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Water soluble glucose derivative of thiocarbohydrazone acts as ionophore with cytotoxic effects on tumor cells

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ABSTRACT

A novel water-soluble ionophore based on the thiocarbohydrazone moiety conjugated with glucose (**GluTch**) was synthesized through a simple two-step procedure. Structural elucidation was carried out in water solution by means of various spectroscopic techniques (NMR, UV–Vis, and CD), electrospray ionization mass spectrometry and density functional theory calculations. The flexible nature of the thiocarbohydrazone moiety of the new glycoderivative compound induced both different coordination motifs and stoichiometry towards copper and zinc. Cytotoxicity assays of the ligands on the human normal keratinocyte NCTC-2544, MDA-MB-231 breast cancer and PC-3 human prostate adenocarcinoma cell lines demonstrated that i) higher activity on cancer cells growth inhibition compared to a normal cell line; ii) the introduction of the glucose unit does not alter the cytotoxic activity of the underivatized ionophore ligand and iii) the presence of copper ion improves the activity of the thiocarbohydrazones.

1. Introduction

Inorganic medicinal chemistry gained favor ever since the accidental discovery of cisplatin as anticancer agent [1]. However, the side effects characterized by diffuse and high toxicity, the risk of intrinsic and acquired resistance, low water solubility of platinum-based drugs induced the development of new anticancer drugs agents based on metal ion different from platinum with lower toxicity and a broader spectrum of activity [2–4].

An emerging concept in this cancer research field takes advantage of metal complexes containing molecules with known therapeutic values as ligands [5]. As a result, the organic moieties protect the metal ion, preventing side reactions in the transfer towards its target, while the modifications of their chemical structures allows for the synthesis of a wide range of metal complexes some of which showed increased cytotoxicity and pharmacokinetic profile [6]. Among the first-row transition metals, copper and zinc complexes have been employed as cytotoxic compounds [7–12], being well known that the homeostasis of these metal ions is altered not only in tumor cells [13] but also in biological fluids and tissues of cancer affected patients [14–18].

"Based upon how the compound's biological effects are altered by an increase in metal concentration" [19], a classification of metal binding ligands has recently been proposed: metal chelators, metal shuttle and metal ionophore. While metal chelators, able to remove extracellular metal ions, possess cytostatic rather than cytotoxic properties [20–23], metal shuttle and metal ionophore ligands show cytotoxic activity, increasing intracellular metal ion levels [24,25].

The metal-shuttle conjugates may exhibit biological activity because the metal dissociates from the complex, or because the conjugate itself is biochemically active whereas ionophore compound activity is amplified by increasing the metal concentration [19].

Different ligands classes have thus been studied, characterized by N—N, N—O, N—S, S—S, N-N-S and N-N-N donor atoms [26] allowing for new application of old metal binding drugs [5].

Thiosemicarbazones are molecules of great interest in medicinal chemistry as display different biological activities: antibacterial, antiviral and antifungal; particular interest has been devoted to their use in neurodegenerative diseases [27] and cancer therapies [28]. The anticancer activity of thiosemicarbazone has not been fully understood yet, but it has mainly been attributed to its ability to act as iron chelating

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agent and so to inhibit ribonucleotide reductase activity [29].

Multiple mechanisms have recently been invoked to rationalize the cytotoxic activity of these compounds *in vitro* and *in vivo* assays [9,30,31].

In addition to the interaction with iron, thiosemicarbazones are able to chelate other bio-metals, such as copper and zinc; [32] more recently, it has become evident that their anticancer activity is not simply due to their chelating properties but rather on their ability to act as "ionophore", increasing the metal ion concentration inside cells [33]. Reactive oxygen species (ROS) production [34] and activation of cascade pathways inducing apoptosis [35,36] characterize the cytotoxic activities of the copper and zinc complexes with these ligands. The zinc supplementation improves thiosemicarbazone activity against human breast adenocarcinoma cells (MCF-7) and copper ions impact on the ability of ligand to transport zinc into the cell. These data indicate a metal depletion mechanism in the cytotoxic effect of un-coordinated thiosemicarbazone ligand confirming several anti-cancer pathways of action associated with the level of available metal ions in the extracellular environment [37].

Different thiosemicarbazone derivatives have been synthesized with the aim to increase their solubility, availability, toxicity and then their effectiveness [38–40]. However, also one of the more investigated molecule as 3-amino-2-pyridinecarboxaldeyde thiosemicarbazone, which has entered > 20 years ago in multicentered clinical trials, has showed many issues including low efficacy in some tumors and toxic side effects [26,41].

The thiocarbohydrazones (TCH) are the higher homologues of thiosemicarbazones (TSC): the extra hydrazine moiety can act both as a supplementary-metal binding site towards transition metal ions and as a reactive group for a further functionalization of the ligand. The interesting coordination chemistry of TCH has mainly been focused on the symmetric derivatives, which are relatively simple to synthesize [42–45]. The high biological activity of the 2-Acetylpyridine TSCs [46–48] and related metal complexes makes the TCHs a reasonable starting point for rational design of new and selective agents for future generations of metal-based anticancer drugs.

However, it is relevant to characterize the complex species formed in aqueous solution and reveal the effective activity of compounds as chelating or ionophore agent. Indeed, the solubilization of a copper or zinc solid-state complex in solution leads to the formation of an equilibrium in which complex species, also different from the crystallized one, are formed depending on pH and other environmental conditions.

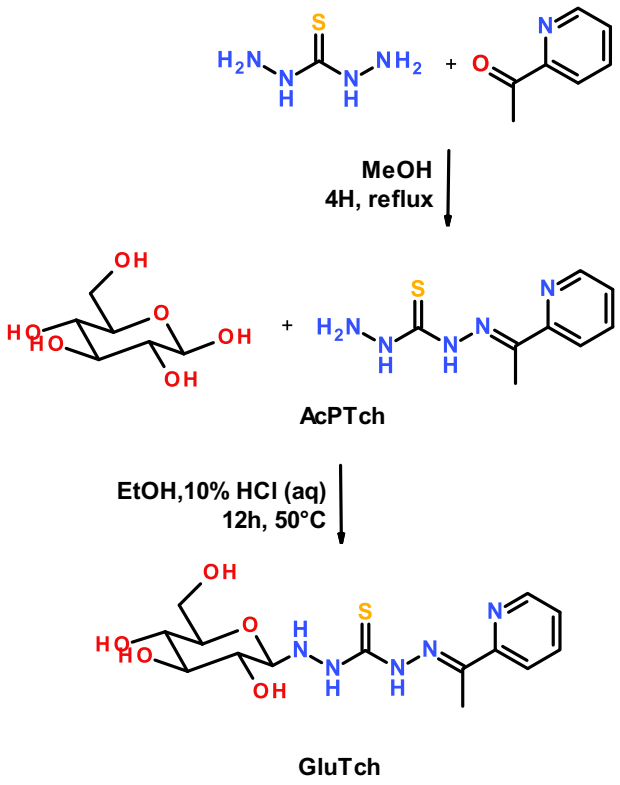
The sugar-conjugation [49] usually improves the drug biocompatibility, solubility and allows for the drug concentration increase in the malignant tumor cells [50,51].

In this paper, we report on the synthesis of an asymmetric water-soluble thiocarbohydrazone as metal delivery system with cytotoxic activity, glycoconjugate of Acetylpyridine thiocarbohydrazone (**GluTch**) (see Scheme 1) potentially able to overcome the limitations of the thiosemicarbazones.

To the best of our knowledge, TCH ligands and their metal complexes formed in aqueous solution have not been characterized.

A combined approach, including mass spectrometry, NMR, IR, UV–vis and Circular Dichroism spectroscopy, was employed for the characterization of the new ligand **GluTch** and its copper(II) and zinc (II) complexes in aqueous solution at physiological pH.

Preliminary evaluation of the cytotoxic activity was performed for the **GluTch** and compared to the acetylpyridine thiocarbohydrazone parent ligand (**AcPTch**), showing that the cytotoxicity is greatly influenced by the presence of the copper ions and results as high as 5-FU, a potent anticancer drug.



Scheme 1. Synthesis of the glucose-functionalized Tch.

2. Materials and methods

2.1. Synthesis

All commercially available chemicals were obtained from Sigma Aldrich or Alfa Aesar and used as received. Thin layer chromatography (TLC) was carried out on silica gel plates (Merck 60, F254). All reactions were carried out under nitrogen atmosphere unless otherwise stated. High purity water (Millipore, Milli-Q Element A 10 ultrapure water) was employed throughout.

2.2. Synthesis of 1-[(E)-1-(2-pyridyl)ethylideneamino]-3-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]amino]thiourea (GluTch)

To a stirred suspension of 1-amino-3-[(E)-1-(2-pyridyl)ethylideneamino]thiourea (**AcPTch**) (0.63 g, 3.0 mmol) in 120 mL of methanol was added β-D-Glucose (0.54 g, 3.0 mmol) and 0.3 mL of 10% HCl(aq). The mixture was heated at 50 °C overnight. The resulting clear orange solution was cooled to room temperature and solvents were removed under reduced pressure. The crude yellow solid was purified by column chromatography on Diol silica gel (eluent CH_2Cl_2/Methanol) to give **GluTch** as pale-yellow solid (0.71 g, 1.9 mmol) in 63.8% yield.

IR (KBr, cm^{-1}): 3400 (broad, OH, NH), 3233, 2923, 1638, 1616 (C=N), 1468, 1229 (C=S), 1079, 1027, 784 (C=S). UV–vis [H_2O, pH = 7.40, λ_{max}, nm (ε, $dm^3\,mol^{-1}\,cm^{-1}$)]: 303 (17446). ^{1}H NMR (499.88 MHz; D_2O, ppm): 8.47 (d, J = 4.9 Hz, 1H, ArH), 7.93 (d, J = 5.0 Hz, 1H, ArH), 7.82 (t, J = 8.0 Hz, 1H, ArH), 7.38 (t, J = 7.9 Hz, 1H, ArH), 4.18 (d, J = 9.0 Hz, 1H, GluCH), 3.82 (dd, J^1 = 12.2 Hz, J^2 = 2.1 Hz, 1H, GluCH), 3.63 (dd, J^1 = 11.8 Hz, J^2 = 5.9 Hz, 1H, GluCH), 3.45 (t, J = 9.1 Hz, 1H, GluCH), 3.36 (m, 1H, GluCH), 3.30 (m,

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