

Antitumor Activity of Py-Im polyamides

Thesis by

Fei Yang

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

California Institute of Technology
Pasadena, California
2014
(Defended July 8th, 2013)

© 2013

Fei Yang

All Rights Reserved

For my family

Acknowledgement

I would like to thank Professor Peter Dervan for his guidance over the past five years. I am especially grateful for the latitude he has given me to pursue new ideas, and for his continued support after many of those ideas led to dead ends. The skills I've learned from the numerous training opportunities he has provided will be instrumental as I navigate my career in science.

Being at Caltech, I've had the opportunity to work with very capable individuals, many of whom I've had the good fortune to collaborate with. Georgi, John, Ben, Jerzy, Jordan, and Jamie, I thank you all for your contributions. In particular, I would like to acknowledge Nick Nickols, who has been my collaborator since the beginning of the animal projects. Our many discussions over hookah helped shape the direction of the work presented herein.

Most of all, I thank my family, without their support this work would not be possible.

Table of Contents

List of Figures and Tables.....	vii
Chapter 1: Introduction: DNA as a therapeutic target in cancer.....	1
Chapter 2: Single-dose pharmacokinetic and toxicity analysis of pyrrole imidazole polyamides in mice.....	14
Chapter 3: Antitumor activity of a pyrrole-imidazole polyamide.....	36
Chapter 4: Animal toxicity of hairpin pyrrole-imidazole polyamides varies with the turn unit	59

List of figures and tables

Chapter 1

Figure 1.1. Transcription and replication dependent mechanisms of DNA repair.....	3
Table 1.1. Selection of FDA approved compounds.....	4
Figure 1.2. Select noncovalent sequence specific minor groove binders.....	7
Figure 1.3. Sequence recognition by hairpin Py-Im polyamides.....	8

Chapter 2:

Figure 2.1. Chemical structures and plasma concentration of polyamides.....	22
Table 2.1. Plasma pharmacokinetic parameters.....	23
Figure 2.2. Urinary and fecal excretion of polyamides.....	24
Figure 2.3. Tissue distribution of polyamides.....	25
Table 2.2. Tissue pharmacokinetic parameters.....	26
Figure 2.4. Animal toxicity of polyamides.....	27
Figure S2.1. Chemical structures of polyamide standards.....	34
Figure S2.2. Stability of polyamides.....	35

Chapter 3:

Figure 3.1. Chemical structures of polyamides 1 and 2	39
Figure 3.2. Global effect of 1 on RNAP2.....	45
Figure 3.3. Cytotoxicity of polyamides 1 and 2 in LNCaP cells.....	47
Figure 3.4. Effects of polyamide 1 on cellular DNA damage response.....	48
Figure 3.5. Effects of polyamide 1 on human prostate cancer xenografts.....	49
Figure S3.1. Heat map of global distribution of RNAP2 over gene bodies.....	56
Figure S3.2. Cytotoxicity and cell uptake of polyamides 1 and 2	57
Figure S3.3. Circulation of polyamide 1	58

Figure S3.4. Animal weights after polyamide treatment.....	58
Table S3.1. Primer sequences used in study.....	58
Chapter 4:	
Figure 4.1. Chemical structures of polyamides 1-4	62
Figure 4.2. Experiment set up of single dose weight curve experiments.....	69
Figure 4.3. Histopathology analysis and serum chemistry of animals treated with 1-4	72
Figure 4.4. Experiment set up of multi dose weight curve experiments.....	74
Figure 4.5. Activity of 3 in LNCaP cells.....	76
Figure 4.6. Activity of 3 against LNCaP xenografts.....	77
Table S4.1. MALDI-ToF analysis of compounds.....	86
Figure S4.1. DNA thermal stabilization of 1-4	86
Figure S4.2. Serum circulation of 1-4	87
Figure S4.3. Characterization of cyclic polyamide 5	88
Table S4.2. Microsomal stability of 1-4	89
Figure S4.4. Chemical structures of 6-9	90
Figure S4.5. Nuclear localization of 6-9 in the liver.....	90