Bioorganic & Medicinal Chemistry 21 (2013) 6554–6564

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and anti-tumor activity of carbohydrate analogues of the tetrahydrofuran containing acetogenins

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ARTICLE INFO

Article history:
Received 8 June 2013
Revised 6 August 2013
Accepted 15 August 2013
Available online 29 August 2013

Keywords:
Carbohydrate
Lipid
Metathesis
Mimetic
Dynamic light scattering

ABSTRACT

The tetrahydrofuran (THF) containing annonaceous acetogenins (AAs) are attractive candidates for drug development because of their potent cytotoxicity against a wide range of tumors and their relatively simple and robust structures. Replacement of the THF segment with a sugar residue may deliver analogues with improved tumor selectivity and pharmacokinetics and are therefore attractive for drug development. As a first test to the feasibility of such structures, a set of such monosaccharide analogues was synthesized and assayed against four human tumor cell lines, cervical (HeLa), breast (MDA-MB231), T-cell leukemia (Jurkat) and prostate (PC-3). Certain analogues showed low micromolar activity that was comparable to a structurally similar, naturally occurring mono-THF acetogenin. A preliminary examination of the structure–activity profile of these carbohydrate analogues suggests that they have a similar mechanism of action as their THF congeners.

1. Introduction

The tetrahydrofuran (THF)-containing annonaceous acetogenins (AAs) are noted for their potent cytotoxicity against a variety of tumor cell lines.[1–3] There is considerable interest in their development as clinical agents.[4,5] Their cytotoxic activity is believed to be connected to their interaction with the reduced nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (complex I), a membrane-bound protein of the mitochondrial electron-transport system.[6] Several reviews on structure activity relationships (SARs) for the THF-containing AAs have been published.[7–10] The AAs generally contain one or more tetrahydrofuran (THF) rings, although a small number contain a tetrahydropyran (THP), and are classified into two major structural subgroups depending on the number and arrangement of the THFs: the mono-THF and the adjacently linked bis-THF acetogenins (Fig. 1). A relatively small number of structures with non-adjacently connected bis-THF or THF–THP motifs comprise a third subgroup. The central ether segment is generally flanked at each terminus by a carbinol center, giving rise to a central polar core. One of the carbinol carbons is connected to a methylated γ-lactone ring by a polymethylene spacer, which may contain one or more hydroxyl groups, and the other carbinol center is linked to a long hydrophobic chain. SAR studies suggest that the butenolide moiety

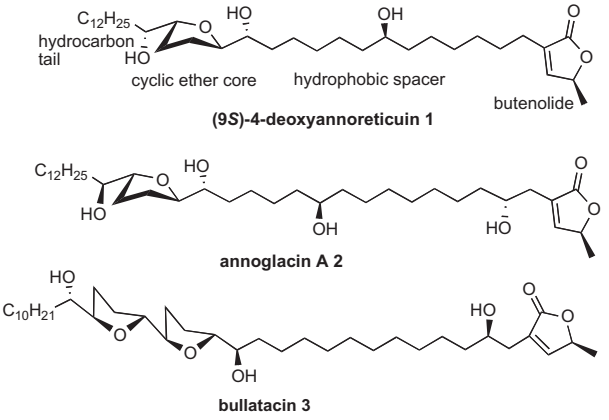

Figure 1. Representative mono- and bis-THF acetogenins.

contributes significantly to anti-tumor activity as analogues in which the alkene is reduced are generally much less active. The cyclic ether core is also important for activity but the high potency observed for frameworks with a diverse arrangement and numbers of the THF rings, suggests that a broad range of core structures that meet a critical volume and polarity requirement may be tolerated. The length and degree of hydroxylation of the hydrocarbon chains are also important, with too long or short chains and a high degree

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http://dx.doi.org/10.1016/j.bmc.2013.08.027

of hydroxylation leading to reduced activity. These SARs are consistent with a cytotoxicity model in which the cyclic ether core sits at the membrane–water interface in the mitochondria and the lipid chains stabilizes a conformation that presents the butenolide to a binding domain on complex I. Interestingly, the structural requirements for activity against complex I appears to be less stringent than the SARs for anti-tumor activity, suggesting that anti-tumor activity may be linked to other mechanisms of action.[11,12] Although individual THF containing AAs show very high selectivity against certain tumors cell lines, their potent activity against mitochondrial ATP production suggests that in general they may be too cytotoxic for clinical application.

Against this backdrop we are interested in analogues of the THF acetogenins in which the cyclic ether core is replaced with a carbohydrate residue. Given the wide variations of the cyclic ether core among highly potent analogues, we speculated that a carbohydrate could be used as a mimic of the cyclic ether core. The structures so generated are to be distinguished from the glycosylated AAs examined by Hocquemiller and co-workers, in which alcohols on natural acetogenins are glycosylated.[13] In the analogues in the present study, the carbohydrate motif is imprinted in the acetogenin framework and may be less likely to interfere with binding to the cellular target. Like the Hocquemiller molecules, their carbohydrate-likeness has important implications for drug design. First, the carbohydrate motif may allow for targeting of tumors that overexpress cell surface lectins or carbohydrate transporters.[14,15] Second, the hydrophilicity of the carbohydrate may help overcome potential problems with low water solubility and non-specific cellular uptake. Third, the easy access to diverse carbohydrates allows for a wide variety of analogues for drug optimization studies. Herein we describe the synthesis and evaluation of the anti-tumor activity of a preliminary set of these new carbohydrate-like AAs.

2. Results and discussion

2.1. Analog design

An α-mannopyranose sugar scaffold was selected because of the relatively simple synthetic chemistry required for the modification of this template (Fig. 2). To mimic the frameworks of the natural AAs, the hydrocarbon chains were appended as an *O*-glycoside at C1 and as a 6-*C*-extended sugar. Structures **4**–**7** in which one or more hydroxyl groups were removed from the sugar ring were prepared, to evaluate whether increasing the density of hydroxyl groups leads to reduced activity, as observed in the naturally occurring THF AAs. The β-galactose **8** derived analog was also of interest to probe how the relative positioning of the two hydrocarbon branches on the sugar scaffold might impact on activity. Finally, to interrogate whether the anti-tumor activity of the new analogues was due to a similar mechanism of action as the natural AAs, structures **9**–**11**, with a reduced butenolide and without the butenolide or the hydrocarbon tail, were prepared.

2.2. Synthesis

A modular plan in which a fixed butenolide alkene **17** and different carbohydrate alkene partners **18**–**23** were connected through an olefin cross metathesis (CM) was conceived. The carbohydrate alkenes were prepared via established carbohydrate transformations from known precursors **12**–**16** (Table 1).

The fully oxygenated mannose-derived alkenes **18** and **23** were prepared from penta-*O*-acetylated mannose **12** (Scheme 1). Thus, treatment of **12** with 9-decenol and $BF_3{\cdot}OEt_2$ gave the 9-decenyl-α-glycoside, which was subjected to standard procedures for acetate hydrolysis and *O*-isopropylidene formation, to give the

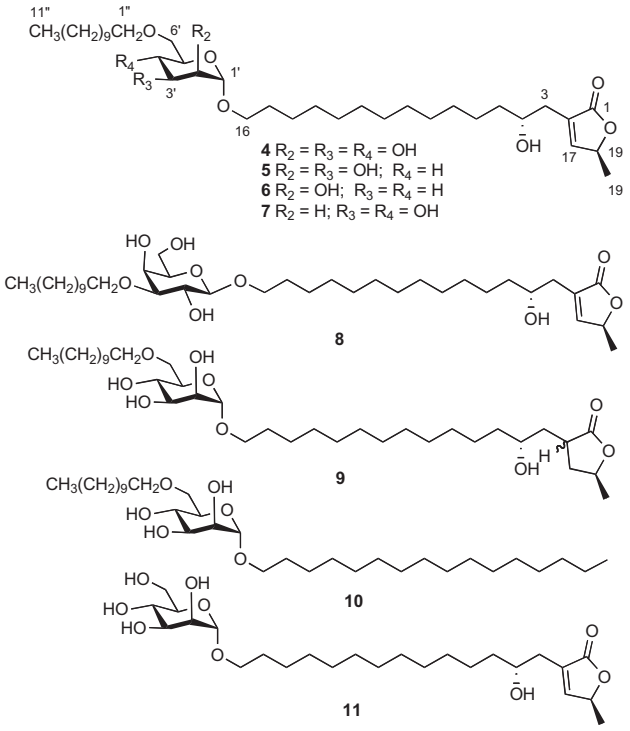

Figure 2. Sugar mimics of mono-THF acetogenins.

2,3,4,6-di-*O*-isopropylidene **23**. Selective removal of the 4,6-*O*-isopropylidene in **23** and sequential formation of the pivalate and ethoxyethyl acetal on the diol **30**, provided **31**. Ester cleavage on **31**, O-alkylation of the resulting alcohol with 1-bromo-undecane, and hydrolysis of the ethoxyethyl acetal protecting group, afforded **18**.

For the 4-deoxy pyranoside alkene **19**, the 2,3-eno-pyranoside **13**[16] was converted via an O-alkylation-alkene dihydroxylation sequence to the 4-deoxy-pyranoside **32**. Hydrogenolysis of **32** and acetylation of the product provided the 1,2,3-tri-*O*-acetyl derivative, which was subjected to the aforementioned glycosidation procedure with 9-decenol. This reaction afforded a single α-glycoside **33**, which after ester hydrolysis and isopropylidination of the derived diol led to the 2,3-*O*-isopropylidene **19**.

The synthesis of the 3,4-dideoxy pyranoside **20** started from the D-glyceraldehyde-derived, alkene **14**.[17] Selective Bu_2SnO-mediated O-alkylation of the primary alcohol in **14** afforded **34**. The hydroxyalkene **34** was next transformed to the *E* α,β-unsaturated ester **35**, through a straightforward sequence of reactions: O-benzylation, ozonolyis of the alkene and reaction of the derived aldehyde with methyl(triphenylphosphoranylidene)acetate. Asymmetric dihydroxylation on **35** using AD-mix α,[18] followed by isopropylidenation of the resultant diol afforded methyl ester **36**. Basic hydrolysis on **36** and Suárez fragmentation on the resulting acid with iodosobenzene diacetate and iodine provided a mixture of acetates **37**.[19] Hydrogenolysis of **37**, treatment of the product with aqueous TFA and standard acetylation of the resulting material led to a mixture of di-*O*-acetates **38**α/β (ca. ratio 100/1). Application of the glycosidation and acetate hydrolysis procedures on **38**α, as previously described, provided **20**.

The 2-deoxy-pyranoside **21** was prepared from commercially available tri-*O*-acetyl-D-glucal **15**. Thus, $Ph_3P{\cdot}HBr$ catalyzed glycosidation of **15** with 9-decenol, followed by ester hydrolysis on the product, provided triol **39** as a single α-glycoside.[20,21] Standard alcohol protecting group processing on **39** and *O*-alkylation as described earlier, provided **40**, and then **21**.