6. Summary, general evaluation and future developments

The contents that can be found in each chapter of this thesis are briefly exposed. Some conclusions are extracted from the results obtained during the course of this research. Proposals are presented for further work in this line of investigation.
6.1. Introduction

Several ruthenium(II) polypyridyl complexes were synthesised, with two main purposes: finding new potential anticancer metallodrugs and getting some insight in their mechanism of action. The obtained results are presented in this thesis. This last chapter gives a brief overview of all the herein described work, and it provides a number of suggestions for further research.

6.2. Summary

It is not possible to understand the role of ruthenium in the field of anticancer metallodrugs without a previous reference to platinum chemistry. With this in mind, a brief historical introduction to cisplatin is given in Chapter 1, followed by an explanation of its mechanism of action and the development of second and third generation platinum anticancer agents. Ruthenium chemistry is presented as a possible alternative to platinum therapy. A classification of ruthenium compounds with proven anticancer activity is provided, and their possible mechanisms of action are discussed, providing examples from relevant literature.

In Chapter 2, the synthesis and characterisation of three carefully-chosen, closely-related ruthenium(II) polypyridyl complexes is described. The structural information deduced from NMR spectroscopy supports the results obtained from the elucidation of the crystal structures. The distinct 3D packing of each of the three complexes is interesting to mention, as well as the formation of a hydrogen-bond net in one of the cases.

The reasons for the choice of these three ruthenium complexes are further detailed in Chapters 3 and 4, in which a kinetic study is described of the reactions between each of these complexes and the DNA model base 9-ethylguanine. A parallel study of the cytotoxicity of each complex sheds some light on the importance of the leaving group and the kinetics, vide infra. Moreover, NMR spectroscopy was proven to be a valuable tool in the study of some interesting temperature-dependant conformational changes in the formed ruthenium-DNA model-base adduct, while CD and LD were the techniques of choice for the study of conformational changes provoked by the tested ruthenium compounds in the DNA molecule.

A look beyond any possible coordinative interactions between the metal atom and the DNA nucleic bases has resulted in Chapter 5, in which other alternative interaction modes are dealt with. The synthesis and characterisation of a possible intercalator and a possible
groove-binder are described, as well as some cell tests. Further work needs to be done in order to establish the mechanism of action of these novel compounds.

Also in Chapter 5, the study of the interaction between potential ruthenium(II) anticancer agents and some selected serum transport proteins, such as transferrin, is discussed. Finally, a reminder is made of the fact that some ruthenium complexes that were proven to be inactive against primary tumours, showed nevertheless an important antimetastatic activity.

The Appendix to this thesis deals with the formation of a planar hydrogen-bonded network of nucleic bases and formate residues in parallel sheets, which are of great theoretical importance and may have applications in nanotechnology.

6.3. Conclusions and future perspectives

Two cytotoxic compounds were considered, Ru(tpy)Cl$_3$ and $\alpha$-[Ru(azpy)$_2$Cl$_2$], with an activity that seems to be due to the formation of intra- and/or interstrand cross-links, in the same way cisplatin does. In this thesis, the design of a complex of formula [Ru(apy)(tpy)L]$_{(2-n)^+}$ (L = leaving group) is described, which was based on the first two, but with an improved water-solubility. However, this new complex is monofunctional; therefore it can only bind to one nucleic base. The new complex was proven to display a moderate and, in some cases, even a high activity against a number of cell lines. Studies were carried out to elucidate its mechanism of action. First, the kinetic factor was taken into account. For that purpose, three variants of the same complex were obtained: those in which the leaving group was a chloro, an aqua residue and an acetonitrile, respectively. All three complexes were capable of binding the DNA model base 9-EtGua in the experimental conditions, although following different kinetics in each case. The differences in the kinetics could not be correlated to the small differences in cytotoxicity. These results seem to suggest that it does not matter how fast these molecules can bind to DNA.

The cytotoxicity of an analogous dinuclear complex with no ability to coordinatively bind to DNA was tested. From the positive results obtained it can be concluded that coordination to DNA is not essential for cytotoxic activity, and it might be that the mechanism of these complexes does not involve DNA at all.

In order to test this last theory, experiments with calf-thymus DNA were carried out. The results from the circular and linear dichroism show an extensive interaction of the dinuclear complex with the DNA molecule, which is clearly different from the way the
mononuclear complex interacted with the nucleic acid. Therefore from these results an interaction with DNA cannot be ruled out as a key step in anticancer activity of this kind of ruthenium compounds.

Finally, two other ruthenium(II) complexes with heterocyclic ligands were studied in search for structure-activity relationships. According to these studies, the azo function might be essential for activity.

To summarize, neither all compounds capable of DNA-coordination are anticancer-active, nor all ruthenium cytotoxic compounds can coordinate to DNA. This observation underlines the importance of alternative ways of DNA recognition. Several strategies for further research in this direction are suggested in this thesis, including some examples of possible candidate compounds (Chapter 5, section 5.1.1).

A better understanding of the mechanisms of action is crucial for the development of new ruthenium drugs. The study of the interactions between a potential metallodrug and DNA is of utmost importance, as well as the interactions between ruthenium complexes and serum transport proteins.

Simultaneously, an effort should be made to improve and standardize the tests used to screen a metallodrug for anticancer activity, including tests of drug uptake and of antimetastatic activity.

6.4. References