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New insights on cytotoxic activity of group 3 and lanthanide compounds: complexes with [N,N,N]-scorpionate ligands

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Abstract

Objectives In this work was to evaluate the cytotoxic activity of a series of monomeric group 3 and lanthanide (N,N,N)-heteroscorpionate-triflate complexes (M (OTf) 2 (cybpamd) (THF)) (Ln = Sc (2), Y (3), La (4), Nd (5), Sm (6), Dy (7), Yb (8); OTf = SO3CF3; cybpamd = N, N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidinate) having octahedral geometry around the metal atoms on the human epithelial lung adenocarcinoma (A549), human melanoma (A375), human cervical epithelial adenocarcinoma, human embryonic kidney (HEK-293) and murine macrophages (J774.A1) cell lines.

Methods All the tested compounds were incubated with cells for 72 h and their growth inhibition assessed by using MTT assay.

Key findings On the cell line HEK-293 complexes 5 and 7 show a reasonable activities, while the murine macrophage cell line (J774.A1), only the scandium 2 complex is not very active. All complexes tested are poorly active on human health adenocarcinoma lung epithelial (A549) and human melanoma (A375).

Conclusions The group 3 and lanthanide (N,N,N)-heteroscorpionate triflate-complexes (M(OTf)2(cybpamd)(THF)) on murine macrophage (J774.A1) cell line, except that of scandium, show a reasonable activity. On human epithelial cervix adenocarcinoma (HeLa) complexes 3, 5 and 6 are significantly more active than cis-platinum, as well as complex 5 is more active on human embryonic kidney (HEK-293) cell line. All the tested complexes are poorly active on human epithelial lung adenocarcinoma (A549) and human melanoma (A375).

The different behaviour of the complexes examined (2–8) let us hypothesize that the cytotoxic activity is related to the molecule as a whole and not only to the ligand or the metal ion separately.

Introduction

After the great success as antitumour drug of *cis*-platin, (*cis*-$(NH_3)_2PtCl_2$), especially in the clinical treatment of ovarian, testicular, neck and head cancers, considerable research activity has been developed to obtain new organometallic compounds that do not present the limits of applicability of the *cis*-platin and of its analogues.[1] In fact, *cis*-platin gives severe toxic side effects, comprising nephrotoxicity and neurotoxicity, which limit the dose that can be given to patients. So, a second generation of platinum drug, carboplatin (1,1-cyclobutanedicarboxylato(2-)-O,O'-platinum(II)), was introduced in clinic in mid-1980s. The compound is devoid of nephrotoxicity along with reduced gastrointestinal tract toxicity and neurotoxicity. Furthermore, carboplatin allows treatment with higher doses of the drug because of its lower toxicity.[2,3]

The third generation of platinum drugs includes oxaliplatin, which overcomes *cis*-platin resistance and is specific for common cancer diseases (colon or rectal that has

metastasized, testicular and ovarian), it has less ototoxicity and nephrotoxicity than cisplatin and carboplatin.[4]

Complexes of many metals in the periodic table have been studied up to now, including those of group 3, which have shown a significant biological activity.[5]

Many of the pharmacological properties of the metals of group 3 are due to their affinity for Ca^{2+} sites in biological molecules and to the formation of a stronger bond with water molecules. Moreover, they can also replace the metal ions Mg^{2+}, Mn^{2+} and Fe^{3+} in the biological processes.[5,6]

The lanthanides can replace calcium in the protein, and so calcium-dependent enzymes can be inhibited or be activated by lanthanides. It has been proposed that the stimulation or the inhibitory effect of the lanthanides may be a function of role of calcium in the native enzyme. If calcium plays a catalytic role, its substitution with a group 3 ion leads to the deactivation of the enzyme, and the degree of inhibition depends on the ionic radius of metal. Instead, if calcium plays a structural role, the replacement with a M^{3+} ion gives, at least, the maintenance of the activity.[5,6]

Accordingly, the mode of action of anticancer-active group 3 metals is related to their possible inhibition of function of ions essential for cells cycle regulation, and antitumour activity is significantly enhanced by complexation with various ligands.

In fact, lanthanide complexes with coumarins exhibited superior activity in various tumour models as compared with the corresponding inorganic salts. Coumarins are an important group of organic compounds that show a wide variety of biological activity as antitumour and antiproliferative effects.[7–10] Complexes of rare earth metal as cerium(III), lanthanum(III) and neodymium(III), having coumarines as ligand, were examined on different cell lines.[11]

Rogers and coworkers reported the cytotoxic activity of acenocumarol samarium(III) and gadolinium(III) complexes against melanoma B16 and fibrosarcoma L929 cell lines.[12]

Lanthanide complexes having macrocyclic monoanionic ligands containing five coordinating nitrogen atoms in the central core, the texaphyrins, have progressed into clinical trials.[13,14]

Furthermore, it has been reported that a series of cerium(III) complexes with bipyridyl or phenanthroline ligands showed an interesting antiproliferative activity against tumoral cell lines. Recently, the lanthanum complex of phenanthroline derivative (KP772) has shown to exert comparable activity with that of *cis*-platin and methotrexate against a wide range of tumour cell lines *in vitro* and a colon carcinoma xenograft model *in vivo*.[15,16]

Recently, it has been evaluated the cytotoxic activity of a significant number of new complexes of metals of group 3 and lanthanides with heterocyclic ligands,[17–23] and we have reported the synthesis and characterization of scandium(III), yttrium(III), samarium(III) and neodymium(III) chloro- or triflate-complexes with tridentate monoanionic quinoline-phenoxy-amine, quinoline-phenoxy-imine and *ansa*-monocyclopentadienyl-imino-pyridine ancillary ligands, and their cytotoxic activity was evaluated on rat glioma (C6), murine fibroscarcoma (WHEI-164) and human embryonic kidney (HEK-293) cell lines.[24]

Hence, the complexation of lanthanides with heterocyclic ligands having more than one donor atom is of great interest in the chemistry of coordination compounds, as the complexation can lead to compounds with significant cytotoxic effects.

Homoscorpionate and heteroscorpionate compounds have shown to be very interesting species for the synthesis of a wide range of stable group 3 elements and lanthanide derivatives. Scorpionates represent attractive and versatile ligands because of their fine-tuning of the electronic and steric properties and consequently for the control of the metal coordination sphere.[25–34]

In a previous study, our group reported the synthesis and characterization of a series of group 3 and lanthanide (N,N,N)-heteroscorpionate complexes with k^3-NNN-coordination of the heteroscorpionate ligand N,N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidinate (cybpamd).[35] In this study, we detail on cytotoxic screening and further investigation of ($Ln(OTf)_2$(cybpamd)(THF)) (where Ln = Sc (2), Y (3), La (4), Nd (5), Sm (6), Dy (7), Yb (8); OTf = SO_3CF_3) and THF = tetrahydrofuran.

Results and Discussion

Chemistry

Synthesis of N,N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidine (cybpamd-H) (1) and ($Ln(OTf)_2$(cybpamd)(THF)) complexes (Ln = Sc (2), Y (3), La (4), Nd (5), Sm (6), Dy (7), Yb (8); OTf = SO_3CF_3) were carried out as follows.

The organic compound N,N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidine (cybpamd-H) (1) was prepared, according to Otero *et al.*,[36] by reacting bis-(3,5-dimethyl-pyrazol-1-yl)methane with butyllithium in THF at low temperature to form *in situ* the corresponding lithium salt, which was subsequently reacted with one equivalent of dicyclohexylcarbodiimide. The quench of the reaction mixture with cold water allowed the formation of the final product, which was purified following common procedures and isolated in high yield (see Figure 1).

The neutral triflate complexes ($Ln(OTf)_2$(cybpamd)(THF)) (Ln = Sc (2), Y (3), La (4), Nd (5), Sm (6), Dy (7), Yb (8); OTf = SO_3CF_3) were all prepared as reported in the literature[35] in ca. 70% yield by allowing to react

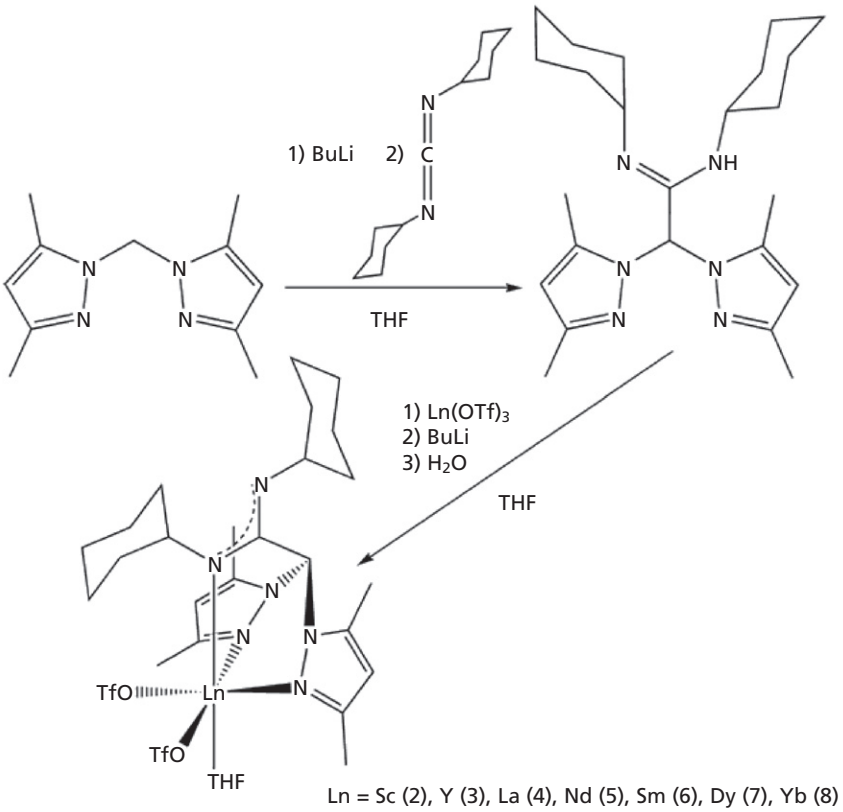

Figure 1 Sunthetic route for the preparation of metal complexes 2–8.

cybpamd-H with a stoichiometric amount of anhydrous $Ln(OTf)_3$ salt in THF and subsequent addition of diluted butyllithium (see Figure 1).

As previously reported,[35] in these complexes, the anionic ligand cybpamd acts as tridentate with the two pyrazole rings, and only one amidinate nitrogen atom strongly bonded to the metal centre. The coordination mode of the two triflate groups appears comprised between κ^1 and κ^2, while the THF molecule results only weakly bonded.

Cytotoxicity studies

The cytotoxic activity of complexes 2–8 have been evaluated on human epithelial lung adenocarcinoma (A549), human melanoma (A375), human epithelial cervix adenocarcinoma (HeLa), human embryonic kidney (HEK-293) and murine macrophage (J774.A1) cell lines. The same studies have been carried out also on the simple lanthanide triflate and on the free ligand, which is N,N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidine (cybpamd-H) (1) at the pH value of the biological experiments. Complexes 4 and 8 were tested against only one cell lines: that is, HEK-293. The IC50 (the half maximal inhibitory concentration) value, expressed as μM, is the concentration of compound that affords a 50% reduction in cell growth as compared with control cells, reported in Table 1. *Cis*-platin is also included for comparison, and it was chosen for testing the susceptibility of our cell lines to cytotoxicity.

All the tested compounds, as detailed into experimental part, were incubated with cells for 72 h and their growth inhibition assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide (MTT) assay.[37]

It is important to note that the cell lines used in this study are very different from each other, as denoted by the different response to *cis*-platin (Table 1). No cytotoxic activity was exerted by $Ln(OTf)_3$ (Ln = Sc, Y, La, Sm, Nd, Dy, Yb) on any of the cell lines used. The free ligand cybpamd-H is active only on HeLa cells. In fact, on this cell line, the free ligand showed a cytotoxic activity comparable with that of *cis*-platin. However, the IC50 values of complexes 2–5 and 6 were lower than that of the free ligand, demonstrating that the presence of metal potentiates the activity. Human embryonic kidney (HEK-293) cell lines are poorly sensitive to the examined compounds, and only complex **7** has a cytotoxic effect comparable with that of *cis*-platin. This result suggests that the cytotoxic activity on HEK-293 cells is strongly dependent upon the ionic radius of the metal ion. Also on murine macrophage (J774.A1) cell lines, a role

Table 1 Effect of complexes 2–8 and of 1 on several cell lines viability

	Cell lines				
Compound	HEK	J774.A1	A375	A549	HeLa
Sc 2	>100	>100	>100	>100	>100
Y 3	>100	76 ± 5.77***	38 ± 3	>100	6.2 ± 0.95***
La 4	>100	—	—	—	—
Nd 5	97 ± 1.3	85 ± 4.3***	>100	62 ± 4.3	12 ± 1.8***
Sm 6	>100	73 ± 1.5***	>100	>100	5.7 ± 0.2***
Dy 7	56 ± 1.12*	70 ± 3.7***	>100	43 ± 4.2	22 ± 1.2
Yb 8	>100	—	—	—	—
$Ln(OTf)_3$[a]	>100	>100	>100	>100	>100
cybpamd-H 1	>100	>100	>100	>100	18 ± 1.6
Cis-Pt	67 ± 4.2	>100	0.49 ± 0.05	6.5 ± 0.2	15 ± 1.2

[a]Ln = Sc, Y, La, Sm, Nd, Dy, Yb. The viability of control cells was designated as 100%, and results were expressed as the concentration (μM) of each complex able to induce the 50% of mortality in each cell line (IC50). Results are expressed as mean ± SEM from at least three independent experiments. Kruskal–Wallis test was used for statistical analysis. *denotes $P < 0.05$, and ***denotes $P < 0.0001$ *versus cis*-Pt on the same cell line.

to the ionic radius can be ascribed, having the scandium complex a very high IC50 (>100 μM), whereas the other examined complexes have an activity superior ($P < 0.001$) to that of *cis*-platin. Finally, human epithelial lung adenocarcinoma (A549) and human melanoma (A375) are poorly responsive to synthesized compounds 2–8, and a concentration almost 10 times higher than that of *cis*-platin was required.

By considering that Ln^{3+} ions are hard acid and that they preferably coordinate hard donor atoms such as oxygenated species, we had the suspect that in the medium used for the tests (dimethylsulfoxide (DMSO)/H_2O), the complexes could not maintain the coordination with the ligand containing only N-donor atoms (less hard then O-donor atoms). The different cytotoxic activity between the free ligand and the corresponding complexes, in particular on the HEK, J774.A1, A375 and A549 cells, suggests that the ligand-metal bonds are almost partially maintained in biological media. On considering that the negative charge of the tridentate ligand is located on the acetamidinate group and that the lanthanide ions give stronger bonds with negatively charged nitrogen atoms, probably, the complexes maintain in solution the acetamidinate–Ln^{3+} interaction, while the coordination sphere is saturated by water and dimethylsufoxide molecules. We have also verified that the acetamidine group is not hydrolysed under the experimental conditions used. To do this, cybpamd-H has been maintained in a DMSO solution containing the 10% of water for 72 h at room temperature. The subsequent extraction (water/CH_2Cl_2) has led to the initial product, as verified by Nuclear Magnetic Resonance (NMR) spectroscopy.

Thus, the different behaviour of the complexes examined (2–8) shows that the cytotoxic activity is related to the molecule as a whole and not only to the ligand or the metal ion.

Experimental Section

Chemistry

The ligand cybpamd-H **1** and the complexes 2–8 were prepared as reported in the literature.[35] All manipulations were carried out under oxygen- and moisture-free atmosphere in a MBraun MB 200 glove-box (MBraun GmbH, Garching, Germany). All the solvents were thoroughly deoxygenated and dehydrated under argon by refluxing over suitable drying agents, while NMR deuterated solvents (Euriso-Top products, Euriso-Top, C.E. Saclay, France) were kept in the dark over molecular sieves. The anhydrous triflate salts $M(OTf)_3$ (M = Sc, Y, La, Nd, Sm, Dy, Yb; OTf = CF_3SO_3) (Strem Chemicals Inc., Kehl, Germany; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used as received, as the organic reactants (Aldrich) required for the synthesis of 1. The synthesis of the desired products was verified by recording their ^1H and ^{13}C (^1H) NMR spectra, using a Bruker Avance 300 spectrometer (Bruker Spectrospin, Zuerich, Switzerland) operating at 300 MHz (^1H) and 75 MHz (^{13}C).

The stability of 1 was tested by dissolving 50 mg of the neutral compound in 30 ml of DMSO and 3 ml of water. The solution was left under stirring for 72 h, then water (100 ml) was added and the product was extracted with dichloromethane (50 ml). The traces of water were removed from the organic solution by addition of anhydrous sodium sulfate. After removal of the solvent by evaporation under reduced pressure, the ^1H NMR was recorded in $CDCl_3$.

Antiproliferative activity

Human epithelial lung adenocarcinoma (A549), human melanoma (A375), human epithelial cervix adenocarcinoma (HeLa), human embryonic kidney (HEK-293) and

murine macrophage (J774.A1) cell lines (3.5×10^4 cells/well) were plated on 96-well microtitre plates and allowed to adhere at 37 °C in a 5 % CO_2 atmosphere for 2 h.

Thereafter, the medium was replaced with 50 µl of fresh medium, and a 75-µl aliquot of serial dilution of each test compound was added and then the cells were incubated for 72 h. In some experiments, serial dilutions of *cis*-platin were added. Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of MTT to formazan, and cells viability was assessed accordingly to the method of Mosmann.[37]

Briefly, 25 µl of MTT (5 mg/ml) were added, and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilized with 100 µl of a solution containing 50% (v:v) N,N-dimethylformamide, 20% (w:v) SDS (sodium dodecyl sulphate) with an adjusted pH of 4.5 (Opipari *et al.*, 1992).[38] The OD of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340, Toronto, Canada) equipped with a 620-nm filter. The viability of each cell line in response to treatment with tested compounds and 6-mercaptopurine was calculated as: % dead cells = 100−(OD treated/OD control) × 100.

Statistical methods

The IC50 was calculated, as described earlier and in the caption of the Table 1, by evaluating the average of three different experiments, each performed in triplicate. Results are expressed as mean ± SEM (standard error of the mean). Kruskal–Wallis test was used for statistical analysis. A value of $P < 0.05$ was considered as statistically significant.

Conclusion

A series of monomeric group 3 and lanthanide (N,N,N)-heteroscorpionate triflate-complexes ($M(OTf)_2$(cybpamd) (THF)) (M = Sc (2), Y (3), La (4), Nd (5), Sm (6), Dy (7), Yb (8); OTf = SO_3CF_3; cybpamd = N,N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidinate), having octahedral geometry around the metal atoms, have been tested to evaluate their cytotoxic activity on: human epithelial lung adenocarcinoma (A549), human melanoma (A375), human epithelial cervix adenocarcinoma (HeLa), human embryonic kidney (HEK-293) and murine macrophage (J774.A1) cell lines. On murine macrophage (J774.A1) cell line, all tested complexes, except that of scandium, show a reasonable activity. On human epithelial cervix adenocarcinoma (HeLa) complexes 3, 5 and 6 are significantly more active than *cis*-platinum, as well as complex 5 is more active on human embryonic kidney (HEK-293) cell line. All the tested complexes are poorly active on human epithelial lung adenocarcinoma (A549) and human melanoma (A375).

It is worth emphasize that the lanthanide triflate compounds show no cytotoxic activity on any of the cell lines tested, and N,N'-dicyclohexyl-2,2-bis-(3,5-dimethyl-pyrazol-1-yl)-acetamidine (cybpamd-H), the used ligand, shows a quite good activity only on human epithelial cervix adenocarcinoma (HeLa), however lower than the complexes **3**, **5** and **6.**

Thus, the different behaviour of the complexes examined (2–8) let us hypothesize that the cytotoxic activity is related to the molecule as a whole and not only to the ligand or the metal ion separately.

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