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CYTOTOXICITY OF RUTHENIUM COMPLEXES

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Cancer

- As we know cancer is a malignant tumor that occurs as a result of abnormal and uncotrolled cellular divisions.
- The first anti-cancer inorganic drug introduced in the clinic was de cisplatin [*cis*-diamminedichloroplatinum(II)].
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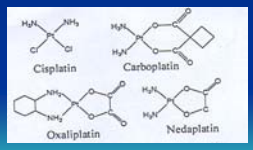
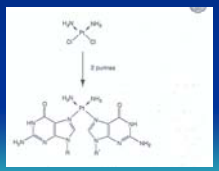
- The only four agents registered for use in the clinic, which do not represent a fundamental breakthrough in cancer treatment are:*cis*-diamine-dichloro-platinum(II) [cisplatin] and its direct analogues *cis*-diamine-(cyclobutane-1,1-dicarboxylato) platinum(II) [carboplatin], [cyclohexane-1,2-diamine-N,N'-oxalate(2-)-*O*,*O*]platinum(II) [oxaliplatin] and cis-diammineglycolato platinum(II) [nedaplatin]



- Cisplatin has limited solubility in aqueous solution and is administered intravenously. There are also significant problems in terms of inducing severe side-efects (especially kidney damage, vomiting/nausea, neurotoxicity and emetogenesis).
- Unfortunately, in general cisplatin analogues have not demonstrated any substantial advantages over cisplatin.

- There is evidence that the success of platinum complexes in killing tumor cells mainly results from their ability to form various types of adducts with DNA.
- Thus the search for metal-based compounds able to bind irreversibly to DNA is generally considered as one of the most attractive directions of research exploited to develop anti-cancer drugs. The cisplatin binds to two purines of DNA:



- Cisplatin reacts with DNA in the cell nucleus, where the concentration of chloride is markedly lower than in extracellular fluids. The drug loses its chloride ligands in media containing low concentrations of chloride to form positively charged mono- and di-aqua species:
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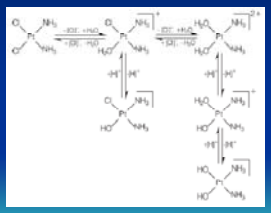
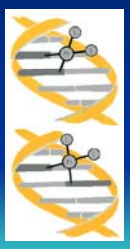


(a)

(b)



- Thus, the limitations associated with the clinical use of platinum drugs agents driven a search for more effective and less toxic alternative anti-tumor agents.
- Thus, much research looking for anti-tumour compounds has been carried out synthesizing and characterizing new non-platinum metal complexes, where the nature of both the metal and the ligand are varied.

- Complexes containing atoms of different metals are used in anticancer therapy for their inhibitory action on cell proliferation.
- One strategy used in this field is to obtain complexes where the metal center acts as a carrier of the real drug (the ligand), protecting it from oxidation or other reactions in the cellular medium.

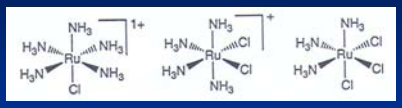
- Free diphosphines are active in several tumor modes in mice, but much less potent than transition metal complexes. The complexes [Au(I) and Cu(I)] are at about 20-fold more potent!
- It is interesting to point out that the activity of the diphosphines, or bridged [Cl-Au(R2P(CH2)nPR´2)-Au-Cl] or chelated complexes against P388 leukemia is lost when the phenyl groups are replaced by ethyl groups or when a cis-CH=CH group in the diphosphine or in the bridged diphosphine complex is replaced by *trans*-CH=CH.
- This means that the characteristic of the complex (the metal and the phosphine) is important for its anti-tumoral activity.

- The metal may play an important role in the mechanism of the cytotoxicity and antitumor activity of the complex:
- 1) it acts as a carrier for the reactivity ligand;
- 2) it protects the ligand of oxidation;
- The characteristic of the metal is indeed very important.
- 1) bis(diphosphine) complexes of Au(I), Cu(I) and Ag(I) are active anti-tumor agents.
- 2) in contrast, the complexes [M(dppe)Cl2] where M=Pd(II) and Pt(II) are inactive.
- 3) Ni(II) bis-(phosphine) complexes should exhibit antitumor activity, because they are kinetically labile, but the coordinated phosphine is liberated from the metal and fastly oxidazed. Since dppeO2 is not cytotoxic, NI(II)-bis(phosphines) must be stabilized if they are to be of biological use.

PERSPECTIVES OF RUTHENIUM COMPLEXES IN CANCER THERAPY

- Ruthenium ions have high affinities for lone pairs of electrons and are thus often complexed by nitrogen atoms of high-field polyazaaromatic ligands like guanine residue;
- Ruthenium can selectively bind biomolecules;
- The synthesis processes of ruthenium complexes are well-developed;
- There are possibilities to adjust complex properties (ligand substitution rate, redox properties, etc.) by varying the ligands coordinated to the metal center.
- **Ruthenium complexes are stable as in solid state, as in solution and their structures are predictable.**

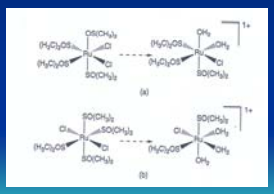
- The first systematic investigation of ruthenium compounds and their antitumor property was done in beginning of 1980s, by Clarke and co-workers who studied chloro-ammino derivatives of the general formula $[Ru(NH_3)_{6-x}Cl_x]^{Y+}$:



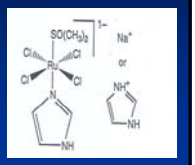
- These compounds were thosen due to their similarities with cisplatin.

Why dimethyl-sulfoxide complexes?

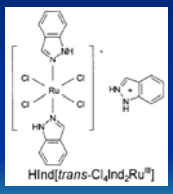
- The solubility of ruthenium complexes can be highly improved by substituting the NH_3 ligands by dimethylsulfoxide (DMSO) molecules. The initial studies with DMSO complexes were performed by Mestroni, Alessio and co-workers on the *cis*- and *trans*-$[Ru(DMSO)_4Cl_2]$ (a and b):



- The NAMI-A is been tested in clinical phase I and has high selectivity for solid tumor metastasis and low host toxicity at pharmacologically active doses.
- It was the first ruthenium to enter clinical trials.
- It has a remarkably low general toxicity and shows marked efficacy against metastases. It does not affect primary tumor growth.



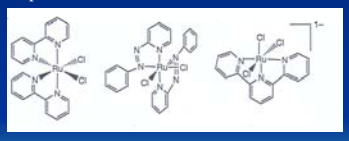
- – $[H_2Ind][trans\text{-}RuCl_4(HInd)_2]$ (HInd = 1*H*-indazole), exhibits anticancer activity, and like the NAMI-A it is activated by reduction [to Ru(II)] in vivo which facilitates DNA binding.



By comparing the general toxicity of ruthenium compounds with platinum drugs the ruthenium has lower toxicity, that is, has been attributed to the ability of ruthenium compounds to specifically accumulate in cancer tissues.

COMPLEXES WITH MIXED CHLORIDE AND N-HETEROCYCLIC LIGANDS

- Several Ru complexes containing chlorides and N-heterocyclic ligands have also been prepared and investigated for their anti-tumor properties on the basis of their similar reactivity as compared to cisplatin.



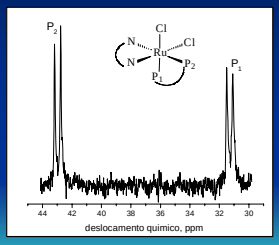
Why phosphine ruthenium complexes?

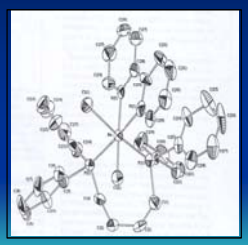
- Phosphine complexes of various transition metals (Au, Ag, Cu, Ru, Rh, Pt, Pd) have been evaluated as potential antitumor agents in various human tumor cell lines. In the Au complexes, for instance, the observed activity has been attributed to the presence of the phosphine ligands, since similar complexes without them had a very low activity[31]. In this sense, the metal center acts as a carrier of the real drug, protecting it from oxidation or other reactions in the cellular medium

Antitumoral activity

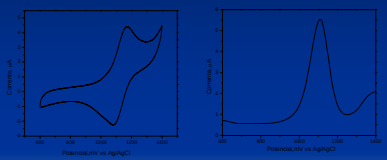
- The cells were exposed to each compound for a period of 24 hours, in order to allow them reach DNA or any other biological target. The new complexes **1** and **2**, the precursors **3** and **4** and the uncoordinated dppb, bipy, Me-bipy and HSpymMe2 ligands were tested against the MDA-MB-231 cells. For comparison, the cytotoxicity of cisplatin was evaluated under the same experimental conditions. The IC50 values, calculated from the dose-survival curves generated by the MTT assay obtained after 24 h drug treatment.

The complexes used in this work were characterized by microanalysis (CNHS), Infrared, UV/Visible, $^{31}P\{1H\}$ spectra, electrochemistry and when possible, by X-ray data. *cis*-[$RuCl_2$(dppb)(bipy)]

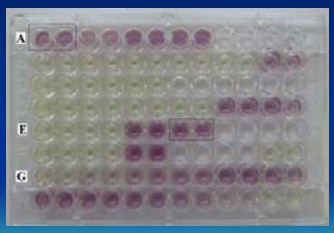




cis-[$RuCl_2$(dppb)(bipy)]



IC50(μM) values for the MDA-MB-231 cell line of Ru complexes, measured in DMSO solution.



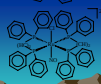
IC50(μM) values for the MDA-MB-231 cell line of Ru complexes, measured in DMSO solution.

- [$Ru(pic)_2(PPh_3)_2$] 20 – 200

- *trans*-[$RuCl(NO)(dppm)_2](BF_4)_2$ **2.00**

- *trans*-[$RuCl(NO)(dppe)_2](PF_6)_2$ **2.00**

- *trans*-[$RuCl(NO)(c\text{-}dppen)_2]Cl_2$ 2,95



- *fac*-[RuCl3(NO)(dppf)] **10 ± 3**

- **[Ru(´SpymMe2`,-*N*,-*S*)**
- **(´SpymMe2`,-*S*)(NO)(dppe)]PF_6** **2 – 20**

- **[RuCl(NO)(dcpe)(Me-bipy)]$(PF_6)_2$** **2 – 20**

- **[RuCl(CO)(dcpe)(Me-bipy)]PF_6** **0.43 ± 0.34**
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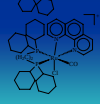
- **[RuCl(CO)(dcpe)(phen)]PF_6** **0.30 ± 0.07**



cis-[$RuCl_2$(dppf)(bipy)] **4.5 ± 0.86**

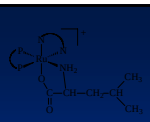
cis*-[$RuCl_2$(dppb)(bipy)] 31.3 ± 19

***cis*-[Ru(pic)(dppm)$_2$]PF_6 0.48 ± 0.13**

***cis*-[Ru(pic)(dppe)$_2$]PF6 0.53 ± 0.16**

[Ru(pic)(dppb)(bipy)]PF_6 4.2 ± 1.5

[Ru(dppb)(bipy)(Leucina-N-O)]PF_6 **4.1 ± 1.6**

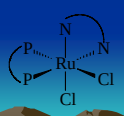


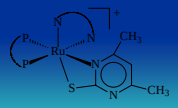
***cis*-[$RuCl_2$(NO)(BPA)] 87 ± 23**

IC50 values for the MDA-MB-231 cell line of free ligands and cisplatin, measured in DMSO solution.

Ligante	IC_{50} (μM)
dppf	97 ± 8
dppb	> 200
Hpic	> 200
HSpymMe2	> 200
Bipy	> 200
Cisplatin	63 ± 5

- In our complexes, much higher activity was achieved when the *cis*-chlorides of the *cis*-[RuCl2(dppb)(N-N)] [IC_{50}= **31.3 ± 19 (μM)]** precursor were replaced by the SpymMe2 ligand, forming the new derivatives *cis*-[Ru(SpymMe2)(dppb)(Me-bipy)]PF6 [IC_{50}= 0,43 ± 0,08 **(μM)]**.
- Even relative to cisplatin (reference metallodrug), our complexes are much more active on the MDA-MB-231 cell line, indicating their potential usefulness as antitumoral agents.





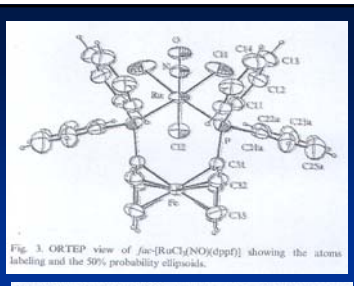


Fig. 3. ORTEP view of *fac*-[$RuCl_3$(NO)(dppf)] showing the atoms labeling and the 50% probability ellipsoids.

IC_{50} values in MDA-MB-231 cell line of *fac*-[$RuCl_3$(NO)(dppf)], $RuCl_3NO \cdot 2H_2O$, free dppf and cisplatin measured in DMSO solutions

Compound	IC_{50} (μM)
fac-[$RuCl_3$(NO)(dppf)]	10 ± 3
$RuCl_3NO \cdot 2H_2O$*	>200
dppf	97 ± 8
cisplatin	63 ± 5

* The maximum utilized concentration was 200 μM.

- **Camundongo C57BL/6 macho com desenvolvimento de melanoma.**

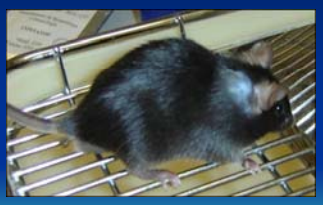
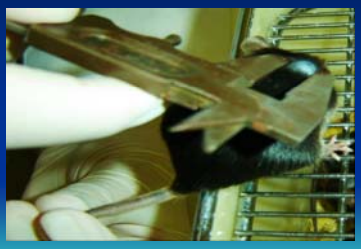
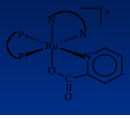
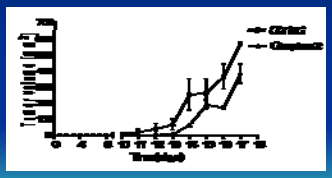


Foto ilustrativa do método usado para medida do volume dos tumores. A foto demonstra como os tumores foram medidos: com um paquímetro em 2 sentidos diferentes, de modo a se obter as medidas do diâmetro menor e do diâmetro maior dos nódulos para posterior aplicação na Equação dos tumores foi usado um paquímetro e o volume (mm^3) calculado com a equação: Volume = [(diâmetro maior) x (diâmetro menor)^2] /2



Blockade effect of complex ***cis*-[$RuCl_2$(NO)(BPA)]** on melanoma growth in C57/BL6 mice. Tumor volume was monitored during 17 days after the complex 2 injection (1mg/Kg).





- In the literature, most ruthenium-containing complexes studied for their antitumor activity have a pair of *cis*-orientated chloro ligands with some reports indicating the possibility of a mechanism involving DNA binding.
- DNA is also a potential target for Ru(II) arene complexes, and they exhibit a high selectivity for binding to N7 of guanine.
- The competitive reactions of N-donor(G^{N7}-DNA) and S-donor(thiol/thioether) ligands for different metals [Pt(II) and Ru(II)] have been suggested in some studies.
- More recently some papers have described that the main factor responsible for the antitumor activity of ruthenium complexes is the binding with serum proteins such as albumin and transferrin, despite that the exact role of such binding plays in the mechanism of the action of metallodrugs remains to be elucidated.