Review Article

Review of Cyanotoxicity Studies Based on Cell Cultures

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Cyanotoxins (CTs) are a large and diverse group of toxins produced by the peculiar photosynthetic prokaryotes of the domain Cyanoprokaryota. Toxin-producing aquatic cyanoprokaryotes can develop in mass, causing “water blooms” or “cyanoblooms,” which may lead to environmental disaster—water poisoning, extinction of aquatic life, and even to human death. CT studies on single cells and cells in culture are an important stage of toxicological studies with increasing impact for their further use for scientific and clinical purposes, and for policies of environmental protection. The higher cost of animal use and continuous resistance to the use of animals for scientific and toxicological studies lead to a progressive increase of cell lines use. This review aims to present (1) the important results of the effects of CT on human and animal cell lines, (2) the methods and concentrations used to obtain these results, (3) the studied cell lines and their tissues of origin, and (4) the intracellular targets of CT. CTs reviewed are presented in alphabetical order as follows: aeruginosins, anatoxins, BMAA (β-N-methylamino-L-alanine), cylindrospermopsins, depsipeptides, lipopolysaccharides, lyngbyatoxins, microcystins, nodularins, cyanobacterial retinoids, and saxitoxins. The presence of all these data in a review allows in one look to advance the research on CT using cell cultures by facilitating the selection of the most appropriate methods, conditions, and cell lines for future toxicological, pharmacological, and physiological studies.

1. Introduction

Cyanotoxins (CTs) are a large and diverse group of toxins produced by the peculiar photosynthetic prokaryotes of the domain Eubacteria, commonly known as cyanobacteria or blue-green algae, and since 1999 named Cyanoprokaryota [1, 2]. Some aquatic cyanoprokaryotes can develop in mass, causing so-called “water blooms” or “cyanoblooms” [3]. When such blooms are formed by toxin-producing cyanoprokaryotic algae, they are considered harmful and are usually abbreviated as Cyanob-HABs. The toxic substances are transported through the food webs and may reach people and animals by drinking water, or through other exposure routes, which include recreational activities or consumption of so-called “seafood”, which includes both freshwater and marine organisms [3–5]. The excretion of toxic compounds may lead to environmental disasters—water poisoning,
extinction of aquatic life, and even to human death [3–5]. Current climate changes and anthropogenic press can intensify and increase the frequency of these hazardous ecological events [3, 6]. Although most research addresses aquatic toxin producers, there is a growing body of evidence on such producers from aeroterrestrial and extreme habitats, and among airborne algae as well, with a considerable number of detected toxins and outlining of additional exposure route through consumption of crops, which have been irrigated by contaminated water [7–8].

Different approaches have been applied to classify CT, two of which are the most common: by the target of their action, or by chemical composition. By target, CT are classified as hepatotoxins, neurotoxins, dermatoxins, and cytotoxins, whereas chemically they are divided in peptides, alkaloids, phosphorylated cyclic N-hydroxyguanine, diaminoacids, and lipopolysaccharides, the last widely recognized as endotoxins. Prolonged use of drinking water, contaminated with low-doses CTs, may have also carcinogenic effect [6]. Thus, microcystin-LR (MC-LR), the most toxic MC, is considered to express tumor promoting effect mainly by violating phosphorylation-dependent regulations of cellular proteins [9, 10]. The pleiotropic downstream mechanisms link MC-LR-dependent inhibition of eucaryotic protein phosphatases (PPs) PP1, PP2A, phospho-PP4, and phospho-PP5 [11] to tumor promotion and neoplastic transformation by cell growth induction, reactive oxygen species (ROS) generation, oxidative stress, mitochondrial DNA impairment, and by the transformation of cell phenotype [9]. Chronic proinflammatory effect of MC-LR alone or a combination with another CT-like cylindrospermopsin (CYN) may additionally stimulate the neoplastic transformation and tumor progression [6, 10].

Cell cultures are very convenient for toxicological studies. They allow to reveal the mechanisms of cytotoxic effects, the affected tissues, intracellular targets, and ways to minimize cytotoxicity [11]. The use of human cell lines in toxicological studies is a fast and effective way to investigate the damaging effects of toxins in humans and to identify the most sensitive tissues.

Although different methods are developed for testing of toxins in cell- and animal-based studies, during the last years, the trials on the use of animals have significantly decreased. This is caused by the high cost of these types of clinical trials and increasing resistance to the use of animals for scientific studies. Therefore, the significance and use of cell lines is gradually increasing.

This review aims to present (1) the important results of the effects of CT on human and animal cell lines; (2) the methods and concentrations used to obtain these results, (3) the studied cell lines, and (4) the intracellular targets of CT. The presence of all these data in a review allows in one look to advance the toxicological and pharmacological studies of CT using cell cultures by facilitating the selection of the most appropriate methods, conditions, and cell lines.

2. Cyanotoxicity on Cell and Cell Cultures

2.1. Cytotoxicity of Aeruginosins (Table 1). Aeruginosin CT contains as a basic structure 2-carboxy-6-hydroxyoctahydroindol that are serine protease inhibitors [12]. They inhibit trypsin-like serine proteases and for this activity are important in the search for new anticoagulants [13].

2.2. Cytotoxicity of Anatoxins (Table 2). Anatoxins-a are two types of low molecular bicyclic amino alkaloids: anatoxin-a (ANTX) and homoanatoxin-a (hANTX). The best known of them is ANTX, which was the first to be identified as a low molecular alkaloid (165 Da). hANTX is a homologue of anatoxin-a with molecular weight 179 Da and has propionyl instead of an acetyl group at C-2. ANTX and anatoxin-a (S) (ANTX(S)) are neurotoxins. ANTX binds competitively to acetylcholine receptors, while anatoxin-a (S) inhibits ace-
ylcholine esterase [2].

2.3. Cytotoxicity of BMAA (Table 3). β-N-methylamino-L-alanine (BMAA) is an environmental nonprotein and toxic amino acid that may harm nervous system via oxidative stress, binding to neuromelanin, forming high toxic metabolites like formaldehyde or inhibiting enzyme activity of glutathione reductase, β-amilase, catalase, and RNase H, and in this way to provoke sporadic neurodegenerative development, such as Alzheimers disease and amyotrophic lateral sclerosis [20, 27, 28]. In addition, BMAA generates a carbamate, which is neurotoxin because it acts as ionotropic and metabolotropic glutamate receptors agonist [21] and references therein.

2.4. Cytotoxicity of CYN (Table 4). CYN is a cyclic quinidine alkaloid combined with hydroxymethyl uracil [49]. It has two epimers, which are equally toxic and are differentiated by the hydroxyl bridge CYN and 7-epi-CYN, and an additional variant 7-deoxy-CYN occurs in natural waters [49]. CYN has been classified mainly as hepatotoxin, but it has also neurotoxic and genotoxic effects and inhibits protein synthesis [3]. It targets kidneys, lungs, heart, spleen, eyes, ovaries, T-cells, neutrophils, and vascular endothelium [50]. CYN may induce oxidative stress, decrease cell viability, and damage mitochondria (discussed by Chichova et al. [35]).

2.5. Cytotoxicity of Depsipeptides (Table 5). Depsipeptides are palmyramide A (Palm A), apratoxin D (AT D), coibamide A (CoA), ichthyopeptins A (Ich A) and B (Ich B), kahalalide F (KF), 4-Fluoro-3-methyl-benzylamino-KF (KF2), morpholin-4-yl-benzylamino-KF (KF4), homodolastatin 16 (HD16), lagunamide C–Lag C, pitipeptolides–Pit A-F, aurilides and wewakpeptins A-D. Depsipeptides show cytotoxic activity and are protease inhibitors selective for chymotrypsin, leukocyte, and pancreatic elastases. They negatively influence the metabolism of human astrocytes [63].
2.6. Cytotoxicity of Lipopolysaccharides (LPS, Table 6). LPS consist of lipid A, the core polysaccharides (mainly glucosamine) and an outer polysaccharide chain, and are common compounds of the cell walls of cyanoprokaryotes and Gram-negative bacteria [49]. They have an inflammatory effect and promote cytokine secretion [3].

2.7. Cytotoxicity of Lyngbyatoxins (Table 7). Lyngbyatoxins were first identified from Moorea producens (formerly Lyngbya majuscula). They are tumor-promoting agents which bind eukaryotic protein kinase C (PKC) isozymes [3].

2.8. Cytotoxicity of MCs (Table 8). MC are cyclic non-ribosomal heptapeptides with low molecular weight (800–1100 Da), which contain several uncommon non-proteinogenic amino acids such as N-methyldehydroalanin (MDHA) derivatives and the uncommon β-amino acid 3-amino-9-methoxo-2,6,8-trimethyldeca-4,6-dienoic acid (ADDA). MC are lipophilic toxins very resistant to hydrolysis, oxidation, and high temperatures. The main route of human exposure is the ingestion of contaminated drinking water, consumption of contaminated food or algal dietary supplements, and body contact, while more occasional routes are hemodialysis and inhalation. MC are classified mainly as hepatotoxins because they block eukaryotic PP (PP1, 2A and phosphoprotein phosphatases PPP4, PPP5) [2] through irreversible covalent binding [97]. Chronic and subchronic exposure to MC seems to be tumor promoting because they can increase the incidence of hepatic tumors in humans. MC could also enhance the oxidative stress. Additional target of MC in high concentrations is the β-subunit of ATP synthase, causing mitochondrial apoptotic signaling. MC have hepatotoxic and tumor promoting action [3].

Table 1: Cytotoxicity of aeruginosins.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|-----------------|--------------|---------|------|
| Huh7 cells | EROD assay, treatment with TNF-α | Aeruginosin-865A 50 and 100 µmol/L | Human hepatoma cell line | Anti-inflammatory activity by inhibition of IL-8 and TNF-α expression; induce expression of cytochrome P<sub>450</sub> 1A (CYP1A) | DNA [12] |
| HLMVEC | IL-8 and ICAM-1 assay upon stimulation with human tumor necrosis factor α (hTNF-α) | Aeruginosin-865 0.1–100 µg/mL/18 h of 0.1 ng/mL hTNF-α stimulated cells | Human lung microvascular endothelial cells | Anti-inflammatory activity by down-regulation of IL-8 (EC<sub>50</sub> : 4.0 ± 1.7 mM) and intercellular adhesion molecule 1 (ICAM-1; 57.8 ± 15.5 mM) | Inhibits NF-kappa B translocation to the nucleus [13] |
| WEHI-133VAR | Lactate dehydrogenase (LDH) cytotoxicity assay | Aeruginosin-865 10–200 µM | Mouse fibrosarcoma cells | Cytotoxic effect of aeruginosin-865 at 200 µM only | [14] |

Abbreviations: EROD – ethoxyresorufin-O-deethylase; hTNFα – human tumor necrosis factor α; ICAM-1 – intercellular adhesion molecule-1; IL-8 – interleukin 8; TNF-α – tumor necrosis factor αα.

Table 2: Cytotoxicity of ANTX, hANTX and ANTX(S).

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|-----------------|--------------|---------|------|
| RAW 264.7, BV-2, N2a | MTT assay, caspase-glo 3/7 assay, ELISA, TNF-α measurement | MC-LR, CYN, ANTX-a | Murine macrophage-like RAW 264.7, immortalised microglial BV-2, neuroblastoma N2a cell lines | CYN, MC-LR and ANTX in a mixture are 3–15 times more potent at inducing apoptosis and inflammation | TNF-α [15] |
| Oocytes, M10 cells | Patch-clamp, <sup>86</sup>Rb<sup>+</sup> influx | ANTX | Xenopus oocytes, human hepatoma cell line | α<sub>7</sub>-nAChR agonist with EC<sub>50</sub> = 0.57 µM (nicotinic current in oocytes), α<sub>4β2</sub>-nAChR EC<sub>50</sub> : 48 nM by <sup>86</sup>Rb<sup>+</sup> influx in M10 cells | α<sub>7</sub>-nAChR, α<sub>4β2</sub>-nAChR, ACh [16, 17] |
| GH<sub>4</sub>C<sub>1</sub> Chromaffin cell culture | 45<sup>Ca</sup><sup>2+</sup> influx, [3H]<sub>ACh</sub> release, HPLC | ANTX water extract, 1–20 mg/mL | Rat anterior pituitary gland, bovine adrenal chromaffin cell culture | hANTX-activated voltage-gated Ca<sup>2+</sup> channels and AChR release; Catecholamine release activation above 0.3 µM ANTX | Voltage-gated Ca<sup>2+</sup> channels, AChR, Secretion of catecholamines [18] |
| GH<sub>4</sub>C<sub>1</sub> Chromaffin cell culture | HPLC | ANTX 0.1–100 µM | Rat anterior pituitary gland, bovine adrenal chromaffin cell culture | Catecholamine release activation above 0.3 µM ANTX | [19] |

Abbreviations: Ach – acetylcholine; AChR – acetylcholine receptor; CYN – cylindrospermopsin; HPLC – high-performance liquid chromatography; MC-LR – microcystin-LR; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; nAChR – nicotinic acetylcholine receptor.
2.9. Cytotoxicity of Nodularins (Table 9). Nodularins (NODs) are cyclic nonribosomal pentapeptides and contain several unusual nonproteinogenic amino acids such as N-methyl-didehydroaminobutyric acid and the ββ-amino acid (all-S, all-E)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA). Ten variants have been discovered with nodularin-R being the predominant toxin variant. NODs are relatively stable compounds, with low sensitivity to light or temperature. NOD affects hepatocytes binding their PPs by noncovalent bonds, which increases the rate of phosphorylation. They are often attributed to gastroenteritis, allergic irritation reactions, and liver diseases. Nodularin-R is the most notorious as a potent hepatotoxin that may cause serious damage to the liver of humans and other animals. NODs have similar effects as microcystins and weak carcinogenicity [3].

2.10. Cytotoxicity of Retinoids from Cyanobacteria (Table 10). Retinol, a novel retinoic acid (RA) analogue 7-hydroxy RA, 4-oxo-RA, and several analogues were identified in cyanobacterial blooms [110]. They act as RA receptors that may cause different malformations, as well as to have a teratogenic effect on aqueous animals.

### Table 3: Cytotoxicity of BMAA.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Ref. |
|-----------|-------|------------|------------------|--------------|-----|
| HepG2 cells, Caco-2 | Isotopically labelled amino acids; metabolic activity; apoptotic and necrotic assays | Human hepatocellular carcinoma and human colorectal epithelial adenocarcinoma cell line | BMAA did not affect the common proteinogenic amino acid metabolic pathways; in the presence of amino acids cellular uptake of BMAA is substantially reduced | [20] |
| SH-SY5Y | LDH assay; qPCR; Western Blot | L-BMAA 1 mM/17 h | Human neuroblastoma cells | Conversion of procaspase-3 (32 kDa) to active caspase-3 p17 and apoptosis | [21] |
| SH-SY5Y | LDH assay; qPCR; Western Blot | L-BMAA 1 mM/17 h and longer for 24–96 h | Human neuroblastoma cells | Misincorporation of L-BMAA protein aggregation, upregulation of lysosomal enzymes and apoptosis; proteolitic stress in prolonged exposure | [22] |
| SH-SY5Y | LDH assay, qPCR; Western Blot | Low L-BMAA (≥0.1 mM)/48 h; high L-BMAA (≥2 mM)/48 h | Human neuroblastoma cells | Low L-BMAA increases protein ubiquitination, 20S proteasomal and caspase 12 activity, stress marker CHOP expression; enhances phosphorylation of eif2α in SH-SY5Y cells; high L-BMAA increases ROS and protein oxidation | [23] |
| OEC | LDH assay, MTS assay, Ca2+ influx assay, DCFDA assay for ROS, DNA damage assay | BMAA 0.1–3 mM/48 h | Rat olfactory ensheathing cells (special glial cells) | Cytotoxic, increases Ca2+ influx, and ROS production; disrupts mitochondrial activity | [24] |
| Primary neurons | LDH assay, MTS assay, Ca2+ influx assay, DCFDA assay for ROS, DNA damage assay | Primary neurons were obtained from 16 to 19 old foetuses and mixed brain cell cultures | BMAA increases Ca2+ influx and DNA damage, enhances production of ROS, disrupts activity of mitochondria | [25] |
| SH-SY5Y, HT22, Neuro-2a | MTT assay, siRNA transfection, flow cytometry for DNA content | Human neuroblastoma cells; mouse hippocampal cell line, mouse neuroblastoma cell line | L-BMAA-induced ER-stress mediated apoptosis via upregulation of ER-stress sentinels, phosphorylation of JNK, p38 and ERK, CHOP activation | [26] |
| SH-SY5Y, MRC-5, HUVEC | Liquid chromatography tandem mass spectrometry, radiolabeled 3H-BMAA assay, LDH assay, | 0.3 mM BMAA and 300 mM L-serine for 96 hours | Human neuroblastoma and human lung fibroblast cell line, human umbilical endothelial cells | BMAA is misincorporated in place of L-serine into human proteins and this is inhibited by L-serine | [22] |

Abbreviations: CHOP – C/EBP homologous protein; DCFDA – 2′,7′-Dichlorofluorescin diacetate assay; ER – endoplasmic reticulum; JNK – c-Jun N-terminal kinase; MTS – 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium.

2.11. Cytotoxicity of saxitoxins (Table 11). Saxitoxin (SXT) is a collective name for a group of more than 20 cyclic nonribosomal peptide molecules, formed by sulphation at different sites of two basic molecules: SXT and neo-SXT. Based on their toxicology, SXT are grouped in three classes—carbamate derivatives, gonyautoxins, N-sulfocarbomoyl derivatives, and decarbomoyl derivatives—decarbamoylsaxitoxin. They have a neurotoxic effect by blocking voltage-gated sodium channels [3].

### 3. Limitations

Studies on cell cultures cannot reveal all possible effects of toxins on the human body. This is due to the following reasons: (1) no matter how many cultures are tested, they will not cover the whole variety of cells in the body; (2) there are often significant differences between the cells in culture,
| Cell type     | Assay                        | Conditions                           | Tissue of origin   | Main effects                                                                 | Targets                                                                 | Ref.   |
|--------------|------------------------------|--------------------------------------|--------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------|--------|
| CaCo-2       | Neutral red uptake          | 1.1 mg/g dw; 0.08–1.25 mg dw/mL/48 h | Immortalized human colorectal adenocarcinoma cell line | Cytotoxicity, EC_{50}: 0.4 ± 0.1 mg dw/mL.                                |                                                                         | [29]   |
| CaCo-2       | Transepithelial electrical resistance (TEER) | CYN 1–10 µM/3–24 h | Immortalized human colorectal adenocarcinoma cell line | 16.7–20.5% intestinal permeability in 24 h; epithelial integrity not significantly altered |                                                                         | [30]   |
| CaCo-2       | Permeability of pseudoepithelial layer | CYN 1.9–48 µM/24–48 h | Immortalized human colorectal adenocarcinoma cell line | Apparent permeability: 3.45 × 10^{-7} cm/s (absorptive), 6.41 × 10^{-7} cm/s (secretive); epithelial permeability (increase): 10-fold (absorptive), 0.7-fold (secretive); |                                                                         | [31]   |
| CaCo-2, NCI-87, HCT-8, HuTu-80, Vero, C3A, HepG2 | MTT assay for cell viability | CYN 0.25–3 µM/1–7 days | Gastro-intestinal and hepatic cell lines | CYN sensitivity decreased in cell lines as follows: Gastric > duodenal > ileal > colonic; EC_{50} is 6.5 ± 3.3 µM for CaCo-2 |                                                                         | [32]   |
| CaCo-2, HepaRG | Cytokinesis-block micronuclear assay | CYN | The same human hepatocyte cell line | CYN increased the frequency of micronuclei in binucleated cells |                                                                         | [33]   |
| CaCo-2       | MTS assay for cell viability | CYN 0.7–96 µM/24–48 h | The same   | Lipid degeneration, mitochondrial damage, nucleolar segregation with altered nuclei, ultrastructure |                                                                         | [34]   |
| HIEC-6       | MTT assay                   | CYN 1–10 µM/24 h | Human intestinal epithelial cell line | Reduced cell viability by 13.4% and 21.8% |                                                                         | [35]   |
| mES          | Real-time PCR (RT-PCR)      | CYN 0–1 µg/mL/24–168 h | Undifferentiated mouse embryonic stem cell | DNA double-strand breaks after 72 h, upregulation of CYP1A1 by CYN and CYN + MC-LR via CDKN1A and GADD45A genes, cells arrested in G0/G1 phase | Oct4 Brachyury Nestin | Reference DNA [36] |
| HepG2        | MTS test, flow cytometry; RT-PCR | CYN | Human hepatocellular carcinoma cell line | DNA double-strand breaks after 72 h, upregulation of CYP1A1 by CYN and CYN + MC-LR via CDKN1A and GADD45A genes, cells arrested in G0/G1 phase | DNA | [37]   |
| Rat hepatocytes | LDH leakage, cysteine, ATP, and GSH assay | CYN | 2.5–5 µM/12 h | Rat hepatocyte cell line | Inhibition of GSH synthesis | GSH, cytochrome P450 | [38]   |
| Mouse hepatocytes | LDH leakage, protein synthesis | CYN | 2.5–5 µM/4–18 h | Mouse hepatocyte cell culture | Inhibition of LDH leakage, max at 0.5 µM CYN; CYN, 1–5 µM lead to 52–82% cell death | GSH, cytochrome P450 | [39]   |
| HepG2        | MTS assay, live/dead staining, qPCR, flow cytometry, confocal z-stack imaging | CYN | 0.125, 0.25, 0.5 µg/mL/72 h | Human hepatocellular carcinoma cell line | DNA double-strand breaks after 72 h, upregulation of CYP1A1 by CYN and CYN + MC-LR via CDKN1A and GADD45A genes, cells arrested in G0/G1 phase | DNA, expression of many enzymes | [40]   |
| WIL2-NS      | Cytokinesis-block micronuclear assay | CYN | 1–7 days | DNA deregulated genes for phase I and II enzymes, for cell proliferation; apoptosis and DNA damage response | DNA, expression of many enzymes | Centromere, micronuclei | [41]   |
| HepG2        | MTS assay, qPCR, flow cytometry | CYN | 0.1–0.5 µg/mL/24–96 h | Human hepatoma cells | Genotoxic effect by DNA double-strand breaks | DNA | [42]   |
| CLC          | AO/EB staining assay and comet assay, flow cytometry, qRT-PCR | CYN 20, 200, 2000 nM/24 h | Common carp (Cyprinus carpio L.) leucocyte cell line | Decreased cell membrane integrity, GSH/GSSG ratio, inhibited cell proliferation, DNA damage, increased ROS and ATP levels (1 µg/mL) | Micronuclei, GSH, ATP, SOD | [43]   |
| HepG2        | MTS assay, qPCR, flow cytometry | CYN 0.5 µg/mL/24 or 72 h, biphenols | Human hepatoma cells | Deregulation of some genes was more pronounced after exposure to the mixture | DNA | [44]   |
| A7r5         | AO/EB staining assay and comet assay, flow cytometry, qRT-PCR | CYN 20, 200, 2000 nM/24 h | Immortalized human colorectal adenocarcinoma cell line | Apparent permeability of the pseudoepithelial cell layer to MC-LR | DNA | [45]   |
| A7r5         | AO/EB staining assay and comet assay, flow cytometry, qRT-PCR | CYN 20, 200, 2000 nM/24 h | Immortalized human colorectal adenocarcinoma cell line | Apparent permeability of the pseudoepithelial cell layer to MC-LR | DNA | [46]   |
### Table 4: Continued.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|------------------|--------------|---------|------|
| LLC-PK1   | Flow cytometry, qRT-PCR | 1.0 μg/mL | Renal epithelial cells derived from proximal tubules | CYN induced necrosis and increased gene expression of Na+/K+–Atpase | Na⁺/K⁺-ATPase activity | [47] |
| Human keratinocytes | LDH leakage, WST-1 cell proliferation assay, Scratch test, crystal violet assay | 1, 10 μg/mL for 24/48h | Primary human keratinocytes | CYN induced cytotoxicity, impaired migration, and inhibition of proliferation | | [48] |

Abbreviations: AO/EB staining – acridine orange/ethidium bromide staining; ATP – adenosine triphosphate; CAT – catalase; GPX – glutathione peroxidase; GSH – glutathione; MTS – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; RP-PCR – reverse transcription polymerase chain reaction; RT-qPCR – quantitative reverse transcription polymerase chain reaction; SOD – superoxide dismutase.

### Table 5: Cytotoxicity of depsipeptides.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|------------------|--------------|---------|------|
| N2a, NCI H-460 | MTT assay | Pal A IC₅₀: 17.2 μM/24 h; 39.7 μM/48 h | Neuro2a mouse neuroblastoma cells; human lung carcinoma cells | Blockage of the voltage-gated sodium channel, modest cytotoxic effects. | Voltage-gated sodium channel | [51] |
| NCI H-460 | MTT reduction | 20 μg/well | Human lung carcinoma cells | Cytotoxicity, IC₅₀: 2.6 nM/48 h | GI-phase cell cycle arrest, apoptosis | [52] |
| 60 cancer cell lines | Flow cytometry | | Human cells from lung, colon, leukemia, melanoma, CNS, ovarian, prostate, breast and renal cancers | Cytostatic and cytotoxic effects – increase the number of cells in G₁, little change in G₂/M and loss of cells in S-phase. GI₅₀ for CoA: 2.8 nM to MDA-MB-231 7.4 nM to LOX IMVI 7.4 nM to HL-60(TB) | Novel unknown mechanism; no effect on tubulin or actin in cytoskeletal assays | [53] |
| MDCK cells infected with influenza virus A/WSN/33/London (H1N1) | Dye uptake assay using neutral red | Ich A and B in nontoxic conc. 12.5–100 μg/mL/30 min. | Canine kidney | Antiviral activity, IC₅₀: 12.5 μg/mL | Non-trypsin protease inhibition | [54] |
| 60 human cancer cell lines (NCI-60 cell lines) | Biokinetics reader, fluorescence detection, acute toxicity determination, MTT assay, hollow fiber assay | | Human leukemia, melanoma, lung, colon, CNS, ovarian, prostate, breast and renal cancer cell lines | | | |
| WHCO1, WHCO6, ME180 | MTT assay | | WHCO1,06–esophageal and ME180–cervical cancer cells | | | |

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WHCO1, WHCO6, ME180

Abbreviations: AO/EB staining – acridine orange/ethidium bromide staining; ATP – adenosine triphosphate; CAT – catalase; GPX – glutathione peroxidase; GSH – glutathione; MTS – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; RP-PCR – reverse transcription polymerase chain reaction; RT-qPCR – quantitative reverse transcription polymerase chain reaction; SOD – superoxide dismutase.
the primary cell lines and the cells in the body tissues in the quantity and quality of expressed proteins (genes expression), metabolic pathways and cell function [113–115]. Therefore, results from cells in culture cannot be directly transferred to the tissue of origin or of which they will form. (3) Numerous regulations are active continuously and simultaneously in the organism, and their cross-influence cannot be simulated in experiments with cell cultures. (4) Parameters like LC50 or ID50 are different for cells in culture and human body.

### Table 5: Continued.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|------------------|--------------|---------|------|
| P388, A549, PC3, HCT8, SK-OV | MTT assay, scintillation counting | P388-murine leukemia, A549-lung carcinoma, PC3-prostate cancer, HCT8 -ileoecal colorectal adenocarcinoma and SK-OV-ovarian cancer cells | Cytotoxicity and antimalarial activity; IC50 for cancer lines: P388–24.4 nM; A549–2.4 nM; PC3–2.6 nM; HCT8–2.1 nM; SK-OV–4.5 nM; IC50 for Plas. Falciparum–0.29 μM Cytotoxicity and antimycobacterial activity against M. tuberculosis. For HT-29 IC50: Pit A–13 μM; Pit B–13 μM; Pit C–67 μM; Pit D–>100 μM; Pit E–75 μM; Pit F–87 μM and for MCF7 IC50: Pit A–13 μM; Pit B–11 μM; Pit C–73 μM; Pit D–>100 μM; Pit E–>100 μM; Pit F–83 μM | Mitochondria-induced apoptosis, lag C selectively binding to the prohibitin | [57] |
| HT-29, MCF7 | MTT assay, disc diffusion assay | HT-29 colon adenocarcinoma, MCF7 breast cancer cells | Cytotoxicity, mitochondria-induced apoptosis Cytotoxicity for NCI-H460 LC50 is: Wew A–0.65 μM; wew B–0.43 μM; wew C–5.9 μM; wew D–3.5 μM; for neuro-2a LC50: Wew A– 0.49 μM; wew B–0.20 μM; wew C–10.7 μM; wew D–1.9 μM | - | [58] |
| HeLa cells | WST-1 assay, Immuno-precipitation 100 nM aurilide | Human cervical cancer cells | Cytotoxicity, mitochondrial-induced apoptosis Mitochondria-induced apoptosis Cytotoxicity for NCI-H460 LC50 is: Wew A–0.65 μM; wew B–0.43 μM; wew C–5.9 μM; wew D–3.5 μM; for neuro-2a LC50: Wew A– 0.49 μM; wew B–0.20 μM; wew C–10.7 μM; wew D–1.9 μM | Prohibitin 1, optic atrophy 1 | [59] |
| NCI–H460, neuro-2a | MTT reduction. | NCI–H460 – human lung tumor, neuro-2a – mouse neuroblastoma cell lines | Cytotoxicity, apoptosis, and inhibition of cell growth. EC50 cytotoxicity is < 100 nM for human U87-MG and SF-295 cells, and for mouse embryonic fibroblasts | Caspase-3, extensive cytoplasmic vacuolization, mTor-independent pathway | [60] |
| 60 human cancer cell lines (NCI-60 cell lines), MEFs | Immunoblot, MTT assay, Trypan blue exclusion, LDH assay, caspase activity assay, autophagy assays, EGF receptor degradation assays | Human cancer cells from leukemia, melanoma, lung, colon, CNS, ovarian, prostate, breast, renal cancers | Cytotoxicity, apoptosis, and inhibition of cell growth. EC50 cytotoxicity is < 100 nM for human U87-MG and SF-295 cells, and for mouse embryonic fibroblasts | Caspase-3, extensive cytoplasmic vacuolization, mTor-independent pathway | [61] |
| HCC2218, UACC-893, T-47D and >50 others | Growth inhibition assay, immune-precipitation study, SEAP secretion assay | Human breast, ovarian, endometrial, pancreatic, skin, lung, and colon cancer cell lines; rat pancreatic exocrine cell line | Cytotoxicity, blocking of cotranslational translocation. IC50 = 5–50 nM for different cell types | Sec61 in the ER membrane | [62] |

Abbreviations: EGF – epidermal growth factor; SEAP – secreted embryonic alkaline phosphatase.
| Cell type | Assay | Conditions | Tissue of origin | Main effects | Ref. |
|-----------|-------|------------|------------------|--------------|-----|
| Microglia | Superoxide anion (O$_2^-$) generation, cell viability by LDH release, thromboxane B$_2$ (TXB$_2$), immunoassay, gelatinase zymography for matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9), rat-specific ELISA for cytokines and chemokines | Microcystis aeruginosa LPS strain UTCC 299; 0.1–100,000 ng/mL/17 h E. coli LPS (0.1–100 ng/mL) as control | Rat neonatal brain microglia | Enhanced O$_2^-$ generation, limited inflammatory mediator generation; MMP-9, macrophage inflammatory protein-2 (MIP-2/CXCL2) release, TXB$_2$, concurrent with maximal O$_2^-$ generation; elevated TXB$_2$, MMP-9, tumor necrosis factor α (TNF-α), interleukin 1-α (IL-1α), and interleukin-6 (IL-6), macrophage inflammatory protein 1α (MIP-1α/CCL3), and MIP-2/CXCL2; LPS activates brain microglia in vitro and the release of O$_2^-$, inflammatory mediators | [64] |
| Microglia | 0.1–100000 ng/mL Oscillatoria sp. LPS; 17 h | Rat neonatal microglia | Classical and alternative activation; pro-inflammatory and anti-inflammatory mediator release Concentration-dependent O$_2^-$, MMP-9, IL-6 TNF-α, MIP-2/CXCL2, CINC-1/CXCL-1, MIP-1α/CCL3, IL-10 release | Cyanobacterial LPS antagonists could be considered as a candidate of protective agents for motor neurons in degenerative diseases | [65] |
| Microglia | Scytonema javanicum and S. ocellatum LPS | Rat neonatal microglia | | | [66] |
| Meningioma cells and meningioma–primary human macrophage | Sandwich immunoassay | Cyanobacterial LPS antagonist (CyP) 1–20 μg/monolayer | Human meningioma cells and meningioma–primary human macrophage cocultures | Cyanobacterial LPS inhibits cytokine production and augments the anti-inflammatory response when combined with benzylpenicillin | [67] |
| Microglia | Immunocytochemical and immunofluorescent assay, ELISA, immunoblotting, live-cell imaging analyses | Cyanobacteria-derived TLR4 antagonist—a highly (95%) purified form of LPS-like molecule from Oscillatoria planktothrix sp. 20 μg/mL for 24 h, Primary cultures from mouse spinal cords | | TLR4 antagonists could be considered as a candidate of protective agents for motor neurons in degenerative diseases | [68] |
| Spleen cells | Hot-water extract of Spirulina platensis | In vitro cultures of murine spleen and thymus cells | | Increased proliferation of spleen cells; enhanced IL-1 production from peritoneal macrophages | [69] |
Table 7: Cytotoxicity of lyngbyatoxins.

| Cell type       | Assay                  | Conditions                                                                 | Tissue of origin          | Main effects                                                                 | Targets                                    | Ref.       |
|-----------------|------------------------|-----------------------------------------------------------------------------|----------------------------|-------------------------------------------------------------------------------|--------------------------------------------|-----------|
| Fibroblasts     |                        |                                                                             |                            |                                                                               |                                            |           |
| FL              | MTT assay, [3H]-thymidine incorporation assay | 15 mg/mL (w/v) of the cyanobacterial extract/4 h or 24 h for MTT test; 24 h for [3H]-thymidine incorporation assay | Human amniotic cells, human | 80% inhibition of cell proliferation, morphology and attachment in 24 h Stimulated MTT reduction after 4 h > 40% vs control cells; decreased cell viability to 32% of controls in 24 h | DNA, cell membrane, cytoskeleton              | [70]      |
| A2058           |                        |                                                                             | Normal amniotic cells, human |                                                                               |                                            |           |
| RD              |                        |                                                                             | Human metastatic melanoma | Cytotoxic in 24 h                                                             |                                            |           |
| 3T3             |                        |                                                                             | Mouse embryonic fibroblasts | 92% inhibition of cell proliferation                                           |                                            |           |
| L1210           | MTT                    | Lyngbyatoxin A and 12-epi-lyngbyatoxin A/18 h                              | Mouse lymphocytic leukemia cell line |                                                                               | PKC isoforms                                | [71]      |
| HL-60 C         | Test of induction of cell adhesion | Lyngbyatoxin A and debromoaplysia toxin/48 h                              | Human promyelocytic leukemia cells |                                                                               |                                            |           |
| DS 19           | Test of inhibition of terminal differentiation | Lyngbyatoxin A and debromoaplysia toxin/48 h                              | Mouse erythroleukemia cells transformed by Friend leukemia virus strain 745A | Inhibition of terminal differentiation in 50% of the cells with 0.35 ng/mL Lyngbyatoxin A and 150 ng/mL debromoaplysia toxin | Cell membrane                              | [72]      |
| Neuro-2a        | MTT                    | 24 h                                                                        | Mouse neuroblastoma cells   |                                                                               |                                            |           |
| CHO             | Patch-clamp            | 0.1–30 μM neo-debromoaplysia toxin G and H                                 | Chinese hamster ovary cells |                                                                               | Voltage-gated potassium channels Kv1.5 (KCNA5) | [74]      |
| Cell type       | Assay                                   | Conditions            | Tissue of origin                                      | Main effects                                                                 | Targets                                      | Ref. |
|----------------|-----------------------------------------|-----------------------|-------------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------|------|
| CaCo-2         | Immuno-localization of MC uptake        | MC-LR 1–75µM/30 min–24 h | Immortalized human colorectal adenocarcinoma cell line | Artificial epithelial cell layer is highly permeable to MC-LR                  |                                               | [45] |
| CaCo-2         | Gene expression, transcriptomics         | MC-LR 10–100µM/4–24 h  | The same                                             | Oxidative stress                                                             | ERK/MAPK and cell cycle pathway molecules    | [75] |
| CaCo-2         | Comet assay, MTT assay (for viability)  | MC-LR 0.2–10µM/4–48 h  | The same                                             | 20% damaged DNA after 0.2µM/4h MC-LR; 40% reduced cell viability after MC-LR 10µM/48h, PP inhibition—3.0 nM MC-LF, 3.8 nM MC-LW, 1.0 nM MC-LR, EC50 of LDH leakage: 25% (50µM MC-LR), 36% (MC-LW), 51% (MC-LF), chromatin cell shrinkage, condensation, membrane blebbing, and cytoskeletal reorganization | DNA                                          | [76] |
| CaCo-2         | Protein phosphatase (PP) inhibition, LDH leakage, cell morphology and proliferation | 1–50µM MC-LR, -LF and -LW for 22–48 h | The same                                             | Facilitated MC uptake in <1h by organic anion transporters, active excretion | PP, cell membrane, chromatin, cytoskeleton   | [77] |
| CaCo-2         | Bradford assay, MTS reduction (for viability), neutral red uptake | MC-LR, –RR and -YR, 50–200µM/6–24 h | The same                                             | Viability—12.5µM/24h; TEER at 50µM/12h and at 12.5µM/24h; apoptosis at 12.5µM/24h; western blot at 12.5µM/24h; occludin; claudin not affected), 25µM/24h; ZO-1; PP2A activity decreases from 12.5µM/24h | Protein synthesis                           | [78, 79] |
| CaCo-2         | Immuno-localization of microcystins      | MC-LR, –RR, 1–50µM/30 min–24 h | The same                                             | Facilitated MC uptake in <1h by organic anion transporters, active excretion | Organic anion transporters 3A1 and 4A1       | [80] |
| HIEC-6         | Cell counting Kit-8 for viability, western blot, TEER, PP2A activity | MC-LR 0–50µM/6–24 h | Human intestinal (colon) epithelial cell line         | Viability—12.5µM/24h; TEER at 50µM/12h and at 12.5µM/24h; apoptosis at 12.5µM/24h; western blot at 12.5µM/24h; occludin; claudin not affected), 25µM/24h; ZO-1; PP2A activity decreases from 12.5µM/24h | PP2A, occludin, claudin                    | [81] |
| HEK293         | Western blot, luciferase assay, rTPCR    | MC-LR 10µM/24 h        | Human embryonic kidney cells                         | PP2A inhibition, enhanced proto-oncogene C-myc expression                   | PP2A, c-Myc protein, proto-oncogene C-myc    | [82] |
| NCC            | PP2A, PP2B, PP2C activity, western blot, Akt, p38, JNK, PI3K assays, genechip analyses; | MC-LR, 0.0001–1.0 µg/ 24 h | Immortalized colorectal crypt cells                  | Constitute activation of Akt/p38 and JNK/MAPK pathways                      | Akt, p38, JNK                                | [83] |
| HBE1, 16HBE14o- | RT-PCR, western blot, RTCA, neutral red uptake | MC-LR 1–20µM/48 h | Human bronchial epithelial cell lines                | No effect on viability, ERK1/2 and p38 activities were not changed          | ERK1/2 and p38 not influenced                | [9]  |
### Table 8: Continued.

| Cell type       | Assay                                | Conditions                  | Tissue of origin                  | Main effects                                                                                           | Targets                                      | Ref. |
|-----------------|--------------------------------------|-----------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------|------|
| DLD-1, HT-29    | Western blot, RT-qPCR, knockdown of SMAD2 by siRNA, migration and invasion assay | MC-LR, 0.1–50 nM/24 h       | Human colorectal cancer cells     | Induction of SMAD2 signal transducer and transcriptional modulating protein expression, its activating phosphorylation by PI3K/Akt, increased migration (epithelial-mesenchymal transition of both cell types) Decreased transcription of mRNA for iNOS, IL-1β, TNF-α, GM-CSF, and IFN-γ; reduced inflammatory response to LPS | PI3K/Akt, SMAD2, iNOS, IL-1β, TNF-α, GM-CSF and IFN-γ | [84] |
| BALB/c          | mRNA                                 | MC-LR, 1–1000 nmol/L/6 h    | Mouse peritoneal macrophages      | decreased transcription of mRNA for iNOS, IL-1β, TNF-α, GM-CSF, and IFN-γ; reduced inflammatory response to LPS | NF-κB, ERK1/2, TNF-α | [85] |
| RAW 264.7 macrophages | Western blot, ELISA, RT-qPCR, Western blot; MTT assay, mitochondrial membrane potential (MMP) | MC-LR, 1–1000 nmol/L/30 min–24 h | Human hepatocellular carcinoma cell line | Microtubules and filamentous actin (cytoskeleton), PP2A/C, p38, | PP2A catalytical and regulatory subunits | [87] |
| HepG2           | MTT assay, PP2A activity, Western blot, proliferation | MC-LR, 0.5–10 μM/24 h       | Human non-small-cells lung cancer cells | Microtubules and filamentous actin (cytoskeleton), PP2A/C, p38, | PP2A catalytical and regulatory subunits | [88] |
| A549            | Western blot, cell detachment, PP2A activity, MTT assay | MC-LR, 0.5–10 μM/24 h       | Human embryonic kidney cells      | PP2A inhibition (>5 μM); PP2A activation (1–2 μM); cell anoikis | PP2A, p38 MAPK, HSP27 | [89] |
| HEK293          | Western blot, cell detachment, PP2A activity, MTT assay | MC-LR, 0.5–10 μM/24 h       | Human embryonic kidney cells      | PP2A inhibition (>5 μM); PP2A activation (1–2 μM); cell anoikis | PP2A, p38 MAPK, HSP27 | [90] |
| PC12            | Western blot, PP2A activity, immunofluorescence | MC-LR, 0.1–10 μM/6 h        | Pheochromocytoma cells of the rat adrenal medulla | Activation of p38 MAPK, JNK and ERK1/2, HSP27-sensitive cytoskeleton reassembly, PP2A inhibition in 6–24 h; activated phosphorylation of tau (by P38 MAPK) and VASP | p38 MAPK, JNK, ERK1/2, PP2A, tau and VASP components of cytoskeleton | [91, 92] |
| HL7702          | PP2A activity, western blot, immunofluorescence | MC-LR, 5 or 10 μM for 30 min to 24 h; | Human normal liver cell line      | p-HSP27, p-VASP and p-cofilin contributed to cytoskeleton change; PP2A inhibition (>0.5 μM); disorder of cytoskeleton | HSP27, VASP, cofilin, PKA, Rac1, PP2A | [93] |
| SMMC-7721       | PP2A activity, western blot, PKA activity and Rac1/Cdc42 activity immunofluorescence, immunoprecipitation | MC-LR, 0.5–10 μM/24 h       | Human liver cancer cell line      | p-HSP27, p-VASP and p-cofilin contributed to cytoskeleton change; PP2A inhibition (>0.5 μM); disorder of cytoskeleton | HSP27, VASP, cofilin, PKA, Rac1, PP2A | [93] |
### Table 8: Continued.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|------------------|--------------|---------|------|
| HepaRG    | RNA quantified by Agilent RNA 6000 Nano kit | MC-LR, –RR 10, 100 and 1000 ng/2 h | Human hepatocyte cell line | Increase of RNA of apoptotic and inflammatory gene; many cellular pathways activated | PI3K/Akt/S6K1, hyperphosphorylation of Bcl-2, Bad, c-Myc and c-Jun | [94] |
| HL7702    | Real-time cell analyzer (RTCA) proliferation, cell cycle analysis, western blot, PP2A activity, immuno-fluorescence MTT and Annexin V/PI assay, ROS and MMP measurements, western blot | MC-LR, 1, 5, 10 µM/1–96 h | Human normal liver cell line | MC-LR promoted HL7702 cell proliferation (36–48 h); activation of Akt/S6K1 cascade; PP2A activity (>1 µM), hyperphosphorylation of Bcl-2, Bad, c-Myc and c-Jun, 1–10 µM PI3K/Akt/S6K1, hyperphosphorylation of Bcl-2, Bad, c-Myc and c-Jun | Caspases | [95] |
| HBE       | MTT and Annexin V/PI assay, ROS and MMP measurements, western blot | MC-LR, 1, 10, 20, 30, 40 µg/mL/24, 48 h | Human bronchial epithelial cells | Inducing mitochondria-dependent apoptosis (1–40 µg/mL), MMP decreases at 10 µg/mL 5 µM MC-LR induced PP2A mRNA expression, p-CREB, expression of NF-κB, IFN-α, and several INF-α-stimulated genes are activated | DNA expression | [89] |
| Huh7      | Micronucleus assay, Flow cytometry, comet assay, DNA damage | NOD, 0.001, 0.01, 0.05, or 0.1 µg/mL/24 h | Human hepatoma cell line | DNA damage >1 µg/mL, apoptosis from 1 µg/mL/48 h NF-κB, p-CREB, DNA | DNA, cellular and mitochondrial membranes | [96] |

Abbreviations: CREB – cAMP responsive element-binding protein; ERK/MAPK – extracellular signal-regulated kinase/mitogen-activated protein kinase; GM-CSF – granulocyte macrophage colony-stimulating factor; IFN-γ – interferon gamma; iNOS – inducible nitric oxide synthase; JNK–c – Jun N-terminal kinases; mRNA – messenger RNA; siRNA – small interfering RNA, VASP – vasodilator-stimulated phosphoprotein.

### Table 9: Cytotoxicity of nodularins.

| Cell type | Assay | Conditions | Tissue of origin | Main effects | Targets | Ref. |
|-----------|-------|------------|------------------|--------------|---------|------|
| CLC       | Fluorometric cell membrane integrity, cell viability and ROS measurements, caspase-glo 3/7 assay, ELISA | NOD, 0.001, 0.01, 0.05, 0.1 µg/mL/24 h | Carp leukocyte cell line and head kidney leukocytes | Cell viability, membrane integrity at 0.1 µg/mL, DNA fragmentation and caspases 3/7 activation at >0.1 µg/mL, ROS increase in 60 min in >0.01 µg/mL, GSH decrease at >0.001/24 h | GSH/GSSG, DNA, membranes, caspases | [98] |
| CLC and kidney leucocytes | Fluorometric cell viability, ROS and nitrogen species (NS) measurements | 0.001, 0.01, 0.05, or 0.1 µg/mL/24 h | Carp leukocyte cell line, kidney leukocytes | Cytotoxicity ≥0.05 µg/mL, ROS and NS increase, expression of TNF-α, IL-10, less TGF-β | DNA expression | [99] |
| HepG2     | Micronucleus assay, Flow cytometry, comet assay, DNA damage | NOD, 1–10 µg/mL, for 6, 12, 24, 48 h | Human hepatoma cell line | DNA damage >1 µg/mL, apoptosis from 1 µg/mL/48 h | DNA, cellular and mitochondrial membranes | [100] |
| HepG2     | RT-PCR, siRNA, flow cytometry, transfection of NF-κB immunoblotting | NOD, 2.5, 5, 7.5, 10 µM/24 h | Human hepatoma cell line | Induces fas receptor (fas) and fas ligand (FasL) expression and apoptosis | NF-κB pathway, fas, FasL | [101] |
| HepG2 and Huh7 | ATF-6 activity qPCR, TNF-α ELISA, immunoblotting | NOD, 0.1, 1, 5 µM for 24, 48 and 72 h | Human hepatoma cell lines | Induction of TNF-α protein, CAAT/enhancer-binding protein-homologous, DNA damage; apoptosis (BAX, BCL2) genes, ROS increase, oxidative stress | TNF-α, ERK 1/2 MAPK, EL-8, CHOP ER-stress—2.5 nM | [102] |
| HepG2     | qPCR, MTT assay, comet assay, cytokinesis micronucleus assay | NOD, 0.01, 0.1 and 1 µg/mL | Human hepatoma cell line | DNA damage; apoptosis (BAX, BCL2) genes, ROS increase, oxidative stress | DNA, ROS | [103] |
4. Perspectives

The use of cell cultures in toxicological studies will remain the main approach due to its speed, relatively low cost, reproducibility, precision with respect to the studied intracellular components, and ethical acceptability. The use of cell cocultures [116–118] and in vitro formed organ-like structures such as artificial neuronal network [119], cardiomyocyte spheroids with contractile activity [120], and organ-on-a-chip systems [121], which are functionally closer to the human body [11], will increase in the future.

5. Conclusion

The presence of all these data on the cytotoxicity of aeruginosins, anatoxins, cylindrospermopsin, depsipeptides, lipopolysaccharides, lyngbyatoxins, microcystins, nodularins, cyanobacterial retinoids, and saxitoxins in a review is a great advantage. It allows the advancement of research on CT using cell cultures by facilitating the selection of the most appropriate methods, conditions, and cell lines for toxicological and pharmacological studies. In addition, it could increase the use of CT in functional studies of their intracellular targets. Therefore, this review allows in one look to advance the toxicological, physiological, and pharmacological studies of CT by the knowledge of their harmful effects with a focus on human and animal health as well as on environmental protection.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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