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Synthetic analogues of cyanobacterial alkaloid cylindrospermopsin and their toxicological activity

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ABSTRACT

Cylindrospermopsin (CYN) is a naturally occurring alkaloid produced by a variety of cyanobacteria and known to induce oxidative stress-mediated toxicity in eukaryotic cells. Despite extensive research on the mechanism of CYN toxicity, an understanding of the structural features responsible for this toxicity and the mechanism by which it can enter the cell are still not clear. It was established that the presence of both the uracil and guanidine groups is essential in biological activity of CYN whilst not much is known in this regard on the role of tether that separates them and the attached hydroxyl group. Therefore, in the present study we have prepared three synthetic analogues possessing uracil and guanidine groups separated by a variable length tether (4–6 carbons) and containing a hydroxyl function in a position orientation to CYN, together with a tetracyclic analogue of CYN lacking the hydroxyl group at C-7. The toxicity of these compounds was then compared with CYN and guanidinoacetate (GAA; the primary substrate in CYN biosynthesis) in an *in vitro* model using human neutrophils isolated from healthy subjects. The lowest activity measured by means of reactive oxygen species generation, lipid peroxidation and cell death was observed for GAA and the tetracyclic analogue. The greatest toxicity was found in an analogue with a 6-carbon tether, but all three analogues and CYN caused rapid onset of redox imbalance. These results add to the general understanding of CYN toxicity and preliminary findings suggest that the –OH group at C-7 may be significant for the cellular transport of CYN and/or be involved in its toxic activity inside the cell, a hypothesis which requires further testing.

1. Introduction

Cylindrospermopsin (CYN) **1**, is a naturally occurring cyanotoxin originating from the cyanobacteria *Cylindrospermopsis raciborskii* (Ohtani et al., 1992). This was the first member of a small group of related alkaloids to be isolated, along with *7-epi*-cylindrospermopsin **2**, *7*-deoxy-cylindrospermopsin **3**, *7-deoxy-desulfo*-cylindrospermopsin **4** and *7-deoxy-desulfo*-12-acetylcylindrospermopsin **5** from this and other cyanobacteria species (Banker et al., 2000; Norris et al., 1999; Wimmer et al., 2014). In 1979 a bloom of *C. raciborskii* occurred and contaminated a local water supply and was thought to be responsible for an outbreak of hepatoenteritis in 1979 (Byth, 1980). The outbreak, coined the “Palm Island mystery disease” was responsible for the hospitalization of 148 people, the vast majority children (Byth, 1980). The highly toxic potencies of CYN in different types of mammalian cells were later extensively demonstrated (Poniedziałek et al., 2012). Over ten freshwater species are currently known to be able to produce **1** including *Aphanizomenon ovalisporum*, *Raphidiopsis curvata* and terrestrial strain of *Hormoscilla pringsheimii* (Fig. 1) (Banker et al., 1997; Li et al., 2001; Rzymski and Poniedziałek, 2015; Bohunicka et al., 2015). The reasons behind its production are yet to be fully elucidated though the toxin is known to be actively released from intact cells, to up-regulate alkaline phosphatase in sympatric phytoplankton and to contribute to allelopathic interactions (Rzymski et al., 2014).

Previous studies have attempted to link the activity of CYN **1** to specific functional groups within the molecule, most notably a study by Sukenik who proposed that the uracil group was partially responsible for its potent toxic activity, reasoning that there might be competitive or inhibitory binding to a catalytic site (Banker et al., 2001). Since this study, there have been only a small number of reports on the synthesis of analogues of the cylindrospermopsin family, most probably due to their structural complexity. However, Runnegar et al. (2002) conducted a structure activity relationship investigation (SAR) utilising a series of simplified synthetic analogues of the natural products. Within this work

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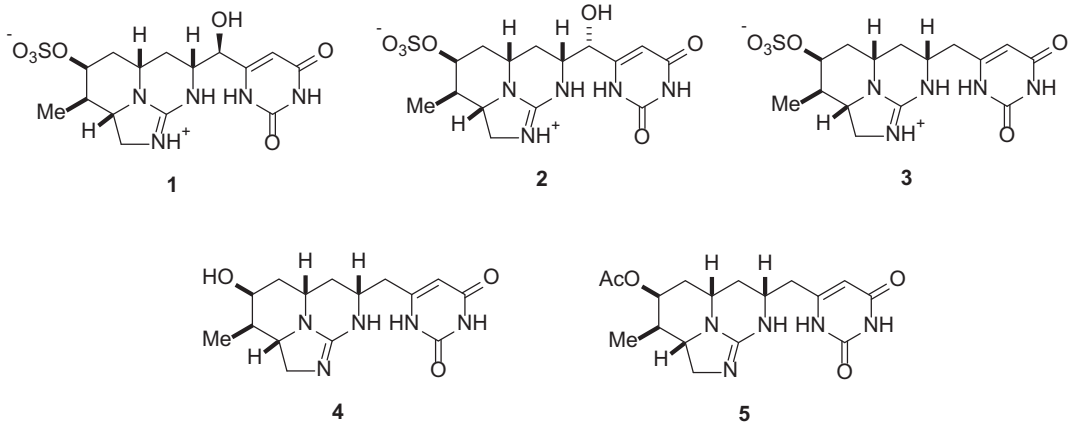

Fig. 1. The cylindrospermopsin alkaloids.

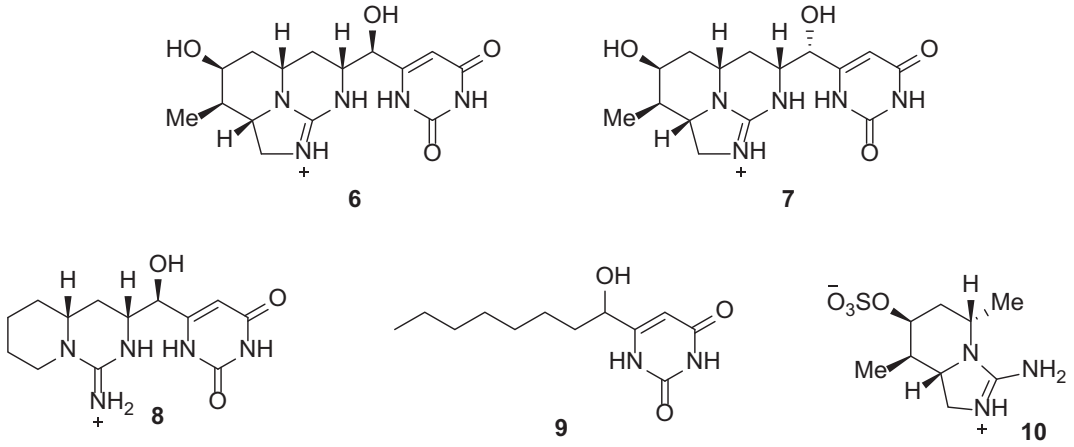

Fig. 2. Cylindrospermopsin analogues.

the unsulfonated analogues ± **6** and **7** were studied along with tricyclic analogue ± **8**. The simplified uracil analogue ± **9** was also investigated, as was the bicyclic sulfonate analogue ± **10**, which lacks the uracil group (Fig. 2) (Runnegar et al., 2002).

Initially, the toxicity of synthetic racemic CYN ± **1** was compared to that of the stereoisomer 7-*epi*-cylindrospermopsin **2**. The results obtained provided evidence to state that the exact stereochemistry of the hydroxyl group located at *C*-7 had little effect on the biological activity or transport of the toxin. Furthermore, a comparison of the activity of ± **1** and **2** alongside the corresponding diols ± **6** and **7** indicated that ± 1 and ± **6** both inhibit protein synthesis with almost identical IC_{50} values of 0.20 and 0.21 μM, whilst 7-*epi*-cylindrospermopsin **2** and diol ± **6** each depleted cell antioxidant glutathione by similar amounts. These results indicate that the sulfate group present within majority of the CYN alkaloids plays no appreciable role in either their biological activity or cellular uptake. Of the other compounds studied, the tricyclic analogue ± **8** demonstrated an inhibitory effect on protein synthesis but only at concentrations between 500 and 1000 times greater than those of **1**. Similarly analogues ± **9** which lacks the guanidine functionality, and ± **10** which lacks the uracil ring displayed no biological activity at concentrations between 800 and 2000 μM (Runnegar et al., 2002). From these results it was concluded, that the presence of both the uracil and guanidine groups are essential and that the guanidine must be contained in a lipophilic environment to enable any biological activity. Shaw also investigated the role of the hydroxyl group located at C-7. This was done by analysing the effect of 7-deoxy-cylindrospermopsin **3** in four different mammalian cell lines, the results demonstrated that **3** displays the same levels of toxicity as CYN **1**. The levels of protein synthesis inhibition were also similar, with IC_{50} values of 340 and 220 nM for **3** and **1** respectively (Neumann et al., 2007). Williams et al. also determined that both toxins inhibit protein synthesis within one order of magnitude of each other and that both have a similar inhibitory effect on cell glutathione (Looper et al., 2005). However, *in vivo* comparison of both compounds revealed that **3** did not produce any toxic effects at levels exceeding the lethal concentrations established for **1** (Norris et al., 1999). Recently it was suggested that exposure to CYN at environmentally relevant concentrations, has the potential to lead to a reduced ability to fight pathogens and therefore increasing susceptibility to potential infections (Poniedziałek et al., 2014a; Poniedziałek et al., 2014b). Further work in this area has sought to elucidate the sequence of events underlying CYN induced oxidative stress in human cells. The toxin has been shown to significantly increase levels of intracellular reactive oxygen species (ROS) and significantly suppresses the activation of superoxide dismutase (SOD) and catalase (CAT) whilst up-regulating glutathione peroxidase (GPx) (Poniedziałek et al., 2015).

It is apparent that it is still unclear as to role of the uracil group in these alkaloids, particularly as to its role in transportation into the cell or if it also plays a key role in the *in vitro* activity. It is however apparent the sulfate group does not play a significant role in either the biological activity or the transport of the CYN alkaloids, but that the hydroxyl group does play a vital role although the hydroxyl-stereochemistry does not seem to be a key factor in this activity (Runnegar et al., 2002; Neumann et al., 2007). With these observations in mind, we were motivated to prepare a series of analogues of **1** which contain the key guanidine, uracil and hydroxyl groups found in **1** separated by a tether. Our plan was to simplify the metabolites by removing the tricyclic ring system and obtaining the structures **11a–c** which are separated by a variable length tether (n = 1–3) (Fig. 3). We eventually compared the bioactive properties of **11a–c** with CYN **1**, a tetracyclic analogue **22** and