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ARTICLE

Positional Isomers of Mannose-Quinoline Conjugates and their Copper Complexes: Exploring the Biological Activity

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8-Hydroxyquinolines show a wide range of pharmacological activities, and some are marketed as therapeutic agents. Despite the significant number of biologically active hydroxyquinolines proposed, there is a continued interest in the development of new active derivatives to overcome the drawbacks associated with their use. Herein, we report the synthesis and characterization of a set of positional isomers of hydroxyquinoline-mannose conjugates. Since in many cases the complexation ability of 8-hydroxyquinoline seems to be responsible for their pharmacological activities, we investigated the capacity of these systems to complex copper(II) ions. We also examined diverse biological activities (antiproliferative, antimicrobial and antioxidant) of the new derivatives and their copper(II) complex and compared them to those of their parent compounds and an analogous glucose-quinoline conjugate. All compounds show antioxidant activity that depends on the regioisomer. Moreover, specific isomers show significant antibacterial activity against *P. aeruginosa* and *S. aureus.* Furthermore only a regioisomer shows a pharmacologically relevant antiproliferative activity against human tumor cells in the presence of copper(II) ions.

Introduction

8-Hydroxyquinolines (HQs) constitute an important class of natural and synthetic compounds with a wide range of applications.[1] Besides their use as analytical tools, HQ derivatives show a wide range of pharmacological activities including anticancer,[2-4] antifungal,[5] antibacterial,[1,6] antiaggregant[7,8] and neuroprotective effects.[9] Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) and PBT2 can suppress the amyloid aggregation and reduce cytotoxicity of Aβ and other proteins involved in neurodegenerative diseases, such as Alzheimer's and Huntington's diseases.[10] Also, 5-carboxy-8-hydroxyquinoline (HQ5, also known as IOX1) is a broad spectrum inhibitor of oxygenase enzymes with excellent potential for the treatment of a panel of human diseases.[11,12] Examples of HQ derivatives that are used in the clinical practice include nitroxoline, an antibiotic to treat urinary tract infections.[13,14] Many of the biological properties of HQs are related to their capacity to bind metal ions, such as copper, zinc and magnesium.[1] HQs act as ionophores, promoting the delivery of metal ions, or as chelators that remove essential metal ions.[1]

Despite the significant number of biologically active HQs proposed, there is a continued interest in the development of new active HQ derivatives because of their limitations resulting from low solubility, toxicity or in some cases low cell permeability.[1]

The glycoconjugation of HQs improves some of their properties as demonstrated by us.[1,15] In particular, the covalent conjugation of β-cyclodextrin with HQ remarkably increases the effects on the inhibition of Aβ aggregation[16,17] whereas the conjugation with galactose and trehalose actively reduces the cytotoxicity of these compounds.[1,18]

Finally, we demonstrated that the glucoconjugation of HQs is a promising strategy for specific targeting of cancer.[19,20] Indeed, a well-known approach for targeting specific cells exploits the interaction between a glycoconjugated drug and carbohydrate-binding proteins (lectins or glucose receptors).[21,22] Molecular recognition involving carbohydrates and lectins (cell-surface proteins) is a crucial interaction in a large variety of biological events, such as viral and bacterial infections, inflammatory and immune response, enzyme trafficking, cell-cell adhesion, cellular migration, cancer metastasis.[23,24]

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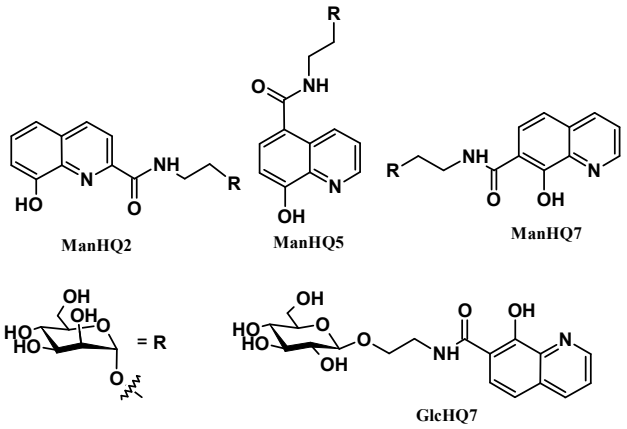

Figure 1. Mannose (ManHQ2, ManHQ5, and ManHQ7) and glucose (GlcHQ7) derivatives of 8-hydroxyquinoline.

Herein we report the synthesis, and characterization of three positional isomers of mannose-HQ conjugates (Figure 1). In particular, we examined the effect of D-mannose (Man) on the biological activity of mannose–quinoline conjugates (ManHQs) comparing them to the parent compounds (Man and HQs) and an analogous glucose-HQ conjugate. Since the complexation ability of HQs seems to be primarily responsible for many of their pharmacological activities, we also investigated the capacity of these systems to complex copper(II) ions. We evaluated the antiproliferative, antioxidant and antimicrobial activity of these new ligands and their complexes in vitro.

Results and discussion

Synthesis and Characterization.

We synthesized three rationally designed mannose-hydroxyquinoline conjugates and evaluated their biological activities. The syntheses started from 2-, 5- and 7-carboxylic-HQ. The sugar residue was introduced through an ethylene spacer using an ether glycosidic linkage to preserve key structural features of the sugar that may be a prerequisite for optimal recognition. At the other end of the chain, HQ moiety was attached through an amide bond. Analogously, we also synthesized a glucose-HQ conjugate to obtain insights on the role of the kind of sugar. The syntheses of the complete set of the positional isomers involved multiple steps in achieving the sugar precursor (Figure 2). α-D-mannose pentaacetate reacted with 2-bromoethanol and the deacylation under Zemplen conditions (NaOMe/MeOH) provided 2'-bromoethyl-α-D-mannopyranoside. To obtain a suitable reactive group for the coupling with HQ carboxylic acid, a nucleophilic substitution of bromine by azide in DMF, followed by the reduction of the azido group provided the desired precursor. The last synthetic step involved the amide coupling reaction between 2'-aminoethyl-α-D-mannopyranoside and the 8-hydroxyquinoline carboxylic acid derivative to obtain the products, ManHQ2, ManHQ5 and ManHQ7. We used 2'-aminoethyl-β-D-glucopyranoside to obtain GlcHQ7.

The products were purified by reversed-phase (C-18) or anion exchange chromatography. All new compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS).

Positive ion ESI-MS spectra (Figures S1-S2) confirm the identity of the products. ESI-MS spectra of ManHQs and GlcHQ7 show a host of peaks due to singly charged ion adducts at *m/z* 395.1 and 417.1 attributable to $[LH+H]^+$ and $[LH+Na]^+$ ions and singly charged dimeric sodium ion adducts $[2LH+Na]^+$ at *m/z* 810.9 (L is a hydroxyquinolinate species). In the negative ion mode ESI spectrum, the base peak can be assigned to the singly charged $[L]^-$ at *m/z* 393.1, probably resulting from the deprotonation of the phenolic group.

As for NMR spectra of ManHQs, the signals due to the sugar moieties are almost superimposable, while the major differences among the spectra can be observed in the aromatic regions, according to the position of substituents on the HQ moiety (Figure 3). In particular, the signals due to the quinoline moiety resonate between 8.9 and 7.1 ppm. The signals of the sugar and the ethylenic chain protons appear in the region 4.9-3.5 ppm. The resonances of the anomeric proton were buried under the HOD signal at 4.8 ppm and were assigned through 2D NMR spectra (Figures S3-S20). The $J_{1,2}$ values of the mannose ring proton (about 1.8 Hz), compared to values reported elsewhere,[25,26] confirm the α configuration at the anomeric carbon for ManHQs. The diastereotopic protons of the ethylenic chain are easily assigned by HSQC, whereas the signals of the other sugar protons appear in the range δ = 4.0 – 3.5 ppm.

The stability of this class of compounds in water and phosphate buffer (10 mM, pH 7) was evaluated by UV-Vis spectroscopy and ESI-MS. We observed that ManHQs and GlcHQ7 are highly stable in water and PBS as evidenced by no change in the UV-Vis and ESI-MS spectra after 72 h.

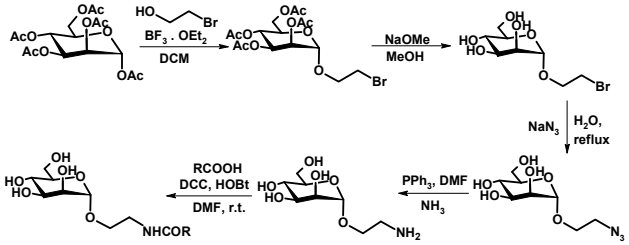

Figure 2. Synthesis scheme of ManHQs. RCOOH is an_8-hydroxyquinoline derivative.

Copper complexes.

The chelating ability of ManHQs was investigated by ESI-MS and UV-vis spectroscopy.

ESI-MS. ESI-MS was used to probe the complex formation between the new HQ derivatives and copper(II) (Figures S21-S22). The main ions observed are reported in Table S1. The ESI-

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MS experiments were performed at pH 7.4 and M/L ratios 1:1 and 1:2 to explore the stoichiometry of metal complexes. In addition to the peak of the ligand, new intense cluster signals indicating the formation of copper complexes (stable isotopes ^{63}Cu and ^{65}Cu in 69:31 ratio, respectively) were observed. In particular, the peak at *m/z* 456.1 can be assigned to the monocharged species $[CuL]^+$ formed by one ligand molecule (L) and one copper atom, since its isotopic distribution is in agreement with the simulated pattern for the molecular composition $C_{18}H_{21}CuN_2O_8$. Furthermore, a singly charged dimeric species resulting from $[CuL_2+H]^+$ (*m/z* 850.1) or $[CuL_2+Na]^+$ (*m/z* 872.1) ions appeared as one of the main peaks in the presence of Cu^{2+}.To a smaller, almost negligible extent, the formation of dinuclear cluster ions, such as $[Cu_2L_2-H]^+$(*m/z* 910.9) was observed at M/L ≥ 1.Overall, CuL^+ and CuL_2 are the primary species over the explored M/L ratios. Finally, it is worth noting that the same species were detected for Man and Glc derivatives. The kind of sugar does not influence copper coordination, as expected.

UV-Vis spectroscopy. UV-Vis spectra of the ligands in water display bands at around 203 nm, in the range 240-282 nm, and in the range 308-352 nm, resulting from π–π* and n–π* transitions (Figure 4). These bands are typically observed in HQ derivatives. Furthermore, the kind of sugar conjugated to HQ does not significantly affect the UV-Vis spectrum of the derivatives as suggested by the similarity between the spectra of GlcHQ7 and ManHQ7.

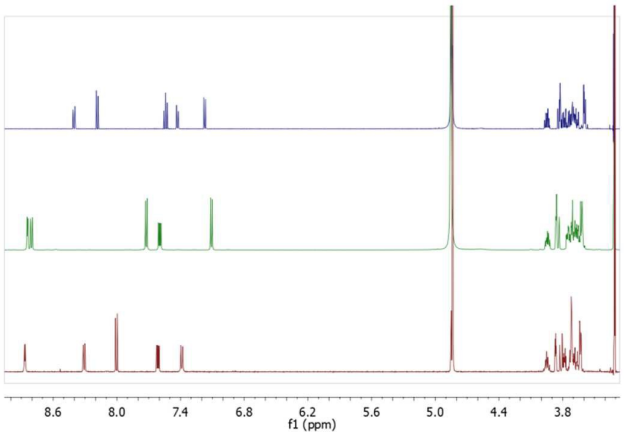

Figure 3. ^1H NMR spectra (500 MHz, CD_3OD) of ManHQ7 (red), ManHQ2 (blue), ManHQ5 (green).

Copper complexes of the mannoconjugates were also characterized by UV-Vis spectroscopy in DMSO/MOPS (pH 7.4, 10 mM) 1:1. MOPS does not bind copper and thus is used for metal complex studies.[27]

The absorption bands of ManHQs are dramatically influenced by the titration with $Cu(ClO_4)_2$.

As for the titration of ManHQ2 with Cu^{2+}, the UV band at 254 nm decreased gradually with concomitant appearance and increase of absorption bands at 269 and 422 nm (Figure 5). These findings have typically been observed for other HQs derivatives when the complexation is associated with the deprotonation of the phenolic group.[28] The molar ratio method suggests the formation of ML and ML_2 species since breaks in the slope of the curve occur at M/L ratios of 1:1 and 1:2. These species were also found for analogous conjugates of HQ2 with trehalose and glucose.[29] On the contrary, the shape of ManHQ7 spectra (Figure S23) shows that, in this compound, the phenolic group is partially deprotonated in the experimental conditions. The deprotonation is completed by the addition of Cu^{2+}, as suggested by the final shape of the spectrum. The molar ratio method was used to determine the stoichiometry of metal-ligand complexes (Figure S25). The plot of the absorbance at 275 nm vs. the equivalents of titrant shows two inflections when about half or one equivalent of Cu^{2+} is added to the ligand solution, indicating the presence of more than one complex species in solution. GlcHQ7 (Figure S24) showed similar behavior to ManHQ7 when titrated with copper(II) ions.

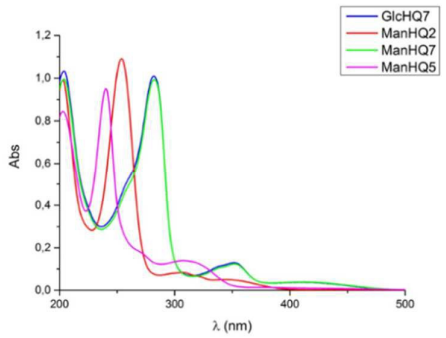

Figure 4. UV-Vis spectra of mannoconjugates (4.0×10^{-5} M) in water.

These compounds can form complex species with Cu^{2+} with a M/L ratio of 1:1 and 1:2, according to ESI-data and other data reported for HQs. The spectra of ManHQ5 were also affected by the addition of Cu^{2+} ions. This compound exhibited similar behavior to ManHQ7 and ManHQ2 and other IOX1 derivatives.[30] Therefore, we can hypothesize the formation of ML and ML_2 complex species with the involvement of N (Py), O (phenolate) for these ligands. As for the ML species of ManHQ2 and ManHQ7, the similarity of the UV spectra of copper complexes to those of other HQ2 and HQ7 derivatives studied by us[29] could suggest the coordination of N of the amido group in the ML species.

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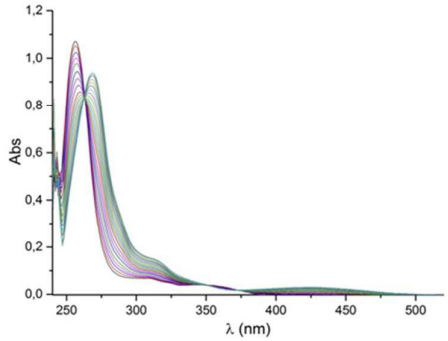

Figure 5. UV-Vis absorption spectra of ManHQ2 upon the addition of Cu^{2+} ions (0-25 equiv).

Biological activity

Antiproliferative activity. We first investigated the antiproliferative effect of the new positional isomers and their parent compounds. The cytotoxicity was evaluated against two cancer cell lines by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. A2780 (ovarian), A549 (lung) cells were treated with the derivatives for 72 h and then the cell viability was evaluated. The IC_{50} values, which represent the concentration of the compound that causes 50% growth inhibition, are reported in Table S2.

The effect of the Cu^{2+} ions on their antiproliferative activity was also investigated since the presence of Cu^{2+} significantly increases the cytotoxicity of HQ and other its derivatives.[2,31] As shown in Table S2, all the compounds showed IC_{50}s > 30 μM (30 μM is the arbitrary limit to define a significant pharmacological antiproliferative activity). The presence of copper ions did not influence the cytotoxicity of the tested compounds except in the case of ManHQ2. These findings suggest that, under these conditions, these derivatives are not cytotoxic. The IC_{50} value of the system Cu^{2+}-ManHQ2 on A2780 cells is 12.7±0.3 μM that is significantly higher than those of HQ2 and the analogous GlcHQ2[31] in the presence of copper(II). Therefore, the kind of sugar in the HQ glycoconjugate could have a role in determining the antiproliferative effect.

Overall, the mannose derivatives are devoid of significant antiproliferative activity. The new derivatives differ from HQ, CQ and other halogenated HQs that are cytotoxic against human cancer cells (IC_{50} values ranging from 0.3 to 8.7 μM).[20]

Antioxidant activity. The overproduction of radical species may cause oxidative damage leading to the development of many diseases such as cancer, neurodegenerative diseases, atherosclerosis, type 2 diabetes mellitus, and cardiovascular diseases.

Since high levels of radical species are toxic to biological organisms, the cells have evolved enzymatic and non-enzymatic antioxidant mechanisms to eliminate ROS and avoid oxidative damage. Both endogenous compounds (e.g., glutathione) and enzymes are designated for the detoxification of ROS. Furthermore, numerous dietary components such as ascorbic acid, carotenoids, and polyphenols are thought to be involved in the antioxidant defense systems.

In epidemiological studies, it has been reported that many antioxidant compounds possess antiatherosclerotic, anticancer, antimutagenic, antibacterial and antiviral activities to greater or lesser extent.[32]

In this contest, the antioxidant activity of the synthesized derivatives was evaluated performing the ABTS radical assay to determine free radical scavenging ability.

The Trolox Equivalent Antioxidant Capacity (TEAC) assay is often used to quantify radicals, which can be scavenged by some antioxidants. It is based on the scavenging of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), converting it into a colorless product. The degree of decoloration induced by antioxidant compounds was measured by UV-vis spectroscopy, and it was compared to that induced by Trolox, a known antioxidant, to obtain the TEAC value.

TEAC values of the HQ glycoconjugates (ManHQ2, ManHQ5, ManHQ7, and GlcHQ7) are shown in Figure 6. TEAC values of the parent mannose (ManEtNH2) and HQ derivatives (HQ2, HQ5, HQ7, and CAHQ2) were also determined for the sake of comparison. Free radical scavenging activity is generally due to the hydroxyl group on aromatic rings. This group is highly reactive and has hydrogen-donating capability.

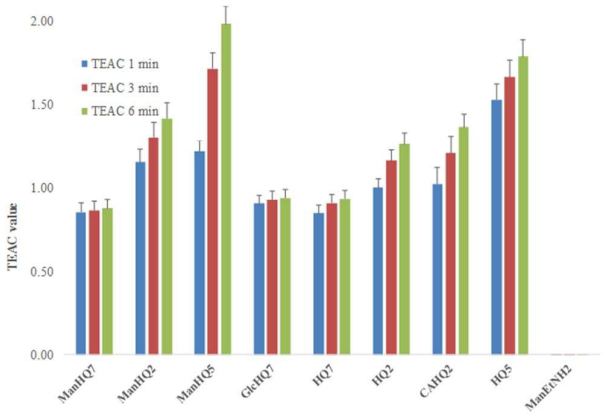

Figure 6. TEAC values at 1, 3, and 6 min for glycoconjugate derivatives. Means are the average of three independent trials, and error bars show standard deviations.

The nature and the position of the substituent may affect the free radical scavenging activity of hydroxyl groups, increasing or decreasing it. Indeed, compounds containing electron-withdrawing, electron-donating, and sterically demanding substituents have a different H-donating ability.

These derivatives possess an excellent ability to scavenge free radical $ABTS^{\bullet+}$ after 1, 3, and 6 min. The antioxidant activity of ManHQs and GlcHQ7 is comparable or superior to that of Trolox. On the other hand, ManEtNH2 does not show a significant antioxidant effect ($TEAC_{6min}$=0.0030 ± 0.0001). The results suggest that the free radical scavenging capacity of ManHQs can be attributed to the hydrogen-donating ability of the hydroxyl groups of the HQ unit, as observed for other HQ

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derivatives. The sugar moiety slightly increases the antioxidant ability of the active group. Nevertheless, the TEAC values are similar to those of analogous compounds with different sugar moiety (ManHQ7, GlcHQ7), indicating that the kind of sugar does not influence the antioxidant activity. ManHQ5 presents the highest capacity to scavenging free radicals (TEAC6min= 1.98 ± 0.09), while ManHQ7 have the lowest scavenging activity ($TEAC_{6min}$ = 0.88 ± 0.05). Because these derivatives are structural isomers, electron-withdrawing and electron-donating effects of the substituent on HQ rings are similar. The differences observed in scavenging activity are probably due to the steric hindrance of the substituent in ortho to the hydroxyl group.

When these compounds were tested in the presence of Cu^{2+}, the scavenging activity changed dramatically. As reported in Figure 7, the TEAC values of the copper complexes of ManHQ5, ManHQ7, and GlcHQ7 are reduced by over 80 % compared to that of free ligand. Unlike the other compounds, TEAC values of ManHQ2 are only slightly affected by the presence of copper ions. The hydroxyl group is involved in the metal binding, resulting in a less available group to react with the radical $ABTS^{\bullet+}$.

Overall, these data suggest that these compounds may have good behavior in protective mechanisms against oxidative stress.

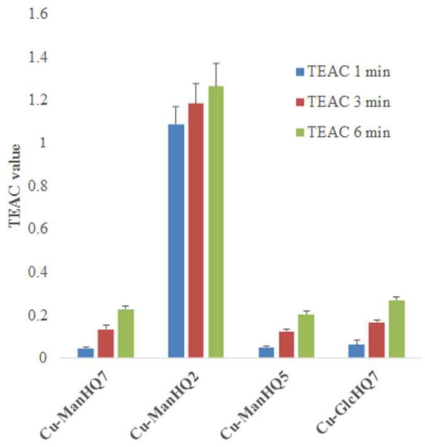

Figure 7. TEAC values at 1, 3, and 6 min for glycoconjugate derivatives in the presence of Cu^{2+}. Means are the average of three independent trials, and error bars show standard deviations.

Antimicrobial activity. Bacterial resistance to conventional antibiotics has increased drastically during the last decades, making difficult the effective treatment of infections.[33,34] This increasing prevalence of multidrug-resistant bacteria urges the development of new antibacterial agents. Some HQ derivatives have been considered as antibacterial drug leads, and some of them, such as nitroxoline and clioquinol, are used as antibiotics.[35,36] The antimicrobial activities of the new HQ mannoconjugates and their parent compounds were evaluated against ten human pathogenic bacteria. Table 1 reports the sensitive strains determined by agar disk diffusion assay.

DMSO was also tested because it was used to solubilize parent HQs; it did not display activity against the tested microorganisms. All compounds were active in a strain-dependent manner except Man and parent HQ2 and HQ7 that did not show antimicrobial activity. The most susceptible strains were *S. sapr ophyticus* UR18, *P. aeruginosa* DSM 939 and *C. albicans* strain 1 that were inhibited by at least four out of nine tested compounds. Overall, disks with active compounds were surrounded by the highest inhibition halo diameters ranging from 7 to 18 mm (Table 1). Among parent HQ compounds, HQ5 was the only compound with antibacterial activity against Gram-positive and Gram-negative strains. Indeed, HQ5 was active against all strains under the assay conditions.

As for the glycoconjugates, ManHQ2 did not display antimicrobial activity. However, HQ derivatives at 2-position of HQ ring are inactive as antimicrobial agents. To further confirm this behavior, we also tested 8-hydroxyquinoline-2-carboxamide (CAHQ2) that also did not display antimicrobial activity.

	C. albicans DSM 1665	*C. albicans strain 1*	*L. monocytogenes LMG 10470*	*E. faecalis OG1RF*	*E. coli ATCC 35401*	*E. coli ATCC 4707*	*P. aeruginosa DSM 939*	*S. aureus LMG 22525*	*H. pylori ATCC43504*	*S. saprophyticus UR18*
Man	0	0	0	0	0	0	8 ± 0	0		
CAHQ2	0	0	0	0	0	0	0	0		
HQ5	13 ± 1	10 ± 0	12 ± 1	7 ± 0	10 ± 0	9 ± 1	7 ± 0	18 ± 1		13 ± 0
ManHQ5	8 ± 1	9 ± 0	0	0	0	0	7 ± 0	8 ± 0		
HQ2	0	0	0	0	0	0	0	0		12 ± 0
ManHQ2	0	0	0	0	0	0	0	0		
GlcHQ7	7 ± 0	10 ± 1	0	9 ± 0	0	0	10 ± 0	0	10 ± 0	14 ± 1
ManHQ7	7 ± 0	8 ± 0	0	0	0	0	11 ± 0	0		
HQ7	0	0	0	0	0	0	0	0		8 ± 0
CHF**	n.d.	n.d.	26 ± 2	23 ± 1	0	27± 2	13 ± 1	28 ± 2	23 ± 1	35 ± 4
CYH†	0	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMSO	0	0	0	0	0	0	0	0		

Table 1. Antimicrobial activity of Man, HQs and the ManHQs determined by agar disk diffusion assay.

*Values represent the average ± standard deviations of inhibitory halo diameters; N = 3; n.d.: not determined; **Disks contain 78.9 µg of ManHQ5, GlcHQ7, ManHQ2 or ManHQ7 , 37.8 µg of HQ5, HQ7 or HQ2, 36.0 µg of Mannose and 30 µg of chloramphenicol (CHF); †Disks contain 10 µg cycloheximide (CYH)

Conversely, ManHQ5, ManHQ7, and GlcHQ7 were active against *P. aeruginosa* and the *C. albicans* strains. Also, ManHQ5 also showed antimicrobial activity against *S. aureus*, while GlcHQ7 was also active against other three pathogenic strains (Table 1). Remarkably, the corresponding parent compounds HQ7, and Man were not active against the same microorganisms. These findings suggest that the glycoconjugation influences the antimicrobial activity of HQ7 derivative. Furthermore, the kind of conjugated sugar seems

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to be crucial against *E. faecalis*, *H. pylori*, and *S. saprophyticus* (GlcHQ7 was active whereas ManHQ7 was inactive); GlcHQ7 and ManHQ7 showed a comparable activity in the remaining strains. Further studies are needed to clarify the precise mechanism of action of the HQ glycoconjugates as antimicrobial agents.

Based on these results, the most active compounds were selected to calculate the minimal inhibitory concentration (MIC) values towards the most susceptible strains. The results (Table 2) show that MIC value for HQ5 is 1.25 mM for *C. albicans* strain 1, *E. faecalis* OG1RF and *S. saprophyticus* UR18. Moreover, the same compound was active when assayed at higher concentrations against the remaining strains. GlcHQ7 shows the lowest MIC value of 1.25 mM only for OG1RF and UR18 strains. Higher MIC values were found for ManHQ5 (Table 2).

The antimicrobial activity of the compounds was negatively affected by copper complexation. Indeed, no inhibition halos were registered when copper complexes of the most glycoconjugated HQs were tested. In addition, a $CuCl_2$ solution did not show any antimicrobial activity at the same concentrations. However, the copper complexes of HQ2 or HQ7 showed antimicrobial activity against *S. aureus* and *P. aeruginosa* at 10 mM, while ligand parent compounds were not active at the same concentration. Similar results were also registered for GlcHQ7 and ManHQ7 against *S. aureus* in the presence of copper(II), even though this strain was inhibited by 10 mM of $CuCl_2$.

Table 2. Minimal inhibitory concentrations (MIC, mM) of HQ derivatives against selected bacterial strains involved in urinary tract infections *.

	C. albicans strain 1	*E. faecalis OG1RF*	*P. aeruginosa DSM 939*	*S. aureus LMG 22525*	*S. saprophyticus UR18*
HQ5	1.25	1.25	5	2.5	1.25
ManHQ5	5	n.d.	10	2.5	n.d.
HQ2	n.d.	n.d.	n.d.	n.d.	5
GlcHQ7	10	1.25	10	n.d.	1.25
ManHQ7	10	n.d.	10	n.d.	n.d.
HQ7	n.d.	n.d.	n.d.	n.d.	10

Conclusions

We synthesized and characterized three new mannoconjugates of 8-hydroxyquinolines (HQs) and explored their biological activity. The new mannoconjugates are the first examples of mannose-HQ derivatives. They can complex copper(II), and form ML and ML_2 and display several biological activities. All compounds are potent antioxidants and this capacity is not affected by the kind of the conjugated sugar, but instead, it depends on the position of the substituent on the quinoline ring. Moreover, the conjugates show significant antibacterial activity against *P. aeruginosa* and *S. aureus*

Unlike other HQ derivatives, the HQ-mannose conjugates are not toxic on human cancer cell lines. Therefore, they could have better biocompatibility and be potentially exploited as antibiotics.

Overall, these results suggest that the HQ mannoconjugates deserve further investigation to better explore their potential as biologically active molecules.

Experimental

Chemicals. Commercially available reagents were used directly unless otherwise noted. 8-Hydroxyquinoline-7-carboxylic acid (HQ7), 8-hydroxyquinoline-2-carboxylic acid (HQ2), 8-hydroxyquinoline-5-carboxylic acid (HQ5), 8-hydroxyquinoline-2-carboxamide (CAHQ2), and mannose pentaacetate were obtained from Sigma-Aldrich or Alfa-Aesar.

2'-aminoethyl-β-D-glucopyranoside was synthesized as reported elsewhere.[29]

Thin Layer Chromatography (TLC) was carried out on silica gel plates (Merck 60-F254). Carbohydrates derivatives were detected on TLC by UV and an anisaldehyde test.

Cu^{2+} stock solutions were prepared by dissolving the corresponding salt in water and titrating the resulting solution with standardized EDTA using murexide.

NMR spectroscopy. ^{1}H 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. The NMR spectra were obtained by using standard pulse programs from Varian library. The 2D experiments (COSY, TOCSY, gHSQCAD, gHMBC) were acquired using 1K data points, 256 increments and a relaxation delay of 1.2 s. The spectra were referred to the solvent signal.

UV-visible spectroscopy. UV-vis spectra were recorded using an Agilent 8452A diode array spectrophotometer. UV/Vis direct titrations were carried out at 25 °C in water/DMSO 50/50 v: v at pH 7.4 (MOPS, 10 mM) using the same spectrophotometer interfaced with a Hamilton burette.

Increasing amounts of Cu^{2+} ion (0-2.5 equivalents) were added with the precision burette into the cell containing a known volume (2 mL) of the ligand solution (4.0×10^{-5} M).

ESI-MS. ESI-MS measurements were performed by a Finnigan LCQ DECA XP PLUS ion trap spectrometer that was equipped with an orthogonal ESI source (Thermo Electron Corporation, USA) operated in positive and negative-ion mode. Cu^{2+} complexes were obtained by adding a solution of $Cu(ClO_4)_2$ to the ligand solution. Different metal-ligand ratios were investigated. Sample solutions were injected into the ion source at a flow rate of 5 µL/ min. The operating conditions, such as capillary exit skimmer voltage and heated capillary temperature were varied to optimize the signal response. Mass spectra were recorded from *m/z* = 100 to 2000. Xcalibur software was used for the elaboration of mass spectra. The

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assignments of the peaks in ESI-MS spectra were made by comparing the observed isotopic patterns with the corresponding predicted isotopic profiles. Note that all listed m/z values refer to the light isotopes (^{1}H, ^{12}C, ^{14}N, and ^{63}Cu).

Trolox equivalent antioxidant capacity assay. The antioxidant ability of HQ derivatives was determined by 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical cation decolorization assay using Trolox as reported by us.[37] All determinations were carried out at least four times to obtain consistent data with statistical error.

Antiproliferative activity assay (MTT assay). The antiproliferative activity of the derivatives was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).[38]

Briefly, the human cell lines A2780 (ovary, adenocarcinoma), A549 (lung, carcinoma) cells were used, and a set of them was treated with $CuCl_2$ (20 μM). The compounds (solubilized in DMSO and then diluted in FCS, fetal calf serum) were added after 6–8 h. The 50% inhibiting concentrations (IC_{50}s) were calculated by the analysis of single concentration-response curves. Each experiment was repeated 4–12 times.

Antimicrobial activity assay. The compounds were assayed for antimicrobial activity against several microbial strains stored in the microbial collection of Institute of Sciences of Food Production (CNR-ISPA), Bari, Italy; these strains belong to microbial species mainly involved in urinary tract infections.[39] The strains were screened using agar disk diffusion assay method according to EUCAST guidelines.[40] Briefly, a sterile cotton swab soaked with 0.5 McFarland solution of each strain was spread on Petri dishes with Muller Hinton agar (Tryptone, 17.5 g/L; Beef extract, 2 g/L; Soluble starch, 1.5 g/L and Agar, 17 g/L. Then, cellulose disks (Oxoid) soaked with 20 μl of 10 mM solution (in DMSO) of each HQ-derived compound were applied in triplicate to the surface of inoculated agar plates. Disks with the same volume of 10 mM $CuCl_2$–HQs and $CuCl_2$–ManHQs (1:1) mixture were also applied to seeded Petri dishes. Blank disks soaked with DMSO and a DMSO solution of 10 mM $CuCl_2$ were used as controls. Moreover, disks containing 30 μg of chloramphenicol (CHF) or 10 μg cycloheximide (CYH) were included. Plates were incubated at 37 °C for 16-20 h. Diameters (nearest millimeter) of inhibition zones around the assayed disks were measured with a caliper from the back of plate held above a dark background.

The systems with the highest activity against most target bacteria were subsequently evaluated for the minimal inhibitory concentration (MIC) by broth microdilution method with 96-well microtiter plates.[41] Briefly, 1 mL of a culture of the assayed strains corresponding to 0.5 McFarland optical density were centrifuged, and the bacterial pellets were suspended with 1 mL of sterile 0.85% NaCl. Finally, 20 μl of 1:100 dilution of each bacterial suspension (corresponding to ca. 1×10^6 CFU/ml) were added to 180 μL of Mueller-Hinton broth containing selected HQ-derived compounds ranging from 0, 1.25, 2.5, 5.0 and 10.0 mM. DMSO was included as a control. The plates were incubated at 37 °C for 24 h. Bacterial growth kinetics were monitored at 600 nm using Varioskan Flash (Thermo Fisher Scientific, Finland). The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism.

Synthesis of 2'-bromoethyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (ManAc-Br). $BF_3 \cdot OEt_2$ (1.8 mL) was added dropwise to a solution of 1.00 g of acetylated mannose (2.6 mmol) and 0.20 mL of 2-bromoethanol (2.8 mmol) in 50 mL of dry CH_2Cl_2 at 0 °C, over a period of 30 min. The progress of the reaction was followed by TLC (3:2 ethyl acetate/ cyclohexane). After 12 h of stirring at room temperature, the reaction mixture was slowly added to ice-cooled water (50 mL). The aqueous phase was extracted with 20 mL of CH_2Cl_2. The combined organic phases were washed with H_2O and dried (Na_2SO_4). The solvent was evaporated under vacuum at room temperature, and the crude product was purified by flash chromatography on silica (3:2 ethyl acetate/hexane), to give 0.87 g (1.9 mmol) of the product. Yield: 75 %. TLC: Rf = 0.69 (3:2 ethyl acetate/hexane).

Synthesis of 2'-bromoethyl-α-D-mannopyranoside (ManEt-Br). ManAc-Br was deacetylated in MeOH with NaOMe, under stirring at r.t.

^{1}H NMR (500 MHz, D_2O) δ (ppm): 4.84 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1), 3.95 (ddd, J = 11.6, 6.3, 5.0 Hz, 1H, β CH_2O), 3.89 (dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, 1H, H-2), 3.86 – 3.76 (m, 2H, β' CH_2O and H-6), 3.74 (dd, $J_{3,2}$= 3.5 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 3.69 – 3.63 (m, 2H, H-5 and H-6'), 3.60 – 3.50 (m, 3H, H-4 α and α' CH_2Br).

^{13}C NMR (125 MHz, D_2O) δ (ppm): 99.8 (C-1), 73.0 (C-5), 70.5 (C-3), 69.9 (C-2), 67.6 (β CH_2O), 66.6 (C-4), 60.9 (C-6), 31.3 (CH_2Br).

Synthesis of 2'-azidoethyl-α-D-mannopyranoside (ManEt-N_3). NaN_3 (2.48 g, 38.1 mmol) was added to a solution of ManOEt-Br (400 mg, 1.39 mmol) in water (20 mL). The reaction mixture was heated under reflux for 20 h and dried under vacuum. The residue was dissolved in water and purified by column chromatography (RP-18) to afford 2-azidoethyl mannoside.

^{1}H NMR (500 MHz, D_2O) δ (ppm): 4.87 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1), 3.93 (dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, 1H, H-2), 3.88 (m, 1H, β CH_2O), 3.85 (dd, $J_{6,6'}$ = 12.4 Hz, $J_{6,5}$ = 2.0 Hz, 1H, H-6), 3.79 (dd, $J_{3,4}$ = 9.3 Hz, $J_{3,2}$ = 3.5 Hz, 1H, H-3), 3.76 – 3.65 (m, 2H, H-6', β' CH_2O), 3.65 – 3.59 (m, 2H, H-4 and H-5), 3.48 (qdd, $J_{\alpha,\alpha'}$ = 13.6 Hz, 6.6, 3.2 Hz, 2H, α and α' CH_2N).

^{13}C NMR (125 MHz, D_2O) δ (ppm): 99.9 (C-1), 72.9 (C-5), 70.1 (C-2 and C-3), 66.5 (C-4 and CH_2O), 61.1 (C-6), 50.2 (CH_2N_3).

Synthesis of 2'-aminoethyl-α-D-mannopyranoside (ManEtNH_2). 2-Azidoethyl-α-D-mannopyranoside (350 mg, 1.40 mmol) was dissolved in DMF (15 mL) with PPh_3, NH_3 (35% solution) was added, and the reaction mixture was stirred until the reduction was

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complete (monitored by TLC). Then, it was dried under low pressure. The residue was dissolved in water and filtered, washed with ethyl acetate-ethanol (1: 1) and the filtrate was concentrated under low pressure. Purification by cation exchange chromatography on a CM-25 Sephadex column using a linear gradient of NH_4HCO_3 (0→0.25 M) gave $ManEtNH_2$ (250 mg, 80%) as a colorless syrup.

^{1}H NMR (500 MHz, CD_3OD) δ 4.79 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1), 3.84 (m, 2H, H-2 and H-6), 3.79 (ddd, $J_{\beta,\beta'}$ = 10.4 Hz, $J_{\beta,\alpha}$ = 6.2, $J_{\beta,\alpha'}$ =4.6 Hz, 1H, β CH_2O), 3.75 – 3.69 (m, 2H, H-6' and H-3), 3.62 (t, J = 9.5 Hz, 1H, H-4), 3.57 – 3.52 (m, 1H, H-5), 3.49 (ddd, $J_{\beta',\beta}$ = 10.4 Hz, $J_{\beta',\alpha}$ = 6.2, $J_{\beta',\alpha'}$ =4.6 Hz, 1H, β' CH_2O), 2.85 (dt, $J_{\alpha,\beta}$ = 6.2 Hz, $J_{\alpha',\beta'}$= 4.2 Hz, 2H, CH_2N).

^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 100.3 (C-1), 73.2 (C-5), 71.0 (C-3), 70.6 (C-2), 68.5 (CH_2O), 67.1 (C-4), 61.6 (C-6), 40.7 (CH_2N).

Synthesis of 2'-[(8-hydroxyquinolyl)-7-carboxyl]aminoethyl]-α-D-mannopyranoside (ManHQ7). 1-hydroxybenzotriazole monohydrate (HOBt, 30.3 mg, 0.22 mmol) and DCC (46.2 mg, 0.22 mmol) were added to a suspension of HQ7 (42.4 mg, 0.22 mmol) in dry DMF (10 mL). After 30 min, $ManEtNH_2$ (50.0 mg, 0.22 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. The crude mixture was dissolved in water, filtered off and purified by anion exchange chromatography on DEAE–Sephadex A-25 using a linear gradient of NH_4HCO_3 (0→0.4 M). Yield: 75 %. TLC: Rf = 0.51 (PrOH/AcOEt/H_2O/NH_3 4:3:2:1).

ESI-MS (+, H_2O) : *m/z* = 395.1 $[M+H]^+$, 417.1 $[M+Na]^+$, 433.1 $[M+K]^+$, 810.9 $[2M+Na]^+$.

ESI-MS (-, H_2O) : *m/z* =393.0 $[M-H]^-$.

^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 8.87 (dd, $J_{2,3}$ = 4.2, $J_{2,4}$ = 1.6 Hz, 1H, H-2 of HQ), 8.32 (dd, $J_{4,3}$ = 8.3, $J_{4,2}$ = 1.6 Hz, 1H, H-4 of HQ), 8.01 (d, $J_{6,5}$ = 8.8 Hz, 1H, H-6 of HQ), 7.62 (dd, $J_{3,4}$ = 8.3, $J_{3,2}$ =4.2 Hz, 1H, H-3 of HQ), 7.39 (d, $J_{5,6}$ = 8.8 Hz, 1H, H-5 of HQ), 4.86 (H-1 of Man, overlapped to H_2O), 3.96 (qd, *J* = 8.6, 7.5, 6.0 Hz, 1H, β CH_2O), 3.87 (dd, $J_{1,2}$ 1.7, $J_{2,3}$ Hz = 3.4, 1H, H-2 of Man), 3.85 – 3.76 (m, 2H, H-3 and H-6 of Man), 3.76 – 3.60 (m, 6H, H-4, H-5, H-6' of Man, β' CH_2O, α and α' CH_2N).

^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 168.5 (C=O), 156.0 (C-8 of HQ), 148.7 (C-2 of HQ), 139.1 (C-9 of HQ), 136.4 (C-4 of HQ), 130.9 (C-10 of HQ), 125.4 (C-6 of HQ), 123.2 (C-3 of HQ), 116.6 (C-5 of HQ), 112.6 (C-7 of HQ), 100.3 (C-1 of Man), 73.4 (C-5 of Man), 71.1 (C-3 of Man), 70.7 (C-2 of Man), 67.3 (C-4 of Man), 65.7 (CH_2O), 61.3 (C-6 of Man), 39.3 (CH_2N).

UV-vis (H_2O): λ/nm (ε/$mol^{-1}cm^{-1}$) 204 (24472), 282 (24398), 352 (3047), 412 (958).

Synthesis of 2'-[(8-hydroxyquinolyl)-2-carboxyl]aminoethyl]-α-D-mannopyranoside (ManHQ2). HOBt (42.4 mg, 0.22 mmol) and DCC (46.2 mg, 0.22 mmol) were added to a suspension of HQ2 (42.4 mg, 0.22 mmol) in dry DMF (10 mL)). After 0.5 h, $ManEtNH_2$ (50.0 mg, 0.22 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. The crude product was purified by filtration, followed by flash chromatography on a C18 reversed-phase column (0 → 100% MeOH). Yield: 70 %; TLC: Rf = 0.61 (PrOH/AcOEt/H_2O/NH_3 4:3:2:1).

ESI-MS (+, H_2O) : *m/z* = 395.0 $[M+H]^+$, 417.1 $[M+Na]^+$, 810.8 $[2M+Na]^+$.

ESI-MS (-, H_2O) : *m/z* =393.1 $[M-H]^-$.

^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 8.42 (d, $J_{4,3}$ = 8.6 Hz, 1H, H-4 of HQ), 8.19 (d, $J_{3,4}$= 8.6 Hz, 1H, H-3 of HQ), 7.55 (dd, $J_{6,5}$= 8.3 Hz, $J_{6,7}$= 7.7 Hz, 1H, H-6 of HQ), 7.44 (dd, $J_{5,6}$ = 8.3 Hz, $J_{5,7}$ = 1.2 Hz, 1H, H-5 of HQ), 7.18 (dd, $J_{7,6}$= 7.7 Hz, $J_{7,5}$= 1.2 Hz, 1H, H-7 of HQ), 4.84 (H-1 of Man, overlapped to H_2O signal), 3.95 (m, 1H, β CH_2O), 3.85 – 3.64 (m, 7H, H-2, H-3, Hs-6 of Man, β' CH_2O, α and α' CH_2N), 3.63 – 3.57 (m, 2H, H-4 and H-5 of Man).

^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 165.3 (C=O), 153.6 (C-8 of HQ), 147.1 (C-2 of HQ), 137.4 (C-4 and C-9 of HQ), 130.1 (C-10 of HQ), 129.2 (C-6 of HQ), 118.5 (C-3 of HQ), 117.5 (C-5 of HQ), 111.3 (C-7 of HQ), 100.5 (C-1 of Man), 73.4 (C-5 of Man), 70.9 (C-2 and C-3 of Man), 67.3 (C-4 of Man), 66.0 (CH_2O), 61.5 (C-6 of Man), 39.1 (CH_2N).

UV-vis (H_2O): λ/nm (ε/$mol^{-1}cm^{-1}$) 203 (25000), 254 (27525), 309 (2096), 343 (1288).

Synthesis of 2'-[(8-hydroxyquinolyl)-5-carboxyl]aminoethyl]-α-D-mannopyranoside (ManHQ5)

The synthesis was carried out as for ManHQ2, starting from HQ5. ManHQ5 was isolated by flash chromatography on a C18 reversed-phase column (0 → 100% MeOH). Yield: 65%. TLC: Rf = 0.28 (PrOH/AcOEt/H_2O/NH_3 4:3:2:1).

ESI-MS (+, H_2O) : *m/z* = 395.2 $[M+H]^+$, 417.1 $[M+Na]^+$, 433.0 $[M+K]^+$, 810.9 $[2M+Na]^+$.

ESI-MS (-, H_2O) : *m/z* =393.1 $[M-H]^-$.

^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 8.84 (dd, $J_{2,3}$ = 4.2, $J_{2,4}$ = 1.5 Hz, 1H, H-2 of HQ), 8.80 (dd, $J_{4,3}$ = 8.6, $J_{4,2}$ = 1.5 Hz, 1H, H-4 of HQ), 7.72 (d, $J_{6,7}$ = 7.9 Hz, 1H, H-6 of HQ), 7.60 (dd, $J_{3,4}$ = 8.6, $J_{3,2}$ = 4.2 Hz, 1H, H-3 of HQ), 7.11 (d, $J_{7,6}$ = 7.9 Hz, 1H, H-7 of HQ), 4.85 (bs, 1H, H-1 of Man), 3.94 (ddd, *J* = 11.2, 6.6, 4.6 Hz, 1H, β CH_2O), 3.89 – 3.81 (m, 2H, H-2 and H-6 of Man), 3.80 – 3.56 (m, 7H, H-3, H-4, H-5 and H-6' of Man, β' CH_2O, α and α' of CH_2N).

^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 169.9 (C=O), 155.4 (C-8 of HQ), 148.2 (C-2 of HQ), 138.3 (C- C-9 of HQ), 134.2 (C-4 of HQ), 128.0 (C-6 of HQ), 126.6 (C-10 of HQ), 123.8 (C-5 of HQ), 122.4 (C-3 of HQ), 109.2 (C-7 of HQ), 100.5 (C-1 of Man), 73.5 (C-5 of Man), 71.3 (C-3

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of Man), 70.8 (C-2 of Man), 67.4 (C-4 of Man), 65.9 (CH_2O), 61.7 (C-6 of Man), 39.5 (CH_2N).

UV-vis (H_2O): λ/nm (ε/$mol^{-1}cm^{-1}$) 203 (21259), 240 (23980), 308 (3501).

Synthesis of 2'-[(8-hydroxyquinolyl)-7-carboxyl]aminoethyl]-β-D-glucopyranoside (GlcHQ7). The synthesis was carried out as for ManHQ7, starting from 2'-aminoethyl-β-D-glucopyranoside.[29] The crude product was purified by filtration, followed by flash chromatography on a C18 reversed-phase column (0 → 100% MeOH). Yield: 78 %. TLC: Rf =0.52 (MeOH).

ESI-MS (+, H_2O) : *m/z* = 395.1 $[M+H]^+$, 417.0 $[M+Na]^+$, 433.1 $[M+K]^+$, 810.9 $[2M+Na]^+$.

ESI-MS (-, H_2O) : *m/z* =393.1 $[M-H]^-$.

^{1}H NMR (500 MHz, D_2O) δ (ppm): 8.64 (m, 1H, H-2), 8.49 (m, 1H, H-4), 7.78 (m, 1H, H-6), 7.67 (m, 1H, H-3), 7.00 (m, 1H, H-5), 4.48 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1 of Glc), 4.07 (ddd, 1H, $J_{\beta,\beta'}$ = 10.7 Hz, $J_{\beta,\alpha}$ = 6.4 Hz, $J_{\beta,\alpha'}$ = 4.2 Hz, β CH_2O), 3.87 (m, 1H, β' CH_2O), 3.83 (dd, 1H, $J_{6,6'}$= 12.4 Hz, $J_{6,5}$ =2.2 Hz, H-6), 3.63 (m, 3H, α CH_2N, H-6'), 3.47-3.26 (m, 4H, H-2, H-3, H-4, H-5 of Glc).

^{13}C NMR (125 MHz, D_2O) *δ* (ppm): 169.2 (C=O), 159.3 (C-8 of HQ), 142.9 (C-4 of HQ), 142.7 (C-2 of HQ), 135.7 (C-9 of HQ), 131.9 (C-10 of HQ), 128.3 (C-6 of HQ), 122.8 (C-3 of HQ), 114.8 (C-7 of HQ), 111.8 (C-5 of HQ), 102.5 (C-1 of Glc), 75.9 (C-5 of Glc), 75.6 (C-3 of Glc), 73.0 (C-2 of Glc), 69.7 (C-4 of Glc), 68.5 (CH_2O), 60.7 (C-6 of Glc), 39.2 (CH_2N).

UV-vis (H_2O): λ/nm (ε/$mol^{-1}cm^{-1}$) 204 (25432) 282 (24938) 352 (3210) 411 (938).

Conflicts of interest

There are no conflicts to declare.

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