

DSM Science & Technology Awards 2004

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Summary Evaluation Work Candidate DSM Award 2004

Design, synthesis and determination of the structure and antitumor properties of new cytostatics on the basis of ruthenium complexes

Dr. Anna C.G. Hotze, MSc; Leiden Institute of Chemistry

1. Introduction

With great pleasure we introduce the nomination of Dr. Anna G.C. Hotze as a candidate for a DSM Award 2004. Anna Hotze was a PhD student who has been working extremely efficient, independent, fast and dedicated on a very exciting research project, where she has reached very important results, parts of which have already appeared in her publications (see list of publications). She defended her PhD thesis on September 30, 2003 *cum laude*.

2. Topic and approach in the thesis research

The PhD thesis of Anna Hotze describes the design and synthesis of new ruthenium coordination complexes that are antitumor active and that can (and in fact do) interact with DNA. Her research topic is well chosen and timely, and the questions addressed in the thesis and in her papers have been evaluated as highly relevant. The applied methodology selected by Dr. Hotze is quite diverse, and includes:

- Ligand design and ligand synthesis. Most notably several derivatives of Azpy, the key ligand.
- Ru(II) and Ru(III) coordination compounds: bis and tris complexes synthesis, characterisation and structure using XRD and high-resolution NMR techniques at variable temperature.
- Study of the antitumor activity of the ruthenium compounds in tumor cell lines and (for a few very active compounds) also in living animals (externally performed in Trieste).
- Study the binding of the ruthenium compounds to DNA and DNA fragments with molecular resolution.

On all of the 4 above-mentioned main topics Dr. Hotze has been studying in an excellent innovative way. In fact within the group only one previous PhD thesis research was done. Also she has made an impressive use of collaboration with some undergraduate students for which she made a really excellent planning and did a great supervision.

The approach in her research is appealing and of use for many people elsewhere. In all cases, the crucial role of both the steric requirements have been very well considered. In the final part of the study the reactivity of the azpy-type ligands is a good concluding approach. She is an extremely stimulating personality for undergraduate students and colleagues; she prepares and presents excellent presentations at conferences.

3. Topic Description

The bis(2-phenylazopyridine)ruthenium(II) complexes topic of the PhD project belong to the class of the chelating heterocyclic ruthenium complexes. As the ligand 2-phenylazopyridine (azpy) is an asymmetric ligand, the coordination of two azpy ligands to one metal results in several isomeric complexes. The so-called α , β and γ isomers of $[\text{Ru}(\text{azpy})_2\text{Cl}_2]$ (fig. 1) are the three main isomers, easily to synthesize. The isomeric $[\text{Ru}(\text{azpy})_2\text{Cl}_2]$ complexes have been tested for their cytotoxicity and show remarkable differences in IC_{50} values (IC_{50} value represents the

concentration of a drug that is required for 50 % reduction of cellular growth) against a variety of human tumor cell lines. The two isomers both with the chloride ligands in a *cis* position show already distinct differences. The α isomer shows a very high cytotoxicity, and is even better than the well-known anticancer agents cisplatin and 5-fluorouracil. The β isomer is a factor 10 less cytotoxic than the α isomer. The isomer with the chloride ligands in a *trans* position, the γ isomer, is also very cytotoxic with IC₅₀ values comparable with the α isomer.

One of the aims of the PhD study concerning the [Ru(azpy)₂Cl₂] complexes was the synthesis and investigation of biological activity of slightly different compounds [RuL₂Cl₂] with L= 4-methyl-2-phenylazopyridine (mazpy) and o-tolylazopyridine (tazpy) (fig. 1) and the mixed ligand compound [RuLL'Cl₂] (with L azpy and L' bpy). The second topic of research is the improvement of the water solubility of the dichlorobis(2-phenylazopyridine)ruthenium(II) complexes. Unfortunately the [Ru(azpy)₂Cl₂] complexes are poorly water soluble, one of the requirements needed for further *in vitro* and *in vivo* testing. The final subject is the interaction of this kind of complexes with DNA model bases in search for structure-activity relationships.

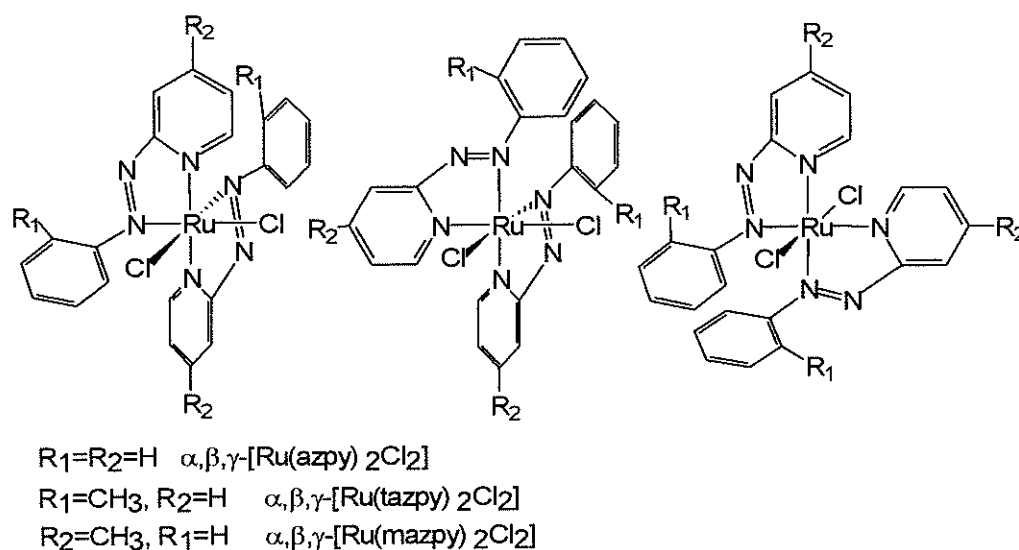


Fig.1: Three important isomers (α left, β center, and γ right) of bis(2-phenylazopyridine)ruthenium(II) complexes (α =Npy *trans*, Nazo *cis*, Cl *cis*; β = Npy *cis*, Nazo *cis*, Cl *cis*, γ =Npy *cis*, Nazo *cis*, Cl *trans*)

The three main isomers α , β and γ -[RuL₂Cl₂] (fig. 1) with L= 4-methyl-2-phenylazopyridine or o-tolylazopyridine have been synthesized and tested for their cytotoxic activity in a panel of human tumor cell lines. The *in vitro* cytotoxicity data of the [RuL₂Cl₂] complexes show the same differences between the several isomers as shown for the parent [Ru(azpy)₂Cl₂] complexes. The α isomer again exhibits a very high cytotoxicity and the β isomer is a factor 10 less active than the α isomer. Finally the *trans* chloride γ isomer of these derivative ligands are as active as the α isomer. There are only slight differences between the cytotoxicity of the methylated azpy complexes and the parent compounds, which are not completely understood. The synthesis of another structural derivative of the [Ru(azpy)₂Cl₂] complexes was inspired by the remarkable difference in activity between the very cytotoxic compound α -[Ru(azpy)₂Cl₂] and inactive *cis*-[Ru(bpy)₂Cl₂].

The mixed ligand complex α -[Ru(azpy)(bpy)Cl₂] has been developed which shows unfortunately a very low cytotoxicity probably caused by the structural and electronic different aspects of this complex related to α -[Ru(azpy)₂Cl₂]. The reason for the various activities of (derivatives of) the [Ru(azpy)₂Cl₂] complexes have been partly explained by different DNA model base binding modes (*vide infra*).

As the dichlorobis(2-phenylazopyridine)ruthenium(II) complexes are not water-soluble the water-soluble nitrate compounds α -[Ru(azpy)₂(NO₃)₂] and β -[Ru(azpy)₂(NO₃)₂] have been developed. Another type of water-soluble analogues has been designed, and they consist of a group of dicarboxylatobis(2-phenylazopyridine)ruthenium(II) complexes (fig. 2), and in particular the 1,1-cyclobutanedicarboxylatobis(2-phenylazopyridine)ruthenium(II), α -[Ru(azpy)₂(cbdca-O,O')], oxalatobis(2-phenylazopyridine)ruthenium(II) and α -[Ru(azpy)₂(ox)] and malonatobis(2-phenylazopyridine)ruthenium(II), α -[Ru(azpy)₂(mal)]. The choice of the carboxylato ligands was in fact inspired by the compound carboplatin, which is known to be less toxic than cisplatin, while being comparatively active as cisplatin.

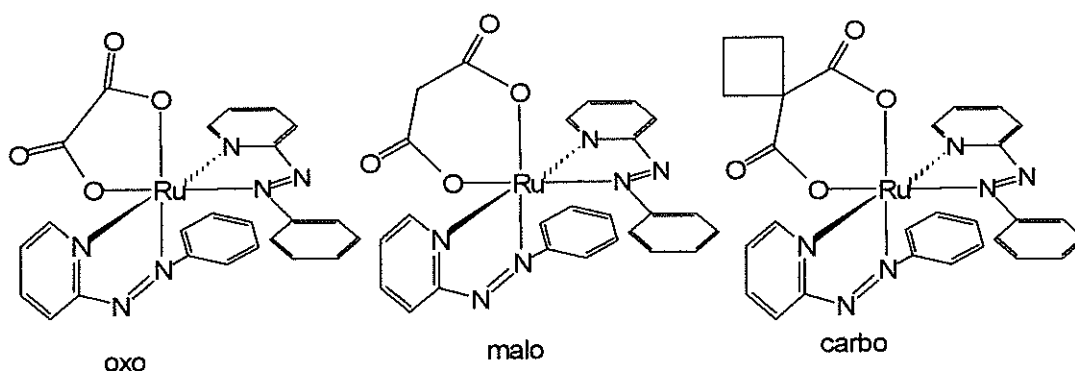


Fig. 2: Water soluble α -carboxylatobis(2-phenylazopyridine)ruthenium(II) species

The water soluble compounds have been successfully synthesized and also characterised by X-ray structure determination, NMR, UV VIS and IR. For example the molecular structure of α -[Ru(azpy)₂(cbdca-O,O')] is redrawn in fig. 3.

The compounds have been investigated for their *in vitro* cytotoxicity, cellular uptake and cell cycle analysis in several tumor cell lines for example the A2780 and A2780cisR cell lines. The A2780cisR is endowed with multifactorial resistance mechanisms, such as decreased uptake, increased glutathion levels and increased DNA repair, and therefore represents a good model for the screening of new anticancer metal-based agents.

The cytotoxic activity of these new water-soluble bis(2-phenylazopyridine)ruthenium(II) carboxylato complexes and of the water soluble α -[Ru(azpy)₂(NO₃)₂] and β -[Ru(azpy)₂(NO₃)₂] have been compared to the cytotoxicity of the parent compounds α -[Ru(azpy)₂Cl₂] and β -[Ru(azpy)₂Cl₂]. Both the nitrate and the carboxylato complexes show a promising high cytotoxicity in the A2780 cell line (IC₅₀ values between 2-10 μ M) and have the important advantage of improved water solubility compared to the parent chloride complexes. The activity is comparable to that of cisplatin, and even higher than the activity of carboplatin. Interestingly, the IC₅₀ values of this series of ruthenium compounds (except the β isomeric compounds) are similar in the cisplatin resistant A2780cisR cell line compared to the normal cell line A2780. This observation strongly suggests that the activity of these compounds might not be influenced by the multifactorial resistance mechanism that affect platinum anticancer agents, and therefore is promising for clinical trials.

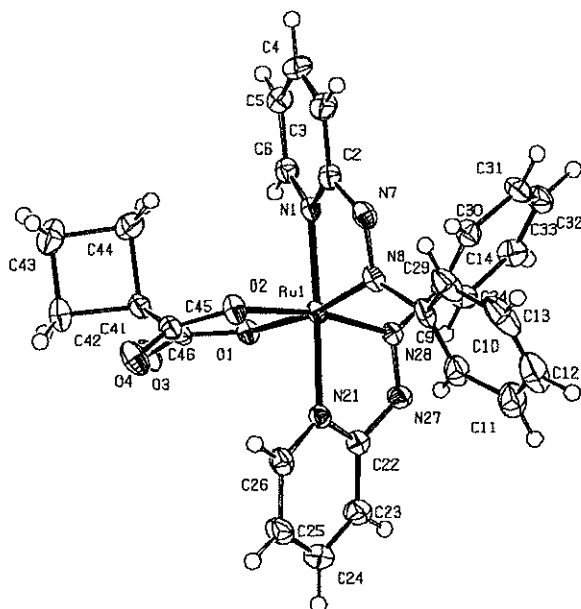


Fig. 3: Molecular structure of the water soluble α -[Ru(azpy)₂(cbdca-O,O')]

Especially the nitrate and the parent chloride complexes show very rapid cellular uptake and consecutively reduction of proliferation after challenging tumor cells for 1 hour with the present ruthenium drugs. A very interesting result was the correlation found between the activity of the compounds and the amount of ruthenium complex inside the cell. For example, after exposure of tumor cells for 1 hour to α -[Ru(azpy)₂Cl₂] and β -[Ru(azpy)₂Cl₂], atomic absorption spectroscopy showed that the 10 times more cytotoxic α isomer appears also in 10 times higher quantities than the β isomer inside the cells.

Because of the strong differences in biological activity between the structurally similar complexes *cis*-[Ru(bpy)₂Cl₂] (inactive) α -[Ru(azpy)₂Cl₂] and β -[Ru(azpy)₂Cl₂] it is important to compare the binding of DNA-model bases to these complexes in a search for structure-activity relationship. Extensive 2D-NMR experiments of the DNA adducts have been performed and show that the DNA-model base 9-Ethylguanine (9-EtGua) coordinates to both *cis*-[Ru(bpy)₂], α -[Ru(azpy)₂] and β -[Ru(azpy)₂] moieties monofunctionally and via the N7 atom. This similar coordination of 9egua would at first sight not explain the differences in cytotoxicity. However, some small, but important changes in flexibility of the coordination to DNA model bases have been observed and explained in terms of (in)activity.

The crystal structure of *cis*-[Ru(bpy)₂(9egua)Cl]PF₆ shows that the keto group of 9-Etgua is positioned “stacked” between two pyridyl rings of the bpy ligands. From NMR data it has been concluded that no rotamers of *cis*-[Ru(bpy)₂(9egua)Cl]PF₆ are present. This is in contrast with the results of the azpy analog mentioned above. In fact, the larger, but more flexible azpy ligand appears to allow more orientations for the guanine derivatives!

The α -[Ru(azpy)₂] moiety being more flexible in its coordination to heterocycles than the *cis*-[Ru(bpy)₂] moiety is in agreement with studies on the rotational behaviour of the smaller model base 1-MeBim in α -[Ru(azpy)₂(1-MeBim)₂](PF₆)₂ and *cis*-[Ru(bpy)₂(1-MeBim)₂](PF₆)₂.

Also in case of the rotational behaviour of the 1-MeBim ligands in β -[Ru(azpy)₂(1-MeBim)₂](PF₆)₂ less flexibility was observed compared to in α -[Ru(azpy)₂(1-MeBim)₂](PF₆)₂ in correspondence with the reduced activity.

Although in the case of *cis*-[Ru(bpy)₂Cl₂] in reaction with 9egua only the monofunctional adduct *cis*-[Ru(bpy)₂(9egua)Cl]Cl could be isolated, the bifunctional binding to two nucleobases in DNA cannot be excluded a priori. The binding of different N-heterocycles to *cis*-[Ru(bpy)₂Cl₂] shows that this complex is a borderline case, in which monofunctional and bifunctional coordination of the heterocycles depend on relatively small sterical differences in the ligands. For this reason the possibility of bifunctional coordination of the α -[Ru(azpy)₂] moiety to DNA *in vivo* should not be excluded and therefore the reaction of α -[Ru(azpy)₂(NO₃)₂] with oligonucleotides is of interest.

Finally, the interaction of *cis*-[Ru(bpy)₂Cl₂] (inactive) α -[Ru(azpy)₂Cl₂] and β -[Ru(azpy)₂Cl₂] with (derivatives) of the DNA base adenine has been studied. The model base coordinates in all cases in the rare neutral imine tautomeric form via both the N6 and N7 atoms. This identical binding mode of adenine to the three complexes, therefore, does not explain the differences in cytotoxicity.

This special binding mode has been further proven by extensive 2D NMR experiments of α -[Ru(azpy)₂(9-MeAde)](PF₆)₂ and with the X-ray structure of α -[Ru(azpy)₂(3-MeAde-H)](PF₆) (fig. 4). Biological consequences of the occurrence of the imine form of 9-MeAde and 3-MeAde upon coordination of 9-MeAde (or 3-MeAde) to ruthenium(II) might be present. Speculatively, the obvious shift towards the imine tautomer of 9-MeAde (and 3-MeAde) presented in this study might result in Adenine-Thymine transversions when these ruthenium compounds would coordinate to DNA.

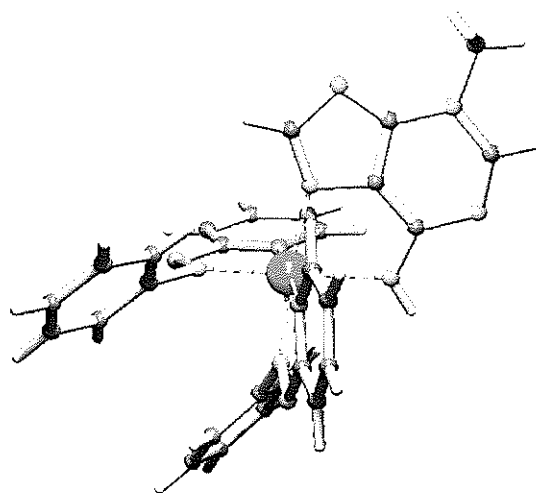


Fig. 4: Molecular structure of the DNA adduct α -[Ru(azpy)₂(3-MeAde-H)](PF₆)

4. Final remarks

In conclusion, by synthesizing structural derivatives of the dichlorobis(2-phenylazopyridine)ruthenium(II) complexes, suitable explanations for differences in cytotoxicity between the several isomers and analogous bpy complexes have been provided. To improve the water solubility of the dichlorobis(2-phenylazopyridine)ruthenium(II) complexes especially the change of anions X in $[\text{Ru}(\text{azpy})_2\text{X}_2]$, using X = nitrate or carboxylate, complexes resulted in novel water soluble compounds with a very high biological activity, encouraging further studies.

DNA model-base binding studies clearly show the monofunctional coordination of guanine and adenine model bases. Synthesis and biological testing of structural derivatives in combination with DNA binding studies will finally bring us a step closer to the development of structure-activity relationships for this class of complexes and to a possible clinical trial.

Leiden; February, 2004

J. Reedijk