

Small Molecule DNA and RNA Binders

From Synthesis to Nucleic Acid Complexes

M. Demeunynck, C. Bailly, W. D. Wilson (Eds.)

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Contents

Volume 1

Preface *xix*

Contributors *xxi*

1	Forty Years On	1
	<i>Michael J. Waring and L. P. G. Wakelin</i>	
1.1	Early Experiments Prior to Molecular Modeling	1
1.2	Formulation of Molecular Models and Mechanisms of Binding to DNA	3
1.3	Specificity of Nucleotide Sequence Recognition	4
1.4	Details at the Atomic and Molecular Levels	6
1.5	Identification of Motifs for Drug Design	9
1.6	Actions on Nucleoproteins, Chromatin, and Enzymes	11
	References	12
2	Targeting HIV RNA with Small Molecules	18
	<i>Nathan W. Luedtke and Yitzhak Tor</i>	
2.1	Introduction	18
2.1.1	Translation	18
2.1.2	RNA Viruses	19
2.2	Small Molecules that Modulate RNA Activity	19
2.2.1	Magnesium (II)	20
2.2.2	Aminoglycosides	21
2.2.3	Ligand Specificity	23
2.2.4	Goals	23
2.3	The RRE and HIV Replication	24
2.4	Determination of RRE–Ligand Affinity and Specificity	25
2.4.1	Fluorescence Anisotropy	26
2.4.2	Solid-phase (Affinity-displacement) Assay	27
2.4.3	Ethidium Bromide Displacement	29
2.5	New RRE Ligands	29
2.5.1	Neomycin–acridine Conjugates	29

2.5.2	Dimeric Aminoglycosides	32
2.5.3	Guanidinoglycosides	32
2.6	Conclusions	36
	Acknowledgments	37
	References	37
3	RNA Targeting by Bleomycin	41
	<i>Sidney M. Hecht</i>	
3.1	Activation of Bleomycin for Polynucleotide Degradation	41
3.2	Bleomycin-mediated Cleavage of Transfer RNAs and tRNA Precursor Transcripts	42
3.3	Other RNA Targets for Bleomycin	44
3.4	Characteristics of RNA Cleavage by Fe·BLM	46
3.5	Chemistry of Bleomycin-mediated RNA Cleavage	50
3.6	Significance of RNA as a Target for Bleomycin	52
	Acknowledgments	54
	References	54
4	Inhibitors of the Tat–TAR Interactions	58
	<i>Chimmanamada U. Dinesh and Tariq M. Rana</i>	
4.1	Introduction	58
4.2	Mechanism of Transcriptional Activation by Tat	59
4.3	Tat–TAR Interactions	61
4.4	RNA as a Small Molecule Drug Target	63
4.5	Ligands for TAR RNA	63
4.5.1	TAR RNA Bulge Binders	63
4.5.2	Targeting Multiple Sites in TAR RNA	65
4.5.3	Targeting RNA with Peptidomimetic Oligomers	66
4.5.3.1	Backbone modification	66
4.5.3.2	D-Peptides	68
4.6	Combinatorial Library Approach in the Discovery of Small Molecule Drugs Targeting RNA	69
4.6.1	Combinatorial Chemistry	69
4.6.2	Split Synthesis	70
4.6.3	Encoding	72
4.6.4	On-bead Screening and Identification of Structure-specific TAR-Binding ligands	73
4.6.5	Ligand Sequence Analysis	74
4.6.6	Heterochiral Small Molecules Target TAR RNA Bulge	76
4.6.7	Inhibition of Tat <i>trans</i> -Activation <i>in vivo</i>	78
4.7	Cyclic Structures as RNA-targeting Drugs	78
4.8	Summary and Perspective	80
	Acknowledgments	80
	References	81

5	DNA and RNA Recognition and Modification by Gly-Gly-His-Derived Metallopeptides	88
	<i>Eric C. Long and Craig A. Claussen</i>	
5.1	Introduction	88
5.1.1	General Considerations	88
5.1.2	Metallopeptides in the Study of Nucleic Acid Recognition	89
5.2	Interactions of Gly-Gly-His-Derived Metallopeptides with DNA	89
5.2.1	Natural Occurrence and Metal-binding Properties	89
5.2.2	Development as a DNA-Cleavage Agent	90
5.2.3	DNA Binding and Modification by Ni(II)-Xaa-Xaa-His Metallopeptides	92
5.2.3.1	Selective minor groove recognition and binding	92
5.2.3.2	Minor groove-directed deoxyribose oxidation	99
5.2.3.3	Nature of the intermediate involved in deoxyribose oxidation	102
5.2.3.4	Generation of minor groove binding combinatorial libraries	104
5.2.3.5	Guanine nucleobase modification/oxidation	108
5.2.4	DNA Strand Scission by Co-Xaa-Xaa-His Metallopeptides	111
5.2.4.1	Activation via ambient O ₂ and light	111
5.2.4.2	Highly selective DNA cleavage via ambient O ₂ activation	112
5.3	Recognition and Cleavage of RNA by Ni(II)-Xaa-Xaa-His Metallopeptides	116
5.4	Summary	118
	Acknowledgements	118
	References	118
6	Salen-Metal Complexes	126
	<i>S. E. Rokita and C. J. Burrows</i>	
6.1	Introduction	126
6.2	Reversible Binding of Simple Metal-Salen Complexes	127
6.3	Nucleic Acid Strand Scission Induced by Simple Metal-Salen Complexes	129
6.3.1	Metal-Salens Activated by Reductants for Strand Scission	130
6.3.2	Metal-Salens Activated by Peracids for Strand Scission	131
6.3.3	Metal Salens Activated by Hydrogen Peroxide for Strand Scission	134
6.3.4	Metal-Salens Activated by Molecular Oxygen for Strand Scission	134
6.4	Covalent Coupling between Simple Nickel-Salen Complexes and Nucleic Acids	136
6.5	Chimeric Metal-Salen Complexes	139
6.6	Conclusion	140
	References	141
7	Charge Transport in DNA	146
	<i>Tashica T. Williams and Jacqueline K. Barton</i>	
7.1	Introduction	146

7.2	DNA Metallointercalators	147
7.2.1	Phenanthrenequinone Diimine Complexes of Rhodium	148
7.2.2	Dipyridophenazine Complexes of Ruthenium	148
7.3	Photophysical Studies of Electron Transport in DNA	150
7.3.1	Electron Transport between Ethidium and a Rhodium Intercalator	150
7.3.2	Ultrafast Charge Transport in DNA: Ethidium and 7-Deazaguanine	151
7.3.3	Base–Base Charge Transport	152
7.4	DNA-mediated Electron Transport on Surfaces	153
7.4.1	Characterization of DNA-modified Surfaces	153
7.4.2	Electrochemical Probe of Redox Reactions of Intercalators	154
7.4.3	Sensing Mismatches in DNA	155
7.5	Long-range Oxidative Damage to DNA	156
7.5.1	Long-range Oxidative Damage at 5'-GG-3' Sites by a Rhodium Intercalator	156
7.5.2	Models for Long-range DNA Charge Transport	158
7.5.3	Sequence Dependence of DNA Charge Transport	159
7.5.4	The Effects of Ion Distribution on Long-range Charge Transport	160
7.5.5	Mismatch Influence on Long-range Oxidative Damage to DNA	162
7.6	Using Charge Transport to Probe DNA–Protein Interactions and DNA Repair	163
7.6.1	DNA-Binding Proteins as Modulators of Oxidative Damage from a Distance	163
7.6.2	Detection of Transient Radicals in Protein/DNA Charge Transport	164
7.6.3	Electrical Detection of DNA–Protein Interactions	165
7.6.4	Repair of Thymine Dimers	167
7.6.5	Oxidative Damage to DNA in Nucleosomes	169
7.6.6	DNA Charge Transport within the Nucleus	170
7.7	Conclusions	171
	Acknowledgements	172
	References	172
8	DNA Interactions of Novel Platinum Anticancer Drugs	178
	<i>Viktor Brabec and Jana Kasparkova</i>	
8.1	Introduction	178
8.2	Modifications by Cisplatin	178
8.2.1	Adducts and Conformational Distortions	178
8.2.2	Effects on DNA Replication and Transcription	181
8.2.3	Cellular Resistance, Repair	182
8.2.4	Recognition of the Lesions by Cellular Proteins	184
8.2.4.1	HMG-domain proteins	184
8.2.4.2	Proteins without an HMG domain	185
8.2.5	Mechanism of Action of Cisplatin	188
8.3	Modifications by Antitumor Analogs of Cisplatin	189
8.3.1	Carboplatin	189

8.3.2	Oxaliplatin	190
8.3.3	Other Analogs	194
8.3.3.1	Bidentate analogs	194
8.3.3.2	Monodentate analogs	196
8.4	Modification by Antitumor Analogs of Clinically Ineffective Transplatin	197
8.4.1	Modifications by Transplatin	199
8.4.2	Analogs Containing Iminoether Groups	199
8.4.3	Analogs Containing Planar Amine Ligand	201
8.4.4	Other Analogs	203
8.5	Modifications by Polynuclear Platinum Antitumor Drugs	204
8.5.1	Dinuclear Compounds	205
8.5.2	Trinuclear Compound	209
8.6	Concluding Remarks	211
	Acknowledgments	212
	References	212
9	Electrochemical Detection of DNA with Small Molecules	224
	<i>Shigeori Takenaka</i>	
9.1	Introduction	224
9.2	Electrochemistry of Nucleic Acids	224
9.3	DNA Labeling Through a Covalent Bond	227
9.4	Electrochemistry of Metal Complexes Bound to DNA	227
9.5	Electrochemistry of DNA-binding Small Molecules	231
9.6	DNA Sensor Based on an Electrochemically Active DNA-binding Molecule as a Hybridization Indicator	233
9.7	Mismatched DNA Detection by Hybridization Indicator	236
9.8	DNA-detecting System using Hybridization Indicator as a Mediator	239
9.9	Application to DNA Microarray	239
9.10	Conclusion	240
9.11	Summary	241
	Acknowledgments	241
	References	241
10	Design and Studies of Abasic Site Targeting Drugs: New Strategies for Cancer Chemotherapy	247
	<i>Jean-Francois Constant and Martine Demeunynck</i>	
10.1	Introduction	247
10.1.1	Importance of Abasic Sites in Cells	247
10.1.2	Structure of Abasic DNA	249
10.1.3	Abasic Site Reactivity	251
10.1.4	Enzymology Of the Abasic Site	252
10.2	Drug Design	252
10.2.1	Introduction	252

10.2.2	Synthesis of the Heterodimers	256
10.2.3	Nuclease Properties	259
10.2.4	Molecules Inducing Multiple DNA Damage	262
10.2.5	Drug–DNA Interaction	264
10.2.6	Enzyme Inhibition	267
10.3	Pharmacological Data	269
	Dedication	272
	Acknowledgments	272
	References	272
11	Interactions of Macrocyclic Compounds with Nucleic Acids	278
	<i>Marie-Paule Teulade-Fichou and Jean-Pierre Vigneron</i>	
11.1	Introduction	278
11.2	Nucleotide Complexation	279
11.2.1	Macrocyclic Polyamines	279
11.2.2	Azoniacyclophanes	280
11.2.3	Cyclobisintercalands	282
11.2.3.1	Acridinium derivatives	282
11.2.3.2	Phenanthridinium derivatives	283
11.2.3.3	Polyamino naphthalenophanes and acridinophanes	284
11.3	Nucleic Acids Complexation	288
11.3.1	Azoniacyclophanes	288
11.3.2	Porphyrin Derivatives	289
11.3.3	Phenanthridinium Derivatives	291
11.3.4	Acridinium Derivatives	291
11.3.4.1	SDM Macrocycle	292
11.3.4.2	BisA Macrocycle	294
11.3.5	Miscellaneous	306
11.3.5.1	Naphthalene diimide derivatives	306
11.3.5.2	Phenazine derivatives	309
11.3.5.3	Aminocalixarenes and aminocyclodextrins	309
11.4	Conclusion and Perspectives	310
	Acknowledgements	311
	References	311
12	Triplex- versus Quadruplex-specific Ligands and Telomerase Inhibition	315
	<i>Patrizia Alberti, Magali Hoarau, Lionel Guittat, Masashi Takasugi, Paola B. Arimondo, Laurent Lacroix, Martin Mills, Marie-Paule Teulade-Fichou, Jean-Pierre Vigneron, Jean-Marie Lehn, Patrick Mailliet, and Jean-Louis Mergny</i>	
12.1	Introduction	315
12.2	Nucleic Acids Samples	317
12.3	Dialysis Results	322
12.4	Induction of Quadruplex Structures	327
12.5	Triplex versus Quadruplex Stabilization	328
12.6	Conclusion and Further Developments	332

12.7	Summary	332
	Acknowledgments	333
	References	333

Volume 2

13	Design and Analysis of G4 Recognition Compounds	337
	<i>Shozeb Haider, Gary N. Parkinson, Martin A. Read, and Stephen Neidle</i>	
13.1	Introduction	337
13.2	Telomeric DNA	340
13.3	Crystal Structures of G-quadruplexes	342
13.3.1	The d(TG ₄ T) Quadruplex	342
13.3.2	The Na ⁺ form of d(G ₄ T ₄ G ₄) <i>Oxytricha nova</i> telomeric DNA	343
13.3.3	The K ⁺ form of d(G ₄ T ₄ G ₄) <i>Oxytricha nova</i> Telomeric DNA	344
13.3.4	The Crystal Structure of the Human Telomere G-quadruplex	345
13.3.5	The r(UG ₄ U) RNA Quadruplex	347
13.4	NMR Studies of Quadruplexes	347
13.5	Quadruplex-binding Ligands	348
13.6	NMR and Modeling Studies of Quadruplex–Ligand Complexes	349
	Appendix. Methodology for Ligand Quadruplex Modeling	351
	References	355
14	Triple Helix-specific Ligands	360
	<i>Keith R. Fox and Richard A. J. Darby</i>	
14.1	Introduction	360
14.2	Triplex-binding Ligands	362
14.2.1	Benzopyridoindole Derivatives	362
14.2.2	Coralyne	365
14.2.3	Naphthylquinolines	366
14.2.4	Bis-amidoanthraquinones	367
14.2.5	Aminoglycosides	369
14.2.6	Other Ligands	369
14.3	Sequence and Structural Selectivity of Triplex-specific Ligands	370
14.3.1	Sequence Selectivity	370
14.3.2	Binding to Different Motifs	370
14.4	Interaction of Duplex-specific Ligands with Triple Helical Nucleic Acids	371
14.4.1	Intercalators	371
14.4.2	Minor Groove Binders	371
14.5	Tethered DNA-binding Agents	373
14.5.1	Tethered Triplex-binding Ligands	373
14.5.2	Tethered Intercalators	374
14.5.3	Other Tethered DNA-binding Agents	374
14.6	Other Uses of Triplex-binding Ligands	375
14.6.1	Relaxing the specificity of triplex formation	375

14.6.2	Triplex Cleaving Agents	375
14.6.3	Antigene Activity	376
	Acknowledgments	376
	References	376
15	Polyamide Dimer Stacking in the DNA Minor Groove and Recognition of T-G Mismatched Base Pairs in DNA	384
	<i>Eilyn R. Lacy, Erik M. Madsen, Moses Lee, and W. David Wilson</i>	
15.1	Introduction: Sequence-specific Recognition of DNA by Synthetic Molecules	384
15.1.1	DNA Sequence Recognition	384
15.1.2	Stacking Behavior of Polyamides and DNA Recognition	386
15.2	T-G Mismatched DNA Base Pairs and their Biological Relevance	389
15.3	Potential Applications for Recognition of Mismatched Base Pairs	390
15.4	Structure of T-G Mismatched Base Pairs	390
15.5	Binding of Imidazole-containing Polyamide Analogs to T-G Mismatches through a Dimeric Binding Motif – Structural Studies	391
15.6	Binding of Imidazole-containing Polyamides to T-G Mismatches through a Dimeric Binding Motif: Thermodynamic and Kinetic Studies	395
15.6.1	Stoichiometry of Complexes	395
15.6.2	Binding Constants and Cooperativity	398
15.6.3	Kinetic Studies	400
15.7	Developing Molecules Capable of Recognizing Mismatches in DNA	404
15.8	Use of the T-G Recognition Motif by Im/Im Pairs to Probe the Effects of the Terminal Head Group on the Stacking of Polyamides	405
15.9	Future Directions	407
15.9.1	Polyamide–DNA Complexes	407
15.9.2	Mismatched Base Pair Recognition	408
	Acknowledgments	408
	Dedication of this Chapter to Professor J. William Lown on the Occasion of his Retirement	409
	References	409
16	Dicationic DNA Minor Groove Binders as Antimicrobial Agents	414
	<i>Richard R. Tidwell and David W. Boykin</i>	
16.1	Introduction	414
16.1.1	Intercalation	415
16.1.2	Minor Groove Binding	416
16.1.3	Netropsin	417
16.1.4	DAPI	419
16.1.5	Berenil	420
16.1.6	Pentamidine	421
16.2	Dicationic Carbazoles and Analogs	422
16.2.1	Introduction	422
16.2.2	DNA Binding of Dicationic Carbazoles and Analogs	423

16.2.3	Antimicrobial Activity of Carbazoles and Related Analogs	427
16.2.4	Pro-drugs of Carbazoles and Related Analogs	429
16.2.5	Synthesis of Carbazoles and Related Analogs	431
16.3	Dicationic Furans	433
16.3.1	Introduction	433
16.3.2	DNA Binding of Furamidine and Analogs	434
16.3.3	Antimicrobial Activity of Furamidine and Analogs	436
16.3.4	Pro-drug Approaches for Furamidine	444
16.3.5	Synthetic Approaches for Furamidine and Analogs	446
16.4	Conclusions	451
	Acknowledgments	452
	References	452
17	Energetics of Anthracycline–DNA Interactions	461
	<i>Jonathan B. Chaires</i>	
17.1	Introduction	461
17.2	Binding Free Energy	463
17.3	Salt Dependency of Daunorubicin Binding to DNA	465
17.4	Binding Enthalpy and the Temperature Dependence of Binding	468
17.5	Thermodynamic Profile for Daunorubicin Binding to Calf-Thymus DNA	471
17.6	Hydration Changes	472
17.7	Substituent Contributions	475
17.8	Isostructural is not Isoenergetic	476
17.9	Parsing the Binding Free Energy	477
17.10	Summary	478
	Acknowledgements	479
	References	479
18	Acridine-4-carboxamides and the Concept of Minimal DNA Intercalators	482
	<i>William A. Denny</i>	
18.1	DNA Intercalation	482
18.1.1	Definition	482
18.1.2	Effect of Chromophore Size	482
18.1.3	Effect of Chromophore Cross-sectional Thickness	484
18.2	Intercalative Binding and Cytotoxicity	484
18.2.1	Correlation of Cytotoxicity with Mode, Strength and Kinetics of Binding	484
18.2.2	The Drive For Tight Binders; Chromophore and/or Side Chain Modulation	486
18.2.3	Mechanism of Cytotoxicity of DNA Intercalators: Topoisomerase Poisoning	486
18.2.4	Pharmacological Drawbacks of Tight DNA Binding: the Concept of “Minimal Intercalators”	487
18.3	Classes of “Minimal Intercalators”	487

18.3.1	Styrylquinolines	487
18.3.2	2-Phenylquinolines	488
18.3.3	Phenylbenzimidazoles	489
18.3.5	Phenazines	490
18.3.4	Dibenzodioxins	491
18.4	Acridinecarboxamides: the Development of DACA	491
18.4.1	9-Aminoacridine-4-carboxamides	491
18.4.2	DACA and Other Acridine-4-carboxamides	493
18.4.2.1	Introduction	493
18.4.2.2	Interaction of DACA with topoisomerase	494
18.4.2.3	Cellular studies with DACA	495
18.4.2.4	Metabolism and pharmacology of DACA	495
18.4.2.5	Clinical studies with DACA	496
18.5	Conclusions	496
	References	497
19	DNA Topoisomerase-targeted Drugs	503
	<i>M. Palumbo, B. Gatto, and C. Sissi</i>	
19.1	Introduction	503
19.2	Structure and Functions of DNA Topoisomerases	503
19.2.1	Type I Topoisomerases	504
19.2.1.1	Structural features	504
19.2.1.2	Catalytic process	505
19.2.2	Type II DNA Topoisomerases	506
19.2.2.1	Structural features	507
19.2.2.2	Catalytic process	508
19.3	Drug Targeted at Topoisomerases	509
19.3.1	Top Poisons	510
19.3.1.1	Top1 poisons	510
19.3.1.2	Top2 poisons	515
19.3.1.3	Sequence specificity of top poisoning	524
19.3.2	Top Inhibitors	525
19.3.2.1	Human top2 inhibitors	525
19.3.2.2	Bacterial top2 inhibitors	525
19.3.3	Mixed Top1/2 Poisons or Inhibitors	527
19.4	Conclusions	529
	References	530
20	Targeting DNA and Topoisomerase I with Indolocarbazole Antitumor Agents	538
	<i>Christian Bailly</i>	
20.1	Introduction	538
20.2	Naturally Occurring Indolocarbazoles	540
20.2.1	Staurosporine and Analogs with a Pyranose Sugar Moiety	540
20.2.2	K252a and Analogs with a Furanose Sugar Moiety	542

20.2.3	Rebeccamycin	543
20.2.4	AT2433	545
20.3	Synthetic Indolocarbazole Derivatives Targeting DNA and Topoisomerase I	545
20.3.1	Influence of Chloro and Bromo Substituents on the IND Chromophore	546
20.3.2	Modification of the Imide Heterocycle	548
20.3.3	Halogenoacetyl Derivatives	549
20.3.4	Glucose and Galactose Derivatives: Stereospecific DNA Recognition	549
20.3.5	From Rebeccamycin to Staurosporine-type Analogs	551
20.3.6	Amino Sugar Derivatives	553
20.3.7	Methylation of the Indole Nitrogen	553
20.3.8	Indolo[2,3- <i>c</i>]carbazole	555
20.3.9	Rebeccamycin Dimers and Conjugates	556
20.4	Design of a tumor-active compound: NB-506	557
20.4.1	From BE13793C to ED-110 and NB-506	557
20.4.2	DNA Binding and Topoisomerase I Inhibition	559
20.4.3	Cytotoxicity and Apoptosis	559
20.4.4	NB-506-resistant Cell Lines	561
20.4.5	Antitumor Activity	562
20.4.6	Inhibition of the Topoisomerase I Kinase Activity	562
20.4.7	A Novel Clinical Candidate: J-107088	564
20.4.8	Biosynthesis	566
20.5	Conclusion	567
	Acknowledgments	567
	References	567
21	Defining the Molecular Interactions that are Important for the Poisoning of Human Topoisomerase I by Benzimidazoles and Terbenzimidazoles	576
	<i>Daniel S. Pilch, Hsing-Yin Liu, Tsai-Kun Li, Edmond J. LaVoie, and Christopher M. Barbieri</i>	
21.1	Human DNA Topoisomerase Type I	576
21.2	Topoisomerase I as a Target for Anticancer Drugs	577
21.3	Benzimidazoles	577
21.3.1	The Extent to which Benzimidazoles Stimulate hTOP1-mediated DNA Cleavage Depends on their Structure	577
21.3.2	Identification of hTOP1 as the Specific Cytotoxic Target of 5N2pMPBZ	579
21.3.3	Viscometric Measurements Reveal that 56MD2pMPBZ Binding Unwinds Negative Supercoils in pUC19, Consistent with an Intercalative Mode of Interaction	580
21.3.4	The Affinity of Benzimidazoles for Duplex DNA is Modulated by the Structure and Electronic Properties of the Substituents on the Benzimidazole Rings	583

21.3.5	Benzimidazole Binding to Duplex DNA Requires the Ligand to be in its Fully Protonated Cationic State	585
21.3.6	DNA Binding Alone is not Sufficient to Impart Benzimidazoles with the Ability to Trap and Stabilize the Cleavable TOP1–DNA Complex	587
21.3.7	A Structural Model for the Ternary hTOP1–5N2pHPBZ–DNA Cleavable Complex that is Consistent with the Current Structure–Activity Database	587
21.4	Terbenzimidazoles	590
21.4.1	The Pattern of hTOP1-mediated DNA Cleavage Induced by Terbenzimidazoles is Distinct from that Induced by CPT	590
21.4.2	TOP1 Poisoning by Terbenzimidazoles is not the Result of Ligand-induced DNA Unwinding	592
21.4.3	TB Derivatives Exhibit Linear Dichroism Properties Characteristic of Minor Groove-directed DNA Binding	593
21.4.4	The Relative DNA-binding Affinities of the Terbenzimidazoles is Correlated with their Relative TOP1-poisoning Activities	594
21.4.5	A Potential Role for Ligand Interactions with the DNA Minor Groove in Stabilization of the TOP1–DNA Cleavable Complex	596
21.4.5.1	5PTB preferentially binds and stabilizes bent versus normal duplex DNA	598
21.4.5.2	5PTB binding does not remove helical bends from kinetoplast DNA	599
	Acknowledgments	600
	References	601

22 Binding and Reaction of Calicheamicin and Other Eneidyne Antibiotics with DNA

Joseph P. Cosgrove and Peter C. Dedon

22.1	Introduction	609
22.2	Sources, Biosynthesis, and Structural Conservation	609
22.3	Mechanisms of Target Recognition	612
22.3.1	Overall Scheme for Binding and Activation	612
22.3.2	Structure of the Calicheamicin–DNA Complex	613
22.3.3	Esperamicin Structure and Function	615
22.3.4	Structure and Dynamics of Calicheamicin Binding Sites	616
22.3.5	Neocarzinostatin Recognition of Bulged DNA Structures	618
22.4	Products of Eneidyne-induced DNA Damage	620
22.4.1	Proportions of Single- and Double-stranded DNA Lesions Produced by Eneidyne	620
22.4.2	Oxidation of the 1'-Position of Deoxyribose and the Biochemistry of the Deoxyribonolactone Abasic Site	622
22.4.3	Oxidation of the 4'-Position of Deoxyribose and the Chemistry of Base Propenal	623
22.4.4	Oxidation of the 5'-Position of Deoxyribose and the Chemistry of Butenedialdehyde	624

22.4.5	Covalent Adducts of Eneidyne with Deoxyribose	624
22.5	Eneidyne-induced DNA Damage in Cells	626
22.5.1	Eneidyne Target Recognition in Chromatin and Cells	626
22.5.2	Molecular and Genomics Approaches to Understanding Cellular Responses to Eneidyne	628
22.6	Summary	630
	Acknowledgments	631
	References	631
23	Devising a Structural Basis for the Potent Cytotoxic Effects of Ecteinasidin 743	643
	<i>Federico Gago and Laurence H. Hurley</i>	
23.1	Introduction	643
23.2	Biological Activity and Characterization of the Active Compounds	643
23.3	Structural Characterization and Synthesis of Ecteinasidins	646
23.4	Structure–Activity Relationships	651
23.5	Structural Characterization of Et 743–DNA Adducts	652
23.6	Structural Studies of Ecteinasidin–DNA Complexes	654
23.7	Molecular Basis for Covalent Reactivity and Sequence Selectivity	660
23.8	The Rate of Reversal of Et 743 from Drug-Modified 5'-AGT is Faster than that from Drug-modified 5'-AGC Sequences	660
23.9	Et 743 can Reverse from its Initial Covalent Adduct Site and Bond to an Unmodified Target Sequence	661
23.10	The Kinetics of the Covalent Modification of 5'-AGC and 5'-AGT Sequences by Et 743 are Similar	662
23.11	The Differences in the Rate of the Reverse Reaction May Be Derived from Structural Differences between Et 743–DNA Adducts at the 5'-AGC and 5'-AGT Sequences	664
23.12	Molecular Targets for Et 743	664
23.12.1	Involvement of Transcription-coupled Nucleotide Excision Repair in Mediating the Cytotoxic Effects of Et 743	665
23.12.2	Suppression of MDR1 Transcription by Et 743	666
23.13	Relationship of Structural Consequences of DNA Modification by Et 743 to Biological Effects	667
23.14	Conclusions	671
	Acknowledgments	672
	References	672
24	The Azinomycins. Discovery, Synthesis, and DNA-binding Studies	676
	<i>Maxwell Casely-Hayford and Mark Searcey</i>	
24.1	Introduction	676
24.2	Isolation of the Azinomycins 1–3	677
24.3	Studies of the Truncated Analog 3	678
24.3.1	Stereoselective Synthesis of the Truncated Azinomycin 3	678

24.3.2	DNA-binding and Biological Activity Studies of the Truncated Fragment and Synthetic Analogs	681
24.4	Studies on the Total Synthesis of the Azinomycin A	683
24.4.1	Synthesis of the 1-Azabicyclo[3.1.0]hex-2-ylidene Dehydroamino Acid Subunit	683
24.4.1.1	Synthesis of the aldehyde unit	684
24.4.1.2	Wadsworth–Horner–Emmons reaction, bromination, and ring closure	684
24.4.2	The Total Synthesis of Azinomycin A	686
24.5	Computational Studies of DNA Binding of the Azinomycins	689
24.6	Experimental DNA-binding Studies and Antitumor Activities of the Full Azinomycin Structures – Is Crosslinking Required for Biological Activity?	690
24.7	Conclusions	691
	Acknowledgments	694
	References	694
25	The Generation and DNA-Interaction of PBD and CBI Libraries	697
	<i>Alison Hardy, Jane M. Berry, Natalie Brooks-Turner, Philip W. Howard, John. A. Hartley, and David. E. Thurston</i>	
25.1	Introduction	697
25.2	Synthesis of Tethered PBD and CBI Constructs	699
25.2.1	Synthesis of Capping Units	699
25.2.2	Attachment of PBD and CBI Capping Units to Solid Support	700
25.3	On-bead DNA Interaction	701
25.3.1	Interaction of Double-stranded DNA to PBDs and CBIs On-bead	701
25.3.2	On-bead DNA Sequence Selectivity	703
25.4	Library Synthesis	704
25.4.1	Library Screening	706
25.5	Conclusion	709
	Acknowledgments	709
	References	709
	Index	711

Preface

The ultimate goal of most organic-medicinal chemists is to see the small molecule that they have synthesized become a useful drug for the treatment of human diseases. Unfortunately, even with modern technology this is an extremely rare event. In most cases, the compounds designed and synthesized (generally with pain and passion) have a brief existence that does not exceed the first biological activity assay. The valley between chemistry and therapeutics is deep and difficult to cross but nevertheless the two disciplines are intimately associated. It is our goal to help construct a bridge between the makers of the small molecules and the users. Over the past two decades, a relatively large number of useful anticancer and anti-parasitic drugs have been discovered or rationally designed based on the principle of nucleic acids recognition. A better understanding of the molecular rules that govern interactions between small molecules and the many sequences and structures of DNA and RNA is pivotal to the development of novel drug candidates. How does the drug adapt to the nucleic acid target (and *vice versa*)? How do nucleic acid structures affect ligand binding? How do small molecules read the genetic information? These types of questions continue to excite our scientific curiosity and the quest for better DNA/RNA binders drives modern researchers much as the search for the Holy Grail did the ancients.

Design and development of nucleic acid targeted drugs is a challenging enterprise but real breakthroughs have been made in recent years and many are reported here. This volume is intended to give the reader an up-to-date view of the current status and expected developments in research involving ligand-nucleic acid recognition. This book was built on a discussion among the three of us on how chemistry, biophysical chemistry and pharmacology serve our field to help design new drugs. Our different but complementary view angles on the subject prompted us to edit this volume focussed on DNA/RNA recognition by a variety of small molecules: peptides, intercalators, groove binders, metal complexes. The various DNA structures that can be targeted by drugs are also considered and the field of natural products is partially covered. Altogether, the 25 chapters of this volume survey most of the drug categories that bind, bond or cleave nucleic acids. The reader will notice the diversity of small molecules mentioned here, from marine products to platinum complexes, from G4-binders to RNA cleaving agents, from abasic site selective agents to aptamers, as well as the panel of biophysical and

biochemical approaches routinely used to investigate the structures and dynamics of drug-nucleic acids complexes. The portraits of specific drug families (anthracyclines, indolocarbazoles, bleomycins, ...) are also thoroughly presented. The amalgam was deliberately chosen to cross ideas of organic chemists and biophysicists and those more interested in the therapeutic end point of the research.

The volume starts with a general introduction (magisterially presented in a British style) and then it flies over the world, from several countries in Europe (Spain, Italy, France, Czech Republic, UK) to the USA, via Japan and New Zealand, illustrating the essential international character of the research (and the friendly atmosphere of the edition). Inevitably we have neglected (mostly for consideration of space) a number of interesting areas that should have been cited here, such as clinical applications. But the gallery of molecules presented in this volume must be considered as a live exhibit to explore and to use for further drug design. Come on in, and like us, become fascinated by the “small molecules” that bind or bite the genetic material in its many forms. We hope you will also find examining this volume an enriching experience.

The enterprise was very exciting and proceeded smoothly (with no delay!) thanks to the enthusiastic contribution of all the authors. We are grateful to everyone for delivering their manuscript on time (and in some cases even well before the deadline!) and for making our task as editors such a memorable one. We also thank our “artist” Christian Coulombeau who kindly drew the front cover, sort of a railway to the future. Finally, we shall dedicate this volume to our colleagues who left the world too quickly to contribute (Marc Leng, David S. Sigman, and Peter A. Kollman in particular).

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1

Forty Years On

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1.1

Early Experiments Prior to Molecular Modeling

The quest to understand specific interactions between drugs and nucleic acids dates back a long time – more than 40 years. Even though the concept of gene targeting could not be explicitly formulated until much later, there were early realizations that DNA could provide a fine receptor for drugs. A major turning point in the history of drug binding to DNA, the publication in 1961 of the intercalation hypothesis by Leonard Lerman [1], in many people's estimation represents the true birth of the subject, but it would be wrong to neglect mention of the contributions of earlier workers. These workers knew they were dealing with drug–nucleic acid interactions and must have had some inkling of the future importance of the topic. Among them were the histologists who employed dyes such as aminoacridines to stain cells and tissue sections, particularly the fluorescent dye acridine orange, whose capacity to cause nuclei to fluoresce bright green while the cytoplasm fluoresced red was a valuable tool in histology and cell biology. Indeed in the researches of these pioneers can be found the first evidence that particular dyes can react differently with different kinds of nucleic acid-containing structures and therefore that the small molecules must be capable of some form of discrimination based upon what we would today call molecular recognition. From the variable and sometimes capricious performance of substances such as acridines employed as stains it could also be surmised that depending upon the solvent conditions a given dye might react in more than one way with its 'receptor,' foreshadowing the concept of heterogeneity in binding that was later to occupy the attention of biophysicists.

At the same time, thanks to the seminal work of Paul Ehrlich half a century earlier, the usefulness of dyes – particularly aminoacridines – as antiseptics and antimalarials was widely recognized so that the connection between cell staining and useful biological activity was more than implicit. Thus it happened at a critical moment that the potency of proflavine as a mutagen was recognized. This led to the brilliant experimental work of Crick, Brenner and colleagues [2] showing that exposure of bacteriophage-infected bacteria to proflavine produced frameshift mutations – a phenomenon that enabled them to deduce the triplet nature of the

genetic code. Meanwhile, the careful experiments of Peacocke and Skerrett [3] on the interaction of proflavine with purified DNA were under way and the first truly quantitative measurements of a reversible drug–DNA binding reaction became available, complete with a proper description of the metachromatic shift in the absorption spectrum, application of spectrophotometry and equilibrium dialysis to determine genuine binding constants, and clear evidence of the occurrence of secondary binding after saturation of the strong primary binding sites had been accomplished.

Now all the elements were in place for Lerman, at that time working in the Cambridge MRC Laboratory of Molecular Biology with Crick and Brenner, to get to work on the intercalation hypothesis. Stone and Bradley [4] disposed of the secondary interaction of acridine orange with nucleic acids by attributing it to the formation of stacked aggregates of dye bound externally to the polyanion.

Two other pre-intercalation areas of endeavor must be mentioned, the first of which is the action of the antibiotic actinomycin D. Actinomycin had been discovered in the 1940s and was the first antibiotic found to be highly active against certain tumors – indeed, through the 1950s and early 1960s it was reckoned to be the most potent anticancer agent available in the arsenal of chemotherapy. The antibiotic was known to be capable of inhibiting nucleic acid synthesis in susceptible cells, a process that was consequently identified as a prime target for anti-cancer chemotherapy. The discovery of mRNA and the process of gene transcription owes much to the earnest work of early cell biologists who showed that actinomycin was an exquisitely selective inhibitor of transcription by virtue of its specific inhibitory action on the newly discovered enzyme RNA polymerase; that in turn was attributable to tight but reversible binding of actinomycin to the double-helical DNA template [5]. These discoveries firmly established the business of ligand–DNA interaction as a matter of concern to biologists, clinicians, and a breed of pharmacologists who later emerged as key players in founding what was to become the illustrious discipline of molecular interactions.

The second area of endeavor, though it had little influence on the development of ideas about reversible ligand–nucleic acid interactions, is the remarkable work of people like Kohn, Brooks, and Lawley on nitrogen mustards and comparable alkylating agents used for cancer chemotherapy [6, 7]. We should recall that nitrogen mustards were the very first chemicals used to treat cancer, prompted by unhappy events that occurred during the Second World War; it is indeed salutary that so evidently worthy a purpose as the alleviation of suffering from one of humanity's most dreaded diseases should have come about in such an inauspicious manner. The determined attentions of a few medically minded individuals capable of grappling with rather complicated and messy chemistry did a lot to clarify the mechanisms of action of these highly reactive substances, and again the critical target turned out to be DNA. Painstaking analysis of the products formed *in vitro* and *in vivo* when cells were exposed to mustards eventually identified the N7 position of the guanine ring as the most reactive (i.e. nucleophilic) site for alkylation of DNA, and the perceived correlation of anticancer activity with the possession of two alkylating centers spaced some five atoms apart led to the concept that bi-functional reactivity must be crucial for therapeutic effect.

1.2

Formulation of Molecular Models and Mechanisms of Binding to DNA

Before we return to the historic turning point at which the intercalation hypothesis was born, it is logical to finish consideration of the early alkylating agents by referring to their identification as crosslinking agents capable of covalently linking the complementary strands of the DNA double helix. At first it was thought that this action would adequately explain their cytotoxic activity through inhibiting the progress of the replication fork, but more recently the possible contribution of *intra*-strand crosslinks and DNA–protein crosslinks has brought this assumption into question [8, 9]. Meanwhile other types of powerful alkylating agents have been discovered that do not necessarily form interstrand crosslinks but are endowed with excellent biological activity. Moreover, an early twist to the tale of covalent reaction with DNA came with the finding that the antibiotic mitomycin C must be activated by reduction prior to forming inter-strand DNA crosslinks; this discovery added impetus to the idea of bioreductive activation of pro-drugs, particularly for cancer treatment, which has become an important focus for the efforts of several groups of drug designers (see Chapter 9). There is also a complex relation between bond-forming and bond-breaking interactions with nucleic acids that can be seen with several DNA-binding compounds described elsewhere in this volume (see Chapters 3 and 23).

A unifying theme that runs through these lines of work, and indeed throughout the volumes of this publication, is the extraordinarily sophisticated chemistry that attends the reaction of many compounds with nucleic acids, not to mention the amazing biosynthetic capabilities of the organisms that produce those substances that are of natural origin. Neither should we belittle the remarkable inventiveness and achievements of the organic chemists who increasingly are succeeding in their efforts to design strategies to come up with novel DNA-reactive compounds for chemotherapy as well as other purposes.

Returning to the historical thread, we go back to the year 1961, which was when the first reasonably explicit model for binding of a drug to the double helix – the intercalation hypothesis – was proposed for the interaction of aminoacridines like proflavine with DNA [1]. It is no secret, though not often appreciated outside laboratories of molecular biology, that the notion of frameshift mutation furnished a degree of inspiration for the model. However, the idea of intercalation was not universally acclaimed: indeed it was greeted with profound skepticism in certain quarters. Lerman's original evidence, drawn from observations of changes in viscosity, sedimentation coefficient, and X-ray diffraction from oriented fibers of DNA, was perfectly reasonable so far as it went. But that was not far enough to satisfy many of the “real” structure solvers, who made it clear that they were not going to believe the postulate unless and until it had been verified by their own favorite “direct” technique as opposed to the admittedly rather indirect evidence adduced by Lerman. One of the present authors remembers conversations including such phrases as “do you believe in intercalation?”, as if it were an article of faith akin to religion. In due course, experiments were devised to verify or disprove the hypothesis, eventually to the satisfaction (or conversion) of the most hardened skeptics.

One early experiment was the circular DNA unwinding test, based upon the generally (but not quite universally) agreed expectation that intercalation must locally unwind the double helix [10]. It worked, and confirmed ethidium together with aminoacridines and several other interesting ligands, including actinomycin, as intercalators [11]. By the same token, antibiotics like netropsin and distamycin were identified as something else: minor groove binders as we now know [11]. It also became clear that different drugs unwind the helix by different angles when they intercalate, and the test even unearthed certain ligands that could unwind the double helix somewhat without apparently intercalating in the usual sense: steroidal diamines and triphenylmethane dyes [12, 13]. Questions still remain to be answered about these ligands. Of course a legacy of this early work is the detailed understanding of higher order structure, especially circularity, of DNA which studies on drug interactions have helped to elucidate. Thirty-five years after it was first shown to unwind circular DNA, ethidium is still routinely used to isolate plasmids.

Perhaps because of the seminal contributions of physical (bio)chemists during the early years of probing mechanisms of drug–nucleic acid interaction, the study of reaction kinetics soon emerged as a powerful tool for throwing light on the forces involved [14]. Don Crothers, an influential advocate of the kinetic approach, used to remark that the study of kinetics was uniquely valuable, if only because it added a new dimension – time – to the analysis of the phenomena. He was absolutely right. A highlight was the discovery that some ligands which bound well but not outrageously tightly to DNA could be characterized by on-rates and off-rates many orders of magnitude slower than ostensibly comparable substances. The anthracycline antibiotic nogalamycin is a good case in point; its slow association and dissociation kinetics are attributable to the disruption of base pairing needed to “thread” its bulky sugar substituents through the double helix [15]. Slow dissociation kinetics have been correlated with improved biological activity, and underlie the success of Phillips’ relatively recent assay for transcription termination at particular drug-binding sites on DNA [16]. Direct ligand transfer between binding sites on DNA without involving complete dissociation from the polymer was evidenced many years ago and has given rise to the “shuffling” concept whereby ligands are supposed to migrate one-dimensionally along a DNA molecule in search of better (tighter) binding sites [17, 18].

1.3

Specificity of Nucleotide Sequence Recognition

Although the value of DNA and, to a lesser extent, RNA as a target for selective drug action had been evident from the outset, it also quickly became apparent that few known drugs showed much, if any, selectivity for binding to particular nucleotide sequences. Yet the holy grail of selectively suppressing gene expression was conceived early on, together with the realization that to attain this end it would be necessary to recognize moderately long stretches of base pairs. Eventually it was

calculated that one might need to recognize a sequence composed of a number of base pairs in the high teens in order to identify a single targeted site in the human genome. The first experiments aimed at examining drug-binding preferences were crude and laborious to say the least, consisting of little more than attempts to detect different levels of binding to nucleic acids from different sources. Scatchard plots were employed to determine affinity constants, together with the frequency of binding sites, initially by simple and inappropriate means that were eventually much improved by better theoretical treatments like those of McGhee and von Hippel [19]. Sometimes the available methods (spectroscopy, equilibrium dialysis, etc.) were simply inapplicable because of the poor aqueous solubility of the ligands under investigation and alternative techniques had to be devised, such as solvent partition analysis used for the quinoxaline antibiotics [20]. Much effort was required just to establish a preference for, say, GC-rich DNA. Then the steady development of chemical methods for polynucleotide synthesis began to extend the range of synthetic, defined sequences available to the investigator and furnished substrates that could be used to examine whether or not a particular sequence would support interaction with a drug of interest.

A quantum leap occurred in the early 1980s with the invention of footprinting methodology in several laboratories at much the same time, using enzymes or Dervan's cleverly designed synthetic reagent MPE-Fe(II) to cut a cloned radio-labeled DNA fragment [21–23]. At a stroke it became possible to identify exactly where the preferred binding sites for a ligand were on a substrate that amounted to a real gene or a chosen fragment of a known gene. Although only semiquantitative at first, methods were quickly developed to adapt the technology to provide passable binding constants so that a true thermodynamic comparison of ligand affinity and capacity to discriminate between different sites could be gained in a single experiment or series of experiments. The power of the footprinting technique can be gauged from the reports of sequence-selectivity to be found in several chapters of this book. With its application, a substantial database of binding affinities for different sequences has been amassed, so that it is now becoming possible to enquire about general mechanisms that underlie the recognition of particular base-pair sequences, such as whether binding occurs predominantly in the major groove, the minor groove (much the most common with small molecules), or occasionally both.

Some workers have focused attention on the distinction between “digital” and “analog” readouts of sequence information, based on the notion that micro-structural variation in the exact parameters of the double helix (groove width, for example) can sensitively reflect nucleotide sequence heterogeneity and therefore afford a means of sequence recognition that is independent of direct, specific contacts with the base pairs themselves. One of the techniques that can throw light on such questions involves looking at the behavior of DNA molecules containing unnatural nucleotide substitutions, which have the effect of shifting, removing, or adding specific base substituents. Such experiments have amply confirmed the dominant role of the 2-amino group of guanine in directing many ligands to their preferred binding sites, and have also thrown light upon related questions like the

role of base pair substituents in modulating groove width, reactivity towards alkylating agents, helix curvature or flexibility, and the sequence-dependent winding of DNA around the histone octamer in nucleosome core particles [24].

While footprinting and related gel methodology continues to play a major role in studies of this sort it has recently been joined by the elegant but simple method of competition dialysis, whereby the relative binding of a test ligand to many different types of nucleic acids can be assessed at the same time [25]. This method is of particular interest for investigating structure-specific binding of drugs to nucleic acids or indeed other polymers, whether natural or synthetic.

1.4

Details at the Atomic and Molecular Levels

Insight into the structure and dynamics of intercalation complexes has progressed by a close synergy between theoretical and experimental approaches, which today has developed to the point at which it is now possible to give a complete molecular description of what a drug–DNA complex looks like at the atomic level, and how its constituent atoms move in solution. The techniques that have proved invaluable in this quest are X-ray crystallography, NMR spectroscopy, quantum chemistry, molecular mechanics, and molecular dynamics. Lerman himself used X-ray fiber diffraction data from proflavine–DNA complexes as part of the initial evidence he marshaled for the intercalation hypothesis [1], and Fuller and Waring adopted the technique to produce the first molecular model of the ethidium–DNA complex [26].

These early attempts at model building took an important step forward at the beginning of the 1970s when Sobell and colleagues solved the crystal structure of a 2:1 actinomycin–deoxyguanosine complex, which enabled them to construct a fairly precise intercalation model based upon purely geometrical constraints [27]. Later in the 1970s the commercial availability of DNA and RNA dinucleoside monophosphates made possible crystallographic and NMR studies of intercalated mini-duplexes of ethidium and aminoacridines such as 9-aminoacridine, proflavine, and acridine orange. The crystallographic studies by Sobell, Neidle, Rich, and their colleagues provided the first truly atomic description of intercalation complexes, and unequivocally proved that the DNA duplex could indeed stretch so as to sandwich acridine and phenanthridine chromophores between two base pairs [28–30].

These were seminal studies that not only provided insight into the fine details of individual drug–DNA complexes, but also revealed modifications to the geometry of the sugar–phosphate backbone generally required to open the intercalation cavity. Armed with the latter information, Neidle and others were able to construct molecular models of suitably modified B- and A-DNA duplexes containing stereochemically sound intercalation cavities [31, 32]. This provided the means for many investigators, Neidle, Pullman, and Hopfinger prominent amongst them, to use