European Journal of Medicinal Chemistry 87 (2014) 745–758

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

A new goniothalamin *N*-acylated aza-derivative strongly downregulates mediators of signaling transduction associated with pancreatic cancer aggressiveness

Rosimeire Coura Barcelos [a, 1], Karin Juliane Pelizzaro-Rocha [b, 1], Julio Cezar Pastre [a, 1], Marina Pereira Dias [b], Carmen Veríssima Ferreira-Halder [b], Ronaldo Aloise Pilli [a, *]

[a] Department of Organic Chemistry, Chemistry Institute, University of Campinas, CP 6154, 13083-970, Campinas, SP, Brazil
[b] Department of Biochemistry, Biology Institute, University of Campinas, 13083-862, Campinas, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 2 July 2014
Received in revised form
5 September 2014
Accepted 8 September 2014
Available online 30 September 2014

Keywords:
Goniothalamin
N-acylated aza-derivatives
Pancreatic cancer
Panc-1 cells
AXL kinase
HIF-1α

ABSTRACT

In this study, a novel concise series of molecules based on the structure of goniothalamin (**1**) was synthesized and evaluated against a highly metastatic human pancreatic cancer cell line (Panc-1). Among them, derivative **8** displayed a low IC_{50} value (2.7 μM) and its concentration for decreasing colony formation was 20-fold lower than goniothalamin (**1**). Both compounds reduced the levels of the receptor tyrosine kinase (AXL) and cyclin D1 which are known to be overexpressed in pancreatic cancer cells. Importantly, despite the fact that goniothalamin (**1**) and derivative **8** caused pancreatic cancer cell cycle arrest and cell death, only derivative **8** was able to downregulate pro-survival and proliferation pathways mediated by mitogen activated protein kinase ERK1/2. Another interesting finding was that Panc-1 cells treated with derivative **8** displayed a strong decrease in the transcription factor (c-Myc), hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) protein levels. Notably, the molecular effects caused by derivative **8** might not be related to ROS generation, since no significant production of ROS was observed in low concentrations of this compound (from 1.5 up to 3 μM). Therefore, the downregulation of important mediators of pancreatic cancer aggressiveness by derivative **8** reveals its great potential for the development of new chemotherapeutic agents for pancreatic cancer treatment.

1. Introduction

Pancreatic cancer is the tenth most commonly diagnosed cancer, and ranks as the fourth cause of deaths related to cancer in the United States [1]. Pancreatic cancer aggressiveness is closely related to quick progression leading to metastasis and, so far, minimal progress has been made to improve diagnosis and treatment. Tumor resection is indicated only when the tumor is confined to the pancreas, without lymphatic or hematogenous spread. Unfortunately, the majority of patients are diagnosed with pancreatic cancer at a locally advanced or metastatic stage, excluding a curative surgical resection [2]. Moreover, conventional therapies, such as chemotherapy and radiotherapy, present low efficacy and have little effect on the development of this malignancy [3]. Therefore, life expectancy of patients diagnosed with pancreatic cancer is very short.

The high heterogeneity of this cancer is mainly due to the dysregulation of multiple pathways governing fundamental cell processes [4]. To date, a number of mediators have been associated with pancreatic cancer aggressiveness. Among them, the receptor tyrosine kinase AXL is upregulated in several types of cancer, including pancreatic cancer, and has been implicated with aggressive phenotypes [5a–d]. Moreover, downstream signaling pathways of AXL contribute to a variety of oncogenic mechanisms,

Abbreviations: GSH, glutathione; AXL, tyrosine–protein kinase receptor; ERK1/2, extracellular signal-regulated kinases 1 and 2; HIF-1α, hypoxia-inducible factor-1α; ROS, reactive oxygen species; BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma protein-2; PARP-1, poly (ADP-ribose) polymerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; STAT3, activator of transcription 3; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; VEGF, vascular endothelial growth factor; RB, retinoblastoma protein; GAS6, growth arrest-specific 6; IAP, inhibitor of apoptosis proteins; BCL-xl, B-cell lymphoma-extra large; Mcl-1, myeloid cell leukemia sequence 1; MTT, thiazolyl blue tetrazolium blue; DMEM, Dulbeco's modified Eagle's medium; FSB, fetal serum bovine; PMSF, phenylmethanesulfonyl fluoride; HF, hydrogen fluoride.

* Corresponding author.
E-mail address: pilli@iqm.unicamp.br (R.A. Pilli).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.ejmech.2014.09.085
0223-5234/

including cell survival, proliferation, migration, invasion, angiogenesis, chemoresistance and metastasis [6], indicating that AXL is a relevant therapeutic target. Besides that, the presence of a hypoxic microenvironment in solid tumors has been pointed out as the cause of resistance to conventional chemotherapy and radiotherapy, as well as an increased predisposition to metastasis [7]. The major transcriptional regulator of the adaptive response to hypoxia is the hypoxia inducible factor-1α (HIF-1α), which has been shown to correlate with metastasis in several tumors, including pancreatic cancer [8a,b]. Constitutively expressing HIF-1α provides resistance to chemo- and radiotherapy, increasing *in vivo* tumorigenicity [9].

Concerning the drug development for cancer treatment, it is clear that new therapeutic strategies are still needed to overcome the resistance and tumor aggressiveness associated with rapid disease progression and metastasis. In this context, natural products remain a source of inspiration for the development of novel chemical entities for cancer treatment [10]. The styryl lactones have been considered as a privileged class of structurally simple compounds with a broad spectrum of biological activities [11]. Among them, goniothalamin (**1**, Fig. 1), originally isolated from several species of *Goniothalamus* (Annonaceae), exhibited antiproliferative and cytotoxic activities against a variety of tumor cell lines, including kidney, prostate, breast, lung, liver cancer and leukemia [12a–e]. *In vivo* studies performed in a solid tumor experimental model in mice confirmed the potential of goniothalamin (**1**) as a lead compound for new chemotherapeutic agents [12e]. The synthesis of goniothalamin derivatives have culminated in the development of compounds with higher potency and selectivity, pointing out the structural requirements for the cytotoxic activity [13a–h]. From these studies, it is evident that the α,β-unsaturated δ-lactone is a key feature due to its ability to act as a Michael acceptor in biological systems [14a,b].

In a recent study from our group [13g], a series of aza-goniothalamin derivatives was prepared and evaluated against a variety of tumor cell lines. The synthesis of aza-analogues is a strategy used to prepare compounds with better bioavailability and several reports in the literature have validated this approach. For example, in view of its increased stability due to isosteric replacement of the lactone by a lactam ring, the epothilone B analogue ixabepilone was approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with metastatic or locally advanced breast cancer resistant to treatment with anthracycline and taxane [15a,b]. From our study, it is evident that the introduction of the lactam ring significantly reduced the antiproliferative activity for all cancer cell lines evaluated. Indeed, no compound in the aza-series displayed significant cytotoxic activity, even at the highest concentration used [13g]. This disappointing result was rationalized in terms of the much lower electron deficiency of the α,β-unsaturated δ-lactam unity, having a deleterious effect on the biological profile of the goniothalamin aza-analogues. In order to check this hypothesis, we proposed that the introduction of acyl groups at the nitrogen atom of aza-goniothalamin would restore or even surpass the cytotoxic activity already observed for goniothalamin derivatives. In fact, recently Raj et al. reported that piperlongumine (**PL**) selectively kills cancer cells, including pancreatic cancer cells, over non-cancerous cells and suggested that it elevates cellular levels of reactive oxygen species (ROS) selectively in cancer cell lines [16a–c]. Later on, the same group reported that the presence of the electrophilic double bond in the dihydropyridin-2-one ring is critical for the cytotoxic effect, and not only the ability to elevate ROS levels, as analogues lacking the above mentioned double bond were able to elevate ROS levels but displayed reduced cytotoxicity. The authors suggested that ROS-independent mechanisms may also contribute to trigger apoptosis and protein glutathionylation associated with piperlongumine's cytotoxicity [16b].

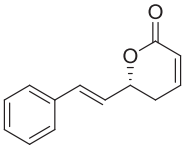

Fig. 1. Chemical structure of the natural styryl lactone (*R*)-goniothalamin (**1**).

In view of our great interest in identifying small molecules able to control the aggressiveness of pancreatic cancer, Panc-1 cells were selected for the screening of new potent antipancreatic cancer agents. Among the pancreatic cancer cell lines, Panc-1 cells are known to be the least differentiated and the most aggressive ones, presenting the highest resistance to the chemotherapeutics available for pancreatic cancer treatment [17].

Taking into consideration the antitumoral effect of goniothalamin (**1**) in a broad spectrum of cancer cell lines, in this study we report the synthesis of a novel concise series of compounds based on the structure of goniothalamin (**1**), namely *N*-acylated aza-goniothalamin derivatives, and its evaluation against the resistant human pancreatic cancer cell line (Panc-1 cells). The effect of goniothalamin (**1**) and its most potent derivative **8** was further investigated on the signal transduction pathways known to be related with the pancreatic tumor aggressiveness.

2. Results and discussion

2.1. Chemistry

Firstly, aza-goniothalamin (**2**) was prepared on gram scale using the conditions described in our previous work [13g]. Then, the preparation of *N*-acylated aza-goniothalamin derivatives **3**–**14** was carried out according to Scheme 1. *N*-Acylation was performed based on the reaction between the anion derived from deprotonation of aza-goniothalamin (**2**) with LHMDS and an acyl chloride or methyl chloroformate. Alternatively, mixed anhydride was generated *in situ* after treatment of the carboxylic acid of interest with pivaloyl chloride. The *N*-acylated derivatives **3**–**11** were obtained in good to excellent yields (53–98%). For phenolic derivatives, removal of the TBS protecting group was accomplished using a solution of hydrogen fluoride pyridine complex (Scheme 1), affording compounds **12**–**14**, which were obtained in good yields (up to 94%).

2.2. Biological evaluation

2.2.1. Effect of goniothalamin (*1*), aza-goniothalamin (*2*) and their derivatives on the Panc-1 cells viability

Goniothalamin (**1**), aza-goniothalamin (**2**) and their derivatives were evaluated for antitumor activity against Panc-1 cells by the MTT reduction assay, after 24 h of treatment. Fig. 2 represents the concentration–response curves for goniothalamin (**1**), aza-goniothalamin (**2**) and *N*-acylated aza-derivatives **3**–**8** and **12**–**14**, while chemical structures and IC_{50} values for each compound are presented in Table 1.

Goniothalamin (**1**) reduced Panc-1 cells viability and displayed an IC_{50} value of 65 μM after 24 h of treatment (Fig. 2). On the other hand, the isosteric substitution of the lactone ring by the corresponding lactam, affording aza-goniothalamin (**2**), completely abolished the *in vitro* antitumor activity of goniothalamin (**1**) (Fig. 2). Indeed, no effect on cell viability was observed when aza-goniothalamin (**2**) was evaluated, even at the highest