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# New Glycoconjugates for the Treatment of Diseases Related to Metal Dyshomeostasis\*\*

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### Introduction

Metal dyshomeostasis is known to be involved in the etiology of a number of pathologies such as Wilson's, Alzheimer's, Parkinson's, and Niemann–Pick diseases and cancer. Neurodegenerative disorders and cancer have long been viewed as among the most enigmatic and problematic issues in biomedicine, and their multifaceted nature have been well-documented. The precise mechanisms responsible for triggering these disorders remain unclear; however, they share multifactorial pathogenic mechanisms related to metal dyshomeostasis and, as a consequence, oxidative stress. Considering the multifactorial nature of these diseases, it is becoming more and more evident that the next generation of drugs must have multiple functions to combat the multiple processes of disease progression; metal ions represent a promising therapeutic target as they could be a starting point to hit multiple targets. On the other hand, especially in the treatment of cancer, drugs should ideally be directed to the site of action in order to decrease their side effects on healthy tissues and enhance their uptake by targeted cells.

The doctoral research described here focused on the synthesis and evaluation of multifunctional molecules that could interfere with different key target points of neurodegeneration and cancer. 8-Hydroxyquinoline (OHQ), a metal chelator, was chosen as a molecular scaffold to build selective prodrugs and multifunctional metal-binding compounds because it has emerged as a privileged structure for new drug candidates. The synthesis, characterization and biological evaluation of new covalent glycoconjugates with OHQ derivatives (Figure 1) are reported in the Thesis. Glycosylation is a successful strategy for improving several features of these systems—in addition to the increase in solubility, a sugar moiety typically confers selectivity and multifunctionality.

## **Glycoconjugated Prodrugs**

New glucoconjugates and galactoconjugates of OHQ derivatives were designed in order to exploit the unique features common to cancer cells, such as the high expression of certain proteins (e.g.,  $\beta$ -glucosidases and glucose transporters) and the high levels of copper(II) ions. These features can be synergistically combined to obtain new compounds that are highly specific for neoplastic cells, minimizing chemotherapeutic side effects.

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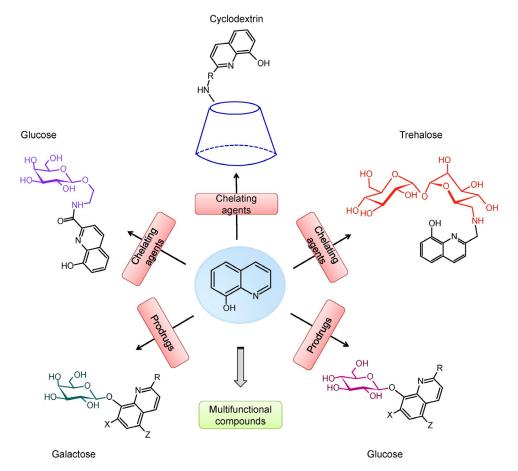


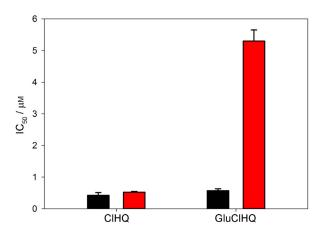
Figure 1. Glycoconjugates of 8-hydroxyquinolines.

The main advantages of these glycosylated compounds are targeting and prevention of side effects due to systemic chelation. The chelating function of these compounds is masked, and they must be subject to hydrolysis through specific  $\beta$ -glucosidases or  $\beta$ -galactosidases to liberate the active aglycone in targeted cells.

The new glycoconjugates were obtained through a one-pot reaction in good yields using a modified Michael procedure. The pure products were characterized by mass spectrometry and NMR, and were then biologically evaluated.

The glucoconjugates exhibit significant antiproliferative activity, with the exception of 2-methyl-8quinolinyl-β-p-glucopyranoside; the activity of the compounds was remarkably enhanced by the presence of copper ions. Furthermore, the results obtained in the presence of a  $\beta$ -glucosidase inhibitor, 2,5-dideoxy-2,5-imino-p-mannitol (DMDP), unequivocally confirm the crucial role of  $\beta$ -glucosidase activity to allow the release of the active quinoline moiety in order to develop the antiproliferative activity (Figure 2). The in vitro cleavage studies suggest that the glucoconjugates are differently hydrolyzed by  $\beta$ -glucosidase. These data are consistent with the results of the antiproliferative activity assay and docking studies. In some cases, the antiproliferative activity of the prodrugs was inferior to that of their parent compounds indicating the incomplete cleavage of the glycosidic bond for these compounds. In particular, 2-methyl-8-quinolinyl-β-p-glucopyranoside, which is inactive, is the compound cleaved over a longer time period in comparison with the other glucosides. These experimental results are supported by docking studies; 2-methyl-8-quinolinyl-β-p-glucopyranoside shows a different binding pose compared with the chlorinated compounds (5-chloro-8-quinolinyl-β-p-glucopyranoside and 5,7-dichloro-8-quinolinyl-β-p-glucopyranoside). More precisely, it is predicted to have the highest binding affinity for the active site of  $\beta$ -glucosidase, suggesting that prodrugs with longer half-life values are more tightly bound to the active site than those with shorter half-life values. It can be deduced as a logical consequence that the glucoconjugates tightly bound to the enzyme are more slowly hydrolyzed.





**Figure 2.** Histograms represent the IC<sub>50</sub> values of 5-chloro-8-hydroxyquino-line (CIHQ) and its glucoconjugate (GluCIHQ) in the presence (■) or absence (■) of the β-glucosidase inhibitor, 2,5-dideoxy-2,5-imino-p-mannitol (DMDP;  $100 \mu M$ ) on A2780 cells. Values are the mean of at least four independent experiments performed in triplicate; error bars represent the SD.

In summary, the glucoconjugates could be actively transported into the cells (e.g., by glucose transporters). The cytosolic  $\beta$ -glucosidase could hydrolyze the compounds that could complex copper(II) ions (present in large amounts in cancer tissues) and exert their antiproliferative action. Free OHQs could have multiple targets causing cancer cell death by apoptosis.

Chemical conjugation of OHQs with galactose leads to new prodrugs with a cytotoxic differential of approximately 100-fold compared with their parent compounds. This degree of prodrug activation is a necessary requirement in developing antibody directed enzyme prodrug therapy (ADEPT). The results reported in the Thesis

strongly suggest that attachment of the galactose residue to OHQ leads to almost complete drug inactivation, and that the glycosidic bond is highly stable under the conditions used in the in vitro assay. Interestingly, 2-amino-8-quinolinyl- $\beta$ -D-galactopyranoside (GalAHQ) and 5-nitro-8-quinolinyl- $\beta$ -D-galactopyranoside (GalNHQ) show pharmacological relevant activity in the presence of Cu<sup>2+</sup>. Their activity is cell-line-dependent, and they could be good candidates for targeted delivery to human ovarian carcinoma, exploiting prodrug monotherapy approach. Unlike glucoconjugates, the galactosides do not easily reach the lysosomal enzymes within the cell and thus they are not rapidly cleaved; the exceptions were GalNHQ and GalAHQ, which were shown to be partially hydrolyzed within 72 h in cell lines where there is an overexpression of galactosidase. However, the presence of exogenous  $\beta$ -galactosidase increases the antiproliferative activity of the galactoconjugates, confirming that the compounds are hydrolyzed by the enzyme and that the latter is able to exert its optimal activity at a pH close to that of the tumor extracellular fluid. To conclude, these prodrugs have interesting properties that render them suitable for ADEPT approach.

## **Chelating Glycoconjugates**

Chelating 8-hydroxyquinoline conjugates with monosaccharides, disaccharides and oligosaccharides have also been synthesized in order to compare their properties to those of the glycosylated prodrugs. Unlike the latter compounds, they are able to complex Cu<sup>2+</sup> and Zn<sup>2+</sup> ions with high stability constants

In particular, the new cyclodextrin-8-hydroxyquinoline conjugates (CyD-OHQ) center on the belief that "one molecule-multiple targets" is an approach with remarkable advantages compared to "one molecule-one target" strategy. CyD-OHQs were synthetized by an amide condensation or a stepwise reductive amination, functionalizing the cyclodextrin at the upper or lower rim with one or two OHQ moieties. The characterization and unequivocal structural assignments of the compounds were achieved by a combination of techniques including circular dichroism, mass spectrometry, UV-vis and NMR spectroscopy.

CyD-OHQs have significant antioxidant capacity on the basis of in vitro antioxidant assays, superior to that of a well-known antioxidant, Trolox. The compounds can complex Cu<sup>2+</sup> and Zn<sup>2+</sup> ions with conditional stability constants in aqueous solution similar or higher to/than those of OHQ (Figure 3). This behavior could be explained by the presence of an exocyclic nitrogen available to complex the metal ion. The metal-ligand (ML) main species with the metal ion bound by the nitrogen atom of the pyridine ring, the phenolate and amino or amide groups can be hypothesized. The exocyclic nitrogen stabilizes the ML species in comparison to OHQ. The conditional stability constant of ML<sub>2</sub> species is significantly lower than the one calculated for OHQ as the presence of the exocyclic nitrogen destabilized this species.



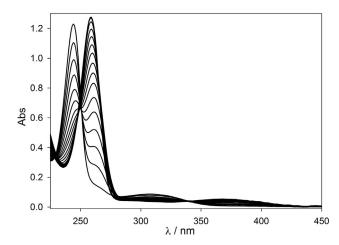
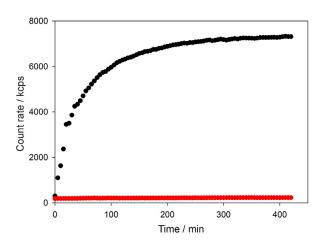


Figure 3. Titration of  $6^A$ -deoxy- $6^A$ -[(8-hydroxyquinolyl)-2-methylamino]-β-cy-clodextrin (CD6RHQ) with Cu(NO<sub>3</sub>)<sub>2</sub>.



**Figure 4.** Time evolution of the normalized scattered intensity of the systems  $Zn^{2+}$ -BLG ( $\blacksquare$ ) and Zn-BLG-CD6RHQ ( $\blacksquare$ ).

Copper(II) complexes of the conjugates exhibit high superoxide dismutase (SOD)-like activity. Moreover, these ligands are also able to strongly inhibit metal-induced protein aggregation as demonstrated by an effective assay that exploits the formation of amyloid fibrils when  $\beta$ -lactoglobulin A (BLG) is heated in the presence of metal ions (Figure 4). Obviously, these features are highly desirable considering the critical involvement of metal ions in protein misfolding and aggregation, which are hallmarks of several neurodegenerative disor-

Antiproliferative studies of the compounds in the absence or presence of Cu<sup>2+</sup> on five different cell lines demonstrated relatively high IC<sub>50</sub> values. These results suggest that these agents could be administered in relatively high concentrations, for use as antioxidant and antiaggregant drugs, if necessary, without damage to healthy tissues. It is clear that the cyclodextrin moiety renders the systems less toxic compared with the respective OHQ derivatives.

These compounds could have great potential as therapeutic agents in the treatment of neurodegenerative diseases related to oxidative stress and metal dyshomeostasis, such as Alzheimer's disease

and especially Niemann–Pick Type C. Apart from improving the aqueous solubility due to the presence of the saccharidic residue, the hybrid systems might have advantageous features including the possibility of forming inclusion complexes with coformulating drugs and/or to include endogenous compounds, such as cholesterol, in order to remove them from the body. Moreover, the presence of the bulky  $\beta$ -cyclodextrin moiety might provide a platform for improved pharmacokinetics of the drug conjugates protecting, for example, from glucuronidation or sulfation of OHQs.

Keywords: antioxidants · cyclodextrin · glycoconjugates · metal complexes · metal dyshomeostasis

### Publications arising from this work:

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