Bioorganic & Medicinal Chemistry Letters 21 (2011) 380–382

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Glycosylated nordihydroguaiaretic acids as anti-cancer agents

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ARTICLE INFO

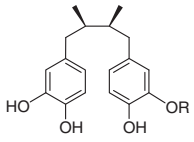
Article history:
Received 19 May 2010
Revised 23 October 2010
Accepted 28 October 2010
Available online 31 October 2010

Keywords:
Nordihydroguaiaretic acid
NDGA
Hepatocellular carcinoma
Triazol-glucose
Triazol-galactose

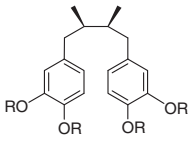
ABSTRACT

Three perglycosylated nordihydroguaiaretic acids (NDGA) were synthesized through the Huiseng 1,3-dipolar cycloaddition reaction. These sugar–NDGA conjugates containing triazole-linkages possessed good solubility in water. NDGA-(triazol-galactose)$_4$ (**12b**) and NDGA-(triazol-glucose)$_4$ (**12c**) were found to act as inhibitors against human hepatocellular carcinoma Hep3B cells in culture.

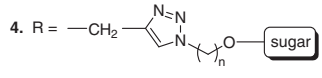
Nordihydroguaiaretic acid (NDGA, **1**), a naturally occurring lignan, is isolated from a desert medicinal plant in the Creosote bush (*Larrea tridentate*). It is known as the inhibitor of lipoxygenase[1] and an anti-oxidation agent.[2] Huang and co-workers[3] reported that 3′-O-methylated-NDGA (i.e., Mal.4, **2**) can inhibit HIV transcription by interfering with binding of transcription factor Sp1 to the promoter of the HIV proviral template. Meanwhile, in vitro, NDGA shows no inhibitory effect on HIV transcription and no effect on Sp1 binding. Thus, a series of NDGA derivatives with modification at the four phenolic hydroxyl groups therein were developed for these purposes.[4–7]



1. R = H (**NDGA**)
2. R = Me (**Mal.4**)



3. R = Me (**M_4N**)



Bioactivities of the O-methylated derivatives of NDGA, especially tetra-*O*-methyl-NDGA (M_4N, **3**), have been examined extensively.[4] M_4N can inhibit human immunodeficiency virus (HIV) Tat transactivation, replication of herpes simplex virus type 1 (HSV-1),[8,9] and human papilloma virus (HPV) E6/E7 promoter activity.[10] It can also arrest growth and induce apoptosis of cancer cells, including mouse C3 tumor cell and five different human xenograph tumors.[11] Moreover, Sp1-dependent transcription of many promoters is inhibited by M_4N, such as the HIV long transcribed region and genes related to code for the cell cycle protein cyclin-dependent kinase (Cdc2) and apoptosis protein survivin.[3,12,13] In addition, M_4N is able to reverse the MDR phenotype of the tumor cells in a xenograft model system; the combination therapy with M_4N and paclitaxel is effective at inhibiting growth of tumors in nude mice.[14] Although the methylated NDGA analogs are potentially good candidates for antiviral and anti-tumor drugs, their water solubility is poor.

Conjugation of carbohydrates with drugs becomes increasingly important in bio-organic chemistry and chemical biology. In addition to facilitation of the active transport of the modified drugs across biological membranes, sugar-conjugation may allow to modify the physico-chemical properties of the conjugated drugs, including polarity, solubility, and stability at physiological pH.[15] In some examples, sugar-conjugated drugs are used in enzyme-specific activation,[16] selective transport of O^6-methylguanine-DNA methyltransferase (MGMT) inhibitors,[17] specific targeted delivery,[18] and providing amphipathic characteristics.[19] There is some precedence of glycosylated drugs, of which physico-chemical

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0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.10.137

properties and therapeutic effects are improved. For examples, carbohydrate–geldanamycin conjugates for enzyme-specific activation increase tumor selectivity.[16,20] Glucose-conjugated MGMT and O^6-methylguanine-DNA methyltransferase are developed as antagonists for selective transport of MGMT inhibitors in order to target glucose transporters that are often over expressed in tumor cells.[17,21] Accordingly, we planned to synthesize new water-soluble NDGA analogs containing sugar units.

We applied the Huisgen Cu-catalyzed 1,3-dipolar cycloaddition between alkynyl NDGA and (azido)glycosides to form triazole linkages. This method was reported by Tornøe,[22] Rostovtsev,[23] and their co-workers. Our targets included five new NDGA derivatives: three of them contained different lengths of triazole-linkage with four galactose or glucose moieties (i.e., **4**); the remaining two were not attached by any sugar moiety. We found that NDGA derivatives with sugar units can be easily dissolved in an aqueous solution or other polar solvents. More important is that NDGA-(triazol-galactose)$_4$ and NDGA-(triazol-glucose)$_4$ exhibited inhibited cell proliferation toward Hep3B cell line.

To obtain perglycosylated NDGA targets **12a–c**, we first treated commercially available NDGA (**1**) with 6.0 equiv of propargyl bromide (**5**), a catalytic amount of 18-crown-6, and 5.5 equiv of potassium carbonate in acetone (Scheme 1).[24] After the solution was heated at reflux, peralkynyl NDGA **6** was generated in 71% yield.

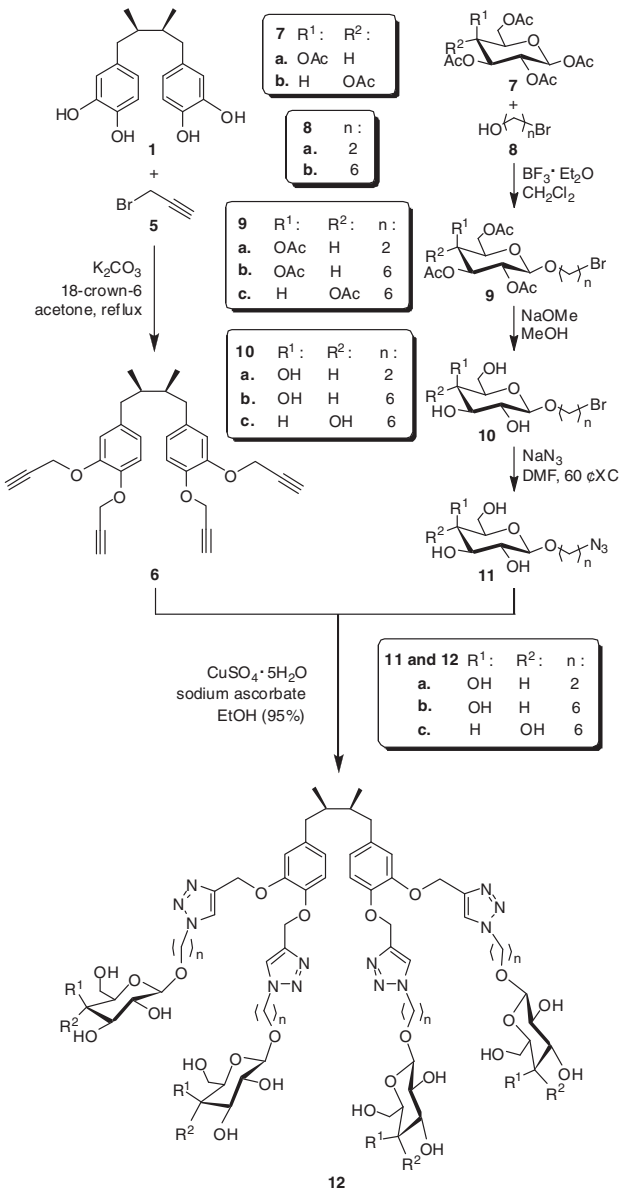
Second, we prepared ω-(azidoalkyl)glycosides **11a–c** from commercially available penta-*O*-acetyl-D-galactose and -glucose (i.e., **7a** and **7b**) by utilizing several published procedures.[25,26] Reactions of **7a,b** in dichloromethane with a bromoalkanol (i.e., **8a** or **8b**) in the presence of boron trifluoride etherate[25] produced the corresponding (bromoalkyl)glycosides **9a–c**, respectively, in 31–45% yields. These products were assigned as the β-anomers because of the large coupling constants ($J_{1,2}$ = 7.2–7.6 Hz) associated with their anomeric protons. Subsequent deacetylation of **9a–c** gave the unprotected glycosides **10a–c**. Treatment of these bromides with sodium azide in DMF[26] produced ω-(azidoalkyl)glycosides **11a–c** in 71–90% yields.

Coupling of peralkynyl NDGA **6** with an excess of (azido)glycosides **11a–c** (6.7–8.4 equiv) was accomplished by the use of $CuSO_4$/sodium ascorbate[23] in 95% EtOH as described previously[27] (Scheme 1). The triazole-linked conjugated products **12a** (57%), **12b** (56%), and **12c** (81%) were obtained with purity >99.5% after purification with Sephadex LH-20 and Sephadex G-15 chromatography. Furthermore, we applied the same conditions to couple **6** with azides **13** (see Scheme 2). The tetratriazole alcohols **14a** and **14b** were obtained and used as reference compounds in bioassays.

The NDGA derivatives M_4N (**3**) and **14a,b** were found insoluble in water. In sharp contrast, we found that the glyco-conjugated NDGA derivatives **12a–c** possessed solubility of 503–624 mg/mL in water. Thus the tetraglycoside moieties attached to NDGA greatly increased the water solubility of the resultant conjugates. Moreover, these conjugates were also found soluble in regular organic solvents, including THF, ether, acetone, ethyl acetate, DMF, chloroform, etc. They remained intact (>99.5%) on bench at room temperature for 6 months.

We have previously reported that tetra-*O*-methyl NDGA (**3**) inhibits the growth of human cancer cell lines hepatocellular carcinoma Hep3B, prostate carcinoma LNCaP, colorectal carcinoma HT-29, breast carcinoma MCF-7, and ovarian carcinoma NCI/ADR-RES in culture effectively with similar potency with $IC_{50} \leqslant 10\ \mu M$.[11,14] We further examined the efficiency of compounds **12a–c** and **14a,b** in these cell lines. Galactosylated NDGAs **12a,b**, glucosylated NDGA **12c**, and nor-glycosyl NDGA **14a,b** were found not effective in the inhibition of human tumor cells HT-29, MCF-7, and NCI/ADR-RES.

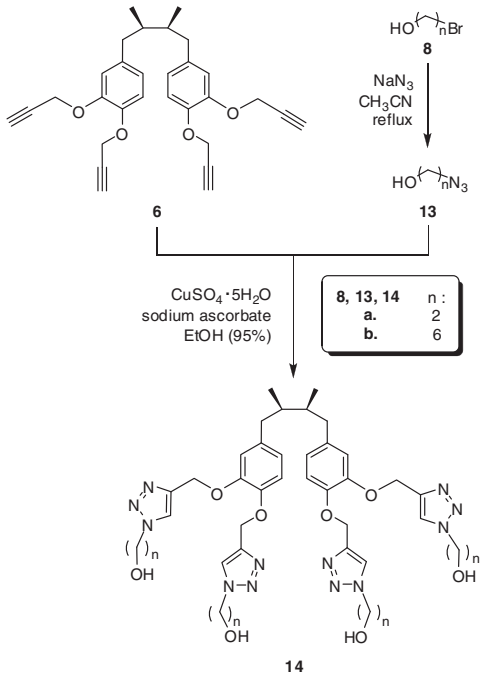
In contrast with these results (especially from **14b**), we found that compounds **12b** and **12c** were able to inhibit the growth of human hepatocellular carcinoma Hep3B cells in culture (Fig. 1 and Table 1). We therefore believe that the glycosidic moieties are essential to the biological activity associated with the conjugated compounds **12b** and **12c** (cf. **14b**). Presence of the ASGP receptors may allow the galactosyl derivative **12b** showing some preference for hepatocytes cell lines.[28] Similarly, the efficacy of the glucosyl derivative **12c** may be due to the glucose transporter.[29] These observations, however, require further experiments to elucidate their mechanisms in detail.



Scheme 1. Syntheses of conjugates of sugar–nordihydroguaiaretic acid.

In conclusion, three glycosylated NDGA derivatives were designed and prepared, which possessed good water solubility ranging from 503 to 624 mg/mL. Two of these glucose- and galactose-conjugated NDGA derivatives exhibited significant inhibition activity toward human hepatocellular carcinoma Hep3B cells.

Results from controlled experiments indicate that these glycosyl moieties were essential to their anti-cancer activity associated with these conjugated compounds. Detailed studies on the uptake of these two sugar-attached NDGA derivatives **12b** and **12c** into the liver cells in targeting human cancer are currently in progress and will be reported separately.



Scheme 2. Syntheses of tetratriazole alcohols **14a** and **14b**.

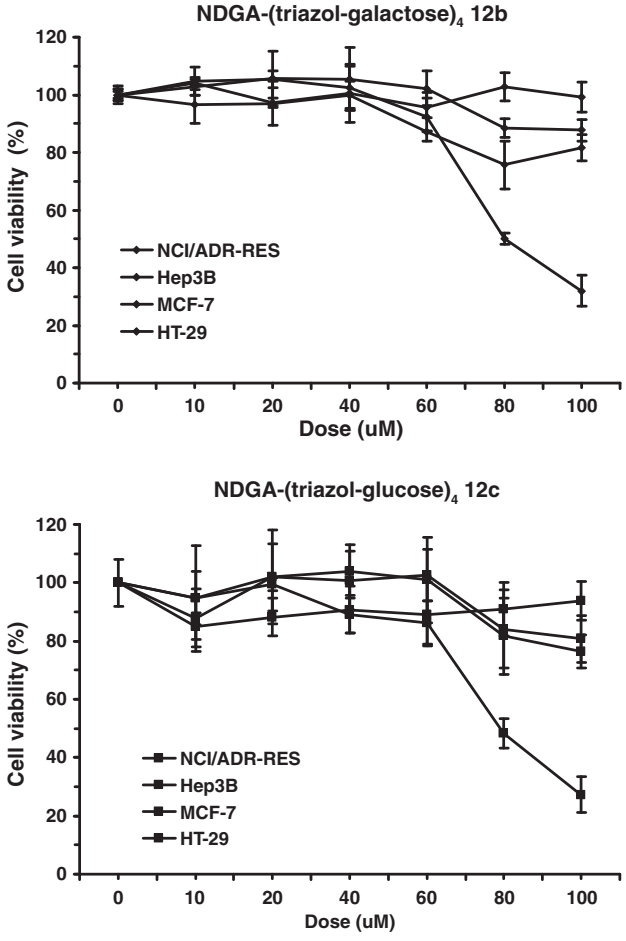


Figure 1. Dose measurement of compounds **12b** and **12c** on their ability to inhibit human tumor cells NCI/ADR-RES, MCF-7, HT-29, and Hep3B in culture.

Table 1
Inhibition of proliferation of human hepatocellular carcinoma cell line by NDGA-(triazol-galactose)$_4$ **12b** and NDGA-(triazol-glucose)$_4$ **12c**

Compounds	IC_{50}[a] (μM), Hep3B
NDGA-(triazol-galactose)$_4$ **12b**	88 ± 3.01
NDGA-(triazol-glucose)$_4$ **12c**	83 ± 2.17

[a] Data are shown as mean values ± S.D. of three independent determinations.

Acknowledgments

This work was partially supported by grants and contributions from the National Science Council of Republic of China to J.R.H. and from Erimos Pharmaceutical, LLC to R.C.C.H.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.137.

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