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

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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

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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 heamatological 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_{50} value of 490 nM against U937 and Jurkat leukemic cell lines, after 24 h. 5,7-Dibromo-*N*-(*p*-trifluoro-methylbenzyl)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	*N*,*N*-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
J	coupling constant
LDP	ligand-directed prodrug
Lit.	literature
LR	low resolution
m	multiplet
m.p.	melting point
m/z	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-carboymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt
NHS	*N*-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_f	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 form TMS

Units Used

mol	mole (6.022×10^{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})
µ	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
°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 21st International Congress for Heterocyclic Chemistry, Sydney, NSW, AUSTRALIA, July 15-20th 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 21st International Congress for Heterocyclic Chemistry, Sydney, NSW, AUSTRALIA, July 15-20th 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 3-7th 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 20-22nd 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 29th, 2006.

8) Kara L. Vine, Marie Ranson and Kirsten Benkendorff. Cytotoxic Activity of Indole Derivatives from the Egg Masses of Marine Muricid Molluscs. Indirubin the Red Shade of Indigo, Les Eyzies-de-Tayac, FRANCE, April 8-13th 2006.

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 4-6th 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, 4th June, 2004.