

**Improving the Biological Activity of
Pyrrole-Imidazole Polyamides**

Thesis by
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For my family

Thank you for always supporting me

Acknowledgements

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Abstract

DNA is nature's blueprint, holding within it the genetic code that defines the structure and function of an organism. A complex network of DNA-binding proteins called transcription factors can largely control the flow of information from DNA, so modulating the function of transcription factors is a promising approach for treating many diseases. Pyrrole-imidazole (Py-Im) polyamides are a class of DNA-binding oligomers, which can be synthetically programmed to bind a target sequence of DNA. Due to their unique shape complementarity and a series of favorable hydrogen bonding interactions that occur upon DNA-binding, Py-Im polyamides can bind to the minor groove of DNA with affinities comparable to transcription factors. Previous studies have demonstrated that these cell-permeable small molecules can enter cell nuclei and disrupt the transcription factor-DNA interface, thereby repressing transcription. As the use of Py-Im polyamides has significant potential as a type of modular therapeutic platform, the need for polyamides with extremely favorable biological properties and high potency will be essential. Described herein, a variety of studies have been performed aimed at improving the biological activity of Py-Im polyamides. To improve the biological potency and cellular uptake of these compounds, we have developed a next-generation class of polyamides bearing aryl-turn moieties, a simple structural modification that allows significant improvements in cellular uptake. This strategy was also applied to a panel of high-affinity cyclic Py-Im polyamides, again demonstrating the remarkable effect minor structural changes can have on biological activity. The solubility properties of Py-Im polyamides and use of formulating reagents with their treatment have also been examined. Finally, we describe the study of Py-Im polyamides as a potential artificial transcription factor.

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List of Abbreviations

A	adenine
Ac	acetyl
Ac ₂ O	acetic anhydride
A ₅₉₅	absorbance maximum
AR	androgen receptor
ARE	androgen response element
A·T	adenine Watson-Crick hydrogen bonded to thymine
ATCC	American Type Culture Collection
β	beta-amino alanine
Boc	<i>tert</i> -butyloxycarbonyl
Boc-Im-OH	(4-[(<i>tert</i> -Butoxycarbonyl)amino]-1-methylimidazole-2-carboxylic acid)
Boc ₂ O	di- <i>tert</i> -butyl dicarbonate
Boc-Py-OBt	[(1,2,3-Benzotriazol-1-yl 4-[(<i>tert</i> -Butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate)
bp	base pair
BSA	bovine serum albumin
°C degrees	Celsius
C	cytosine
C·G	cytosine Watson-Crick hydrogen bonded to guanine
calc'd	calculated
Cbz	carbobenzyloxy
ChIP	chromatin immunoprecipitation
cm	centimeter
Da	Dalton
dATP	2'-deoxyadenosine triphosphate
DABA	diaminobutyric acid
DCM	dichloromethane
dex	dexamethasone
DFO	deferoxamine
DHT	dihydrotestosterone
DIEA	N,N-diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
Dp	N,N-dimethylaminopropylamine
DPPA	diphenylphosphoryl azide
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbant assay
EMSA	elecrophoretic mobility shift assay
ESI	electrospray ionization
Et ₂ O	diethyl ether

List of Abbreviations

Ex	excitation
FBS	fetal bovine serum
FITC	fluorescein isothiocyanate
Fmoc	fluorenylmethyloxycarbonyl
γ -DABA	γ -2,4-diaminobutyric acid
G	guanine
G·C	guanine Watson-Crick hydrogen bonded to cytosine
GABA	γ -aminobutyric acid
HIF-1 α	hypoxia inducible factor 1 α
Hr	hour(s)
Hp	3-hydroxypyrrrole
HPLC	high-performance liquid chromatography
HRE	hypoxic response element
Im	N-methylimidazole
IPA	isophthalic acid
K _a	association constant
K _d	dissociation constant
λ	wavelength
LN ₂	liquid nitrogen
<i>m/z</i>	mass to charge ratio
μ	micro (1×10^{-6})
M	molar
m	milli (1×10^{-3})
Max	Myc associated protein X
max	maximum
MALDI	matrix-assisted LASER desorption/ionization
min	minute(s)
mol	mole(s)
mRNA	messenger ribonucleic acid
MS	mass spectrometry
N	A, T, G, or C
n	nano (1×10^{-9})
n-BuLi	n-butyl lithium
NF- κ B	nuclear factor- κ B
OBt	hydroxytriazole ester
p	pico (1×10^{-12})
PCR	polymerase chain reaction
PSA	prostate-specific antigen
Py-Im	pyrrole-imidazole
qPCR	quantitative polymerase chain reaction
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction

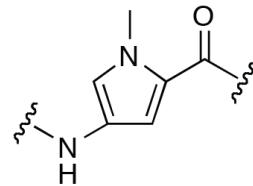
List of Abbreviations

PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
Py	N-methylpyrrole
PyBOP	(benzotriazol-1-yloxy)tritylolidinophosphonium hexafluorophosphate
R	guanine or adenine
RCF	relative centrifugal force
RIPA	radio immunoprecipitation assay
RNA	ribonucleic acid
RNAi	ribonucleic acid interference
RT	reverse transcription
siRNA	small interfering ribonucleic acid
Smad	Sma and Mad-related protein
T	thymine
T·A	thymine Watson-Crick hydrogen bonded to adenine
t-BuOH	<i>tert</i> -butanol
TF	transcription factor
TFA	trifluoroacetic acid
TFO	triplex-forming oligonucleotides
THF	tetrahydrofuran
T _m	midpoint of transition temperature
TOF	time-of-flight
TFRE	transcription factor response element
tri/triamine	3,3'-diamino-N-methyldipropylamine
U	uracil
UV	ultraviolet
VEGF	vascular endothelial growth factor
Vis	visible
W	adenine or thymine

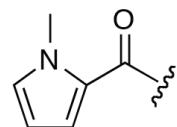
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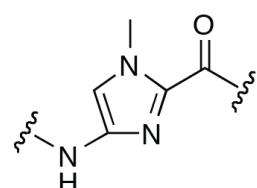
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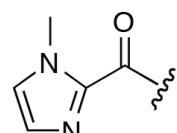
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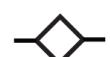
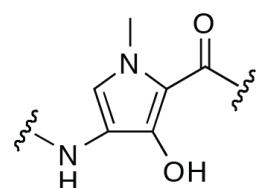
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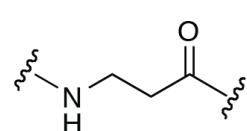
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-Hp-



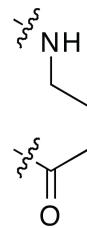
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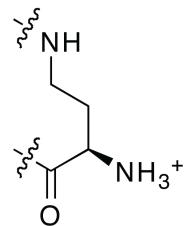
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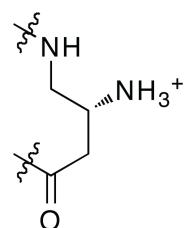
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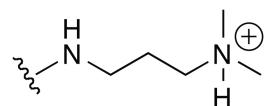
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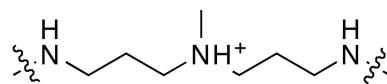
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-Dp



-tri-



-IPA

