# Synthetic Studies of Biologically Active Natural Products – Ascididemin and 6-Substituted 2-Pyranones

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# Abstract

Synthetic studies of two classes of natural products, ascididemin and 6-stryl-4-methoxy-2-pyranone, are described. Compounds produced in these studies were submitted to a number of biological assays.

The marine alkaloid ascididemin has considerable activity against *Mycobacterium tuberculosis* but unfortunately possesses significant cytotoxity and poor solubility. Analogues of ascididemin were prepared with variations at the 6-position and, in many instances, with replacement of the nitrogen at the 8-position with a carbon. Substituents were introduced that explored steric bulk and also enhanced aqueous solubility. Two new general routes were developed for the preparation of amide analogues at the 6-position of ascididemin. A set of 6-amidostyryl-8-deaza-ascididemin analogues showed considerable activity (MIC MTb 0.2-0.7 μM), good solubility and modest to good selectivity (SIs from 6 to 125).

2-Pyranone natural products **1.14** and **1.15** have been reported to exhibit modest in vitro activity against *Mycobacterium tuberculosis* and the related 2-pyranones pseudopyronines A (**1.27**) and B (**1.28**) and analogues are reported to have anti-parasitic activity. To explore the structure activity relationship of these compounds a library of 6-substituted-4-methoxy-2-pyranones was prepared. Significant in vitro activity against *Plasmodium falciparum* was observed for several members of the compound library (*e.g.* **3.55** and **3.61** with $IC_{50}$ values of of 1.3 and 3.6 μM, respectively). Biomimetic photo-dimerisation of several styryl pyrones was explored and isolated dimers submitted to assays. X-ray crystal structures of two of the monomers were used to rationalise the resulting dimer structures. These dimerised pyrones were found to be generally more active against *P. falciparum* and less toxic than their monomers and had the best selectivity of the pyrone library evaluated.

# Declaration and preface

This is to certify that:

1) the thesis comprises only my original work towards the PhD except where indicated below;

2) due acknowledgment has been made in the text to all other material used.

Biological testing of compounds was performed by various collaborators:

Dr. Helena Boshoff and Dr. Clifton E. Barry III at the National Institute of Allergy and Infectious Diseases, NIH, USA tested compounds in *Mycobacterium tuberculosis* H37Rv assays.

Dr Ronan O'Toole, School of Biological Sciences, Victoria University of Wellington tested compounds in *Escherichia coli*, *Mycobacterium smegmatis* and HL-60 assays;

Ms Gill Ellis, Department of Chemistry, The University of Canterbury evaluated P388 activities of compounds.

Drs Marcel Kaiser and Reto Brun, Department of Parasite Chemotherapy of the Swiss Tropical and Public Health Institute, Basel, Switzerland evaluated compounds against *Trypanosoma brucei rhodesiense, Leishmania donovani*, *Plasmodium falciparum* and an L6 rat skeletal myoblast cell line.

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# Abbreviations

| | |
|---|---|
| **Ac** | acetyl |
| **ACT** | artemisinin-based combination therapy |
| **ATCC** | American Type Culture Collection |
| **ATR** | attenuated total reflectance |
| **br** | broad |
| **BCG** | bacillus Calmette-Guérin |
| **BSA** | bovine serum albumin |
| **$C_8$** | octyl-derivatized silica |
| **$C_{18}$** | octadecyl-derivatized silica |
| **CHO** | Chinese hamster ovary |
| **CI** | chemical ionization |
| **COSY** | gradient correlation spectroscopy ($^1$H-$^1$H) |
| **d** | doublet |
| ***dn*** | n deuterium atoms present in the molecule |
| **DCC** | *N*,*N'*-dicyclohexylcarbodiimide |
| **DCU** | *N*,*N'*-dicyclohexylurea |
| **DNA** | deoxyribonucleic acid |
| **DMAP** | 4-dimethylaminopyridine |
| **DMF** | dimethylformamide |
| **DMF-DEA** | dimethylformamide diethyl acetal |
| **DEPT** | distortionless enhancement by polarisation transfer |
| **DMSO** | dimethylsulfoxide |
| **DOTS** | directly observed therapy short course |
| **$EC_{50}$** | half maximal effective concentration |
| **$ED_{50}$** | 50 % effective dose concentration |
| **EI** | electron impact |
| **ELISA** | enzyme-linked immunosorbent assay |
| **ESI** | electrospray ionization |
| **Et** | ethyl |
| ***et al.*** | et alia |
| **FAB** | fast atom bombardment |

| | |
|---|---|
| **g** | gram |
| **h** | hours |
| **$H_{37}$Ra** | an avirulent strain of *Mycobacterium tuberculosis* |
| **$H_{37}$Rv** | a well characterised virulent strain of *Mycobacterium tuberculosis* |
| **HIV** | human immunodeficiency virus |
| **HMBC** | gradient heteronuclear multiple-bond correlation |
| **HPLC** | high performance liquid chromatography |
| **HR** | high resolution |
| **HRP2** | histidine-rich protein II |
| **HSQC** | gradient heteronuclear single-quantum correlation |
| **Hz** | hertz |
| **$IC_{50}$** | 50% inhibitory concentration |
| **IR** | infrared |
| ***J*** | coupling constant |
| ***$J_{AB}$*** | coupling constant between atoms A and B |
| **L** | litre |
| **LH20** | Sephadex LH20 |
| **M** | molarity |
| **m** | metre |
| **m** | milli |
| **m** | multiplet (NMR) |
| ***m*** | meta |
| **MDR** | multidrug-resistant |
| **Me** | methyl |
| **MeCN** | acetonitrile |
| **MeOH** | methanol |
| **MIC** | minimum inhibitory concentration |
| **min** | minutes |
| **m.p.** | melting point |
| **MS** | mass spectrometry |
| **MTb** | *Mycobacterium tuberculosis* |
| ***m/z*** | mass to charge ratio |
| **n** | nano |
| **N** | normal |

| | |
|---|---|
| **NBS** | N-bromosuccinimide |
| **NCI** | National Cancer Institute |
| **NMR** | nuclear magnetic resonance |
| **NOE** | Nuclear Overhauser Effect |
| **NOESY** | Nuclear Overhauser Effect Spectroscopy |
| ***o*** | ortho |
| ***p*** | para |
| **ppm** | parts per million |
| **$R_f$** | retention factor |
| **RNA** | ribonucleic acid |
| **ROESY** | rotating-frame nuclear Overhauser enhancement spectroscopy |
| **r.t.** | room temperature |
| **s** | singlet |
| **SAR** | structure-activity relationship |
| **SI** | Selectivity Index |
| ***sp.*** | species |
| **t** | triplet |
| ***tert*** | tertiary |
| **TB** | tuberculosis |
| **TBDPS** | *tert*-butyldiphenylsilyl |
| **$Tf_2O$** | trifluoromethanesulfonic anhydride |
| **TFA** | trifluroacetic acid |
| **THF** | tetrahydrofuran |
| **TLC** | thin layer chromatography |
| **TMS** | trimethylsilyl |
| **UV** | ultraviolet |
| **W** | watt |
| **WHO** | World Health Organization |
| **XDR** | extensively drug-resistant |
| **Å** | angstrom |
| **2D** | two dimensional |
| **$^1$H NMR** | proton nuclear magnetic resonance |
| **$^{13}$C NMR** | carbon-13 nuclear magnetic resonance |
| **$\delta_A$** | NMR chemical shift in ppm downfield from a standard for nucleus A |

| | |
|---|---|
| **μ** | micro |
| $\Delta^n$ | chemical bond from position n to n + 1 |
| $\nu_{max}$ | Wavenumber with local maximal intensity |