Synthesis and Evaluation of Norcantharidin and Acrylonitrile Derivatives as Potential Anti-Cancer Agents

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B.Sc. (Hons)

Thesis Presented for the Degree of
Doctor of Philosophy (Chemistry)

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Department of Chemistry

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April 2012

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each senior co-author; and endorsed by the Faculty Assistant Dean of Research Training, attesting to my contribution to the joint publications.

Mark Tarleton

Acknowledgements

I would like to thank my supervisors Professor Adam McCluskey and Dr Warwick Belcher. Thank you for giving me the opportunity to work on this project and supplying me with a very well equipped research lab to work in. Also your help, guidance, patience, and understanding have also been much appreciated.

Thank you to biologists Dr Jennette Sakoff and Dr Jayne Gilbert from the Department of Medical Oncology at the Mater Hospital Newcastle, for running the biological assays for my many samples and returning the data as quick as possible.

I would also like to thank Dr Mike Bowyer and Dr Ken Zimmerman for taking time during the initial stages of my undergraduate degree to show me what can be achieved with the right amount of time and effort.

The Medicinal Chemistry Group has grown during my time and as a result, the list of people that need thanking is significant. To all the post docs that have had an influence in the synthetic or literature components of my research namely Dr Mark Robertson, Dr Chris Gordon, Dr Fiona Deane, Dr Joey Ambrus, and Dr Cecilia Russell thankyou for making a shared lab such a positive experience. I would also like to thank Kelly-Ann Young, that lab would not run without you, and to Lacey Hizartzidis, I have taught you everything I know, mostly not chemistry related, and I think I have taught you too well.

To all the technical staff I would like to extend my thanks especially to Vicki Thompson for allowing me access to the undergraduate lab equipment and reagents when needed.

Finally thank you to all my family and friends from the Nambucca/Valla area for all their encouragement and ability to make me forget about what I do during the week in particular Liam, Tim, Mitch, Carli, Manrat, Muela, Jason, Jay, Lee, Cattanach, Legend and to those at university Will, Chris, Andrew, Nat, and Deb.

List of publications included as part of the thesis

- Campbell, B. E., Tarleton, M., Gordon, C. P., Sakoff, J. A., Gilbert, J., McCluskey, A, Gasser, R. B. (**2011**), Norcantharidin analogues with nematocidal activity in *Haemonchus contortus*. *Bioorganic and Medicinal Chemistry Letters*, 21, 3277-3281.
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Contents

Chapter 5 – Appendix

List of Abbreviations

Adenosine Diphosphate	**ADP**
Asialoglycoprotein receptor	**ASGP-R**
Adenosine Triphosphate	**ATP**
Cdk-activating kinase	**CAK**
Cyclin-dependant kinases	**Cdks**
Deoxyribonucleic Acid	**DNA**
Estrogen Receptor	**ER**
Growth Inhibition 50	**GI_{50}**
Dissociation constant for an enzyme inhibitor complex	**K*i***
Lethal Dose 50	**LD_{50}**
National Cancer Institute	**NCI**
Protein 16	**p16**
Protein 21	**p21**
Protein 27	**p27**
Protein 53	**p53**
Protein Phosphatases	**PPs**
Protein Phosphatase 1	**PP1**
Protein Phosphatase 2A	**PP2A**
Protein Phosphatase 2B	**PP2B**
Protein Phosphatase 2C	**PP2C**
Protein Phosphatase 4	**PP4**
Protein Phosphatase 6	**PP6**
Protein Phosphatase 7	**PP7**
Retinoblastoma protein	**Rb**
Structure Activity Relationships	**SAR**
Tumour Suppression Genes	**TSG**

Breast carcinoma	**MCF-7 (ER +ve), MDA-MB231 (ER –ve)**
Colon carcinoma	**HCT116, HT29, WiDr, SW480, HCT-8**
Glioblastoma	**SJ-G2**
Haematopoietic carcinoma	**L1210, HL60**
Hepatocellular carcinoma	**Hep-3B, Hep-1**
Kidney carcinoma	**G401**
Leukaemia	**K-562, KG1a**
Liver carcinoma	**Be17402, SMMC-7721**
Lung carcinoma	**H460, A549**
Neck and head carcinoma	**KB**
Neuroblastoma	**BE2-C**
Oesophageal carcinoma	**ECA109**
Osteosarcoma	**143B**
Ovarian carcinoma	**A2780, ADDP, HO-8910**
Pancreatic carcinoma	**Panc-1**
Prostate carcinoma	**DU145**
Skin carcinoma	**A431**
Stomach carcinoma	**SGC-7901**

Abstract

Treating cancer by targeting protein phosphatases, namely protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) is a novel 'fighting fire with fire' strategy. There are numerous small molecule inhibitors known to achieve this. Most important to this study, cantharidin, and its demethylated analogue, norcantharidin, offer the simplest structure for subsequent modification. The added benefit of effective membrane permeability, makes these compounds ideal candidates for further development. In contrast to most other anticancer drugs, these compounds stimulate the production of white blood cells by bone marrow, while other anticancer drugs that have the unwanted side effect of inducing myelosuppression.

Cantharidin displays kidney toxicity which has prevented its use in mainstream oncology. However, norcantharidin is void of kidney toxicity allowing its development for the treatment of cancer. These biologically active compounds have been shown to have multiple uses such as the treatment of warts. Norcantharidin analogues have also been shown to display anti-parasitic activity against nematode *Haemonchus contortus*, the barbers pole worm, an intestinal parasite that affects livestock industries.

Preliminary analysis of a norcantharidin derivative with a single reduced carbonyl group displayed selectivity towards HT29 (colon; GI_{50} = 14μM) and SJ-G2 (glioblastoma; GI_{50} = 15μM) when tested against the NCI 60 cell line panel. Intrigued by this finding, multiple small focused libraries were synthesised and assessed in order to compile structure activity relationships (SAR). Interestingly an analogue with an isopropyl ether showed promise with strong selectivity towards HT29 (colon; GI_{50} = 19μM) and SJ-G2 (glioblastoma; GI_{50} = 21μM) cell lines but completely void of activity (>100μM) against all seven remaining carcinoma cell lines tested.

Norcantharidin analogues were also tested for anti-parasitic activity against *Haemonchus contortus*, the barbers pole worm, with multiple analogues displayed activity against *Haemonchus contortus* with associated LD_{50s} between 25-40 μM. The observed hit-rate of 5.6% associated with this screening of norcantharidin analogues is far higher than conventionally used drug screening methods usually employed. As part of a toxicity pre-filter, all new anti-parasitic compounds are screened against a panel of ten cancer cell lines to ensure the end user was not subjected to toxic compounds being applied in a non-ideal environment such as farming communities. Surprisingly, analogues from a small acrylonitrile library, originally used as an internal standard, displayed high levels of cytotoxicity.

Subsequent focused library development based on the acrylonitrile scaffold produced multiple broad spectrum cytotoxic compounds with average GI_{50} values of 1.1-2.1 μM across the nine carcinoma cell lines examined. Interestingly, some acrylonitrile compounds showed a high degree of specificity towards MCF-7 (breast carcinoma) cells of up to 543 fold over the other carcinoma cell lines tested. Some of these compounds were further shown to selectively target estrogen dependent MCF-7 cells that over express the estrogen receptor (ER+ve) over estrogen receptor negative carcinoma (MDA-MB231) and non-malignant breast tissue (MCF10A) up to 268 and 126 fold respectively.

With the high throughput of synthesised analogues, flow chemistry methodologies were developed in order to alleviate some of the associated issues with synthetic medicinal chemistry such as reproducibility between batches and difficulty in scale up for live animal studies. Along with effectively producing specific acrylonitrile derivatives in high purity and yield, these procedures were further developed in other projects leading to the discovery of the most potent protein phosphatase inhibitors developed within the research group.