

**Biological Activity of a Py-Im Polyamide Androgen Receptor Antagonist**

Thesis by

John W. Phillips

In Partial Fulfillment of the Requirements  
for the Degree of  
Doctor of Philosophy

California Institute of Technology  
Pasadena, California  
2011

(Defended May 31<sup>st</sup>, 2011)

© 2011

John W. Phillips

All Rights Reserved

*To Valerie.*

*For all those late nights and long weekends.*

**Acknowledgements.**

I would like to thank Peter Dervan for providing a superlative training environment for my six years at Caltech. The mentorship and teaching philosophy you have demonstrated over the years have been invaluable. I would also like to thank the members of my committee, Dennis Dougherty, Bil Clemons, and Judy Campbell for their support and interest in my graduate work.

I have had the privilege of working with supremely talented colleagues in the Dervan lab, for which I am also grateful. I would particularly like to thank my intramural collaborators: Carey Hsu, Jim Puckett, Michelle Farkas, Christian Döse, Dave Chenoweth, Dan Harki, and Ben Li. Thanks also to Nick Nickols, a senior graduate student who gave me the best introduction to graduate-level research that I could have ever hoped for. Jevgenij Raskatov also deserves my gratitude for participating in our many scintillating scientific discussions.

I would also like to thank Kenneth Karanja, my collaborator and coauthor in the Campbell lab. His expertise, keen insight, and unexpected interest helped me close the final chapter of my graduate research, and just in the nick of time, too.

Caltech is home to a number of staff scientists whose fine work has contributed to this thesis. I would like to single out Shelley Diamond for her dedication and professionalism. Her expertise in flow cytometry was instrumental to my work on the mechanism of polyamide cytotoxicity. Her decades of experience and high standards for data quality helped me tremendously.

**Abstract.**

Py-Im polyamides are cell-permeable, programmable, sequence-specific, DNA minor groove-binding small molecules. When designed to bind a DNA sequence that matches the consensus DNA-binding sequence of a transcription factor, they can be used to block the binding of that transcription factor to its response element *in vitro* and in cell culture. We have used this approach to inhibit the genotoxic activity of the endogenous transcription factors HIF1 $\alpha$ , glucocorticoid receptor (GR), and androgen receptor (AR). In this work, we report the completion of a library of hairpin Py-Im polyamides targeted to all possible 5'-WGNNNW-3' (W = A or T) sequences. These compounds bind their target DNA sequences with high affinity. One compound from this set targets the sequence 5'-WGWWCW-3', which matches the DNA binding consensus sequence of GR and AR and has been shown to inhibit the gene regulatory activity of these proteins in cell culture. Herein, we show that a cyclic derivative of this compound maintains its activity against AR-driven gene expression in hormone-sensitive LNCaP prostate cancer cells. As androgen receptor signaling is crucial to prostate cancer growth and metastasis even in its recurrent form, we next examine the activity of the AR/GR antagonist in a tissue culture model of castration-resistant prostate cancer. In this model, the polyamide retains its activity against AR-driven mRNA expression, but it fails to inhibit the binding of AR to its response element. The polyamide-mediated repression is also accompanied by significant cell stress and cytotoxicity, which are explored in the final two chapters of this thesis. The former investigates a role for polyamides as inhibitors of DNA Topoisomerase II. Despite *in vitro* evidence indicating polyamides prevent Topoisomerase II binding, no evidence for this is found in cell culture. The final chapter reveals that polyamide-mediated cytotoxicity is likely due to inhibition of DNA synthesis. This occurs at concentrations similar to those used for transcription factor inhibition, suggesting that S-phase disturbance accompanies efforts to regulate gene expression with polyamides.

**Table of Contents**

List of Figures and Tables.....	vii
Chapter 1: Introduction.....	1
Chapter 2: Completion of a Programmable DNA-Binding Small Molecule Library.....	27
Chapter 3: Cyclic Pyrrole-Imidazole Polyamides Targeted to the Androgen Response Element.....	49
Chapter 4: Characterization of Py-Im Polyamide Androgen Receptor Antagonists in Hormone-Refractory Prostate Cancer Cells .....	72
Chapter 5: Py-Im Polyamides Inhibit DNA Topoisomerase II Activity <i>In Vitro</i> by Disrupting Enzyme Binding.....	92
Chapter 6: Mechanism of Polyamide-Induced Cytotoxicity in Prostate Cancer Cells .....	111

**List of figures and tables.**

## Chapter 1

Figure 1.1.	Chart depicting the genome size and number of protein-coding genes of several eukaryotes .....	2
Figure 1.2.	Signal transduction converges on transcription factors .....	3
Figure 1.3.	Structural diversity of DNA-binding proteins .....	4
Figure 1.4.	Composite model of cooperative assembly of transcription factors mediated by allosteric interactions on the Interferon- $\beta$ enhancer .....	6
Figure 1.5.	AR signaling in prostate cancer .....	8
Figure 1.6.	Two different strategies for the inhibition of the transcription factor HIF1 $\alpha$ .....	9
Figure 1.7.	Engineered zinc finger proteins for control of transcription.....	10
Figure 1.8.	Structure of B-form DNA .....	12
Figure 1.9.	Hydrogen-bonding pattern of the four Watson-Crick base pairs in the major and minor groove.....	13
Figure 1.10.	Sequence-specific, minor groove-binding natural products and their target sequences.....	14
Figure 1.11.	Schematic of an 8-ring hairpin polyamide designed to distinguish all four Watson-Crick base pairs.....	15
Figure 1.12.	Cell permeability and nuclear localization of polyamides in live MCF7 breast cancer cells.....	16
Figure 1.13.	Structural basis for allosteric inhibition of major groove-binding transcription factors by minor groove-binding polyamides....	17
Figure 1.14.	Py-Im polyamide inhibitors of HIF1 $\alpha$ and nuclear hormone receptor signaling.....	19

## Chapter 2

Figure 2.1.	Model for the complex formed between hairpin polyamide <b>24</b> and its match DNA sequence .....	31
Figure 2.2.	Plasmid design for pCFH2, pCFH3, pCFH4, pCFH5, pPh2, and pMFST .....	33
Figure 2.3.	Quantitative DNase I footprint titration experiments .....	34
Table 2.1.	$K_a$ ( $M^{-1}$ ) values reported are the mean values from at least three DNase I footprint titration experiments .....	35
Table 2.2.	Equilibrium association constants $K_a$ ( $M^{-1}$ ) .....	37
Table 2.3.	Equilibrium association constants $K_a$ ( $M^{-1}$ ) .....	39
Figure 2.4.	Quantitative DNase I footprint titration experiments .....	41

## Chapter 3

Figure 3.1.	Chemical structures for cyclic and hairpin polyamides .....	52
Scheme 3.1.	Preparation of <b>10</b> and <b>11</b> .....	53
Scheme 3.2.	Preparation of <b>1</b> , <b>2</b> , and <b>3</b> .....	54
Table 3.1.	$T_m$ values for polyamides <b>1-5</b> .....	55
Figure 3.2.	Targeting the ARE with DNA-binding polyamides .....	56

## Chapter 4

Figure 4.1.	Disrupting the AR/ARE interface in HRPC .....	75
Table 4.1.	$IC_{50}$ values for inhibition of PSA mRNA expression .....	77
Figure 4.2.	A Py-Im polyamide antagonist of AR-ARE binding inhibits expression of AR-target gene PSA .....	78
Figure 4.3.	Inhibition of AR occupancy at the FKBP5 intronic enhancer .....	79
Figure 4.4.	Inhibition of prostate cancer cell growth and induction of cytotoxic response following treatment with ARE-targeted polyamide <b>1</b> .....	80
Table 4.2.	Cytotoxicity $IC_{50}$ values in LNCaP and LN-AR cells in	

	response to treatment with <b>1</b> .....	81
Figure 4.5.	Caspase 3/7 activation accompanies PSA downregulation in unstimulated LN-AR cells .....	81
Figure 4.6.	Stabilization of p53 in response to polyamide treatment.....	82
Chapter 5		
Figure 5.1.	Chemical structure and binding preferences of the Py-Im polyamides used in this study .....	96
Figure 5.2.	<i>In vitro</i> DNA relaxation assay demonstrating polyamide-mediated, dose-dependent inhibition of Top2 $\alpha$ -p170 catalytic activity without cleavage complex formation.....	97
Figure 5.3.	Polyamides <b>1</b> and <b>2</b> inhibit Top2 $\alpha$ -p170 binding <i>in vitro</i> .....	98
Figure 5.4.	Dose-dependent induction of cytotoxicity by polyamides <b>1</b> and <b>2</b> in DU145 (wt) and Top2 knockdown cell lines .....	100
Table 5.1.	Cytotoxicity IC <sub>50</sub> values ( $\mu$ M) of compounds <b>1</b> and <b>2</b> in DU145 and DU145-shTop2 cell lines. ....	100
Figure 5.5.	Polyamide treatment causes S-phase arrest in DU145 and DU145-shTop2 $\alpha$ cells.....	101
Chapter 6		
Figure 6.1.	Chemical structure and DNA binding preferences of the Py-Im polyamides used in this study .....	113
Table 6.1.	Summary of cytotoxicity IC <sub>50</sub> values of 5'-WGWWCW-3' (W = A or T) polyamides in AR-expressing, AR-overexpressing, and AR-negative prostate cancer cell lines .....	116
Figure 6.2.	Polyamide treatment induces apoptosis in DU145 cells.....	117
Figure 6.3.	Polyamide treatment causes S-phase arrest .....	119
Figure 6.4.	Polyamide treatment does not induce DNA damage or activate the DNA-damage induced S-phase checkpoint .....	120