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Novel hydroxyamides and amides containing D-glucopyranose or D-fructose units: Biological assays in MCF-7 and MDST8 cell lines

Elisabete P. Carreiro [a,*], Ana R. Costa [b,c], Maria M. Cordeiro [b], Rute Martins [b], Tiago O. Pires [b], Mafalda Saraiva [b], Célia M. Antunes [b,c,d], Anthony J. Burke [a,b]

[a] Centro de Química de Évora, IIFA, Universidade de Évora, Colégio L.A. Verney, 7000 Évora, Portugal
[b] Departamento de Química, Universidade de Évora, Rua Romão Ramalho, 59, 7000 Évora, Portugal
[c] Instituto de Ciências Agrarias e Ambientais Mediterrânicas (ICAAM), Apartado 94, 7002-554 Universidade de Évora, Portugal
[d] Center For Neurosciences and Cell Biology, University of Coimbra, Portugal

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ABSTRACT

A novel library of 15 compounds, hydroxyamides and amides containing a β-D-glucopyranose (D-Gluc) or a β-D-fructose (D-Fruc) units was designed and synthesized for antiproliferative assays in breast (MCF-7) and colon (MDST8) cancer cell lines. Twelve of them were hydroxyamides and were successfully synthesized from β-D-glucuronic acid (D-GluA). Six of these hydroxyamides which were acetylated hydroxy-β-D-glucopyranuronamide **2a**–**2f** (1st Family) and the other six were their respective isomers, that is, hydroxy-β-D-fructuronamide **3a**–**3f** (2nd Family), obtained by acid–base catalyzed isomerization. These compounds have the general structure, D-Gluc—C=ONH—CHR—$(CH_2)_n$—OH and D-Fruc—C=ONH—CHR—$(CH_2)_n$—OH, where R = an aromatic, alkyl or a hydrogen substituent, with $n = 0$ or 1. Eight of these contained a chiral aminoalcohol group. Three compounds were amides containing a D-glucopyranose unit (3rd Family). SAR studies were conducted with these compounds. Antiproliferative studies showed that compound **4a**, the bromo-amide containing the β-D-glucopyranose ring, potently inhibits the proliferation of the MDST8 cells. Five compounds (**2e**, **2f**, **3d**, **3e**, and **3f**) were shown to potently selectively inhibit the proliferation of the MCF-7 cells. Compound **4b** was the only one showing inhibition in both cell lines. In general, the more active compounds were the amides and hydroxyamides containing the β-D-fructose moiety, and containing an alkyl group or hydrogen. Half-inhibitory concentrations (IC_{50}) of between 0.01 and 10 μM, were observed.

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Cancer is currently one of the most lethal diseases in the world. Chemotherapy is the most common treatment used for this disease, which is usually integrated with surgery and radiation therapy. For this reason cancer drug discovery is extremely important. Carbohydrates are one of the most abundant biomolecules on our planet, and currently carbohydrate scaffolds are being developed for new drugs, as they are intricately involved in inter- and intracellular communication processes.[1] Synthetic and natural compounds containing the D-glucuronic acid unit have wide ranging pharmacological activities, that include antibiotic, antiviral, antibacterial and antitumoral activity. Gougerotin, Bagougeramine A and B are some examples[2] of biologically active compounds containing a 4-amido-glucuronamide motif. β-Glucuronides are used like prodrugs for selective cancer chemotherapy, the active drug is released by the action of endogenous intracellular β-glucuronidase enzymes (they show elevated concentrations in tumors compared to normal tissues), and this reduces the toxicity of the chemotherapy process.[3] Recently, El-Nezhawy et al.[4] synthesized and evaluated antitumoral activity of some novel acetylated and deacetylated D-glucuronic acid derivatives and screened them for antitumor activity using MCF-7, TK-10 and UACC-62 cell lines. Although a molecular target was not indicated, these studies revealed some promising compounds for human breast adenocarcinoma (MCF-7) growth inhibition such as *N*-(pyridine-4-yl)-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronamide (**A**) and allyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**B**) (Fig. 1). Hydroxamic acid derivatives play a very important role in cancer therapy, they are known to be inhibitors of histone deacetylases (HDACs).[5] For example, suberanilohydroxamic acid – SAHA (vorinostat) (Fig. 1) is one of most clinically advanced HDAC inhibitors.[6] A large number of preclinical studies demonstrate that SAHA can induce growth arrest, differentiation and apoptosis in a wide range of cancer cell lines by re-expression of genes (i.e., thioredoxin-binding

* Corresponding author. Tel.: +351 266745312; fax: +351 266702306.
E-mail address: bete_carreiro@yahoo.com (E.P. Carreiro).

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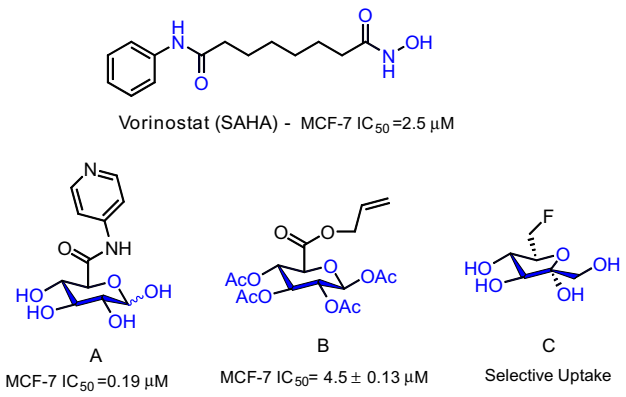

Figure 1. Some compounds with anticancer activity.

protein-2).[7] SAHA contains a hydroxamic acid group (R–CO-NH-OH), which coordinates with Zn and can also form hydrogen bonds with the amino acids residues of the HDAC active site, containing the triad; Tyr306, His142, His143.[8] The IC_{50} value obtained for SAHA against MCF-7 was 2.5 μM.[9]

Fructose derivatives also have an important biological application, such as for fructose transporter GLUT5[10] inhibition and anticonvulsant activity. Fluorescent fructose derivatives were used as diagnostic reagents for breast cancer.[11] Based on recent research, the overexpression of GLUT5 is an intrinsic characteristic of some breast cancer cell lines, but not for normal tissue.[12] This selective expression could represent a potential therapeutic target. A few examples of the successful application of this concept, employing selective uptake of fructose analogues 6-deoxy-6-fluoro-D-fructose (**C**) (Fig. 1)[11a] and their phosphorescent metal complexes have appeared in the literature.[13] Most workers in this field synthesize these fructose analogues by isomerization of glucose. Various methods have been described, such as with enzymes,[14] Lewis acids,[15] zeolites[16] and ion-exchange resins.[17]

Our goal was to synthesize three novel families, two of which consisted of hydroxyamide derivatives of D-glucuronic acid, 1st Family (compounds **2a–2f**) and the 2nd Family (compounds **3a–3f)**. The 3rd Family (compounds **4a–4c)** was composed of amide derivatives of D-glucuronic acid containing a bromo or pyrrolidine ring on the amide tether (Fig. 2). We decided to introduce pyrrolidine rings into these molecules given that this unit behaves generally as an important biostere, present in many molecules active against cancer.[18] Our molecules were designed based on the two biomolecular targets described above. In the case of HDAC inhibition, the hydroxyamide and amide attached to the glucopyranose unit, the –OH and –NH– were designed to function like a zinc-binding group, forming a H-bond with key H-bonding amino acid residues in the active site. In the case of the fructose derivatives, they were designed as GLUT5 transporter inhibitors. The fructose analogues are expected to be recognized by the GLUT5 receptor and the hydroxyl groups important for H-bonding and binding in the central cavity.[19] The hydroxyl group is crucial for establishing H-bonds in the active site of the potentials biological targets, thus improving the compounds inhibitory effect and consequently its antitumor activity. Eight of the 12 compounds in our library have a stereogenic center in the hydroxyamide side chain group. These molecules were evaluated in antiproliferative tests in the human breast adenocarcinoma (MCF-7) and colon adenocarcinoma (MDST8) cell lines. 5-Fluorouracil (5-FU), which is an antineoplasic agent, was used as a bench-mark.

The 1st and 3rd Families of compounds **2a–2f** and **4a–4c**, respectively, were successfully synthesized according to the general procedure reported in Scheme 1. Compound **1**, 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronic acetic anhydride was synthesized from commercial D-glucuronic acid by treating it with AcO_2/I_2 using the method described by Tosin and Murphy.[20] Using the method initially developed by El-Nezhawy et al.,[4] compound **1** was reacted with the hydroxyamines **I.a–f** or amines **II.a–c** furnishing the corresponding amidoalcohols **2a–2f** and amides **4a–4c**, in very good yields, between 24% and 85%. The structure of the compounds **2a–2f** and **4a–4c**, was confirmed by NMR spectroscopy.

^{1}H NMR analysis confirmed the structures of all the acetylated compounds **2a–2f** and **4a–4c**, in general for all compounds, the anomeric proton appears as a doublet around 5.70 ppm (J = 8 Hz) which corresponds to the diaxial orientation for both the anomeric proton and the adjacent proton (H-2), thus confirming the β-anomeric configuration.

^{13}C NMR was used also to characterize the acetylated compounds. These compounds were characterized by a signal at 91.2 ppm corresponding to the anomeric carbon of the β-D-glucopyranose ring.

The second Family, was synthesized by isomerization of compounds **2a–2f**, (Scheme 2) using NaOMe to deprotect the acetyl groups and at the same time opening the pyran ring to form the aldose (1st step) or the enolate. The proposed mechanism is depicted in Scheme 3. The strong acid resin (H^+), Amberlite IR120, was used for ionic exchange of the Na^+ with H^+, further keto-enol tautomerism to form the ketone followed by cyclization onto the ketone (2nd Step) was proposed to give **3c**.

NMR studies were conducted to probe the reaction mechanism. Compound **2c** was dissolved in MeOD and NaOMe (5.5 equiv) and analyzed by ^{1}H NMR. After 30 min a strong signal at 10 ppm appeared indicating the presence of the aldehyde or enolate (as the base could probably form the enolate under these conditions). Amberlite IR120 (H^+) was then added, analysis by ^{1}H NMR showed the presence of a mixture of anomers, consisting of 80% β-D-fructose and 20% of α-D-fructose with the presence of a vestigial quantity of D-glucopyranose isomers. We used anhydrous conditions to avoid amide hydrolysis. The isomerization of compounds **2a–f** also showed an 8:2 mixture of the β/α anomers by ^{1}H NMR spectroscopy.

All our compounds were evaluated for their antiproliferative activity against two cell lines, human breast adenocarcinoma (MCF-7) and human colon adenocarcinoma (MDST8). Batches of cells were cultured for 72 h with the compounds in the concentration range of 1×10^{-9}–1×10^{-5} M. Cell proliferation was determined using Cell Counting Kit (Sigma). 5-FU was used as positive control for both cell lines. Whenever possible the IC_{50} values, corresponding to a 50% inhibition of proliferation, were determined by fitting the results to a dose–response growth sigmoidal curve using Origin Software (OriginLab Corporation). The antiproliferative results are representative of n = 2–3 independent experiments with 5 replicates each. 5-FU showed IC_{50} values of 6.4×10^{-8} M and 5.2×10^{-7} M for MDST8 and MCF-7, respectively (data not shown). At the highest concentration used, 1×10^{-5} M, 5-FU inhibited colon and breast cell growth by 83 and 60%, respectively (Fig. 3).

Antiproliferative activity at a concentration of 1×10^{-8} M (50% of inhibition) in the MDST8 cell line was only observed in the case of **4a** containing a Br, this compound had more than 90% of an antiproliferative effect at 1×10^{-5} M, and was more active than 5-FU Hydroxy-D-fructuronamide derivative **3c** with an isopropyl substituent, had 80% of an antiproliferative effect at 1×10^{-5} M against MDST8 cell line, and was more active than its isomer **2c**. Compound **3d** had an antiproliferative effect in the MCF-7 cell line and showed similar inhibition as 5-FU. In the case of those compounds with aromatic substituents, like **2a** and **3a** (benzyl) and **2b** and **3b** (phenyl), they didn't show any antiproliferative activity in the MDST8 and MCF-7 cell lines. **2e–f** and **3e–f**, were shown to

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