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STRUCTURAL REQUIREMENTS FOR BIOLOGICAL ACTIVITY OF THE MARINE ALKALOID ASCIDIDEMIN.

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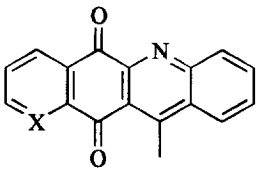
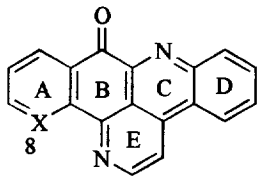
Abstract. Comparison of the biological activities observed for ascididemin (**2**) and synthetic precursors/analogs has established the importance of N-8 in ring A, and a completed ring E, to topoisomerase II enzyme inhibition, human tumor cytotoxicity and antifungal/antibacterial properties. The results also suggest the presence of multiple mechanisms of toxicity by **2** towards mammalian cell systems.

The search for new pharmaceuticals from the marine environment has resulted in the isolation of an ever increasing number of alkaloids based upon the pyrido[2,3,4-*kl*]acridine skeleton.[1] These structurally related compounds typically exhibit a wide range of potentially useful biological activities, including tumor cytotoxicity and fungal growth inhibition. However, due to the random array of structures isolated and the paucity of directly comparable reports of biological activities, it is extremely difficult to define the structural requirements of these activities. In order to address this issue, we have recently initiated a structure-activity study of the pyrido[2,3,4-*kl*]acridine-based alkaloid ascididemin (**2**).



(**1**) X = N
(**3**) X = CH



(**2**) X = N
(**4**) X = CH

Ascididemin has previously been reported to exhibit *in vitro* tumor cytotoxicity (L1210, IC_{50} 1.4 μM)[2] and to inhibit topoisomerase II enzyme.[3] To assess the influence of the ring A nitrogen and the presence of ring E on the biological properties of **2**, we have prepared **1** - **4** using established procedures[4,5] and evaluated them in a range of biological assays.

Ascididemin (**2**) exhibited cytotoxicity to a range of *in vitro* tumor cell-lines, including mouse leukemia (P388), human colon (HCT116) and human breast (MCF7) (Table 1). A ten-fold differential toxicity (hypersensitivity factor, HF) towards the DNA-double strand break repair deficient Chinese Hamster Ovary (CHO) cell-line xrs-6[6] versus the repair competent wild type BR1[7] was observed. This result, in conjunction with the previously observed *in vitro* inhibition of topoisomerase II enzyme,[3] is indicative of a mechanism of action involving poisoning of the enzyme by stabilization of the DNA-topoisomerase II cleavable complex.[8] No differential toxicity (HF_2 : Table 1) was observed between wild type BR1 and the DNA-single strand break repair deficient CHO cell-line EM9.[9] As topoisomerase I enzyme inhibiting compounds e.g. camptothecin, typically exhibit hypersensitivity factors in this assay, we conclude that ascididemin does not interfere with the correct functioning of this particular DNA-processing enzyme.

Table 1. Evaluation of the *In Vitro* Cytotoxic Activities of Compounds **1** - **4.**

	1	**2**	**3**	**4**
P388 IC_{50} (μM)	56	0.4	39	1.6
HCT116 IC_{50} (μM)	n.t. [a]	0.3	n.t.	>350
MCF7 IC_{50} (μM)	n.t.	0.3	n.t.	>350
xrs-6 IC_{50} (μM) (HF) [b]	n.t.	0.03 (10)	n.t.	18 (1)
EM9 IC_{50} (μM) (HF_2) [c]	n.t.	0.3 (1)	n.t.	18 (0.2)
BSC-1 [d]	3+ [e]	4+	-	-

[a] n.t.: not tested.

[b] HF: hypersensitivity factor = (IC_{50} BR1/IC_{50} xrs-6).

[c] HF_2 = (IC_{50} BR1/IC_{50} EM9).

[d] BSC-1: African Green Monkey kidney cell-line. 80 μg of the test compound was applied to a 6 mm paper disc and incubated with the BSC-1 cell-line growing in continuous culture in a 16 mm well for 24 h at 36 °C in an atmosphere containing 5% CO_2.

[e] Zones of cytotoxicity were measured microscopically as excess radii from the disc and indicated by '-', none detectable; '3+', 3.5-4.5 mm; '4+', 4.5-5.0 mm.

Ascididemin was also toxic to the non-malignant African Green Monkey kidney cell-line BSC-1 (Table 1), bacteria *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative) and fungi *Candida albicans* and *Cladisporium resinae* (Table 2). No activity was observed against the fungus *Trichophyton mentagrophytes* and the Gram negative bacterium *Pseudomonas aeruginosa*.

Comparison with the results observed for the synthetic precursor **1** indicate the importance of ring E to the antitumor and antifungal/antibacterial properties of ascididemin. Formation of ring E in **2** results in a dramatic increase in cytotoxicity (P388) as well as enhanced *B. subtilis*, *E. coli*, *C. albicans* and *C. resinae* inhibiting properties. This structural change however, results in only a moderate increase in toxicity to the non-malignant

BSC-1 cell-line. Similar trends underlying the cytotoxic and antifungal[5] importance of ring E were observed between the deaza-analogues **3** and **4**.

Table 2. Evaluation of the Antimicrobial Activities of Compounds **1** - **4**.[a]

	1	**2**	**3**	**4**
Bacillus subtilis	10	14	3	8
Escherichia coli	0	10	0	0
Pseudomonas aeruginosa	0	0	0	0
Candida albicans	1	11	0 [b]	12 [c]
Trichophyton mentagrophytes	0	0	0	10
Cladisporium resinae	6	10	0	0

[a] 120 μg of the test compound was applied to a 6 mm paper disc, and incubated with the various organisms for 18 h at 35 °C. Zones measured as excess radii in millimeters.

[b] Literature[5] MIC 25 μg/mL.

[c] Literature[5] MIC 0.39 μg/mL.

Of most interest however, was the comparison between the biological activities of **2** and **4**. Antibacterial/antifungal properties of **2** are clearly modulated by the presence, or absence, of ring A N-8. While N-8 is a requirement for *E. coli* and *C. resinae* growth inhibition, its absence is essential for the inhibition of *T. mentagrophytes*. Similar anti-candidal properties of **2** and **4** indicate this biological activity is independent of carbon or nitrogen substitution at position 8.

Deaza-ascididemin (**4**) was found to have greatly reduced cytotoxicity to the human colon and breast tumor and non-malignant CHO and BSC-1 cell-lines. Significantly, the absence of xrs-6 differential toxicity (HF=1) indicates that **4** is incapable of inducing DNA double-strand breaks, probably due to inability to inhibit topoisomerase II enzyme. Further studies are required to show whether this is, in-turn, a result of the loss of 1,10-phenanthroline-type metal co-ordinating ability of rings A-B-E in compound **2**. Despite the loss of mammalian topoisomerase II enzyme inhibiting properties, with concomitant abolition of *in vitro* human solid tumor cytotoxicity, activity against the P388 murine leukemia cell-line was still evident. This observation supports the presence of additional mechanisms of action for this class of molecule in mammalian cell systems.[1]

We are currently working to define, and optimise, the topoisomerase II-cleavable complex stabilizing pharmacophore of ascididemin (**2**), and to define further cellular targets of this cytotoxic agent.

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