Searching for intracellular targets of oxindolimine-copper(II) or zinc(II) complexes with antitumor activity

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In this work, we are interested on verifying the most probable targets inside the cell for imine-copper(II) and zinc(II) complexes that have shown remarkable antitumor properties, in previous studies. These complexes have been previously synthesized and characterized. The ligands are derivatives of isatin, an oxindole found in human fluids as a metabolite of tryptophan. They are capable of binding to DNA,[1] causing oxidative damage through the generation of reactive oxygen species (ROS). They can also inhibit some specific proteins, as human topoisomerase I,[2] cyclin-dependent kinases (CDKs),[3] and proteasome. Moreover, they can act on mitochondria as uncoupling agents. We intend through a systematic study to identify specific proteins present in the cellular membrane, organelles or in the nucleus that by interaction with these compounds could explain a significant inhibition of their functions, being responsible for the antitumor activity observed.

Based on the cytotoxicity data obtained, after incubation for 24h at 37°C, it was possible to observe that copper is important for the toxicity of the compound and, moreover, there is no significant difference between the IC_{50} values for the human sarcoma lines MES-SA and resistant MES-SA/DX5, cultivated in the presence of doxorubicin 0,05 µM. Therefore, this line doesn't exhibit resistance to the compounds studied. A possible explanation for this could be the damage to the proteins present in the cell membrane, that are responsible for the pump out of the drug. In an assay with rhodamine, it was possible to observe that P-glycoprotein, overexpressed in the resistant line, remains active in the MES-DX5 cells treated with the copper or zinc complexes. By analysis of the mitochondrial membrane potential, after incubation with the metal complexes, it was observed that these compounds are able to decrease it, and this effect is dependent on the number of ligands and on the metal coordinated.

Cell lines	MES-SA	MES-SA/DX5
isaepy	>150	>150
Cu(isaepy)(H_2O)](ClO_4	15,3 ± 2,3	12,6 ± 0,7
[Cu(isaepy)$_2$](ClO_4)$_2$	7,1 ± 0,3	6,4 ± 1,0
[Zn(isaepy)Cl_2]	110,7 ± 2,9	128,9 ± 3,1
Zn(isaepy)$_2$](ClO_4)$_2$	112,2 ± 3,2	123,4 ± 1,7

In these experiments, $1x10^4$ cells of MES-SA, MES-SA/DX5, were plated in a 96 wells plate, using McCoy culture medium for MES-SA, MES-SA/DX5, supplemented with 10% FBS. After 24 hours of plating, medium was replaced by solutions containing compounds at concentrations in the range 150 µM to 5 µM, and the incubation was maintained for 24 h. After this period, the medium culture was replaced again by a medium containing MTT, that remained for 3 h. Subsequently, absorbance of each solution was verified at 570 nm.

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