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Ternary copper(II) complexes with hippurate derivatives and 1,10-phenanthroline: Synthesis and biological activity

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ABSTRACT

Several new Cu–hippurate derivative–phenanthroline ternary complexes have been prepared. The X-ray structure of one of them, $[Cu(hip)(phen)_2]^+\cdot(hip^-)$ (**2**) (where hip is hippurate and phen is 1,10-phenanthroline) has been solved. The structure of this new compound shows important differences (3D-pattern) to other similar related complexes (2D-pattern). A study of the biological activity of $[Cu(hip)(phen)_2]^+\cdot(hip^-)\cdot 2H_2O$ (**2**), $[Cu(BGG)(phen)_2]^+\cdot(BGG^-)\cdot 6H_2O$ (**3**), $[Cu(B^IGG)_2(phen)](H_2O)$ (**4**) and $[Cu(I\text{-}hip)(bpy)_2]^+\cdot(I\text{-}hip^-)\cdot 3.5H_2O$ (**5**) (where I-hip is *ortho*-iodohippurate, BGG corresponds to benzoylglycilglycine, and B^IGG is *ortho*-iodobenzoylglycilglycine) is included and compared with the anti-proliferative activity of $[Cu(I\text{-}hip)(phen)_2]^+\cdot(I\text{-}hip^-)\cdot 7H_2O$ (**1**) previously described, resulting in a greater cytotoxic activity of the compounds with 1,10-phenanthroline instead of those with 2,2′-bipyridyl, in the same way that removing iodine substitution or lengthening the peptidic chain diminishes the activity of compounds compared with **1**. The presence of an *ortho*-iodine group and the direct bond between Ar–CO and glycine moieties yield to the best results.

1. Introduction

Cancer is on the way to be the first cause of death in industrialized countries. Actually, about one half of all the diagnosed cancers result in the death of the patient. By this reason, the development of new anticancer therapies is one of the fundamental goals in medicinal chemistry.

Since many years a lot of researchers have actively investigated copper compounds for cancer therapy based on the assumption proposal that endogenous metals may be less toxic. Recently the activity of copper complexes as anticancer agents has been reviewed but few it is known about mechanisms [1,2]. Also recent works have demonstrated that the copper transporters Ctr1, Atp7a and Atp7b regulated the cellular pharmacology of cisplatin by mediating its uptake and reflux [3]. Furthermore, recent finding indicates that Atox1, a key chaperone that imports Cu from Ctr1 and delivers it to Cu exporters Atp7a and Atp7b in the secretory compartment, can also bind DNA and regulate transcription [4].

Copper phenanthroline (or related ligands) derivatives show important biological activities. These include chemical nuclease, antitumoural, antimycobacterial, antifungal and antimicrobial activity [5–20]. This activity is related with the presence of $[Cu(phen)_2]^+$ produced by the reduction of the parent copper(II) compound [5,6,18]. The nuclease activity has been improved using the ligand "clip-phen" that links two molecules of phenanthroline [19,20] and improves the stability constant of the complex by chelate effect. In some cases the apoptosis mechanism like in liver carcinoma cell line was observed induced by copper-1,10-phenatholine complexes [15] or in human cervical epidermoid carcinoma with a 3,4,7,8-tetramethyl-1,10-phenanthroline copper complex [19,20]. Other ligands have also been considered like bis(phosphite) [21].

In a previous paper [22] we have observed that the ternary copper complex $[Cu(I\text{-}hip)(phen)_2]^+\cdot(I\text{-}hip^-)\cdot 7H_2O$ cleaves DNA and induced cell death in lung cancer cells, possibly due to $[Cu(phen)_2]^+$ complex that is formed in solution by an internal captodative

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reduction process of the former complex. This ternary complex does not require, under physiological conditions, the presence of: (i) thiols and hydrogen peroxide; (ii) reductants and air; or (iii) irradiation with UV or visible light [14,15], which are very usual with other types of Cu(II) ternary complexes.

For this reason, we have prepared several Cu–hippurate derivatives–1,10-phenanthroline ternary complexes in order to compare the biological activity of all of them and to know the influence of the *ortho*-iodine groups and/or the distance between the aromatic system and the glycine moiety (Scheme 1).

Thus, in the present study, we describe the synthesis of some new copper-phenanthroline derivatives with different hippurate ligands and the X-ray structure of $[Cu(hip)(phen)_2]^+\cdot(hip^-)\cdot 2H_2O$. Moreover, the biological activities of all the series are presented and compared. On the other hand, by means of cell proliferation assays and high resolution mass spectroscopy we will try to confirm the mechanism of $[Cu(phen)_2]^+$ formation.

2. Experimental

2.1. Analysis and physical measurements

Elemental microanalyses were carried out using a Carlo Erba model 1108 microanalyzer. IR spectra in the solid state (KBr pellets) were measured on a Brucker IFS 66 spectrometer. High-resolution mass spectroscopy with electro-spray ionization (ESI-HRMS) was focused in ethanol on an AUTOSPEC 3000 with PEG-600 and PEG-900 as standards for exact mass determination. ^{1}H NMR spectra were recorded at room temperature on a Bruker AMX 300 (300 MHz). Proton chemical shifts in deuterated dimethylsulfoxide (DMSO-d_6) were referenced to DMSO-d_6 (δ: 2.47 ppm).

Electrochemical data were obtained by cyclic voltammetry under nitrogen at 25 °C using acetonitrile (HPLC grade) as solvent and tetrabutylammonium hexafluorophosphate (0.1 M) as supporting electrolyte. The measured potentials were referred to an Ag/AgNO3 (0.1 M in acetonitrile) electrode separated by a medium porosity fritted disc. A platinum wire auxiliary electrode was used in conjunction with a platinum disc TACUSSEL-EDI rotatory electrode (3.14 mm^2). Cyclic voltammograms of 10^{-3} M solutions, previously deoxygenated with gaseous N_2, of the samples in acetonitrile were run and the measured potentials were then referred to ferrocene, which was used as internal standard to facilitate the interpretation of the results. The experiments were carried out at scan rate $v = 0.1$ V s^{-1}, from -1 to +1 V and following a reduction forward step and a subsequent oxidation reverse step to complete the cycle.

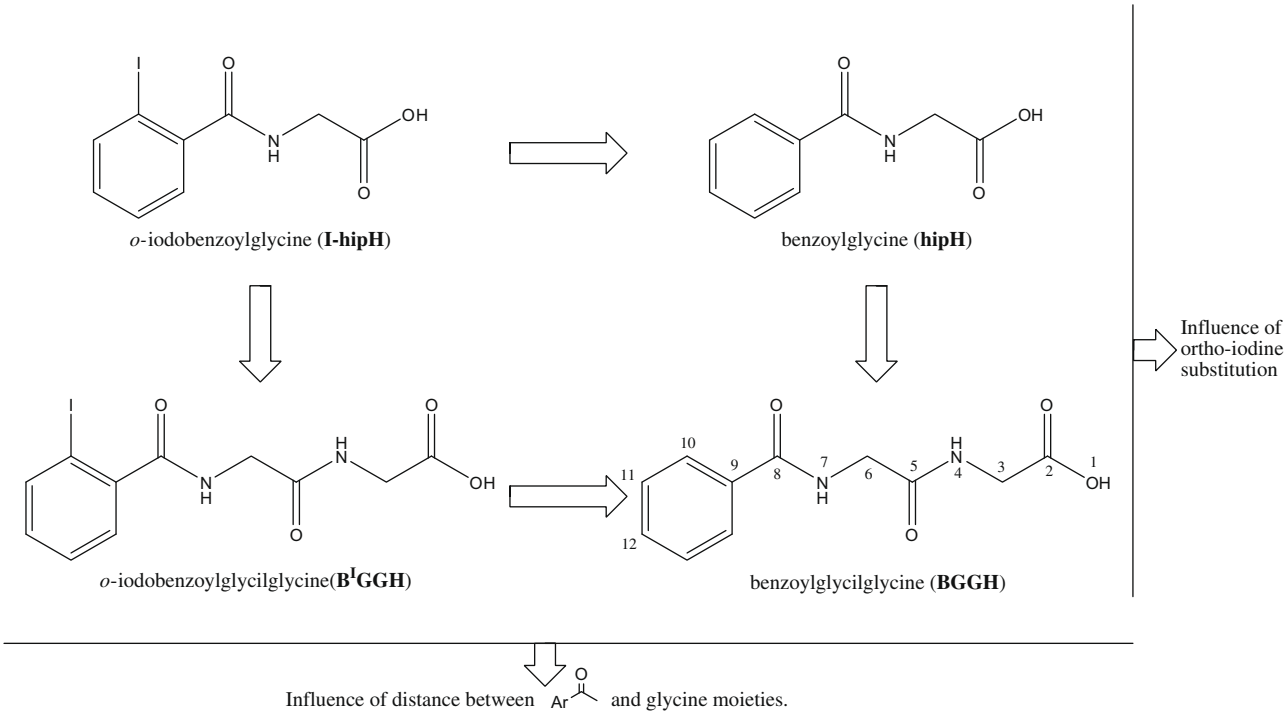
Cyclic voltammetry was performed with the compound **2** and **5** and was compared with the described previously for compound **1** and the monomeric $[Cu(phen)_2(acetato\text{-}O,O')][NO_3]\cdot 2H_2O$ which X-ray structure confirms that the metal ion is coordinated to the same donor atoms that the parent complex [22]. It is quite important to write down that the solution of compounds **2** and **5** partially discoloured immediately after solution in deoxygenated acetonitrile, while compound **1** became totally colourless and $[Cu(phen)_2(acetato\text{-}O,O')][NO_3]\cdot 2H_2O$ did not lose colour after solution.

Reagents were used as received from Sigma or Aldrich. *o*-Iodobenzoylglycilglycine (B^IGG) was previously synthesized [23] and benzoylglycilglycine (BGG) was prepared in a similar manner (see below).

2.2. Synthesis of benzoylglycilglycine (BGG)

About 3.53 g (42 mmol) of sodium bicarbonate were added to a solution of 2.64 g (20 mmol) of glycilglycine in 35 ml of water. The mixture was stirred for 1 h until complete solution and then 2.3 ml (20 mmol) of benzoyl chloride were added. The reaction was stirred for 4 h, and then neutralized with HCl 1 N, stirred for 15 min, and the resulting precipitate was filtered off and washed with cold water. The neutralization-washing process was repeated three times and finally the white precipitate was dried at room temperature (yield: 65%).

White powder. *Anal.* Calc. for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.86. Found: C, 55.97; H, 5.19; N, 11.83%. IR (cm^{-1}): 433w,



Scheme 1. Formulae and numeration of the four N-protected amino acid ligands used in this paper.