First examples of H₂S-releasing glycoconjugates: stereoselective synthesis and anticancer activities

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ABSTRACT: H₂S-donors are currently emerging as promising therapeutic agents in a wide variety of pathologies, including tumors. Cancer cells are characterized by an enhanced uptake of sugars, such as glucose. Therefore, novel glycoconjugated H₂S-donors were synthesized, so that high concentrations of H₂S can be selectively achieved therein. Dithiolethione- or isothiocyanate-portions were selected for their well-known H₂S-releasing properties in the presence of biological substrates. A synthetic procedure employing trichloroacetimidate glycosyl donors was applied to produce, in a stereoselective fashion, C₁-glycoconjugates, whereas C₆-glycoconjugates were obtained by a Mitsunobu-based transformation. The resulting molecules were then tested for their anti-cancer effects on human pancreas adenocarcinoma ascites metastasis cell line AsPC-1. The most potent inhibitors of cell viability (**6aβ** and **7b**) proved to release H₂S inside the AsPC-1 cells and to alter the basal cell cycle.

INTRODUCTION

H₂S gasotransmitter is nowadays widely acknowledged to play key roles in pathophysiological processes involving cardiovascular, neurodegenerative, inflammatory and neoplastic diseases.[1] As a matter of fact, over the past few years, several novel H₂S-donor molecules with perspective therapeutic activities have been designed and synthesized, due to their potential roles in treating several pathologies, including inflammation, cardiovascular diseases and cancer.[2,3]

As far as tumors are concerned, the controversial mechanism of action of H₂S on tumor biology is still poorly understood. In fact, low or high H₂S cellular concentrations can oppositely influence tumor growth. In fact, endogenous H₂S can induce angiogenesis and regulate mitochondrial bioenergetics, so that cell cycle progression is accelerated, and apoptosis is inhibited (pro-cancer effect). On the contrary, high concentrations of exogenous H₂S produce a pro-apoptotic effect by inducing cell cycle arrest and, therefore, suppressing tumor growth (anti-cancer effect).[4]

The purpose of this study is to develop H₂S-donor molecules with an enhanced uptake in cancer cells, so that they can deliver high amounts of hydrogen sulfide in the cytoplasm and produce an anti-cancer effect. In order to do so, we decided to exploit the well-established propensity of cancer cells to take up large amounts of sugars, especially glucose and fructose, by means of an overexpression of membrane glucose transporters (GLUTs).[5] In the past, we successfully targeted LDH-inhibitors into cancer cells by including their molecules in glucose-[6] or other non-glucose glycoconjugates,[7] since the sugar portion can be recognized by GLUTs and, therefore, efficiently taken up by cancer cells. Therefore, we designed new hybrid molecules containing two functional portions that consist in a glucose-mimic unit coupled to well-known H₂S-donor functionalities. Among the numerous H₂S-donor portions included in H₂S-donating compounds reported in the literature, we decided to utilize either dithiolethione derivatives (ADT analogues) or aryl/alkyl-isothiocyanate moieties, due to their proven ability to release hydrogen sulfide in cells and to the synthetic accessibilities to chimeric molecules containing them.[8-10] As for the sugar-mimicking portion, glucose, galactose, and mannose were reported to be good substrates of the sugar transporters in cancer cells.[11] Therefore, we designed chimeric compounds that where prepared by means of glyco-conjugation reactions at anomeric or at C6 positions of the sugar analogue with a suitably derivatized H₂S-releasing unit. The resulting molecules were then tested for their anti-proliferative effects on human pancreas

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adenocarcinoma ascites metastasis cell line AsPC-1. Their capacity to inhibit cell viability was assessed as the primary endpoint and, for the most active compounds, the intracellular H₂S release and the alteration of AsPC-1 basal cell cycle were measured.

RESULTS AND DISCUSSION

Chemistry. We designed and synthesized H₂S-releasing glycoconjugates, characterized by a glycosidic portion, such as (+)-D-glucopyranose, (+)-D-galactopyranose, (+)-D-mannopyranose, or 1-methyl-α-D-glucopyranose, linked to an H₂S-releasing moiety via an acetal- or ether-bond (Figure 1). ADT-OH (**1**) and isothiocyanate portions (**2** and **3**) were selected among the large series of H₂S-releasing compounds, since they have been widely demonstrated to efficiently release H₂S under physiological conditions.[9] These active units were, therefore, conjugated with fully *O*-protected glycosyl derivatives, in order to improve their drug uptake by cancer cells. An additional advantage deriving from the glyco-conjugation of these H₂S-releasing units is the improvement of their aqueous solubility.
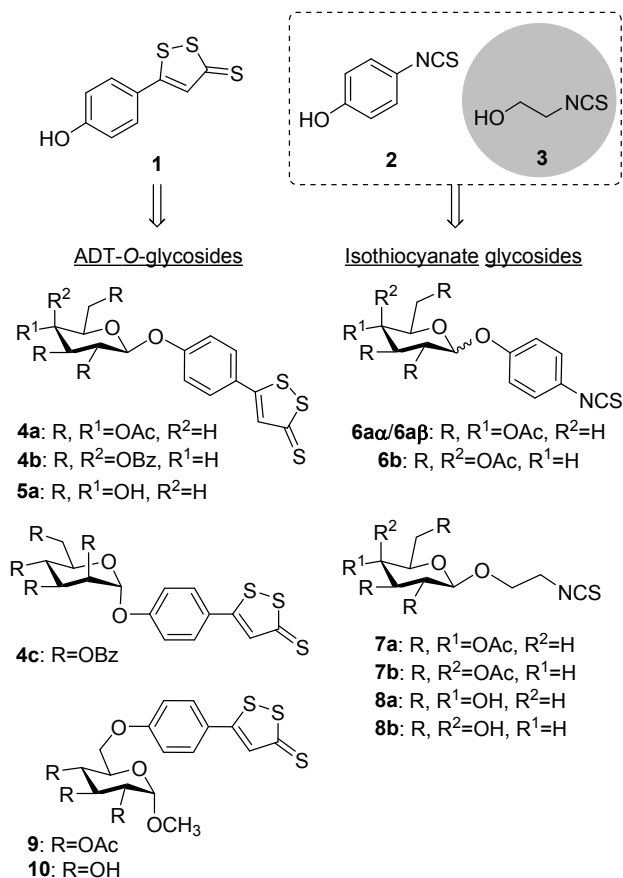

4a: R, R¹=OAc, R²=H
4b: R, R²=OBz, R¹=H
5a: R, R¹=OH, R²=H

4c: R=OBz

6aα/6aβ: R, R¹=OAc, R²=H
6b: R, R²=OAc, R¹=H

7a: R, R¹=OAc, R²=H
7b: R, R²=OAc, R¹=H
8a: R, R¹=OH, R²=H
8b: R, R²=OH, R¹=H

9: R=OAc
10: R=OH

Figure 1. H₂S-releasing moieties and related glycoconjugates.

Synthesis of glycosyl acceptors. Hydroxyl glycosyl acceptor **1** (ADT-OH) was synthesized from its methyl analogue, the anethol-trithione (ADT-OCH₃), as previously described by Curphey *et al.*[12] On the other hand, the synthesis of the aryl and alkyl isothiocyanate

compounds **2** and **3** (Figure 1) was initially planned by using amino alcohol precursors **11** and **12** (Scheme 1) as starting material, and by proceeding with a suitable method for converting both aromatic and alkyl amine into isothiocyanate moieties.[13] Therefore, amino alcohols **11** and **12** were treated with a base, such as triethylamine (Et₃N), and an excess of carbon disulfide at 0 °C. Then, hydrogen peroxide (H₂O₂) was added as a dehydrosulfurization agent at the same temperature. This procedure allowed the synthesis of aryl derivative **2** with a 65% yield. The introduction of the isothiocyanate group was confirmed by ¹H-/¹³C-NMR and FT-IR analysis (Figure S1 in the Supporting Information), showing a distinctive ¹³C-NMR peak at 157 ppm and a broad IR band at 2110 cm⁻¹. On the other hand, the same procedure applied to alkyl amino alcohol **12** resulted instead in the formation of an undesired 2-thiooxazolidone derivative **13** (Scheme 1), as demonstrated by NMR analysis and IR spectra: no signals around 155 ppm in the ¹³C NMR spectra and no IR-peaks associated to the NCS group asymmetric stretching between 2000 and 2100 cm⁻¹ were observed. Indeed, the 2-hydroxy-isothiocyanate **3** totally cyclized via an intramolecular attack (Scheme 1A) of the free OH group to the electrophilic center of the isothiocyanate portion.[14,15] Due to the high reactivity of the flexible alkylisothiocyanate portion, a different synthetic strategy via a stable and small glycosyl acceptor, **3a**, was implemented starting from compound **12**, and the generation of the NCS portion was postponed to a later stage of the synthetic pathway (see Scheme 3). The selective conversion of nucleophilic NH₂ residue into *N*-Boc was performed under anhydrous conditions in the presence of di-*tert*-butyl dicarbonate (Boc₂O) and Et₃N (Scheme 1B).
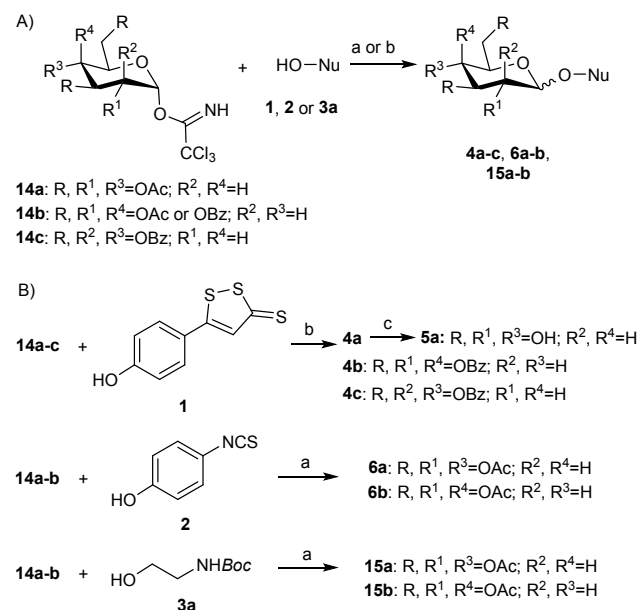
Scheme 1: Synthesis of compound 2, cyclization of the alkyl derivative 3 (A), and synthesis of the new glycosyl acceptor 3a (B)[a]
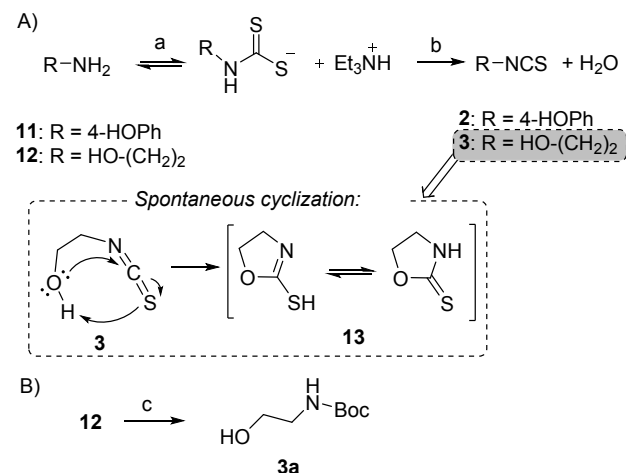
[a] Reagents and Conditions: (a) CS₂, Et₃N, THF, 0 °C; (b) H₂O₂, 0 °C; (c) Boc₂O, Et₃N.

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1-*O*-Glycoconjugated H$_2$S donors. A general glycosylation method using the *O*-glycosyl trichloroacetimidates (TCA) **14** as the classical glycosyl donor and the hydroxylated nucleophiles, such as compounds **1**, **2**, and **3a**, as the glycosyl acceptors was employed for the synthesis of the series of 1-substituted glycosyl derivatives **4**, **6**, and **15** (Scheme 2).[16,17]

Scheme 2. General glycosylation reaction with TMSOTf (Condition a), or with Sc(OTf)$_3$ (Condition b) as catalyst (A); glycosylation of compound 1, 2 and 3a (HO-Nu) and deacetylation step of compound 4a (B)[a]

14a: R, R^1, R^3=OAc; R^2, R^4=H
14b: R, R^1, R^4=OAc or OBz; R^2, R^3=H
14c: R, R^2, R^3=OBz; R^1, R^4=H

4a-c, 6a-b, 15a-b

5a: R, R^1, R^3=OH; R^2, R^4=H
4b: R, R^1, R^4=OBz; R^2, R^3=H
4c: R, R^2, R^3=OBz; R^1, R^4=H

6a: R, R^1, R^3=OAc; R^2, R^4=H
6b: R, R^1, R^4=OAc; R^2, R^3=H

15a: R, R^1, R^3=OAc; R^2, R^4=H
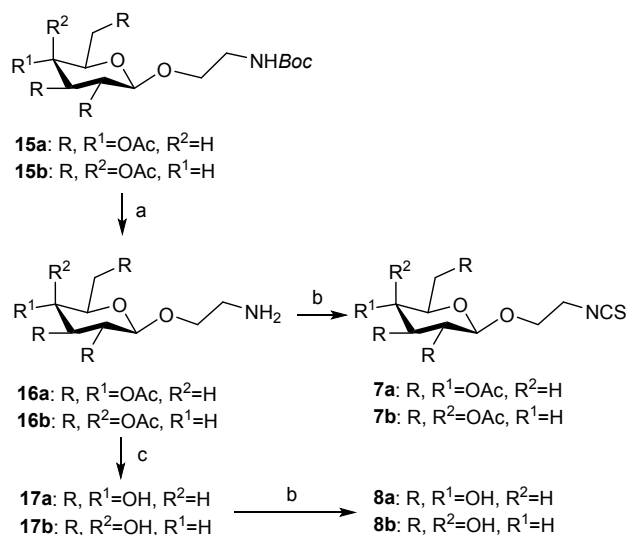**15b**: R, R^1, R^4=OAc; R^2, R^3=H

[a] Reagents and Conditions: (a) TMSOTf, AW300, dry DCM (for compounds **4c**, **6a**, **6b**, and **15a**, **15b**); (b) Sc(OTf)$_3$, AW300, dry DCM (for compounds **4a** and **4b**); (c) 0.33 M MeONa, MeOH.

Three α-trichloroacetimidate (α -TCA) derivatives **14a**, **14b**, and **14c**, opportunely protected with acyl or benzoyl functionalities at C2, C3, C4, and C6 positions, were obtained starting from commercially available (+)-D-glucopyranose, (+)-D-galactopyranose, or (+)-D-mannopyranose.[18, 19] Treatment of these intermediates with glycosyl acceptors **1**, **2**, or **3a** (HO-Nu, Scheme 2), in dry CH$_2$Cl$_2$ and in the presence of AW300 molecular sieves (4 Å) and a suitable Lewis acid afforded anethole trithione derivatives (**4a**, **4b** and **4c**), the fully-*O*-acyl-glycopyranoside aryl isothiocyanates (**6a-α**, **6a-β**, and **6b**), and the alkyl-*N*-Boc gluco- and galacto- pyranosides (**15a** and **15b**). Specifically, the glycosylation process efficiently proceeded by using TMSOTf as the catalyst in the case of glycosyl acceptors **2** and **3a** (conditions *a*, Scheme 2). Instead, in the case of compound **1**, the glycosylation proceeded with satisfactory yields only when using Sc(OTf)$_3$ as the catalyst (conditions *b*, Scheme 2).[20] Overall, the stereochemistry of the glycosylation process during the glycosidic bond formation is generally influenced by the neighbouring participating group at C2 position of the glycosyl donor, which usually leads to the 1,2-*trans*-

glycoside.[21] Indeed, *O*-acyl- or *O*-benzoyl- α-TCA provided a β-stereoselective glycosylation in case of gluco- and galacto-derivatives **4a**, **4b**, **6b**, **15a**, and **15b**. On the other hand, a α-stereoselectivity resulted from the mannopyranosyl derivative **4c**, which, contrarily to the previously mentioned donors, contains the acyl participating group linked at C2 position on the β-face of the sugar. In the case of glycosyl-*p*-hydroxyaryl-isothiocyanate **6a**, in spite of the presence of the participating acetyl group at C2 position, the glycosylation process afforded a mixture of α- and β-glucopyranoside derivatives (**6aα/6aβ**, 3:7 ratio), probably due to the formation of the oxonium ion in the reaction process.[22] The glycosylation reaction for the glucopyranose aryl-isothiocyanate **6a** and the β-galactopyranosyl analogue **6b** were performed in 52% (3:7 ratio for the α/β anomers) and 36% yields, respectively. Also in this case, the presence of the NCS group was confirmed by IR spectra displaying intense and broad peaks between 2060-2130 cm^{-1}, and by ^{13}C NMR analysis, which showed the typical signal at around 155 ppm (Figure 1 and Table S1 of Supporting Information). Fully-*O*-acylated compound **4a** was also converted to its *O*-deprotected analogue **5a**, by using a freshly prepared solution of 0.33 M MeONa/MeOH.

Since the isothiocyanate moiety would readily decompose under Zemplén deprotection conditions, no further deprotection treatments were carried out on glycopyranosyl aryl-isothiocyanates **6a** and **6b**,[23] which were then tested as acylated glycoconjugated-H$_2$S donors (see Biopharmacology). Furthermore, it was not possible to synthesize the isothiocyanate portion on the fully deprotected *p*-amino-aryl glycoside by following a procedure similar to that described in Scheme 3 (which was successfully utilized for the preparation of compounds **16a** and **16b** and their final transformations through steps c and b of Scheme 3).[13]

**Scheme 3. Synthesis of the final isothiocyanate derivatives 7a, 7b, and 8a, 8b**[a]

15a: R, R^1=OAc, R^2=H
15b: R, R^2=OAc, R^1=H

16a: R, R^1=OAc, R^2=H
16b: R, R^2=OAc, R^1=H

7a: R, R^1=OAc, R^2=H
7b: R, R^2=OAc, R^1=H

17a: R, R^1=OH, R^2=H
17b: R, R^2=OH, R^1=H

8a: R, R^1=OH, R^2=H
8b: R, R^2=OH, R^1=H

[a] Reagents and Conditions: (a) TFA, DCM, 0 °C-RT; (b) CS$_2$, Et$_3$N, H$_2$O$_2$, 0 °C; (c) MeONa, MeOH.

The synthesis of the corresponding aliphatic isothiocyanate derivatives started from *N*-Boc-glycoconjugates **15a** and **15b**, which were first deprotected by trifluoroacid acid (TFA) for 2.5 hours, yielding free amino derivatives **16a** and **16b** (Scheme 3), which were then treated with an excess of CS$_2$, Et$_3$N as the base, and H$_2$O$_2$ for the sulfurylation step,[13] affording final compounds **7a** and **7b** with low non-optimized yields (18% and 15%, respectively). This may be ascribed to the partial loss of material during aqueous workup, as well as to the sensitivity that carbohydrates often display towards oxidizing agents, such as hydrogen peroxide. Nevertheless, we were able to extend the alkyl isothiocyanate series to the fully *O*-deprotected NCS-analogues without affecting the NCS group itself. In fact, the common deacetylation procedure of compounds **16a** and **16b** gave fully *O*-deprotected-aliphatic amines **17a** and **17b** as pure compounds, which were then submitted to the same sulfurization conditions previously described, to afford the corresponding isothiocyanate glycopyranosides, **8a** and **8b**. Also for compounds **7a**, **7b**, **8a**, and **8b**, NMR and FT-IR analysis (Table S1 of Supporting Information) confirmed the presence of the NCS group, showing a distinctive ^{13}C-NMR peak around 165 ppm, and a broad IR band between 2110-2130 cm^{-1}.

6-*O*-Glycoconjugated H$_2$S donors. New glucoderivatives **9** and **10**, substituted at C6 position with the H$_2$S donor moiety, were also designed and synthesized starting from the opportunely protected primary alcohol **18** (Scheme 4). Compounds **1** and **18** were treated under Mitsunobu conditions to afford acetylated derivative **9** in 61% yield.[24] The deprotected analog **10**, characterized by free OH at C2, C3, and C4 positions, was also obtained in high yield (94%) starting from **9** upon classical treatment with a 0.33 M MeONa/MeOH solution.

Scheme 4. Synthesis of the 6-O-ADT-glycopyranosyl derivatives 9 and 10[a]

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9: R=OAc

10: R=OH
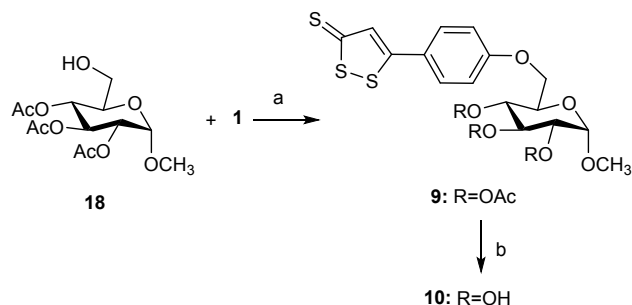
[a] Reagents and Conditions: a) DMEAD, PPh$_3$, THF, 0 °C; b) MeONa, MeOH.

Biopharmacology. Hydrogen sulfide has been demonstrated to induce inhibitory effects on tumor growth. Hence, the development of novel H$_2$S-donors and H$_2$S-hybrid molecules able to release this gasotrasmitter may represent a promising strategy for the design of new classes of antineoplastic compounds. Furthermore, H$_2$S-donors efficacy is related to their ability to reach the tumor cells, cross the cell membrane and accumulate into the cells, leading to the intracellular H$_2$S release and, thus, promoting cytotoxic effects. Therefore, different H$_2$S-donor moieties, such as ADT-OH (compound **1**), 4-isothiocyanophenol (compound **2**) and 2-oxazolidinethione (compound **13**), and their novel glycoconjugated derivatives prepared as described above, were evaluated for their cell inhibitory effect on viability, using pancreatic adenocarcinoma tumor cells (AsPC-1). These cells are characterized by an almost complete resistance to the currently known therapeutic strategies and by the overexpression of several glycolytic effectors, including GLUT1.[26]

Reference dithiolethione derivative **1** led to a decrease in cancer cell viability by about 89 % at the concentration of 1 mM. The cytotoxicity profile of compound **1** is not clearly concentration-dependent. Indeed, in the concentration interval between 100 µM and 300 µM, there is a threshold value: under this threshold no evident cytotoxic activity is observed, while there is a strong abolishment of cell viability above this level. Compound **2** is characterized by the isothiocyanate portion, recently described as H$_2$S-donor moiety.[26,27] This compound presents an evident antiproliferative profile: it exhibits a complete abolishment of cancer cell viability at the concentration of 1 mM and a clear concentration-dependent fashion, with a pIC$_{50}$ value of 4.03 ± 0.04. Compound **13**, a cyclization product of 2-hydroxy-isothiocyanate (Scheme 1), showed a complete lack of activity in this assay. Such a behavior may be linked to the slower H$_2$S-releasing kinetic profile of this thiocarbamate derivative, when compared to isothiocyanate moieties (Figure S2).[26,28]

Dithioletione derivative **1** was used as starting moiety for the synthesis of two glycoconjugated derivatives functionalized in position 6, peracetylated methyl glucoside **9** and its deprotected analog **10**, as well as for the synthesis of C1-functionalized glucoconjugate **5a**.

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Unfortunately, **5a** proved to be highly insoluble in the media utilized in our bioassays and, therefore, could not be properly evaluated. Compound **10** showed an antiproliferative effect only when incubated at the concentration of 1 mM, with an efficacy of about the 79 %. At lower concentrations the inhibitory activity was completely lost, with no concentration-dependent response. Thus, the analysis of the results underlined that the addition of the glycosidic portion to compound **1** did not alter the compound **10** activity profile, but led to a reduction in term of potency, since the pIC_{50} was 3.08 ± 0.44 (Figure 2). Therefore, it is possible to hypothesize that, in spite of the glucosidic portion, the presence of the dithiolethione portion in position 6 made compound **10** unable to exploit the glucose transporter to enter the cell. Its peracetylated precursor **9**, showed a slightly better antiproliferative activity than that of **10**, with concentration-dependent profile comparable to that of reference compound **1** with a pIC_{50} of 3.80 ± 0.04 (Figure 2). These results seem to indicate that compound **9** enters the cell by passive diffusion through the cell membrane, without exploiting the GLUT-mediated facilitated transportation inside the cell.

Arylisothiocyanate-containing derivatives **6aβ** and **6b** were obtained by conjugation of **2** with acetyl-D-glucopyranoside and acetyl-β-D-galactopyranoside portions, respectively. Compound **6aβ** showed a marked improvement in the cell viability inhibitory effect compared to non-conjugated isothiocianate **2**, with a clear concentration dependent profile and a value of pIC_{50} of 4.48 ± 0.04 (Figure 2). Therefore, the glucoconjugation in this case seems to favorably affect the internalization of **6aβ** inside the cell. On the other hand, galacto-derivative **6b** showed an antiproliferative effect that is superimposable to its non-conjugated counterpart **2**, displaying a pIC_{50} value of 4.09 ± 0.03 and a concentration-dependent response (Figure 2). These data revealed that only the conjugation to a glucose-derived portion, such as that of **6aβ**, induces a noteworthy increase of the cytotoxic effect of the aryl-isothiocyanate portion **2**, whereas the introduction of a galactoside moiety (**6b**) does not produce any significant improvement.
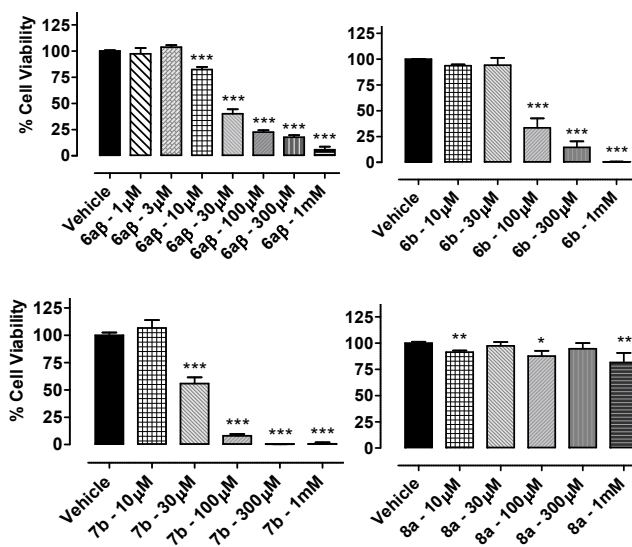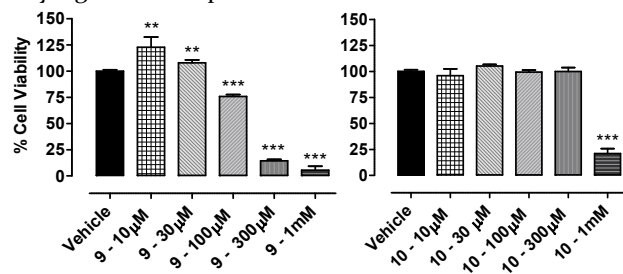
Figure 2. Inhibition of cell viability induced by glycoconjugated derivatives 9, 10, 6aβ, 6b, 7b and 8a in AsPC-1 after 72 hours of incubation. The histograms show the viability of AsPC-1 cells, treated with vehicle or the indicated compound at the concentration of 10 μM, 30 μM, 100 μM, 300 μM and 1 mM. Data are expressed as a percentage of the value of absorbance recorded for AsPC-1 treated with vehicle. Vertical bars represent the standard errors (n = 9). The asterisks indicate a statistically significant difference from the value of cell viability observed for cells treated with vehicle (*p<0.05, **p<0.01, ***p<0.001).

Finally, alkylisothiocyanate derivatives **8a** and **7b**, containing glucose- or peracetylated galactose-portions, respectively, showed antipodal activities in this test. In details, glucose-derivative **8a** resulted to be totally ineffective in inhibiting cell viability, whereas acetylgalactose-derivative **7b** displayed a marked cytotoxicity against AsPC-1 tumor cells in concentration-dependent manner with a pIC_{50} value of 4.45 ± 0.02 (Figure 2). Here again, the lack of the antiproliferative effect of **8a** denotes a lack of sufficient internalization of this molecule by GLUTs. On the contrary, the remarkable activity associated to peracetylated galactose derivative **7b** would further support a non-GLUT-mediated accumulation of this compound inside cancer cells, as seen above for the couple of dithiolethiones **9** and **10**. Of course, this range of pIC_{50} values would not support the use of these compounds as single agents. Rather, as it generally happens for most anticancer drugs, their perspective use should be included in combination therapies.

In order to confirm that the cytotoxic effects of the two most active compounds, **6aβ** and **7b**, can be, at least partially, ascribed to their ability to deliver hydrogen sulfide inside the cell, AsPC-1 cells were incubated with these compounds at different concentrations (10 μM, 30 μM and 100 μM) and the intracellular H_2S release was measured at different times (Figure 3).
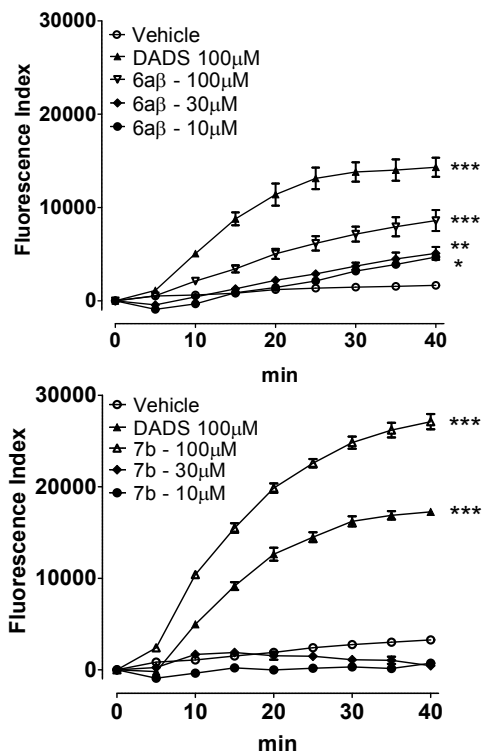
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Figure 3. Fluorometric recording of Compound 6aβ and 7b intracellular H₂S-release inside AsPC-1. Fluorometric recordings of H₂S released by vehicle, DADS 100 μM and **6aβ** (upper panel) or **7b** (lower panel) for 40 minutes: the increase of H₂S is expressed as Fluorescence Index (FI). The vertical bars represent the standard error (n = 9). The asterisks indicate a statistically significant difference from vehicle (*p<0.05, **p<0.01, ***p<0.001).

In this experiment diallyldisulfide (DADS) was utilized as a reference "slow" H₂S donor.[29] Both **6aβ** and **7b** showed a clear time-dependent H₂S intracellular release up to 40 minutes, thus supporting their ability to efficiently cross the cell membrane and their profile as slow H₂S-releasing agents, which is a desirable feature for this type of application. Compound **6aβ** released H₂S in a finely modulated dose-dependent fashion, which could already be appreciated at 10 μM. On the contrary, **7b** displayed a threshold-type behavior, with a massive release of H₂S only at 100 μM, which, by the way, remarkably exceeded that of reference compound DADS at the same concentration. These results seem to favorably compare with those found in the cell viability assay where **7b** proved to have a stronger effect than **6aβ** at 100 μM, whereas at 30 μM the reduction of cell viability by **6aβ** was more evident than that caused by **7b** (Figure 2). It should be noticed that H2S-release by compounds **6aβ** and **7b** in HASMC non-cancerous cells was found to be significantly lower (Figure S3, Supporting Information), thus supporting a preferential uptake of these two molecules by cancer cells.

Finally, the inhibition of cell cycle progression has been evaluated after 72-hour treatment with **6aβ** and **7b** at 30 μM, a concentration that is close to their respective IC_{50}

values measured in the cell viability inhibition assay. Both the compounds induced a significant, although modest, increase in S phase with a decrease in G0/G1 phase (Figure 4A and 4B). These results seem to further support a common mechanism of action of both compounds on the cell cycle and a substantial propensity to inhibit cell duplication, although we do not have any further evidence about the way these compounds arrest the cell cycle at this stage.

In conclusion, we developed a preliminary series of the first examples, to the best of our knowledge, of synthetic H₂S-releasing anticancer glycoconjugates (although different glycoconjugated isothiocyanates had been described in natural products[30] or in synthetic intermediates[31]). Their synthesis required the complex assembly of carbohydrate portions with well-established H₂S-releasing moieties. These new chemical entities were designed with the purpose of efficiently delivering hydrogen sulfide to cancer cells and, therefore, exerting an antiproliferative effect. Some of these compounds proved to noticeably inhibit cancer cell viability and to efficiently release H₂S intracellularly with a desirable slow kinetic. The analysis of the cell cycle progression of cancer cells treated with these compounds revealed a common effect with a significant increase in the S phase subpopulation at the expenses of the G0/G1 portion. The most active compounds (**6aβ** and **7b**), both containing an isothiocyanate portion, proved to be more active than their non-conjugated counterpart. Much to our surprise, these active compounds bear *O*-acetyl groups in the monosaccharide moiety, whereas non-acetylated analogues were substantially inactive. Therefore, a simple GLUT-promoted uptake could not explain the mechanism of cellular internalization of these compounds. Studies are currently ongoing in order to further determine the way these conjugates enhance the intracellular delivery of H₂S.
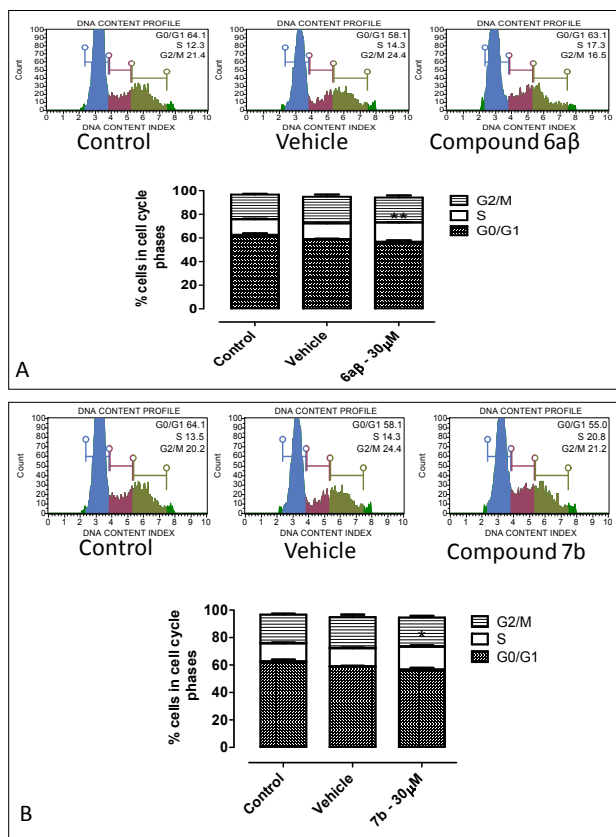
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Figure 4. Inhibition of cell cycle progression induced by compound 6aβ and compound 7b after 72 hours. The histograms indicate the percentage of AsPC-1 cells in G0/G1, S, G2/M cell cycle phases after no treatment (Control), treatment with vehicle, compound **6aβ** 30 μM (**A**) and compound **7b** 30 μM (**B**) after 72 h of incubation. The vertical bars represent the standard errors (n = 9). The asterisks indicate a statistically significant difference from the percentage of AsPC-1 cells in different cell cycle phases after the treatment of 72 h with the vehicle (*p<0.05, **p<0.01).

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, compound characterization data, NMR-, FT-IR- and mass-spectra, additional cell-based assays.

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Author Contributions

The manuscript was written through contributions of all authors, who have given approval to its final version.

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ABBREVIATIONS

GLUT, glucose transporter; LDH, lactate dehydrogenase; ADT, anethole dithiolthione; RT, room temperature; TCA, trichloroacetimidate; THF, tetrahydrofuran; TFA, trifluoroacetic acid; TMSOTf, trimethylsilyl trifluoromethanesulfonate; DCM, dichloromethane; DMEAD, di-2-methoxyethyl azodicarboxylate; FI, fluorescence index; DADS, diallyldisulfide.

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