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Original article

Differential effect of heterocyclic D-ribofuranoside derivatives on human prostate cancer cell viability and cell cycle progression

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ABSTRACT

New D-ribofuranoside derivatives containing two five membered heterocycles, isoxazole and triazole or two triazole rings, were synthesized. The final products as well as the synthetic precursors were physically and spectroscopically characterized. These new diheterocyclic derivatives together with other D-riboside compounds were assessed for their impact on PC3 cell line viability. We found that exposure of prostate cancer cells to some of these compounds caused a significant inhibition of cell growth and a G_0/G_1 cell cycle arrest, which was concomitant with alterations in the expression of proteins involved in cell cycle progression. Furthermore, the inhibitory activity was improved in di-heterocycles when the carbohydrate moiety was protected with a cyclopentylidene group compared to the isopropylidene analogues.

1. Introduction

The development of potent and effective antitumoral agents has become one of the most intensely studied topics of contemporary medicinal chemistry. In this context, we have been interested in exploiting the synthesis and biological properties of five membered heterocyclic rings linked to carbohydrate moieties. In a previous work, we have reported the synthesis of some 1,2,4-triazole D-ribose derivatives (Fig. 1) and their antiproliferative activity, *in vitro*, against a BW 5147 lymphoma cell line [1].

Among these structures, the synthesized compounds containing a 1,2,4-triazole ring linked by sulfur to the carbohydrate moiety (compounds **1**, **4**, **6** and **8**) displayed a moderate antiproliferative activity against this cell line, but compounds **1** and **6** showed a strong inhibitory behavior in the range of measured concentrations. The structures with 3-thiobenzyl-5-substituted-1,2,4-triazole ring (compounds **2**, **3**, **5**, **7** and **9**) led to compounds with a biphasic behavior, meanwhile the deprotected compounds (**4**, **5**, **8** and **9**) showed a reduction in the antiproliferative activity [1].

It is well known that a large number of compounds with important biological activities contain a 1,2,3-triazole ring, for example, β-lapachone based 1,2,3-triazole derivatives were highly active ($IC_{50} < 2$ μM) for HL-60 and MDA-MB435 cancer cell lines [2]. Moreover, series of 1,2,3-triazole 1,5-disubstituted analogs of combretastatin A-4 exhibited potent cytotoxic activity in the nanomolar range in several cancer cell lines as well as a moderate tubulin inhibitory activity in the low micromolar range [3,4].

The histone deacetylase inhibitors (HDAC) have been revealed as a promising new class of anticancer agents that act through a variety of mechanisms [5–7]. The novel HDAC inhibitor MHY219, a suberoylanilide hydroxamic acid-like (SAHA-like), showed an important antiproliferative effect in prostate cancer cells [8]. However, compounds, which were several folds more potent than SAHA, were obtained when the amide group in SAHA-like inhibitors is replaced by a triazole ring [9].

On the other hand, the isoxazole ring is known for its medicinal importance and form the basis of several drugs, such as zonisamide (anti-convulsant) [10], valdecoxib (COX-2 inhibitor) [11] and leflunomide (a disease-modifying antirheumatic drug) [12]. In addition, an isoxazole derivative from curcumin has been reported as a good antioxidant and COX inhibitory agent [13] and showed greater activity against both MCF-7 and MCF-7R cell lines than its natural product precursor [14]. Promising properties as a heat

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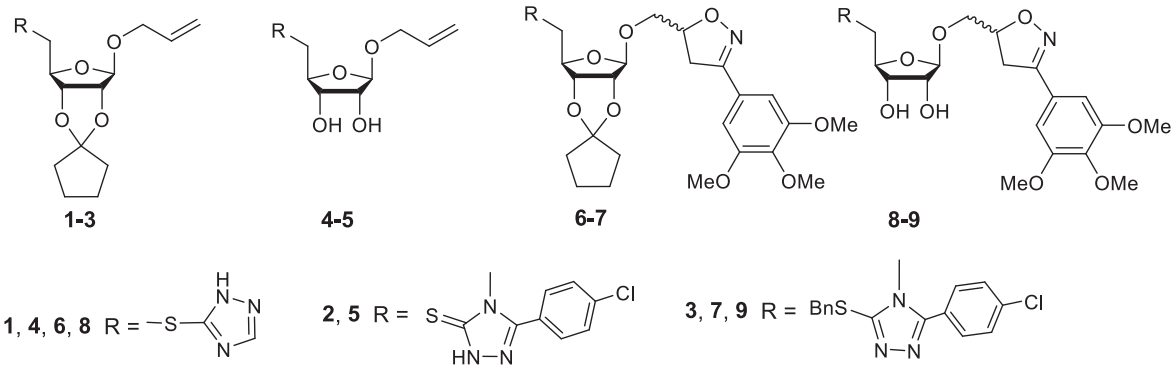

Fig. 1. Chemical structures of 1,2,4-triazole D-ribose derivatives.

shock protein 90 (HSP90) inhibitor have been reported for a diaryl resorcinylic isoxazole amide derivative, with antiproliferative potency quite similar to the clinical drug 17-AAG [15]. HSP90 is a molecular chaperone required for the folding and post-translational stability and function of several signal transducing proteins referred as "client" proteins [16]. Many known oncoproteins are client proteins of HSP90, thus targeting HSP90 has emerged as an interesting avenue for cancer therapeutics. In particular, these inhibitors have appeared in the prostate cancer (PCa) scene [17], since several HSP90 client proteins are implicated in the pathogenesis of PCa.

In the present work, we report the synthesis of new lipophylic diheteroaromatic D-ribose derivatives having an isoxazole or a 1,2,3-triazole ring at the anomeric carbohydrate position using two different protective groups (cyclopentylidene or isopropylidene). These diheterocyclic derivatives together with the most promising molecules (**1**, **6**), that we previously described [1], were screened against the PC3 cells (human PCa cell line) and evaluated the impairment of cell growth and cell cycle progression.

2. Materials and methods

2.1. Chemistry

The syntheses were carried out using reagents as purchased, without further purification. Solvents were reagent grade and, in most cases, dried and distillated before use according to standard procedures. Analytical TLC was conducted on Silica Gel 60G (Merck) on precoated plates and visualization was made by UV light and ethanol/sulfuric acid (10:1) or cerium molybdate followed by heating. Column-chromatographic separations were performed on Silica Gel (240–400 mesh, Merck). Elemental analysis was performed on an Exeter Analytical CE-440 elemental analyzer. Optical rotations were recorded at 20 °C on a Perkin Elmer 343 polarimeter, and melting points were uncorrected. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer, operating at 200, 50 MHz respectively; or a Bruker AMX-500 spectrometer, operating at 500, 125 MHz respectively. Assignments of the ^{1}H and ^{13}C NMR spectra were confirmed with the aid of two dimensional techniques ^{1}H, ^{13}C (COSY, HSQC, HMBC). Chemical shifts (δ) are reported in parts per million downfield from tetramethyl silane as internal standard. Compounds **1**, **6** and **8** were synthesized as we previously reported [1].

2.1.1. General procedure for the synthesis of propargyl derivatives (10, 11)

Powered D-ribose (6.05 g, 40.30 mmol) and anhydrous cuprous sulfate (12.9 g) were suspended in a mixture of cyclopentanone (110 mL) or acetone (96 mL) and propargyl alcohol (28 mL) containing a catalytic amount of H_2SO_4 (0.3 mL). The resulting mixture was stirred at 40 °C for 48 h and then neutralized with $NaHCO_3$, filtered, and the solvents were evaporated. The crude product was extracted with ethyl acetate and washed with brine, dried (Na_2SO_4), and the solvent was evaporated to give the crude product, which was purified by flash chromatography (cyclohexane:acetone) to obtain compound **10**, **11**.

2.1.1.1. Propargyl 2,3-O-cyclopentylidene-β-D-ribofuranoside (10). Compound **10** was obtained as a colorless oil (22.77 mmol, 57%); $[\alpha]_D^{20}$ −100.2 (*c* 1.1, chloroform). IR υ_{max} 3474 (υ O–H), 3284 (υ C_{sp}–H), 2957 (υ C_{sp3}–H), 2125 (υ C_{sp}–C_{sp}), cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.26 (s, 1H, H-1), 4.74 (dd, 1H, $J_{3,2}$ 6.0 Hz, $J_{3,4}$ 0.5 Hz, H-3), 4.57 (d, 1H, $J_{2,3}$ 6.0 Hz, H-2), 4.42 (t, 1H, $J_{4,5}$ 3.6 Hz, H-4), 4.27 (d, 2H, *J* 2.3 Hz, C$\underline{H}_2$-C≡CH), 3.70 (ddd, 1H, $J_{5b,5a}$ 12.3 Hz, $J_{5b,OH}$ 4.4 Hz, $J_{5b,4}$ 3.2 Hz, H-5b), 3.61 (ddd, 1H, $J_{5a,5b}$ 12.6 Hz, $J_{5a,OH}$ 8.6 Hz, $J_{5a,4}$ 4.2 Hz, H-5a), 2.86 (dd, 1H, $J_{OH,5a}$ 8.9 Hz, $J_{OH,5b}$ 4.6 Hz, OH), 2.48 (t, 1H, *J* 2.4 Hz, ≡CH), 1.95–1.63 (m, 8H, cyclopentylidene protons); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 122.0 (quaternary carbon of cyclopentylidene ring), 107.5 (C-1), 88.4, 85.7, 81.3 (C-2, C-3, C-4), 78.5 (CH$_2$–$\underline{C}$≡CH), 75.4 (≡CH), 64.0 (C-5), 55.3 ($\underline{C}$H$_2$-C≡), 35.8, 23.7, 23.2 (cyclopentylidene carbons). EIMS *m/z* 254 $[M]^{+\bullet}$ (16), 253 (11), 225 (100), 199 (75), 169 (93). Anal. Calcd for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Found: C, 61.54; H, 7.29.

2.1.1.2. Propargyl 2,3-O-isopropylidene-β-D-ribofuranoside (11). Compound **11** was obtained as a colorless oil (21.36 mmol, 53%); $[\alpha]_D^{20}$ −112.0 (*c* 1.0, chloroform). IR υ_{max} 3466 (υ O–H), 3284 (υ C_{sp}–H), 2941 (υ C_{sp3}–H), 2118 (υ C_{sp}–C_{sp}), 1076 (υ C_{sp3}–O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.25 (s, 1H, H-1), 4.80 (d, 1H, $J_{3,2}$ 6.0 Hz, H-3), 4.63 (d, 1H, $J_{2,3}$ 5.9 Hz, H-2), 4.41 (t, 1H, $J_{4,5}$ 3.5 Hz, H-4), 4.27 (d, 2H, *J* 2.4 Hz, C$\underline{H}_2$-C≡CH), 3.70 (dd, 1H, $J_{5b,5a}$ 12.4 Hz, $J_{5b,4}$ 3.2 Hz, H-5b), 3.61 (dd, 1H, $J_{5a,5b}$ 12.6 Hz, $J_{5a,4}$ 4.2 Hz, H-5a), 2.48 (t, 1H, *J* 2.4 Hz, ≡CH), 1.47 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 112.4 (quaternary carbon of isopropylidene group), 107.8 (C-1), 88.7, 86.0, 81.5 (C-2, C-3, C-4), 78.5 (CH$_2$-$\underline{C}$≡CH), 75.5 (≡CH), 64.0 (C-5), 55.3 ($\underline{C}$H$_2$-C≡), 26.5, 24.9 (CH_3). EIMS *m/z* 228 $[M]^{+\bullet}$ (16), 227 (29), 173 (100), 169 (50), 137 (40), 131 (71). Anal. Calcd for $C_{11}H_{16}O_5$: C, 57.88; H, 7.07. Found: C, 58.23; H, 7.22.

2.1.2. General procedures for the synthesis of tosyl derivatives (12, 13)

To a solution of propargyl derivative **10** or **11** (21.40 mmol) dissolved in anhydrous pyridine (10 mL), tosyl chloride (26.2 mmol) was added with continuous stirring. The mixture was kept at room temperature during overnight. Then, the reaction mixture was dissolved in methylene chloride and extracted with water, hydrogen chloride (5%), sodium bicarbonate (5%) and finally washed with water. The organic layer was dried with Na_2SO_4 (anhydrous) and the solvent was evaporated. The crude product