Journal of Inorganic Biochemistry 105 (2011) 1373–1382

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Conjugates of ferrocene with biological compounds. Coordination to gold complexes and antitumoral properties

M. Concepción Gimeno [a,*], Helen Goitia [a], Antonio Laguna [a], M. Elvira Luque [a], M. Dolores Villacampa [a], Catarina Sepúlveda [b], Margarida Meireles [b]

[a] Departamento de Química Inorgánica, Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), CSIC-Universidad de Zaragoza, Pedro Cerbuna 12, E-50009 Zaragoza, Spain
[b] Departamento de Química e Bioquímica,CQB, Faculdade de Ciências da Universidade de Lisboa Campo Grande, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 14 February 2011
Received in revised form 22 July 2011
Accepted 23 July 2011
Available online 29 July 2011

Keywords:
Ferrocene bioconjugates
Gold
Antitumor properties
Amino acid esters
Biological ligands

ABSTRACT

Several bioconjugates of ferrocene with biological compounds such as aminoacid esters and related species have been prepared by reaction of chlorocarbonyl ferrocene with the corresponding amino acid ester (histidine methyl ester, tryptophan methyl ester, methionine methyl ester and lysine ethyl ester) or histamine or prolinamide in the presence of NEt_3. The reaction of the tryptophan or prolinamide ferrocene conjugates with [Au(acac)(PR_3)] (acac = acetylacetonate) results in the substitution of the proton of the cyclic NH groups by the fragment $AuPR_3^+$ affording the complexes [Au(FcCO-tryptophan-OMe)(PR_3)] or [Au(FcCO-prolinamide)(PR_3)] (Fc = ferrocenyl group). The reaction of FcCO-Met-OMe with [Au(OTf)(PR_3)] (OTF = trifluoromethysulfonate) or [Au(C_6F_5)$_3$(OEt_2)] yields the gold(I) or gold(III) derivatives [Au(FcCO-Met-OMe)(PR_3)]OTf or [Au(C_6F_5)$_3$(FcCO-Met-OMe)], respectively. Cytotoxicity studies towards several cancer lines such as MCF-7, HeLa or NIE-115 have been performed. The ferrocene bioconjugates show no activity whereas the gold complexes exhibit antiproliferative effect. Preliminary studies of interaction of compounds with cells were carried out with the goal of increasing our knowledge on the mechanism of action of these potential drugs.

1. Introduction

In the last years the bioorganometallic chemistry of ferrocene has aroused a great interest and its study has been encouraged by its potential biological applications [1–4]. Several chemical properties have made ferrocene an attractive molecule to study because of its stability toward moisture and air, hydrophobicity and lipophilicity, easy chemical modification and functionalization, as well as its redox properties [5–7]. Many bioconjugates of ferrocene with amino acids [8–10], peptides [11–17], proteins and DNA [18] have been synthesized and their potential applications in electrochemical sensor devices and immunoassay reagents have been studied [19–22]. Ferrocene itself and its derivatives have been used as cytotoxic and antianemic agents within the area of medicinal applications [23]. The ferrocenyl group has been incorporated into the structure of a number of biologically active molecules such as antibiotic [24,25], anticancer [26,27], or malaria drugs [28–31] resulting in an increase of the activity. Thus the ferrocene moiety has proven to be a successful addition to penicillin and cephalosporine, enhancing their antibacterial activity, to tamoxifen, increasing its antitumor properties, or to known malaria therapeutics, increasing their efficacy toward chloroquine-resistant strains of the parasite. Furthermore, the antiproliferative activity of compounds based on polyphenols is dramatically activated by the introduction of the ferrocene group [32–35].

The anticancer properties of metallic compounds have been demonstrated with the great success of the platinum anticancer drugs and more recently with the introduction into clinical trials of ruthenium complexes for the cancer treatment, or the already mentioned ferroquine for the treatment of malaria. Other metallic compounds such as gold derivatives have attracted much attention because of their strong antiproliferative effects [36–40]. Gold(I) complexes comprising aromatic tertiary phosphines and diphosphines [41–44], water-soluble derivatives [45,46] and gold(III) species [47–51] have been studied for its cytotoxic activity.

Here we report the synthesis of several bioconjugates of ferrocene with biological ligands and further coordination of some of them to gold compounds in order to check whether the association of the two factors, bioconjugate of ferrocene and gold compounds, exerts a cooperative effect resulting in more significative antitumor activity. Several gold(I) and gold(III) complexes have been prepared and their cytotoxicity towards several cancer lines, two human cell lines such as MCF-7 (derived for breast cancer), HeLa (derived from cervical cancer) and one murine cell line NIE-115 (derived from mouse sympathetic ganglion neurons of C-1300 mouse) has been determined. Biological assays and studies of interactions of compounds with cells and with DNA were

* Corresponding author. Tel.: +34 976762291; fax: +34 976761187.
E-mail address: gimeno@unizar.es (M.C. Gimeno).

0162-0134/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2011.07.015

carried out for the purpose of increasing our knowledge on the mechanism of action of these potential drugs.

2. Results and discussion

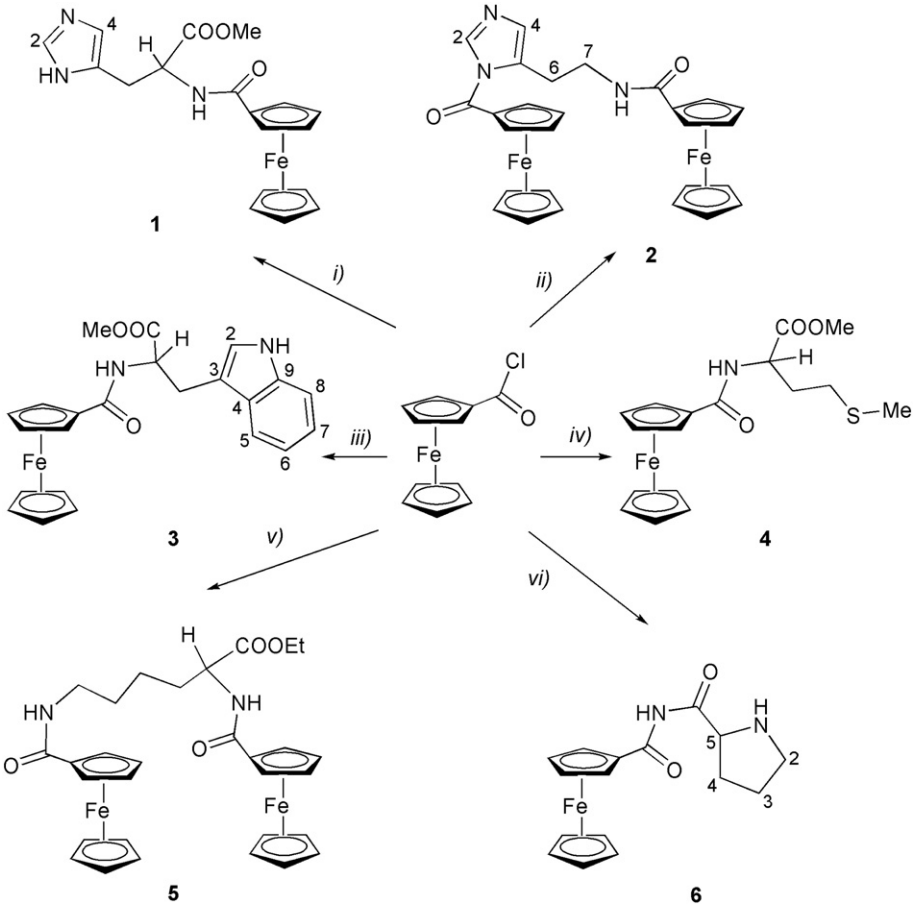
2.1. Synthesis and spectroscopic characterization

The ferrocene conjugates resulted from the coupling of chlorocarbonyl-ferrocene with the corresponding amino acid ester or related species in the presence of NEt_3 (see Scheme 1) in moderate to high yield. Enantiomerically pure L amino acid esters were used in all the cases. The reaction of chlorocarbonylferrocene with histidine methyl ester, tryptophan methyl ester, methionine methyl ester or prolinamide was carried out in a 1:1 molar ratio to afford the monosubstituted derivatives FcCO-histidine-OMe (**1**), FcCO-tryptophan-OMe (**3**), FcCO-Met-OMe (**4**) or FcCO-prolinamide (**6**). The reaction with histamine methyl ester and lysine ethyl ester has been carried out in a 2:1 molar ratio, since the corresponding equimolar reaction produces a mixture of the mono- and disubstituted compounds, to give FcCO-histamine-COFc (**2**) and FcCO-Lis-OEt-COFc (**5**). Compound **4** with the methionine methyl ester was reported previously by Metzler-Nolte et al. who synthesized it from ferrocene carboxylic acid activated with EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), and HOBt (hydroxybenzotriazole) in the presence of the amino acid ester [15]. The ^{1}H NMR spectra of compounds **1**–**6** present the expected resonances for all the protons (see experimental section); the ferrocene moieties exhibit one singlet for the unsubstituted cyclopentadienyl ring and two multiplets for the α and β protons of the substituted cyclopentadienyl ring and in compounds **2** and **5** inequivalent ferrocenyl units appear. The ^{13}C{^{1}H} spectra also agrees with the proposed formulation and the resonances.

Gold(I) and gold(III) derivatives have been prepared with some of these ferrocene bioconjugates in order to test their biological activity in comparison with the starting products. We have chosen the species with cyclic amines and the methionine derivative as the most suitable to coordinate gold fragments. The reaction of FcCO-tryptophan-OMe with one equivalent of [Au(acac)(PR_3)] (acac = acetylacetonate) produces the abstraction of the proton from the cyclic NH group with formation of acetylacetone, and the incorporation of the isolobal fragments $AuPR_3^+$ giving the complexes [Au(FcCO-tryptophan-OMe)(PR_3)] (PR_3 = PPh_3 (**7**), PPh_2py (**8**))(see Scheme 2). Two different phosphine ligands have been used with the aim of discerning the influence of substitution of a phenyl for a pyridine ring in the phosphine ligand. The ^{1}H NMR spectra show the expected resonances for the coordinated ligand with the exception of the cyclic NH protons which have disappeared. In the ^{31}P{^{1}H} NMR spectra only one resonance for the PPh_3 or PPh_2py ligands is observed.

Similarly the treatment of FcCO-prolinamide with one equivalent of [Au(acac)(PR_3)] results in the abstraction of the proton of the cyclic NH group and formation of the compounds [Au(FcCO-prolinamide)(PR_3)] (PR_3 = PPh_3 (**9**), PPh_2py (**10**)). The ^{1}H NMR spectra show the absence of the cyclic NH proton although the other resonances for the coordinated ligand are present. In the ^{31}P{^{1}H} NMR spectra a single resonance for the phosphorus atom is observed.

The reaction of FcCO-Met-OMe with two gold(I) derivatives containing the tryphenylphosphine and the 2-pyridyldiphenylphosphine as ligands, [Au(OTf)(PR_3)], or the gold(III) species [Au(C_6F_5)$_3$(OEt_2)] progresses with coordination of the metal, probably to the sulfur atom of the methionine, affording the gold(I) [Au(FcCO-Met-OMe)(PR_3)]OTf (PR_3 = PPh_3 (**11**), PPh_2py (**12**) or the gold(III) species [Au(C_6F_5)$_3$(FcCO-Met-OMe)] (**13**). The ^{1}H NMR spectra for complexes **11** and **12** show the expected resonances for the ferrocenyl-methionine ligand coordinated to



Scheme 1. i) L-histidine methyl ester, ii) histamine, iii) L-tryptophan methyl ester, iv) L-methionine methyl ester, v) L-lysine ethyl ester , vi) L-prolinamide.