UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

FACULTAD DE CIENCIAS DEL MAR
UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

GRADO EN CIENCIAS DEL MAR

Search for marine natural products with cytotoxic activity.

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0. Abstract

A bioprospecting work has been carried out on marine microalgae, from which a strain of *Chrysoreinhandia giraudii* was selected for its anti-proliferative activity against a panel of 6 cancer cell lines. This job shows the preliminary results of the fraction of the selected extract.

In addition, a bibliographic review of marine microalgae has been researched, those belonging to the Cyanobacteria Phylum. Cyanobacteria are emerging as an important source of novel bioactive secondary metabolites; such as apratoxins, dolastatin, and curacin A. These compounds have biological activities such as antioxidant, antitumor, anticancer and antiproliferative activity. This review is an attempt to consolidate the latest studies and critical research in this field, and to showcase the immense competence of cianobacteria as bioactive metabolite producers.

1. Introduction

Cancer remains one of the most lethal diseases worldwide. There is an urgent need for new drugs with novel mechanism of action and thus considerable research has been conducted for new anticancer drugs from natural sources, especially from microalgae. Cancer is a genetically abnormal disease, in which changes in the DNA sequence alter the structure, function and expression of proteins that control essential cellular processes such as growth, proliferation and apoptosis. It is one of the main causes of death among population aged 30 to 64 and the second leading cause of death with more than 7 million deaths per year [1]. If no action is taken, the number of people diagnosed with cancer is expected to increase to about 23.6 million of new cases by 2030 [2]. Chemotherapy, or administration of cytotoxic drugs, is the most common alternative in anticancer therapy and the most indicated in cases of metastasis. However, cancer chemotherapy has been undermined by the fact that the drugs currently used are relatively toxic or sometimes ineffective by increased resistance. This fact raises the necessity to look for new sources of drugs in natural molecules with potential antiproliferative activity, including those of marine origin [3].

Currently, marine natural products and their derivatives represent an important starting point for the discovery of new molecules with application in cancer chemotherapy, due to the great biological diversity of marine ecosystems. Oceans cover around the 70 % of the surface of the planet, and marine environments represents about 95% of the biosphere, and thus, its biodiversity by far exceeds that presented in the terrestrial environment [4]. The metabolic and physiological capabilities of marine organisms allow them to survive in a complex habitat which give them enormous potential for the productivity of unique metabolites that are not found in terrestrial environments. Mechanisms against predators, inter-species communication, competition for space, food availability, presence or absence of light, salinity, pressure or levels of oxygen, have led marine organisms to produce secondary metabolites for being able to adapt to the environment [5]. To emphasize this potential, Yondelis (trabectedine), isolated from the tunicate Ecteinascidia turbinata is one of the first molecules of marine origin to reach market for treatment of soft tissue sarcoma [6].

Marine biotechnology is an emerging discipline that encompasses the applications of biotechnology tools on the use of marine natural resources. Drug discovery represents one of

the most promising and highly visible outcomes of marine biotechnology research. Marine biotechnology has great potential for discovery of new chemical entities that can aid in the prevention and treatment of cancer. After 1980, biotechnology emerged as a field that provided direction to the study of marine sources, aiming at applications such as drug development [7]. Additionally, the advances in modern biotechnology on nutrition and cancer have shown that it is possible to obtain benefits from certain nutrients and bioactive compounds from foods against the development and evolution of various types of cancer [8].

This work was carried out jointly between the group TQDS of Universidad de Las Palmas de Gran Canaria (ULPGC) and the group of Marine Products of Universidad de La Laguna (ULL).

2. Objectives

Based on the importance of marine biotechnology to access novel bioactive molecules, the main objective of this experimental work was:

-To explore the chemical content of an extract of *Chrysoreinhardia giraudii* with antiproliferative activity through a bio-guided isolation process.

However, due to the suspension of presential activities caused by the current health situation (COVID-19), the experimental activities were suspended and this objective was only partially achieved. Consequently, this TFT has been reformulated and a new objective related to the previous one is proposed:

-To complete a detailed and updated review of the natural marine products present in marine microalgae, those belonging to the Cyanobacteria Phylum, with antiproliferative activity, for the treatment of various types of cancer, delving into the source, structure, mechanism of action, biological active substances and cytotoxic profile.

3. Methodology

3.1 Bibliographic revision on marine microalgae with antiproliferative activity

An analysis of the scientific literature in the area of chemical and biomedical sciences was carried out using specialized databases, specifically, Scifinder Scholar, a tool that contains all the bibliographic information from Chemical Abstract Services (CAS). This platform includes periodicals and international patents. Additionally, other sources such as institutional web pages or scientific social networks (ResearchGate) allows the access to researchers' profile and their publications.

The bibliographic search was carried out from the 5 th of April until the 28 th of June 2020.

The following search criteria were selected:

-Species/gender typology, structure and active biological function.

-Release date: To cover everything that has been published on the mentioned topic.

-Keywords: "marine cytotoxic", "marine natural products", "marine drugs", "microalgae" and "antiproliferative", as well as the specific names of each of the marine microalgae with cytotoxic activity.

Other databases that have been entirely conclusive to do this work are Scopus and Algaebase data, which have allowed to issue up-to-date information about the citotoxity activity of the marine natural products mentioned in this work. Access to such databases has been made indirectly from the section of the website itself or through the download of articles from the website.

3.2 Experimental part

3.2.1. Biological source: *Chrysoreinhandia Giraudii*

This benthic species, first described in the genus Tetraspora, and later transferred to the genus Phaeocystis Lagerheim (Haptophyceae), belongs in fact to the genus Pulvinaria Reinhard (*Chrysoreinhardia Billard*) as suggested by Bourrelly (1957). They belong to the protist kingdom. and known by the name of chrysophytes. Autotrophic organisms whose pigments are chlorophyll a, chlorophyll c and various carotenoids, including fucoxanthin. The reserve substance is a carbohydrate called chrysolamine. They do not have a cell wall or, where appropriate, it consists of cellulose with silica particles in some cases, or of silica in its entirety, as in diatoms [9]. Also, it is known as Golden algae and represent a diverse group, with mainly unicellular organisms, most of them with flagella: although others do not possess flagelos, and there are also amoeboid individuals [10].

3.2.2. Morphology

The mucilaginous light brownish colonies are up to 15 mm in diameter (Fig 1.). Cells are hemispherical to spherical, 7-10 µm in diameter and are embedded in a homogeneous non-stratified mucilage (palmelloid stage) or in a stratified mucilage (gloeocystis stage) (Fig. 1). Numerous granules are present at the periphery of the cell. Cells contain two yellow-brown chloroplasts with pyrenoids and one or several lipidic granules. Reproduction is by vegetative cell division and by zoospore formation [11]

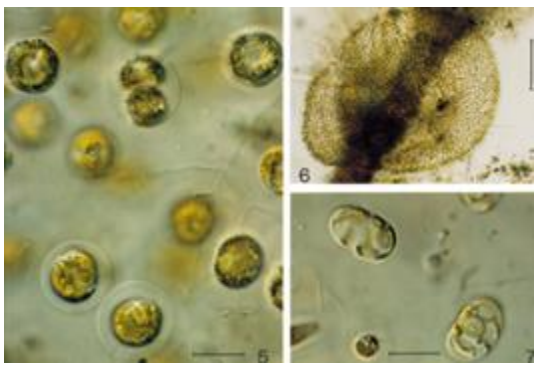


Figure 1: *Chrysoreinhardia giraudii* [11].

3.2.3. Background/Habitat and ecology

Chrysoreinhardia giraudii was first collected at Alga in Revellata Bay in June 1979 from the rhizomes of *Posidonia oceanica* , at a depth of 3 m by one of the authors (Coppejans and Boudouresque 1983) but was inconspicuous until 1989 [12]. The development of C. giraudii is maximal at the end of spring and in summer. It generally appears in macroscopically visible aggregates in May and disappears in September. *C. giraudii* extends to a depth of at least 38 meters, but is often sparser below 20 m. The maximum development is usually at shallow depths in relatively calm and well-lit situations in agreement with Verlaque's (1987) classification in the 'groupe photophile infralittoral thermophile [13]. There it can form a layer several mm thick (Fig 2.) that replaces the macroalgae. In more exposed situations it may, however, start deeper and have its maximum at depths of 15-20 m.

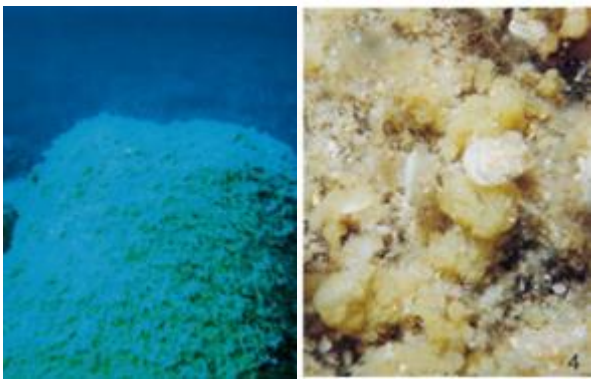


Figure 2: *Chrysoreinhardia giraudii* (colonies) [13]

3.2.4. Extract preparation and fractionation

In the search for bioactive compounds, 5 microalgae strains (2 Cyanobacteria and 3 eukaryotic algae) selected at the Spanish Bank of Algae (BEA) were grown in two different nutrient medium (F/2 and seawater diluted centrate - SDC) in 80 - 400 L photobioreactors under outdoor natural conditions. SDC was used to carry out bioremediation experiments under the framework of the EU Project H2020-SABANA. The dried biomass of both cultures were labeled as:

-SAB-GC-313 Med: Centrate 1%

-SAB-GC-313 Med: F/2

And the microalgae strains were the following:

-BEA0313B *Chrysoreinhardia giraudii* (Pelagophyceae)

-BEA0912B *Anabaena* sp. (Cyanobacteria)

-BEA0912B *Dolychospermum* sp. (Cyanobacteria)

-BEA0069B *Halochlorella rubescens* (Chlorophyceae)

-BEA0046B *Parachlorella* sp. (Trebouxiophyceae)

The only extract obtained with moderate cytotoxic activity was found in BEA0313B *Chrysoreinhardia giraudii* (Pelagophyceae). The strain BEA0313B *Chrysoreinhardia giraudii* (Pelagophyceae) was selected among a collection of microalgal extracts to possess from good to moderate cytotoxic activity against the A549 (IC50 13 mm), HBL-100 (IC50 32 mm), HeLa (IC50 41 mm), SW1573 (IC50 5.2 mm), T-47D (IC50 75 mm), WiDr (IC50 48 mm), cancer cell lines.

To prepare the extracts, both samples were extracted using a mixture acetone-methanol (1:1) for 48 hours. Then, the organic solvent was filtered on filter paper and the solution evaporated on a rotavapor to remove the solvent (Fig 3.) (BÜCHI Rotavapor R-114) obtaining the crude extracts.

Figure 3: BÜCHI Rotavapor R-114.

From the biomass of SAB-GC-313 Med: F/2 (20.82 grams), 0.904 grams of crude extract were obtained. In the case of SAB-GC-313 Med: Centrate 1%, dry biomass of 13.08 grams, yielded 0.483 grams of extract.

Once the extracts were weighed, we proceeded to fractionate the extracts by gel filtration chromatography (Fig 4.) with Sephadex LH-20 as stationary phase, and methanol as mobile phase. The column dimensions were: 3 cm internal diameter and a length of 32 cm of Sephadex. The total height of the column was 35 cm.

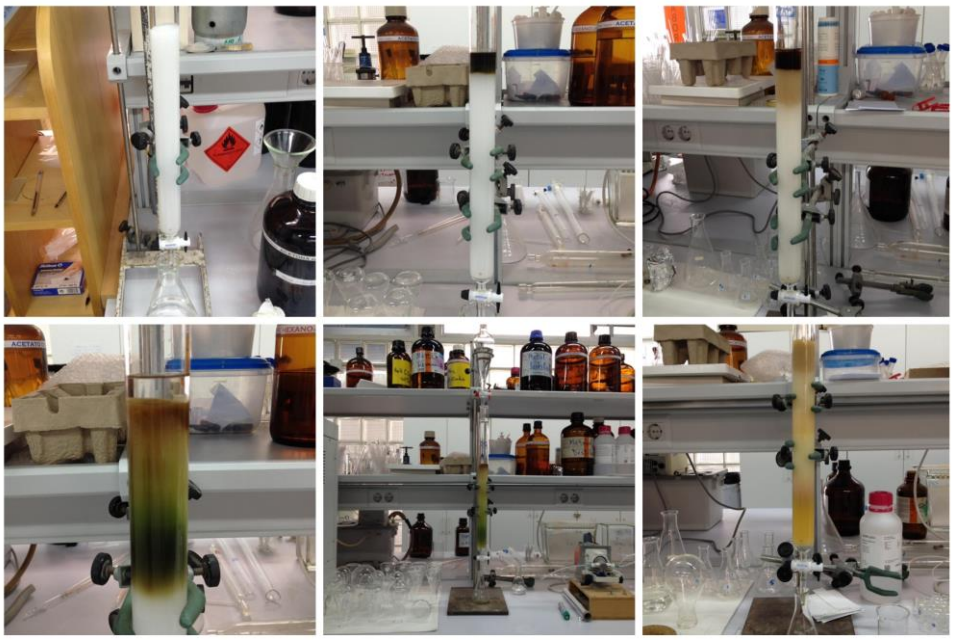


Figure 4: Column chromatography with Sephadex LH-20.

Analytical TLC (thin layer chromatography) was used to monitor the chromatography and combine fractions with the same composition (Fig 5). TLC were performed on silica gel plates (Fig 6.) (0.25mm thickness) using mixtures of hexane / acetone. The TLC plates were developed by spraying Oleum reagent [sulfuric acid (4%) + acetic acid (80%) + water (16%)] and heated for 2-3 minutes.

Figure 5: Fractions obtained from Sephadex LH-20 chromatography.

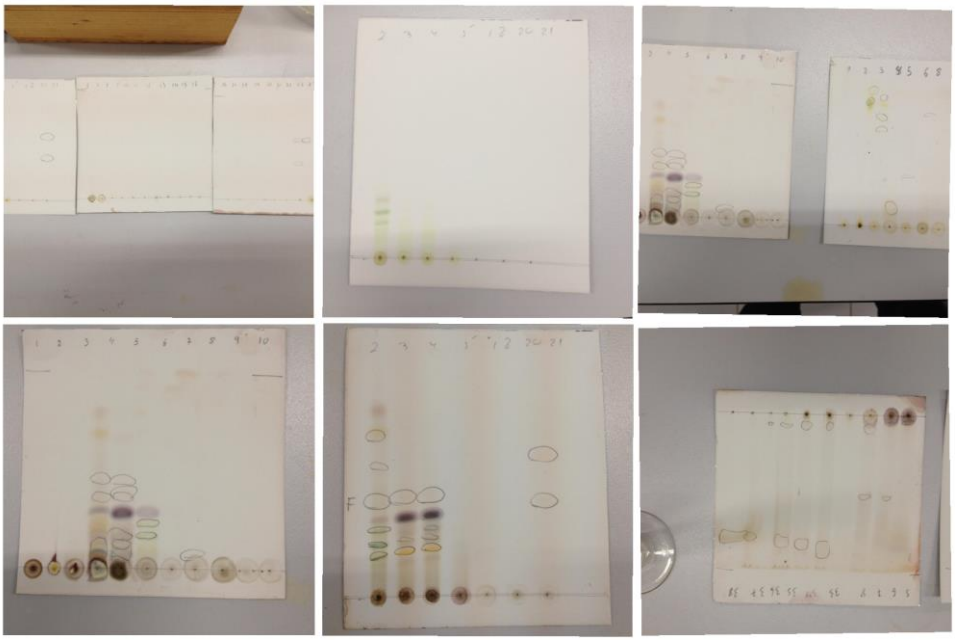


Figure 6 : Analytical TLC performed on silica gel plates.

With each extract we reach ten final fractions that we send to Universidad de La Laguna (ULL) to determine cytotoxic activity. The active fractions must then be subjected to chromatographic and spectroscopic procedures (^{1}H-NMR and ^{13}C-NMR) to determine the structure of the active compounds. At this stage, the experimental work had to be suspended due to the current health situation caused by COVID-19, therefore the fractions have been kept at -20 ºC for preservation and use after the situation allows it.

3.2.5. Antiproliferative test

The organic extract/s (and the chromatographic fractions obtained) were evaluated for their in vitro antiproliferative activity against a panel of representative human solid tumour cell lines (A549 (lung); HBL-100 (breast); SW1573 (lung); HeLa (cervix); T-47D (breast); WiDr (colon)) select the potentially active extract/s (Fig 7.)

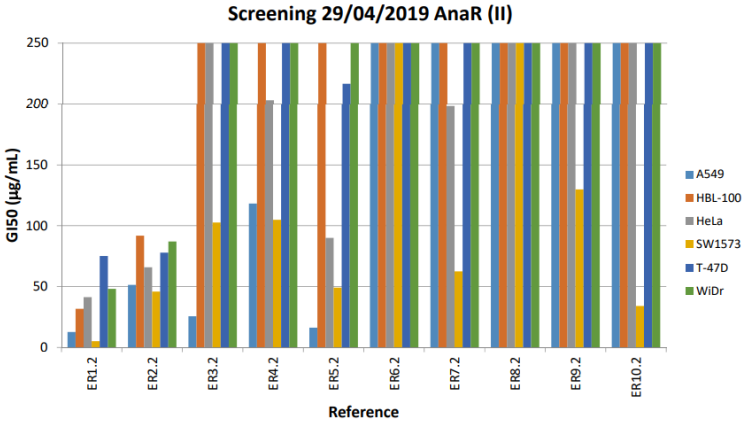


Figure 7 : Antiproloferative activity of microalgal extracts. ER1.2 corresponds to strain BEA0313B, which shows the best values of half growing inhibition (GI50) against a panel of sixcancercelllines.

Bibliographic review

4.1 Cyanobacteria: Source of bioactive compounds.

Marine microalgae are a diverse group of microorganisms which play an essential role in ecosystems as producers of photosynthetic activity. These organisms are considered as “cell factories” to produce a great variety of bioactive substances with different applications [14]. Among the main algal groups are those belonging to Phyla Cyanobacteria (blue-green algae), Dinophyta (dinoflagellates), Chlorophyta (green algae), Phaeophyta (brown algae), Rhodophyta (red algae) and Chryptophyta (golden brown algae). Of the above-mentioned Phyla, Cyanobacteria have been credited with the majority of bioactive compounds which will be the subject of Cyanobacteria are a group of Gram-negative bacteria and one of the richest sources of new bioactive compounds with antiproliferative activities [15]. Also known as blue-green algae, these microorganisms are prolific sources of more than 400 novel metabolites, particularly unique, biologically active peptide and polyketide metabolites [16], effective at either killing cancer cells by inducing apoptotic death or affecting cell signaling via activation of the protein kinase C (PKC) family [17]. The exact number of species is not yet known. That

makes them valuable candidates for molecular development in new pharmaceutical applications. Furthermore, the metabolites produced by cyanobacteria show other interesting biological activities, besides anti-cancer and anti-tumor activities. However, the main emphasis is given to research on drug discovery for human diseases such as cancer and AIDS. Cyanobacteria are potential sources of molecules with anti-tumor and anti-proliferative activity. The latter is the ability of some substances to inhibit the uncontrolled development of cancer cells. Cyanobacteria could be a good dietary supplement or even a good drug to prevent cancer disease. Many of its components have demonstrated their anticancer role.

This bibliographic review is exclusively focused on Phylum Cyanobacteria and analyzes the isolated molecules, the natural source as well as their biological activity and mechanisms of action. The scientific literature described in this work follow a bioactivity criterion.

Table 1: Cont.

Cyanobacteria: Anti-microtubule agents.

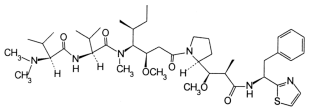
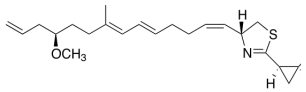
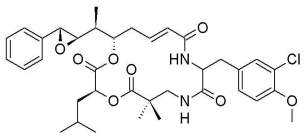
Compound	Chemical Structure	Source/Species	Biological Activity	Mechanism of Action	References
Dolastatin 10		*Symploca* sp.	Antimicrotubule	Microtubule inhibitor that can arrest cell mitotic division.	[18]
Curacin A		*Lyngbya majuscula*	Antimicrotubule/ Antiproliferative	Inhibited tubulin polymerization and selective inhibitory activity against leukemia and Burkitt lymphoma cell lines.	[22]
Cryptophycin-1		*Nostoc* sp.	Antimicrotubule/ Antitumor	Involves binding at the microtubule ends, leading to the disruption of cell mitosis.	[24]

Table 2: Cont.

Cyanobacteria: Activity against human HELa cancer cells.

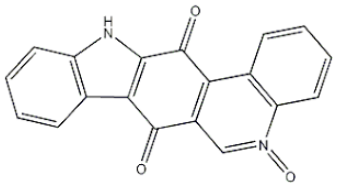
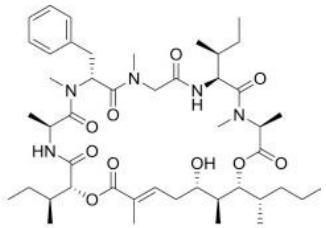
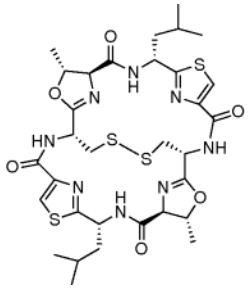
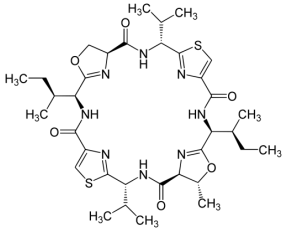
Compound	Chemical Structure	Source/Species	Biological activity	Mechanism of action	References
Calothrixin-A		*Calohtrix*	Anticancer	Inhibit the growth of human HELa cancer cells.	[28]
Odamide		*Okeanis* sp	Anticancer	Inhibit the growth of human cervical cancer cells.	[31]

Table 3: Cont.

Cyanobacteria: Antitumor activity against carcinoma cells.

Compound	Chemical structure	Source/ Species	Biological activity	Mechanism of action	References
Ulithiacyclamide		*Prochloron spp.*	Antitumor	Potent cytotoxic activity against human nasopharyngeal carcinoma cell line.	[32]
Patellamide A		*Lissoclinum patella*	Antitumor	Cytotoxic agent against human nasopharyngeal carcinoma cell line.	[33]

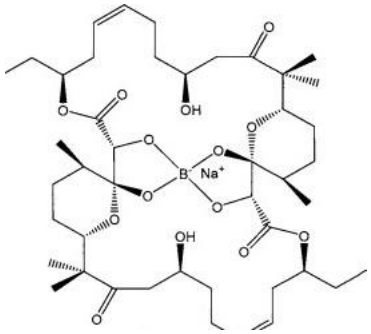
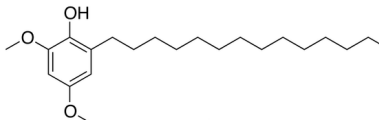
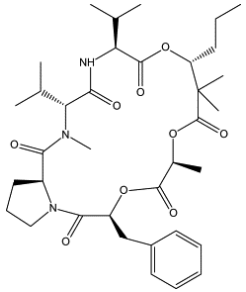
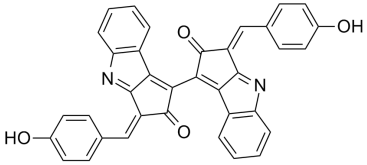
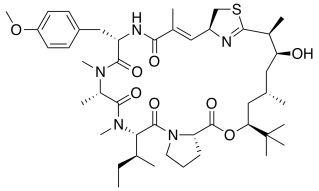
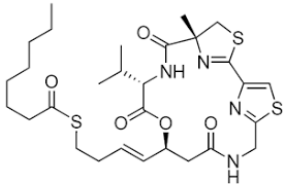
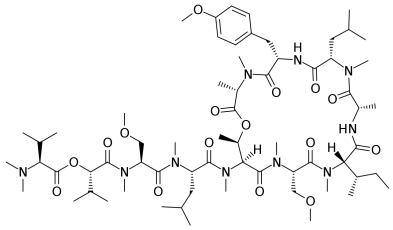
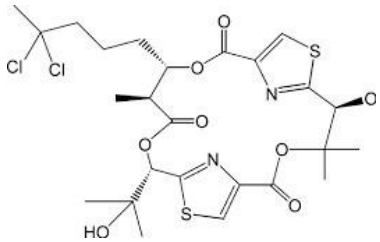
Borophycin		*Nostoc linckia and Nostoc spongiaeforme var. tenue*	Antitumor	Potent cytotoxicity against human epidermoid carcinoma (LOVO) and human colorectal adenocarcinoma (KB) cell lines.	[34]
Hierridin B		*Cyanobium* Sp	Antitumor	Colon adenocarcinoma cells.	[36]
Palmyramide A		*Moorea producens*	Antitumor	Block the sodium channel in neuro-2a cells and cytotoxic activity in H-460 human lung carcinoma cells.	[37]

Table 4: Cont.

Cyanobacteria: Antiproliferative activity.

Compound	Chemical structure	Source/ Species	Biological activity	Mechanism of action	References
Scytonemin		*Nostoc, Scytonema, Calothrix, Lyngbya,* etc...	Antiproliferative	Regulates mitotic spindle formation as well as enzyme kinases involved in cell cycle control, and to also inhibit the proliferation of human fibroblasts and endothelial cells.	[40]
Apratoxins		*Lyngbya* sp	Antiproliferative	Exhibited apoptotic activity against acute myeloid leukemia cells without affecting non-malignant cells, e.g., hepatocytes and cardiomyoblasts..	[41]

Largazole		*Symploca* spp	Anti-proliferative activity	Inhibits histone deacetylases (HDACs)/ growth inhibitory activity.	[44]
Coibamide A		*Leptolyngbya / Caldora penicillata*	Antitumor cytotoxity	Inhibits VEGFA/VEGFR2 expression and suppreses tumor growth.	[45]
Hectochclorin		*Lyngbya mujuscule*	Antiproliferative	Inhibits the growth of human cell lines by hyper-polymerization of actin.	[46]

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4.2. Cyanobacteria: Anti-microtubule agents.

Cyanobacteria biosynthesize anticancer compounds, such as dolastatin 10, curacin A, dolastatin 15, and cryptophycin (Tab 1.) , which target tubulin assembly or actin filaments of eukaryotic cells. This family of peptides have been clinically tested for the treatment of cancer and are considered as lead structures for the synthesis of a number of synthetic analogs and derivatives [18].

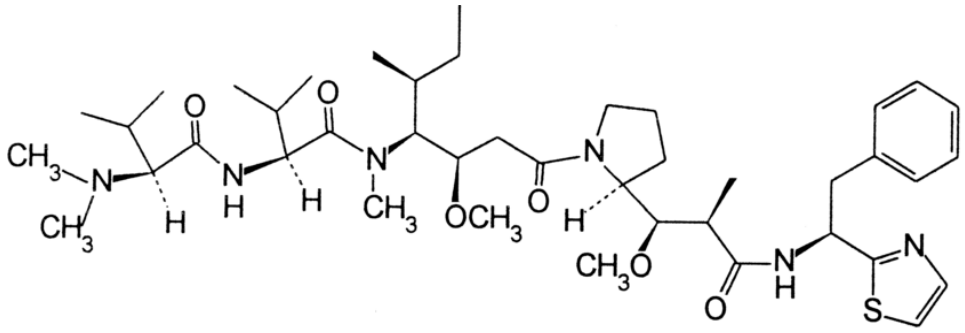


Figure 8 : Dolastatin 10 chemical structure [18]

Dolastin 10 is a strong microtubule inhibitor that can arrest cell mitotic division**.** It was originally isolated from the sea hare *Dolabella auricularia*, and it is actually a cyanobacterial metabolite isolated from a *Symploca sp*. [18]. It is a pentapeptide containing four unique amino acids- dolavaline, dolaisoleucine, dolaproline, and dolaphenine (Fig 8.). It is a potent antiproliferative agent. It binds to tubulin on the rhizoxin-binding site and affects microtubule assembly arresting the cell into G2/M phase [18]. However, in clinical tests, its Phase II trial was discontinued because of the development of peripheral neuropathy in 40% of the patients [19]. Hence an analog of dolastatin 10, TZT-1027, which differs from dolastatin 10 only in the absence of the thiazoline ring from the dolaphenine amino acids, was found to be effective [20]. Another member of the dolastatin family, dolastatin 15, is a linear peptide acting against various cancer cell lines. It acts directly to the alkaloid site on tubulin and blocks the transition into M phase. Likewise, then Dolastatin 10, it was undertaken into no clinical trials for representing unexpected results. Currently, ILX-651, an analog has successfully passed Phase II.[21]

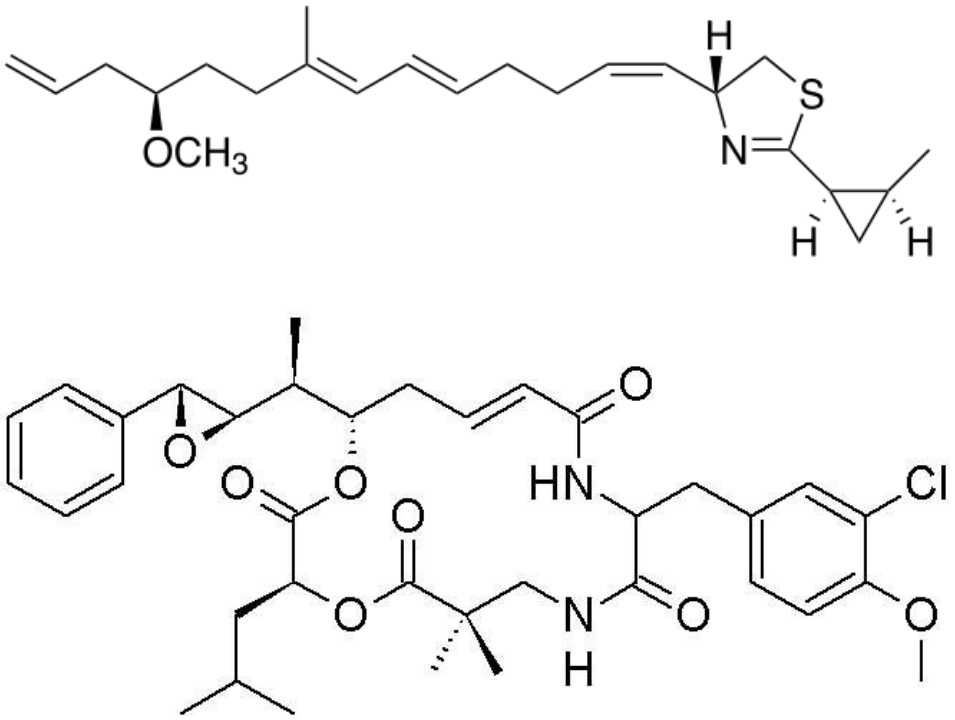


Figure 9: Cryptophycins and curacin A chemical Structure [22,25].

Curacin A (Fig 9.), isolated from the organic extracts of Curacao collections of *Lyngbya majuscula* [22], is an exceptionally potent anti-proliferative agent that inhibited tubulin polymerization and also exhibited selective inhibitory activity against leukemia and Burkitt lymphoma cell lines (IC50 = 9 nM and 200 nM) [23]. In addition, curacin A contains a prominent thiozoline ring joint to a cyclopropyl which is important for the biological activity of the compound. It has been described as a powerful antiproliferative cytotoxic compound with notable antitumor activity against renal, colon, and breast cancer cell lines [24].

Potent cytotoxicity was displayed by cryptophycins (Fig 9.), isolated from *Nostoc* sp. GSV 224, against tumor cells in vitro at nanomolar concentration (human tumor cell lines KB and LOVO with IC50 = 0.005; 0.003 ng/mL, respectively) and in vivo against human solid tumors (colon, pancreatic ductal and mammary adenocarcinomas) [25]. It has also shown efficacy against L1210 leukemia cells, ovarian and drug-resistant breast cancer cells. The mechanism of

action of cryptophycins involves binding at the microtubule ends, leading to the disruption of cell mitosis [26].

A synthetic derivative, Cryptophycin-8, was evaluated for preclinical activity against subcuttaneous tumors in human origins. It was less potent than crypthophycin-1; although it was more soluble and had greater therapeutic efficacy [27]. Cryptophycin-8 has demostrated a powerful anticancer property that is especially useful in the chemotherapy of drug- resistant tumors.

4.3. Cyanobacteria: Activity against human HELa cancer cells (Tab 2.).

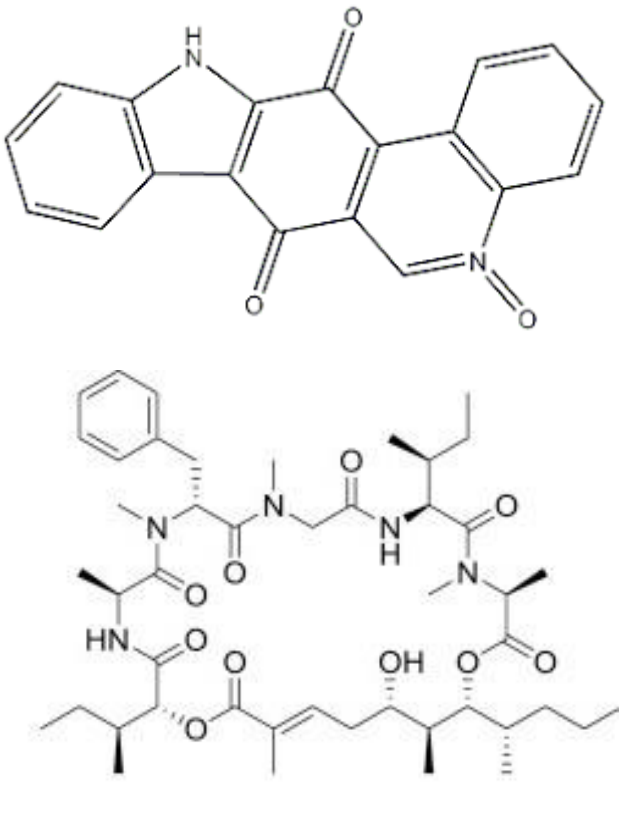


Figure 10: Calothrixin-A and Odamide [28,31]

Calothrixins A and B are pentacyclic alkaloids containing an indoor [28] phenantridine ring system (Fig 10.). These metabolites were isolated from two strains of cyanobacteria of genus *Calothrix* sp. which showed potent anticancer activity against human HeLa cancer cells in a dose-dependent manner at nanomolar concentrations (IC50 40 and 350 nM, respectively) in

vitro studies [29]. Additionally, cell-extracts of *Calothrix* sp. also showed inhibition in vitro of a chloroquine-resistant strain of the malaria parasite *Plasmodium falciparum* [30].

Odamide (Fig 10.) is a newly discovered cyclic depsiptide from *Okeanis* sp, showing strong cytotoxicity against HELa S3 human cervical cancer cells [31].

4.4. Cyanobacteria: Antitumor activity against carcinoma cells (Tab 3.).

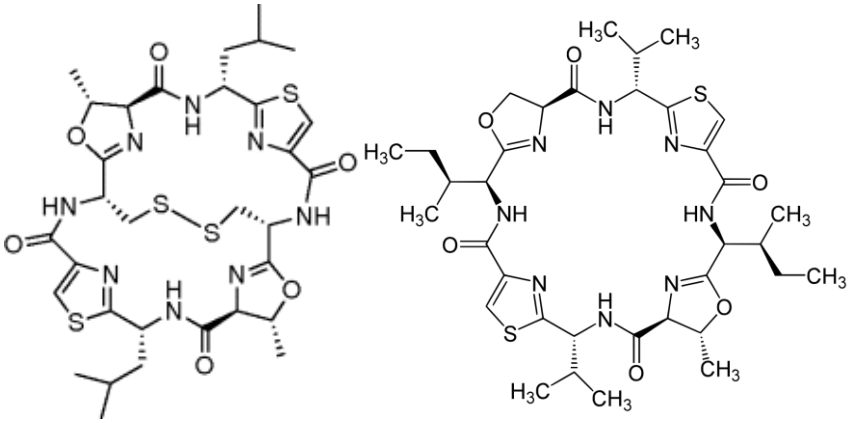


Figure 11 : Ulithiacyclamide and patellamide A[32,33].

Ulithiacyclamide and patellamide A (Fig 11.) , produced by cyanobacteria of genus *Prochloron* spp. and *Lissoclinum patella* [32], exhibited potent cytotoxic activity against a human nasopharyngeal carcinoma cell line at IC50 value of 17 and 3000 ng/mL, respectively [33].

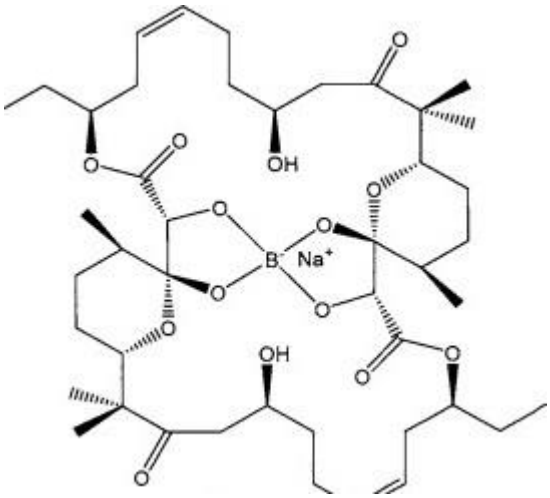


Figure 12: Borophycin isolated from *Nostoc linckia and N. spongiaeforme var. tenue* [34].

Borophycin (Fig 12.) , a boron-containing metabolite isolated from marine cyanobacterial strains of *Nostoc linckia and N. spongiaeforme var. tenue* [34], attributed potent cytotoxicity against human epidermoid carcinoma LOVO and human colorectal adenocarcinoma KB cell lines [35].

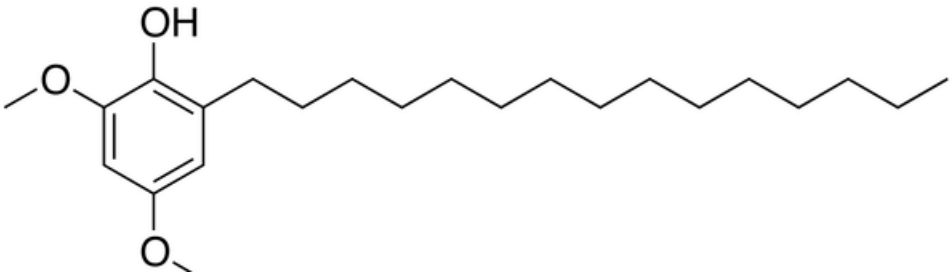


Figure 13: Hierridin B isolated from *Cyanobium* sp. [36].

The long chain poliketide hierridin B (Fig 13.) was isolated from the picocyanobacterium *Cyanobium* sp. LEGE 06113 and exerted moderated cytotoxicity towards HT-29 colon adenocarcinoma cells [36].

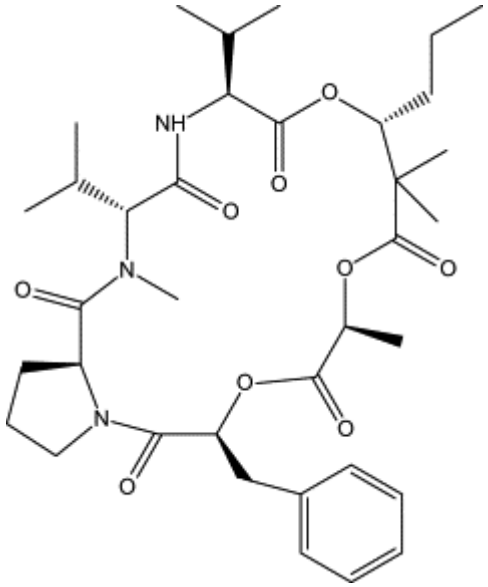


Figure 14: Palmyramide A isolated from *Moorea producens* [37].

Palmyramide A (Fig 14.), isolated from the filamentous cyanobacteria *Moorea producens* is an antitumor agent which block the sodium channel in Neuro-2a cells and cytotoxic activity against H-460 human lung carcinoma cells [37].

4.5 Cyanobacteria: Antiproliferative activity (Tab 4.).

Scytonemin (Fig 15.) is present in the extracellular matrix of many strains of blue-green algae. This pigmented aromatic indole alkaloid is a protein serine/threonine kinase inhibitor of the cell division cycle 25C (cdc25C) in a dose-dependent manner with an IC50 of 2.3 µM, where significant inhibition was observed at concentrations as low as 300 nM [38]. This compound regulates mitotic spindle formation as well as enzyme kinases involved in cell cycle control, and to also inhibit the proliferation of human fibroblasts and endothelial cells [39].

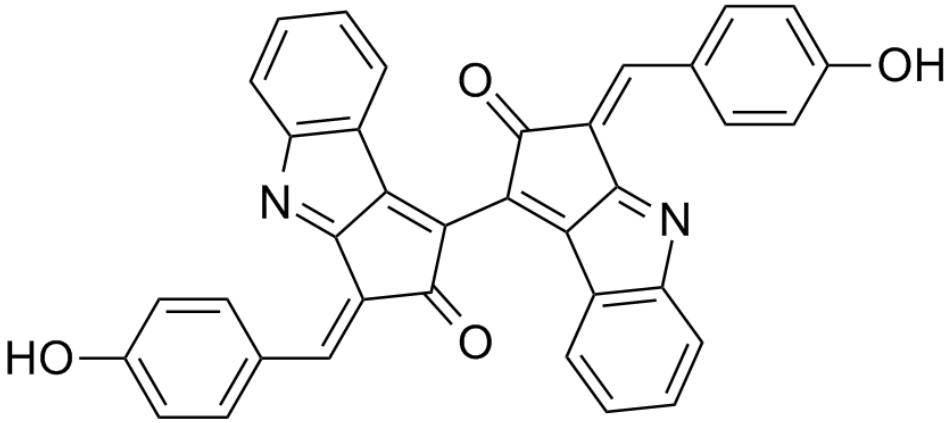


Figure 15: Scytonemin, isolated from *Nostoc*, *Scytonema*, *Calothrix*, *Lyngby*a cyanobacterium [40]

Apratoxins represent are another class of cyanobacterial peptide-plyketide (Fig 16.) natural products inhibitors a variety of cancer cell lines at nanomolar dose levels. Various strains of cyanobacteria exhibited apoptotic activity against acute myeloid leukemia cells without affecting non-malignant cells, e.g., hepatocytes and cardiomyoblasts [41].

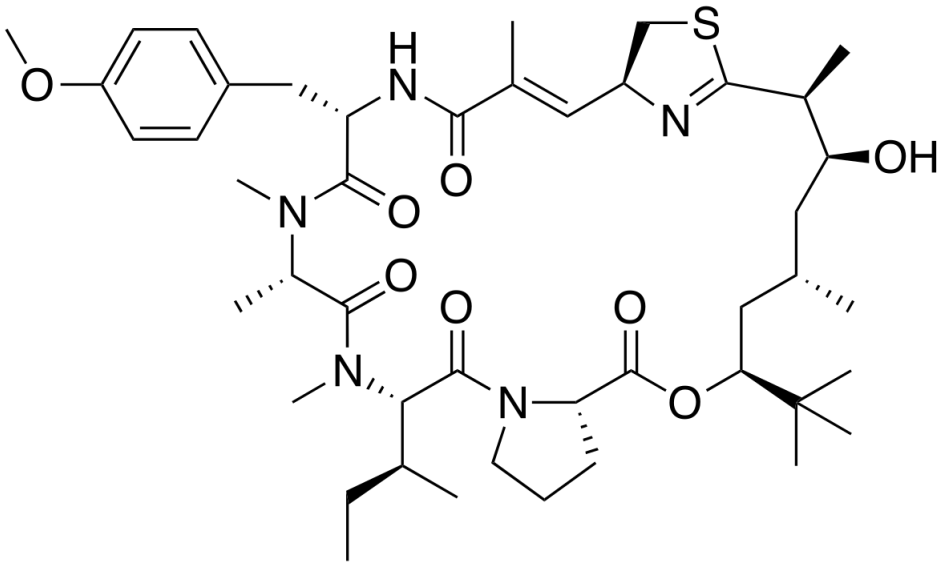


Figure 16: Apratoxin A chemical structure [41].

Largazole represented a unique chemical scaffold derived from *Symploca* spp. with impressive anti-proliferative activity [42]. It is a cyclic dipeptide (Fig 17.) marine natural product with strong potency as inhibitor of histone deacetylases (HDACs) [43]. It possesses highly differential growth-inhibitory activity. The impressive structure and biological activity of largazole have attracted a huge interest to the chemistry community. The desing of largazole analogues is ian expanding fiel of study due to thir remarkable potential as novel anticancer therapeutics [44].

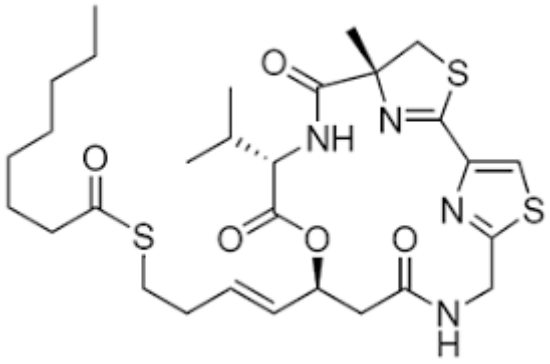
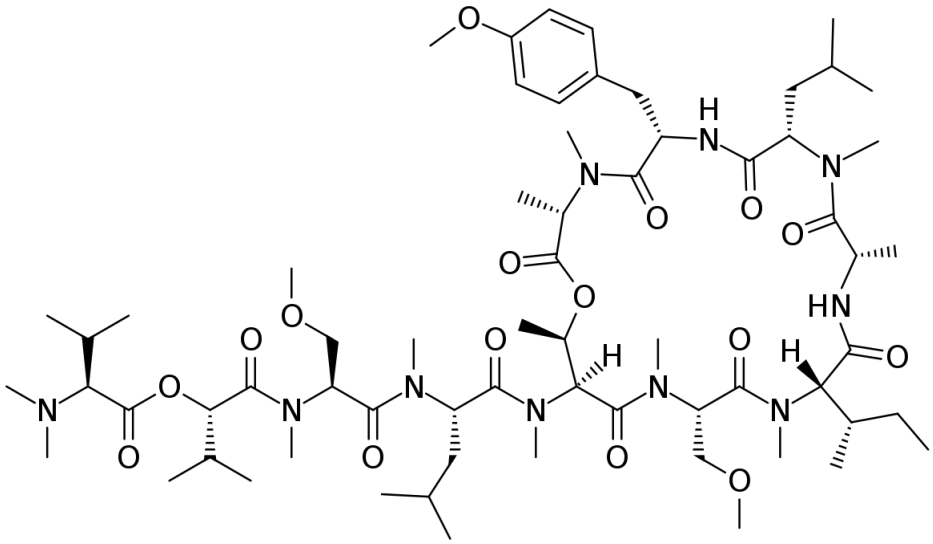


Figure 17 : Largazole chemical structure [44].

The dipeptide coibamide A (Fig 18.) is a promising anti-cancer agent with a new potential mechanism of action. Derived from a strain of *Leptolyngbya* (pantropical cyanobacteria *Caldora penicillata*, exhibited significant cytotoxicity against NCIH460 lung and mouse neuro-2a cells (LC50 < 23 nM) [45]. Coibamide A inhibits VEGFA/VEGFR2 expression and suppreses tumor growth in glioblastona senografts.



Figrue 18: Coibamide A chemical structure [45]

Another interesting compound with antifungal and cytotoxic activities is hectochlorin (Fig 19.) obtained from cyanobacterium *Lyngbya majuscula*. This chlorinated lipopeptide inhibits the growth of human cell lines by hyper-polymerization of actin [46].

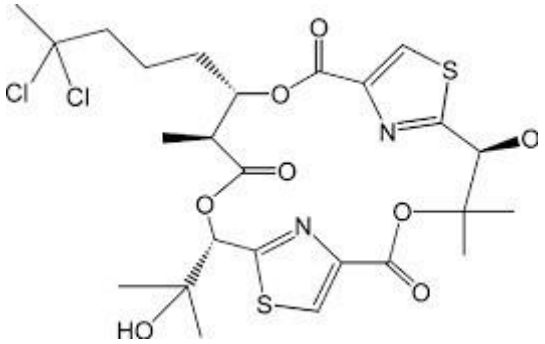


Figure 19: Hectochlorin chemical structure [46].

This all compound shows anticancer activity against various types of cancer cells with different mechanisms hence it is recommended further investigation of their potential biological activities and clinical uses.

5. Conclusions

· Marine microalgae represent an important part of the total existing biodiversity. They are key candidates for the search for new bioactive molecules potentially useful to treat health diseases such as cancer, among others.

· The bioprospection study of a collection of marine microalgae extracts allowed us to identify an extract of *Chrysoreinhardia giraudii* BEA0313B with promising antiproliferative activity, showing to be particularly active for the SW15 cell line with a (GI50 5.2 mm), among a panel of six different cancer cell lines. It is planned to continue this research work to identify the active metabolites.

· The bibliographic analysis reveals that Phylum Cyanobacteria constitute a huge and unique group of photosynthetic bacteria producers of bioactive molecules obtained that show a broad spectrum of activities, such as antitumor, anticancer, antiviral and antiproliferative effects.

· Marine biotechnology is a very important tool that allows the sustainable development of marine biodiversity to give access to new pharmaceuticals.

· In this review we summarize the contribution and effects of marine microalgae to treat cancer in several ways. We discussed the pharmaceutical prospects and his potential as a source of new anticancer leads. It is clear that marine microalgae are promising in providing a platform for improving the anti-cancer therapeutic strategies

6. Acknowledgements

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Descripción detallada de las actividades desarrolladas durante la realización del TFT.

La realización del TFT se ha propuesto en dos partes; una primera parte basada en la actividad desarrollada en el laboratorio durante las prácticas externas; y otra parte en una búsqueda bibliográfica del potencial anticancerígeno de los productos naturales aislados de cianobacterias.

La parte experimental consistió en preparar una serie de extractos de microalgas para ensayar su actividad anticancerígena, y posteriormente someter el extracto activo a una separación cromatográfica biodirigida, con el objeto de determinar los productos naturales responsables de su actividad. El estudio y análisis se realizó bajo la supervisión de Elsa María Rodríguez Pérez y Ana Raquel Díaz Marrero (tutora y co-tutora del TFG).

La búsqueda bibliográfica sobre el potencial anticancerígeno del phylum Cyanobacteria se realizó a través de varias páginas webs, bases de datos y artículos científicos, usando palabras claves como: Marine Microalgae, cytotoxic activity o Cyanobacteria.

• Formación recibida (cursos, programas informáticos, etc.)

Desde el punto de vista de la capacitación recibida, el estudio me ha ayudado a aprender varias técnicas espectroscópicas y cromatográficas que se aplican comúnmente en la investigación de productos naturales marinos.

Por otro lado, la investigación bibliográfica de productos naturales, derivados de cianobacteria, me ha hecho comprender el inmenso campo, aún por conocer, que tiene esta área de investigación, y por tanto me ha alentado y motivado a seguir formándome en este entorno.

He recibido consejos útiles durante este trayecto y propuestas interesantes como el Congreso IMS. Además, he necesitado utilizar programas informáticos para el dibujo de moléculas químicas.

• Nivel de integración e implicación dentro del departamento y relaciones con el personal.

Durante mi estancia en el laboratorio tuve en todo momento una muy buena relación con el resto de los compañeros. Tenía acceso completo a las instalaciones donde pude organizar mi propio trabajo con libertad de horarios. Mis tutoras han estado presentes en todo momento, tanto para las explicaciones de los procedimientos de las actividades de laboratorio, cómo resolver dudas, etc.

Por lo tanto, estoy agradecido por el buen nivel de integración y participación que yo

he experimentado.

• Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFT

Destacar positivamente: la implementación del trabajo práctico, donde aprendí a lidiar con la instrumentación de laboratorio y con los procedimientos técnicos descritos en el manuscrito; además del aprendizaje y la adquisición de nuevos conocimientos en el campo de los productos naturales marinos ; fue bueno aprender de mis tutoras que han demostrado una gran disponibilidad, especialmente en lo que respecta a preguntas y correcciones del manuscrito.

Entre los aspectos negativos mencionar la presentación obligatoria del proyecto en un idioma extranjero, en este caso el inglés. Aunque tenga nociones del idioma, no deja de representar una gran dificultad para mí; la principal razón es que durante la carrera no se ha realizado ninguna presentación sobre este idioma. Por otro lado, decir que no deja de ser un “challenge” y una prueba de superación personal.

• Valoración personal del aprendizaje conseguido a lo largo del TFT.

Durante la realización del TFG he aprendido muchas cosas, entre las ya mencionadas anteriormente: a buscar , comparar , descartar y seleccionar información de recursos bibliográficos; aprender sobre la forma en que escribir un manuscrito; y especialmente enfatizar la adquisición de nuevos conocimientos relacionados con química orgánica y la motivación infinita desde el principio del TFT por parte de mis tutoras, Elsa María Rodríguez Pérez y Ana Raquel Marrero Díaz.