Bioorganic & Medicinal Chemistry Letters 24 (2014) 5606–5611

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Thio-sugar motif of functional CARB-pharmacophore for antineoplastic activity. Part 2 ☆

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ARTICLE INFO

Article history:
Received 25 August 2014
Revised 27 October 2014
Accepted 29 October 2014
Available online 4 November 2014

Keywords:
Functional CARB-pharmacophore (FCP)
Thio-sugars
1-4-*S*-thiodisaccharides
Cancer cell lines

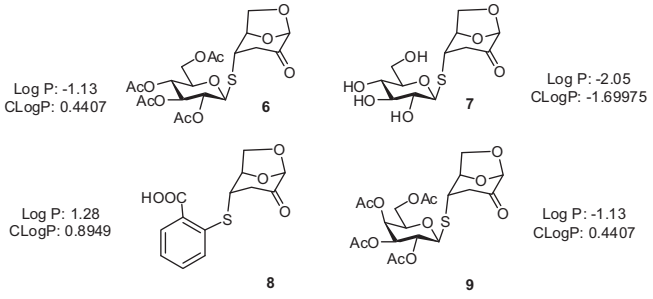
ABSTRACT

Diverse functionalized representatives of (1-4)-*S*-thiodisaccharides, **6**–**9** were synthesized and assessed for cytotoxicity and apoptosis against human cancer cell lines (A549, LoVo, MCF-7 and HeLa). The FCP **6** was more active against MCF-7 cells (i.e., an estrogen-dependent breast cancer line), whereas other (1-4)-*S*-thiodisaccharides showed strongest activity against A549 cells (i.e., a lung adenocarcinoma line). We propose to use a concept of functional 'CARB-pharmacophores' when evaluating a potential for the compounds' general antineoplastic activity. Future studies will determine the reasons for cell-type specificity of these compounds. The thio-sugar motif appears to be a promising lead for future developments.

The 'sugar code' concept assumes the interaction of carbohydrates with cellular components.[1,2] This concept is the basis of therapy employing carbohydrate-derived chemicals as drugs. The 'carb' pharmacological potential is enhanced by the addition of pharmacophores and functional groups. We proposed an idea of '*functional CARB-pharmacophore*' (FCP) as a new approach to examine biological response from functionalized sugar derivatives.[3] CARB-pharmacophore (FCP) functionalized with sulfur atoms (thio-sugars) have garnered significant attention. Several reviews[4–6] suggest that thio-sugars may have therapeutic potential in the treatment of a variety of pathological conditions such as infectious diseases including HIV-1,[7] cancer[8] and diabetes[9–11] (α-glycosidase inhibitor activity).

In previous studies we designed several functionalized carbohydrates that are biologically active as α-fucosidase and α-glucosidase inhibitors,[9,10] and potentially as anticancer therapeutics.[11,12]

Our previous studies showed that functionalized carbohydrates can decrease cancer cell viability, and some were taken under consideration as potential anticancer therapeutics because of their cell growth inhibitory activity.[12] In the newly selected group of FCPs we examined precursors of previously functionalized sulfones and sulfoxides of (1-4)-thiodisaccharides.[12]



Scheme 1. (1-4)-*S*-thiodisaccharides **6**–**9**.

In this study we present the results of research conducted on four cancer cells lines on the cytotoxicity and apoptosis induction ability of selected derivatives bearing a thio-sugar motif. Due to screening character of our study we decided to use cell lines representing various types of cancer including hormone-dependent and hormone-independent cancers. Those representative sets of cancer cell lines are commonly used in cytotoxicity studies and are well described including their genetic profile.

We consider FCPs as 'carbohydrate compounds containing specific chemical functional groups that render these compounds potentially therapeutically useful'.

The selected (1-4)-*S*-thiodisaccharides **6**–**9** are depicted in Scheme 1. Compounds **6**, **7**, and **9** were synthesized earlier[11,12]

☆ For Part 1. See Ref. 3.

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http://dx.doi.org/10.1016/j.bmcl.2014.10.095
0960-894X/

via the thio-click approach, whereas compound **8** was prepared exclusively for this study.[13] The rationale to synthesize compound **8** is the potential influence of the aromatic substituent at C-4, bearing –COOH group for the anticipated different and overall response from the cell proliferation assay. Additionally, phenyl carboxyl substituent is well known bioisosters group and pharmacophore present in many anti-inflammatory drugs. Additionally, there are no QSAR data for the thio linked molecules **6**–**9**.

The thiodisaccharides **6**–**9**, exhibit a broad range of log*P* values, which is very useful for the assessments of this parameter's role in their cytotoxicity level. The calculated log*P* and *C*log*P* values clearly indicate their level of water solubility and fit the Lipinski's[14–17] 'rules of five' for small molecules.

The crystal structure of the thiodisaccharide **6** (Fig. 1) clearly revealed the geometry of the molecule and the presence of a sulfur bridge at C1–C4.

Additionally, the calculated dihedral angles (Fig. 2) are in the expected ranges of 110°, 100° and 109°, respectively.

In contrast the dihedral angles calculated for disaccharide **8** are in the different range of 128°, 113° and 97° (Fig. 3). The anticipated different value of the dihedral angles of sulfur bridges of compound **6** and **8** is an important factor for the overall assessment of cytotoxicity.

Consequently, the obvious difference in the dihedral angles and stereochemical orientation of the 1-4-thio bridge is reflected in the much higher cytotoxicity for **6** and lower for **8**.

We believe that this important new observation and solid cytotoxicity data will help to formulate the plausible hypothesis on the relationship between value of the dihedral angles and cytotoxicity pointing out the differences in accessibility of both molecules **6** and **9** to the cancer cell lines.

However, this initial hypothesis must be further verified on the next set of functionalized thio-sugars pharmacophores.

The apoptosis induction for **6** exceeds that for **8** on all tested cell cultures.

The functional pharmacophore as defined[18] plays an important role in the development of carbohydrate therapeutics and has been continuously improved and published.[19–24]

The cytotoxicity of functional CARB-pharmacophore **6**–**9** was tested at nine different concentrations (⩽2.2 mM) on four cell lines: lung, cervix, mammary gland-breast and colon carcinoma (A549, HeLa, MCF-7 and LoVo, respectively).

We chose the particular dose range of FCPs for two reasons. Firstly it fits NCI recommendations for a potential anticancer drug. Secondly it is similar to the dose range used in our previously published studies as we expected the similar cytotoxicity. The characteristics of the studied cancer cells lines are presented in Table 1. Notice that functional CARB-pharmacophore containing thio-sugar motifs inhibited cell growth of all examined cell lines in micromolar concentrations as presented in Figure 4.

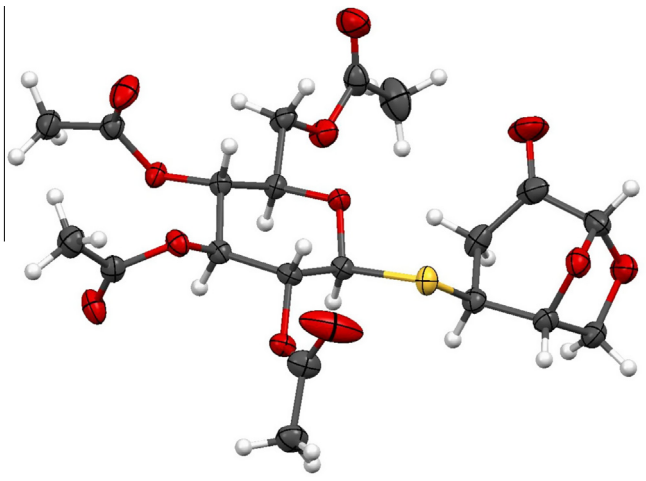

Figure 1. X-ray crystal structure of thio-disaccharide **6**.

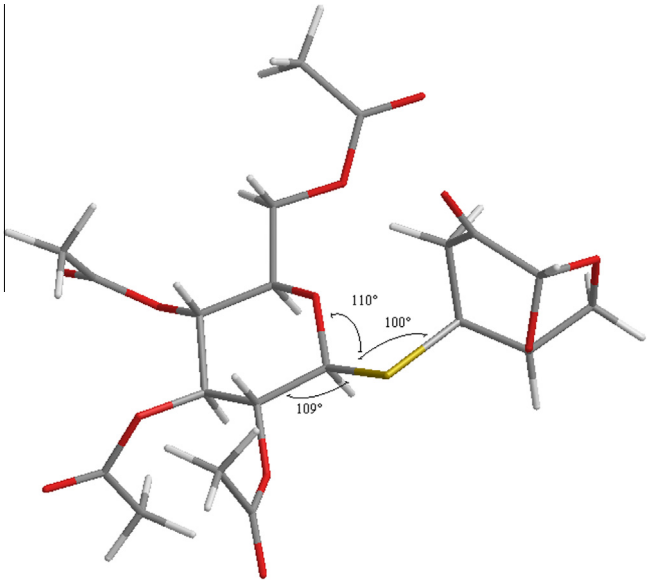

Figure 2. Bond angles of 1-4-thio bridge of disaccharide **6**.

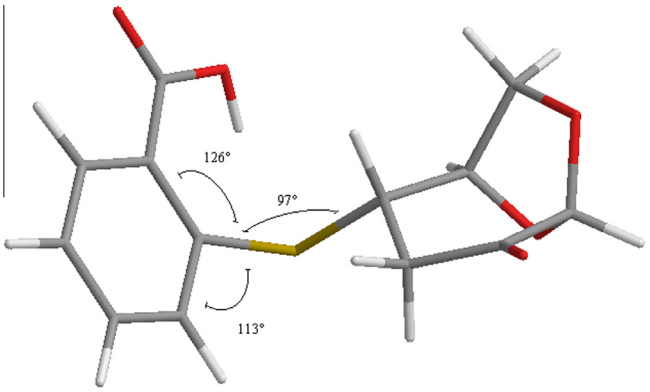

Figure 3. Bond angles of 1-4-thio bridge of disaccharide **8**.

We found that the MCF-7 cell line was most sensitive to FCP as compared with other studied cancer cell lines (Table 2). The FCP **6**, which has four hydroxyl groups in the sugar ring protected by acetyl groups, exhibits the highest cytotoxic activity (IC_{50} 47.1 μM). The hydroxyl group deprotection by removing the acetyl groups (FCP **7**) and/or substitution of glucose with galactose (FCP **9**) did not increase the cytotoxicity of compounds but increased its solubility. A similar cell killing scheme (FCP **6** was always the most toxic FCP) was observed with other studied cancer cell lines. FCP **8** represents another groups of thio-sugars equipped with benzoic acid instead of a second sugar molecule. It was also cytotoxic against cancer cell lines with micromolar IC_{50} values (Table 2). Anticancer agents kill cancer cells using a large variety of mechanisms. A common manifestation of anticancer drug activity is the induction of apoptosis. To test this possibility we employed a well-established and accepted apoptotic assay that is based on the translocation of phosphatidylserine to the outer leaflet of the plasma membrane. The phosphatidylserine was marked with green fluorescent dye by Annexin V/FITC complex. To distinguish