European Journal of Medicinal Chemistry 70 (2013) 411–418

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

# Synthesis of 1,3-thiazine-2,4-diones with potential anticancer activity

Misael Ferreira [a,1], Laura Sartori Assunção [b,1], Fabíola Branco Filippin-Monteiro [b], Tânia Beatriz Creczynski-Pasa [b,1], Marcus Mandolesi Sá [a,*,1]

[a] Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil
[b] Departamento de Ciências Farmacêuticas, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil

ARTICLE INFO

*Article history:*
Received 1 September 2013
Received in revised form
3 October 2013
Accepted 7 October 2013
Available online 14 October 2013

*Keywords:*
1,3-Thiazine-2,4-dione
Anticancer activity
Leukemia
Apoptosis

ABSTRACT

2-Amino-1,3-thiazin-4-ones were subjected to acetylation followed by mild acid hydrolysis to give compounds containing the 1,3-thiazine-2,4-dione core. The potential of these *S*,*N*-containing heterocycles as antitumor agents against human cancer cell lines, among other types, was evaluated. The results show that phenyl- and naphthyl-substituted thiazinediones presented selective antitumoral activity against leukemia cells. These compounds caused cell death with DNA fragmentation and the mechanism of action seems to involve caspase cascade activation, imbalance in intracellular $Ca^{2+}$ and mitochondrial metabolism, and/or endoplasmic reticulum stress.



## 1. Introduction

Cancer is one of the most prevalent causes of death in several countries [1]. Despite the huge efforts to implement novel chemotherapeutic strategies for the treatment of different types of cancer, these diseases remain one of the major concerns worldwide. Consequently, there is an urgent need to find unexplored classes of substances with selective action against cancer cells. The regulation of the apoptotic pathways associated with cell death is known as an important approach to understand a great variety of medical illnesses, including cancer [2,3]. Therefore, the identification of apoptosis inducers to combat cancer cells represents an attractive strategy to the discovery and development of potential antitumor agents [4,5].

Many heterocyclic compounds containing the S–C–N framework are related to an extensive spectrum of biological activities [6–8]. In particular, thiazolidine-2,4-diones (TZDs, Fig. 1) are an important group of *S*,*N*-containing heterocycles, which are high-affinity ligands for peroxisome proliferator-activated receptor gamma (PPARγ) [9,10], a family of nuclear receptors that play a pivotal role in regulating gene expression. TZDs, such as the glitazones, are recognized for their ability to display antihyperglycemic activity and have been widely employed for the treatment of diabetes [11–13]. Conversely, recent studies have shown that TZDs also reduce multiple types of cancer, including breast, colon, lung, prostate, and stomach [14–18]. However, biological activity related to 1,3-thiazine-2,4-diones (Fig. 1), the 6-membered heterocycles homologous to TZD, is less frequently reported [19,20], the only exception being 5-ethyl-6-phenyl-1,3-thiazine-2,4-dione (Dolitrone [21]), an anesthetic employed as a pain-killer.

In previous research, we explored cell death pathways, mainly via apoptosis [22–24], including studies involving the development of mild and efficient methodologies to construct the 1,3-thiazine core [25,26] from allylic bromides derived from the Morita–Baylis–Hillman (MBH) reaction [27]. Consequently, we focused on the development of a facile strategy for the synthesis of 1,3-thiazine-2,4-diones and carried out a broad study on their involvement in apoptotic processes related to antitumor activity.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic plan employed to prepare the target 1,3-thiazine-2,4-diones **1** is presented in Scheme 1 and begins with the allylic bromide **2**, a readily available precursor obtained through the

* Corresponding author. Tel.: +55 48 37216844; fax: +55 48 37216850.
*E-mail address:* marcus.sa@ufsc.br (M.M. Sá).
[1] As first authors, M.F. and L.S.A. participated considerably in the parts of the study related to chemistry and biology, respectively, along with the senior researchers M.M.S. and T.B.C.P.

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.10.017

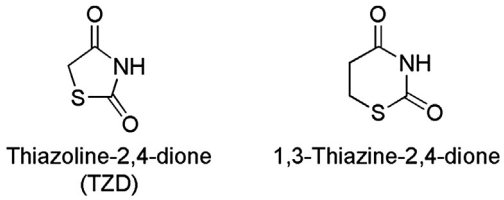



**Fig. 1.** Bioactive heterocyclic compounds containing the S–C–N framework.

reaction of α-methylene-β-hydroxyesters **3** (MBH adducts [27]) with LiBr in acidic medium at room temperature [28–30]. Treatment of the allylic bromides **2** with thiourea in a 3:1 mixture of acetone:water at room temperature followed by the addition of a mild aqueous base to the pre-formed isothiouronium salts **4** gave high yields of the corresponding 2-amino-1,3-thiazin-4-ones **5** as insoluble solids [25,26].

Attempts to directly transform the key 2-aminothiazin-4-one **5a** into thiazine-2,4-dione **1a** by hydrolysis in acidic medium [31,32] led to modest yields (up to 30%). In view of this shortcoming, a two-step (*one-pot*) method had to be developed, which consisted of the initial acetylation of 2-aminothiazin-4-one **5a** followed by mild hydrolysis of the acetylated intermediate. The 2-aminothiazin-4-one **5a** was then acetylated using acetic anhydride in ethanol to give an approximately 1:1 mixture of two (out of four) possible acetylated isomers **6**–**9** (Scheme 1), as noted by the two singlets corresponding to distinct acetyl groups at 2.03 and 2.27 ppm in the $^1$H NMR spectrum of the crude reaction. All modifications to the reaction parameters led to mixtures of acetylated products that underwent slow hydrolysis after prolonged exposure to air, while attempts to obtain an isolated product by column chromatography in silica gel failed due to extensive decomposition. On the other hand, the simple addition of aqueous HCl to the pre-formed mixture of acetylated products (**6**, **7**, **8**, and/or **9**) promoted clean and convergent hydrolysis to 5-benzylidene-1,3-thiazine-2,4-dione **1a** in 82% yield (Table 1).

This sequential acetylation/hydrolysis protocol was then extended to other thiazinones **5** with the exclusive formation of the expected 1,3-thiazine-2,4-diones **1** (Scheme 1 and Table 1; see also the Supplementary information). One of the most remarkable aspects of this methodology is its simplicity in terms of setting up the reaction as well as the subsequent work-up and purification steps, affording high-purity products in good isolated yields.

### 2.2. Cell toxicity

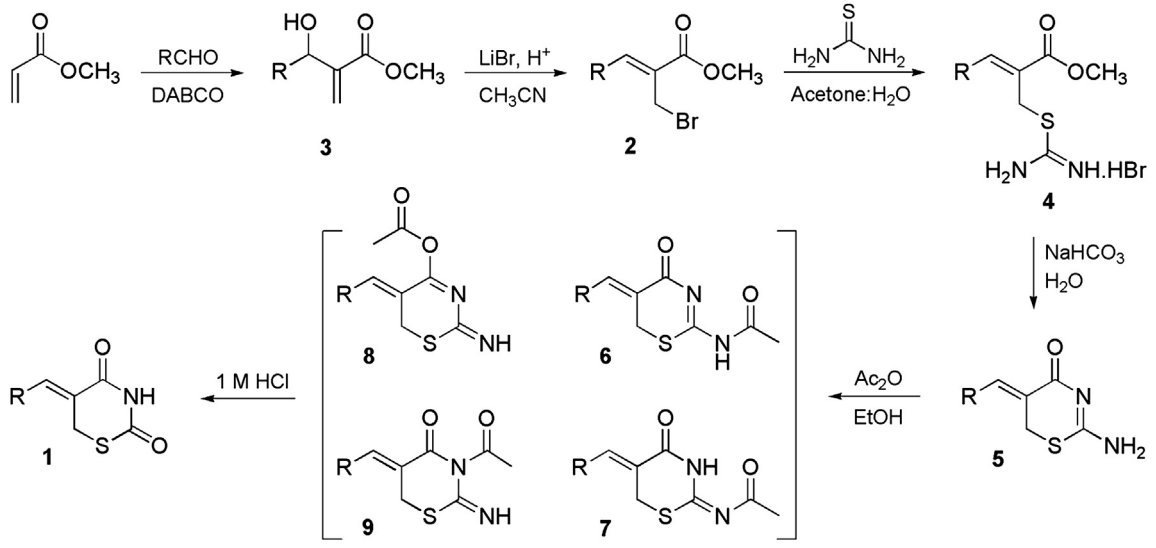
Thiazinediones **1a**–**h** were tested in cancer cell lines that are widely used as *in vitro* cancer models, including L1210 (murine lymphocytic leukemia), CCRF-CEM (human acute lymphoblastic leukemia), B16F10 (murine melanoma), MDA-MB-231 (human breast cancer) and a non-tumoral cell line Vero (kidney fibroblast). The $CC_{50}$ (concentration of the compound which caused 50% of cell death) for each cell line was determined by the MTT method followed by non-linear regression. In addition, the selectivity index (SI) was calculated based on the equation:

$$SI = \frac{CC_{50}\ \text{non tumoral cells}}{CC_{50}\ \text{tumoral cells}}$$

The results are summarized in Table 2. Compounds **1a** and **1g** demonstrated toxic effect at lower concentrations not only against leukemia (L1210) cells (35 and 28 μM, respectively) but also toward melanoma (B16F10) cells (50 and 45 μM, respectively). In addition, higher selectivity (SI ≥ 2.0) was observed in both cases, compared with the other screened thiazinediones. Because compounds **1a** and **1g** showed high selectivity toward leukemia and melanoma, among other cancer cell lines, cell cycle analysis by flow cytometry was performed to evaluate possible alterations in these cells. Cells were treated with 35 μM of **1a** and 28 μM of **1g** for 24 h. As shown in Fig. 2, compounds **1a** and **1g** caused cell fragmentation in leukemia cells, but no alterations were seen in melanoma cells. In the light of these findings, the subsequent investigation on the mechanism of action was carried out using leukemia cells.

#### 2.2.1. Mechanism of action

To investigate whether the observed cell death caused by **1a** and **1g** in leukemia cells was due to necrosis or apoptosis, quantitative and qualitative evaluations were carried out. Initially, a morphological evaluation employing fluorescence microscopy with double-staining acridine orange/ethidium bromide was performed. Acridine orange (AO) easily permeates the cell membrane and results in a green fluorescence of both the nucleus and cytoplasm. Ethidium bromide (EB), however, does not permeate the




**Scheme 1.** Synthesis of 1,3-thiazine-2,4-diones.