Studies in Marine Natural Product Synthesis, Isolation and Ecology

A Thesis Presented to the University of Auckland for the Degree of

Doctor of Philosophy

by

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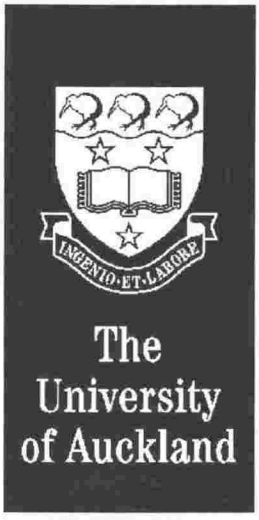

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Abstract

Previous studies indicated the marine pyridoacridone alkaloid ascididemin possessed a unique biological profile. Investigation of synthetic routes to ascididemin led to the discovery that ascididemin precursors possessed a wide range of biological activities. One precursor possessed hollow fiber *in vivo* antitumoral activity and continuing *in vivo* studies at the NCI, using subcutaneous xenograft assays, are in progress. A crystal structure of a precursor indicates that these tetracyclic heterocycles are planar, suggesting intercalation as a mechanism of antitumoral action. Ascididemin was synthesized by two novel ring E forming reactions. The superior methodology was also useful in the preparation of analogues, such as kuanoniamine A. Ascididemin possessed promising hollow fiber *in vivo* antitumoral activity but was poorly active in a subcutaneous xenograft study. Ascididemin was incapable of exerting antitumoral activity at a distance and further analogues were prepared to address this problem. Ten analogues were prepared, with all the non-bromine containing analogues selected for *in vivo* evaluation at the NCI. Two ring A analogues were prepared by a then-new synthetic route, including the antiviral natural product 11-hydroxyascididemin. 11-Methoxyascididemin was selected for hollow fiber evaluation. Two bromine containing ring D analogues were prepared, including the antifungal natural product 2-bromoleptoclinidinone. A crystal structure on 2-bromoleptoclinidinone was the first determined on a pyridoacridone alkaloid and the molecule was planar, further supporting an intercalative mechanism of action. Due to non-selective antitumoral cytotoxicity, ring D analogues are not useful antitumoral agents. Six carbon-based ring E analogues were prepared by novel methodology. All 6-substituted analogues assessed were selected for *in vivo* antitumoral evaluation. Hollow fiber antitumoral activity decreased with bulk of the substituent. 6-Methylascididemin has been selected for subcutaneous xenograft studies. The 5-substituted analogue prepared gave the best *in vitro* antitumoral profile of all alkaloids in this study and has been selected for *in vivo* evaluation. Ring E substituted N-8-deaza-ascididemin analogues possessed no antitumoral activity, highlighting the importance of the 1,10-phenanthroline-like bay region of ascididemin in antitumoral activity. Another four structurally novel, quinoid containing alkaloids have been selected for *in vivo* evaluation. While ascididemin was the only compound capable of topoisomerase IIα cleavable complex stabilization, related alkaloids possessed a similar level of inhibitory action against this enzyme. This further supports intercalation as the dominant mechanism of action for pyridoacridone alkaloids.

Ecological roles of four natural pyridoacridone alkaloids were assessed. Alkaloids were species specific antifeedant agents against important consumers. These alkaloids may have a long term detrimental effect on predator physiology, due to the well established ability of these alkaloids to interfere with cell proliferation. Ascididemin elicited avoidance responses in numerous marine species. Ascididemin has no antifouling activity against macrofoulers. Microbiological assessment of

ascididemin, 11-hydroxyascididemin and 2-bromoleptoclinidinone indicated that modification of the ascididemin chromophore leads to the directing of antimicrobial activity towards a different phyla of parasites. Pyridoacridone alkaloids may be part of a non-antibody based immune system. All studies point to pyridoacridone alkaloids enhancing the eventual reproductive success of the organism.

Biological and chemical evaluation of 29 New Zealand ascidians has been performed. Significant biological activity was detected in ten ascidians. Novel metabolites isolated were 2-(3'-bromo-4'-hydroxyphenol)ethanamine (*Cnemidocarpa bicornuta*) and 1,3-dimethylguanine (unidentified ascidian). Known metabolites isolated were 1,3-dimethylisoguanine (*Cnemidocarpa bicornuta*) and rubrolides A, B and C (unidentified ascidian). The survey highlighted the importance of overexpressed purine bases in ascidian metabolism. No physiological roles for these overexpressed purines are as yet apparent. Our study of NZ ascidians has led to the isolation of many compounds previously isolated from sponges. The widespread distribution of such metabolites gives credence to the theory that common metabolite-generating genes are present in both phyla, due to the evolutionary success of these genes.

Two optically active 9-(5-*S*-methyl-5-sulfinyl-lyxofuranosyl)-9*H*-purin-6-amine (lyxosyl-MTAS) nucleosides were isolated from the nudibranch *Doriopsis flabellifera*. This is the first report of any lyxosyl-MTAS nucleoside as either a natural or synthetic product.

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