European Journal of Medicinal Chemistry 53 (2012) 398–402

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of new 2-galactosylthiazolidine-4-carboxylic acid amides. Antitumor evaluation against melanoma and breast cancer cells

Arménio C. Serra [a,*], António M. d'A. Rocha Gonsalves [a], Mafalda Laranjo [b,c], Ana M. Abrantes [b,c], Ana C. Gonçalves [c,d], Ana B. Sarmento-Ribeiro [c,d], M. Filomena Botelho [b,c]

[a] Departamento de Química, Universidade de Coimbra, Rua Larga, 3004 535 Coimbra, Portugal
[b] Unidade de Biofísica, IBILI, Faculdade de Medicina da Universidade de Coimbra, Azinhaga de Santa Comba, 3000 548 Coimbra, Portugal
[c] CIMAGO, Faculdade de Medicina da Universidade de Coimbra, Rua Larga, 3004 Coimbra, Portugal
[d] Unidade Curricular de Biologia Molecular Aplicada, Faculdade de Medicina da Universidade de Coimbra, Azinhaga de Santa Comba, Celas, 3000 548 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 8 November 2011
Received in revised form
2 April 2012
Accepted 4 April 2012
Available online 13 April 2012

Keywords:
Thiazolidine
MCF7 cancer cells
A375 Melanoma
Cytotoxicity
Triple negative

ABSTRACT

A set of 2-galactosylthiazolidine-4-carboxylic acid amides was synthesized with different length for the carbon chain amide moiety. The cytotoxicity of the molecules was evaluated against A375 melanoma and MCF7 breast cancer cell lines. For the derivatives tested, the one that contains a C_{16} amide carbon chain is the most active with an IC_{50} of 17.0 μM for A375 and 5.8 μM for MCF7. This compound also shows cytotoxicity in the triple negative cancer cell line HCC1806. The selectivity of the compounds was assessed by comparing the cytotoxicity in cancer cell line versus in a fibroblast cell line. Flow cytometry studies show the activation of apoptotic pathways and also DNA damages with blockage of the cell cycle in the S-phase and appearance of peaks in G0/G1-phase.

1. Introduction

The thiazolidine ring (**1**) [1] and related cycles such as thiazolidinone (**2**) [2] thiazoline (**3**) [3] and thiazole (**4**) [4] (Fig. 1) are known to be part of biologically active compounds with diversified properties: antioxidant [5], mucolytic [6], antimicrobial [7], neuroprotective [8], neuraminidase and hydroxysteroid dehydrogenase 1 inhibitors [9], protecting agent against hepatotoxicity [10], urotoxicity [11] and γ-radiation induced toxicity [12]. Also, particularly noteworthy is the cytotoxicity activity of 2-arylthiazolidine carboxylic acid derivatives which showed to be effective against melanoma and prostate tumors cells in *in vitro* studies [13] and also against A375 melanoma cells in *in vivo* experiments [14].

Experimental results [14] and QSAR studies [15] pointed to a positive effect of the presence of a long-chain carboxamide group at position 4 of the thiazolidine ring. The mechanism of action of these derivatives is unknown [14]. The first studies [13] suggested some inhibitory role of lysophosphatidic acid action [16] but this molecule has no effect on cellular proliferation. Instead, it has some role in cancer cellular migration [17].

Most of the cancer cell lines over-express glucose transporters due to high energy demands of uncontrolled cell growth [18]. Thereby, the incorporation of sugar parts in chemical entities in order to get better biological effects is a common practice in several anticancer approaches [19]. In this work we prepared a series of a 2-galactosylthiazolidine derivatives with 4-carboxamide substituents with different carbon chain lengths and studied their cytotoxicity activity against two cell lines.

2. Chemistry

The straightforward condensation of L-cysteine (**5**) with D-galactose (**6**) following described procedures [20] gives the corresponding 2-galactosylthiazolidine-4-carboxylic acid (**7**) as a mixture of diastereoisomers due to generation of a chiral center at C-2. Treating this derivative with a mixture of acetic anhydride/sodium acetate originates the penta-acetylgalactosylthiazolidine lactone (**8**). From the reaction of **8** with several alkylamines corresponding alkylamides (**9**–**12**) with moderate to good yields (80–89%) were obtained (Scheme 1).

* Corresponding author. Tel.: +351 239 854480; fax: +351 239 826069.
E-mail address: armenio.serra@gmail.com (A.C. Serra).

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doi:10.1016/j.ejmech.2012.04.003

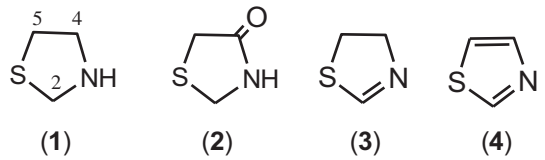


Fig. 1. Structure of compounds of thiazolidine family.

3. Results and discussion

The cytotoxicity of the amide derivatives (**9**–**12**) was evaluated against A375 melanoma and MCF7 breast cancer cells. Increasing concentrations of compounds from 1 μM to 100 μM were incubated with cells during 48 h followed by analysis of the cell proliferation using the MTT assay [21] (Fig. 2).

The results show that compounds cause a concentration-dependent inhibition of cell proliferation, being more active against MCF7 cell line than with A375 cell line (Fig. 2; Table 1). There are significant differences in the anti-proliferative activity between compounds for each cell line (A375: $p < 0.001$; MCF7: $p < 0.001$). Pair-wise comparison shows that compound **11** with the C_{16} carbon chain is the most active (A375: $p < 0.05$; MCF7: $p < 0.01$) and compound **9** with a C_{12} carbon chain is the least active compound. A similar behavior between chain length and cytotoxicity activity was previously observed for a prostate cancer cell line. Also it is not expected that the possible presence of different diastereoisomers for each compound influence the cell viability as showed in the case of other thiazolidines [14].

The evaluation of the selectivity of the compounds was carried out comparing the cytotoxicity of compounds against a fibroblast cell line (HFF1). Regarding the selectivity index (SI), compounds are more selective for MCF7 cells than for A375 cells. Compound **9** with the shortest C_{12} carbon chain is the least selective of all. Compound **11** showed the highest selectivity, 5.8 for MCF7 cells and 1.8 for A375 cells.

Triple negative breast cancer is a very aggressive cancer with relatively poor prognosis that has a high incidence in younger woman. Its specific characteristics make it resistant to current and hormonal based therapies [22]. The most active compound **11** was tested against HCC1806 triple negative breast cancer cell line (Fig. 3) and showed to be less active than for MCF7 cell line. Nevertheless, the value of 12.6 ± 1.8 μM for the IC_{50} is encouraging taking into account this particular type of cancer.

The activity of compound **11** compares favorably with known standard chemotherapeutic agents with 48 h incubation time. In this condition docetaxel presents IC_{50} of 11.9 μM and 8.4 μM for MCF7 and HCC1806 respectively. With A375 cells with this incubation time dacarbazine shows an IC_{50} greater than 500 μM [23].

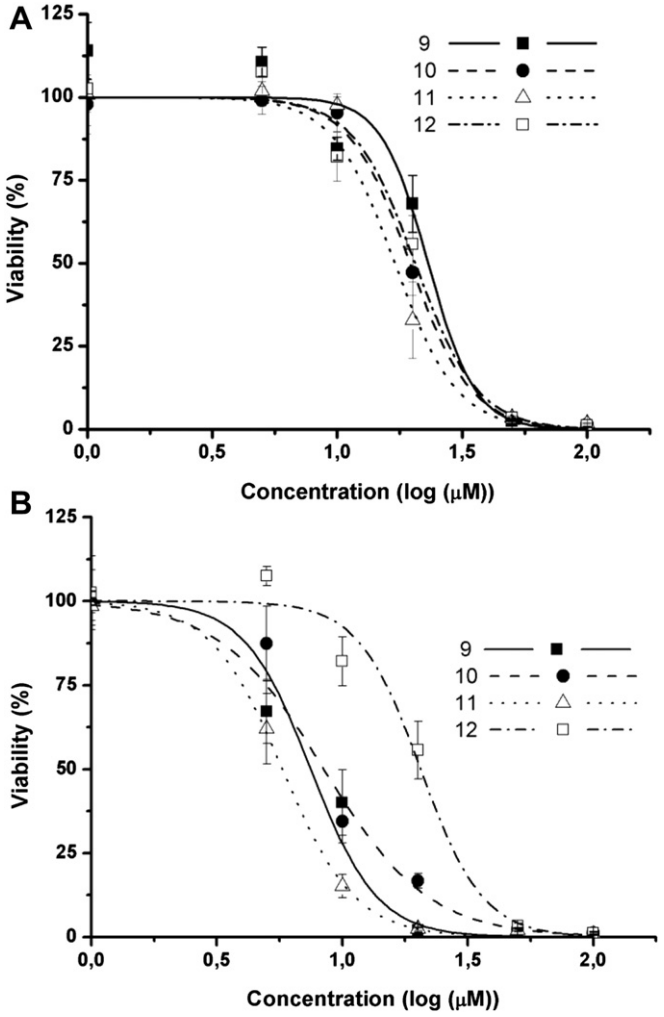
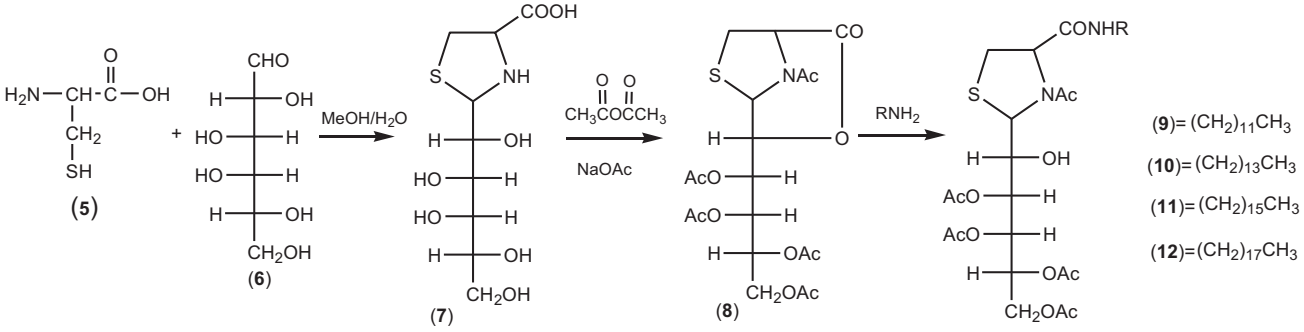


Fig. 2. Results of cytotoxicity (MTT assay) of compounds **9**–**12** against A375 (A) and MCF7 (B) cancer cells. Viability values were calculated using control cultures incubated only with DMSO (<1%). Results are presented as mean ± SD ($n = 6$).

Flow cytometry analysis was performed to evaluate the effect of compound **11** on viability and cell cycle progression. A375 cell cultures were incubated during 48 h with compound **11** at a concentration of 17 μM and 30 μM. Cell viability was accessed using annexin-V and propidium iodide incorporation (Fig. 4) [24]. For cell cycle analysis PI/RNase solution (PI/RNase, immunostep, Spain) was used as described by the supplier. Statistical analysis was performed by the Mann–Whitney test using the PASW Statistics 18.



Scheme 1. Synthesis of penta-acetylgalactosylthiazolidine amides (**9**–**12**).