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Group 11 complexes with amino acid derivatives: Synthesis and antitumoral studies

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ABSTRACT

Gold(I), gold(III), silver(I) and copper(I) complexes with modified amino acid esters and phosphine ligands have been prepared in order to test their cytotoxic activity. Two different phosphine fragments, PPh_3 and PPh_2py (py = pyridine), have been used. The amino acid esters have been modified by introducing an aromatic amine as pyridine that coordinates metal fragments through the nitrogen atom, giving complexes of the type $[M(L)(PR_3)]^+$ or $[AuCl_3(L)]$ (L = L-valine-N-(4-pyridylcarbonyl) methyl ester (**L1**), L-alanine-N-(4-pyridylcarbonyl) methyl ester (**L2**), L-phenylalanine-N-(4-pyridylcarbonyl) methyl-ester) (**L3**); M = Au(I), Ag(I), Cu(I), PR_3 = PPh_3, PPh_2py). The *in vitro* cytotoxic activity of metal complexes was tested against four tumor human cell lines and one tumor mouse cell line. A metabolic activity test (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT) was used and IC_{50} values were compared with those obtained for *cisplatin*. Several complexes displayed significant cytotoxic activities. In order to determine whether antiproliferation and cell death are associated with apoptosis, NIH-3T3 cells were exposed to five selected complexes (Annexin V+ FITC, PI) and analyzed by flow cytometry. These experiments showed that the mechanism by which the complexes inhibit cell proliferation inducing cell death in NIH-3T3 cells is mainly apoptotic.

1. Introduction

In the last decades chemotherapeutic research based on metal complexes has increased considerably, since the good results with platinum(II) compounds as *cisplatin*, the most widely used metal-based antitumor drug. Gold complexes have been largely studied and have gained growing interest because of their high cell growth inhibition [1–4]. Gold(I) complexes with several ligands such as phosphines, thiolates, N-heterocyclic carbenes [5–9], or gold(III) species [10–14] have been studied for their cytotoxic activities. Many of these derivatives have shown promising cytotoxic activities, in some cases overcoming cisplatin resistance to specific cancer cells. In addition they have shown a mechanism of action clearly different from that of platinum drugs. Several targets have been identified for gold complexes; the inhibition of thioredoxine reductase being one of the more important to date [2,15–21]. In spite of the great number of reported gold complexes which show activity in the biological systems, not many gold derivatives with amino acids have been reported [22,23]. We have previously reported on the synthesis and functionalization of gold(I) phosphine nicotinic acid thiolate with amino acids providing complexes with very good cytotoxic activity [24,25].

Silver(I) complexes were used as therapeutic compounds from the middle ages mainly as antimicrobial agents. They have recently regained attention as promising antitumor drugs, as shown in recent reviews [26,27]. Several silver(I) derivatives with O-, N-, P- or S- donor ligands or N-heterocyclic carbene ligands have shown high cytotoxic behavior against diverse human tumor cells [28–34]. Mechanisms of action of silver(I) derivatives are not yet clear. However interactions of the complexes with DNA and thiol groups of the proteins have been observed [26,27].

Copper is an essential trace metal for organisms. This plays an important role to catalyze metabolic oxidations and transfer electrons in some enzymes as superoxide dismutase or cytochrome-c oxidase. The international agency for research on cancer has not classified copper as a human carcinogen. Copper complexes have been investigated as potential antitumor agents because they may be less toxic for normal cells than for cancerous ones. However, as all essential trace metals, copper become toxic at slightly higher concentrations than the optimal level. Principal toxicity may be the result of its redox activity (Cu(I)/Cu(II)) and its affinity for heteroatoms of proteins or other

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biomolecules. An important number of copper complexes, specially copper(II), have been screened for their antitumor properties. They have shown encouraging perspectives and their mechanism of action has been investigated [35]. Although copper(I) complexes have been less studied compared to copper(II), an important number of them have showed potent cytotoxic activity. Copper(I) complexes with P-donor ligands [36–41] or N-heterocyclic carbene ligands [42] have shown strong cytotoxic activities. Research has indicated possible differences (in the mechanisms of action) between copper complexes and antitumor platinum derivatives.

Synthesis of gold, silver and copper complexes with modified amino acid groups could be interesting in view of the possible biological properties. The amino acids can serve as good carriers to deliver the metallic atoms to the biological target. In cancerous cells the amino acid receptors are overexpressed and consequently the complexes could be more selective to abnormal cells. Therefore, it was thought that it would be a good idea to try to introduce metallic ions into the tumor cells by amino acid derivatives as ligands, to facilitate the cell permeation. In addition, it was considered that these ligands would be more biocompatible and therefore reduce side effects *in vivo*.

L1–L3 ligands and the corresponding *meta* piridyl-aminoacid derivatives (Scheme 1) were previously prepared to synthesize a series of *fac* tricarbonyl rhenium diimine complexes [43]. The amino acid methyl esters derivatives were modified by introducing a pyridine group which enhances the ability to coordinate metal atoms. Their applications in fluorescent microscopy cell imaging in the MCF-7 cell line were reported. These derivatives showed a good uptake but with a markedly different effect on the cells. Interestingly, after irradiation, rhenium complexes with *para* amino acid derivatives induced damage in the cell structure and cell death was observed. The cells treated with the complexes with *meta* amino acid derivatives remained healthy.

This caused us to consider the hypothesis that coinage metal complexes synthesized with the *para* and *meta* substituted pyridine amino acid derivatives could have interesting cytotoxic properties. Here, we report on the synthesis, structural characterization and on the study of the biological activity of several group 11 metal complexes with the *para* derivatives. Unfortunately, it was not possible to measure the cytotoxic activity of gold(I) derivatives with the *meta* pyridyl-amino acid ligands, due to their poor stability in solution at room temperature. The complexes decomposed to metallic gold in a few hours. The *in vitro* cytotoxic activities of metal complexes were tested against four tumor human cell lines, A-549 (human lung), Hep-G2 (human liver cancer), HeLa (human cervix epithelial carcinoma), MCF-7 (breast cancer) and one tumor mouse cell line, NIH-3T3 (mouse fibroblast) showing strong antitumor activity *in vitro*. Further experiments have been carried out in order to determine the mechanism for which the complexes inhibit cell proliferation and cause cell death.

2. Results and discussion

2.1. Synthesis and spectroscopic characterization

Amino acid derivatives **L1–L3** were synthesized by reaction of commercially isonicotinic acid chloride with the corresponding amino acid methyl ester compounds, in the presence of triethylamine to allow the condensation reaction (Scheme 1), by following literature precedents [43,44]. Spectroscopic information is shown in the Experimental section.

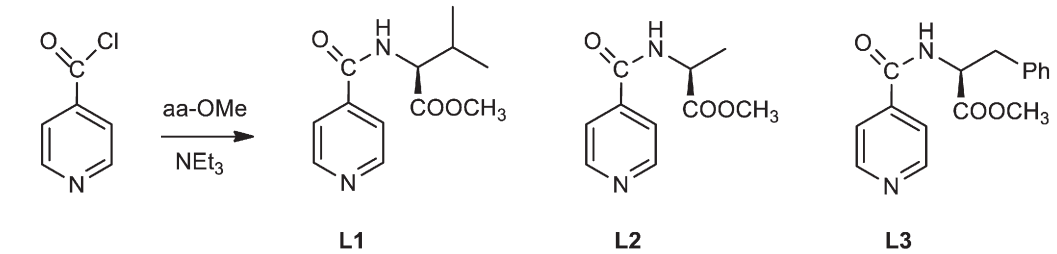
The coordination chemistry of these ligands towards group 11 metal complexes has been studied. Gold(I) compounds **1–6** have been prepared by reaction of **L1–L3** with an equimolecular amount of the corresponding metal phosphine complexes [Au(OTf)(PPh_3)] or [Au(OTf)(PPh_2py)] (OTf = CF_3SO_3, trifluoromethanesulfonate), whereas the gold(III) species **7–9** have been obtained by the reaction of the ligands with $AuCl_3 \cdot nH_2O$ (Scheme 2).

In the same manner, the reaction of **L1–L3** with the silver or copper complexes [Ag(OTf)(PR_3)$_n$] (n = 1, 2) or [Cu(NO_3)(PPh_3)$_2$] has afforded complexes **10–18** and **19–21**, respectively (Scheme 3).

They have been characterized by means of IR, elemental analysis and NMR spectroscopy. Assignments of the ^{1}H NMR and ^{13}C NMR signals were made on the basis of 2D ^{1}H COSY and HSQC spectra. Complete spectroscopic information of the complexes has been collected in the Experimental section.

The ^{1}H NMR spectra of the compounds in acetone-d^6 presented the expected resonances for all the protons (see Experimental section). They showed, in Au(I), Au(III) and Ag(I), a low-field shift of the pyridine protons H(2) and H(3), the nearest to the nitrogen atom, compared to those found in the free ligands, that confirms the coordination through the pyridine nitrogen atom to the metal. The ^{13}C{^{1}H} NMR also showed a low-field shift of the pyridine carbons C(2) and C(3). ^{1}H NMR spectra of Cu(I) complexes 19 (L1), 20 (L2) and 21 (L3) showed the resonances for the corresponding amino ester derivative without no significant changes to those observed in the free amino acid ester ligands. The phenyl resonances indicated that only one phosphine group is coordinated to the copper metal in each compound.

The ^{31}P{^{1}H} NMR spectra of gold(I) and copper(I) compounds consist on one singlet from the phosphorus atom coordinated to the metal. The silver complexes with PPh_3 are fluxional in solution. Their ^{31}P{^{1}H} NMR spectra at room temperature display one broad resonance for complexes **11, 14, 16** and **17** or two broad resonances for complexes **10** and **13**. This kind of fluxionality is typical in the coordination chemistry of silver and can be attributed to exchange phenomena involving the neutral ligands. In the low temperature spectra the expected complex in each reaction was identified as the major product. At 203 K the ^{31}P{^{1}H} NMR spectra of the mono-phosphine complexes **10, 13** and **16**



aa-OMe = L-valine methyl ester (**L1**)

aa-OMe = L-alanine methyl ester (**L2**)

aa-OMe = L-phenyl alanine methyl ester (**L3**)

Scheme 1. Depiction of selected ligands and their precursor.