

Contents

1	Introduction	1
1.1	Ribosomal Antibiotics: Function, Activity and Selectivity	3
1.2	Pleuromutilins: A Class of PTC Ribosomal Antibiotics for Clinical Use	4
1.3	Structural Basis for Cross-Resistance Between Ribosomal PTC Antibiotics.	6
1.4	Oligonucleotides as Ribosomal Inhibitors and as Tools for Structural and Functional Study.	8
1.5	Minimal Ribosomal Components with PTC Structure and Function	10
	References	12
2	Methods	19
2.1	Structural Study of Pleuromutilin Antibiotics	19
2.1.1	Crystallization and Data Collection	19
2.1.2	Data Processing, Structure Solution and Refinement	19
2.2	Comparative Structural Analysis to Reveal the Structural Basis for Cross-Resistance Between PTC Antibiotics.	20
2.3	Antisense Oligonucleotides for Targeting Functional Ribosomal Centers.	22
2.3.1	Database Construction	22
2.3.2	In Vitro Transcription–Translation System for Ribosome Activity Assay.	24
2.3.3	Antisense Oligonucleotides Nomenclature	25
2.4	Minimal Ribosomal Components with PTC Structure and Function.	25
2.4.1	In Vitro RNA Transcription	25
2.4.2	Study of Dimerization Tendency.	25
2.4.3	Electrophoresis Mobility Shift Assay.	26

2.4.4	Size Exclusion Chromatography for the Separation Between Dimer and Monomer	26
2.4.5	Radiolabeling of Substrates for Peptidyl Transferase Activity Assay	27
2.4.6	Assay for Peptidyl Transferase Activity	27
2.4.7	RNA Two-Dimensional Structure Prediction	28
2.5	Numbering, Sequence Alignment, and Images	28
	References	28
3	Results.	31
3.1	The Structures of D50S/Pleuromutilins Complexes	31
3.2	Structural Basis for Cross Resistance Between Ribosomal PTC Antibiotics.	35
3.3	Oligonucleotides as Ribosomal Inhibitors and as Tools for Structural and Functional Study.	38
3.3.1	Correlation between IC ₅₀ and Various ODN Parameters.	38
3.3.2	Effect of ODN Length.	39
3.4	Minimal Ribosomal Components with PTC Structure and Function	40
3.4.1	Construct Design	40
3.4.2	Study of Dimerization Tendency.	46
3.4.3	Functional Characterization: Assay for Peptidyl Transferase Activity	51
	References	52
4	Discussion	55
4.1	Pleuromutilins.	55
4.1.1	Induced-Fit Mechanism for Pleuromutilin Binding	55
4.1.2	C14 Extension is Located in the PTC Void	56
4.1.3	Pleuromutilins Resistance.	57
4.1.4	Pleuromutilins Selectivity Acquired by Remote Interactions	58
4.2	Structural Basis for Cross-resistance Between Ribosomal PTC Antibiotics.	58
4.2.1	Resistance to PTC Antibiotics is Frequently Acquired by Mutating Remote Nucleotides	58
4.2.2	U2504 at the Crossroad of Remote Mutations Networks that Hamper Binding of PTC Antibiotics	59
4.2.3	Second Layer Nucleotides	61
4.2.4	Third Layer Nucleotides	62
4.2.5	Resistance to Various PTC Antibiotics Mediated by the Same Nucleotides	64

4.3 Oligonucleotides as Ribosomal Inhibitors and as Tools for
Structural and Functional Studies 66

4.4 Minimal Ribosomal Components with PTC Structure
and Function. 67

References 71