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

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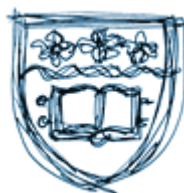
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# **An Investigation into the Cytotoxic Properties of Isatin-Derived Compounds: Potential for use in Targeted Cancer Therapy**

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

14<sup>th</sup> September 2007

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## 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 *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 *N*-alkyl and *N*-arylalkylation, highlighting the importance of N1 substitution for cytotoxic activity. 5,7-Dibromo-*N*-(*p*-methylbenzyl)-isatin (**39**) was the most active compound overall and exhibited an IC<sub>50</sub> value of 490 nM against U937 and Jurkat leukemic cell lines, after 24 h. 5,7-Dibromo-*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 *N*-alkylisatin series of compounds found the *p*-trifluoromethylbenzyl derivative (**54**), together with 9 other representative molecules to destabilise microtubules and induce morphological cell shape changes *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*-benzylisatin **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.



## Abbreviations

|            |  |
|------------|--|
| ATP        | adenosine triphosphate                 |
| CDK        | cyclin-dependant kinase                |
| d          | doublet                                |
| DCC        | dicyclohexylcarbodiimide               |
| dd         | doublet of doublets                    |
| ddd        | doublet of doublets of doublets        |
| DMF        | <i>N,N</i> -dimethylformamide          |
| DMSO       | dimethyl sulfoxide                     |
| DNA        | deoxyribose nucleic acid               |
| dt         | doublet of triplets                    |
| EDTA       | ethylenediaminetriacetic acid          |
| EI         | electron impact                        |
| ESI        | electrospray ionisation                |
| EtOH       | ethanol                                |
| FCS        | foetal calf serum                      |
| HPLC       | high performance liquid chromatography |
| HR         | high resolution                        |
| HRMS       | high resolution mass spectrometry      |
| Hz         | Hertz                                  |
| i.v.       | intravenous                            |
| <i>J</i>   | coupling constant                      |
| LDP        | ligand-directed prodrug                |
| Lit.       | literature                             |
| LR         | low resolution                         |
| m          | multiplet                              |
| m.p.       | melting point                          |
| <i>m/z</i> | mass to charge ratio                   |
| MDR        | multi-drug resistance                  |

|                |   |
|----------------|---|
| MeOH           | methanol  |
| MS             | mass spectrometry   |
| MTD            | maximum tolerated dose  |
| MTS            | 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 <i>H</i> -tetrazolium, inner salt |
| NHS            | <i>N</i> -hydroxysuccinamide  |
| NMR            | nuclear magnetic resonance  |
| OD             | optical density   |
| p.i.           | post injection  |
| PAI-2          | plasminogen activator inhibitor type 2  |
| PBS            | phosphate buffered saline   |
| PI             | propidium iodide  |
| ppm            | parts per million   |
| R <sub>f</sub> | retention factor  |
| RME            | receptor mediated endocytosis   |
| RPMI-1640      | Roswell Park Memorial Institute   |
| RT             | room temperature  |
| s              | singlet   |
| SAR            | structure activity relationship   |
| SD             | standard deviation  |
| SDS-PAGE       | sodium dodecyl sulfate polyacrylamide gel electrophoresis   |
| SEM            | standard error of the mean  |
| td             | triplet of doublets   |
| THF            | tetrahydrofuran   |
| TLC            | thin layer chromatography   |
| uPA            | urokinase-type plasminogen activator  |
| UV/Vis         | ultraviolet/visible spectrum  |
| δ              | chemical shift in ppm downfield from TMS  |

### Units Used

|                    |  |
|--------------------|--|
| mol                | mole ( $6.022 \times 10^{23}$ particles)   |
| MW                 | molecular weight: mass of 1 mole (g/ mole) |
| Da                 | Dalton: unit of molecular weight (g/mol)   |
| g                  | gram                                       |
| k                  | kilo ( $10^3$ )                            |
| m                  | milli ( $10^{-3}$ )                        |
| $\mu$              | micro ( $10^{-6}$ )                        |
| n                  | nano ( $10^{-9}$ )                         |
| L                  | Litre                                      |
| M                  | Molar: concentration mole/L                |
| v/v                | concentration expressed as volume ratio    |
| m                  | metre                                      |
| h                  | hour                                       |
| min                | minutes                                    |
| sec                | seconds                                    |
| $^{\circ}\text{C}$ | degrees Celsius                            |
| K                  | Kelvin                                     |
| rpm                | revolutions per minute                     |
| $\times g$         | gravity force of rotation                  |

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## List of Thesis Publications and Conference Abstracts

- 1) Vine, K. L., Locke, J. M., Ranson, M., Benkendorff, K., Pyne, S. G. and Bremner, J. B. (2007) *In vitro* Cytotoxicity Evaluation of Some Substituted Isatin Derivatives. *Bioorg. Med. Chem.*, **15**, 2, 931-8.
- 2) Vine, K. L., Locke, J. M., Ranson, M., Pyne, S. G. and Bremner, J. B. (2007) An Investigation into the Cytotoxicity and Mode of Action of Some Novel *N*-alkyl Substituted Isatins *J. Med. Chem.*, **50**, 21, 5109-77.
- 3) Julie M. Locke, Kara L. Vine, Marie Ranson, Stephen G. Pyne, and John B. Bremner. The Serendipitous Synthesis of 6-Hydroxyisatins. The 21<sup>st</sup> International Congress for Heterocyclic Chemistry, Sydney, NSW, AUSTRALIA, July 15-20<sup>th</sup> 2007.
- 4) Lidia Matesic, John B. Bremner, Stephen G. Pyne, Julie M. Locke, Marie Ranson and Kara L. Vine. Isatin Derivatives as Novel Anti-Cancer Agents. The 21<sup>st</sup> International Congress for Heterocyclic Chemistry, Sydney, NSW, AUSTRALIA, July 15-20<sup>th</sup> 2007
- 5) Kara L. Vine, Julie M. Locke, John B. Bremner, Stephen G. Pyne and Marie Ranson. *N*-alkylisatins: Potent Anti-Cancer Agents. RACI Drug Design Amongst the Vines, Hunter Valley, NSW, AUSTRALIA, Dec 3-7<sup>th</sup> 2006.
- 6) Kara L. Vine, Julie M. Locke, John B. Bremner, Stephen G. Pyne and Marie Ranson. Substituted Isatins as Small Molecule Anti-Cancer Agents. Inaugural HMRI Cancer Conference, New Therapeutics, Newcastle, NSW, AUSTRALIA, Sept 20-22<sup>nd</sup> 2006.
- 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 29<sup>th</sup>, 2006.

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- 9) Kara L. Vine, John B. Bremner, Stephen G. Pyne, Kirsten Benkendorff and Marie Ranson. A Cytotoxic Marine Natural Product as a Novel Anti-Tumour Agent and Potential for use in Targeted Cancer Therapy. Inaugural HMRI Cancer Conference, New Therapeutics, Newcastle, NSW, AUSTRALIA, Oct 4-6<sup>th</sup> 2004
- 10) Kara L. Vine, Marie Ranson and Kirsten Benkendorff. Cures from the Deep: The Cytotoxicity of Indole Derivatives from the Egg Masses of the Marine Mollusc *Dicathais Orbita*. Australian Health Management Group Medical Research Week Symposium, Wollongong, NSW, AUSTRALIA, 4<sup>th</sup> June, 2004.