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Inhibition-based biosensor for cyanide detection – a preliminary study

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interaction with DNA should be expected for these compounds. Reports on the biological activity of similar gold(III) compounds are relatively scarce in the literature. Only recently, a Au(III) 1,2-dithiolene cyclometalated complex had proved its potential against Gram-positive bacteria [2]. In this study we evaluated two related Au(III) complexes containing N-alkyl-1,3-thiazoline-2-thione dithiolate ligand, $[Au(R\text{-}thiazdt)_2]^{-1}$ (R = ethyl-**1**; R = hydroxyethyl-**2**) [3a,b] as antitumor and antimicrobial agents. The compounds were assessed *in vitro* towards cisplatin sensitive ovarian cancer cells (A2780), bacteria and fungus of clinical importance such as *Staphylococcus aureus* and *Candida*. Spectroscopic studies were also performed to evaluate the interaction with DNA.
Materials and methods: The gold complexes were synthesised as previously described [3a,b]. The cytotoxic activity against the A2780 ovarian cancer cells was assessed by the IC_{50} determined by the MTT assay. The antimicrobial activities of complexes **1** and **2** were assessed by the MIC values towards the Gram+ *S. aureus*, and the fungal strains *C. glabrata* and *C. albicans*, using reported methods [4,5]. The ability of compounds to bind to DNA was assessed by fluorescence spectroscopy using ethidium bromide (EB) as the fluorescence probe.
Results: Complexes **1** and **2** presented high cytotoxic activity in the cisplatin sensitive A2780 cells even superior than cisplatin. Complex **1** was able to inhibit the growth of *S. aureus* and both *Candida* strains, while **2** was much less active. The fluorescence studies revealed a weak interaction of compounds with CT-DNA, in contrast with that found for cisplatin, although the interaction of **2** is somewhat stronger than that found for **1**.
Discussion and conclusions: Results evidenced the importance in what way minor modifications of the Au dithiolate structure can result in loss of activity in particular the antimicrobial activity. In contrast with cisplatin, DNA is not the main target involved in their mode of action. Further studies are needed to explore other potential targets and the mechanism of action.

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ABSTRACT
Introduction: The acute toxicity of cyanide along with its continue industrial use makes this substance of environmental concern [1]. Titration, spectrophotometry and ISE are the standard detection methods. However, they are complex and need sample pre-treatment [2]. To overcome these, another approach is using biosensors. To this end, we developed a disposable inhibition-based biosensor with a multi-heme nitrite reductase (*cc*NiR) coupled to graphite leads.

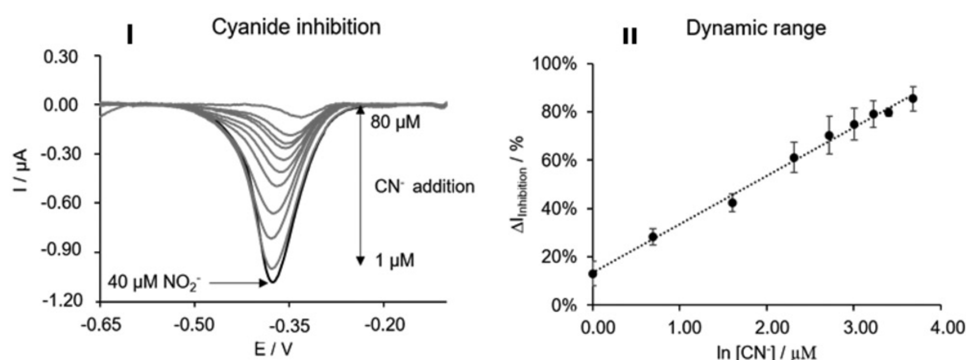

Figure 1. (I) Square wave voltammograms with baseline correction of one of the WE used in the study. Enzymatic inhibition by cyanide among 1–80 µM in the presence of 40 µM of nitrite. (II) Calibration curve ($y = 0.200x + 0.135$; $R^2 = 0.995$) for CN^- quantification, with the dynamic range 1 to 40 µM. Results are expressed as triplicate average with the corresponding standard deviation.

Materials and methods: Electrochemical measurements were carried out in a conventional electrochemical cell, composed by a three-electrode system. The reference was an Ag/AgCl electrode, and the counter electrode was a Pt wire. The working electrode (WE) was in-house made using a graphite lead with the ccNiR (from bacteria D. *desulfuricans* ATCC 27774; stored in 0.05 M phosphate buffer, pH 7.6) immobilised by drop cast at the WE surface. The electrochemical technique used was square wave voltammetry. Electrochemical cells contained 0.1 M KCl in 0.1 M Tris–HCl buffer (pH 7.6) as supporting electrolyte. Dissolved oxygen was removed by a biochemical system (GOx, catalase and glucose).
Discussion and conclusions: In Figure 1 we can observe the decrease in catalytic activity due to the presence of cyanide. The biosensor dynamic range comprises the maximum value imposed by the European Union, 1.92 µM (98/83/EC directive), which does not happen with other cyanide biosensors [3]. Furthermore, a graphite lead cost 0.35€ and each of them can be split, allowing a very low-cost biosensor. Given that enzyme inhibition is reversible [4], the sensor can be used more than one time, if we optimise the enzyme immobilisation method.

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Modification of ZnO nanoparticles with silanes enables their application as anticancer agents

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