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New 8-hydroxyquinoline galactosides. The role of the sugar in the antiproliferative activity of copper(II) ionophores

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ABSTRACT

8-Hydroxyquinoline derivatives and their metal complexes have recently awakened interest as promising therapeutic agents in cancer therapy. We have previously synthesized and evaluated glucoconjugated 8-hydroxyquinolines as copper ionophores activated by β-glucosidases.
In order to further evaluate the crucial role of the sugar, we designed and synthesized a series of new galactoconjugates of 8-hydroxyquinolines and investigated their biological properties in comparison with the 8-hydroxyquinoline analogs. The effect of copper(II) ions on their biological activities was evaluated. In particular, two compounds possess a pharmacologically relevant antiproliferative activity against specific tumor cells in the presence of copper(II) ions. Furthermore, the antiproliferative activity of the selected galactosides was successfully investigated in the presence of β-galactosidase as a preliminary model of antibody directed enzyme prodrug therapy.

1. Introduction

8-Hydroxyquinoline (OHQ) is a privileged molecule in medicinal inorganic chemistry. A large number of OHQ derivatives, such as cloxyquin, clioquinol and nitroxoline are of considerable interest for their unique properties including anti-microbial, anti-cancer and anti-neurodegenerative activities [1].

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ), the best known member of this family, was developed as an effective amebicide for treating diarrhea. In the 1970s, it was suspected as the cause of an epidemic of subacute myelo-optic neuropathy (SMON) and was withdrawn from the market. The explanation for this side effect is not clear, but it has been suggested that the mechanism of action of CQ in causing SMON is associated with its ability to chelate metal ions such as copper. It has long been known that copper deficiency can cause myeloneuropathy [2] Nevertheless, the metal chelating ability of OHQs has also been invoked to explain the biological properties of these molecules as potential agents for the treatment of Alzheimer's disease and cancer [3,4].

In particular, CQ directly induces cell death in malignant cells at micromolar concentrations and also exhibits anti-cancer activity in vivo [5] and CQ induces apoptosis in cancer cells through a caspase-dependent apoptotic pathway. Other OHQ derivatives have also been tested as anticancer compounds and it has been reported that the interaction with copper(II) ion is a prerequisite for their anticancer activity, as well as for CQ [6]. Experimental evidences suggest that OHQ and CQ act as anticancer agents through proteasome inhibition in the presence of copper even if a copper-independent mechanism has also been described [4].

Metal dyshomeostasis has been observed in cancer tissues, in particular elevated copper levels have been found [7,8] even if there is still insufficient information on how cellular transformation drives copper accumulation. Copper plays a pivotal role in promoting angiogenesis [9,10] that is required for tumor growth and metastasis. The elevated intracellular copper levels predispose cancer cells to ionophore sensitivity and a number of copper coordinating compounds (OHQs, dithiocarbamates and thiosemicarbazones) have been investigated as potential anticancer therapeutics [11–13]. In this way copper(II) homeostasis in tumor cells could be modulated and/or the copper(II) complexes could exert their cytotoxic action [14].

We have recently demonstrated that glycoconjugation is a versatile and powerful strategy for improving many of their features including drug targeting, solubility, and biological activities [15–18]. In particular, we had previously synthesized and evaluated the glucosides of OHQs in order to obtain prodrugs selectively activated by glucosidase enzymes in tumor cells. In these compounds, the presence of the sugar unit prevents copper(II) binding reducing the possibility of the systemic complexation of essential metal ions.

Sugars are among the most attractive systems and have been widely used as vectors for prodrug design [19]. In fact, the conjugation with sugars may introduce notable biological and physicochemical properties to drugs, such as improvement of the water solubility, reduction of undesirable toxicity and enhancement of cellular recognition

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exploiting either sugar-specific transporters or sugar-lectin specific interactions [20].

Glucose has been used with successful results [21] in drug design but, in some cases other carbohydrates have been preferred over it [1,22]. Galactose (Gal) is a widely used sugar, which has been conjugated with amines such as dopamine [23] to improve the crossing of the blood–brain barrier (BBB) through hexose transporters. Moreover, galactose has been conjugated with superoxide dismutase to enhance its localization in the liver and reduce the oxidative stress related to ischemia [24]. Among the galactoconjugates, the prodrugs of Duocarmycin analogs [25] are considered the best examples of anticancer compounds suitable for enzyme-activated prodrug therapies, fulfilling the main criteria required for this approach.

The use of enzyme-activated prodrugs based on sugars represents a promising strategy to overcome the drawbacks of the chemotherapeutic agents: the active enzyme may be present and/or overexpressed in the tumor cells as in PMT (prodrug monotherapy) [26,27] or delivered to the tumor site, as in ADEPT (antibody directed enzyme prodrug therapy). In particular, the latter firstly requires the selective delivery of the enzyme to the tumor cells through antibody–antigen recognition. In the second phase, prodrugs are administrated and the enzyme is able to activate the prodrugs at the site of interest, achieving high site selectivity [28]. This aspect is of particular importance for the treatment of cancer cells avoiding cytotoxic effects on healthy tissues.

ADEPT and PMT have been widely investigated as therapies to deliver drugs specifically to cancer tissue and have shown promise in both animal studies and clinical trials [29,30].

Several galactoside prodrugs [31] have appeared as potential candidates to achieve selective chemotherapy of solid tumors in combination with antibody-β-galactosidase conjugates.

β-Galactosidase is almost undetectable in serum, which makes this enzyme very attractive for ADEPT as its low levels would minimize the premature release of active aglycone [31].

Furthermore, the recombinant forms of the human galactosidases, alpha and beta Agalsidase are currently applied in the treatment of diseases related to defects in glycolipid breakdown (Fabry's and Gaucher's diseases) [32] and therefore the usage of galactosidase enzymes has a successful clinical history.

On the basis of the interest in galactose conjugation and the challenge of achieving drug selectivity, here we report the synthesis, the characterization and the biological activities of new galactose conjugates of OHQ derivatives (Fig. 1). We evaluated the antiproliferative activities of the new compounds against tumor cells in comparison with their parent OHQ. The effect of Cu^{2+} ions on their antiproliferative activities was also investigated. The comparison between the glucosides and galactosides of OHQs confirms the mechanism of action proposed elsewhere [15,16] for the former systems and highlights the importance of the identity of the sugar residue in the glycoconjugation strategy. Galactose conjugates have to be subjected to hydrolysis by specific β-galactosidases to liberate the active aglycone, which may exert its anticancer activity upon the copper(II) complexation. We also tested the activities of the galactose conjugates on cancer cell lines in the presence of β-galactosidase in order to evaluate their suitability for ADEPT.

2. Materials and methods

Commercially available reagents were used directly, unless otherwise noted. 8-Hydroxyquinoline (**1**), 2-methyl-8-quinolinol (**2**), 2-amino-8-hydroxyquinoline (**3**), 5-nitro-8-hydroxyquinoline (**4**), 5-chloro-8-quinolinol (**5**), 5,7-dichloro-8-quinolinol (**6**), 5-chloro-7-iodo-8-hydroxyquinoline (**7**), 8-hydroxyquinoline-β-D-galactopyranoside (Gal**1**) and β-Galactosidase from *Escherichia coli* were purchased from Sigma-Aldrich. TLC was carried out on silica gel plates (Merck 60-F254).

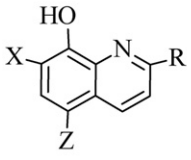
The copper(II) complexes were prepared by adding a $CuSO_4$ standardized solution to a ligand water solution.

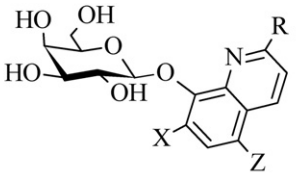
2.1. NMR spectroscopy

^1H and ^{13}C NMR spectra were recorded at 25 °C with a Varian UNITY PLUS-500 spectrometer at 499.9 and 125.7 MHz respectively. NMR spectra were obtained by using standard pulse programs from Varian library. 2D experiments COSY (correlation spectroscopy), TOCSY (total correlation spectroscopy), gHSQCAD (gradient heteronuclear single quantum coherence), gHMBC (heteronuclear multiple quantum correlation) were acquired using 1 K data points, 256 increments and a relaxation delay of 1.2 s. The spectra were referred to the solvent signal.

2.2. UV–vis and circular dichroism (CD) spectroscopy

UV spectra were recorded with an Agilent 8452A diode array spectrophotometer.





Compound	X	Z	R
1	H	H	H
2	H	H	CH_3
3	H	H	NH_2
4	H	NO_2	H
5	H	Cl	H
6	Cl	Cl	H
7	I	Cl	H

Compound	X	Z	R
Gal**1**	H	H	H
Gal**2**	H	H	CH_3
Gal**3**	H	H	NH_2
Gal**4**	H	NO_2	H
Gal**5**	H	Cl	H
Gal**6**	Cl	Cl	H
Gal**7**	I	Cl	H

Fig. 1. OHQs and galactose conjugates.