An investigation into the cytotoxic properties of isatin-derived compounds: potential for use in targeted cancer therapy

Kara Lea Vine
University of Wollongong, kara@uow.edu.au

UNIVERSITY OF WOLLONGONG
COPYRIGHT WARNING
You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author.

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

Recommended Citation

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
NOTE

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.
An Investigation into the Cytotoxic Properties of Isatin-Derived Compounds: Potential for use in Targeted Cancer Therapy

A thesis submitted in fulfillment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

From

School of Biological Sciences
UNIVERSITY OF WOLLONGONG

By
Kara Lea Vine, B.Biotech (Hons)
2007
Declaration

The work described in this thesis does not contain any material that has been submitted for the award of any higher degree in this or any other University and to the best of my knowledge contains no material previously published or written by any other person, except where due reference is made in the text of this thesis.

Kara Lea Vine

14th September 2007
Acknowledgements

My sincere thanks to my supervisory ‘committee’ A. Prof. Marie Ranson, Prof. John Bremner, Dr. Kirsten Benkendorff and Prof. Stephen Pyne for your continued support and encouragement. You have all helped me on my PhD journey in so many ways, both on an academic and personal level and for this I am truly grateful. For helping me build fences and having a laugh along the way, I would also like to thank Dr. Julie Locke, for which without her synthetic skills, this thesis would not have been possible. Thank you also to Dr. Christopher Burns (Cytopia, Vic) and Dr. Laurent Meijer (CNS, France) for the compound screening and Dr. Renate Griffith (Newcastle University, NSW) for assistance with related work. A big thank you also to Dr. Larry Hick, Sister Sheena McGhee and Prof. Alistair Lochhead for running mass spectrometry samples, taking blood and help with histopathological analysis of tissue sections (in that order). Thank you to the University of Wollongong for financial support through a University Cancer Research grant and University Postgraduate Award (UPA).

For continued support in the lab and the start of new friendships I would also like to thank the Ranson (including Dave) and Bremner research groups (special thanks to Joey for running my MS samples). To Tamantha, Tracey and Laurel, thank you for all of your advice and help during the animal studies. To the ‘Lay-dees’ (Christine, Elise, Jill, Martina, Amanda, Carola, Anna) and Justin for your continued friendship, support and laughter, I couldn’t have done it without you!

Thank you to my wonderful family for your patience, support and love. And last but not least, thank you to my loving and inspirational husband Shane, for your endless encouragement and belief in me. I made it here because of you!
Abstract

The increased incidence of multidrug resistance (MDR) and systemic toxicity to conventional chemotherapeutic agents suggests that alternative avenues need to be explored in the hope of finding new and effective treatments for metastatic disease. Considering natural products have made enormous contributions to many of the anticancer agents used clinically today, the cytotoxic molluscan metabolite tyrindoleninone (1) and its oxidative artifact, 6-bromoisatin (5), were initially used as templates for drug design in this study. Structural modifications to the isatin scaffold afforded a total of 51 isatin-based analogues, 21 of which were new. Cytotoxicity screening of the compounds against a panel of hematological and epithelial-derived cancer cell lines \textit{in vitro}, found the di- and tri-bromoisatins to be the most potent, with activity observed in the low micromolar range. Interestingly compound activity was enhanced by up to a factor of 22 after \textit{N}-alkyl and \textit{N}-arylalkylation, highlighting the importance of \textit{N}1 substitution for cytotoxic activity. 5,7-Dibromo-\textit{N}-(p-methylbenzyl)-isatin (39) was the most active compound overall and exhibited an IC$_{50}$ value of 490 nM against U937 and Jurkat leukemic cell lines, after 24 h. 5,7-Dibromo-\textit{N}-(p-trifluoromethylbenzyl)isatin (54) was also of interest, considering the potent cell killing ability displayed against a metastatic breast adenocarcinoma (MDA-MB-231) cell line. Investigation into the molecular mode of action of the \textit{N}-alkylisatin series of compounds found the \textit{p}-trifluoromethylbenzyl derivative (54), together with 9 other representative molecules to destabilise microtubules and induce morphological cell shape changes \textit{via} inhibition of tubulin polymerisation. This resulted in cell cycle arrest at G2/M and activation of the effector caspases 3 and 7, ultimately resulting in apoptotic
cell death.

Further investigations into the pharmacological profile of compound 54 in vivo, found it to be moderately efficacious (43% reduction in tumour size compared to vehicle control treated mice) in a human breast carcinoma xenograft mouse model. Although histopathological analysis of the bone marrow in situ after acute dosing found only mild haematopoietic suppression, analysis of biodistribution via SPECT imaging found large amounts of activity also in the gut and liver.

In an effort to reduce non-target organ up-take and thus increase accumulation of drug in the tumour, the N-benzyloisatin 54 was derivatised so as to contain an acid labile imine linker and was conjugated to the targeting protein PAI-2 (a naturally occurring inhibitor of the urokinase plasminogen activation system) via amide bond formation with free lysine residues. The conjugate was found to contain an average of 4 molecules of 54 per protein molecule without affecting PAI-2 activity. Hydrolytic stability of the PAI-2-cytotoxin conjugate at pH 5-7 as determined by UV/Vis spectrophotometry, was directly correlated with the lack of activity observed in vitro, suggesting a need to investigate cleavable linker systems with enhanced lability in the future. Despite this, PAI-2 conjugated to the cytotoxin 5-FUdr through a succinate linker system, showed enhanced and selective uPA-mediated cytotoxicity, in two different breast cancer cell lines which varied in their expression levels of uPA and its receptor. This suggests that PAI-2-cytotoxin based therapies hold potential, in the future, as new therapeutic agents for targeted therapy of uPA positive malignancies, with limited side effects.
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CDK</td>
<td>cyclin-dependant kinase</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>ddd</td>
<td>doublet of doublets of doublets</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribose nucleic acid</td>
</tr>
<tr>
<td>dt</td>
<td>doublet of triplets</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetriacetic acid</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FCS</td>
<td>foetal calf serum</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HR</td>
<td>high resolution</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>$J$</td>
<td>coupling constant</td>
</tr>
<tr>
<td>LDP</td>
<td>ligand-directed prodrug</td>
</tr>
<tr>
<td>Lit.</td>
<td>literature</td>
</tr>
<tr>
<td>LR</td>
<td>low resolution</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>$m/z$</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>MDR</td>
<td>multi-drug resistance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MTD</td>
<td>maximum tolerated dose</td>
</tr>
<tr>
<td>MTS</td>
<td>3-(4,5-dimethylthiazol-2-yl)-5-(3-carboethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinamide</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>p.i.</td>
<td>post injection</td>
</tr>
<tr>
<td>PAI-2</td>
<td>plasminogen activator inhibitor type 2</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PI</td>
<td>propidium iodide</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>R_f</td>
<td>retention factor</td>
</tr>
<tr>
<td>RME</td>
<td>receptor mediated endocytosis</td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>SAR</td>
<td>structure activity relationship</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>td</td>
<td>triplet of doublets</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>uPA</td>
<td>urokinase-type plasminogen activator</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>ultraviolet/visible spectrum</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift in ppm downfield from TMS</td>
</tr>
</tbody>
</table>
## Units Used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mol</td>
<td>mole (6.022 × 10²³ particles)</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight: mass of 1 mole (g/ mole)</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton: unit of molecular weight (g/mol)</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>k</td>
<td>kilo (10³)</td>
</tr>
<tr>
<td>m</td>
<td>milli (10⁻³)</td>
</tr>
<tr>
<td>μ</td>
<td>micro (10⁻⁶)</td>
</tr>
<tr>
<td>n</td>
<td>nano (10⁻⁹)</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>M</td>
<td>Molar: concentration mole/L</td>
</tr>
<tr>
<td>v/v</td>
<td>concentration expressed as volume ratio</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>sec</td>
<td>seconds</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>× g</td>
<td>gravity force of rotation</td>
</tr>
</tbody>
</table>
Table of Contents

Declaration .................................................................................................................. ii
Acknowledgements .................................................................................................... iii
Abstract ................................................................................................................... iv
Abbreviations ........................................................................................................... vi
List of Tables ............................................................................................................... xv
List of Figures ........................................................................................................... xvi
List of Schemes ......................................................................................................... xix
List of Thesis Publications ....................................................................................... xx

CHAPTER 1
Drug Design and Development: Advances in the Area of Targeted Cancer Therapy...................................................................................................................... 2

1.1 General Introduction ............................................................................................ 2

1.2 The Molecular Biology of Cancer: a Disease of Deregulated Proliferation and Cell Death ........................................................................................................... 3
  1.2.1 The Cell Cycle ................................................................................................. 5
  1.2.1.1 Cell Cycle Mutations in Cancer................................................................. 9
  1.2.2 Apoptosis ..................................................................................................... 10
  1.2.2.1 Apoptotic Aberrations in Cancer............................................................. 13

1.3 Current Treatment Strategies: Promises and Pitfalls ........................................... 15
  1.3.1 Conventional Chemotherapy and Systemic Toxicity .................................... 15
  1.3.2 The Emergence of Multi-Drug Resistance (MDR) ....................................... 16

1.4 Revival of Natural Product Research .................................................................. 17
  1.4.1 The Marine Environment as a Source of Novel Anticancer Agents ............ 23
  1.4.1.1 Cytotoxic Molecules from Marine Molluscs and their Egg Masses ......... 27
  1.4.2 Obstacles in the Prevention of Marine Natural Products as Drugs ............... 29

1.5 Targeted Cancer Therapy ..................................................................................... 31
  1.5.1 Small Molecule Inhibitors ............................................................................ 31
  1.5.1.1 Targeting Cell Signaling Pathways and their Receptors ......................... 31
1.5.1.2 Problems Associated with Small Molecule Targeted Therapies.........34
1.5.2 Ligand-Directed Prodrug Therapies.............................................................35
   1.5.2.1 Acid-Labile Linker Systems..................................................................37
      1.5.2.1a Ligand-Directed Prodrugs Containing cis-Aconityl Linkers...39
      1.5.2.1b Ligand-Directed Prodrugs Containing Carboxylic Hydrazone
         Linkers..........................................................................................................39
      1.5.2.1c Esters..................................................................................................41
      1.5.2.1d Other Acid-Labile Linkers..................................................................42
   1.5.2.2 Lysosomally Degradable Linkers..........................................................42
   1.5.2.3 Carrier Molecules.......................................................................................43
      1.5.2.3a Antibodies............................................................................................43
      1.5.2.3b PAI-2 and the Urokinase Plasminogen Activation System........45
1.6 Rationale and Project Objectives......................................................................48

CHAPTER 2
General Materials and Methods........................................................................51

2.1 Materials.........................................................................................................51
   2.1.1 Chemicals...................................................................................................51
   2.1.2 Cells Lines and Culture Reagents.............................................................51

2.2 General Organic Chemistry Methods............................................................52

2.3 General Cell and Protein Analysis Methods................................................53
   2.3.1 Cell Lines and Tissue Culture....................................................................53
      2.3.1.1 Human Cancer Cells..............................................................................53
      2.3.1.2 Untransformed Human Cells.................................................................54
         2.3.1.2a Blood Collection..................................................................................54
      2.3.1.2b Isolation of Human Mononuclear Cells (MNC): Density
         Centrifugation.................................................................................................54
   2.3.2 Cell Viability Assays...................................................................................55
      2.3.2.1 MTS Assay.............................................................................................55
      2.3.2.2 Propidium Iodide (PI) Staining and Flow Cytometry..........................57
   2.3.3 Apoptosis Detection Systems....................................................................57
      2.3.3.1 Caspase-3/7 Assay................................................................................57
   2.3.4 Protein Analysis methods..........................................................................59
      2.3.4.1 Protein Concentration Assay.................................................................59
      2.3.4.2 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
         (SDS-PAGE).......................................................................................................59

CHAPTER 3
From Tyrindoleninone to Isatin: Synthesis and in vitro Cytotoxicity Evaluation
of Some Substituted Isatin Derivatives.............................................................62
3.1 Introduction .............................................................................................................62
  3.1.1 Reported Syntheses of Tyrindoleninone Derivatives ...........................................63
  3.1.2 Isatins as Anticancer Agents ...............................................................................64
  3.1.3 Rationale and Aims ............................................................................................66

3.2 Materials and Methods ..........................................................................................67
  3.2.1 General .............................................................................................................67
  3.2.2 Chemical Synthesis ...........................................................................................68
    3.2.2.1 Attempted Synthesis of 2-methylthioindoleninone (29c) ........................68
    3.2.2.2 Attempted Synthesis of Tyrindoleninone (1) and Brominated Derivatives ...........................................................................................................70
    3.2.2.3 Attempted Synthesis of Tyrindoleninone (1) via Methylation of a Thioamidine Intermediate ....................................................................................70
    3.2.2.4 Synthesis of Substituted Isatin Derivatives ...............................................71
  3.2.3 Biological Activity ............................................................................................75
    3.2.3.1 In vitro Cytotoxicity Evaluation of Isatin Derivatives ....................................75
    3.2.3.2 Investigations into Cancer Cell Specificity .....................................................76
    3.2.3.3 Preliminary Mode of Action Studies ...............................................................76

3.3 Results and Discussion ...........................................................................................78
  3.3.1 Chemistry ..........................................................................................................78
  3.3.2 Biological Activity ............................................................................................83

3.4 Conclusions ............................................................................................................92

CHAPTER 4
An Investigation into the Cytotoxicity and Mode of Action of Some N-Alkyl Substituted Isatin ..........................................................96

4.1 Introduction .............................................................................................................96
  4.1.2 Anticancer Activity of N-Alkylated Indoles .........................................................98
  4.1.3 Rationale and Aims ............................................................................................99

4.2 Materials and Methods ..........................................................................................101
  4.2.1 General .............................................................................................................101
  4.2.2 Chemical Synthesis ...........................................................................................102
    4.2.2.1 General Method for the Alkylation of Isatin ................................................102
    4.2.3 Biological Activity and SAR ...........................................................................103
    4.2.3.1 In vitro Cytotoxicity Evaluation of N-alkyl Isatin Derivatives ......................103
    4.2.4.2 Investigations into Cancer Cell Specificity ......................................................103
  4.2.4 Mode of Action Studies .......................................................................................104
    4.2.4.1 Apoptosis Investigations .................................................................................104
      4.2.4.1a Whole Cell Staining: Propidium Iodide (PI) ..............................................104
      4.2.4.1b Activation of Apoptotic Caspases ...............................................................104
CHAPTER 5
A Preliminary in vivo Assessment of Some N-Alkylisatins.................................141

5.1 Introduction...........................................................................................................141
  5.1.1 Efficacy of Synthetic, Small Molecule Tubulin Binders.................................142
  5.1.2 Rationale and Aims.........................................................................................144

5.2 Materials and Methods..........................................................................................144
  5.2.1 General...........................................................................................................144
  5.2.2 Chemical Synthesis..........................................................................................146
    5.2.2.1 Attempted synthesis of 5-(tributylstannyl)isatin (64)...............................146
    5.2.2.2 Synthesis of N-(p-methoxybenzyl)-5-(tributylstannyl)isatin (65)..............146
    5.2.2.3 Synthesis of 5,7-Dibromo-N-[4’-(tributylstannyl)benzyl]isatin (66)...........147
    5.2.2.4 Synthesis of N-(p-methoxybenzyl)-5-([123]I)iodoisatin (67)......................148
    5.2.2.5 Synthesis of 5,7-dibromo-N-[4’-(123)I]iodobenzyl]isatin (68)....................149
  5.2.3 In Vivo Studies................................................................................................150
    5.2.3.1 Preliminary Toxicological Assessment.....................................................151
      5.2.3.1a Dose Tolerance......................................................................................151
      5.2.3.1b Acute Toxicity.....................................................................................151
    5.2.3.2 Tumour Models.......................................................................................152
      5.2.3.2a Human Epithelial, Mammary Gland Adenocarcinoma
(MDA-MB-231) Xenograft in Nude Mice.................................................................152
      5.2.3.2.b Human Amelanotic Melanoma (A375) Xenograft in Nude Mice.............152
5.2.3.2.3 Rat 13762 MAT B III Mammary Adenocarcinoma in F344 Fisher Rats..........................................................153
5.2.3.3 Tumour Growth Delay: Efficacy in a Human Mammary Tumour Model.................................................................153
5.2.3.4 Histopathology......................................................................................................................................................154
5.2.3.5 Statistical Analyses..............................................................................................................................................155
5.2.3.6 Single Photon Emission Computed Tomography (SPECT) Imaging of Human Melanoma and Rat Mammary Tumour Models.................................................155

5.3 Results and Discussion..................................................................................................................................................157
5.3.1 Chemistry.................................................................................................................................................................157
5.3.2 In Vivo Studies.........................................................................................................................................................160
5.3.2.1 Toxicological Evaluation........................................................................................................................................160
5.3.2.2 Evaluation of Efficacy in MDA-MB-231 Tumour Xenografts.............................................................167
5.3.2.3 Single Photon Emission Computed Tomography (SPECT) Imaging.......................................................172

5.4 Conclusions.................................................................................................................................................................178

CHAPTER 6
A Preliminary Investigation into Targeted Drug Delivery via Receptor Mediated Endocytosis ........................................180

6.1 Introduction.................................................................................................................................................................180
6.1.1 Serum Proteins as Carriers in Drug Targeting Strategies...............................................................181
6.1.2 Rationale and Aims..................................................................................................................................................183

6.2 Materials and Methods...............................................................................................................................................185
6.2.1 General.................................................................................................................................................................185
6.2.2 Chemical Synthesis.................................................................................................................................................186
6.2.2.1 Conjugation of 2′-deoxy-5-fluoro-3′-O-(3-carbonylpropanoyl)uridine (5-FUdrsucc) to PAI-2........186
   6.2.2.1a Activation of the ester.........................................................................................................................................186
   6.2.2.1b Conjugation to PAI-2........................................................................................................................................186
6.2.2.2 Conjugation of 5,7-dibromo-3-[m-(2′-carboxymethyl)-phenylimino]-N-(p-trifluoromethyl)isatin to PAI-2...187
   6.2.2.2a Activation of the ester.........................................................................................................................................187
   6.2.2.2b Conjugation to PAI-2........................................................................................................................................187
6.2.2.3 Characterisation of Protein-Cytotoxin Conjugates.................................................................188
   6.2.2.3a Electrospray Ionisation Mass Spectrometry (ESI-MS).............................................................................188
   6.2.2.3b PAI-2: uPA Complex Formation.....................................................................................................................188
6.2.2.4 Hydrolysis Studies.................................................................................................................................................189
6.2.2.5 In vitro Cytotoxicity Evaluation.......................................................................................................................189
   6.2.2.5a Addition of Exogenous uPA.......................................................................................................................190
6.2.2.6 Statistical Analyses.............................................................................................................................................190
6.3 Results and Discussion

6.3.1 Chemistry

6.3.2 Biological Evaluation

6.4 Conclusions

CHAPTER 7
Conclusions and Future Directions

REFERENCES

APPENDICES

THESIS PUBLICATIONS
List of Tables

Table 1.1 Overexpression of the cell cycle kinases..........................................................9
Table 1.2 The annual incidence of human cancers and Bcl-2 overexpression..............14
Table 1.3 All anticancer agents approved for clinical use by the FDA between the 1940s and 2002.................................................................19
Table 1.4 Status of selected marine-derived compounds in clinical and preclinical trials.................................................................................................................24
Table 1.5 FDA approved small molecule inhibitors ......................................................34
Table 1.6 FDA approved monoclonal antibodies (mAb)............................................36
Table 3.1 Cytotoxicity IC50 (µM) of isatin derivatives 4-26 on U937 cells...............85
Table 3.2 Cytotoxicity of di- and tri-substituted isatin derivatives against various cancer cell lines.................................................................................................................91
Table 3.3 IC50 (µM) mean graph for 5,7-dichloroisatin.............................................93
Table 4.1 Chemical structures of the N-alkylated isatins (compounds 33-60) ..........100
Table 4.2 Cytotoxicity of compounds 33-60 on U937, Jurkat and MCF-7 cells........113
Table 4.3 Physiochemical properties of selected N-alkylisatins.................................118
Table 4.4 Cytotoxicity of N-alkyl isatins against various cancer cell lines ..........119
Table 4.5 Enzyme and cell based inhibitory activity of compounds 39, 45, 48, 54, 59 and 60 on CDK5, GSK3, DYRK1A, JAK1, JAK2 and c-FMS.................................138
Table 5.1 Protocol for SPECT imaging of radiotracer 67 and 68 in female Balb/c (nu/nu) melanoma xenografts.................................................................157
Table 5.2 Protocol for SPECT imaging of radiotracers 67 and 68 in F344 Fisher rats bearing 13762 MAT B III mammary adenocarcinoma............................157
Table 6.1 The effect of PAI-2-5-FUdrsucc and unconjugated cytotoxins 5-FUdr and 5-FUdrsucc on MDA-MB-231 and MCF-7 cells................................200
Table 6.2 The effect of PAI-2-CF3imine and unconjugated cytotoxins 54 and 72 on MDA-MB-231 and MCF-7 cells.........................................................204
List of Figures

Figure 1.1 A schematic representation of the development of a benign tumour into a metastatic malignant tumour.................................................................4
Figure 1.2 The cell cycle and associated checkpoints........................................6
Figure 1.3 Phases of the cell cycle......................................................................8
Figure 1.4 Molecular pathways involved in apoptosis.........................................12
Figure 1.5 The percentage of marine natural products isolated from various phyla...26
Figure 1.6 Examples of the brominated and non-brominated compounds present in the hypobranchial gland and egg masses of muricid mollusces..........................27
Figure 1.7 Structure of Gemtuzumab ozogamicin (Mylotarg).............................30
Figure 1.8 Cancer pathways for exploitation in targeted therapy........................32
Figure 1.9 Internalisation of a ligand-drug conjugate via RME............................38
Figure 1.10 Structures of representative acid-labile drug conjugates...............40
Figure 2.1 Cellular conversion of the CellTiter 96 Aqueous One Solution Cell Proliferation Assay Reagent...............................................................56
Figure 2.2 Cleavage of the non-fluorescent Caspase substrate Z-DEVD-R110 by Caspase-3/7............................................................58
Figure 3.1 Adult Muricid mollusces Dicathais orbita, amongst freshly laid egg capsules..............................................................63
Figure 3.2 Some halogenated derivatives of isatin with reported anticancer activity...65
Figure 3.3 Chemical structures of the isatin-based compounds 4-26 that were screened for cytotoxic activity in this study.................................................................67
Figure 3.4 Viability of U937 cells after treatment with various concentrations of 5,6,7-tribromoisatin (19) over time.................................................................86
Figure 3.5 Cell associated fluorescence of U937 cells after treatment with 5,6,7-tribromoisatin (19) for 24 h.................................................................87
Figure 3.6 Activation of caspases 3 and 7 in Jurkat cells after treatment with various concentrations of 5,6,7-tribromoisatin (19).................................................................87
Figure 3.7 Viability of U937 cells after treatment with different concentrations of compounds 20, 21, 24-26.................................................................89
Figure 3.8 Viability of U937 cells and freshly isolated PBLs after treatment with 5-bromoisatin (7)................................................................................91
Figure 3.9 Viability of U937, Jurkat, HCT-116, MDA-MB-231 and PC-3 cells after treatment with 5,6,7-tribromoisatin (19).................................................................92
Figure 4.1 The reactivity of isatin.................................................................96
Figure 4.2 Examples of some 3-substituted indolin-2-ones with reported anticancer activity................................................................................97
Figure 4.3 Recently reported N-alkylated indoles with anticancer activity........99
Figure 4.4 Measurement of tubulin polymerisation using the fluorescence based tubulin polymerisation assay.................................................................107
Figure 4.5 Principle for the AlphaScreen assay................................................110
Figure 4.6 Viability of U937 cells after treatment with 40, 41, 42, 43 and 44........116
Figure 4.8 Cancer cell line selectivity.................................................................................................................................120
Figure 4.9 Activation of the effector caspases 3 and 7 in Jurkat, U937 and PBL cells after treatment with various N-alkylisatinats.................................................................122
Figure 4.10 Morphological evaluation of nuclei stained with Diff Quik.................................................................123
Figure 4.11 The effect of N-alkylisatins 39 and 54 on the cell cycle.................................................................124
Figure 4.12 Morphological effects of compound 39 on U937 cells.................................................................126
Figure 4.13 Morphological effects of compound 53 U937 cells.................................................................127
Figure 4.14 Morphological effects of compound 59 U937 cells.................................................................128
Figure 4.15 Morphological effects of compound 53 Jurkat T-cells.................................................................129
Figure 4.16 A comparison of the morphological effects exhibited by U937 and Jurkat cells.................................................................130
Figure 4.17 The morphological effects of the commercial anticancer agents vinblastine, paclitaxel and 5-fluorouracil U937 cells.................................................................131
Figure 4.18 Examples of indole derivatives that inhibit tubulin polymerisation.................................................................132
Figure 4.19 The effect of various N-alkylisatins and commercial anticancer agents on tubulin polymerisation.................................................................133
Figure 4.20 The effect of 54 on the stability of microtubules in U937 cells.................................................................135
Figure 5.1 Examples of synthetic small molecule microtubule inhibitors in preclinical and clinical development.................................................................144
Figure 5.2 Average weight change from day zero and percent survival of mice treated with 45.................................................................163
Figure 5.3 Acute toxicity organ profile of 54 over time.................................................................165
Figure 5.4 H & E stained tissue preparations after treatment with 54.................................................................166
Figure 5.5 H & E stained tissue preparations treatment with 54.................................................................167
Figure 5.6 Efficacy of 54 in a breast carcinoma xenograft mouse model .................................................................169
Figure 5.7 Average weight change from day zero and percent survival of mice treated with 54.................................................................170
Figure 5.8 H & E stained mammary MDA-MB-231 tumours after treatment with DMSO or 54.................................................................172
Figure 5.9 SPECT imaging of 123I labeled compounds 67 and 68 in an athymic female Balb/c (nu/nu) melanoma xenograft.................................................................175
Figure 5.10 SPECT imaging of 123I labeled compounds 67 and 68 in F344 Fisher rats bearing 13762 MAT B III mammary adenocarcinoma.................................................................177
Figure 5.11 Tumour uptake of 123I labeled compounds in F344 Fisher rats bearing 13762 MAT B III mammary adenocarcinoma.................................................................178
Figure 6.1 ESI-MS of PAI-2-5-FUdrsucc.................................................................193
Figure 6.2 SDS PAGE showing PAI-2-5-FUdrsucc:uPA complexation.................................................................194
Figure 6.3 SDS PAGE showing PAI-2-CF3imine:uPA complexation.................................................................197
Figure 6.4 UV absorption spectrum of transferrin and transferrin-CF₃imine conjugates under different pH conditions.................................................................198
Figure 6.5 The in vitro cytotoxicity of PAI-2-5-FUdrsucc against MDA-MB-231 and MCF-7 cells........................................................................................................201
Figure 6.6 Average weight change from day zero and percent survival of mice treated with 70 and PAI-2-5-FUdrsucc.................................................................203
Figure 6.7 The in vitro cytotoxicity of PAI-2-CF₃imine against MDA-MB-231 and MCF-7 cells........................................................................................................205
Figure 7.1 A cytotoxicity, SAR summary for the N-alkylisatin derivatives.........211
List of Schemes

Scheme 3.1 Method of synthesis of tyrindoleninone derivatives from isatin...............64
Scheme 3.2 Proposed method for the synthesis of 2-methylthioindoleninone (29c).....69
Scheme 3.3 Proposed method for the synthesis of tyrindoleninone (1) using
Lawesson’s Reagent.................................................................................................70
Scheme 3.4 A retrosynthetic scheme for the synthesis of tyrindoleninone (1) ..........80
Scheme 3.5 A proposed method for the synthesis of 2-methylthioindoleninone (29c)..81
Scheme 3.6 Synthesis of 15c..................................................................................82
Scheme 4.1 General method for the N-alkylation of isatin....................................102
Scheme 5.1 Preparation of 65................................................................................158
Scheme 5.2 Synthesis of 69....................................................................................158
Scheme 5.3 Synthesis of 67 and 68 by oxidative radiohalogenation....................160
Scheme 6.1 Schematic representation of PAI-2-cytotoxin targeted delivery via
receptor mediated endocytosis..................................................................................184
Scheme 6.2 Preparation of 70 from 2′-deoxy-5-fluorouridine (5-FUdr)...............191
Scheme 6.3 Activation of 5-FUdrsucc (70) to form the active ester 71 and
conjugation to PAI-2...............................................................................................192
Scheme 6.4 Preparation of 72..................................................................................195
Scheme 6.5 Activation of 72 to form the ester 73.................................................196
List of Thesis Publications and Conference Abstracts


7) Kara L. Vine, Julie M. Locke, John B. Bremner, Stephen G. Pyne and Marie Ranson. Substituted Isatins as Small Molecule Anti-Cancer Agents RACI Natural
Products Group Symposium, University of Wollongong, NSW, AUSTRALIA, Sept 29th, 2006.

