MARINE CYANOBACTERIA

ISOLATED FROM THE PORTUGUESE COAST AS A SOURCE OF BIOACTIVE COMPOUNDS: CYTOTOXICITY AGAINST HUMAN TUMOUR CELLS AND NORMAL CELLS

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Resumo

As cianobactérias marinhas constituem uma fonte promissora de compostos com potencial antitumoral. Nos últimos anos, um arsenal de compostos tem sido revelado, alargando a gama de novos compostos ou estruturas de base ao desenvolvimento de novas drogas. Neste projeto tivemos como objetivo avaliar o potencial de cianobactérias da coleção LEGE-CC como fonte de compostos antitumorais. Foi também nosso objetivo avaliar os mecanismos que conduzem à inibição do crescimento celular nomeadamente o envolvimento da apoptose. O projeto resultou na seleção de estirpes promissoras com potencial bioativo. Os compostos hierridina B, hierridina C e bartolosidas foram isolados. A hierridina B foi citotóxica para a linha celular de adenocarcinoma do cólon HT-29, reduzindo a atividade mitocondrial e paragem do ciclo celular. A hierridin C não revelou citotoxicidade. No entanto, este composto foi testado quanto à sua atividade antiplasmodial, de onde resultou uma patente.

Abstract

Marine cyanobacteria have proved to be an important source of potential antitumour drugs. In recent years, a plethora of compounds were isolated revealing a great scope for the discovery of novel compounds or lead structures for the development of new drugs. In this project we aimed to evaluate the potential of cyanobacteria strains of the LEGE-CC as a source of anticancer compounds. It was also our objective to evaluate the mechanisms that potentially leads to the inhibition of cell growth namely the involvement of apoptosis. The project led to the selection of promising strains with bioactive potential. The compounds hierridin B, hierridin C and bartolosides were isolated. Hierridin B was cytotoxic to the HT-29 colon adenocarcinoma cell line by reducing the mitochondrial activity and induced a cell cycle arrest. Hierridin C did not reveal any cytotoxicity. However, this compound was tested for its antiplasmodial activity which resulted in a patent.

1. Introduction and aim

Cyanobacteria are prokaryotes with diverse morphological, physiological and biochemical properties. At marine environments they are considered one of the most important components of the microbial communities, both at open ocean and along the shores. Marine cyanobacteria produce structurally diverse products, including terpenes, glycosides polyketides, peptides, and lipopeptides with pharmacological bioactivities ranging from anti-inflammatory, antioxidant, antitumor, antimicrobial and antiparasitic, among others (Demay et al., 2019). The potential of marine cyanobacteria as anticancer agents has however been the most explored and, besides cytotoxicity in tumor cell lines, several compounds have emerged as templates for the development of new anticancer drugs (Costa et al., 2012). The project as a result of the collaboration between the Interdisciplinary Center for Marine and Environmental Research (CIIMAR | UP), the Polytechnic School of Health of Porto (ESS | P. PORTO) and the group of pharmacology of the Faculty of Dental Medicine, University of Porto (FMD | UP). Central to this project is the cyanobacteria culture collection of the Blue Biotechnology and Ecotoxicology – LEGE-CC- https://lege.ciimar.up.pt/ mainly constituted by cyanobacteria isolated from the Portuguese coast. The project included twenty-eight marine cyanobacteria strains that were tested for cytoxicity against tumor and normal cell lines. The main objective

was to evaluate the capability of the cyanobacteria to produce compounds with anticancer activity in order to infer about their potential interest as a source of therapeutic agents.

2. General methodology

Twenty-eight cyanobacteria strains of the picoplanktonic and filamentous marine cyanobacteria genera Nodosilinea, Cyanobium, Synechocystis, Synechococcus, Leptolyngbya, Pseudanabaena and Romeria of the LEGE CC were selected. Strains were extensively cultured under laboratory conditions in order to obtain biomass for the preparation of organic extracts (Costa et al., 2013).

Cyanobacteria extracts were tested for cytotoxicity in the HT-29 human colon adenocarcinoma, SH-SY5Y neuroblastoma, T47D and SK-BR-3breast carcinoma, PC-3 prostate adenocarcinoma, RKO colon carcinoma, HepG2 hepatocellular carcinoma and MG-63 osteosarcoma. Cellular viability was evaluated by the reduction of the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and the LDH Release Assay (Costa et al., 2013). For the most interesting strains a bioassay-guided fractionation was further performed and the fractionation of the fraction that revealed more cytotoxicity was conducted. Fractions were prepared using VLC (Afonso et al., 2016; Leao et al., 2013; Leao et al., 2015).

For strains LEGE 06113 LEGE 06155 we investigate the mechanisms undergoing the cytotoxic effects by a genomic and proteomic approach. From these cyanobacteria strains the mechanisms involved in cytotoxicity on the RKO human colon cancer cell line were evaluated by employing real-time PCR to analyse gene expression of genes involved in cell cycle (CCNB1, CCNE, P21CIP) and apoptosis (BAD, BCL-2) and by two-dimensional gel electrophoresis for protein expression. From strain LEGE06113 the compounds hierridin B and C were isolated.

3. Results

Results from the cytotoxicity screening revealed that more than 25% of the strains induced a decrease in cell viability between 70-90% (figure 1). 59% of the strains induced a decrease in cell viability below 50% and were considered as no cytotoxic. However, the majority of the tested cyanobacterial strains were capable to induce cytotoxicity in, at least, one of the cell lines (Costa et al., 2013)

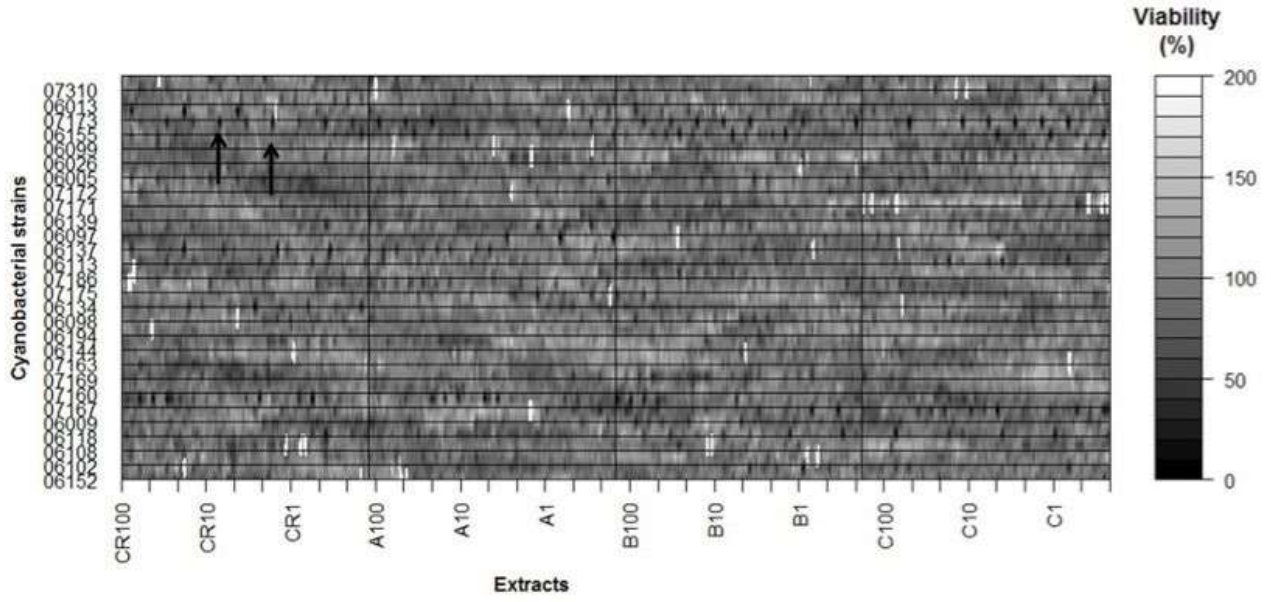

Figure 1. Results of all individual experiments of the MTT assay. The percentage of cell viability was calculated relatively to the control. Dark dots (arrows as example) are indicative of strong and moderate cytotoxicity.

Concerning the mechanisms undergoing the cytotoxic effects on the RKO human colon of the strains LEGE06113 and LEGE06155, RT-PCR results using multiple reference gene normalization showed an increased in the BCL-2 mRNA expression and a decreased the CCNB1 mRNA expression (Fig. 2 and 3). This result supported the hypothesis that there is an interaction with the progression of the cell cycle, since CCNB1 is involved in progression of the cell cycle from G2 to mitosis (M) and the lower expression of this target gene reduce the cells ability to progress in the cell cycle. Progression on cell cycle was also evaluated by flow cytometry and results point also to alterations in the cell cycle. For strain LEGE 06155 an increase in cells in G2/M occurred (Fig.4), which is supported by the decreased the CCNB1 mRNA expression. These results point to a cell cycle arrest at G2/M (Freitas, Martins, Campos, et al., 2016).

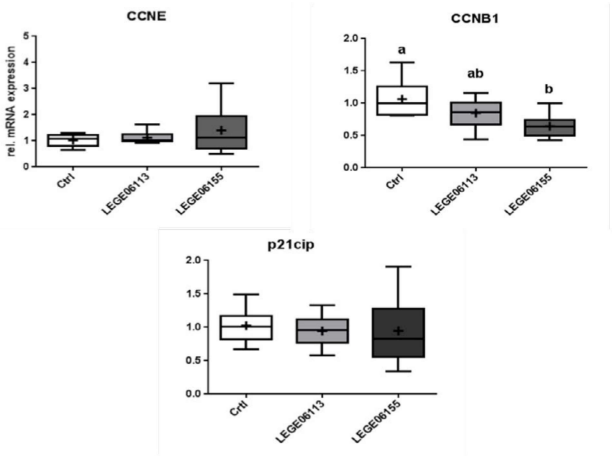

Figure 2. Relative mRNA expression from selected cell cycle genes, CCNE, CCNB1 and P21CIP. CCNB1 showed significant mRNA expression according to the fraction B of the cyanobacterial strain.

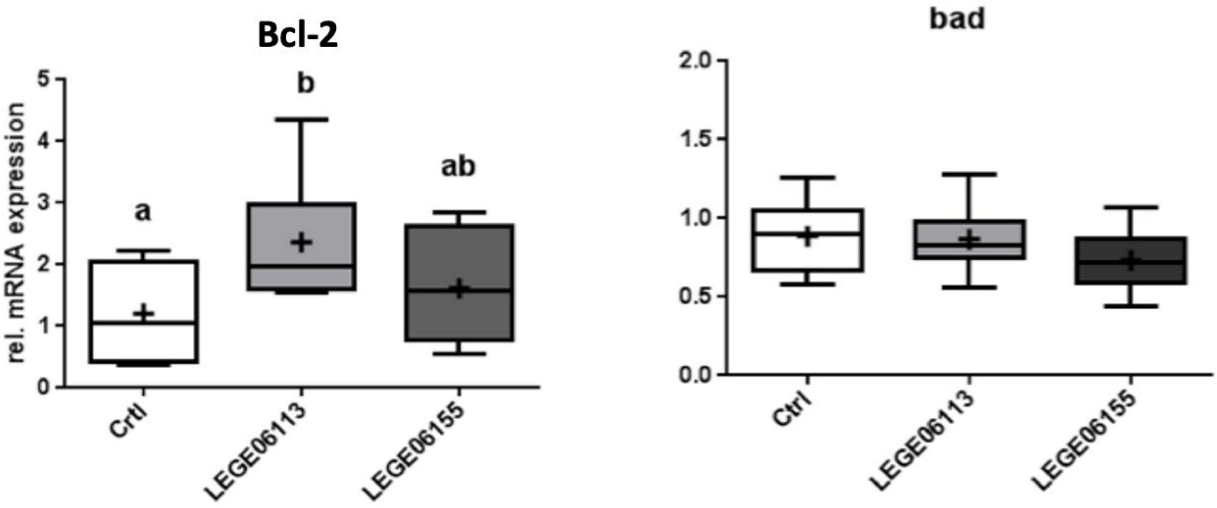

Figure 3. Relative mRNA expression from selected apoptosis genes, BCL-2 (anti-apoptotic) and BAD (pro-apoptotic). BCL-2 showed significant mRNA expression according to the fraction B of the cyanobacteria strains.

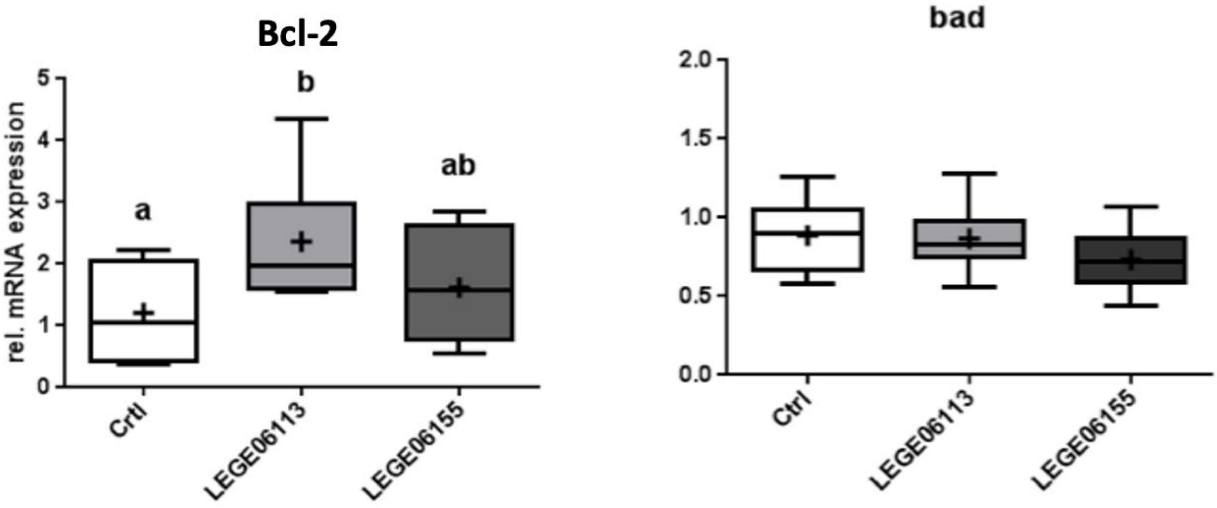

Figure 4. Percentage of RKO cells in S and G2/M phase of the cell cycle. The number of cell in G2/M was significantly higher and in S phase significantly lower in RKO treated with strain LEGE06155 which are indicative of cell cycle arrest.

By applying this bioassay-guided fractionation the crude lipophilic extract from strain LEGE 06113 was fractionated using vacuum-liquid chromatography (VLC). The 1H NMR spectrum (500 MHz, CDCl3) of one of the most non-polar fractions contained two sharp singlets at d3.85 and d3.76, suggestive of aromatic methoxy groups, which led us to further investigate this fraction and ultimately obtain a compound after purification by reversed-phase (RP) HPLC. The identity of the purified metabolite was confirmed as hierridin B, previously isolated from a Phormidium ectocarpi strain (Leao et al., 2013).

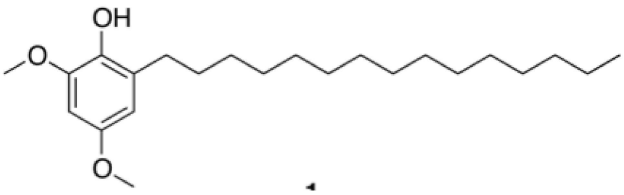

Figure 5. Structure of hierridin B . doi:10.1371/journal.pone.0069562.g001

The cytotoxic activity of hierridin B was exclusively observed on the HT-29 colon adenocarcinoma cell line with an IC50 of 100.2 µM (Leao et al., 2013).

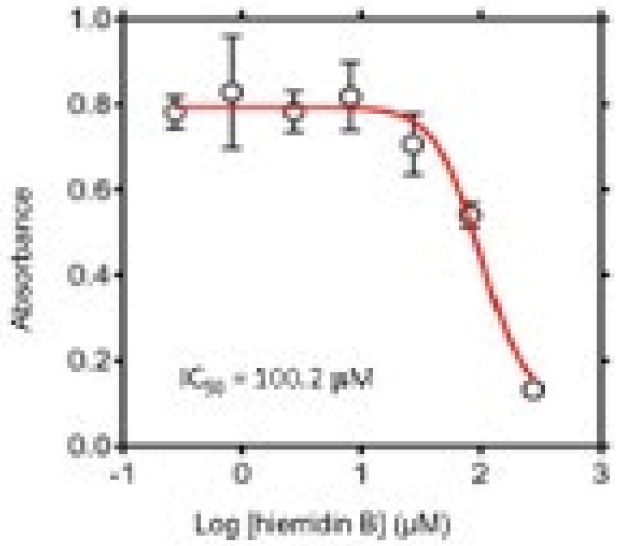

Figure 6. Cytotoxicity of hierridin B isolated from the cyanobacteria strain Cyanobium sp. LEGE06113 in HT-29 human colon adenocarcinoma cells.

The exploration of its mode of action suggested that hierridin B reduced strongly the mitochondrial activity, and induced a cell cycle arrest, which led finally to cell death. Furthermore, an effect was observed on VDAC1 protein expression, which might disturb the formation of mitochondrial channels (Freitas, Martins, Costa, et al., 2016).

Hierridin C was isolated for the first time. Hierridin C did not reveal cytotoxicity against the cells tested however, its antiplasmodial activity was more potent in comparison to its homolog (hierridin C IC50 1.5 ± 0.1µM against 3D7 strain of Plasmodium falciparum and IC50 2.3 ± 0.7µM against Dd2 strain) (Costa et al., 2019). Hierridin C is now in a patent (Leão, P., Rosário, M.M., Costa, M., Vasconcelos, V., Nogueira, F., Domingues, V. (2016) WO2016207869A1.

A group of four bartolosides (A, B C and D), which belong to the chemical class of alkylresorcinols, were isolated from LEGE 06102 and LEGE 06155. Bartoloside A had an IC50 of 21 µM against HT-29. Bartoloside B had an IC50 of

9.5 μM against the PC-3 (Leao et al., 2015). Bartolosides E-K were also isolated, and no cytotoxicity was found (Afonso et al., 2016).

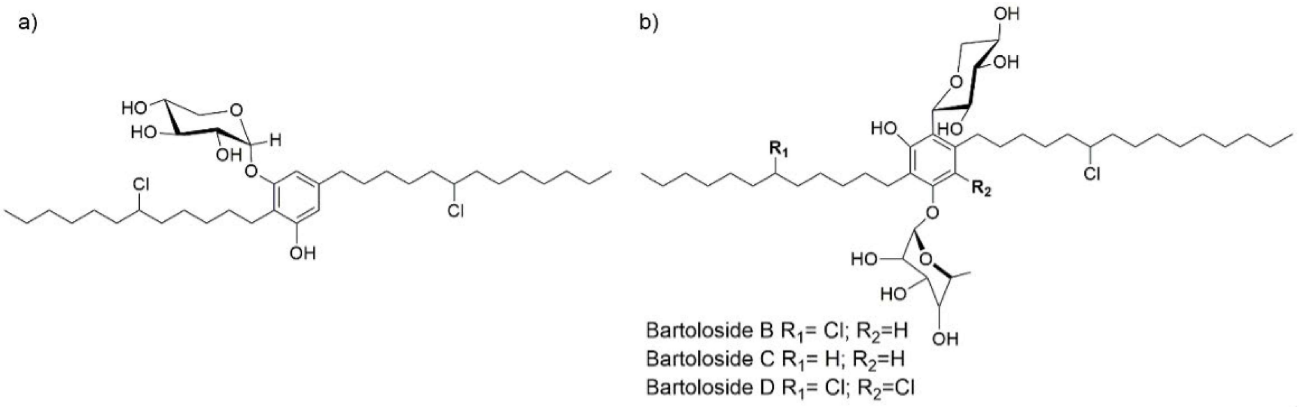

Figure 7. Chemical structures of a) bartoloside A. b) bartoloside B, C and D skeleton.

4. Final remarks

The identification of new sources of natural products is an important step in drug discovery. In this sense, the results from this screening highlight the potential of marine cyanobacteria genera as a source of interesting bioactive compounds.

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