and Hf…little or no reactivity



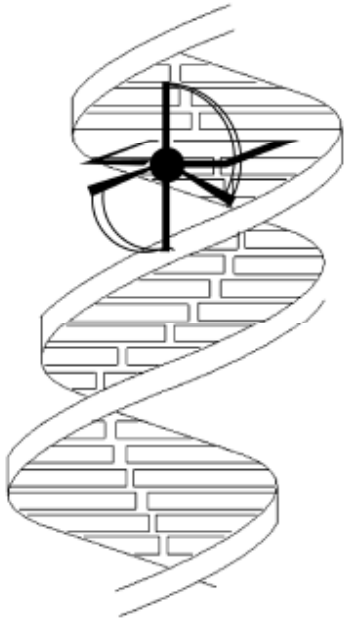
Z = CH_2, CHMe, SiHMe, $SiEt_2$, $GeMe_2$

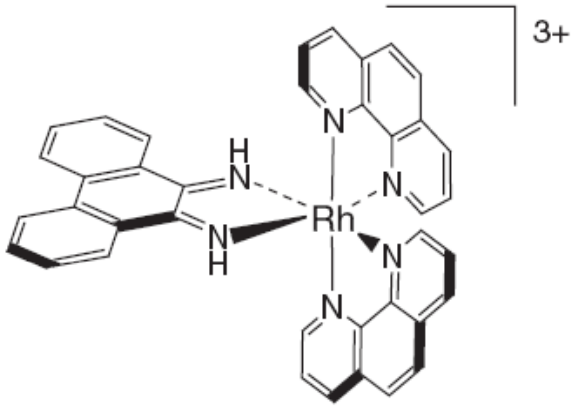
The mechanism of action of the metallocene dihalides remains uncertain but the effectiveness of $CpTiCl_2$ against cis-platin resistant cell lines suggests a different mechanism of action…

DNA-intercalators as anti-cancer drugs…

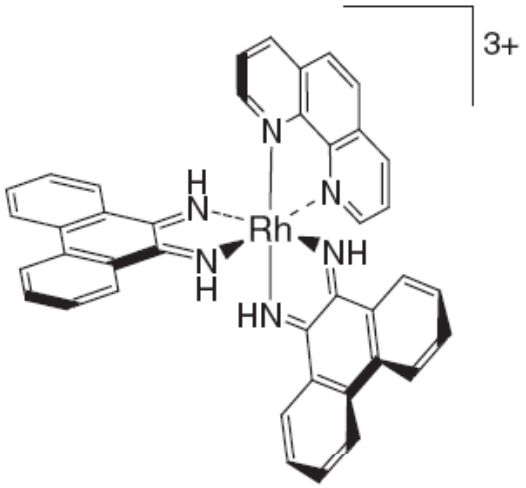
3 types of interactions:

1) **electrostatic**
2) **hydrophobic**
3) **intercalation**

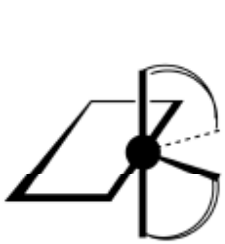




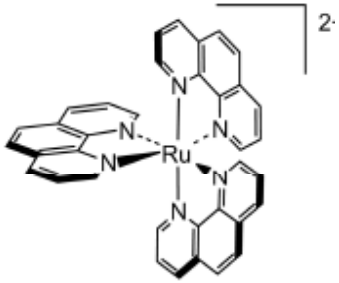
Δ-[Rh(phi)(phen)$_2$]$^{3+}$



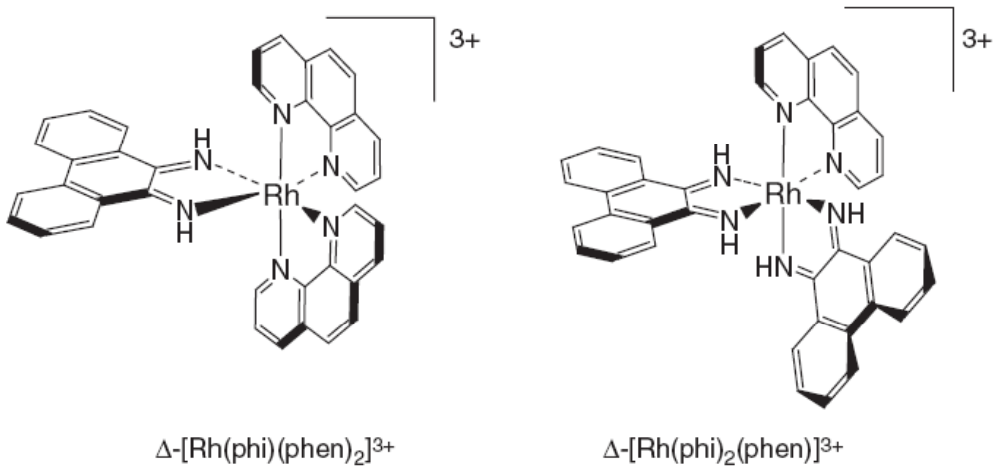
Δ-[Rh(phi)$_2$(phen)]$^{3+}$



Represents



Δ-[Ru(phen)$_3$]$^{2+}$



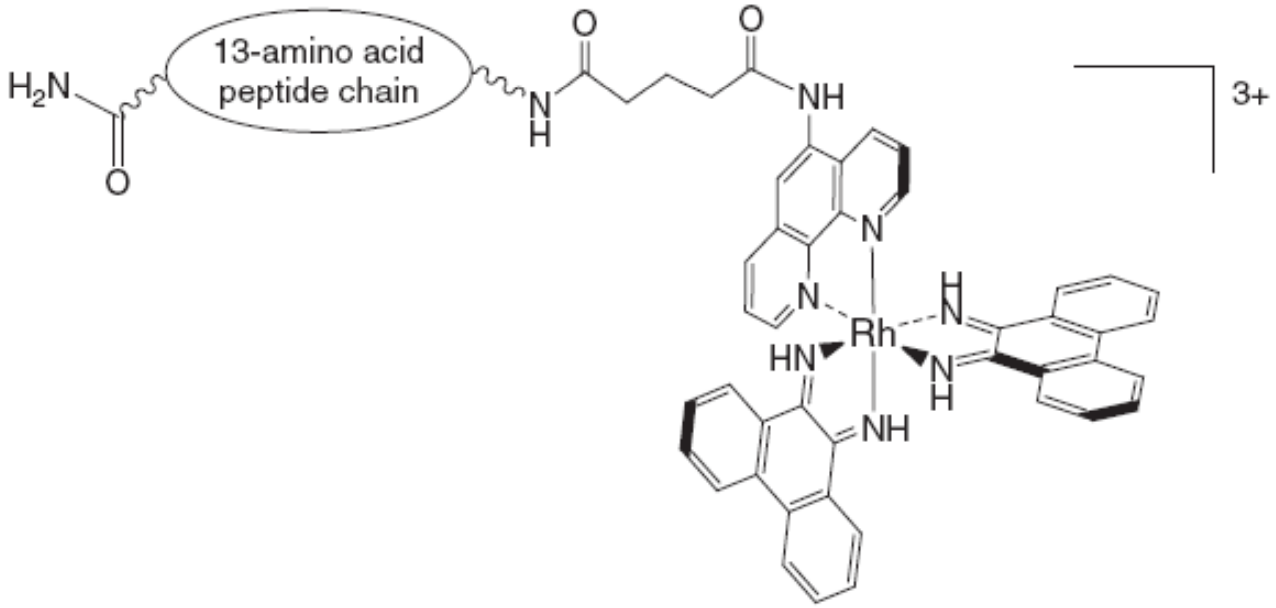
Δ-[Rh(phi)(phen)$_2$]$^{3+}$ Δ-[Rh(phi)$_2$(phen)]$^{3+}$

An important feature of such complexes is their chirality and this affects their DNA binding. Usually right-handed D-isomers preferentially bind to right-handed DNA.

The preference of many metallointercalators for binding in the major groove of DNA is an important feature, uncommon among synthetic agents.

The reactivity of the metal centre is also an important feature and much of the early work in this area exploited the photo-oxidation properties of certain metal complexes. As an example **photolysis** of complexes [Rh(phi)(phen)$_2$]$^{3+}$, or [Rh(phi)$_2$(phen)]$^{3+}$, bound to DNA in the absence of dioxygen produces 3'- and 5'-phosphate termini as well as free bases. This is consistent with a mechanism involving radical reactions. The supposition is that **light induced ligand to metal charge transfer** in the DNA bound metal complex produces, for example, [Rh^{2+}(phi$^{\cdot+}$)(phen)$_2$]$^{3+}$ containing **a ligand radical which can then extract a hydrogen from a deoxyribose unit**….initiates a sequence of events leading to DNA strand cleavage in a similar but not identical fashion to the interaction with activated bleomycin.

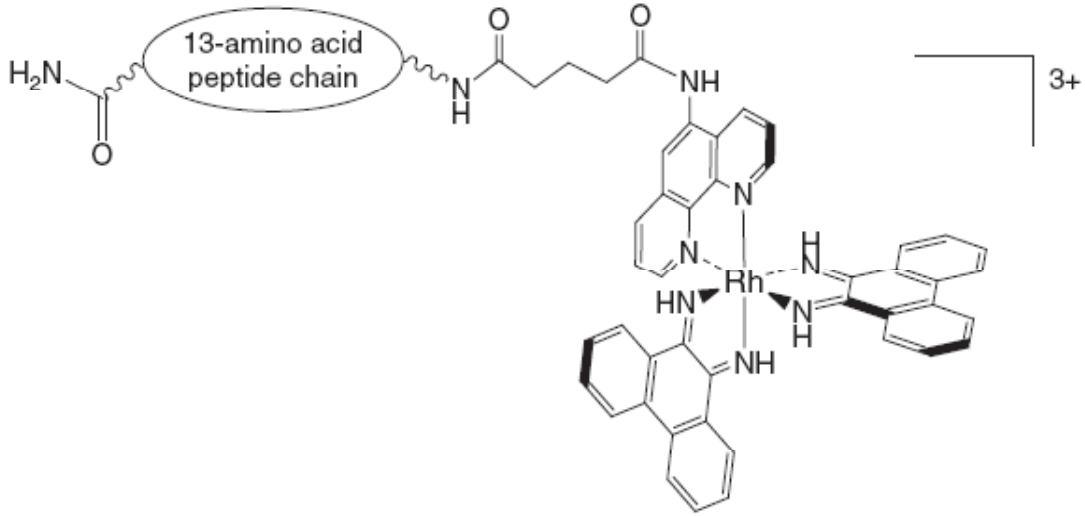
"*Chimera*" complexes



Contain a metal complex capable of acting as a DNA intercalator (*non-specific*) attached to a polypeptide capable of recognising a specific base sequence in DNA.

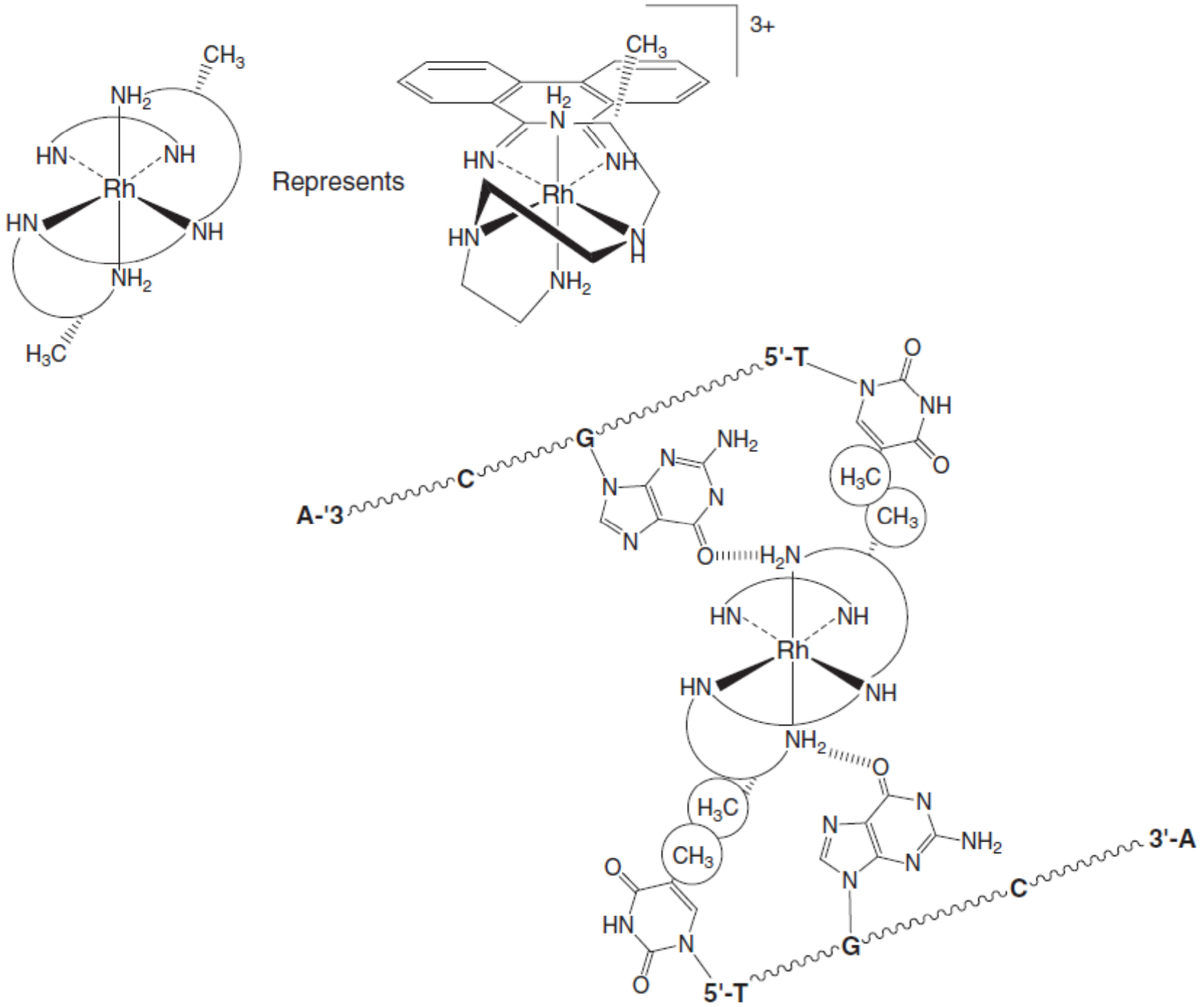
But, lack of rigidity is a potential problem for predicting their behaviour and achieving high sequence specificity

A kind of reverse DNA recognition strategy to that used by bleomycin is employed. The metal complex region acts as a non-specific DNA intercalator while the peptide sequence is a DNA binding domain able to recognise *5'*-CCA-*3'* sequences.



There is particular interest in metallointercalators for DNA, which can effect **oxidative or hydrolytic cleavage**. However, the lack of rigidity in these 'chimeras' is a potential problem for predicting their behaviour and achieving high sequence specificity. **Work on molecules which combine the redox activity of metal centres, the structural selectivity of the metal complex and the biological activity of domains taken from protein or oligonucliotides is in its infancy**.

Nonetheless the chimera approach offers a potentially powerful and selective means of modifying DNA in a medical context. Initially in vitro DNA manipulation applications will be much easier to establish and will presumably precede developments of in vivo therapy.


Represents
3+
CH3
NH2
HN
NH
Rh
H3C
H2
N
5'-T
G
C
A-'3
O
NH
H2N
3'-A