

Synthesis, Molecular Docking Analysis and Evaluation of Antibacterial and Antioxidant Activities of Quinoline Analogs



Digafie Zeleke Koricho

A Dissertation Submitted to

The Department of Applied Chemistry

School of Applied Natural Science

**Presented in Partial Fulfillment of the Requirement for the Degree of
Doctor of Philosophy in Applied Chemistry (Organic Synthesis)**

Office of Graduate Studies

Adama Science and Technology University

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Adama, Ethiopia

Synthesis, Molecular Docking Analysis and Evaluation of Antibacterial and Antioxidant Activities of Quinoline Analogs

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Declaration

I hereby declare that this Dissertation entitled “Synthesis, Molecular Docking Analysis and Evaluation of Antibacterial and Antioxidant Activities of Quinoline Analogs” is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this thesis have been duly acknowledged through appropriate citations.

Digafie Zeleke



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We, the supervisor(s) of this dissertation, hereby certify that I/we have read and revised the dissertation entitled “Synthesis, Molecular Docking Analysis and Evaluation of Antibacterial and Antioxidant Activities of Quinoline Analogs” prepared under our guidance by Digafie Zeleke submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Philosophy in Applied Chemistry (Organic Synthesis). Therefore, we recommend the submission of the dissertation to the department for further review and defense.

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Table of Contents

ACKNOWLEDGEMENTS	v
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF SCHEMES.....	xiii
LIST OF APPENDIXES.....	xv
ABBREVIATIONS	xx
CHAPTER ONE	1
1. INTRODUCTION	1
1.1 Background of the study.....	1
1.2 Statement of the problem	3
1.3 Objectives.....	4
1.3.1 General objective	4
1.3.2 Specific objectives	5
1.4 Significance of the study	5
1.5 Delimitations of the study	5
CHAPTER TWO	7
2. LITERATURE REVIEW.....	7
2.1 Bioactive molecules	7
2.2 The chemistry of quinoline.....	8
2.2.1 Electrophilic reactions of quinoline	9
2.2.2 Substitution of leaving groups	10
2.2.3 Substitution of hydrogen.....	10
2.3 Synthesis and bioactivity of quinolines.....	11
2.3.1 The classical methods of quinoline synthesis	11
2.3.1.1 The Skraup reaction.....	11
2.3.2 Bioactivities of quinolines	22
2.4 Synthesis and bioactivities activities of quinolones	24
2.4.1 The general synthesis methods of fluoroquinolones.....	29
2.4.2 The antibacterial activities of fluoroquinolones.....	31
2.4.3 The anticancer properties of fluoroquinolones	33
2.5 Synthesis of 2-chloroquinoline-3-carbaldehydes and its analog using Vilsmeier-Haack reagent.....	34

2.6	Schiff bases.....	36
2.6.1	The mechanism of Schiff base formation	37
2.7	The role of hybrid molecules in improving bioactivity	38
2.8	Stilbenes: their Chemistry and application.....	39
2.8.1	The Synthesis of stilbene analogs	40
2.8.2	The biological activities of stilbenes.....	42
2.9	Radical scavenging activity	43
2.9.1	DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity.....	44
2.10	<i>In-silico</i> drug-likeness predictions.....	45
2.11	Molecular docking	46
3.	MATERIALS AND METHODS.....	48
3.1	General	48
3.2	Lists of chemicals, reagents and apparatus.....	49
3.3	Antibacterial activity assay.....	50
3.4	DPPH radical scavenging activity	50
3.5	<i>In-silico</i> Drug-Likeness and toxicity predictions	51
3.6	Molecular docking analyses of synthetic compounds against <i>E. coli gyrase</i> and <i>S. aureus topoisomerase IV</i>	51
3.7	Molecular docking analysis against human <i>topoisomerase IIα</i>	52
3.8	Synthesis of some novel quinoline derivatives	53
3.9	Synthesis of the stilbenes and pinacol of quinolines	59
3.9.1	Synthesis of (Z)-2-chloro-3-styrylquinoline (211)	59
3.9.2	Synthesis of (Z)-3-styrylquinolin-2(1H)-one (213)	59
3.9.3	Synthesis of (Z)-2-methoxy-3-styrylquinoline (214).....	60
3.9.4	Synthesis of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215).....	61
3.9.5	Synthesis of (Z)-2-chloro-8-methyl-3-styrylquinoline (216).....	61
3.9.6	Synthesis of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)	62
3.9.7	Synthesis of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (219)	62
3.9.8	Synthesis of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222).....	63
3.9.9	Synthesis of methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224).....	64

3.10	Synthesis of Novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs	65
3.10.1	Synthesis	65
3.10.1.1	Synthesis of 2-phenylquinoline-4-carboxylic acid (62)	65
3.10.1.2	Synthesis of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225)	66
CHAPTER FOUR.....		71
4.	RESULTS AND DISCUSSION	71
4.1	Synthesis and antibacterial, antioxidant, and molecular docking analysis of some novel quinoline derivatives	71
4.1.1	Synthesis of Quinoline-3-carbaldehyde and its derivatives	71
4.1.2	Synthesis of quinoline-stilbenes and pinacol of quinolines	73
4.2	Antibacterial activities synthesized compounds.....	82
4.2.1	Antibacterial activity of quinoline-3-carbaldehyde derivatives	83
4.2.2	Antibacterial activities of quinoline-stilbenes and pinacol of quinolines	85
4.2.3	Antibacterial activities of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs	88
4.3	Radical scavenging activities of compounds.....	91
4.3.1	Radical scavenging activity of quinoline-3-carbaldehyde derivatives	92
4.3.2	The Radical Scavenging Activities of quinoline-stilbenes and pinacol of quinolines	94
4.4	Molecular docking value of compounds (199 , 227 and 235) against human peroxidoxin 5 (PDB ID:1HD2)	98
4.5	In silico Pharmacokinetics (Drug-likeness) study	99
4.5.1	In silico Pharmacokinetics (Drug-likeness) study of quinoline-stilbenes and pinacol of quinolones	100
4.6	In silico molecular docking study	103
4.6.1	In silico molecular docking study of quinoline-3-carbaldehyde derivatives against <i>E. coli</i> DNA gyrase B	103
4.6.2	In silico molecular docking study of quinoline-stilbenes and pinacol of quinolones <i>E. coli</i> DNA gyrase A.....	107
4.6.3	In silico molecular docking evaluation of quinoline-stilbenes and pinacol of quinolones against human <i>Topoisomerase IIα</i> (PDB ID: 4fm9).....	110
4.6.4	In silico Molecular docking study of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against <i>E-coli</i> DNA gyrase A	114

4.6.5	Molecular docking study of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against human <i>topoisomerase IIα</i>	122
5.	CONCLUSION AND RECOMMENDATIONS.....	128
5.1.	Conclusion.....	128
5.1.	Recommendations	129
	REFERENCES.....	130
	APPENDIXES	146

LIST OF TABLES

Table 1. The four generation fluoroquinolones.....	29
Table 2. The inhibition zone of quinoline-3-carbaldehyde derivatives in mm (mean $\pm$ SD). .	84
Table 3. Antibacterial activities of quinoline-stilbenes and pinacol of quinolines	86
Table 4. The antibacterial activities of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs	89
Table 5. Percentage inhibition of quinoline-3-carbaldehyde and its derivatives and ascorbic acid	92
Table 6. Percent radical scavenging activity and IC ₅₀ values of quinoline-stilbenes and pinacol of quinolines.....	95
Table 7. The % inhibition of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs	97
Table 8. Molecular docking value of compounds (199 , 227 and 235) against human peroxidoxin (PDB ID: 1HD2).....	99
Table 9. In-Silico drug-likeness predictions of quinoline-stilbenes and pinacol of quinolones computed by SwissADME.....	101
Table 10. In Silico drug-likeness predictions of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs by SwissADME	101
Table 11. Molecular docking value of quinoline-3-carbaldehyde derivatives.....	104
Table 12. Molecular docking study of quinoline-stilbenes and pinacol of quinolones against E.coli DNA gyrase A (PDB ID 1ZI0).....	109
Table 13. Molecular docking values of quinoline-stilbenes and pinacol of quinolones against Human Topoisomerase II α (PDB ID: 4fm9).....	112
Table 14. Molecular docking value of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against E.coli DNA gyrase A (PDB ID: 6F86).....	116
Table 15. Molecular docking values of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against S. aureus topoisomerase IV (PDB ID 2INR).....	117
Table 16. Molecular docking values of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against Human Topoisomerase II α (PDB ID: 4fm9).....	124

LIST OF FIGURES

Figure 1. The structure of urea and some natural products.....	1
Figure 2. The structure of quinoline scaffold.....	9
Figure 3. Chemical structures of Quinoline-based antimalarial (QBAs).....	23
Figure 4. Quinoline based anticancer agents.....	24
Figure 5. Some synthetic antibiotics	25
Figure 6. Progress in the development of fully synthetic quinolone antibacterials, 1960–1999	26
Figure 7. The chemical structure of some of first-generation quinolones.	26
Figure 8. The chemical structure of some of second generation fluoroquinolones	28
Figure 9. Structure of new generation fluoroquinolones	34
Figure 10. The structure of cis and trans Stilbene.....	39
Figure 11. The Structure of resveratrol	40
Figure 12. Antibacterial activity of resveratrol structural analogues	43
Figure 13. The inhibition zone of the synthetic compounds in mm (mean $\pm$ SD) at 500 μ g/mL.	85
Figure 14. Antibacterial activity of quinoline-stilbenes and pinacol of quinolines at 500 μ g/mL	88
Figure 15. The inhibition zone (mm) of synthetic compounds at 0.1 μ g/ μ L. The error bar indicates the standard deviation	91
Figure 16. The Percentage inhibition of free radical DPPH by the synthetic compounds.....	93
Figure 17. Percent radical scavenging activity of of stilbenes and pinacol of quinolines	95
Figure 18. The percent inhibition of the compounds and the standard.....	97
Figure 19. The binding interactions of Ciprofloxacin against E.coli DNA gyraseB (PDB ID: 6F86).	105
Figure 20. The binding interactions of compound 198 against E.coli DNA gyraseB (PDB ID: 6F86	106
Figure 21. The binding interactions of compound 203 against E.coli DNA gyraseB (PDB ID: 6F8)	107
Figure 22. The binding interactions of compound 219 against E.coli DNA gyrase A (PDB ID: 1ZI0).....	110
Figure 23. The binding interactions of compound 224 against E.coli DNA gyrase A (PDB ID: 1ZI0).....	110
Figure 24. The binding interactions of vosaroxin against Human Topoisomerase II α (PDB ID: 4fm9).	113
Figure 25. The binding interactions of 211 against Human Topoisomerase II α (PDB ID: 4fm9).	113
Figure 26. The binding interactions of 217 against Human Topoisomerase II α (PDB ID: 4fm9).	113
Figure 27. The binding interactions of 224 against Human Topoisomerase II α (PDB ID: 4fm9).	114
Figure 28. The binding interactions of 225 against E. coli DNA gyrase A (PDB ID: 6F86).	118
Figure 29. The binding interactions of 226 against E. coli DNA gyrase A (PDB ID: 6F86).	118

Figure 30. The binding interactions of 227 against E.coli DNA gyrase A (PDB ID: 6F86).	119
Figure 31. The binding interactions of 228 against E.coli DNA gyrase A (PDB ID: 6F86).	119
Figure 32. The binding interactions of 62 against E.coli DNA gyrase A (PDB ID: 6F86).	120
Figure 33. The binding interactions of 230 against E.Coli DNA gyrase A (PDB ID: 6F86).	120
Figure 34. The binding interactions of 232 against E.coli DNA gyrase A (PDB ID: 6F86).	121
Figure 35. The binding interactions of 235 against E.coli DNA gyrase A (PDB ID: 6F86).	121
Figure 36. The binding interactions of 236 against E.coli DNA gyrase A (PDB ID: 6F86).	122
Figure 37. The binding interactions of 62 against Human Topoisomerase II α (PDB ID: 4fm9).	125
Figure 38. The binding interactions of 225 against Human Topoisomerase II α (PDB ID: 4fm9).	125
Figure 39. The binding interactions of 226 against Human Topoisomerase II α (PDB ID: 4fm9).	126
Figure 40. The binding interactions of 227 against Human Topoisomerase II α (PDB ID: 4fm9).	126
Figure 41. The binding interactions of 230 against Human Topoisomerase II α (PDB ID: 4fm9).	126
Figure 42. The binding interactions of 232 against Human Topoisomerase II α (PDB ID: 4fm9).	127
Figure 43. The binding interactions of 235 against Human Topoisomerase II α (PDB ID: 4fm9).	127
Figure 44. The binding interactions of 236 against Human Topoisomerase II α (PDB ID: 4fm9).	127

LIST OF SCHEMES

Scheme 1. Nitration and sulfonation of quinoline	10
Scheme 2. Displacement of chlorine by Ethoxide	10
Scheme 3. Substitution of Hydrogen by Hydroxyl	11
Scheme 4. The Mechanism of Skraup quinoline synthesis.....	12
Scheme 5. General Doebner-von Miller reaction	13
Scheme 6. The Mechanism of Doebner-von Miller quinoline synthesis	14
Scheme 7. General route Friedländer quinoline synthesis	15
Scheme 8. General route Friedländer reaction from an o-nitro aldehyde or ketone	16
Scheme 9. The Mechanism of Friedländer Quinoline Synthesis	17
Scheme 10. The mechanism of Pfitzinger synthesis	18
Scheme 11. Synthesis of functionalized quinaldine-4-carboxylates.....	19
Scheme 12. One-pot synthesis of quinoline-4-carboxylic acid derivatives in aqueous medium	19
Scheme 13. Doebner quinoline synthesis	20
Scheme 14. The mechanism of Doebner quinoline synthesis.....	20
Scheme 15. The general reaction of Conrad-Limpach quinoline synthesis.....	20
Scheme 16. The Proposed mechanism of Conrad-Limpach quinoline synthesis	21
Scheme 17. The general Combes quinoline synthesis	21
Scheme 18. The mechanism of Combes quinoline synthesis	22
Scheme 19. The mechanism of Gould-Jacobs quinolone synthesis.....	29
Scheme 20. Synthesis of some quinolones	30
Scheme 21. Synthesis of fluoroquinolones from fluorinated benzoyl derivatives.....	31
Scheme 22. Synthesis of 2-chloroquinoline-3-carbaldehyde (148)	35
Scheme 23. The Mechanism of 2-chloroquinoline-3-carbaldehyde synthesis via Vilsmeier reagent	36
Scheme 24. The General reaction of Schiff base	36
Scheme 25. The mechanism of Schiff base formation.....	37
Scheme 26. The betaine mechanism for the Wittig reaction	41
Scheme 27. The proposed mechanism for Wittig reactions and vinylphosphine oxide formation in alcohol	41
Scheme 28. Synthesis of stilbene analogues by (HWE) reaction in the presence and absence of PTC	42
Scheme 29. Reaction of 1,1-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant.	44
Scheme 30. Synthesis of 2-chloroquinoline-3-carbaldehyde and its derivatives.....	72
Scheme 31. Synthesis of 2-chloro-8-methylquinoline-3-carbaldehyde and its derivatives	73
Scheme 32. Synthesis of quinoine stilbenes and Schiff bases (211-215)	75
Scheme 33. Synthesis of 8-methylquinoline stilbenes (219-219)	75
Scheme 34. Synthesis of 8-methylquinoline stilbenes and pinacol of quinoline (222 and 203)	76
Scheme 35. Synthesis of [2,3'-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid (62 , 225 and 226).....	78
Scheme 36. Synthesis of [2,3'-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid (227 and 228).....	78
Scheme 37. Synthesis of 2-(o-tolylimino)propanoic acid (230)	79
Scheme 38. Synthesis of 2-chloroquinoline-3-carbaldehyde analogs (232 , 235 and 236)	80

Scheme 39. Proposed mechanism of formation of 2-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)	81
Scheme 40. Proposed mechanism of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (236)	82
Scheme 41. Proposed radical scavenging mechanism of compound 199	94

LIST OF APPENDIXES

Appendix 1. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-Chloroquinoline-3-carbaldehyde (196)	146
Appendix 2. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Chloroquinoline-3-carbaldehyde (196)	146
Appendix 3. The DEPT (100 MHz, CDCl_3) spectrum of 2-Chloroquinoline-3-carbaldehyde (196)	147
Appendix 4. The IR spectrum of 2-chloroquinoline-3-carbaldehyde (196)	147
Appendix 5. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-methoxyquinoline-3-carbaldehyde (197)	148
Appendix 6. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-methoxyquinoline-3-carbaldehyde (197)	148
Appendix 7. The DEPT (100 MHz, CDCl_3) spectrum of 2-methoxyquinoline-3-carbaldehyde (197)	149
Appendix 8. The IR spectrum of 2-methoxyquinoline-3-carbaldehyde (197)	149
Appendix 9. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-Ethoxyquinoline-3-carbaldehyde (198)	150
Appendix 10. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Ethoxyquinoline-3-carbaldehyde (198)	150
Appendix 11. DEPT (100 MHz, CDCl_3) spectrum of 2-Ethoxyquinoline-3-carbaldehyde (198)	151
Appendix 12. The FTIR spectrum of 2-Ethoxyquinoline-3-carbaldehyde (198)	151
Appendix 13. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-thiocyanatoquinoline-3-carbaldehyde (199)	152
Appendix 14. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-thiocyanatoquinoline-3-carbaldehyde (199)	152
Appendix 15. The IR spectrum of 2-thiocyanatoquinoline-3-carbaldehyde (199)	153
Appendix 16. The ^1H NMR (400 MHz, $\text{DMSO}-d_6$) spectrum of 2-Chloroquinoline-3-carboxylic Acid (200)	153
Appendix 17. The ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) spectrum of 2-Chloroquinoline-3-carboxylic Acid (200)	154
Appendix 18. The DEPT-135 (100 MHz, $\text{DMSO}-d_6$) spectrum of 2-Chloroquinoline-3-carboxylic Acid (200)	154
Appendix 19. The IR spectrum of 2-chloroquinoline-3-carboxylic acid (200)	155
Appendix 20. The ^1H NMR (400 MHz, CDCl_3) of spectrum 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (203)	155
Appendix 21. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (203)	156
Appendix 22. The DEPT-135 NMR (100 MHz, CDCl_3) spectrum of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (203)	156
Appendix 23. IR spectrum of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (203)	157
Appendix 24. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-Chloro-8-methylquinoline-3-carbaldehyde (206)	157
Appendix 25. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Chloro-8-methylquinoline-3-carbaldehyde (206)	158

Appendix 26. The DEPT-135 (100 MHz, CDCl ₃) spectrum of 2-chloro-8-methylquinoline-3-carbaldehyde (206).....	158
Appendix 27. The IR spectrum of 2-Chloro-8-methylquinoline-3-carbaldehyde (206).....	159
Appendix 28. The ¹ H NMR (400 MHz, CDCl ₃) spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (207).....	159
Appendix 29. The ¹³ C NMR (100 MHz, CDCl ₃) spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (207).....	160
Appendix 30. The DEPT-135 (100 MHz, CDCl ₃) spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (207).....	161
Appendix 31. The IR spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (207)	161
Appendix 32. The ¹ H NMR (400 MHz, CDCl ₃) spectrum of (2-methoxy-8-methylquinolin-3-yl)methanol (208).....	162
Appendix 33. The ¹³ C NMR (100 MHz, CDCl ₃) spectrum of (2-methoxy-8-methylquinolin-3-yl)methanol (208)	162
Appendix 34. The DEPT-135 (100 MHz, CDCl ₃) spectrum of (2-methoxy-8-methylquinolin-3-yl) methanol (208)	163
Appendix 35. The IR spectrum of (2-methoxy-8-methylquinolin-3-yl) methanol (208)	163
Appendix 36. The ¹ H NMR(400 MHz, CDCl ₃) spectrum of (2-Ethoxy-8-methylquinolin-3-yl)methanol (209).....	164
Appendix 37. The ¹³ C NMR (100 MHz, CDCl ₃) of (2-Ethoxy-8-methylquinolin-3-yl)methanol (209).....	164
Appendix 38. The DEPT-135(100 MHz, CDCl ₃) spectrum of (2-Ethoxy-8-methylquinolin-3-yl)methanol (209).....	165
Appendix 39. The IR spectrum of (2-Ethoxy-8-methylquinolin-3-yl)methanol (209).....	165
Appendix 40. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-2-chloro-3-styrylquinoline (211)	166
Appendix 41. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-2-chloro-3-styrylquinoline (211)	166
Appendix 42. The DEPT-135(100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-2-chloro-3-styrylquinoline (211).....	167
Appendix 43. The IR spectrum of (Z)-2-chloro-3-styrylquinoline (211)	167
Appendix 44. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-3-styrylquinolin-2(1H)-one (213)	168
Appendix 45. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-3-styrylquinolin-2(1H)-one (213)	168
Appendix 46. The DEPT-135 (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-3-styrylquinolin-2(1H)-one (213)	169
Appendix 47. The IR spectrum of (Z)-3-styrylquinolin-2(1H)-one (213).....	169
Appendix 48. ¹ H NMR (400 MHz, CDCl ₃) spectrum of (Z)-2-methoxy-3-styrylquinoline (214).....	170
Appendix 49. ¹³ C NMR (100 MHz, CDCl ₃) spectrum of (Z)-2-methoxy-3-styrylquinoline (214).....	170
Appendix 50. The DEPT-135 (100 MHz, CDCl ₃) spectrum of (Z)-2-methoxy-3-styrylquinoline (214).....	171
Appendix 51. The IR spectrum of (Z)-2-methoxy-3-styrylquinoline (214)	171

Appendix 52. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215).....	172
Appendix 53. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215).....	172
Appendix 54. The DEPT-135 (100 MHz, DMSO- <i>d</i> ₆) spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215).....	173
Appendix 55. The IR spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215).....	173
Appendix 56. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (216).....	174
Appendix 57. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (216).....	174
Appendix 58. The DEPT-135 (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (216).....	175
Appendix 59. The IR spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (216)	175
Appendix 60. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)	176
Appendix 61. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)	177
Appendix 62. The DEPT-135 (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217).....	177
Appendix 63. The IR spectrum (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)	178
Appendix 64. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (219)	178
Appendix 65. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (219)	179
Appendix 66. The DEPT-135 (100 MHz, DMSO- <i>d</i> ₆) spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (219).....	180
Appendix 67. The IR spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (219)	180
Appendix 68. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222).....	181
Appendix 69. ¹³ C NMR (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222).....	182
Appendix 70. The DEPT-135 (100 MHz, DMSO- <i>d</i> ₆) spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222).....	182
Appendix 71. The IR spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222).....	183
Appendix 72. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of Methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224).....	184
Appendix 73. ¹³ C NMR(100 MHz, DMSO- <i>d</i> ₆) spectrum of Methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224).....	187
Appendix 74. The IR spectrum of Methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224)	187
Appendix 75. The ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225).....	188

Appendix 76. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225)	188
Appendix 77. The DEPT (100 MHz, DMSO- d_6) spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225).....	189
Appendix 78. The IR spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225)	189
Appendix 79. The ^1H NMR (400 MHz, DMSO- d_6) spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (226)	190
Appendix 80. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (226)	190
Appendix 81. The DEPT (100 MHz, DMSO- d_6) spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (226)	191
Appendix 82. The IR spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (226)	191
Appendix 83. The ^1H NMR (400 MHz, DMSO- d_6) spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (227)	192
Appendix 84. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (227)	192
Appendix 85. The DEPT-135 (100 MHz, DMSO- d_6) spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (227)	193
Appendix 86. The IR spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (227)	193
Appendix 87. The ^1H NMR(400 MHz, DMSO- d_6) of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (228)	194
Appendix 88.The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (228).....	194
Appendix 89.The DEPT-135 (100 MHz, DMSO- d_6) spectrum of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (228).....	195
Appendix 90. The IR spectrum of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (228)	195
Appendix 91. The ^1H NMR spectrum (400 MHz, DMSO- d_6) of 2-phenylquinoline-4-carboxylic acid (62).....	196
Appendix 92. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2-phenylquinoline-4-carboxylic acid (62).....	196
Appendix 93.The DEPT-135 (100 MHz, DMSO- d_6) spectrum of 2-phenylquinoline-4-carboxylic acid (62).....	197
Appendix 94. The IR spectrum of 2-phenylquinoline-4-carboxylic acid (62).....	197
Appendix 95. The ^1H NMR (400 MHz, DMSO- d_6) spectrum of 2-(o-tolylimino)propanoic acid (230)	198
Appendix 96. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2-(o-tolylimino)propanoic acid (230)	198
Appendix 97. The DEPT-135 NMR (100 MHz, DMSO- d_6) spectrum of 2-(o-tolylimino)propanoic acid (230)	199
Appendix 98. The FTIR spectrum of of 2-(o-tolylimino)propanoic acid (230)	199
Appendix 99. The ^1H NMR (400 MHz, DMSO- d_6) spectrum of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (232).....	200

Appendix 100. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (232)	200
Appendix 101. The DEPT-135 Spectrum of (100 MHz, DMSO- d_6) 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (232)	201
Appendix 102. The IR spectrum of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (232)	201
Appendix 103. The ^1H NMR (400 MHz, CDCl_3) spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)	202
Appendix 104. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)	202
Appendix 105. The DEPT-135 (100 MHz, CDCl_3) spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)	203
Appendix 106. The IR spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)	203
Appendix 107. The ^1H NMR (400 MHz, CDCl_3 and CD_3OH) of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (236)	204
Appendix 108. The ^{13}C NMR (100 MHz, CDCl_3 and CD_3OH) spectrum of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (236)	204
Appendix 109. The DEPT-135 (100 MHz, CDCl_3 and CD_3OH) of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (236)	205
Appendix 110. The IR spectrum of NMR of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (236)	205

ABBREVIATIONS

AFV: Acidic food vacuoles

ADMET: absorption, distribution, metabolism, excretion, and toxicity

BBB: the blood–brain barrier

cLogP: logarithm of octanol-water partition coefficient

DABCO:1,8-diazabicyclo(2,2,2)-Octane

DBU: 1,8-diazabicyclo[5,4,0]-undec-7-ene

DES: diethylsulphate

DMF: *N,N*-dimethylformamide

EMME: diethylethoxy methylene melonate

HBA: Hydrogen bond acceptor

HBD: Hydrogen bond donor

HCQ: hydroxychloroquine

HWE: Horner-Wadsworth-Emmons

log K_p: logarithm of skin permeation value

MFQ: Mefloquine

MIC: Minimum inhibitory concentration

MTB: Mycobacterium tuberculosis

NRB: number of rotatable bonds

PPA: polyphosphoric acid

QBA: quinoline based antimalaria

QN: Quinine

QNX: Quinacrine

QSAR: quantitative structural activity relationship

SMILE: simplified molecular-input line-entry system

TPSA:topological polar surface area

TMSCl:Tetra methyl silyl chloride

USFAD:United states Food and Drug Administration

ABSTRACT

Quinoline scaffold has been used to synthesize antibacterial, antimalarial and anticancer drugs. It is easily amenable to introduce various bioisosteres on the scaffold and for developing different bioactive molecules. Thus this project was designed to synthesize novel quinoline analogs and evaluate their antibacterial and antioxidant activities. In this dissertation, 26 quinoline analogs and 2 Schiff bases were synthesized. The structures of the synthesized compound were established using spectroscopic techniques. The drug-likeness properties of eighteen of the synthesized compounds were evaluated and results showed that all the eighteen synthesized compounds satisfy Lipinski's rule of five with zero violations. All of the synthesized compounds were also screened for their antibacterial activities using paper disc diffusion against three Gram positive and three Gram negative bacteria strains. Ciprofloxacin and DMSO was used as a positive and negative control, respectively. Compound **225** (19.3 ± 3.1 mm), **227** (20.7 ± 1.5 mm) and **228** (19.7 ± 1.5 mm) were among those that showed comparable antibacterial mean inhibition zone compared to ciprofloxacin (22.0 ± 1.0 mm). The in silico molecular docking study of the synthesized compounds were evaluated against DNA gyrase for Gram negative and topoisomerase IV for Gram positive bacterial strains. Compounds **199**, **219**, **225**, **227**, **228** and **230** displayed good docking scores and were in a good agreement with the in vitro antibacterial analyses. The radical scavenging activities of the compounds were evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method. The findings of this assay indicated that some of them possess strong to moderate antioxidant properties compared to ascorbic acid. Two of them, compound **227** ($IC_{50} = 1.25$ μ g/mL) and **235** ($IC_{50} = 1.755$ μ g/mL) were stronger than ascorbic acid in radical scavenging activities presumably due to the presence of easily transferable electron with in the molecules. The molecular docking values of the two compound against human peroxidoxin 5 were also significant. The in silico anticancer properties of the compounds were evaluated by docking against human topoisomerase II α , most of them showed stronger binding energy than the reference drug (Voreloxin). Thus, compounds **211**, **213**, **216**, **217**, **224**, **225**, **226** and **228** might have better anticancer activities. Among the scaffolds developed in this study, various bioisosteres can be attached on quinoline-stilbenes and [2,3'-biquinoline]-4-carboxylic acid scaffold to improve their bioactivities. Therefore, these scaffolds may serve as leads to develop effective drug candidates if further research is being conducted on them.

Key words: Quinoline, Antibacteria, Antioxidant, DNA gyrase, and Topoisomerase II α

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Scientific discoveries and their application have changed the world has changed dramatically in the last two centuries. The advent of organic synthesis as marked by Wöhler's synthesis of urea in 1828 was one of them (Cohen and Cohen 1996). The latter part of the twentieth century showed great advances in the area of new synthetic methodology, which brought organic synthetic methodology to higher levels of elegance, practicality and efficiency (Nicolaou 2014; Nicolaou, Rigol, and Yu 2019).

The development of experimental methods for practical chemistry and the discoveries of naturally occurring substances such as urea (1), quinine (2), morphine (3) and strychnine (4) (Figure 1) in the late eighteenth and early nineteenth centuries laid the foundations and provided the impetus for the emergence of organic synthesis (Nicolaou 2014). Soon after its occurrence, the advent of organic synthesis gave birth first to the dye industry and then to the pharmaceutical industry with the synthesis and commercialization of mauve (or mauveine) (5) and acetylsalicylic acid (aspirin) (6), respectively, triggering these industrial revolutions (Figure 1) (Nicolaou and Hale 2012).

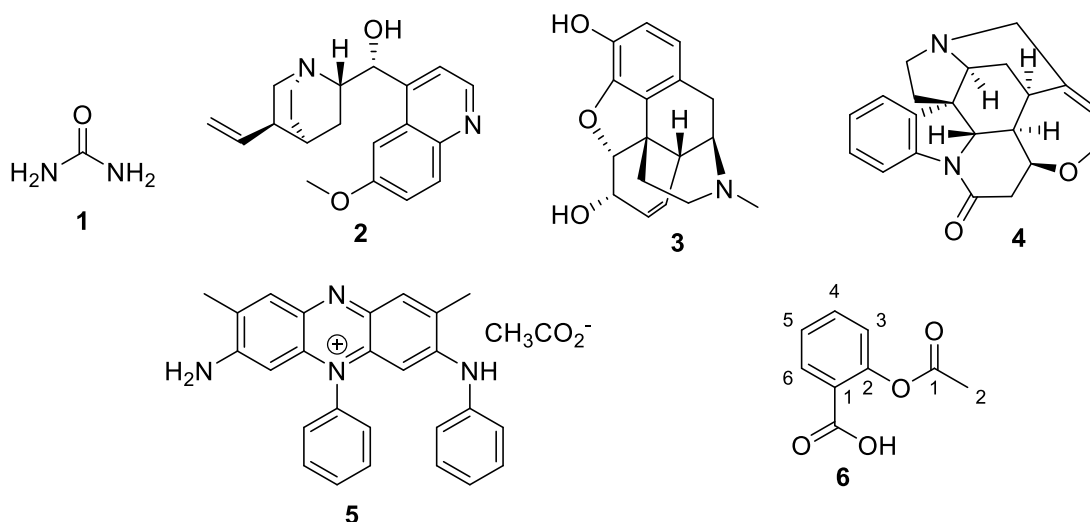


Figure 1. The structure of urea and some natural products

Even the current society, active substances are needed to control various illnesses which continually challenge mankind badly and deserves effective treatment. Active substances are small bioactive molecules which played therapeutic and preventing roles. They may be phytochemicals or artificially synthesized substances (Rotella 2016). Medicinal and organic chemists employ different synthetic methodologies in the synthesis of various effective bioactive molecules. Organic synthetic methodology has also been used in modifying and optimizing of bioactive secondary metabolites to improve their efficacy, bioavailability and to minimize their side effects (Baillie and MacCoss 2004).

Infectious diseases are caused by both parasite (Protozoa and worms) and microorganisms (viruses, bacteria, and fungi) (Piękowski 2019). A pathogenic organism is an organism which is able to causing diseases in a host (person) (Panayidou, Ioannidou, and Apidianakis 2014). Bacterial infections is the main killer of all the pathogenic microorganisms (Hu et al. 2015). According 1996 the World Health Report, infectious diseases kill over 17 million people a year (Go and Bar n.d. 1996).

The discovery of antibiotics in the early 20th century was the main corner stone in fighting infectious disease caused by pathogenic microorganisms. Antibiotics now save millions of lives every year around the world (Aminov 2010). However, the control of infectious diseases is seriously threatened by the steady increase in the number of micro-organisms that are becoming resistant to the exiting antimicrobial agents. Resistant infections lead to increased morbidity and prolonged hospital stays, as well as to prolonged periods during which individuals are infectious and can spread their infections to other individuals. The evolution of drug resistance is facilitated by a number of factors, including increasing use of antibiotics and antimalarial agents, insufficient controls on drug prescribing, inadequate compliance with treatment regimens, poor dosing, lack of infection control, increasing frequency and speed of travel, which lead to the rapid spread of resistant organisms. Insufficient awareness of patients, physicians, and/or even government authorities have been found to contribute to the increasing drug resistance (Jackson, Czaplewski, and Piddock 2018; Powers 2004; Rendon et al. 2016). Antimicrobial drug resistance mechanisms fall into four main categories: limiting uptake of a drug, modifying a drug target, inactivating a drug, and active drug efflux (C Reygaert 2018). Because of differences in their morphology, there is variations in the types of mechanisms used

by Gram negative bacteria as opposed to Gram positive bacteria. Gram negative bacteria make use of all four main mechanisms, whereas Gram positive bacteria less commonly use limiting the uptake of a drug (C Reygaert 2018).

Bacterial resistance to the existing drugs is increasing, yet drug development in this arena is decreasing due to lack of funds. Thus, there is clearly a need for new, safe, and more effective antimicrobials to combat disease due to resistant pathogens in serious and life-threatening diseases and which in turn has opened up a new area of research for medicinal and organic chemists. Besides the development of completely new agents possessing chemical characteristics that clearly differ from those of existing ones, there is another approach, molecular hybridization-involving the combination of two or more biologically active heterocyclic systems in a single molecular scaffold (File 2005; Irbas 2012; Powers 2004).

Among the pharmacologically important heterocyclic compounds, a quinoline scaffold has become an important construction motif for the development of new drugs. Quinoline and its derivatives possess many types of biological activities including antimalarial (Parhizgar 2017), antibiotic (Lam et al. 2014; Verbanac et al. 2016), anticancer (Rashad et al. 2010; Shobeiri et al. 2016), anti-inflammatory (Rizk et al. 2005), antihypertensive (Shang et al. 2018), and anti-HIV (Shruthi, Subramanian, and Eswaran 2020).

Herein an attempt was made to synthesis some analogs of quinoline using Vilsmeier-Haack, Wittig, Doebner, nucleophilic substitution, oxidation, reduction and hydrolysis reactions. *In vitro* antibacterial evaluation, DPPH antioxidant assay, and molecular docking analysis of the synthetic compounds computed and incorporated.

1.2 Statement of the problem

Life threatening systemic infections continues to be incredible main challenges in the health care. The microbial self-defense genetic mutations increased multi-drug resistant strains of pathogens causing limitless infection diseases (Marisa L. Winkler, Krisztina M. Papp-Wallace, Andrea M. Hujer et al. 2014; Momoh et al. 2019). Because of the failure of many of marketed antibiotic agents and the high incidence of microorganism resistance to them, there is an urgent demand for search for safer and more effective antimicrobial drugs. The modification of some of functional group of the existing antibiotics and developing new drug candidates are crucial strategy to mitigate infections caused by multi-drug resistant strains of pathogens. Synthetic

antibiotics based on quinolone scaffold have saved millions of lives and investigation is going on to synthesize more effective and safe analogs (Zehiroglu and Ozturk Sarikaya 2019). Quinoline remains an important scaffold to develop new drugs owing to its easily modifiable nature, several bioisosteric grouped can be introduced on it to overcome the drug resistance developed by pathogens (Zehiroglu and Ozturk Sarikaya 2019).

Besides pathogens, excess oxidative stress also affect human health. Free radicals and oxidants produced either from normal cell metabolism or from external sources (Pham-Huy 2019). Depending on their concentration in the body, free radicals be either harmful or helpful to the body. At moderate concentration, they are necessary for the maturation process of cellular structures and defense system. Phagocytes discharge free radicals to combat pathogenic microbes (Pham-Huy 2019).

However, high concentration of free radicals causes chronic and degenerative illness including autoimmune disorders, premature aging, cancer, cardiovascular and neurodegenerative diseases (Pham-Huy 2019). The human body produces normally antioxidant to counteract oxidative stress.

But there situations where our body needs external source of antioxidants to neutralize excess free radicals. Thus consuming a wide variety of antioxidant sources are important to provide the body with the complete protection against free-radical damage (Zehiroglu and Ozturk Sarikaya 2019). Compounds exhibiting antioxidant activity helps to keep more of the body's cells healthy and less susceptible to becoming cancerous. Quinoline analogs are known to have antioxidant activities and their antioxidant activity have been evaluated.

Therefore this research was designed to synthesize novel quinoline analogs that have antibacterial and antioxidant properties to solve challenges mentioned above.

1.3 Objectives

1.3.1 General objective

The general objective of this project was to synthesize novel quinoline analogs and investigate their *in vitro* antibacterial and antioxidant activities as well as to evaluate their molecular docking analysis against selected protein targets.

1.3.2 Specific objectives

- ✓ To synthesize quinoline-3-carbaldehyde, quinoline-stilbene and biquinoline-4-carboxylic acid analogs
- ✓ To characterize the synthesized compounds using spectroscopic methods including IR, UV-Vis and NMR
- ✓ To evaluate the antibacterial activities of the synthesized compounds using paper disc diffusion against three Gram negative (*E. coli*, *S. typhimurium*., *P. aeruginosa*) and three Gram positive bacterial strains (*S. aureus*, *B. subtilis*, *S. pyogenes*)
- ✓ To evaluate the radical scavenging activities of the synthetic compounds using DPPH assay
- ✓ To conduct molecular docking study of the synthetic compounds against *E. coli* DNA gyrase and *S. aureus* topoisomerase IV
- ✓ To conduct the molecular docking study of the synthesized compounds against human topoisomerase II α and Human peroxiredoxin 5

1.4 Significance of the study

Drug discovery process typically involves target identification and design of suitable drug molecules against these targets. Drug design and synthesis involves many partners both in the design, synthesis and evaluation processes. The scanning and quantifying of the biological activity needs partners from medicine and microbiological department. The design, synthesis and evaluation methodologies developed herein might be used as a references for researchers who intend to investigate on related synthetic protocols. The results of the present research might be useful in search for drug lead candidates for treating various infectious diseases, and in drug design and development antibacterial and antioxidant drugs.

1.5 Delimitations of the study

Drug development is a cumbersome process which involves huge amount of investment in terms of money and time. Research for designing and synthesizing drug molecules need sophisticated laboratory apparatuses. The design and synthesis of these bioactive molecules delimited in varieties and only paper disc diffusion was used for *in vitro* evaluation of the antimicrobial activities. Other important bioassay techniques including determination of

minimum inhibitory concentration, cytotoxicity assay, *vivo* antibacterial assay, and both *in vitro* and *vivo* anticancer assays were not included because of the following constraints

- ✓ Shortage of budget
- ✓ Shortage of laboratory apparatus and equipment
- ✓ In Ethiopia, there is no any laboratory which is able to conduct *in vitro* anticancer analysis for the time being
- ✓ Most of the required chemicals are not only expensive but also they are not available at the local market.
- ✓ There are no NMR and IR spectroscopic instruments in ASTU, and the structural establishment of the synthetic compounds were conducted at the Chemistry Department of Addis Ababa University which added financial burden and delay to get the spectra on time

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Bioactive molecules

Since ancient times, people have explored and found in nature remedies for various diseases. Traditionally, people pay a special attention to plants which attributes them the properties of living beings (soul, feeling, hearing and sight) (Segneanu et al. 2017). Plants are not the only sources of these bioactive materials; animal and other marine lives are also the source of several bioactive molecules (Kang et al. 2013). Bioactive molecules are basically those secondary metabolites exhibiting therapeutic, preventing, toxicological and immune stimulating activities (Segneanu et al. 2017). Synthetic modification of bioactive natural products greatly improved their bioactivity (Kang et al. 2013). Bioactive natural products were not enough to treat the continuous outpouring of various pathogenic infections. Aging, immunosuppression and invasive surgical procedures have increased the risk of contracting severe infections, while globalization is contributing to the rapid spread of multidrug-resistant pathogens (Monciardini et al. 2014).

However, the difficulty and high cost of isolation of novel structures and antimicrobial agents for medical uses became serious barrier in the area (Demain 1999). Meanwhile, new medicines are continually needed because of (1) the rise of resistant pathogens, (2) the evolution of new diseases (e.g., AIDS, Hanta virus, Ebola virus etc. (3) the existence of naturally drug resistant bacteria strains and (4) the toxicity of some of the current compounds (Demain 1999). Thus, improving the bioactivity of natural products by structural modifications and synthesizing new bioactive molecules continues to be the hottest area of researches for years (Barzic and Ioan 2015). Except the huge efforts, antibiotics unfortunately have not won the war against infectious microorganisms, because of the rate at which pathogenic microbes develop resistance. It is evident that the war will continue for a long time and thus the search for new drugs must not cease.

All antibiotics used in human therapy since the dawn of the antibiotics era in the early 1900s can be divided into three distinct categories according to how they ultimately manufactured on large scale. These categorizations follow (Demain 1999; Wright, Seiple, and Myers 2014);

Natural products: compounds manufactured directly by large-scale fermentation of bacteria or fungi.

Semisynthetic Compound: are manufactured by chemical synthesis using natural product as starting material.

Fully synthetic compound: Compounds that are manufactured by fully synthetic routes.

In papers the word “antibiotics and drug” are used exchangeably. Strictly speaking antibiotics are the chemicals obtained naturally or synthetically produced to act against certain bacteria that cause infections (Adeniyi Adefegha 2019). If they act against a specific bacteria only, they are termed as narrow spectrum whereas those act on a number of bacteria are termed as broad spectrum antibiotics drugs are defined as a chemical substance that are used to prevent or cure diseases in humans, animals and plants. So the term drug has a broader meaning (Adeniyi Adefegha 2019; Balaban et al. 2019). Generally, drugs act by interfering with biological processes, so no drug is completely safe.

Among drug scaffolds, the quinoline scaffold is found in the structure of many drugs and in rational drug design in medicinal chemistry for the discovery of novel bioactive molecules. Persistent efforts have been made over the years to develop novel congeners with superior biological activities and minimal potential for undesirable side effects from quinoline (Musiol 2017).

2.2 The chemistry of quinoline

Among heterocyclic compounds, quinoline (7) (Figure 2) is a privileged scaffold that appears as an important construction motif for the development of new drugs. It was discovered by Runge in 1834 as one of the many components extracted from coal tar (Ramann and Cowen 2015b). Quinoline also known as 1-aza-naphthalene or benzo[*b*]pyridine is a heterocyclic aromatic organic compound having molecular formula C_9H_7N , characterized by a double-ring structure that contains a benzene ring fused to pyridine at two adjacent carbon atoms. It is a weak tertiary base. It can form salt with acids and displays reactions similar to those of pyridine and benzene. It shows both electrophilic and nucleophilic substitution reactions. It is nontoxic to humans on oral absorption and inhalation (Marella et al. 2013). Quinoline nucleus occurs in several natural compounds such as cinchona alkaloids and pharmacologically active substances

displaying a broad range of biological activities. Quinoline (7) (Figure 2) has been found to possess antimalarial (Foley and Tilley 1998), anti-bacterial (Lucia 2012) antifungal (J. Zhang et al. 2019), anthelmintic (Shang et al. 2018), cardiotoxic (Abdelwahid et al. 2019), anticonvulsant (Muruganantham et al. 2004), anti-inflammatory (de la Guardia et al. 2018) and analgesic activities (Marella et al. 2013).

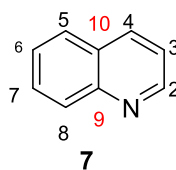
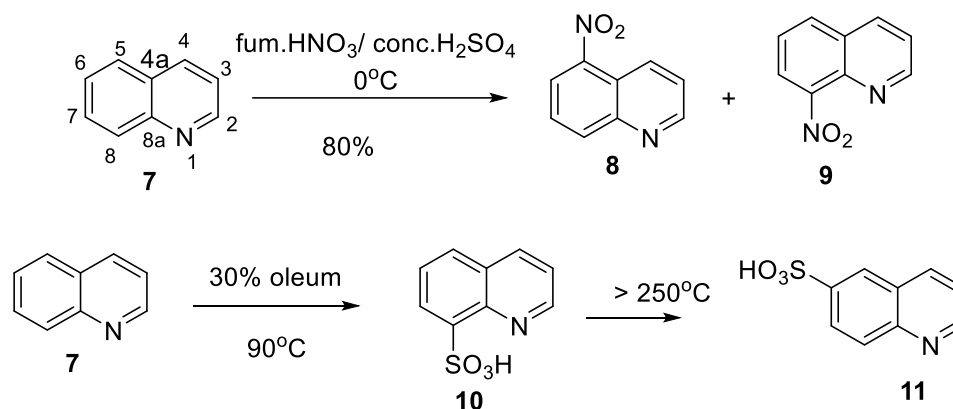


Figure 2. The structure of quinoline scaffold

2.2.1 Electrophilic reactions of quinoline

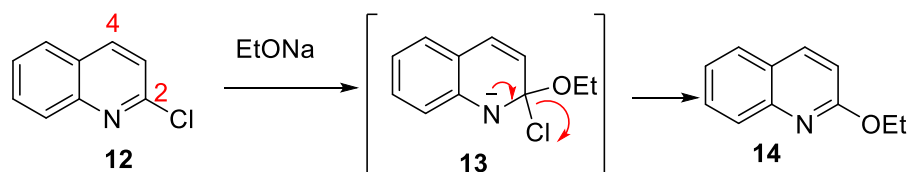
In quinolines, the benzene ring is relatively electron rich than pyridine ring and all standard electrophilic substitutions occur on it (Scheme 1). Similar to the tendency for naphthalene to be attacked most rapidly at the 1-position, such electrophilic substitutions of quinolines and isoquinolines take place only at the 5- and 8-positions. It is important to realize that these substitutions probably involve attack on quinolinium (reaction occurs via the ammonium salt). Electrophilic substitution reactions are faster than those of pyridine but slower than those of naphthalene (Joule 2010). The positional selectivity for proton exchange is partly mirrored in nitrations, quinoline gives approximately equal amounts of 5 - and 8 - nitro – quinolones whereas sulfonation of quinoline gives largely the 8-sulfonic acid, reactions at higher temperatures produce the 6-isomers, under thermodynamic control, for example both quinoline 8 - sulfonic acid and quinoline 5 - sulfonic acid are isomerised to the 6 – acid (Joule 2010). The 5/8-positin intermediates have one additional resonance contributor with disturbing the pyridine ring.



Scheme 1. Nitration and sulfonation of quinoline

2.2.2 Substitution of leaving groups

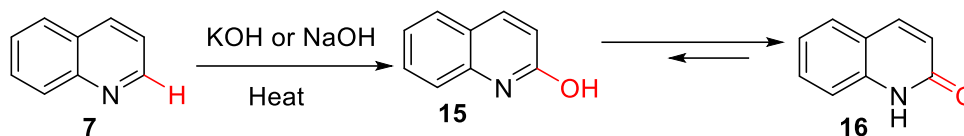
Nucleophilic displacement of leaving groups from the quinoline-2- and -4-positions occurs under quite mild conditions *via* the two-step, addition–elimination mechanism. Halogens at other positions are best regarded as being exactly like those in benzene compounds (Scheme 2) (Joule 2010).



Scheme 2. Displacement of chlorine by Ethoxide

2.2.3 Substitution of hydrogen

As the intermediates for nucleophilic addition to the heterocyclic ring still contain a complete benzene ring, with its associated resonance stabilization, nucleophilic addition is easier in these bicyclic systems than in pyridines – less resonance stabilisation is lost in proceeding to the intermediate. For example quinoline can be hydroxylated at C-2 by heating with potassium hydroxide or sodium hydroxide-no such transformation is possible with pyridines (Scheme 3). The products exist in the carbonyl tautomeric forms (Joule 2010).



Scheme 3. Substitution of Hydrogen by Hydroxyl

2.3 Synthesis and bioactivity of quinolines

Quinoline and its fused heterocyclic derivatives endowed with diverse pharmacological activities. The quinoline scaffold is widely present in several natural and synthetic molecules and endowed with a variety of therapeutic activities (Olusegun et al. 2012). Quinoline and its derivatives form an important class of heterocyclic compounds, and hence many researchers have developed various synthetic methods (Kumar, Bawa, and Gupta 2010). Quinolines are being synthesized both by classical and various new non-classical methods which involve metallic or organometallic reagents (Kumar, Bawa, and Gupta 2010). The general classical method of quinoline synthesis includes Skraup (Denmark and Venkatraman 2006; Ramann and Cowen 2015a; Wu et al. 2006), Doebner-von Miller (Denmark and Venkatraman 2006; Ramann and Cowen 2015a), Friedländer (Li et al. 2010), Pfitzinger (Knight, Porter, and Calaway 1944; Lu et al. 2017; Shvehgheimer 2004), Conrad-Limpach (Z. Wang 2010a), and Combes (Sloop 2009; Yamashkin, Yudin, and Kost 1992) synthesis.

A large number of quinoline derivatives have been prepared and showed significant biological activities (Heravi, Asadi, and Azarakhshi 2014), such as anti-malarial (Joshi and Viswanathan 2006), anti-bacterial (Nayyar et al. 2007), anti-inflammatory (Savini et al. 2001), anticancer (Chou et al. 2010), antidiabetic (Edmont et al. 2000), anti-asthmatic (Dubé et al. 1998), anti-hypertensive (Muruganantham et al. 2004), antialzheimer (Camps et al. 2001), and anti-platelet potencies (Ko et al. 2001). It has also been found that they are HIV-1 replication inhibitors, tyrosine kinase inhibitors and CysLT (LTD4) receptor antagonists (Heravi, Asadi, and Azarakhshi 2014). These compounds can also be applied as chemo- and fluorescent sensors for fluoride ions (Heravi, Asadi, and Azarakhshi 2014).

2.3.1 The classical methods of quinoline synthesis

2.3.1.1 The Skraup reaction

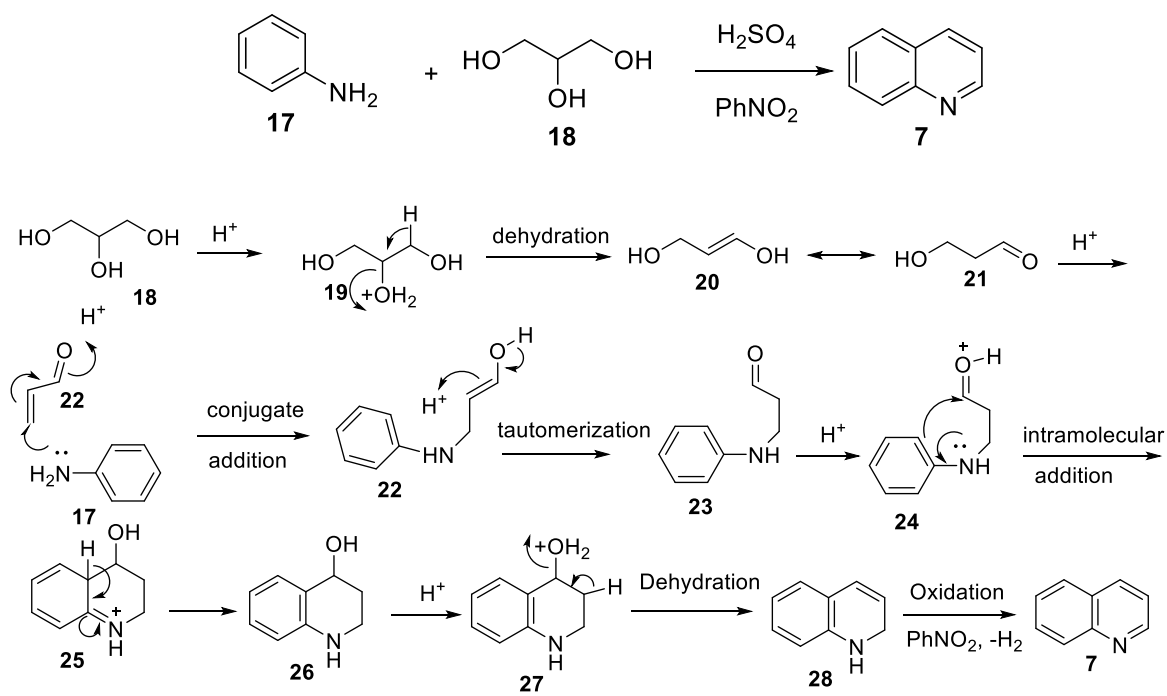
The Skraup reaction was first studied in 1880 by Zdenko Hans Skraup (1850-1910), a Czech scientist born in Prague city of Czech Republic. Skraup's synthesis is a chemical reaction used

to synthesize quinolines by the condensation of glycerine in the presence of a strong acid (conc. H_2SO_4) and an oxidizing agent like nitrobenzene (Saggadi, Luart, Thiebault, Polaert, et al. 2014). It involves heating aniline or a substituted aniline with glycerol in the presence of sulphuric acid, ferrous sulphate and nitrobenzene. This procedure has the advantage of a simple experimental procedure and the use of glycerol as major by-product of the biodiesel industry (Saggadi, Luart, Thiebault, and Polaert 2014).

The Skraup synthesis of quinoline occupies a position of interest in organic chemistry because it offers a ready method of synthesis of the quinoline group of compounds. Several modifications have been proposed since the method was inaugurated in 1880. The most important advance has been the enlargement of the yield. The ferrous sulfate modification is outstanding in this phase of the development (Eisch 1989; Ronald R. Eva, Bahama 2000).

The mechanism of Skraup reaction

The mechanism of the Skraup reaction involves initial dehydration of the glycerol to give acrolein, which undergoes a 1,4-addition by the aniline. The resulting β -anilinopropionaldehyde is then cyclized to a dihydroquinoline, which is finally oxidized to give the product (Scheme 4) (Ramann and Cowen 2015b; Ronald R. Eva, Bahama 2000).



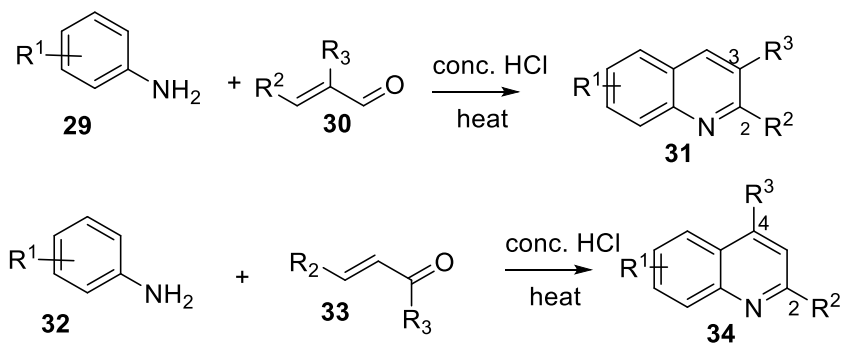
Scheme 4. The Mechanism of Skraup quinoline synthesis

2.3.1.2 Improvement of Skraup reaction

Most of classical reactions including Skraup reaction, suffer from inefficiency, harsh reaction conditions, and toxic reagents. To minimize the drawbacks of Skraup reaction, a lot of initiatives have been undertaken by several researchers. The improvements include microwave irradiation, ionic liquid media, and novel annulation partners, all of which have shown increased reaction efficiency and improved yield of the heteroring-unsubstituted quinoline products (Saggadi, Luart, Thiebault, and Polaert 2014). Similarly, modifications to other synthetic routes have been implemented, with the quinoline products displaying a wide variety of substitution patterns.

2.3.1.3 Doebner-von Miller reaction

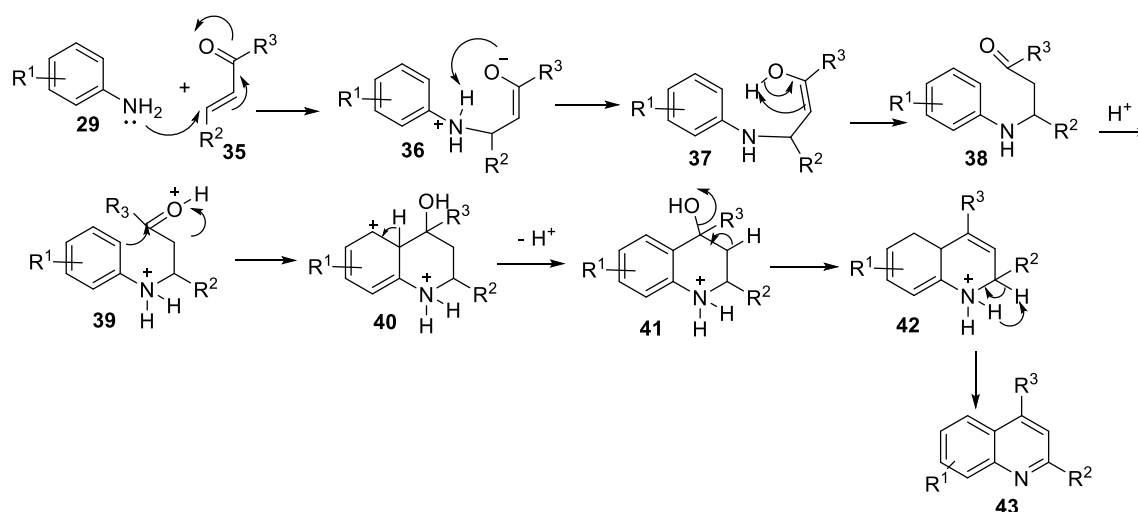
The Doebner-von Miller reaction was initially reported by Doebner and von Miller in 1881 (Weyesa and Mulugeta 2020) as a modification of the original Skraup Reaction, and it was subsequently modified by Beyer in 1886 (Mills, Harris, and Lambourne 1921; Z. Wang 2010b). It is an acidic condensation between primary aromatic amines (e.g., anilines) and α,β -unsaturated carbonyl compounds (mostly α,β -unsaturated aldehydes) to give 2,3-disubstituted quinolines (Scheme 5). The Skraup-Doebner-von Miller synthesis, which is often carried out using protic acids or Lewis acids, gives predominantly 2-substituted quinolines from the reaction of 3-substituted α,β -unsaturated carbonyl compounds. One plausible explanation for this regioselectivity is that the reaction proceeds *via* 1,4-addition of anilines to α,β -unsaturated carbonyl compounds, followed by dehydrative ring closure and oxidation *via* route I (Scheme 5) (Z. Wang 2010b).



Scheme 5. General Doebner-von Miller reaction

The mechanism of the Doebner-Miller synthesis of quinaldines has been the subject of many investigations, leading to the currently accepted mechanism which involves an aldol condensation forming an α,β -unsaturated aldehyde followed by Michael addition of the aromatic amine (Scheme 6) (Forrest, Dauphinee, and Miles 1969).

It is evident that the condensation of aniline with a substituted acrolein to yield quinoline involves the loss not only of water but of two hydrogen atoms as well (Manske 1942). Therefore, the illustrative mechanism is proposed here for the reaction between a simple aniline and acrolein using iodine as an oxidizing reagent (Scheme 6).



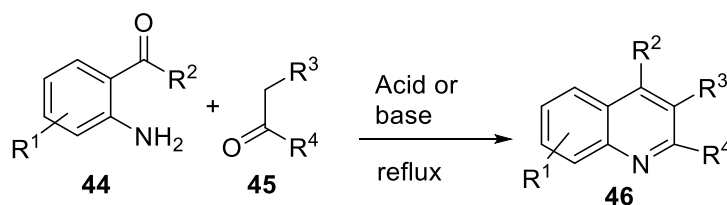
Scheme 6. The Mechanism of Doebner-von Miller quinoline synthesis

The Doebner-von Miller reaction have undergone various improvements since its discovery in 1881 (Weyesa and Mulugeta 2020). The Doebner-Miller reaction has found a great deal of synthetic utility for the preparation of substituted quinaldines (2-methylquinolines) (Weyesa and Mulugeta 2020). Unlike the closely related Skraup synthesis of quinolines, the Doebner-Miller reaction is experimentally much simpler, and not nearly as hazardous to run. However, the method does suffer from some major disadvantages (Weyesa and Mulugeta 2020). The yields reported are usually low owing to the many by-products formed in the reaction (Weyesa and Mulugeta 2020). Among the improvements, addition of an equimolar amount of zinc chloride (Weyesa and Mulugeta 2020), using the diethyl acetal of acrolein as an annulation partner (Ramann and Cowen 2015a), application of a continuous flow in water is a rapid and

green route for the synthesis (Weyesa and Mulugeta 2020) were illustrative examples of these modifications.

2.3.1.4 Friedländer quinoline synthesis

The Friedländer synthesis deals with the formation of a quinoline derivative by the condensation of an aromatic *o*-aminoaldehyde or *o*-aminoketone with an aldehyde or a ketone containing a methylene group α - to the carbonyl moiety, under basic or acidic conditions (Muchowski and Maddox 2004). Friedlander annulations are generally carried out either by refluxing an aqueous or alcoholic solution of the reactants in the presence of a base or acid or by heating a mixture of the reactants at 150-220 °C in the absence of a catalyst (Scheme 7) (Pe et al. 2009).



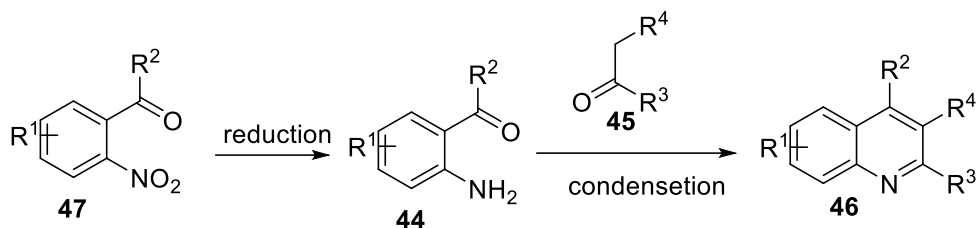
Scheme 7. General route Friedländer quinoline synthesis

The development of the heteroatom-directed *o*-metalation of aromatic systems, (Snieckus 1990) which has provided facile access to a variety of *o*-aminoaldehydes and *o*-aminoketones, has made the Friedländer reaction one of the most reliable of all quinoline syntheses. Basic reaction conditions are particularly efficient for the generation of quinoline derivatives from ketones and *o*-aminoaldehydes, whereas the condensation of ketones with *o*-aminoketones is best effected in acidic media (Muchowski and Maddox 2004).

The most notable advantage of the Friedländer synthesis is that the positions of the substituents in the homocyclic ring are fixed by the structure of the *o*-aminocarbonyl component, and the regiochemistry of the substituents in the heterocyclic ring is predictable because nonsymmetrical ketones usually give rise to the quinoline derived from the thermodynamic enolate as the major or exclusive product (Muchowski and Maddox 2004).

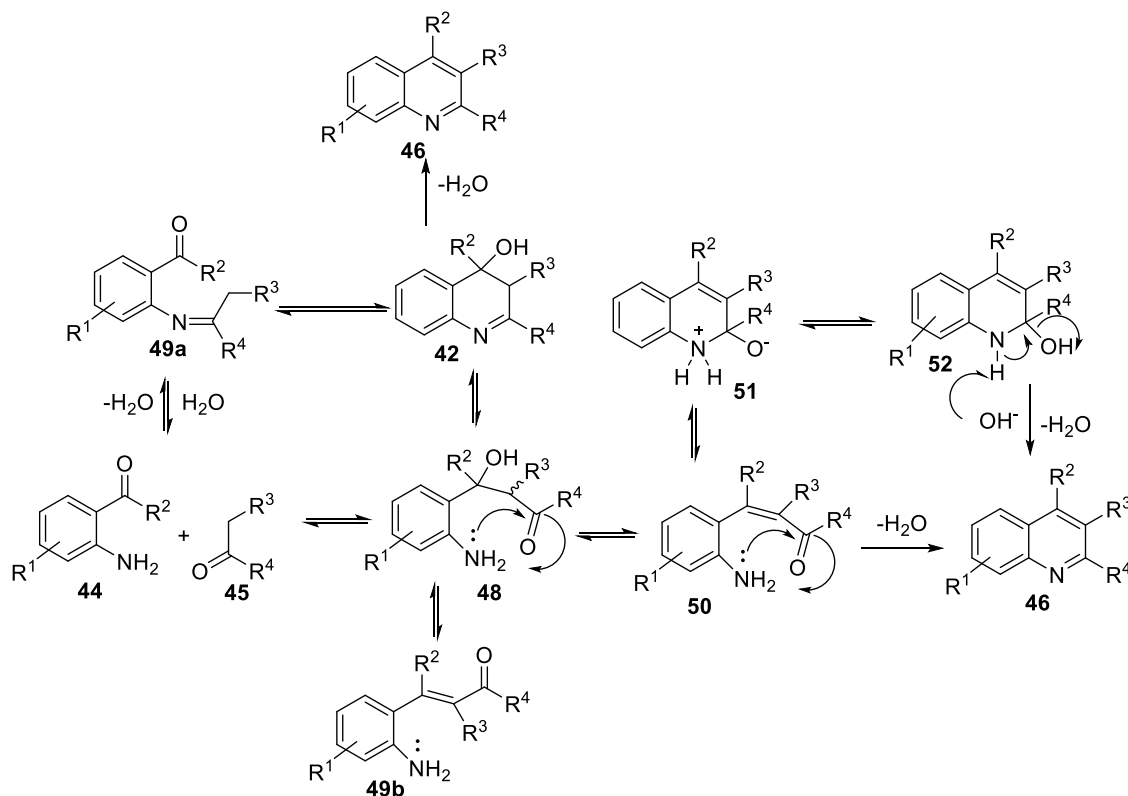
The Friedländer reaction may be achieved from *o*-nitrocarbonyl component via two steps: reduction of an *o*-nitro aldehyde or ketone 47 into an *o*-amino aldehyde or ketone 44 followed

by condensation of this intermediate with a ketone or aldehyde **45** (Scheme 8)(Heravi, Asadi, and Azarakhshi 2014)



Scheme 8. General route Friedländer reaction from an o-nitro aldehyde or ketone

Although the features of the Friedlander reaction are generally well understood, its mechanism has not been unambiguously established (Pe et al. 2009). Experimental reports support two different mechanistic routes. Starting from 2-aminosubstituted aromatic carbonyl compound **44** and the carbonyl partner **45**, one pathway involves the initial formation of the Schiff base **49a** followed by an intramolecular aldol reaction to give the hydroxy imine **50** followed by loss of water to produce the quinoline **46** (Scheme 9). Alternatively, it has been proposed that the initial rate-limiting step is an intermolecular aldol reaction to afford product **48**, which gives the quinoline via the same intermediate **51** and **52** (Scheme 9) (Muchowski and Maddox 2004; Pe et al. 2009).



Scheme 9. The Mechanism of Friedländer Quinoline Synthesis

2.3.1.5 Scope and the improvement the Friedlander reaction

The particular value of the Friedlander reaction is largely due to the broad range of functional group compatibility. Regarding the 2-amino carbonyl component, a wide range of functional groups is tolerated on the aromatic ring system. With respect to the ketone partner, a number of different types have been employed including symmetrical ketones and ketones with additional activating group alpha to the carbonyl function (Pe et al. 2009). However, the Friedlander reaction exhibited regioselectivity with unsymmetrical ketones (Pe et al. 2009).

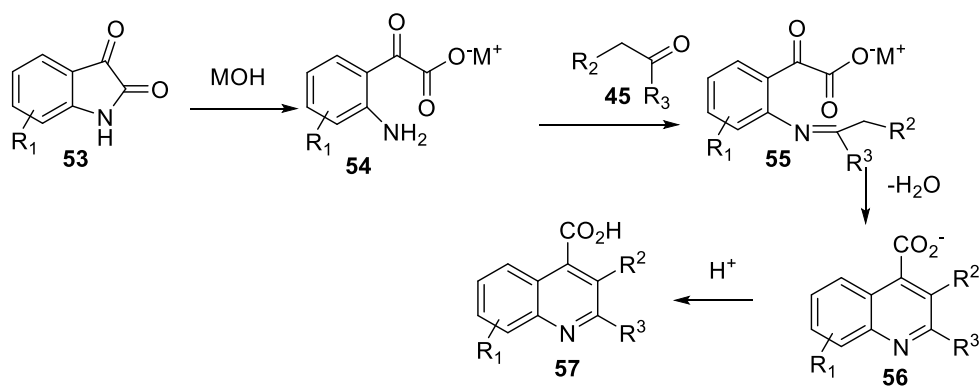
The classical Friedlander reaction has been improved and applied to 2-nitro-substituted aromatic carbonyl derivatives. This synthesis is usually carried out via a two-step procedure (Scheme 8). After the aldol-like condensation with the active methylene component, the reduction of the nitro group to the amine produces in situ the reactive intermediate that cyclizes to give the quinoline, avoiding the usual preparation and isolation of the potentially unstable 2-amino aromatic carbonyl component, which can readily undergo self-condensation reactions (Chelucci and Thummel 1999; Muchowski and Maddox 2004).

A convenient one-pot route for the Friedlander synthesis of quinolines using various starting materials (*o*-nitroarylcarbaldehydes) (Li et al. 2007, 2010) and various catalysts such as sulfonic acid functionalized ionic liquid in combinatorial approach (Akbari et al. 2010), multifunctional catalysis of a ruthenium-grafted hydrotalcite (Motokura et al. 2004) etc. are among the latest development in Friedlander synthesis of quinolines.

2.3.1.6 Pfitzinger quinoline synthesis

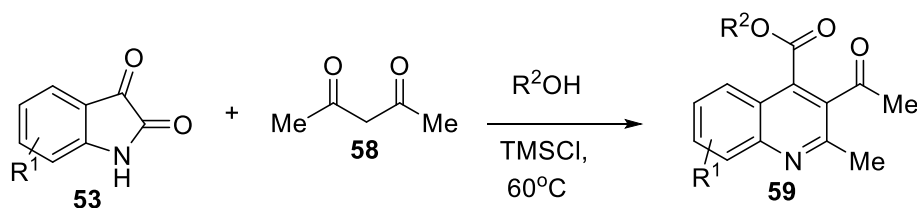
The formation of derivatives of 4-quinolinecarboxylic (cinchoninic) acid as a result of the reaction of isatin (**51**) or its derivatives with ketones containing the $-\text{CH}_2\text{CO}-$ group in the presence of sodium hydroxide or potassium hydroxide was first discovered at the end of the nineteenth century by Pfitzinger (Shvehkgeimer 2004). Pfitzinger quinoline synthesis involves isatin and its derivatives on the one hand and various ketones on the other hand and it was used to synthesize biologically active substances with good yields from readily available materials (Shvehkgeimer 2004).

The reaction of isatins with ketones leading to 4-quinolinecarboxylic acids is carried out in the presence of strong nucleophiles (sodium hydroxide or potassium hydroxide). The isatins **53** are converted by the action of alkalis into the salts of isatoic acids **54**, which condense with ketones with the release of water, forming the salts **55**. The latter undergo cyclization through the CO and CH_2 groups and are converted into the salts of 4-quinolinecarboxylic acids **56**, the treatment of which with acids (usually acetic) gives the required compounds **57** (Scheme 10) (Shvehkgeimer 2004).



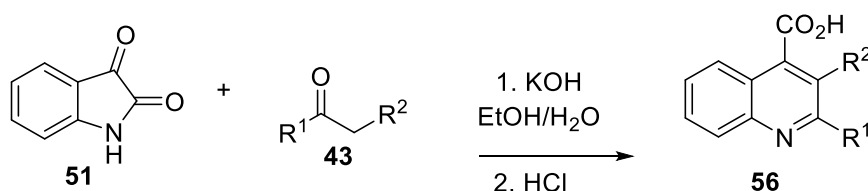
Scheme 10. The mechanism of Pfitzinger synthesis

The classic Pfitzinger chemical reaction is commonly performed in a two step process based on strong base and subsequently acidified with strong acid, which can only generate quinoline-4-carboxylic acids and is unable to obtain the quinoline-4-carboxylates (Lu et al. 2017). Therefore, selective synthesis of the quinoline-4-carboxylate motifs under mild reaction condition (strong base and acid-free) has remained a daunting challenge, especially through one-step method (Lu et al. 2017). Among the latest improvement Lu. *et al* (2017), developed an improved one-step cascade protocol Pfitzinger reaction for the synthesis of highly functionalized quinaldine-4-carboxylates, from isatins with 1,3-dicarbonyl compounds in alcohols mediated by TMSCl (Scheme 11) (Lu et al. 2017).



Scheme 11. Synthesis of functionalized quinaldine-4-carboxylates

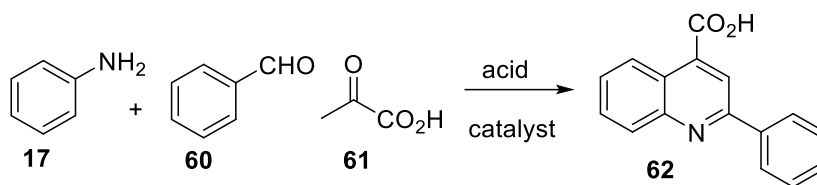
Lv *et al* (2012) reported a simple one-pot synthesis of quinoline-4-carboxylic acid derivatives by an improved Pfitzinger reaction of isatins with various ketones and catalysts in aqueous medium (Scheme 12) (Lv et al. 2013).



Scheme 12. One-pot synthesis of quinoline-4-carboxylic acid derivatives in aqueous medium

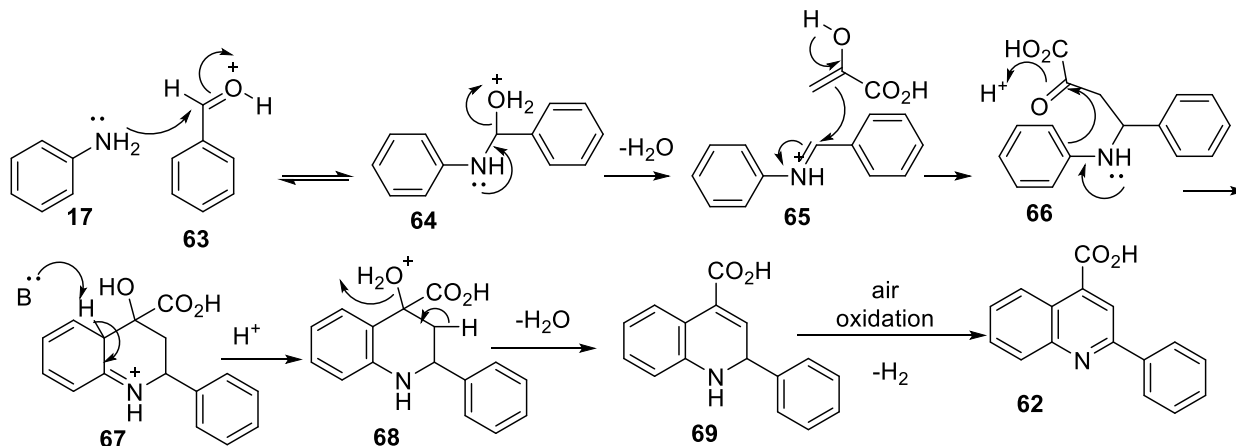
2.3.1.7 Doebner quinoline synthesis

The Doebner quinoline synthesis comprises coupling of an aniline, pyruvic acid, and an aldehyde to provide a quinoline-4-carboxylic acid (Scheme 13). Recently, there have been several reports about modified Doebner reaction for preparing diverse quinoline-4-carboxylic acids via solid-phase synthesis (L. M. Wang et al. 2009).



Scheme 13. Doebner quinoline synthesis

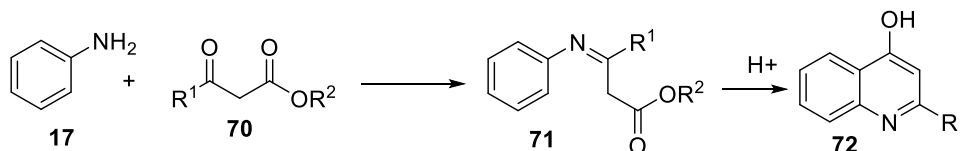
The proposed mechanism of the Doebner synthesis was shown in Scheme 14.



Scheme 14. The mechanism of Doebner quinoline synthesis

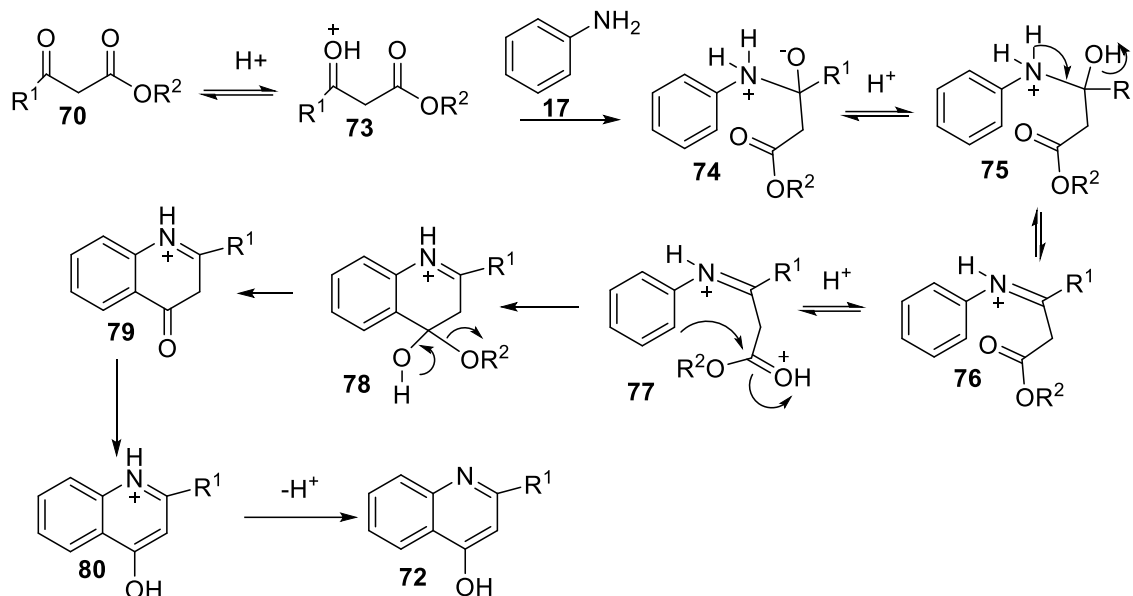
2.3.1.8 Conrad-Limpach quinoline synthesis

Conrad-Limpach quinoline synthesis dealt with the synthesis of 4-quinolones (Scheme 15) (Brouet et al. 2009; Z. Wang 2010a). In Scheme 15, the condensation of primary aromatic amines with β -ketoesters, the amino group might also react at the ester group of β -ketoesters to form β -ketoacetamide intermediates (isomerize to ketoacetanilides), which cyclize to 2-hydroxyquinolines; this type of conversion was initially discovered by Knorr in 1886 (Z. Wang 2010a).



Scheme 15. The general reaction of Conrad-Limpach quinoline synthesis

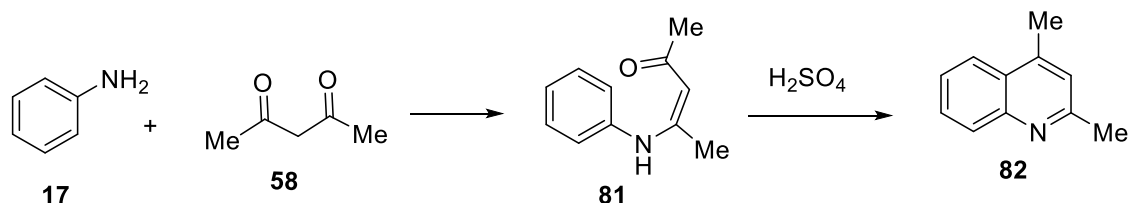
The proposed mechanisms of Conrad-Limpach quinoline synthesis was shown in Scheme 16



Scheme 16. The Proposed mechanism of Conrad-Limpach quinoline synthesis

2.3.1.9 Combes quinoline synthesis

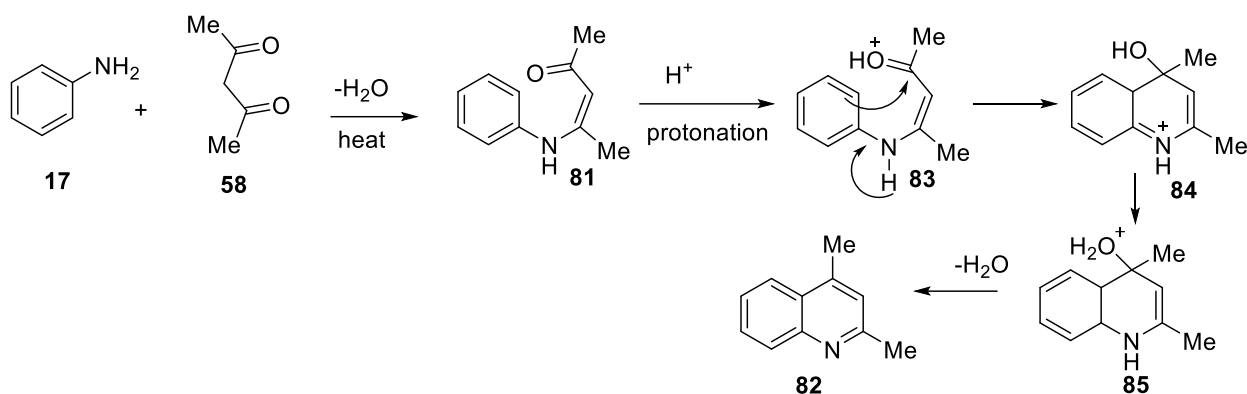
The Combes reaction consists of the condensation of aromatic amines with 1,3-diketone compounds. (Yamashkin, Yudin, and Kost 1992) The acid catalyzed condensation of aromatic amine like aniline with acetyl acetone (1,3-diketone), keto aldehyde or dialdehyde to furnish quinoline skeleton is regarded as Combes quinoline synthesis or Combes synthesis (Scheme 17) (Born 1972).



Scheme 17. The general Combes quinoline synthesis

During the reaction, enamine is formed which cyclises. The strong electron withdrawing group ($-NO_2$) does not allow cyclisation. The rearrangement is observed when ketoaldehyde is used during cyclisation. The reagents used are $ZnCl_2$, acetic acid, P_2O_5 or $POCl_3$ as well as sulphuric acid, PPA, lactic acid, chloroacetic acid, con. hydrochloric acid, p-toluenesulphonic acid, hydrofluoric acid etc (Surrey 1961).

The mechanism of the reaction involves formation of enamine by dehydration and protonation of ketone and cyclisation followed by loss of water (Scheme 18) (Born 1972; Sloop 2009).



Scheme 18. The mechanism of Combes quinoline synthesis

2.3.2 Bioactivities of quinolines

Several quinoline analogs exhibited antimicrobial potency that diverged from good to excellent (Desai, Patel, and Dave 2017). For example, Desai *et al* (2016) developed a quinoline analog that showed highest inhibition at MIC = 12.5 $\mu\text{g ml}^{-1}$ against some Gram-negative bacterium (*E. coli*) and against Gram positive bacterium (*S. aureus*) (Desai, Patel, and Dave 2017). Insuasty *et al* (2019) reported quinoline hybrids showing some degree of antifungal activity with MIC values between 15.6 and 250 $\mu\text{g/mL}$ (Insuasty *et al.* 2019). Insuasty D. *et al* (2019) reported that molecules with MICs >250 $\mu\text{g/mL}$ to be considered as inactive; in the range of 250 to 62.5 $\mu\text{g mL}^{-1}$, with moderate or low activity, and molecules with MICs $\leq 31.25 \mu\text{g mL}^{-1}$ were considered high activity (Clinical and Laboratory Standards Institute (CLSI) 2008; Insuasty *et al.* 2019).

2.3.2.1 Antimalarial properties of quinoline analogs

The discovery of quinine (2) is considered the most serendipitous medical discovery of the 17th century and malaria treatment with quinine marked the first successful use of a chemical compound to treat an infectious disease (Achan *et al.* 2011). Quinine (2) which was a component of the bark of the cinchona tree, was used to treat malaria from as early as the 1600s, when it was referred to as the “Jesuits’ bark,” “cardinal’s bark,” or “sacred bark” (Achan *et al.* 2011). It was isolated in 1820 as the active ingredient and replaced the crude bark the treatment (Wiesner *et al.* 2003). Although quinine has relatively low efficacy and tolerability,

it played a historical role in the development of quinoline alkaloids, and still plays an important role in the treatment of multi-resistant malaria (Wiesner et al. 2003).

Quinoline-based antimalarial (QBA) compounds are weak bases that accumulate in the acidic food vacuoles (AFVs) of *Plasmodium falciparum* parasites. The chemical structures of several QBAs are displayed in Figure 3. Chloroquine (**87**), the prototypical antimalarial compound, interferes with ferriprotoporphyrin IX detoxification in the AFVs of *P. falciparum*, thereby causing parasite death. In addition to AFVs, CQ has been shown to accumulate in the acidic lysosomes of tumor cells, thereby disrupting autophagy (Golden et al. 2015; Martinez, Krake, and Poggi 2018).

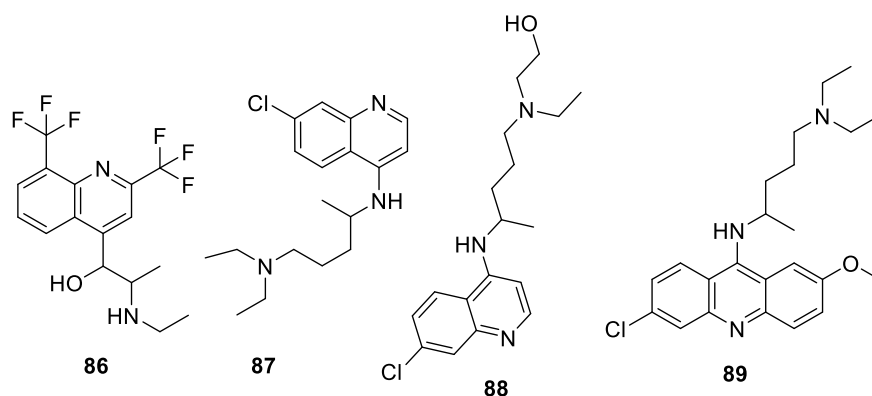


Figure 3. Chemical structures of Quinoline-based antimalarial (QBAs)

Quinoline-based antimalarial compounds, such as Chloroquine (CQ) (**87**), hydroxychloroquine (HCQ) (**88**), quinine (QN) (**2**), mefloquine (MFQ) (**86**), and quinacrine (QNX) (**89**), are mainstay therapies that effectively kill *P. falciparum* parasites. **87** a commonly prescribed QBA that harbors weakly basic properties, traverses erythrocyte and *P. falciparum* cellular membranes down a pH gradient to accumulate inside AFVs and disrupt heme detoxification, thereby causing parasite death. **88** has antimalarial activity similar to that of **87**; however, since **88** has a lower associated risk of ocular toxicity, it is clinically preferred over **87** when high doses are necessary for the treatment of malaria (Golden et al. 2015; Martinez, Krake, and Poggi 2018).

2.3.2.2 Antitumor activities of quinoline analogs

Quinoline alkaloids are important N-based heterocyclic aromatic compounds with a broad range of bioactivities. Several synthetic quinoline analogues were reported to show antibacterial

and anticancer properties. Camptothecin (**90**), topotecan (**91**), zanthosimuline (**92**), huajiaosimuline (**93**) and irinotecan (**94**) are quinoline based anticancer drugs (Figure 4). Some quinoline alkaloids were also isolated and evaluated for their cytotoxic activities. Among them, Chen and coworkers (1994) isolated two pyranoquinoline alkaloids, zanthosimuline (**92**) and huajiaosimuline (**93**) from the root bark of *Zanthoxylum simulans* (Figure 4). In cytotoxicity testing, zanthosimuline exhibited a general cytotoxic response to various cultured human cancer cell lines, such as leukaemia cells (P-388 cells) (EC_{50} 5.20 μ M). However, huajiaosimuline produced a more selective cytotoxic activity profile and was especially effective against estrogen receptor-positive breast cancer ZR-75-1 (EC_{50} 11.1 μ M) and P-388 (EC_{50} 9.80 μ M) cells. The two compounds also induced the expression of cellular markers associated with cell differentiation in cultured HL-60 cells (Garuti et al. 2000).

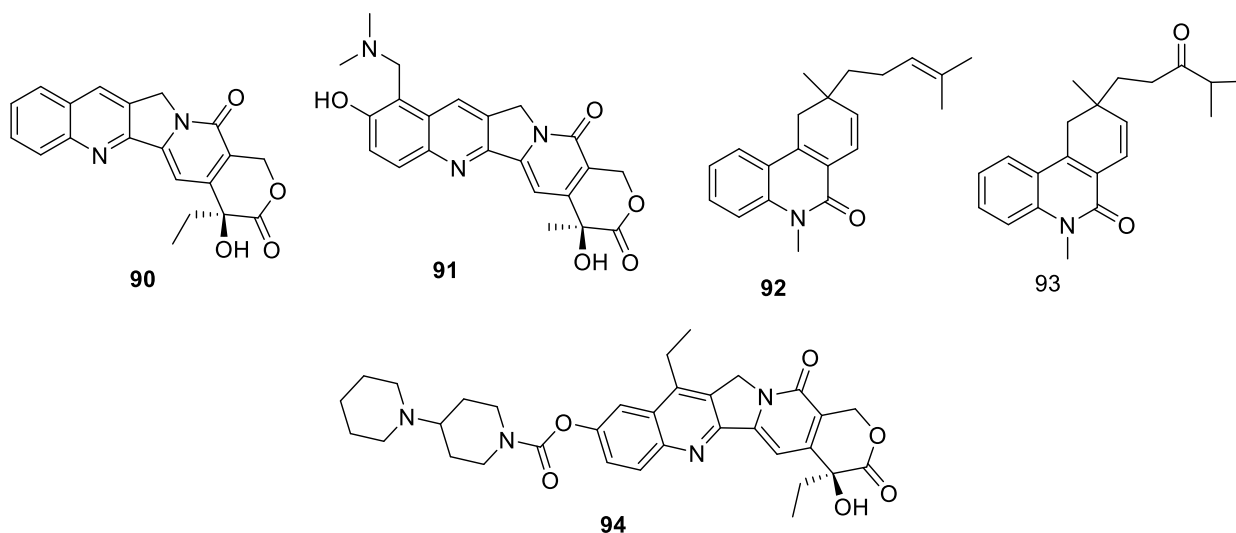


Figure 4. Quinoline based anticancer agents.

2.4 Synthesis and bioactivities activities of quinolones

The origins of the quinolone class lie in the use of **87** as an antimalarial agent. A compound isolated from the commercial preparation of chloroquine proved to have antibacterial activity and was modified to produce the first marketed quinolone, **95** (patented in 1962) (Lucia 2012), which had moderate activity against gram-negative organisms and was used for treating urinary tract infections. In 1968, Kaminsky and Melfezzer discovered an oxolinic acid, which was lately approved by the United States Food and Drug Administration (USFDA) (Sharma, Jain, and Jain 2009).

Structurally quinolone is a bicyclic structure, which contains two fused rings (A and B) with aromatic or heteroaromatic structure. The ‘A’ ring (4-pyridinone) is a ring with absolute necessity for its bioactivity: an unsaturation in position 2-3, a free acid function in position 3 and a substituent at nitrogen in position 1 (Figure 5) (Lucia 2012). **95** was developed in 1960s by George Lescher and co-workers at SterlingWinthrop Research Institute when a by-product from an earlier synthesis of the antimalarial drug **87** was included in a new screening program (Wright, Seiple, and Myers 2014). Fully synthetic approaches to antibacterial drug discovery (which began with the arsenicals and sulfa drugs) have also led to important new classes of antibiotics and large numbers of approved drugs. The most widely appreciated examples may be the quinolones, carbapenems (**96**), and oxazolidinones (**97**), but the development of these families occurred well after the discovery of four other important fully synthetic antibacterials—chloramphenicol (**98**), metronidazole (**99**), trimethoprim (**100**), and fosfomycin (**101**) (Figure 5) (Wright, Seiple, and Myers 2014).

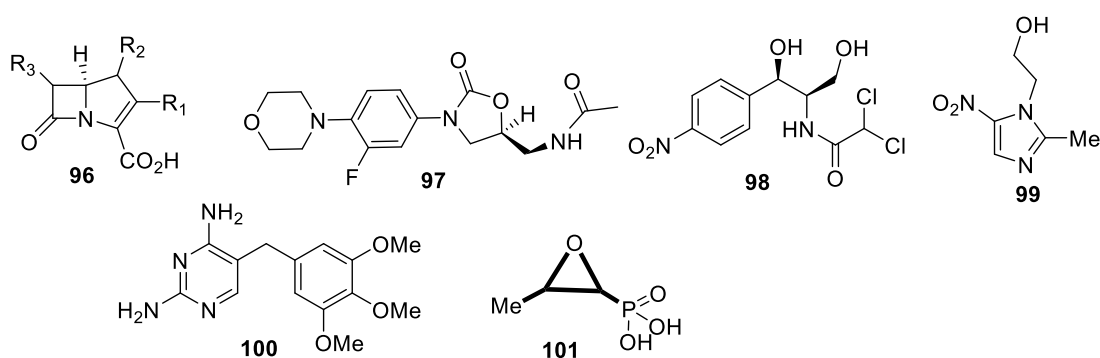


Figure 5. Some synthetic antibiotics

It has been estimated that more than 10 000 quinolones or structurally related agents have been synthesized as part of quinolone antibacterial research and development, resulting in the approval of more than 25 fully synthetic antibiotics of this class (Brighty and Gootz 2000). Fluoroquinolones are still the most widely used antibacterial agent, which are characterized by broad spectrum of antibacterial activities and excellent oral bioavailability. The enzyme DNA gyrase and topoisomerase IV were the target for the fluoroquinolones (Brighty and Gootz 2000) The general trend in the development of synthetic quinolone antibacterial is summarized below (Figure 6.)

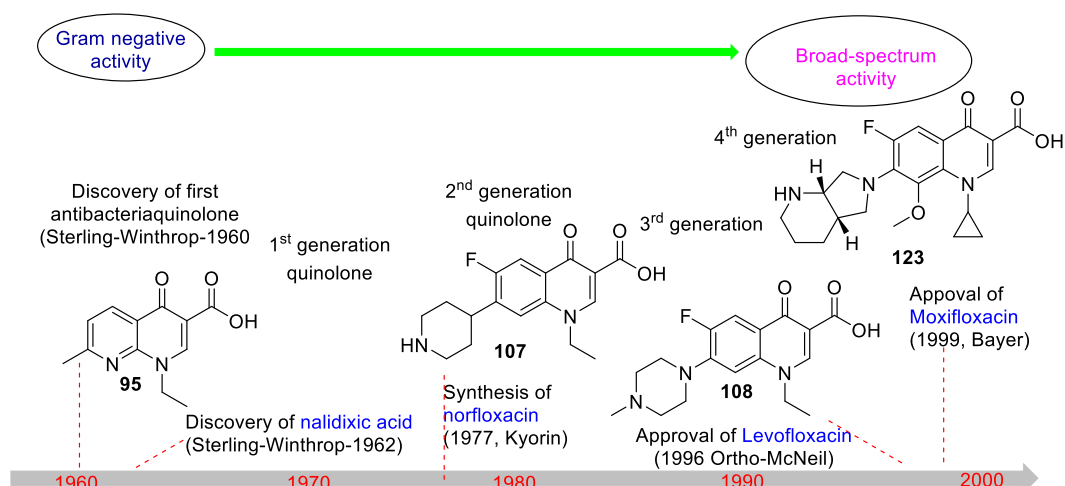


Figure 6. Progress in the development of fully synthetic quinolone antibacterials, 1960–1999

Since then, a large gamma of derivatives from common scaffold were synthesized, which could be grouped by: naphthyridine (**95**) crinoline (cinoxacin) (**102**), pyrido-pyrimidine pipemidic acid (**103**); piromidic acid (**104**) and quinolones (oxolinic acid (**105**), miloxacin (**106**) (Figure 7) etc. These derivatives, with differentiated structures, have two common pharmacological properties, which allowed them to be classified as first generation biologically active derivatives with quinolone structure. The two common characteristics for first generation quinolones are (Lucia 2012);

- a narrow antibacterial spectrum, designed especially for enterobacteriaceae;
- a pharmacokinetic which allows for rapid elimination and reduced tissue absorption, only allowing them to be used as urinary antiseptics

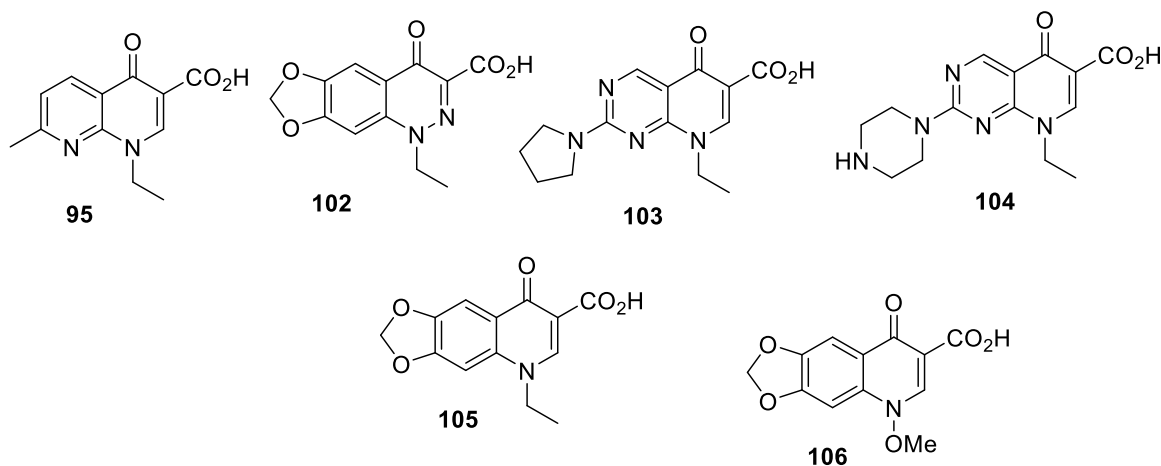


Figure 7. The chemical structure of some of first-generation quinolones.

Research continued to improve pharmacokinetics and pharmacodynamics profiles of quinolones and Flumequine was the first fluoroquinolone which was patented in 1973, after which numerous fluoroquinolone derivatives have been patented and are still used today. These are norfloxacin (**107**), pefloxacin (**108**), enoxacin (**109**), fleroxacin (**110**), ciprofloxacin (**111**) and ofloxacin (**112**) (Figure 8) (Appelbaum and Hunter 2000; Sharma, Jain, and Jain 2009). An advantage of these compounds over previous ones is their broad spectrum activities. A big revolution was made in 1980's when an analog of **95**, enoxacin (**109**) was derived with significantly increased spectrum of activity against both Gram negative or Gram positive bacteria (Sharma, Jain, and Jain 2009).

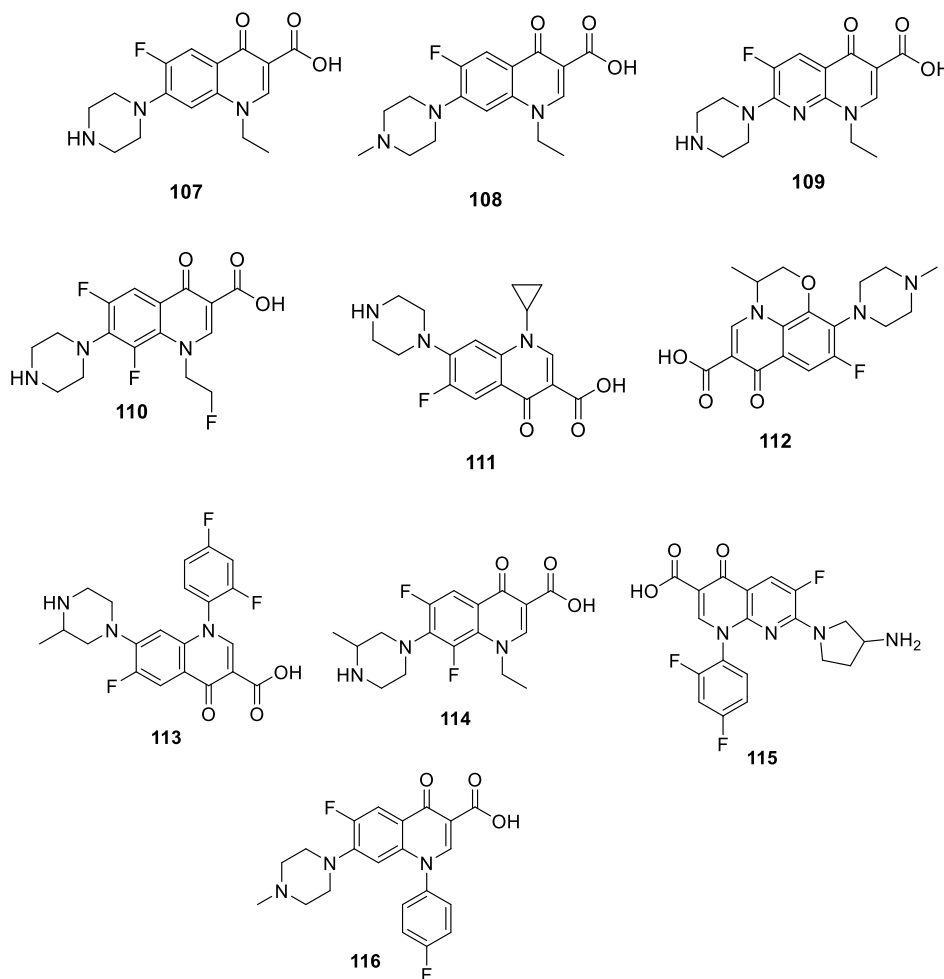


Figure 8. The chemical structure of some of second generation fluoroquinolones

All clinically important compounds of fluoroquinolone class are 6-fluorinated analogues of **95** (Moshirfar et al. 2008). The main chemical feature that distinguishes fluoroquinolones from the early quinolones is the presence of various substituents at 1st-N, carboxylic acid at 3rd C, Oxo at 4th C, fluorine at 6th-C and piperazinyl substituent at 7th -C. (Scholar 2002) Quinolones and fluoroquinolones are classified on the basis of their chemical structure, their spectrum of activity and their pharmacokinetic profiles (Table 1) (Kocsis, Domokos, and Szabo 2016; Sharma, Jain, and Jain 2009).

Recently, various third and fourth generation fluoroquinolones (FQs) have been developed and patented for use (Scoper 2008). These new generations show a more potent and broader-spectrum antibacterial activities and better pharmacokinetic properties relative to the earlier

FQs. However, some of them, exhibit moderate-to-severe toxicity which involve their restriction, suspension or even withdrawal from the market (Figure 9) (Scoper 2008).

Table 1. The four generation fluoroquinolones

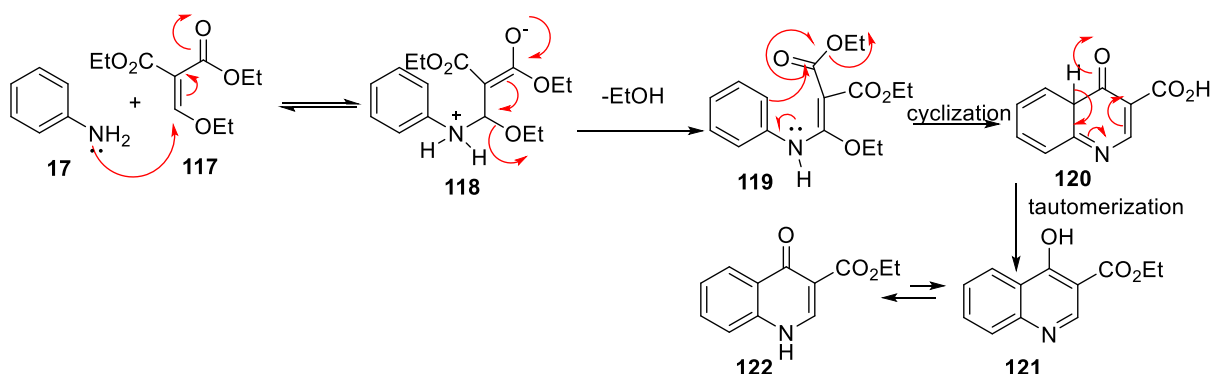
First generation	Second generation	Third generation	Fourth generation
Nalidixic Acid (95)	Norfloxacin (107)	Sparfloxacin (137)	Trovafloxacin (144)
	Fleroxacina (110)	Grepafloxacin (138)	Clinafloxacin (145)
	Ciprofloxacin (111)		Moxifloxacin (143)
	Enoxacin (108)		Gatifloxacin (142)
	Lomefloxacin (114)		
	Ofloxacin (112)		

Among all fluoroquinolones, norfloxacin and ciprofloxacin have been safely administered for various several bacteria related diseases for decades.

2.4.1 The general synthesis methods of fluoroquinolones

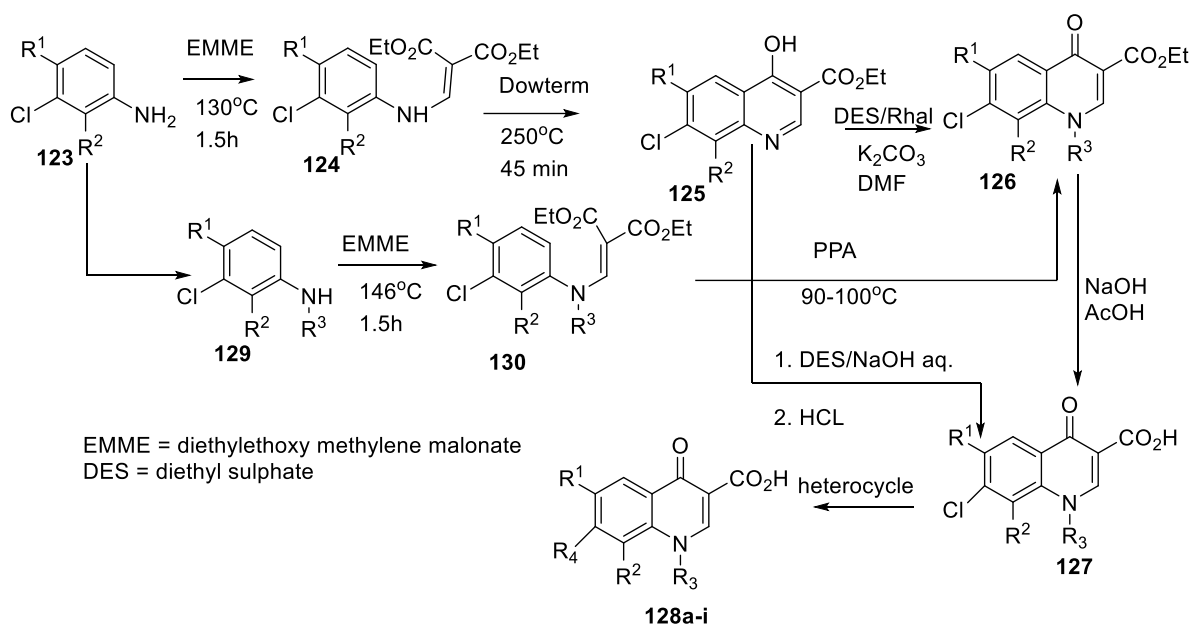
2.4.1.1 Gould-Jacobs method

Basically, there are two principal strategies for the construction of the quinolin-4-one nucleus both proceeding via enaminones intermediates, the Gould-Jacobs and the Bayer route (Charushin et al. 2014; Leyva-Ramos et al. 2017). Gould-Jacobs (1939) is most common synthetic methodology to prepare quinolone derivatives (Gould and Jacobs 1939). It involves a thermal or acid-catalysed (Gould and Jacobs 1939) cyclization of anilinomethylene malonates (Scheme 19). This cyclisation is essentially a modification of the classical Conrad-Limpach synthesis of 4-quinolones from anilines and acetoacetic esters (Conrad and Limpach 1887).



Scheme 19. The mechanism of Gould-Jacobs quinolone synthesis

To illustrate the application of the Gould-Jacobs quinolone synthesis with specific examples, nine quinolones developed by Pintilie L. (2009) are given below (Scheme 20) (Pintilie et al. 2009).

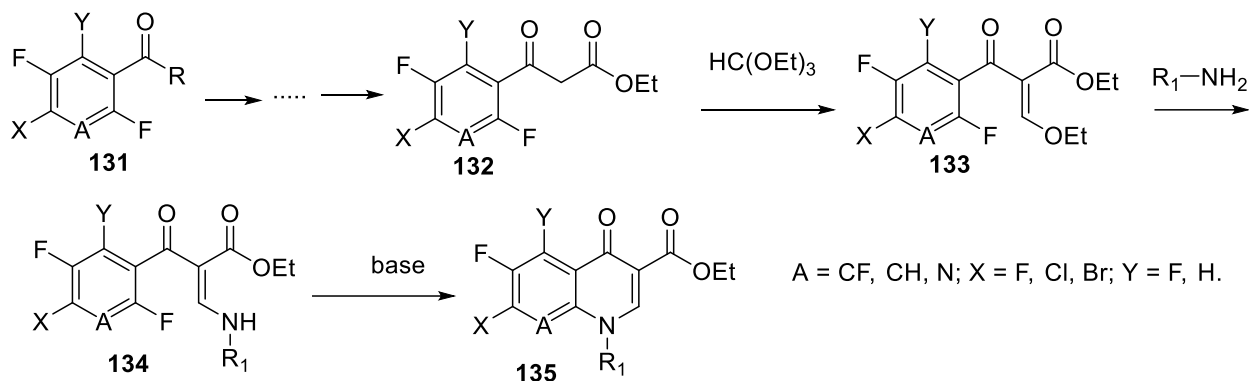


Compound	R ¹	R ²	R ³	R ⁴
128a	F	H	ethyl	3-methylpiperidin-1-yl
128b	Cl	H	ethyl	3-methylpiperidin-1-yl
128c	F	H	ethyl	3-methylpiperidin-1-y
128d	F	H	isopropyl	3-methylpiperidin-1-yl
128e	F	H	isopropyl	morpholin-1-yl
128f	F	H	ethyl	morpholin-1-yl
128g	Cl	H	ethyl	morpholin-1-yl
128h	Cl	H	ethyl	3-methylpiperidin-1-yl
128i	F	Cl	ethyl	morpholin-1-yl

Scheme 20. Synthesis of some quinolones

The second approach suggests use of fluorine-containing benzoyl derivatives (**131** A = CF or CH) or their nicotinoyl analogs (**131**, A = N) as building-blocks (Scheme 21). The key intermediates in this case are benzoyl- or pyridinoyl acrylates **133**. (Charushin et al. 2014) Cyclization of enaminones **134** can be carried out by heating the reaction mixture in DMF in

the presence of potassium carbonate, or in ethyl acetate with NaH. Other basic conditions can also be applied, including organic amines or amidines, 1,4-diazabicyclo[2.2.2]-octane (DABCO) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Charushin et al. 2014).



Scheme 21. Synthesis of fluoroquinolones from fluorinated benzoyl derivatives

2.4.2 The antibacterial activities of fluoroquinolones

Since the discovery **95**, a nonfluorinated agent, which was used clinically for treatment of urinary tract infections in 1962, with in decades, new fluoroquinolones, also called quinolones, 4-quinolones, carboxyquinolones, or quinolone carboxylic acids, have been developed. The typical new fluoroquinolones include norfloxacin (**106**), pefloxacin (**107**), enoxacin (**108**), fleroxacin (**109**), ciprofloxacin (**110**), ofloxacin (**111**), temafloxacin (**112**), lomefloxacin (**113**), tosufloxacin (**114**), difloxacin (**115**) and others (Table 1). These drugs differ significantly from the earlier agents in having increased potency, a broader spectrum of antibacterial activities, and decreased selection of resistant bacteria. Many of them also exhibit favorable pharmacokinetic properties, permitting treatment of systemic bacterial infections (Wolfson and Hooper 1989; Y.X. Furet 1991). Several newly developed fluoroquinolones are more active against Gram-positive bacteria than the presently marketed fluoroquinolones (Figure 8 & 9). Their main biological targets are the DNA *gyrase* (*Gyr*) and *topoisomerase IV* (*Top4*), two enzymes belonging to the type *II topoisomerase* family (Sissi and Palumbo 2003). These enzymes are essential in all bacteria and are expressed in prokaryotic organisms only, features that make them ideal antibacterial drug targets. Indeed, the prominent selectivity of quinolones in poisoning the bacterial type II topoisomerases versus the corresponding mammalian enzymes represents the rational basis for their safe use in antibacterial chemotherapy (Sissi and Palumbo 2003). They essentially block the topoisomerase catalytic cycle when the protein is covalently

linked to the cleaved DNA (cleavage complex). Stabilization of the enzyme-DNA complex has been confirmed to block the progression of the replicative machinery and to create DNA lesions that induce bacterial SOS response (Wentzell and Maxwell 2000).

Indeed, the prominent selectivity of quinolones among the new fluoroquinolones, WIN 57273 (**136**), the most potent compound *in vitro* on a weight basis, is 16 to 128 times more active than ciprofloxacin against various *Staphylococci*, *Streptococci*, *Enterococcus spp*, *Corynebacterium spp.*, *Listeria monocytogenes* and *Bacillus spp.* temafloxacin (**112**), tosfloxacin (**114**), BMY 40062 (**137**), PD 117558 (**138**), PD 127391 (**139**), and sparfloxacin (**134**) (Figure 9) also show enhanced *in vitro* efficacy against these species. These drugs also possess increased activity against various anaerobes, notably *Clostridium perfringens*, *Clostridium difficile* and the *Bacteroides fragilis* group. Mycobacterium tuberculosis, rapidly growing mycobacteria other than Mycobacterium chelonae, and Mycobacterium leprae are often susceptible to quinolones displaying bactericidal activity which is potentially useful for curing difficult-to-treat mycobacteriosis. In addition, a number of new products, notably those containing a cyclopropyl group, are more active than reference fluoroquinolones against *Mycobacterium leprae*. Sparfloxacin (**140**), BMY 40062 (**137**) and WIN 57273(**136**) (Figure 9) compare favorably with older fluoroquinolones in killing of intracellular *Legionella spp.*, and several of the newer compounds have greater antichlamydial potency. Improved antibacterial activity has also been found against *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Acinetobacter spp.* and *Pseudomonas maltophilia*. By contrast, the newer quinolones have similar or less activity against *Pseudomonas aeruginosa* and *Enterobacteriaceae*. Recently, pefloxacin (**107**), ciprofloxacin (**110**) and ofloxacin (**111**) (Figure 8) were found to be active against protozoa, including *Plasmodium spp.*, *Trypanosoma cruzi* and *Leishmania donovani*. I hope, in the near future, more specific research testing unusual pathogens may lead to the identification of quinoiones with more selective activity (Y.X. Furet 1991).

Among all bacterial pathogens, Mycobacterium tuberculosis (MTB) deserves special attention. Tuberculosis (TB) remains the leading cause of mortality due to bacterial pathogen *Mycobacterium tuberculosis*. It is estimated that nearly 1 billion people will become newly infected, over 150 million will become sick, and 36 million will die worldwide every three years if control is not further strengthened (Senthilkumar, Dinakaran, and Yogeewari 2010).

Among fluoroquinolones, ciprofloxacin (**111**), ofloxacin (**112**) and levofloxacin (**113**) are frequently used for the treatment of tuberculosis (TB) including multi-drug resistant TB (MDR-TB) as components of second-line regimens. Two new generation 8-C-methoxy fluoroquinolone, gatifloxacin (**142**) and moxifloxacin (**143**) (Figure 9), currently in Phase III clinical trials for Mycobacterium tuberculosis (MTB) and MDR-MTB (Figure 7) (T. Zhang et al. 2015). Interestingly a lot of other newly developed fluoroquinolones also showed excellent in vitro activity against MTB with MIC of ranging 0.08 to 16.6 μ M in their first phase screening against MTB (Gorzynski, Gutman, and Allen 1989; Senthilkumar, Dinakaran, and Yogeewari 2010).

2.4.3 The Anticancer properties of fluoroquinolones

Since the discovery of **95**, quinolones became a point of interest for researchers all over the world. In addition to antibacterial potential, some quinolones showed variable biological activities, including anticancer, antifungal, antiviral, and anti-inflammatory activities (Abdel-Aal et al. 2019). Cancer is continuing to be a major health problem worldwide and is the leading cause of human death (Abdel-aziz et al. 2016). Anticancer drugs are broadly categorized either as cytotoxic (cell killing drugs) or as cytostatic (anti-proliferative drugs), both of which lead to reduction in tumor growth (Yadav and Talwar 2019). As anticancer agents, quinolones comprise promising candidates; some of them reached different stages of clinical trial phases (Advani et al. 2010). Advani RH. et al (2010) studied Voreloxin (**143**) in details and revealed that it has an acceptable safety profile with clinical activity in patients with relapsed/refractory solid tumors (Figure 7) (Advani et al. 2010). Abdel-Aziz *et al* (2016) evaluated sixteen different quinolones based on norfloxacin (**107**) and ciprofloxacin (**111**) (Figure 8) scaffolds for their antitumor activities, where most of the tested compounds exhibited significant antitumor activities (Abdel-aziz et al. 2016).

Investigation of existing quinolone drugs for their antitumor potential against different types of cancers revealed that they possess anticancer activities through inhibition of mammalian DNA topoisomerases in addition to other mechanisms (Abdel-Aal et al. 2019). Inhibition of eukaryotic Top 2 correlates with cytotoxicity in those cells, and clinically-relevant fluoroquinolones are known to be safe and tolerated at concentrations that far exceed their cytotoxic threshold without bearing cytotoxic, genotoxic or carcinogenic potential. Since they

also mediate antitumor activities in humans at clinically achievable concentrations, how they perform these extended roles is unclear (Idowu 2017). Just to mention few of them, ciprofloxacin (**111**) exhibited a significant activity and induced apoptosis in different cancer cell lines (S et al. 2011); lomefloxacin (**114**) reduced the viability of the human melanoma COLO829 cells (Beberok et al. 2017); and trovafloxacin (**144**) combated metastasis and spread of leukemia cells to other body tissues, and could lengthen the survival of laboratory animals (Thadepalli et al. 2005). However, there are also reports that showed the newer generation fluoroquinolones, Gatifloxacin (**142**) and Moxifloxacin (**143**) (Figure 9) were able to suppress the proliferation of pancreatic cancer cells and induce apoptosis by causing S/G2 phase cell cycle arrest (Yadav et al. 2015).

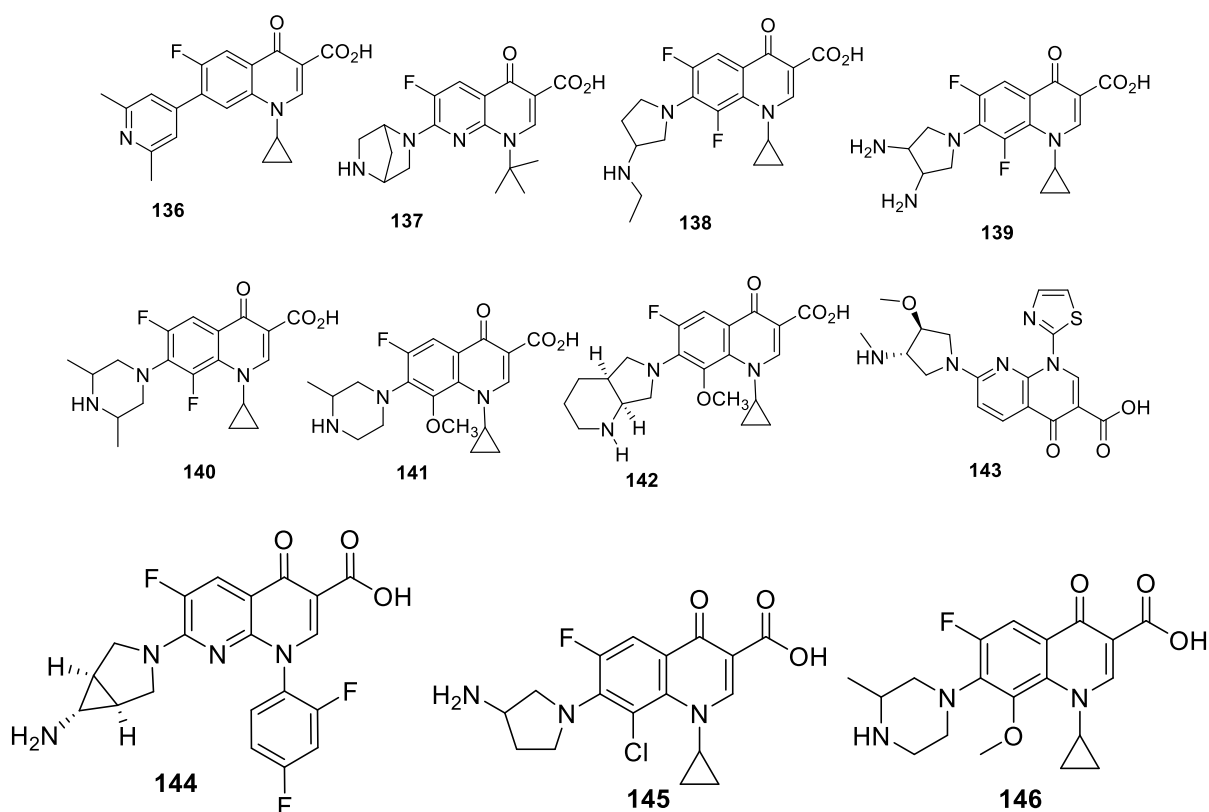


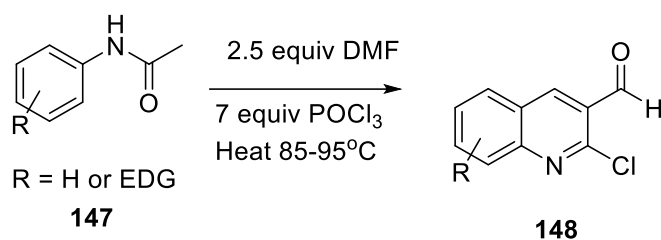
Figure 9. Structure of new generation fluoroquinolones

2.5 Synthesis of 2-chloroquinoline-3-carbaldehydes and its analog using Vilsmeier-Haack reagent

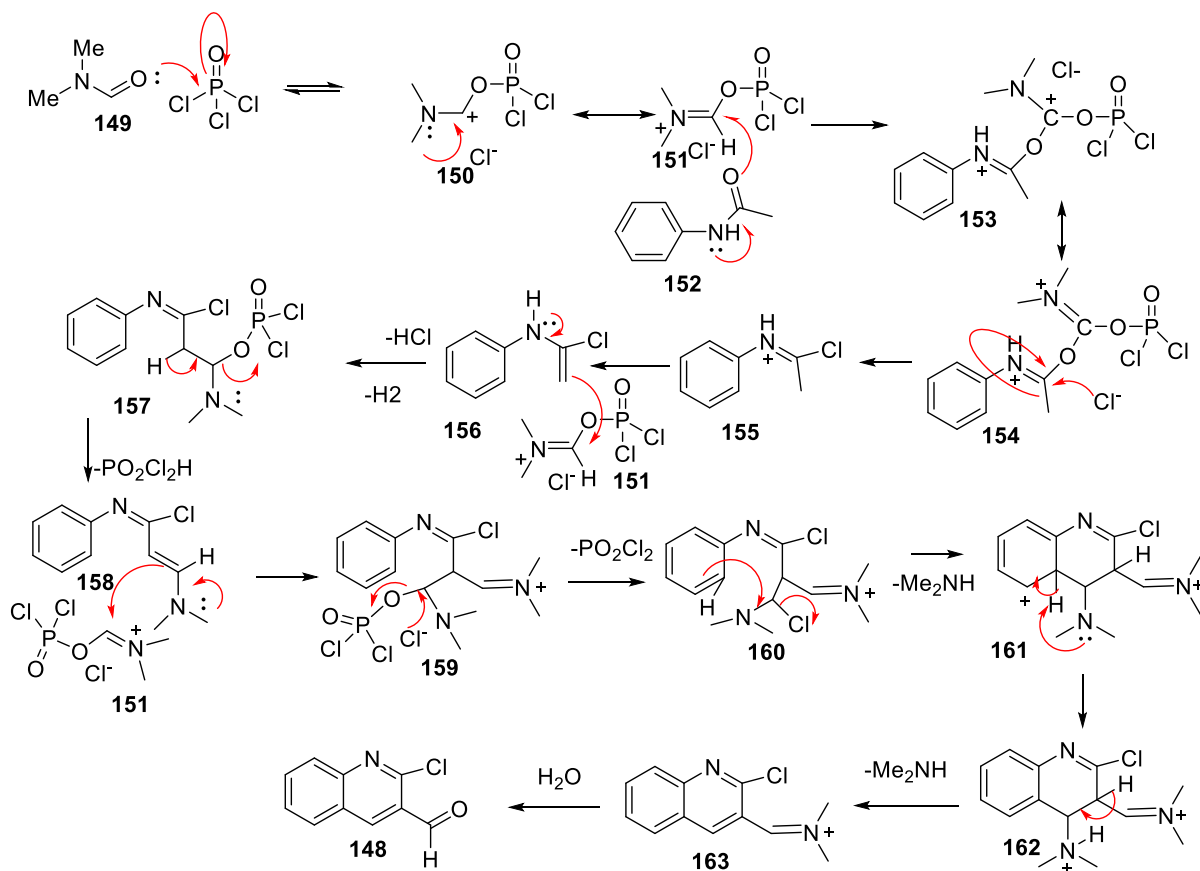
(Chloromethylene)dimethyliminium chloride (Vilsmeier reagent, VR) is well known as a versatile synthetic tool for the formylation of electron-rich aromatics, chlorination of alcohols,

conversion of carboxylic acid into the corresponding acid chloride and so on (Kimura and Matsuura 2013; Su et al. 2010). Typical preparation methods for VR is the treatment of *N,N*-dimethylformamide (DMF) with chlorinating agents such as phosphoryl trichloride, thionyl chloride, phosgene, or oxalyl chloride (Kimura and Warashina 2017). Those chlorinating agents are not suitable though for large scale synthesis due to the expensive or hazardous nature of the reagents or byproducts (Kimura et al. 2012).

VR also found application in the synthesis of a large number of heterocyclic compounds. Otto Meth-Cohn *et al* (1981), showed the treatment of acetanilide and its activated analogs with dimethylformamide in phosphoryl chloride under reflux in a drying tube fitted flask afforded quinoline-3-carbaldehyde in good yield (Scheme 22). (Meth-Cohn, Narine, and Tarnowski 1981). Quinoline-3-carbaldehyde analogs demonstrated considerable biological and pharmacological activities as antimicrobial (Farghaly et al. 1990), anti-inflammatory (Herencia et al. 1998), antimalarial (Sekar and Prasad 1998) and antiviral activities (Abdel-Wahab and Khidre 2013). Recently, Hamama *et al* (2018) proposed mechanism of for synthesis 2-chloroquinoline-3-carbaldehyde (**148**) via Vilsmeier-Haack reagents (Scheme 22) (Hamama et al. 2018).



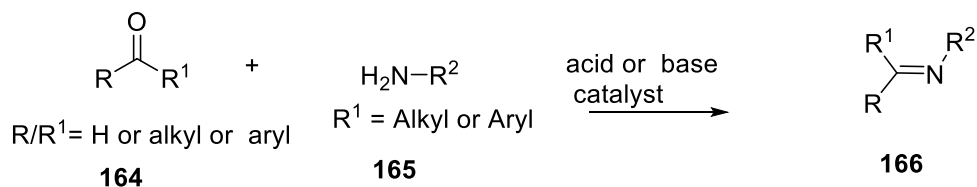
Scheme 22. Synthesis of 2-chloroquinoline-3-carbaldehyde (**148**)



Scheme 23. The Mechanism of 2-chloroquinoline-3-carbaldehyde synthesis via Vilsmeier reagent

2.6 Schiff bases

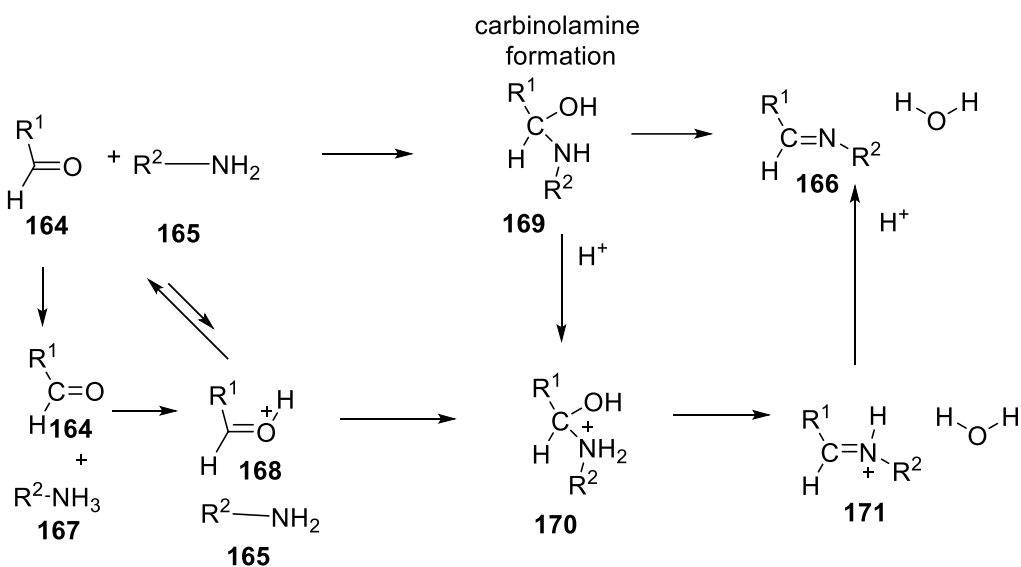
Schiff bases are the compounds containing azomethine group ($-\text{HC}=\text{N}-$). They are condensation products of ketones or aldehydes (**164**) with primary amines (**165**). Formation of Schiff base generally takes place under acid or base catalysis (Scheme 24). Schiff bases are considered as a very important class of organic compounds and have a wide application in many biological aspects, proteins, visual pigments, enzymatic aldolization and decarboxylation reactions. Moreover, some Schiff-bases exhibited antibiotic, antiviral and antitumor agents because of their specific structures (Al-Garawi, Tomi, and Al-Daraji 2012).



Scheme 24. The General reaction of Schiff base

2.6.1 The mechanism of Schiff base formation

Imines can readily be synthesized through the reversible reaction of amines (**165**) with aldehydes or ketone (**164**). This reaction proceeds through an addition step which forms a carbinolamine intermediate (**170**), which is then dehydrated to the imine in the rate-determining step (Scheme 25). The released water is usually removed from the system to shift the equilibrium towards the products. The reaction rate is quite sensitive to pH: moderate amounts of acid greatly accelerate (Santerre 1953), but excess acid prevents it (Jencks 1959).



Scheme 25. The mechanism of Schiff base formation

Schiff bases appear to be important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base (Al-Garawi, Tomi, and Al-Daraji 2012). There are many interesting studies on the Schiff bases or compounds derived from amino acids. There are also reports which indicates various Schiff base with good antibacterial activities in good range when compared to control drug (Ampicilin) (Al-Garawi, Tomi, and Al-Daraji 2012; Antony, Arun, and Manickam 2019; Fattuoni, Vascellari, and Pivetta 2020).

2.7 The role of hybrid molecules in improving bioactivity

The hybridization of biologically active molecules is a powerful method for drug discovery used to target a variety of diseases. It helps to get better drugs for the treatment of a number of illnesses including cancer, malaria, tuberculosis and AIDS. Hybrid drugs can provide combination therapies in a single multi-functional agent and, by doing so, be more specific and powerful than conventional classic treatments (Bérubé 2016). The molecular hybridization (MH) is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates (Lazar et al. 2004; Viegas-junior et al. 2007). Designing hybrid drugs, by covalently linking two distinct chemical moieties with multiple effects, is a common strategy in today's search for new treatment of various diseases. In recent years, various structurally diverse hybrid molecules were reported with better bioactivity (News et al. 2010). Considering the different approaches regarding to the development of hybrid molecules (C. Chen et al. 2005), they are defined as a chemical entity with two (or more than two) structural domains which acts on different/same biological functions or targets via different mode of action." Dual activity of hybrid molecule indicates that it acts as two distinct pharmacophores inside the biological system (News et al. 2010).

Classification of hybrids:

On the basis of types of linkers connecting the two pharmacophores, hybrid molecules can be classified as follows:

- 1) **Conjugate hybrids:-** molecules in which both the pharmacophores are joined through a metabolically stable linker which is not a part of either of the individual pharmacophores (Dechy-cabaret and Benoit-vical 2000).
- 2) **Cleavage conjugate hybrids:-** molecules joined through a linker designed to be metabolized inside the biological system to release the two pharmacophoric units that interact independently with different targets (Grellepois and Grellier 2005).
- 3) **Fused hybrids:-** molecules, in which the size of the linker is decreased / removed such that the framework of the pharmacophores is in contact (Fikkert et al. 2003).

4) **Merged hybrids:-** have their frameworks merged by taking advantage of the common pharmacophore in the structures of the starting compounds, which give rise to smaller and simpler molecules (Fikkert et al. 2003).

2.8 Stilbenes: their Chemistry and application

The name for stilbene (1,2-diphenylethylene) was derived from the Greek word ‘*stilbos*, which means shining. There are two isomeric forms of 1,2-diphenylethylene: (E)-stilbene (trans-stilbene) (**172**), which is not sterically hindered, and (Z)-stilbene (cis-stilbene) (**173**) (Figure 10), which is sterically hindered and therefore less stable. (Synthesis, Methods, and Development 2003) Stilbene (1,2-diphenylethene) does not exist in nature itself, but its derivatives as plant secondary metabolites are present in various plant species and some of them are considered phytoalexins (Z. A. Khan, Iqbal, and Shahzad 2017).

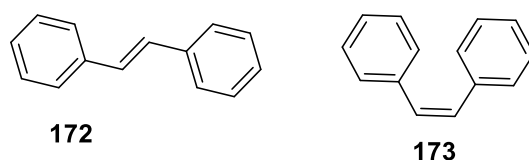


Figure 10. The structure of cis and trans Stilbene

Stilbene is a relatively unreactive colorless compound practically insoluble in water. It possesses intense absorption and fluorescence properties, which correspond to the excitation of pi-electrons of the conjugated ethenediyl group to p-orbitals, as well as reversible, trans-cis photoisomerization (photochromism). Stilbenes are widely used in the manufacture of industrial dyes, dye lasers, optical brighteners, phosphors, scintillators, and other materials (Synthesis, Methods, and Development 2003). Natural stilbenes are characterized by several biological activities such as defense mechanism in plants, fungicidal activity, cardioprotective effects, antioxidant, diabetes control, antimicrobial and anticancer activities *etc* (Bizzarri et al. 2019). Especially, hydroxylated stilbenes have attracted the attention of many scientific groups because of their remarkable biological properties and therapeutic potentials including anti-inflammatory, antioxidative, lipid-lowering, radical scavenging, neuroprotection, anticarcinogenic, antiviral, and also platelet aggregation inhibition activity. One of the best representative compounds of this group is trans-3, 5, 4'-trihydroxy stilbene (resveratrol) (**174**) (Figure 12), a phytoalexin found in grapes, fruits and vegetables of several plants (Reddy, Prakash, and Diwakar 2015). Research also has revealed that the E form or trans exhibits more

potent anticancer activity compared to the Z form or cis form. (Z. A. Khan, Iqbal, and Shahzad 2017)

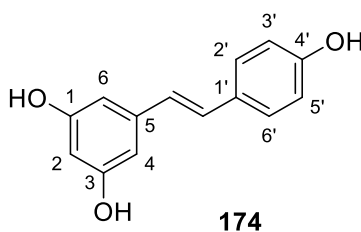


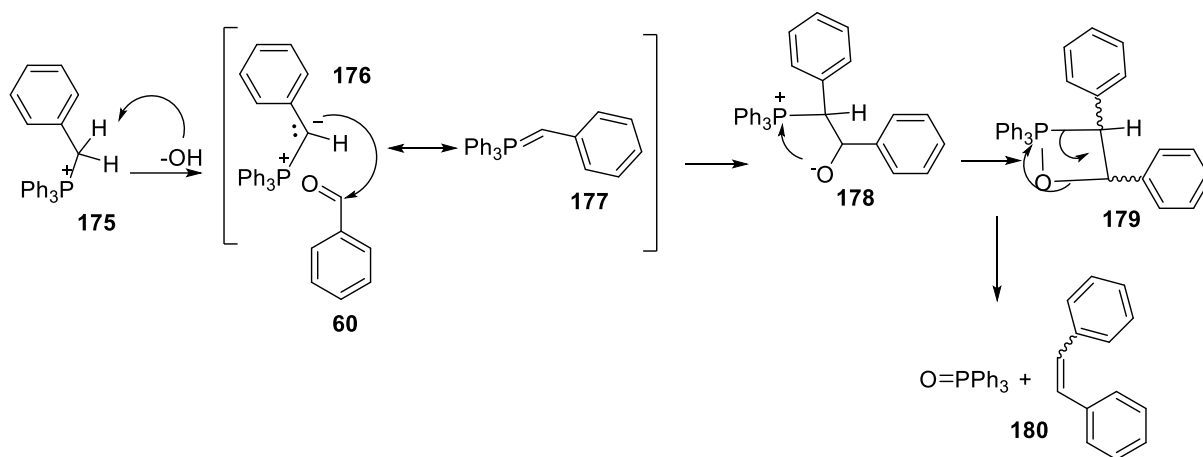
Figure 11. The Structure of resveratrol

2.8.1 The Synthesis of stilbene analogs

The non-availability of naturally occurring stilbenes in sufficient quantities attracted the development of synthetic methodologies for their preparation at large scale such as Wittig or Horner–Wadsworth–Emmons (HWE) olefination, Perkin aldol condensation, transition metal coupling, i.e., Mizoroki–Heck, Negishi, Stille, Sonogashira, Suzuki–Miyaura, Grubbs & McMurry, Knoevenagel–Doebner, Ramberg–Bucklund reactions (Z. A. Khan, Iqbal, and Shahzad 2017).

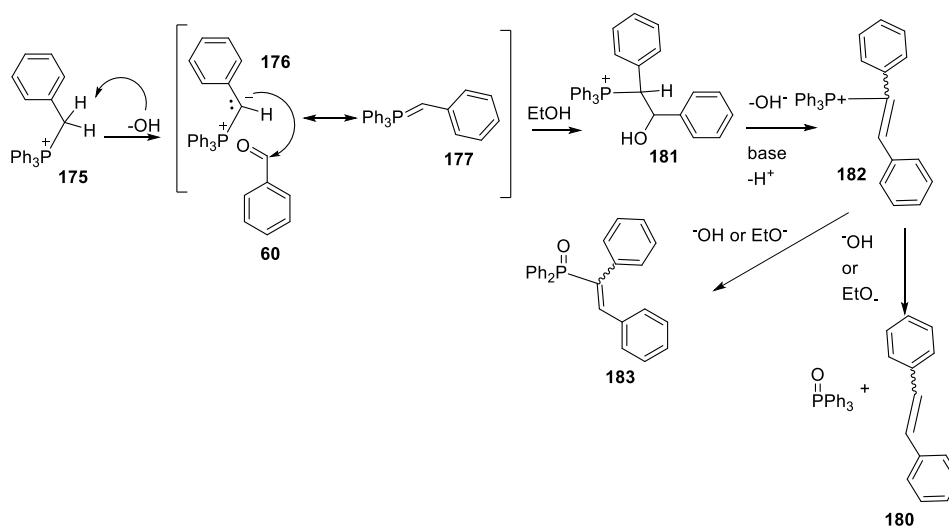
2.8.1.1 Synthesis of stilbene via Wittig reaction

The Wittig reaction is perhaps the most commonly used method for the synthesis of alkenes (Schweizer et al. 1971b). There are a number of features of the Wittig reaction that makes attractive in synthesis; it is regiospecific, reaction conditions are usually mild, the starting materials are frequently easily obtainable, ylides are tolerant of a number of other functional groups, and the stereoselectivity of the reaction can often be directed towards the desired alkene isomer by expedient choice of the nature of the reactants used. The mechanism of the Wittig reaction has long been a contentious issue in organic chemistry. Even now, more than 50 years after its announcement, its presentation in many modern undergraduate textbooks is either overly simplified or entirely inaccurate (Schweizer et al. 1971b). For example Johnson was able to enumerate a total of eight mechanisms. Here only the Betaine and Schweizer mechanism has been discussed (Scheme 26 and 27).



Scheme 26. The betaine mechanism for the Wittig reaction

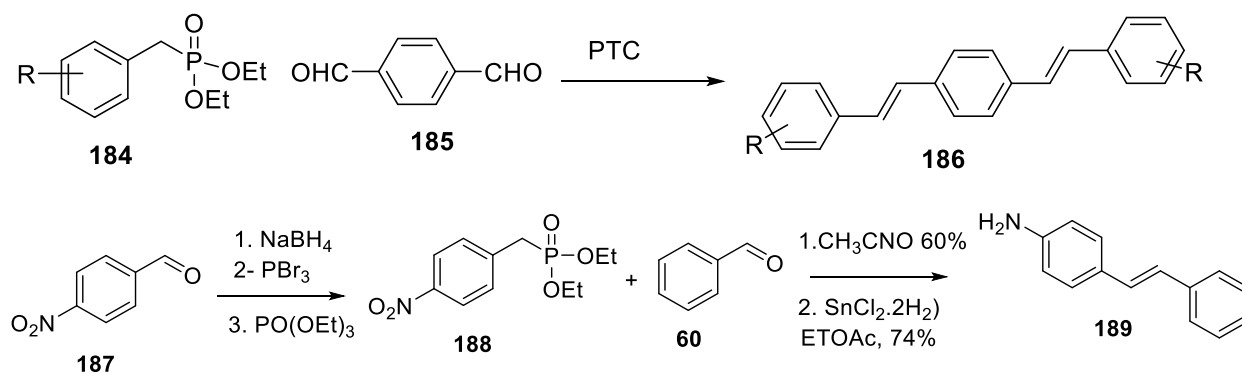
Essential features of Schweizer mechanism is on the basis of the observation that certain semi-stabilised and stabilised phosphonium ylides and conjugated carbonyl compounds reacted in alcohol solvents to give vinylphosphine oxides (**182**) in addition to the expected alkene (**180**) and phosphine oxide products. Schweizer *et al.*, proposed that, these reactants initially form a betaine, which becomes protonated by the alcohol, and then undergo net elimination of water to give a vinylphosphonium salt (**181**). This could undergo nucleophilic attack at phosphorus by either alkoxide or hydroxide. The resultant alkoxy or hydroxyl phosphonium salt could expel either the vinyl group as alkene, giving triphenylphosphine oxide (**156**) (the expected Wittig product), or benzene, giving vinyl phosphine oxide (**183**) (Scheme 27) (Schweizer *et al.* 1971a).



Scheme 27. The proposed mechanism for Wittig reactions and vinylphosphine oxide formation in alcohol

2.8.1.2 Synthesis of stilbene analogues by the Wittig/Horner–Wadsworth–Emmons (HWE) reaction

In synthesis of Stilbene analogs, the methodological selection depends on several factors such as the low atom economy, the (E-Z)-ratio, cost of solvents, the cost of catalyst etc. The Horner-Wadsworth-Emmons (HWE) olefination is the synthesis of olefins from the condensation between carbonyl compounds (both aldehydes and ketones) and carboanions derived from alkyl phosphine oxides (Horner's condition), phosphonates (Wadsworth and Emmons's condition), phosphonamides (Corey), and their thiono counterparts (Corey) (Synthesis, Methods, and Development 2003). In fact, it is the further modification of the Wittig reaction (Schweizer et al. 1971b). The Wittig/HWE olefinations undergoes several improvements such as concert with metal catalyzed cross-coupling reactions, Domino Wittig/HWE—cycloaddition and Wittig-Michael transformations. Wittig/HWE with phase transfer catalysts is simplicity, involves inexpensive reagents, conducted under mild conditions, high reaction rate and high selectivity to the desired product (Scheme 28) (Description and The 1961).



Scheme 28. Synthesis of stilbene analogues by (HWE) reaction in the presence and absence of PTC

2.8.2 The biological activities of stilbenes

Natural stilbenes especially resveratrol (**174**) play important roles in plant protection by acting as both constitutive and inducible defenses. However, their exogenous applications on crops as fungicidal agents are challenged by their oxidative degradation and limited availability (Jian, He, and Song 2016). In fact, natural stilbenes e.g. resveratrol (**174**) (Figure 12) appear to act as constitutive and inducible defenses in response to fungal infections such as *Botrytis cinerea*, as well as to abiotic stresses and plant growth regulators. Consequently, a positive correlation between stilbenes production potential and disease resistance in plants has been well established

(Jian, He, and Song 2016). Stilbenes superior to resveratrol in antibacterial activity were identified in latest study. One study revealed dimerization, halogenation and hydroxy group in conjunction with methoxy group resulted in the best antibacterial molecules. Design of stilbene based drugs would be benefitted with the outcome and rationale presented in the current investigation. The analogues 4-bromoresveratrol and pinosylvin (**190**) exhibited higher inhibition than resveratrol, when evaluated against Gram negative bacteria (Singh et al. 2019).

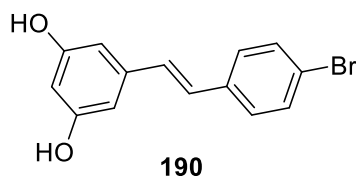


Figure 12. Antibacterial activity of resveratrol structural analogues

2.9 Radical scavenging activity

Free radicals are atoms, groups of atoms or molecules that have one or more unpaired electrons in the outermost orbital. They are chemically highly reactive and tend to seek electron pairs to be able to bind to achieve stability. Free radicals are constantly attacking body's cells including normal cells for obtaining electron pairs (Don 2017). Free radicals react with biological compounds in body continuously and if they do not stop, they can damage body cells and result in harmful effects to body health. This free radical reaction will also cause various diseases such as cancer, heart, cataract, premature aging, and other degenerative diseases (Don 2017).

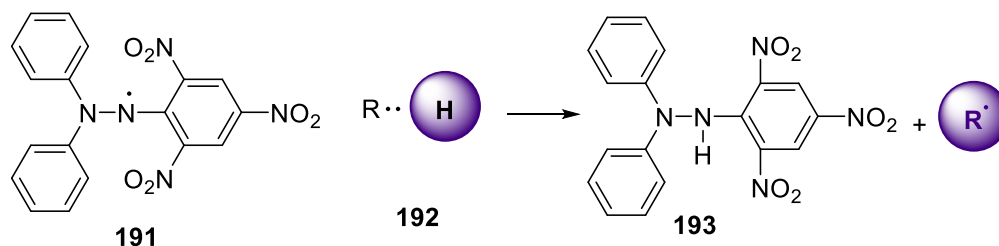
There are various sources of free radicals, they may be originate outside our body such as motor vehicle pollution, cigarette smoke, and ultraviolet light. In addition, free radicals can also come from within body itself i.e, during the process of respiration, oxidative enzyme, as well as oxidative phosphorylation in mitochondria. Oxygen is undeniably essential for the survival for all living things. Approximately, 5% of inhaled oxygen is reduced to oxygen derived free radicals in normal physiological and metabolic processes (hydroxyl radical ($\cdot\text{OH}$), the superoxide radical ($\text{O}_2^{\cdot-}$) and hydroperoxyl radical ($\text{HO}_2^{\cdot}$) (Hangun-Balkir and McKenney 2012). Thus, even if we avoid free radicals from outside the body, they will automatically be generated from normal biological processes of body. To overcome this, antidote of free radicals or antioxidants are needed that serve to inhibit or stop the negative effects caused by free radicals (Don 2017). Antioxidants are substances that neutralize the harmful free radicals in our

bodies. Antioxidants act as “free radical scavengers” and hence prevent or slow the damage done by these free radicals. Their function is as a reducing agent, which ultimately removes free radical intermediates and prevents further oxidation by being oxidized themselves. Fruits and vegetables are known as good sources of antioxidants, such as retinol (Vitamin A), ascorbic acid (Vitamin C), α -tocopherol (Vitamin E), carotenoids, flavonoids, tannins, and other phenolic compounds (Hangun-Balkir and McKenney 2012). Because of this, in some countries antioxidant substances are utilized as food additives (Himamura et al. 2014).

A number of assays have been introduced for the measurement of the total antioxidant activity of substances. DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity, ABTS (2,2-azinobiz-3-ethylbenthiazoline-6-sulfonic acid) radical cation scavenging activity, FRAP (ferric reducing antioxidant power), and hydrogen peroxide (H_2O_2) radical scavenging activity are among these methods (Sivaramakrishnan et al. 2017).

2.9.1 DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity

This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical 1,1-diphenyl-1-picrylhydrazyl (DPPH) (**191**). **191** is reduced to the corresponding hydrazine (**193**) when it reacts with hydrogen or electron donors (Contreras-Guzmán and Strong 1982; Sánchez-Moreno 2002). Antioxidants react with free radicals by different mechanisms—hydrogen atom transfer (HAT) or single electron transfer mechanism (SET); or the combination of both HAT and SET mechanisms being primary examples. The HAT reaction is a concerted movement of a proton and an electron in a single kinetic step (Scheme 29) (Liang and Kitts 2014).



Scheme 29. Reaction of 1,1-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant.

191 possesses a purple color, with a maximum absorption at 519 nm in ethanol; hence, scavenging the DPPH radical by antioxidant substances will result in a decrease in absorption readings over time; the extent of decrease in **191** absorption being proportional to the

concentration of radicals that are being scavenged (Liang and Kitts 2014). Measurements are made using a UV-visible spectrophotometer at room temperature, and the scavenging capacity is reported as the percentage of **191** radical inhibition (eq. 1.).

$$\% \text{ DPPH radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \text{ -----(EQ1)}$$

Where A_0 the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

2.10 *In-silico* drug-likeness predictions

According to statistics, the success rate of candidate compounds found in preclinical detection is about 40%, while the rate of candidate compounds entering the market is only 10% due to their poor biopharmaceutical properties (poor chemical stability, poor solubility, poor permeability and poor metabolic) (Tian et al. 2015). The time-consuming nature and enormous resources required in performing *in vivo* studies to determine the drug likeness of a compound have urged to develop computational techniques as a viable alternative. Christopher Lipinski and coworkers reported the earliest report on decisive criteria for identifying drug-like compounds at Pfizer in the mid-1990s (Agoni et al. 2020). Thus, generating worthy computational methods for prediction of drug-likeness of bioactive molecule is very important to increase the success of drug discovery and development. According to Walters and Murcko (1999) (Walters, Murcko, and Murcko 1999), drug-like compounds are molecules which contain functional groups and/or have physical properties consistent with the majority of known drugs, and hence can be inferred as compounds which might be active biologically or might show therapeutic potential. Lipinski (2000) define those compounds as ‘drug-like’, which have sufficiently acceptable ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties to survive through the Phase I clinical trial (C.-Y. Jia et al. 2020). The concept of drug-likeness, generated by taking into consideration the structural, physicochemical, biochemical, pharmacokinetic (PK), and toxicity characteristics of a compound; thus, a drug-like compound possesses sufficiently acceptable ADME properties, as well as sufficiently acceptable toxicity properties (C.-Y. Jia et al. 2020; C. Y. Jia et al. 2020). Currently, there are

various available online resources for drug-likeness prediction, including integrated databases, drug-likeness evaluating servers, and resources for drug-likeness based on natural products and special drugs.

Thus, understanding these properties has become an integral part of drug discovery and has enabled the accurate selections of hits that are suitable starting points for the identification of new clinical candidates (Agoni et al. 2020). Lipinski *et al.* (2012) (Lipinski et al. 2012) pointed out that leads obtained through high throughput screening (HTS) tend to have higher molecular weights and greater lipophilicity than leads in the pre-HTS era. Lipinski's Rule of 5 was developed to set 'drug ability' guidelines for New molecular entities (NMEs) (Lipinski 2016). The Lipinski "Rule of Five" states that compounds are likely to have good absorption and permeation in biological systems and are more likely to be successful drug candidates if they meet the following criteria (X. Chen et al. 2020): five or fewer hydrogen-bond donors (HBD), ten or fewer hydrogen-bond acceptors (HBA), molecular weight (MW) less than or equal to 500, and Calculated logP ((cLogP)) less than or equal to 5 to survive through the Phase I clinical trials were considered drug-like (Huang et al. 2019). As a guideline, the Lipinski's RO5 is conceptually simple and straightforward to implement, hence its widespread adoption. However, it does not apply to natural products or substrates of biological transporters (Agoni et al. 2020). Further research has added two more conditions: a polar surface area (PSA) of $\leq 140 \text{ \AA}^2$ and ten or less than rotatable bonds (Rot B) which are correlated with drug permeability and flexibility, respectively (C. Y. Jia et al. 2020).

2.11 Molecular docking

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small-molecule ligands to the appropriate target binding site (S. U. Khan et al. 2020). It assists in the process of computer-aided drug designing by considering every possible conformation of the protein and ligand molecule (S. U. Khan et al. 2020). Molecular docking techniques aim to predict the best matching binding mode of a ligand to a macromolecular partner (Salmaso and Moro 2018). Ligand binding to macromolecules plays a key role in biology and medicine as guiding mechanism in biological processes. The binding of drugs to proteins and DNA has led to a vast structural studies using both experimental and theoretical methods. Because of the major role

of DNA in replication and transcription, DNA has been a major target for antibiotic, anticancer and antiviral drugs (Rohs et al. 2005). Nucleic acid binding drugs are known for treatment of various diseases such as cancer, malaria, AIDS and other viral, bacterial and fungal infections (Rohs et al. 2005). The different modes of drug binding to DNA include intercalation between adjacent base pairs, intrusion into the minor groove and into the major groove. Intercalation and minor-groove binding are the predominant DNA-binding modes of small ligands (Rohs et al. 2005).

Molecular docking or computer-simulated ligand binding is a powerful technique for investigating intermolecular interactions. It is an attractive tool to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by introducing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand (Dar and Mir 2017).

The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy. The net predicted binding free energy (ΔG_{bind}) is revealed in terms of various parameters, hydrogen bond (ΔG_{hbond}), electrostatic (ΔG_{elec}), torsional free energy (ΔG_{tor}), dispersion and repulsion (ΔG_{vdw}), desolvation (ΔG_{desolv}), total internal energy (ΔG_{total}) and unbound system's energy (ΔG_{unb}). Therefore, good understanding of the general ethics that govern predicted binding free energy (ΔG_{bind}) provides additional clues about the nature of various kinds of interactions leading to the molecular docking (Agarwal, Chadha, and Mehrotra 2015; Dar and Mir 2017).

3 CHAPTER THREE

3. MATERIALS AND METHODS

3.1 General

All solvents and organic reagents were purchased from LOVA CHEMIE PVT LTD. All analytical grade solvents and reagents were used without further purification. Melting points were determined using capillary tubes with Japson analytical melting point and Thiele tube apparatus and are uncorrected. The NMR and IR spectroscopy analyses were conducted at the Chemistry Department of Addis Ababa University. The NMR spectra of the compounds were obtained using NMR Bruker Avance 400 spectrometer operating at 400 and 100 MHz using DMSO-*d*₆, CDCl₃ and CD₃OH as a solvent and chemical shifts (δ) were reported in ppm and the coupling constants (*J*) are reported in Hertz. The IR of the compounds were recorded using KBr pellets on a Perkin-Elmer BX IR Spectrometer (400-4000 cm⁻¹). UV-Vis spectra were determined using double beam UV-VIS Spectrophotometer(SM-1600 Spectrophotometer)) using methanol as solvent. Analytical thin-layer chromatography was conducted on a 0.2 mm thick layer of silica gel GF₂₅₄ (Merck) on aluminum plate and the spots were visualized using UV lamp at 254 and 366 nm. Silica gel gravity column chromatography was carried out using 100 mesh silica gel.

3.2 Lists of chemicals, reagents and apparatus

The lists of chemicals, reagents and apparatus used during experiments of this research were list below.

Chemicals		Apparatus
Methanol	Potassium hydroxide	Beakers
ethanol	Silica gel 100-200 mesh	Buchner funnel
Ethyl acetate	Hydrochloric acid	Condenser
Acetonitrile	Moist copper	Erlenmyer flask
chloroform	Nitric acid	Filter flask
Ethanol amine	Potassium dichromate	Funnel
benzaldehyde	Copper oxide	Graduate Cylinder
acetic anhydride	Zinc powder	hood
Pyridine	Aluminum powder	Magnetic Stirrer
Acetic acid	Copper sulfate	Rota vapor
aniline	Phosphorusoxy chloride	RB flasks
Benzyltriphenylphosphonium chloride	Calcium chloride	Separatory Funnel
Acetone	Sulfuric acid	Test tubes
<i>n</i> -hexane	Sodium sulfate	Thermometer
Pyruvic acid	Sodium hydroxide	UV lamp 254/366 nm
2-methylaniline	Sodium thiosulfite	Vacuum pump
p-nitroaniline		Wire gauze

3.3 Antibacterial activity assay

Six strains of bacterial species, three Gram-positive bacteria *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC6633), *Streptococcus pyogenes*, (ATCC19615) and three Gram-negative bacteria *Escherichia coli* (ATCC, 25922), *Salmonella typhimurium* (ATCC 13311), *Pseudomonas aeruginosa* (ATCC 27853) which were provided by Adama Public Health Research & Referral Laboratory Center were used to evaluate antibacterial activities of the synthetic compounds. The antibacterial efficacies of the compounds were tested by using the disc diffusion method. The microbial cultures were grown overnight at 37 °C in nutrient broth, adjusted to 0.5 McFarland standard using distilled water and lawn inoculated onto Mueller-Hinton agar (MHA) plates. The synthetic compounds were dissolved in DMSO and adjusted to a desired concentration for the three different experiments as described in the discussion section. Sterile filter paper discs of 6 mm diameter were soaked in 1 mL DMSO solution of the compounds at various concentration for each of the experiments. Then, the saturated paper discs were placed on the centre of each MHA plate. Ciprofloxacin was the standard drug used as positive control and DMSO was as negative control. The plates were then inverted and incubated for 24 hours at 37°C and the zone of inhibition was recorded. The results were expressed as the mean of three measurements.

3.4 DPPH radical scavenging activity

The radical scavenging activities of methanolic solutions of the synthetic compounds was evaluated using DPPH and compared with ascorbic acid. All synthetic compounds were separately dissolved in methanol and serially diluted using 0.004% methanolic solution of DPPH to furnish five different concentration for each sample as indicated in the discussion section. The mixtures were incubated at 37°C for 30 min in the dark. Then, the absorbance was measured with double beam spectrophotometer at 517 nm (SM-1600 Spectrophotometer). The DPPH radical scavenging rate of each sample was calculated using the following formula (Verbanac et al. 2016):

$$\text{Percentage inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) 100$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance measurement in the presence of the test or standard sample

The control DPPH solution was prepared by mixing 1mL 0.004% DPPH with 4 mL methanol. The results were presented in percent radical scavenging activity using tables and line graphs. The IC₅₀ values of the sample were determined by drawing percent inhibition versus concentration on excel and by changing the scatter diagram in to linear with an equation and R square values. Then from the linear equation ($y = mx + b$), where y-axis is percent radical scavenging, m is the slope and x-axis is concentration of the antioxidant and IC₅₀ value will be calculated using the following equation

$$IC_{50} = \frac{50-b}{m}$$

R square value indicates the quality of the measurement. For good quality data $R^2 \gg 0.9$

3.5 *In-silico* Drug-Likeness and toxicity predictions

In silico drug-likeness is a prediction that concludes whether a particular pharmacological agent has properties consistent with being an orally active drug. This prediction is based on an already established concept by Lipinski *et al* (1997), called Lipinski rule of five.(Lipinski 2016) The structures of synthesized compounds were changed to their canonical simplified molecular input line entry system (SMILE) then submitted to SwissADME tool to estimate *in silico* pharmacokinetic parameters and other molecular properties based on the methodology reported by Amina *et al.*, (2016) (Daina, Michielin, and Zoete 2017). The analyses of the compounds were compared with that of clinical drug (vosoroxin), and only those compounds without any violation of the rules were used for the molecular docking analysis.

3.6 Molecular docking analyses of synthetic compounds against *E. coli* gyrase and *S. aureus* topoisomerase IV

To investigate the mode of interaction between the *E. coli* gyrase and synthetic compounds in a 3D projection, the compounds were docked within the binding site of the protein. AutoDock Vina protocol was used to dock the proteins (PDB ID: 1ZI0, PDB ID: 6F86 and PDB ID: 2XCT) and synthetic compounds into the active site of the protein.(Adejoro et al. 2020) The 2D chemical structures of compounds were drawn using Chem Office tool (Chem Draw 16.0) assigned with proper orientation followed by the energy minimization of each molecule using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock

Vina, in order to carry out the docking simulation (Adejoro et al. 2020). The crystal structure of receptor molecule *E. coli* DNA gyrase (PDB ID 1ZIO) and *S. aureus* topoisomerase IV complex with Ciprofloxacin and DNA (PDB ID: 2XCT) were downloaded from protein data bank. The protein preparation was done using the reported (Narramore et al. 2019) standard protocol by removing the co-crystallized ligand, deleting water molecules, adding polar hydrogens and cofactors, then the target protein file was prepared by leaving the associated residue with protein by using Auto Preparation of target protein file Auto Dock 4.2 (MGL tools1.5.6). The graphical user interface program was used to set the grid box for docking simulations. To surround the region of interest in the macromolecule, grid was used. The best docked conformation between the compounds and the protein was explored with the docking algorithm provided with Auto Dock Vina (Carr et al. 2006; Schoeffler and Berger 2005). During the docking process, a maximum of nine conformers were considered for each ligand. The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by Discovery suite visualizer. The ligands are represented in different colour, H-bonds and the interacting residues are represented in stick model representation

3.7 Molecular docking analysis against human topoisomerase II α

To study the interactions and binding affinity between the human topoisomerase II α and synthetic compounds in a 3D fashion, the compounds were docked within the binding site of the protein. AutoDock Vina was used to dock the proteins (PDB ID: 4fm9) and compounds into the active site of protein.(Jadhav and Karuppayil 2017) The chemical structures of the compounds were drawn using Chem Office tool (Chem Draw 16.0) assigned with proper orientation followed by the energy minimization of each molecule using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina, to carry out the docking simulation. The graphical user interface program was used to set the grid box for docking simulations. To surround the region of interest in the macromolecule, grid was used. The best docked conformation between the compounds and the protein was explored with the docking algorithm provided with Auto Dock Vina (Allouche 2012). During the docking process, a maximum of nine conformers were considered for each ligand (Allouche 2012). The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by Discovery studio visualizer. The

ligands are represented in different color, H-bonds and the interacting residues are represented in ball and stick model representation.

3.8 Synthesis of some novel quinoline derivatives

Ten (10) quinoline-3-carbaldehyde derivatives were also prepared and their antioxidant, and antibacterial properties were evaluated using the methods discussed below. Also presented below is the molecular docking analysis conducted using AutoDock 4.2 (MGLTools 1.5.6).

3.8.1 Synthesis (Scheme 30 and 31)

3.8.1.1 Synthesis of acetanilide (195)

In 250 mL round flask, aniline (**17**) (20 mL), acetic anhydride (**194**) (22 mL), zinc powder (0.2 g), and acetic acid (23 mL) were added. The mixture was boiled under reflux using water condenser for an hour. Then, it was cooled to room temperature and poured into 200 mL of crushed ice water. The solid product was collected by suction filtration. The product was white powder; yield was 20.1 g (69 %), mp 112–113°C.

3.8.1.2 Synthesis of 2-chloroquinoline-3-carbaldehyde (195)

N,N-dimethylformamide (20 mL, 0.26 mol) was added to a 100mL round-bottom flask guarded with drying tube; it was cooled to 0°C using ice bath. Then, phosphorus oxychloride (70 mL, 0.75 mol) was added dropwise to it from dropping funnel guarded by drying tube while being stirred by magnetic stirrer. The addition was done during 30 minutes. Then acetamide (13.5 g, 0.1 mol) was added to it. After 5 minutes, the dropper funnel was replaced by air condenser with guarding tube at its end, and the mixture was heated for 22 hours on oil bath at 85–90°C. Then it was cooled to room temperature, poured into a beaker containing 400mL crushed ice water, and stirred for 20 minutes. The yellow solid product was collected by suction filtration and washed with 100 mL cold water. The crude yield was 11.5 g (60%) and was recrystallized from ethyl acetate. The final pure product was yellow crystal and Yield was 9.3 g(48.5%); mp 146–148°C; R_f = 0.22 (n-hexane : EtOAc = 9 : 1); UV-Vis (MeOH); λ_{max} = 280 nm; IR (ν cm^{-1} , KBr): 3035 (CH-arom.), 1693 (C=O aldehyde), 1621 (quinoline C=N str.), 599 (aromatic C=C str.); 1H NMR (400 MHz, $CDCl_3$) δ_H 7.65 (1H, m, H-6), 7.88 (1H, m, H-7), 7.97 (1H, d, J = 8.25 Hz, H-8), 8.07 (1H, d, J = 8.25 Hz, H-5), 8.74 (1H, s, H-4), and 10.54 (1H, s, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 126.3 (C-3), 126.5 (C-8), 128.2 (C-6), 128.6 (C-4a), 129.7 (C-5), 133.6 (C-7), 140.3 (C-4), 149.6 (C-8a), 150.1 (C-2), and 189.1 (C-9).

3.8.1.3 Synthesis of 2-methoxyquinoline-3-carbaldehyde (197)

A 100 mL two-neck round-bottom flask was charged with methanol (10 mL), *N,N*-dimethylformamide (10 mL), 2-chloroquinoline-3-carbaldehyde (0.5 g, 0.0026 mol), and potassium carbonate (0.67 g, 0.0048 mol); the mixture was refluxed using water condenser for 6 hours; and the progress of reaction was monitored by TLC. After completion of the reaction, methanol was removed by distillation, and the remaining DMF mixture allowed to cool to room temperature, and added to 100mL ice cold water. The solid product was collected by suction filtration and washed with excess ice-cold water. The amount of product was 0.44 g (90.2%). Gray powder; mp 106–108°C; R_f = 0.32 (n-hexane:EtOAc = 9 : 1; UV-Vis $\lambda_{\max}$ (MeOH) 295 nm; IR(ν cm⁻¹, KBr): 3065.4 (aromatic C-H str.), 2919.3 (aliphatic C-H str.), 2847 (aliphatic C-H str.), 1673 (aldehyde C=O str.), 1620 (quinoline C=N str.), 1599 and 1579 (aromatic C=C str.); ¹H NMR (400 MHz, CDCl₃) δ_H 4.22 (3H, s, H-10), 7.45 (1H, t, J = 7.3 Hz, H-7), 7.76 (1H, t, J = 7.7 Hz, H-6), 7.85 (2H, m, H-5, H-8), 8.60 (1H, s, H-4), and 10.49 (1H, s, H-9); ¹³C NMR (100 MHz, CDCl₃) δ_C 55.9 (C-10), 120.0 (C-3), 124.4 (C-6), 125.1 (C-4a), 127.1 (C-8), 129.8 (C-5), 132.6 (C-7), 140.0 (C-3), 149.0 C-8a), 161.2 (C-2), and 189.4 (C-9).

3.8.1.4 Synthesis of 2-Ethoxyquinoline-3-carbaldehyde (198)

A 100 mL two-neck round-bottom flask was charged with 2-chloroquinoline-3-carbaldehyde (0.5 g, 0.0026 mol), potassium carbonate (0.6 g, 0.0044 mol), ethanol (10 mL), and *N,N*-dimethylformamide (10 mL), and the necks were fitted with water condenser and stopper. The mixture was refluxed for 5 hours and the progress of the reaction was monitored with TLC. At the end, the ethanol was removed by distillation, and the remaining cold mixture was poured into 100 mL crushed ice water. The solid mass was collected by suction filtration. Yield was 0.35 g (67.3%); white powder; mp 63–65°C, R_f = 0.38 (n-hexane: EtOAc = 9: 1), UV-Vis $\lambda_{\max}$ (MeOH) 300 nm; IR (ν cm⁻¹, KBr) 3429 (OH str.), 3055 (aromatic C-H str.), 2930.9 (aliphatic C-H str.), 2874.45 (aliphatic C-H), 1683 (C=O str.), 1621 (quinoline C=N str.), 1589.6 and 1566 (aromatic C=C str.); ¹H NMR (400 MHz, CDCl₃) δ_H 1.53 (3H, t, J = 7.05 Hz, H-11), 4.89 (2H, q, J = 7.05 Hz, H-10), 7.43 (1H, t, J = 7.3 Hz, H-7), 7.72 (1H, t, J = 7.3 Hz, H-6) 7.84 (2H, d, J = 8.37 Hz, H-8, H-5), 8.59 (1H, s, H-4), and 10.61 (1H, s, H-9); ¹³C NMR (100 MHz, CDCl₃) δ_H 14.5 (C-11), 62.4 (C-10) 120.0 (C-3), 124.3 (C-4a), 124.9 (C-6), 127.3 (C-8), 129.8 (C-5), 132.5 (C-7), 139.7 (C-4), 149.1 (C-8a), 161.2 (C-2), and 189.07 (C-9).

3.8.1.5 Synthesis of 2-thiocyanatoquinoline-3-carbaldehyde (199)

Potassium thiocyanate (0.25 g, 0.0026 mol), 2-chloro-8-methyl quinoline-3-carbaldehyde (0.5 g, 0.0026 mol), and potassium carbonate (0.65 g, 0.0047 mol) were added to 100 mL two-neck round-bottom flask containing N,N-dimethylformamide (20 mL). A water condenser was placed on one of its neck, and the other was closed with glass stopper and then refluxed for 3 hours, and the progress of the reaction was monitored by TLC. The system was cooled to room temperature and poured into 50 mL crushed ice water. The solid product was collected with suction filtration and washed with 10 mL cold water. Yield was 0.43g (83.6%); orange powder; mp 134–136°C; $R_f = 0.44$ (n-hexane: EtOAc = 2 : 1); UV-Vis $\lambda_{\max}$ (MeOH) 255 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3045 (aromatic C-H str.), 2919.9 (aliphatic C-H str.), 2182 (cyanide S-C $\equiv$ N str.), 1683 (aldehyde C=O str.), 1610 (quinoline C=N str.), 1572 (aromatic C=C str.), 1512 and 1340 (O=N-O str.); ^1H NMR (400 MHz, CDCl_3) δ_{H} 7.50 (1H, *d*, *J* = 7.8 Hz, H-8), 7.61 (1H, *d*, *J* = 8.9 Hz, H6), 7.72 (1H, *d*, *J* = 8.8 Hz, H-7), 7.85 (1H, *d*, *J* = 8.9 Hz, H-5), 8.74 (1H, *s*, H-4), 10.65 (1H, *s*, H-9); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} 87.6 (C-10), 121.1 (C-3, C-6), 122.4 (C-6, C-8), 125.8 (C-4), 127.7 (C-5), 129.7 (C-7), 132.8 (C-8a), 135.6 (C-4a), 163.6 (C-2), and 188.9 (C-9).

3.8.1.6 Synthesis of 2-chloroquinoline-3-carboxylic Acid (200)

Sodium hydroxide solution (1 mL, 10%) was added to a suspension of 2-chloroquinoline-3-carbaldehyde (0.5 g, 0.0026 mol) in water (20 mL). Then a saturated solution of potassium permanganate in water was added dropwise until a definite purple color remains after shaking the solution. The mixture was acidified with 10% sulfuric acid, and a saturated oxalic acid was added to destroy the excess permanganate solution. The carboxylic acid precipitate was isolated by suction filtration. Yield was 0.34 g (59.3%); white powder, mp 202–204°C; $R_f = 0.63$ (EtOAc: dichloromethane: methanol = (3: 2: 3); UV-Vis $\lambda_{\max}$ (MeOH) 280 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3440–2483 (broad –OH str. of acid), 1725 (C=O str), 1621 (quinoline C=N str.), 1540 and 1496.6 (aromatic C=C str.); ^1H NMR (400 MHz, DMSO-d_6) δ_{H} 7.72 (1H, *t*, *J* = 7.0 Hz, H-6), 7.92 (1H, *t*, *J* = 7.0 Hz, H-7), 7.98 (1H, *d*, *J* = 7.8 Hz, H-8), 8.15 (1H, *d*, *J* = 7.8 Hz, H-5), 8.93 (1H, *s*, H-4); ^{13}C NMR (100 MHz, DMSO-d_6) δ_{C} 130.65 (C-3), 131.10 (C-4a), 132.83 (C-8), 133.21 (C-5), 134.50 (C-6), 137.92 (C-7), 146.93 (C-4), 151.62 (C-8a), 152.5 (C-2), and 170.90 (C-9).

3.8.1.7 Synthesis of 4-nitrophenol (202)

Concentrated sulfuric acid (5.5 mL) was added to a 100 mL beaker containing water (7.5 mL). To the solution, finely powdered p-nitroaniline (3.5 g, 0.025 mol) and 15 g of finely crushed ice were added and stirred until the p-nitroaniline converted into a homogeneous paste. It was cooled to 0–5°C by immersion of the beaker in crushed ice. A cold solution of sodium nitrite (1.8 g) in 4 mL of water was added to it dropwise while keeping temperature below 5°C during the diazotization process. While the diazotization reaction was in progress, a mixture of 16.5 mL concentrated sulfuric acid in 15 mL of water was heated to just boiling in 100 mL round flask, and the diazonium solution was added to it dropwise within 5 minutes. After additional 5 minutes of boiling, the mixture was cooled in ice bath while being stirred continuously. The solid mass was collected by suction filtration and washed with 10 mL of cold water. The crude product was recrystallized in 6M hydrochloric acid. The yield of yellow crystal product was 2.1 g (60%). The melting point was 112–113°C. Purity was analyzed with TLC, and its authenticity was ascertained by comparing its melting point with literature value (literature value is 113.8°C).

3.8.1.8 Synthesis of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (203)

p-Nitrophenol (0.36 g, 0.0026 mol), 2-chloro-8-methylquinoline-3-carbaldehyde (0.5 g, 0.0026 mol), potassium carbonate (0.67 g, 0.0047 mol), and moist copper powdered (0.1 g, 0.0016) were added to a 100 mL two-neck round-bottom flask containing *N,N*-dimethylformamide (20 mL). A water condenser was fitted into one of the necks, and the other one was closed with glass stopper. The mixture was refluxed for 5 hours with the progress followed by TLC. After being cooled to room temperature, it was added to 100mL crushed ice water. The solid product was separated by suction filtration and washed with 5% sodium hydroxide solution (50 mL). Yield was 0.61 g (81.3%); yellow powder, mp 177–179°C; R_f = 0.67 (n-hexane: EtOAc = 2: 1), UV-Vis λ_{max} (MeOH) 285 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3055 (aromatic C-H str.), 1703 (C=O str.), 1615 (quinoline C=N str.), 1577 (aromatic C=C str.), 1512 and 1340 (O=N-O str.); ^1H NMR: (400 MHz, CDCl_3) δ_{H} 6.97 (2H, *t*, J = 7.4 Hz, H-8, H-9), 7.04 (2H, *d*, J = 8.4 Hz, H-4, H-7), 7.55 (2H, *t*, J = 7.4 Hz, H-1, H-10), 7.96 (2H, *d*, J = 8.0 Hz, H-3, H-11); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} 111.4 (C-11a, C-12a), 117.8 (C-1, C-4, C-9), 119.6 (C-7, C-10, C-10a), 131.0 (C-3, C-8), 137.0 (C-2, C-6a, C-11), 162.2 (C-4a, C-5a), 175.0 (C-12).

3.8.1.9 Synthesis of 2-Methylacetanilide (205)

To a 250 mL round-bottom flask, o-toluidine (22 mL, 22.2 g, 0.207 mol), acetic anhydride (23 mL, 24.84 g, 0.24 mol), acetic acid (23 mL, 24.15 g, 0.40 mol), and zinc powder (0.2 g, 0.003 mol) were added. The mixture was refluxed for an hour using water condenser. It was cooled to room temperature and added to 200 mL crushed ice water. The product was collected by suction filtration. Yield 21.2 g (69%); white powder; mp 108–110°C (Lit. 109–112°C).

3.8.1.10 Synthesis of 2-Chloro-8-methylquinoline-3-carbaldehyde (206)

To a 100 mL round-bottom flask equipped with drying tube, *N,N*-dimethylformamide (20 mL, 18.88 g, 0.26 mol) was added, and the mixture was cooled to 0°C using ice bath. Then phosphorus oxychloride (70 mL, 115.15 g, and 0.75 mol) was added dropwise from dropping funnel guarded by drying tube which was fitted to flask with adaptor while being stirred by magnetic stirrer for 30 minutes. Next, *N*-(o-tolyl)acetamide (13.5 g, 0.1 mol) was added to it, the dropper funnel was replaced by air condenser with guarding tube, and the mixture was heated for 22 hours on oil bath at 85–90°C. After that, it was cooled to room temperature and poured into a beaker containing 400 mL crushed ice water, while being stirred with glass rod. The yellow solid product was collected by suction filtration and washed with cold water. The crude yield was 9.2 g (44.77%), and it was recrystallized from ethyl acetate. The yield of the final of product was 6.8 g (33.09%); pale yellow crystal; mp 136–140°C; $R_f = 0.30$ (n-hexane: EtOAc = 9 : 1), UV-Vis $\lambda_{\max}$ (MeOH) 295 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3223, 3025 (arom-C-H.), 2956 (alip-C-H), 2837 (alip-C-H), 1683.3 (C=O str.), 1621 (quinoline C=N str.), 1579 (aromatic C=C str.); ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.80 (3H, s, H-10), 7.53 (1H, t, H-6), 7.70 (1H, d, H-7), 7.78 (1H, d, H-5), 8.70 (1H, s, H-4), 10.6 (1H, s, H-9); ^{13}C NMR (100 MHz, CDCl_3): δ_{C} 17.8 (C-10), 126.0 (C-3), 126.5 (C-6), 127.5 (C-5), 127.8 (C-4a), 133.6 (C-7), 136.9 (C-8), 140.4 (C-4), 148.7 (C-8a), 149.4 (C-2) and 189.5 (C-9).

3.8.1.11 Synthesis of 8-methyl-2-oxoquinoline-3-carbaldehyde (207)

2-Chloro-8-methylquinoline-3-carbaldehyde (0.5 g, 0.0026 mol) was added to a mixture of 6M HCl (10 mL) and glacial acetic acid (10 mL). The mixture was refluxed for 3 hours, at which the TLC showed complete disappearance of the starting material. Then the acetic acid mixture was removed with distillation under reduced pressure. The solid residue was washed with cold water and allowed to dry in wood cupboard. The yield of the product was 395 mg (79%); yellow

powder; mp 176–178°C; R_f = 0.41 (n-hexane:EtOAc = 1: 1); UV-Vis $\lambda_{\max}$ (MeOH) 375 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3429 (O-H str.), 3179.5 (NH str.), 3023 (aromatic C-H), 2919.8 (aliphatic CH-str'), 2837.2 (aliphatic C-H) 1673 (C=O str.), 1610 (quinoline C=N str.), 1558 and 1465 (aromatic C=C str.); ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.56 (3H, s, C-10), 7.23 (1H, t, J = 7.5 Hz, H-6), 7.51 (1H, d, J = 7.32 Hz, H-7) 7.61 (1H, m, J = 7.7 Hz, H-5), 8.49 (1H, s, H-4) and 10.48 (1H, s, H-9); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} 16.7 (C-10), 117.9 (C-6), 123.3 (C-3, C-8), 129.1 (C-4a, C-7), 135.0 (C-5), 139.1 (C-8a), 144.2 (C-4), 162.7 (C-2), 190.00 (C-9).

3.8.1.12 Synthesis of (2-methoxy-8-methylquinolin-3-yl)methanol (208)

A gram of sodium (0.043 mol) was dissolved in 10mL methanol, and 2-chloro-8-methylquinoline-3 carbaldehyde (0.5 g, 0.0026 mol) and 10mL of *N,N*-dimethylformamide were added to it. The mixture was refluxed for 6 hours, at which TLC analysis showed complete disappearance of the aldehyde. After removal of methanol using distillation, the remaining content was cooled to room temperature and added to 100mL ice-cold water. The precipitate was collected by suction filtration. Yield of the product was 0.26 g (53%); gray powder, mp 98–99°C; R_f = 0.51 (n-hexane: EtOAc = 3 :1), UV-Vis $\lambda_{\max}$ (MeOH) 315 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr): 3470 (br-alcohol CHO-H str.), 3013 (aromatic C-H str.), 2930.9 (aliphatic C-H str.), 1625 (quinoline C=N str.), 1589.6 and 1475.5 (aromatic C=C str.); ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.80 (3H, s, H-10), 4.11 (3H, s, H-9), 4.79 (2H, s, H-1), 7.28 (1H, t, J = 7.58 Hz, H-6), 7.42 (1H, d, J = 7.0 Hz, H-7), 7.58 (2H, d, J = 8.34.Hz, H-5), 7.86 (1H, s, H-4); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} 18.1 (C-10), 51.3 (C-9), 61.9 (C-1), 123.8 (C-3), 124.1 (C-6), 124.2 (C-4a), 125.0 C-5), 129.4 (C-7), 135.1 (C-8), 135.9 (C-4), 144.7 (C-8a), 160.2 (C-2). The product was washed repeated with hexane and some hexane impurity was observed on NMR spectra of **208**.

3.8.1.13 Synthesis of (2-ethoxy-8-methylquinolin-3-yl)methanol (209)

Sodium (1 g, 0.043 mol) was dissolved in ethanol (10 mL), and 0.5 g (0.0024 mol) of 2-chloroquinoline-3-carbaldehyde and 10mL of *N,N*-dimethylformamide were added to it. The mixture was refluxed for 5 hours, at which TLC showed complete disappearance of the aldehyde. The ethanol was removed by distillation, and the remaining content was cooled to room temperature and added to 100 ml cold water. The precipitate was collected by suction filtration. Yield was 0.43 g (82.7%); dull brown; mp 85–87°C; R_f = 0.54 (n-hexane: EtOAc = 3: 1); UV-Vis $\lambda_{\max}$ (MeOH) 315 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3502–3148 (OH str.), 3025 (aromatic C-

H str.), 2930.9 (aliphatic C-H str.), 1625 (quinoline C=N str.), 1582 (aromatic C=C str.); ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.5 (3H, *t*, *J* = 7.01 Hz, H-10), 2.74 (3H, *s*, H-11) 4.63 (2H, *q*, *J* = 7.01 Hz, H-9), 4.48 (2H, *s*, H-1), 7.26 (1H, *t*, *J* = 7.04 Hz, H-6), 7.47 (1H, *d*, *J* = 6.89 Hz, H-7), 7.56 (1H, *d*, *J* = 7.80 Hz, H-5), 7.93 (1H, *s*, H-4); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} 13.7 (C-10), 19.3 (C-11), 61.5 (C-1), 61.8 (C-9), 123.0 (C-9), 124.2 (C-6), 124.90 (C-4a), 125.0 (C-5), 130.3 (C-7), 135.0 (C-8), 135.8 (C-4), 144.7 (C-8a), 158.3 (C-2).

3.9 Synthesis of the stilbenes and pinacol of quinolines

Schemes 33-35 portrayed the synthesis of stilbenes and pinacol of quinolones.

3.9.1 Synthesis of (Z)-2-chloro-3-styrylquinoline (211)

A solution prepared by dissolving 0.4g pellets of KOH in 1 mL water was added dropwise to a mixture of 2-chloroquinoline-3-carbaldehyde (0.5g, 2.6 mmol) and benzyltriphenylphosphonium chloride (1.01g, 2.6 mmol) dissolved in 10 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. The stirring was continued for 5 hrs at which TLC analysis showed the complete consumption of 2-chloroquinoline-3-carbaldehyde. Then the mixture was poured to 100 mL crushed ice water and the precipitate was collected with suction filtration. The crude product was (0.62 g, 89.8%). The product (300 mg) was purified using silica gel column chromatography (*n*-hexane: acetate 9:1) as an eluent. Yield was (106.5 mg, 35.5%); white powder; mp 58-60°C; R_{f} = 0.63 (*n*-hexane:EtOAc = 9:1); UV-Vis λ_{max} (MeOH) = 375 nm; IR (ν cm^{-1} , KBr): 3035 (CH-arom.), 1635 (C=C alkene), 1621 (quinoline C=N str.), 599 (aromatic C=C str.); ^1H NMR (400 MHz, DMSO- d_6): δ_{H} 6.72 (1H, *d*, *J* = 12.25 Hz, H-10), 6.91 (1H, *d*, *J* = 12.25 Hz, H-9), 7.16 (2H, *m*, H-4', H-5'), 7.44 (2H, *m*, H-6, H-7), 7.56 (1H, *m*, H-3'), 7.66 (1H, *m*, H-2'), 7.78 (2H, *m*, H-5, H-6'), 7.94 (1H, *t*, *J* = 7.45 Hz, H-8) and 8.14 (1H, *s*, H-4); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 125.7 (C-9), 127.2 (C-4), 127.4 (C-5), 127.9 (C-6), 128.0 (C-3), 128.3 (C-4'), 128.4 (C-8), 129.0 (C-2', C-6'), 129.1 (C-3', C-5'), 131.2 (C-10), 133.7 (C-4), 135.9 (C-7), 138.9 (C-11), 146.6 (C-8a), and 149.9 (C-2).

3.9.2 Synthesis of (Z)-3-styrylquinolin-2(1H)-one (213)

Firstly, 2-oxo-1,2-dihydroquinoline-3-carbaldehyde was prepared by refluxing 2-Chloroquinoline-3-carbaldehyde (1.0 g, 5.2 mmol) in a mixture of 6M HCl (10 mL) and glacial acetic acid (15 mL) with occasional monitoring with TLC. After 3 hours, the excess acetic acid

was removed by distillation under reduced pressure. Then the residue was added into 50 mL crushed ice cold water and the precipitate was collected by suction filtration, washed three times with 10 mL cold water, and allowed to dry in a wood cupboard. Aqueous solution of KOH (1 mL, 40%) was added dropwise in to the mixture of 2-oxo-1,2-dihydroquinoline-3-carbaldehyde (0.45 g, 2.6 mmol) and benzyltriphenylphosphonium chloride (1.01g, 2.6 mmol) dissolved in 10 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. After stirring for 4 hrs, the mixture was poured to a 100 mL crushed ice water and the precipitate was collected with suction filtration. The crude product (0.55g, 85%) was purified by silica gel (50 g) column chromatography using (*n*-hexane: ethyl acetate (8:1) as an eluent. Yield, 40.5%; dull yellow powder; mp 178-180°C; R_f = 0.29 (*n*-hexane:EtOAc = 6:1); UV-Vis $\lambda_{\max}$ (MeOH) = 400 nm; IR (νcm^{-1} , KBr) 3429 (N-H str.), 3023 (aromatic C-H), 1673 (alkene C=C str.), 1610 (quinoline C=N str.), 1558 and 1465 (aromatic C=C str.); ^1H NMR (400 MHz, DMSO- d_6): δ_{H} 6.56 (1H, *d*, J = 12.55 Hz, H-9), 6.75 (1H, *d*, J = 12.55 Hz, H-10), 7.09 (1H, *t*, J = 7.07 Hz, H-6), 7.23 (1H, *d*, J = 7.05 Hz, H-7), 7.32 (5H, *m*, H-2', H-3', H-4', H-5', H-6'), 7.44 (1H, *d*, J = 6.64 Hz, H-5), 7.59 (1H, *d*, J = 7.3 Hz, H-8). 7.66 (1H, *s*, H-4) and 11.97 (1H, *s*, H-1); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 115.3 (C-8), 119.3 (C-4a), 122.4 (C-3), 125.4 (C-4), 127.0 (C-6), 127.9 (C-9), 128.9 (C-2', C-6'), 129.0(C-3'. C-5'), 129.3 (C-4'), 130.6(C-7), 132.2 (C-5), 136.9(C-1'), 137.5(C-10), 138.7 (C-8a) and 161.8 (C-2)

3.9.3 Synthesis of (Z)-2-methoxy-3-styrylquinoline (214)

Aqueous solution of KOH (1 mL, 40%) was added dropwise to a mixture of 2-methoxyquinoline-3-carbaldehyde (0.50 g, 2.6 mmol) synthesized by the above procedure (Section 2.5.1.3.) and benzyltriphenylphosphonium chloride (1.01g, 2.6 mmol) dissolved in 10 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. The stirring was continued for 8 hrs at which TLC analysis showed the complete consumption of the reactant. Then, the mixture was poured to 100 mL crushed ice water and the precipitate was collected with suction filtration. The crude product (0.6 g, 88%) was purified by silica gel (50 g) column chromatography using *n*-hexane:ethylacetate (9:1) as an eluent. Yield was 0.192g (28.3%); gray powder; mp 138-140°C; R_f = 0.52 (*n*-hexane:EtOAc = 9:1); UV-Vis $\lambda_{\max}$ (MeOH) = 322 nm; IR($\nu\text{ cm}^{-1}$, KBr): 3065.4 (aromatic C-H str.), 2919.3 (aliphatic C-H str.), 2847 (aliphatic C-H str.), 1630 (alkene C=C str.), 1620 (quinoline C= N str.), 1599 and 1579 (aromatic C=C str.); ^1H NMR (400 MHz, CDCl_3): δ_{H} 3.97 (3H, *s*, -OCH₃), 6.65 (1H, *d*, J =

11.96 Hz, H-10), 6.78 (1H, *d*, *J* = 11.96 Hz, H-9), 7.21 (4H, *m*, H-3, H-5', H-4', H-6), 7.34 (1H, *m*, H-6'), 7.42 (1H, *m*, H-2'), 7.61 (2H, *m*, H-7, H-8), 7.78 (1H, *d*, H-5) and 7.91 (1H, *s*, H-4); ¹³C NMR (100 MHz, CDCl₃): δ_C 54.1(-OCH₃), 122.1 (C-3), 124.6 (C-4a), 124.7 (C-9), 126.9 (C-2'), 127.1 (C-8), 127.9 (C-5), 128.0(C-6'), 128.8 (C-6, C-5', C-3'), 129.29 (C-4'), 130.0 (C-10), 132.7 (C-4), 136.7 (C-7), 137.7 (C-1'), 145.6 (C-8a), and 160.2 (C-2)

3.9.4 Synthesis of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215)

2-Chloroquinomoline-3-carbaldehyde (0.5 g, 2.6 mmol) was added to 10 mL 2-aminoethan-1-ol in a 100 mL round bottom flask and heated to 90-95°C for an hour in a water bath. The completion of the reaction was monitored by TLC. The resulting mixture was cooled to room temperature and added to 100 mL cold ice water. The precipitate was separated by suction filtration and washed with 20 mL cold water. Yield was 0.59g, 84%; yellow powder; mp 80-82°C; R_f = 0.25 (*n*-hexane: Methanol = 7:3); UV-Vis λ_{max} (MeOH) = 390 nm; IR(ν cm⁻¹, KBr): 3525-3510 (O-H and N-H), 3065.4 (aromatic C-H str.), 2919.3 (aliphatic C-H str.), 2847 (aliphatic C-H str.), 1623 (imine C=N str.), 1620 (quinoline C= N str.), 1599 and 1579 (aromatic C=C str.) ¹H NMR (400 MHz, DMSO-d₆): δ_H 3.65 (8H, *d*, H-11, H-12, H-14, and H-15), 4.72 (1H, *s*, OH), 4.92 (1H, *s*, OH), 7.19 (1H, *t*, *J* = 7.25Hz, H-6), 7.55(2H, *m*, H-5, H-8), 7.72 (1H, *d*, *J* = 8.36 Hz, H-7), 8.21 (1H, *s*, H-4), 8.5(1H, *s*, H-9), and 9.55 (1H, *s*, NH); ¹³C NMR (100 MHz, DMSO-d₆): δ_C 43.4(C-14), 60.5 (C-12), 61.2(C-15), 63.7(C-11), 117.2 (C-3), 121.9 (C-8), 122.4(C-4a), 125.7 (C-5), 128.9(C-6), 131.5 (C-7), 143.0 (C-4), 148.3(C-8a), 155.4 (C-2), and 163.8 (C-9) ; DEPT-135 δ_C 43.4(C-14 negative), 60.5 (C-12 negative), 61.2(C-15 negative), 63.7 negative (C-11), 121.9 (C-8), 125.7 (C-5), 128.9(C-6), 131.5 (C-7), 143.0 (C-4), and 163.8 (C-9)

3.9.5 Synthesis of (Z)-2-chloro-8-methyl-3-styrylquinoline (216)

1 mL 40% aqueous KOH was added dropwise to a mixture of 2-Chloro-8-methylquinoline-3-carbaldehyde (0.53g, 2.6 mmol) synthesized by the procedure in section 2.5.1.10 and benzyltriphenylphosphonium chloride (1.01g, 2.6 mmol) dissolved in 10 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. The stirring was continued for 8 hrs at which TLC analysis showed the disappearance of the reactant. Then the mixture was poured to 100 mL crushed ice water and the precipitate was collected with suction filtration.

The crude product was 0.64g (88.2%) was purified over silica gel (50g) column chromatography using *n*-hexane:ethyl acetate (9:1) as an eluent. Yield 33%; a yellow gummy; $R_f = 0.7$ (*n*-hexane:EtOAc = 9:1); UV-Vis $\lambda_{\max}$ (MeOH) = 375 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3025 (arom-C-H.), 2956 (alip-C-H), 2837 (alip-C-H), 1630 (alkene C=C str.), 1621 (quinoline C=N str.), 1579 (aromatic C=C str.); ^1H NMR (400 MHz, DMSO- d_6): δ_{H} 7.36 (1H, *m*, H-4'), 7.44 (4H, *m*, H-5', H-3', H-9, H-10), 7.52 (H, *dd*, H-6'), 7.61 (1H, *dt*, H-2'), 7.66 (2H, *m*, H-6, H-7), 7.85 (1H, *dd*, H-5), 7 and 8.78 (1H, *s*, H-4); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 17.5 (C-11), 123.1 (C-6), 126.4 (C-6), 126.4 (C-9), 127.4 (C-5, C-8) 127.7. (C-9), 127.8 (C-4'), 128.1 (C-6'), 129.4 (C-3', C-5'), 129.7 (C-4a), 131.1(C-2'), 133.9 (C-7), 135.4(C-4), 135.8(C-8), 136.8 (C-1'), 145.7 (C-2) and 148.7 (C-8a).

3.9.6 Synthesis of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)

Aqueous solution of KOH (1 mL, 40%) was added dropwise to a mixture of 8-methyl-2-oxo-1,2-dihydroquinoline-3-carbaldehyde (0.50 g, 2.6 mmol) prepared by procedure in section 2.5.1.11 and benzyltriphenylphosphonium chloride (1.01g, 2.6 mmol) in 10 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. The stirring was continued for 7 hrs at which TLC analysis showed the complete consumption of the reactant. Then the mixture was poured to 100 mL crushed ice water and the precipitate was collected with suction filtration. The crude product was 0.55g (85%) was purified by silica gel (50 g) column chromatography using solvent *n*-hexane: ethylacetate 8:1 as an eluent. Yield 36.8%. dull yellow powder; mp 60-62°C; $R_f = 0.38$ (*n*-hexane:EtOAc = 6:1); UV-Vis $\lambda_{\max}$ (MeOH) = 375 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr) 3429 (N-H str.), 3023 (aromatic C-H), 2919.8 (aliphatic CH-str'), 2837.2 (aliphatic C-H) 1630 (alkene C=C str.), 1610 (quinoline C=N str.), 1558 and 1465 (aromatic C=C str.) ^1H NMR (400 MHz, DMSO- d_6): δ_{H} 2.56 (3H, *s*, C-11), 7.11 (1H, *t*, $J = 8.46$ Hz, H-6), 7.24-7.42(5H, *m*, H-10, H-7, H-5, H-3', H-5'), 7.69 (4H, *m*, H-2', H-4', H-6', H-9), 8.23 (1H, *s*, H-4) and 11.10 (1H, *s*, H-1); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 17.7 (C-11), 119.9 (C-8), 122.37 (C-6), 123.4 (C-4), 123.6 (C-5), 123.7 (C-9), 126.5(C-4'), 127.0 (C-2', C-6'), 128.3 (C-4a), 128.4 (C-3', C-5'), 131.3 (C-7), 133.7 (C-3). 136.1 (C-10), 137.7 (C-1'), 136.7 (C-8a) and 162.1 (C-2)

3.9.7 Synthesis of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (219)

8-methyl-2-((4-nitrophenyl)amino)quinoline-3-carbaldehyde was synthesized by adding *p*-Nitroaniline (0.36 g, 0.0026 mol), 2-chloro-8-methylquinoline-3-carbaldehyde (0.5 g, 0.0026

mol), and potassium carbonate (0.67 g, 0.0047) to a 100 mL two-neck round-bottom flask containing *N,N*-dimethylformamide (20 mL). A water condenser was fitted into one of the necks, and the other one was closed with a glass stoppers. The mixture was refluxed for 5 hours, meanwhile the progress of the reaction was attended by TLC. After being cooled to room temperature, it was added to 100 mL crushed ice water. The solid product was separated by suction filtration and washed with 20 mL 5% HCl solution (50 mL) and allowed to dry in air. Then aqueous solution of KOH (1 mL, 40%) was added dropwise to the mixture of 8-methyl-2-((4-nitrophenyl)amino)quinoline-3-carbaldehyde (0.68g, 2.2 mmol) and benzyltriphenylphosphonium chloride (0.86g, 2.2 mmol) dissolved in 15 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. The stirring was continued overnight until TLC analysis showed the complete conversion of the reactant. Then, the mixture was poured to 150 mL crushed ice water and the precipitate was separated by suction filtration and washed with 30 mL cold water. The crude product (0.76 g, 90.9%) was purified by silica gel (50 g) column chromatography using (*n*-hexane: ethylacetate (7:1) as an eluent. Yield was 0.099g (10.8%). Pink powder; mp 150-152°C; R_f = 0.61 (*n*-hexane:EtOAc = 7:3); UV-Vis λ_{max} (MeOH) 315 nm, 319 nm; IR (vcm⁻¹, KBr) 3055 (aromatic C-H str.), 1620 (alkene C=C str.), 1615 (quinoline C=N str.), 1577 (aromatic C=C str.), 1512 and 1340 (O=N-O str.); ¹H NMR (400 MHz, DMSO-d₆): δ 2.36 (3H, s, -CH₃), 6.78 (1H, *d*, *J* = 12.75 Hz, H-10), 6.88 (1H, *d*, *J* = 12.75 Hz, H-9), 7.21-7.78 (22H, *m*, H-5, H-6, H-7, H-9, H-12, H-13, H-14, H-15, H-16, H-18, H-22 and their stereoisomers), 8.14 (1H, *d*, H-6'), 8.26 (2H, *m*, H-19 and its stereoisomer), 8.33 (2H, *m*, H-21 and its stereoisomers) and 8.72 (1H, s, NH), ¹³C NMR (100 MHz, DMSO-d₆): δ_C 17.4(-CH₃), 121.1 (C-9), 122.3(C-18, C-21), 122.7 (C-6), 123.6 (C-5), 127.3 (C-19, C-22), 128.1 (C-14), 128.8 (C-12, C-16), 129.3 (C-13, C-15), 130.5 (C-10), 133.8 (C-7), 135.0 (C-3, C-4a), 136.9(C-8, C-11), 137.1 (C-20), 140.0 (C-4), 143.6 (C-17), 157.2 (C-8a) and 159.2 (C-2) and peaks due to their stereoisomers were also seen.

3.9.8 Synthesis of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222)

First, (4-((3-formyl-8-methylquinolin-2-yl)methyl)phenyl)acetamide was prepared by mixing *N*-(4-hydroxyphenyl)acetamide (0.36 g, 2.4 mmol), 2-chloro-8-methylquinoline-3-carbaldehyde (0.5 g, 2.4 mmol), and potassium carbonate (0.67 g, 4.7 mmol) to a 100 mL round-bottom flask containing 20 mL DMF. An air condenser was fitted to neck. The mixture

was refluxed for 5 hours and the disappearance of the reactant was monitored by TLC. It was cooled to room temperature and poured to 100 mL crushed ice water. The precipitate was separated by suction filtration and washed with 50 mL of 5% sodium hydroxide solution and allowed to dry in air. Then aqueous solution of KOH (1 mL, 40%) was added dropwise to a mixture of N-(4-((3-formyl-8-methylquinolin-2-yl)methyl)phenyl)acetamide (0.64g, 2.0 mmol) and benzyltriphenylphosphonium chloride (0.78g, 2.0 mmol) dissolved in 15 mL DMF in 100 mL round bottom flask while being stirred with magnetic stirrer. The stirring was continued for 16 hours at which TLC analysis showed the complete conversion of the reactant. Then the mixture was poured to 150 mL crushed ice water and the precipitate was separated by suction filtration and washed with 30 mL cold water. The crude product 0.72g (91.1%) was purified by silica gel (50 g) column chromatography (with n-hexane: ethyl acetate (7:4) as eluent. Yield was 0.180 g (19.1%); white powder; mp 178-180°C; R_f = 0.42 (n-hexane:EtOAc = 7:5); UV-Vis λ_{max} (MeOH); IR ($\nu_{cm^{-1}}$, KBr) 3281.8 (N-H str), 3055 (aromatic C-H str.), 1645.7 (NHC=O) 1620 (alkene C=C str.), 1615 (quinoline C=N str.), 1577 (aromatic C=C str.); 1H NMR (400 MHz, DMSO-d₆): δ_H 2.3 (3H, s, H-24) 2.33 (3H, s, 25), 6.81 (1H, d, J = 12.75 Hz, H-10), 6.89 (1H, d, J = 12.75 Hz, H-9), 7.08(2H, d, J = 8.97 Hz, H-18, H-22), 7.25 (5H, m, H-6, H-13, H-14, H-15, H-16), 7.42 (1H, m, H-12), 7.63(2H, m, H-21, H-19), 7.68 (1H, d, J = 8.97, H-5), 8.06(1H, s, H-4) and 8.66 (NH); ^{13}C NMR (100 MHz, DMSO-d₆): δ_C 17.4(C-25), 24.4 (C-24), 120.0 (C-8, C-22), 122.3(C-19, C-21), 124.2 (C-6), 125.1(C-5), 125.5 (C-3, C-4a), 127.2 (C-9), 128.0 (C-28), 128.9 (C-12, C-16), 129.3 (C-13), 130.3 (C-15), 133.3 (C-7), 134.7(C-20) 136.4 (C-8), 137.0 (C-11), 139.2 (C-10), 143.9 (C-17), 148.9 (C-8a), 158.6 (C-2) and 168.6 (C-23).

3.9.9 Synthesis of methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224)

2-methoxy-8-methylquinoline-3-carbaldehyde (6 g, 3 mmol), aluminum powder (0.16 g, 6 mmol) and potassium hydroxide 1.1g, 18 mmol) were added to 10 mL methanol in 100 mL round bottom flask closed with a glass stopper. The flask was mounted over a magnetic stirrer and the reaction mixture was stirred for 12 hours. The progress of the reaction was monitored by TLC. The reaction mixture was filtered to remove unreacted aluminum powder and 50 mL water was added to the filtrate. The precipitate was collected by suction filtration and air dried. The crude product (0.53 g) was charged to a silica gel (50g) column chromatography using *n*-

hexane: ethylacetate (8:1) as eluent. Yield 16.1%. White powder; mp 202-204°C; $R_f = 0.4$ (*n*-hexane: EtOAc = 7:3); UV-Vis $\lambda_{\max}$ (MeOH) 325 nm; IR ($\nu_{\text{cm}^{-1}}$, KBr): 3470 (br-alcohol -CHO-H str.), 3013 (aromatic C-H str.), 2930.9 (aliphatic C-H str.), 1625 (quinoline C=N str.), 1620 (aromatic C=C str.) 1589.6 and 1475.5 (aromatic C=C str.); ^1H NMR (400 MHz, DMSO- D_6): δ_{H} 2.65 (3H, s, H-10), 3.99 (3H, s, H-11), 5.20 (1H, *d*, $J = 7.51$ Hz, H-9), 5.25 (1H, *d*, $J = 7.51$ Hz, H-12), 7.31 (1H, *t*, $J = 8.13$ Hz, H-6), 7.51 (1H, *d*, $J = 8.11$ Hz, H-7), 7.72 (1H, *d*, $J = 8.11$ Hz, H-5), and 8.28 (1H, s, H-4); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 17.3 (C-10), 52.9 (C-11), 69.2 (C-9), 117.2 (C-6), 124.79 (C-3), 125.33 (C-5), 127.1 (C-4a), 128.9 (C-7), 133.8 (C-8), 136.7 (C-4), 143.6 (C-8a), and 158.26 (C-2).

3.10 Synthesis of Novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs

The synthesis of [2,3'-biquinoline]-4-carboxylic acid and quinoline-3-carbaldehyde analogs was outlined in Scheme 37, 38 and 39.

3.10.1 Synthesis

2-Chloroquinoline-3-carbaldehyde and 2-chloro-8-methylquinoline-3-carbaldehyde were prepared by literature report method. (Meth-Cohn et al. 1981; Zehiroglu and Ozturk Sarikaya 2019)

3.10.1.1 Synthesis of 2-phenylquinoline-4-carboxylic acid (62)

Benzaldehyde (2 mL, 0.020 mol), and pyruvic acid (1.5 mL, 0.020 mol) were added to glacial acetic acid (15 mL) and the mixture was refluxed for an hour with stirring. To the mixture, aniline (1.9 mL, 0.02 mol) was added and refluxed for additional 10 hours. It was cooled to room temperature and basified with 5% aqueous NaOH. Then it was filtered by gravity filtration and the filtrate was acidified with 5% aqueous HCl. It was cooled in an ice bath. The precipitate was collected by suction filtration. The crude product was recrystallized from methanol. The yield was 3.10 g (62.2%); Yellow powder; mp 212-214°C: IR ($\nu_{\text{cm}^{-1}}$, KBr): 3389 (*br*, acid-OH.), 3040 (aromatic C-H), 2927 (C-H str.), 2848 (C-H str.), 1709 (acidic C=O), 1607 (aromatic C=C), 1584; ^1H NMR (400 MHz, DMSO- d_6): δ_{H} 7.56 (3H, *m*, H-3', H-4', H-5'), 7.72 (1H, *t*, $J = 7.02$ Hz, H-6), 7.87 (1H, *t*, $J = 7.02$ Hz, H-7), 8.25 (3H, *m*, H-8, H-2', H-6'), 8.44 (1H, s, H-3) and 8.63 (1H, *d*, $J = 7.91$ Hz, H-5); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 119.9 (C-3), 123.9 (C-5), 125.9 (C-4a), 128.0 (C-2', C-6'), 128.6 (C-6), 129.3 (C-8) 129.5 (C-3', C-

5'), 130.8 (C-4'), 131.2(C-7), 137.5 (C-4), 131.1 (C-7), 139.0 (C-1'), 148.8(C-8a), 156.2(C-2), and 167.8(C-9).

3.10.1.2 Synthesis of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225)

2-chloroquinoline-3-carbaldehyde (0.38 g, 0.002 mol) and pyruvic acid (0.15 mL, 0.002 mol) were added to 15 mL glacial acetic acid in 100 mL round bottom flask. The mass was mounted on a magnetic stirrer and refluxed for an hour while being stirred. Aniline (0.17 mL, 0.002 mol) was added to the reaction mixture and then refluxed for additional 8 hours. The mixture was cooled to room temperature and the precipitate was separated by suction filtration. The crude yield (569 mg, 85%) was purified over silica gel column chromatography with CH₂Cl₂:MeOH (9:1) as eluent. Yield 54%; Yellow powder; mp 204-206°C; IR(ν cm⁻¹, KBr): 3423- 2521 (*br*, acid-OH.), 2979 (C-H str.), 2838 (C-H str.), 1675 (acidic C=O), 1584 (aromatic C=C); ¹H NMR (400 MHz, DMSO-d₆): δ_{H} 7.26 (1H, *t*, *J* = 7.21 Hz, H-6'), 7.45 (1H, *m*, *J* = 7.25 Hz, H-6), 7.60 (H, *m*, H-7'), 7.73 (H, *m*, H-7), 7.86 (1H, *m*, H-5'), 7.92 (1H, *d*, *J* = 7.14 Hz, H-5), 8.19 (1H, *d*, *J* = 7.4 Hz, H-8'), 7.87 (1H, *d*, *J* = 7.4Hz, H-8), 8.89 (1H, *s*, H-4'), 8.98 (1H, *s*, H-3) and 12.26 (1H, *s*, O-H); ¹³C NMR (100 MHz, DMSO-d₆): δ_{C} 115.5 (C-3), 119.6 (C-5), 122.8 (C-4a), 123.4 (C-6'), 124.3 (C-8'), 125.9 (C-6), 128.4 (C-3'), 129.1 (C-4'a), 129.6 (C-5'), 130.1 (C-8), 130.4(C-7), 132.0 (C-7), 136.2(C-4), 139.8(C-4'), 141.2(C-8'a), 148.7 (C-8a), 154.3 (C-2'), 161.7 (C-2), and 168.2 (C-9).

3.10.1.3 Synthesis of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (226)

2-chloro-8-methylquinoline-3-carbaldehyde (0.41 g, 0.002 mol) and pyruvic acid (0.15mL, 0.0026 mol) were added to 15 mL glacial acetic acid in a 100 mL round bottom flask. The mixture was refluxed for an hour on stirring by magnetic stirrer. To the mixture, *o*-toluidine (0.18 mL, 0.002 mol) was added and refluxed for additional 8 hours. The precipitate formed after cooled overnight was separated by suction filtration. The product was 557 mg (76.6%) which was purified by silica gel column chromatography using CH₂Cl₂:MeOH (18:1) as eluent. Dark powder; mp 182-184°C; IR(ν cm⁻¹, KBr): 3569-2577 (*br*, acid-OH.), 3141 (aromatic C-H) 2904 (C-H str.), 2848 (C-H str.), 1663 (acidic C=O), 1630 (imine C=N), 1562 (aromatic C=C); ¹H NMR (400 MHz, DMSO-d₆): δ_{H} 2.20 (3H, *s*, H-10), 2.43 (3H, *s*, H-11), 6.37 (1H, *d*, *J* = 7.96 Hz, H-6'), 6.49 (1H, *m*, *J* = 6.88 Hz, H-6), 6.91 (H, *m*, H-7'), 7.01 (2H, *m*, H-5', H-7), 7.26 (1H, *d*, *J* = 7.96 Hz, H-5), 7.38 (1H, *d*, *J* = 6.88Hz, H-5'), 7.67 (1H, *s*, H-3), and 11.01

(1H, s, O-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 17.8 (C-10), 18.6 (C-11), 109.8(C-3), 116.4(C-5), 119.5 (C-6'), 122.1 (C-4'a), 122.4 (C-6), 123.6 (C-3'), 126.1(C-5'), 127.1 (C-4'a), 127.2 (C-7'), 128.2 (C-7), 129.6 (C-8), 130.3 (C-8'), 130.9(C-4), 131.3 (C-4'), 135.9 (C-8'a), 136.6(C-8a), 146.5(C-2), 162.8(C-2'), and 170.3(C-9).

3.10.1.4 Synthesis of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (227)

2-chloro-8-methylquinoline-3-carbaldehyde (1.70 g, 0.008 mol) was refluxed for 4 hours in a mixture of methanol (15 mL), potassium bicarbonate (1.10 g, 0.008 mol) and DMF (10 mL). The methanol was removed by distillation and the residue was added to 100 mL crushed ice water. The precipitate was separated by suction filtration and was washed with excess cold water. The purity of 2-methoxy-8-methylquinoline-3-carbaldehyde was checked with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-8-methylquinoline-3-carbaldehyde (0.4 g, 0.002 mol) was added to pyruvic acid (0.15 mL, 0.002 mol) in 15 mL glacial acetic acid. The mixture was refluxed for an hour while being stirred. Aniline (0.17 mL, 0.002 mol) was added to the reaction mixture and the refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The product was then collected by filtration after cooling to room temperature. The crude product (650 mg, 72.5%) was purified over silica gel column chromatography using CH_2Cl_2 :MeOH (8:1) as eluent. The yield was 160 mg (53.3 %;) Gray powder; mp 202-204°C; IR(ν cm^{-1} , KBr): 3456 -2644 (*br*, acid-OH.), 2938 (C-H str.), 2848 (C-H str.), 1709 (acidic C=O), 1630 (imine C=N), 1585 (aromatic C=C); ^1H NMR (400 MHz, DMSO- d_6): δ_{H} 2.68 (3H, s, H-10), 4.11 (3H, s, H-11), 7.37 (1H, *t*, J = 7.53 Hz, H-6'), 7.59 (1H, *d*, J = 6.72 Hz, H-7'), 7.74 (H, *m*, H-6'), 7.86 (2H, *m*, H-5', H-7), 8.20 (1H, *d*, J = 7.53 Hz, H-8), 8.51 (1H, *s*, J = 6.88 Hz, H-3), and 8.74 (2H, *m*, H-4', H-5); ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} 17.8 (C-10), 53.9 (C-11), 123.6(C-3'), 123.8(C-3), 124.0 (C-4'a), 124.8 (C-6') 125.2 (C-5), 125.9 (C-4a), 126.8 (C-5'), 128.5(C-6), 130.2 (C-8), 130.5 (C-7'), 131.5 (C-7), 134.6 (C-4), 136.7 (C-8'), 140.9(C-4'), 145.2 (C-8'a), 149.0(C-8a), 154.6(C-2), 158.6(C-2'), and 168.2(C-9).

3.10.1.5 Synthesis of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (228)

2-Methoxy-8-methylquinoline-3-carbaldehyde (0.40 g, 0.002 mol) was mixed with pyruvic acid (0.15 mL, 0.002 mol) in 15 mL glacial acetic acid and refluxed for an hour with stirring. Then, *o*-toluidine (0.15 mL, 0.002 mol) was added to the reaction mixture and the reflux was continued for additional 10 hours. It was cooled to room temperature and precipitate was separated by suction filtration. The crude yield (579.7 mg, 80.7%) was purified over silica gel chromatography using CH₂Cl₂:MeOH (18:1) as eluent. Yield 154 mg (51.3%); Gray powder; mp 97-99°C; IR(ν cm⁻¹, KBr): 3377.9-2531.8 (*br*, acid-OH.), 2982.0 (C-H str.), 2903.7 (C-H str.), 1721.2 (acidic C=O), 1641.0 (imine C=N), 1584.7 (aromatic C=C); ¹H NMR (400 MHz, DMSO-d₆): δ_{H} 2.69 (3H, s, H-10), 2.83 (3H, s, H-11), 4.13 (3H, s, H-12), 7.39 (1H, *m*, H-6'), 7.60 (2H, *m*, H-6, H-7'), 7.71 (1H, *m*, H-7), 7.742 (1H, *m*, H-5'), 8.51 (2H, *d*, H-4', H-5, *J* = 8.28 Hz) and 8.81 (1H, s, H-3); ¹³C NMR (100 MHz, DMSO-d₆): δ_{C} 17.8 (C-10), 18.6 (H-11), 53.9 (C-12), 123.0 (C-3'), 123.7 (C-3), 123.9 (C-5), 124.1 (C-4'a), 124.8 (C-6'), 125.3 (C-4a), 126.8 (C-6), 128.2 (C-5'), 130.4 (C-7), 131.1 (C-7), 134.6 (C-8), 137.1 (C-8'), 137.6 (C-4), 141.0 (C-4'), 145.2 (C-8'a), 147.8 (C-8a), 153.0 (C-2), 158.7 (C-2'), and 168.4 (C-9).

3.10.1.6 Synthesis of 2-(*o*-tolylimino)propanoic acid (230)

2-thiocyanatoquinoline-3-carbaldehyde (0.43 g, 0.002 mol) and pyruvic acid (0.15 mL, 0.002 mol) was added to glacial acetic acid (15 mL) and the mixture was refluxed for an hour with stirring. Then to mixture, *o*-toluidine (0.18 mL, 0.002 mol) was added and refluxed for additional 10 hours. The mixture was allowed to cool to room temperature and basified with 5% aqueous NaOH. Then it was filtered by gravity filtration and the filtrate was acidified with 5% aqueous HCl. It was cooled in an ice bath. The precipitate was collected by suction filtration and purified over silica gel column chromatography using CH₂Cl₂:MeOH (9.5; 0.5) as an eluent to furnish not the desired product **229** but the undesired product **230**. The yield was 125 mg (48.5%); Yellow powder; mp 285-287°C; IR(ν cm⁻¹, KBr): 3385 (*br*, acid-OH), 3042 (aromatic C-H), 2927 (C-H str.), 2848 (C-H str.), 1720 (carboxylic C=O), 1674 (imine C=N), 1607 (aromatic C=C), 1584; ¹H NMR (400 MHz, DMSO-d₆): δ_{H} 2.06 (3H, s, H-3), 2.22 (3H, s, H-7), 7.06 (1H, *t*, *J* = 7.90 Hz, H-4'), 7.14 (1H, *t*, *J* = 7.90 Hz, H-5'), 7.19 (1H, *d*, *J* = 8.41 Hz, H-3), 7.39 (1H, *d*, *J* = 8.41 Hz, H-6), and 9.29 (1H, s, H-N=); ¹³C NMR (100 MHz, DMSO-d₆): δ_{C}

18.2 (C-3), 23.7 (C-7), 125.4 (C-5', C-6'), 126.3(C-4'), 130.7 (C-3'), 132.0 (C-2'), 136.0(C-1') and 168.6(C-1, C-2).

3.10.1.7 Synthesis of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (232)

2-chloroquinoline-3-carbaldehyde (0.5 g, 2.6 mmol) was added to ethanolamine (10 mL) in a 100 mL round bottom flask and heated in an oil bath at 100°C for 2 hours. After cooling it to ambient temperature, the mixture was poured into 100 mL crushed ice water. The precipitate was collected by suction filtration and allowed to dry in air (0.62 g, 2.4 mmol). The dried product was refluxed in 20% H₂SO₄ (10 mL) for 2 hours. It was cooled to room temperature and added into crushed ice water (50 mL). The precipitate was collected by suction filtration. The yield was 0.46 g (89%). It was a yellow powder and decomposed without melting at 130°C. IR(ν cm⁻¹, KBr): 3367(alcohol-OH), 2938 (C-H-str.), 2870 (C-H str.), 1652 (aldehyde C=O), 1630 (imine C=N) 1562(aromatic C=C): ¹H NMR (400 MHz, CDCl₃): δ _H, 3.65 (4H, H-10, H-11), 4.96 (1H, s, O-H), 7.26 (1H, s, H-8), 7.55-7.84 (3H, m, H-6, H-7, H-5), 8.24 (1H, s, H-4), 8.68(1H, s, H-9), and 10.02 (1H, s, N-H); ¹³C NMR (100 MHz, DMSO-d₆): δ _C 43.1 (C-10), 60.1 (C-11), 117.7 (C-3), 122.1(C-4a), 122.8(C-8), 126.2 (C-6), 130.2 (C-7), 134.0 (C-5), 149.7 (C-4), 151.6(C-8), 154.7 (C-2), and 194.8(C-9)

3.10.1.8 Synthesis of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)

4-Iodoaniline (3 g, 0.014 mol) was refluxed in mixture of acetic anhydride (10 mL) and acetic acid (10 mL) for two hours in a 250 mL round bottom flask. After cooling to room temperature, it was added to 100 mL crushed ice water and the precipitate was collected by suction filtration. The yield was 3.3 g. Then a Vilsmeier reagent was prepared by adding *N,N*-dimethylformamide (5 mL, 0.09 mol) to a 100 mL round-bottom flask guarded with drying tube; it was cooled to 0°C using ice bath. Then, phosphorus oxychloride (59 mL, 0.63 mol) was added dropwise to it from dropping funnel guarded by drying tube while being stirred by magnetic stirrer. This addition was done for 30 minutes. Then *N*-(4-iodophenyl)acetamide (3.3 g, 0.013 mol) was added to it. After 5 minutes, the dropper funnel was replaced by air condenser with guarding tube at its end, and the mixture was heated for 24 hours on oil bath at 85–90°C. Then it was cooled to room temperature, poured into a beaker containing 150 mL crushed ice water, and stirred for 20 minutes. The brown solid product was collected by suction filtration and washed with 50 mL cold water. TLC analysis revealed that the product (1.29 g) was a mixture of two

compounds. The product was suspended in hot EtOAc and the soluble part was found to be 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (**235**) (125 mg, 0.48 mmol, 3.7%). Whereas the insoluble part was identified as compound **236** (1.16 g, 96.3%). Brown crystal; mp 154-156°C; IR(ν cm⁻¹, KBr): 2927 (C-H-str.), 2848 (C-H str.), 1686(aldehyde C=O), 1608(imine C=N) 1573(aromatic C=C): ¹H NMR (400 MHz, CDCl₃ & CD₃OH): δ_{H} 7.77 (1H, *d*, *J* = 8.67 Hz, H-9), 7.92 (1H, *m*, H-8), 8.10 (1H, *m*, H-7), 8.35 (1H, *s*, H-5), 8.61 (1H, *s*, H-4) and 10.53 (1H, *s*, H-11); ¹³C NMR (100 MHz, DMSO-d₆): δ_{C} 93.7 (C-6), 124.9 (C-3), 126.8 (C-4a), 128.1(C-8), 130.0(C-10), 138.2 (C-4), 138.7(C-5), 138.9 (C-9), 142.2 (C-7), 148.4 (C-8a) 150.6 (C-2), and 188.8(C-11).

The NMR result of compound **236** (96.3%) revealed as it is N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (**236**). A brown powder; mp, 128-130°C; IR(ν cm⁻¹, KBr): 2966.1 (C-H-str.), 2922.0 (C-H str.), 1709.3 (formamide C=N(Me)₂), 1607.5 (imine C=N) 1578.0(aromatic C=C): ¹H NMR (400 MHz, DMSO-d₆): δ_{H} 3.34 (3H, *s*, CH₃), 3.48 (3H, *s*, CH₃), 7.72(2H, *m*, H-2, H-6), 7.75 (2H, *d*, *J* = 7.20Hz, H-3, H-5), and 8.65 (1H, *s*, H-8); ¹³C NMR (100 MHz, DMSO-d₆): δ_{C} 37.0 (CH₃), 43.6 (CH₃), 90.3 (C-4), 121.3 (C, C-2, C-6), 137.1 (C-1), 138.6(C, C-3, C-5), 130.0(C-10), 138.2 (C-4), 138.7(C-5), and 153.5(C-8).

CHAPTER FOUR

4. RESULTS AND DISCUSSION

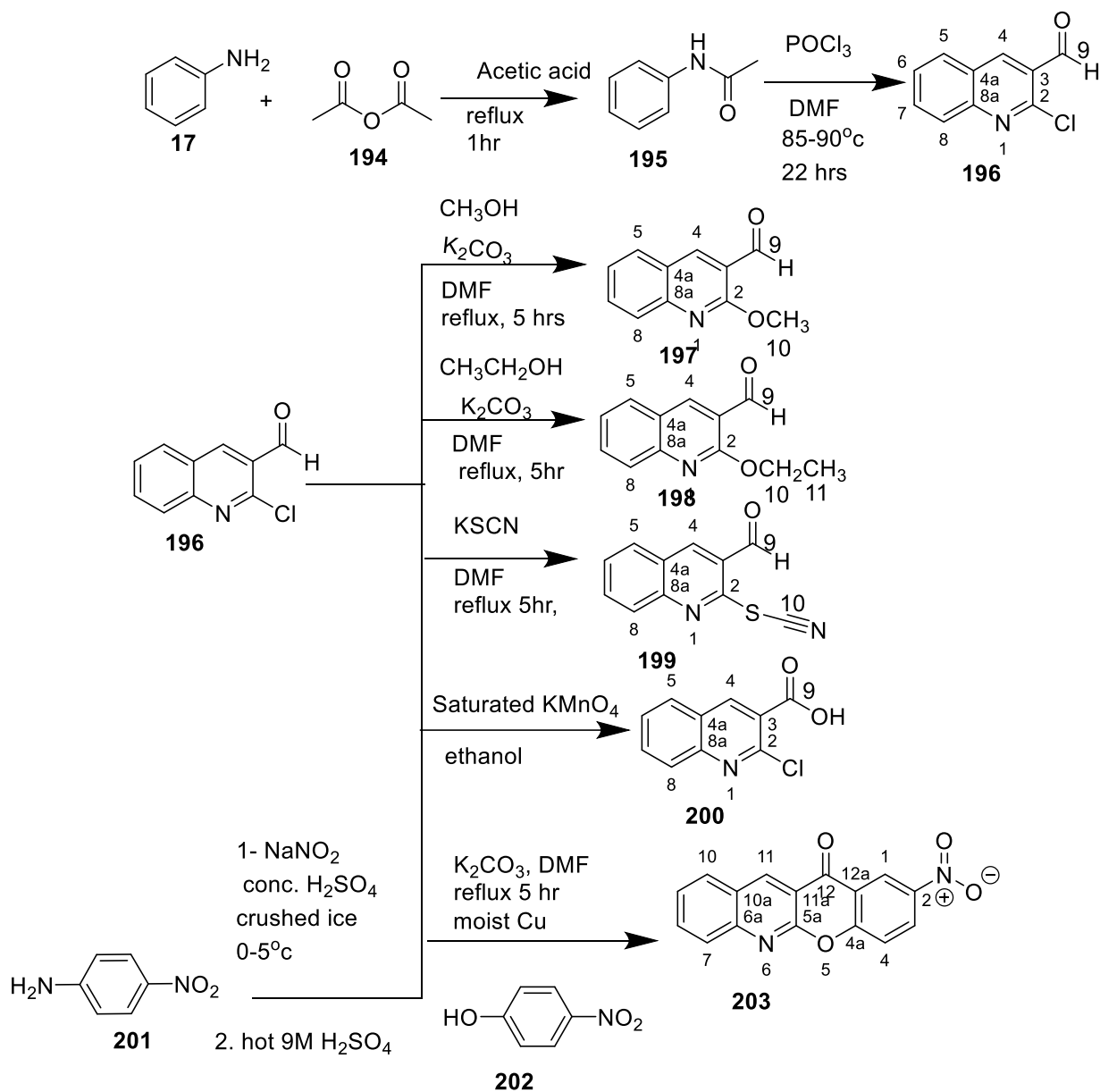
This chapter comprises of synthesis novel quinoline derivatives, quinoline-stilbene and pinacol of quinolines and [2,3'-biquinoline]-4-carboxylic acid. *In vitro* evaluation of the antibacterial, radical scavenging activities, drug-likeness prediction and molecular docking studies of the synthesized compounds have also be incorporated.

4.1 Synthesis and antibacterial, antioxidant, and molecular docking analysis of some novel quinoline derivatives

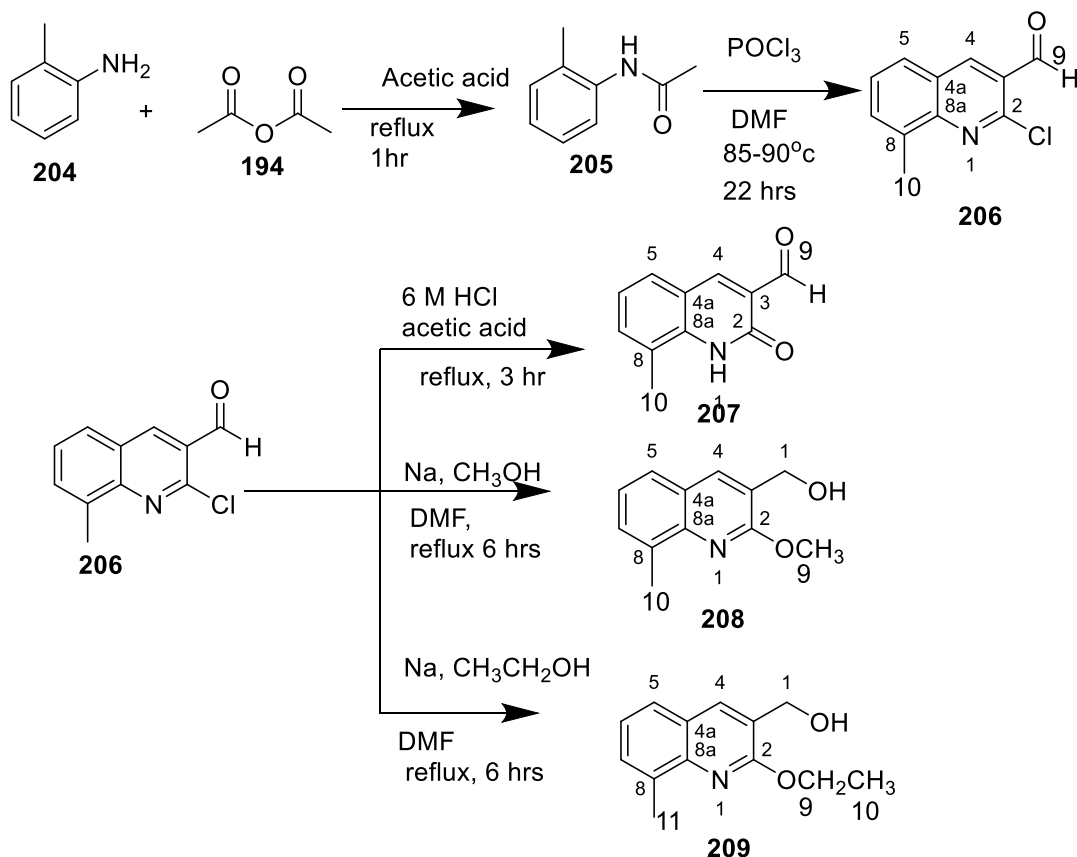
4.1.1 Synthesis of Quinoline-3-carbaldehyde and its derivatives

In an attempt to generate quinoline derivatives which have antibacterial properties, various quinoline derivatives (Scheme 30 and 31) were prepared using various reaction conditions. In the process, acetanilide (**195**) and *N*-(*o*-tolyl)acetamide (**205**) were prepared by refluxing the corresponding aniline (**17** and **204**) in acetic acid and acetic anhydride (**194**) mixture. Then 2-chloroquinoline-3-carbaldehyde (**196**) and 2-chloro-8-methylquinoline-3-carbaldehyde (**206**) were prepared from the acetanilide by the Vilsmeier approach.(Meth-Cohn et al. 1981) The chlorine in position 2 of quinolines was substituted by various nucleophiles by refluxing **196** and **206** in *N,N*-dimethylformamide with various nucleophilic reagents in basic medium. *N,N*-dimethylformamide deemed appropriate solvent in this process, because of its attractive features including high dielectric constant, its aprotic nature, wide liquid range, low volatility, dissolve all polar reactants and able to supply sufficient activation energy for the reactions because of its high boiling point (153°C). Furthermore, it is also miscible in water which eases the isolation of the desired product by adding the reaction mixture into cold ice-water which causes water insoluble product to precipitate out.(Jarwal and Thankachan 2015a, 2015b) Potassium carbonate was used to induce the basic medium since it is weak base and poor nucleophile, it doesn't interfere in most nucleophilic substitution reaction. Refluxing **206** in 6M HCl and acetic acid (1: 1) mixture afforded 8-methyl-2- oxoquinoline-3-carbaldehyde (**207**). The carbaldehyde functional group of quinoline was oxidized to carboxylic acid by permanganate method and was subsequently reduced to alcohol using metallic sodium. The overall sequence of reactions used in the synthesis of various targeted quinoline derivatives are

illustrated in Schemes 30 and 31. The structure elucidation of the synthesized compounds was accomplished using UV-Vis, FTIR, and NMR spectroscopic methods.



Scheme 30. Synthesis of 2-chloroquinoline-3-carbaldehyde and its derivatives



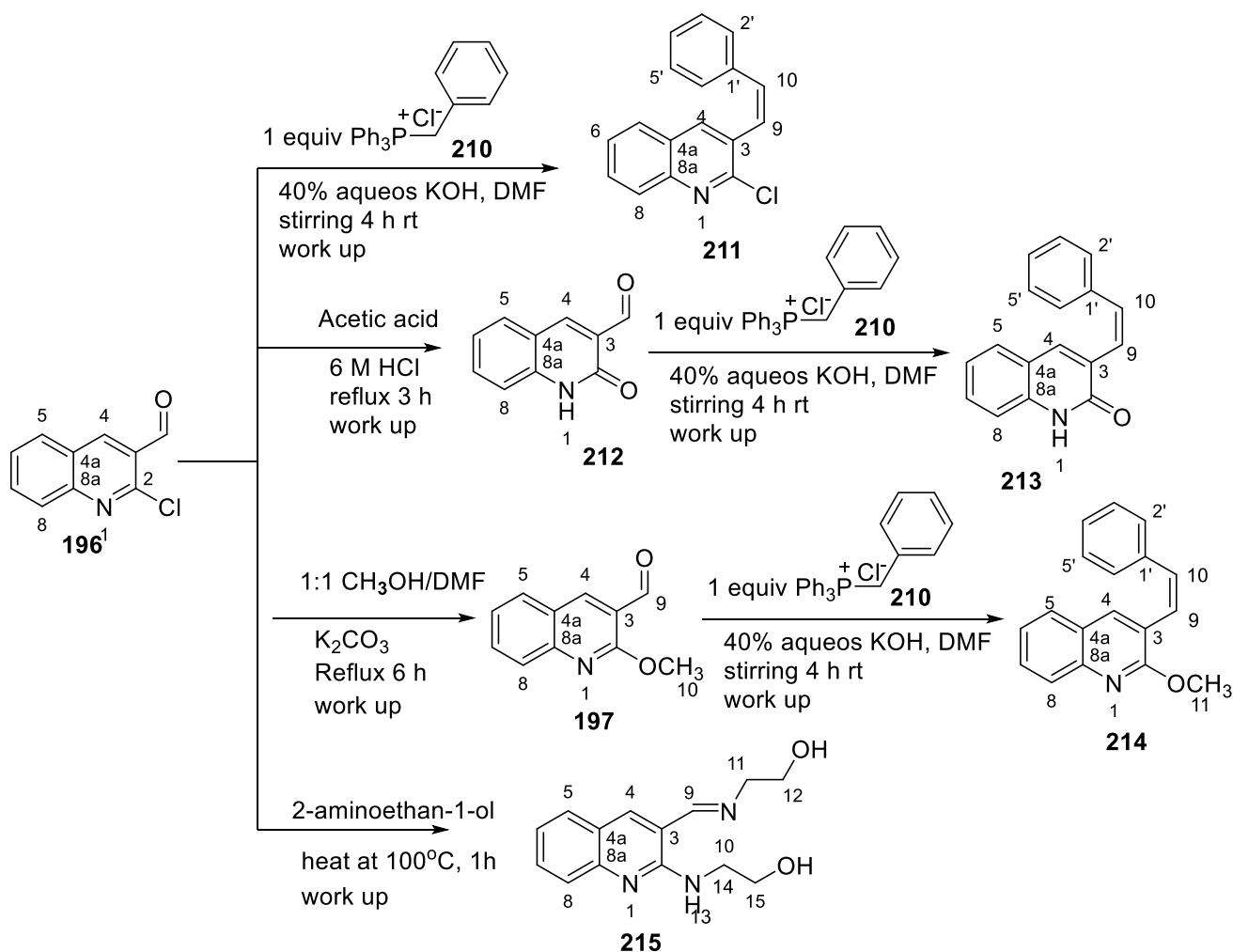
Scheme 31. Synthesis of 2-chloro-8-methylquinoline-3-carbaldehyde and its derivatives

4.1.2 Synthesis of quinoline-stilbenes and pinacol of quinolines

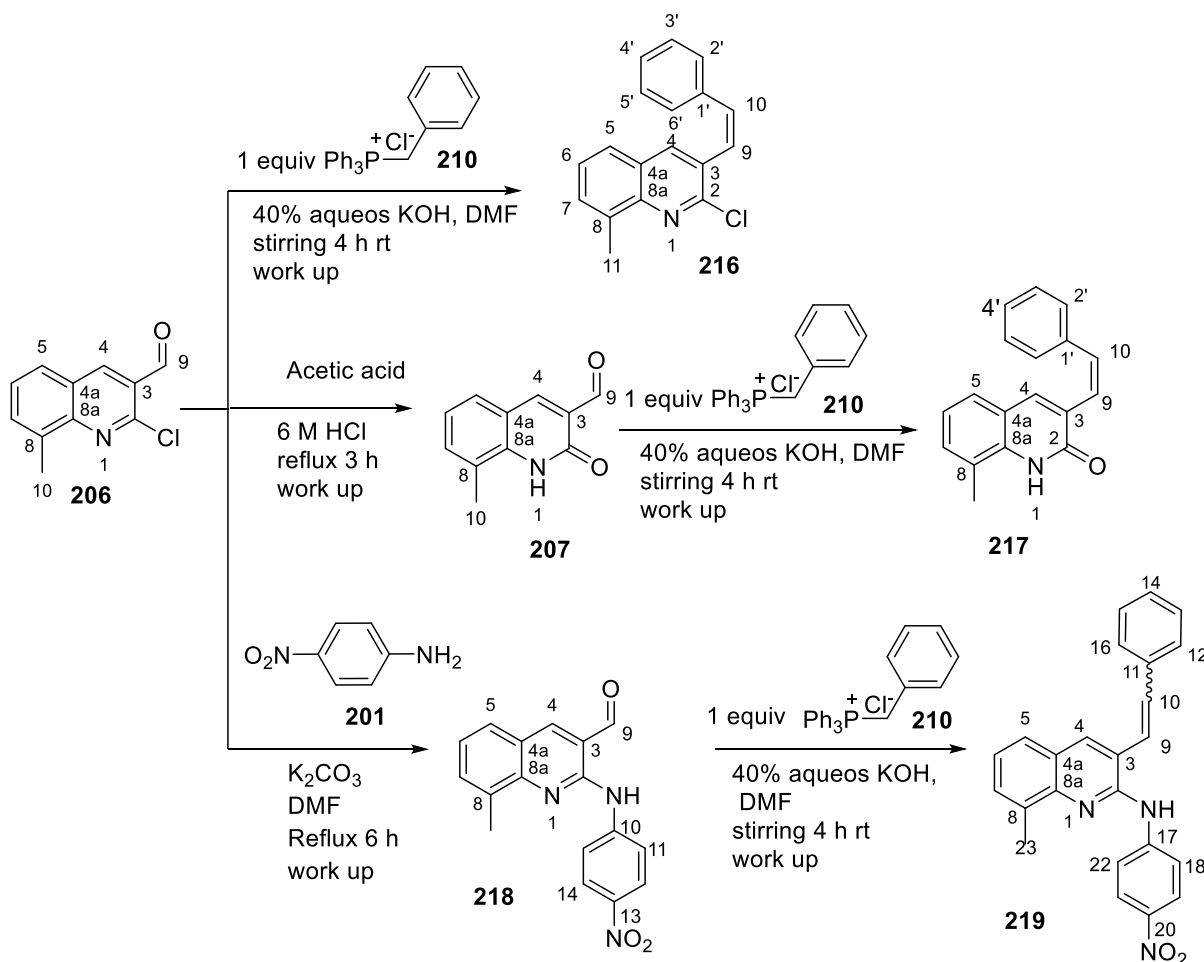
Quinoline-stilbenes (a type of merged hybrid of quinoline and stilbene) and pinacols of quinolines (fused hybrid of quinoline) were synthesized. In process of drug discovery, the hybridization of biologically active molecules is a powerful tool (Bérubé 2016). Hybridization provides better drugs for the treatment of a number of illnesses including malaria, tuberculosis, cancer and AIDS. Hybrid drugs can afford combination therapies in a single multi-functional unit (Bérubé 2016).

After securing the crucial intermediates **196** and **206** (Scheme 30 and 31), attention was given to the synthesis of a series of quinoline-stilbenes and their substituted analogs using Wittig reaction. Subsequent replacement of chlorine by various nucleophiles were achieved in N,N-dimethyl formamide (DMF) as solvent and K_2CO_3 as a base. Compound **212** was prepared by refluxing **196** in a mixture of acetic acid and 6 M aqueous hydrochloric acid. The desired products was obtained in excellent yields after removing acetic acid by distillation under

reduced pressure. An attempt to displace the chlorine atom in compound **196** with 2-aminoethan-1-ol furnished the unexpected compound **215** in excellent yield (84%). This might be *via* a classical condensation reaction of the amine group of 2-aminethanol with carbonyl of compound **196**. The other desired products were made by reacting derivatives of compounds **196** and **206** with benzyltriphenylphosphonium chloride using DMF as a solvent and KOH as a base. The synthesized are mainly the *cis* isomers which were confirmed by the coupling constant of the olefinic double bonds in the stilbenes. This agreed very well with previous products of Wittig reactions done at ambient temperature in DMF/KOH which gives the *cis* stilbenes in good yield (Bergelson, Barsukov, and Shemyakin 1967). However, compound **219** was produced as a mixture of *cis* and *trans* stereoisomers. Further attempt to purify **219** using silica gel column chromatography was unsuccessful since the spots of the two stereoisomers overlapped in all TLC profiles (Scheme 32 and 33).



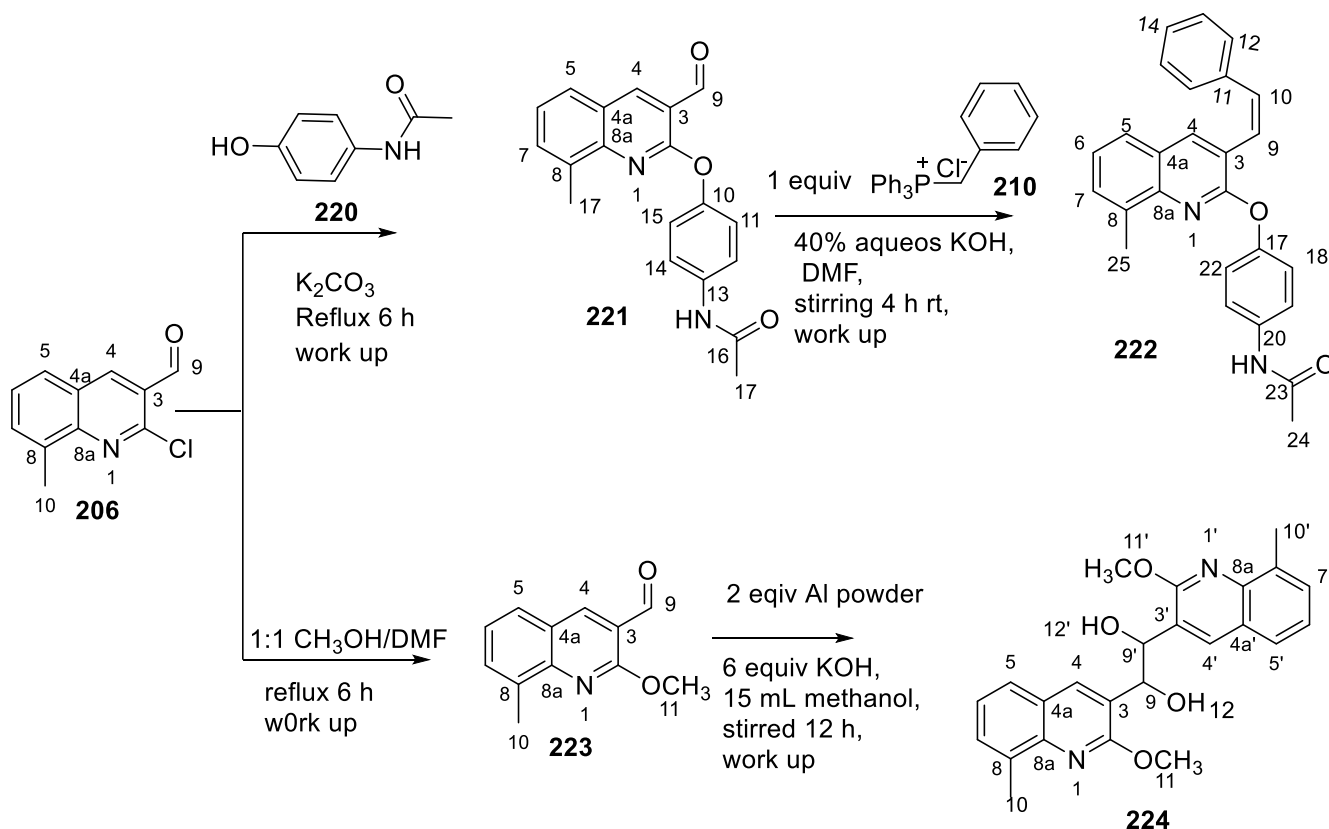
Scheme 32. Synthesis of quinoindoline stilbenes and Schiff bases (**211-215**)



Scheme 33. Synthesis of 8-methylquinoindoline stilbenes (**219-219**)

Pinacolization reaction can be used to couple two bioactive molecules with an aldehyde functional group. Pinacolization of carbonyls has been reported by using a number of reagents such as Mg-MgI_2 , (Gomberg and Bachmann 1927) Zn-ZnCl_2 , transition metals, actinides and lanthanides (Khurana et al. 1996). Ti^{II} and Ti^{III} reagents have also received considerable attention although olefination is a competing reaction with these reagent (Khurana et al. 1996). Among the various pinacolization agents of aromatic carbonyl compounds, aluminium powder with potassium hydroxide in methanol was chosen and applied in the synthesis since it was reported to provide coupling rapidly (Khurana et al. 1996; Khurana and Sehgal 1994; Yuan, Wang, and Li 2006). In this reaction, a ratio of 1:2:6 equivalent of **223**: aluminum powder: KOH were used in the process (Scheme 34) (Sotto *et al.* 2015). Methanol was chosen as solvent

because the reaction was fast and the side products were minimum (Khurana and Sehgal 1994). The crude product was purified by using silica gel column chromatography.



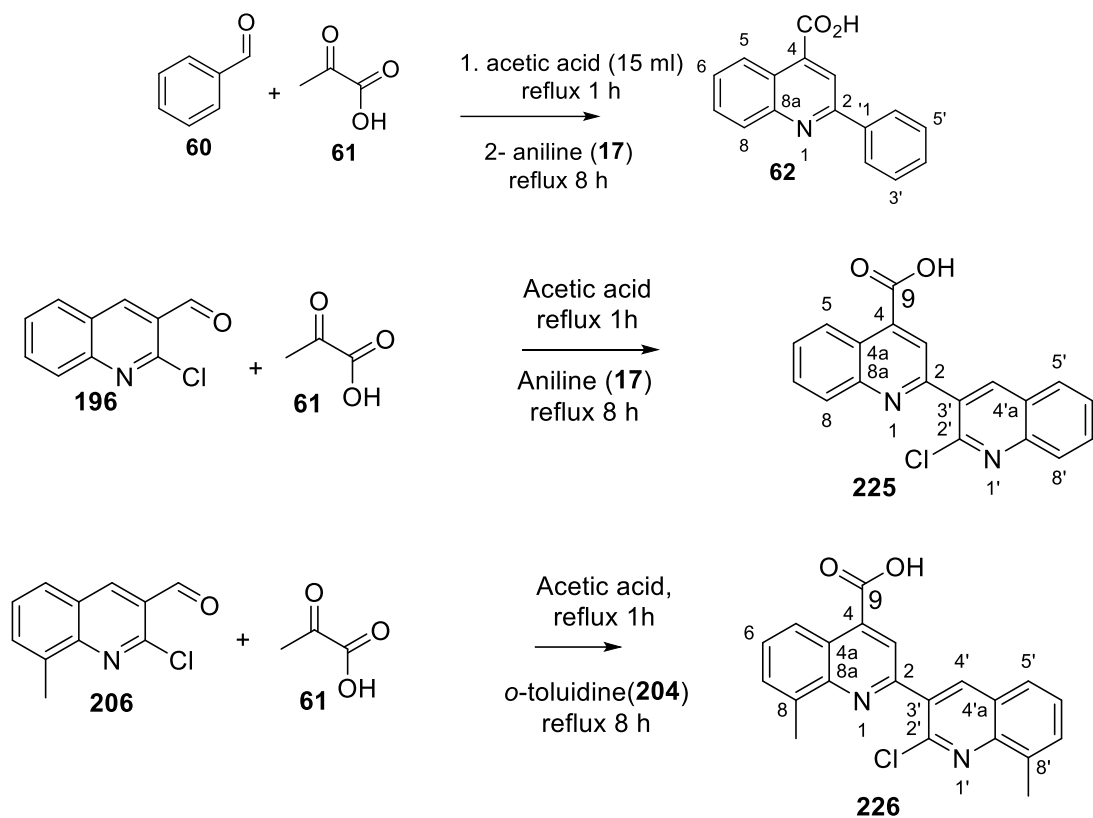
Scheme 34. Synthesis of 8-methylquinoline stilbenes and pinacol of quinoline (**222** and **224**)

4.1.3 Synthesis of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs

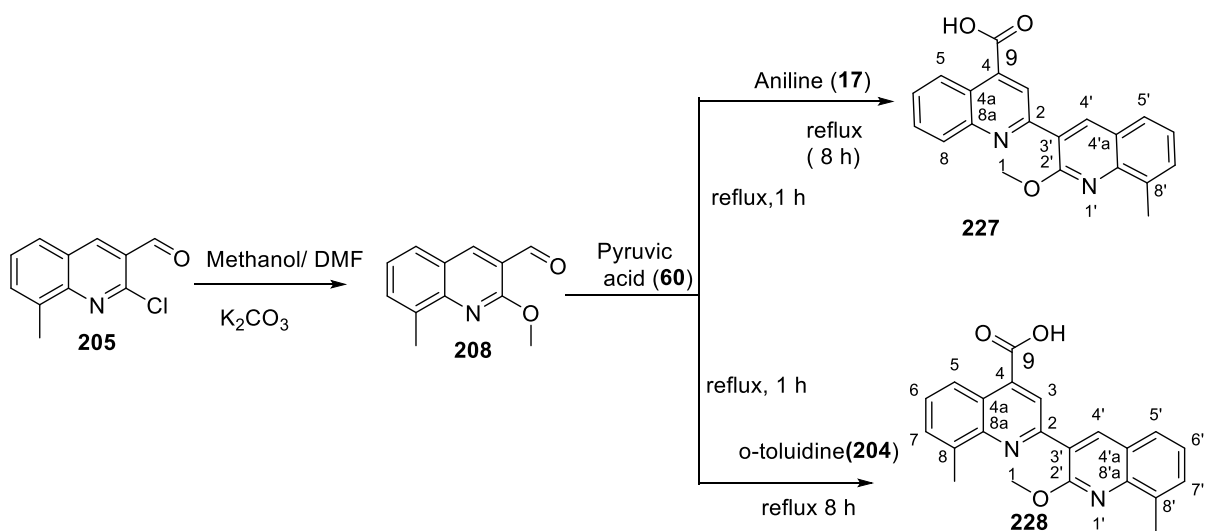
In the third part, a new series of [2,3'-biquinoline]-4-carboxylic acids, 2-phenylquinoline-4-carboxylic acid and additional quinoline-3-carbaldehyde derivatives were synthesized by employing the Vilsmeier, Doebner, condensation, hydrolysis and nucleophilic substitution reactions (Scheme 35-36).

In synthesis, **196** and **206** were treated separately with pyruvic acid, aniline and *o*-toluidine to synthesis four new (2,3'-biquinoline)-4-carboxylic acids by using Doebner quinoline synthesis approach (Scheme 35). (Forrest, Dauphinee, and Miles 1969; Wu et al. 2006) Furthermore, 2-phenylquinoline-4-carboxylic acid (**62**) was also prepared by the same procedure from benzaldehyde (**60**), aniline (**17**), and pyruvic acid (**61**) in order to compare the antibacterial activity of biquinoline-4-carboxylic acid analogs with mono quinoline-4-carboxylic acid

(Scheme 35). The Doebner method, introduced by Oscar Doebner in 1887, combines aniline with an aldehyde and pyruvic acid to give 2-substituted quinoline-4-carboxylic acid (Ramann and Cowen 2016). Low yield, longer reaction times, harsh reaction condition and requirement of large amount of organic solvent are the typical limitations of Doebner method (L. M. Wang et al. 2009). Various solvent systems including absolute ethanol (Patel et al. 2017), acetic acid (X. Wang et al. 2016), and solvent free reactions (X. Wang et al. 2016) as well as different acid catalysts including sulfuric acid, trifluoro acetic acid and Lewis acid (L. M. Wang et al. 2009) were employed by various researchers to overcome these limitations. A recent report showed the replacement of trifluoroacetic acid with acetic acid and using excessive acetic acid as the solvent instead of ethanol provides better yield (X. Wang et al. 2016). Based on this and other related reports, four new [2,3'-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid (Scheme 35 and 36) were synthesized in glacial acetic acid. The synthesis of compounds **62**, **225**, **226**, **227**, and **228** (Scheme 35 and 36) were achieved by refluxing the corresponding quinoline-3-carbaldehyde analogs with pyruvic acid in acetic acid for an hour followed by treatment with aniline or o-toluidine. These compounds were purified using silica gel column chromatography with CH₂Cl₂:MeOH as eluent in good yields. On the other hand, refluxing a mixture of benzaldehyde and pyruvic acid in glacial acetic acid for an hour followed by treatment with aniline gave 2-phenylquinoline-4-carboxylic acid (**62**) in 62.2% yield

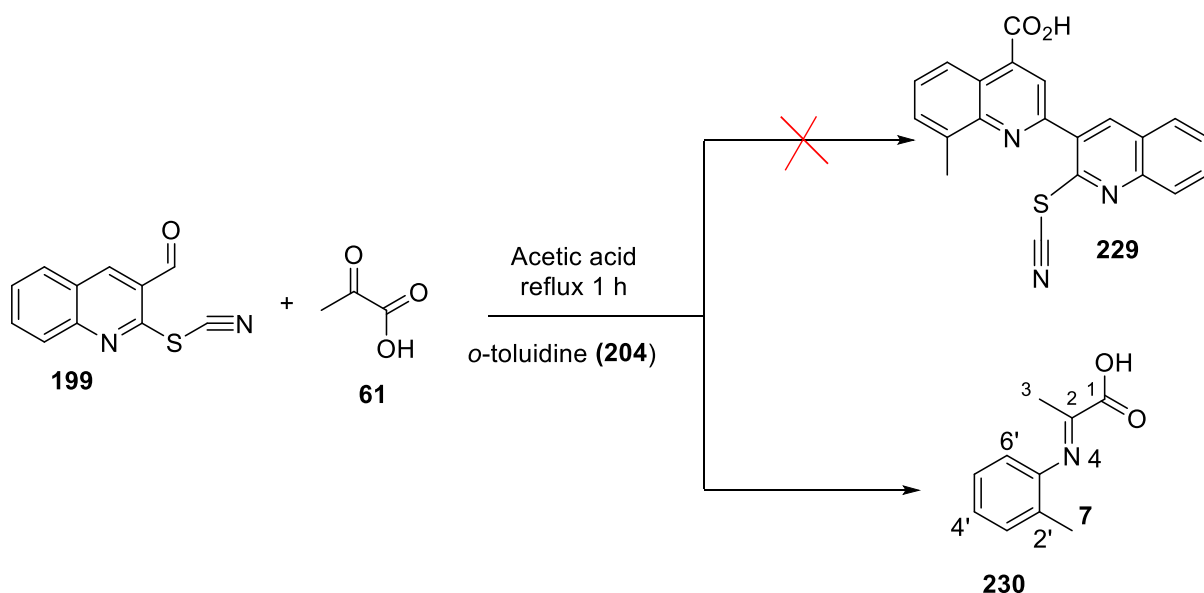


Scheme 35. Synthesis of [2,3'-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid (**62**, **225** and **226**)



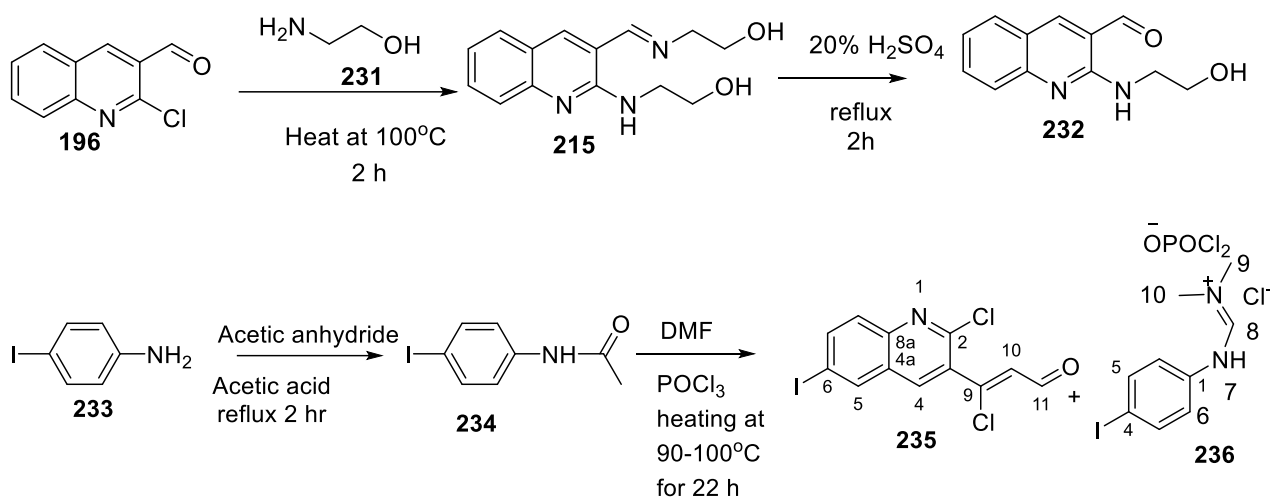
Scheme 36. Synthesis of [2,3'-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid (**227** and **228**)

Even though, glacial acetic acid was used both as solvent and catalyst, Doebner method is very sensitive both to electronic and steric effects. When attempt was made to synthesis compound **229** using the above procedure from **199**, *o*-toluidine (**204**), and pyruvic acid (**61**), was failed. The major product was identified using NMR as compound **230** which was a Schiff base formed between *o*-toluidine and pyruvic acid (Scheme 37).



Scheme 37. Synthesis of 2-(*o*-tolylimino)propanoic acid (**230**)

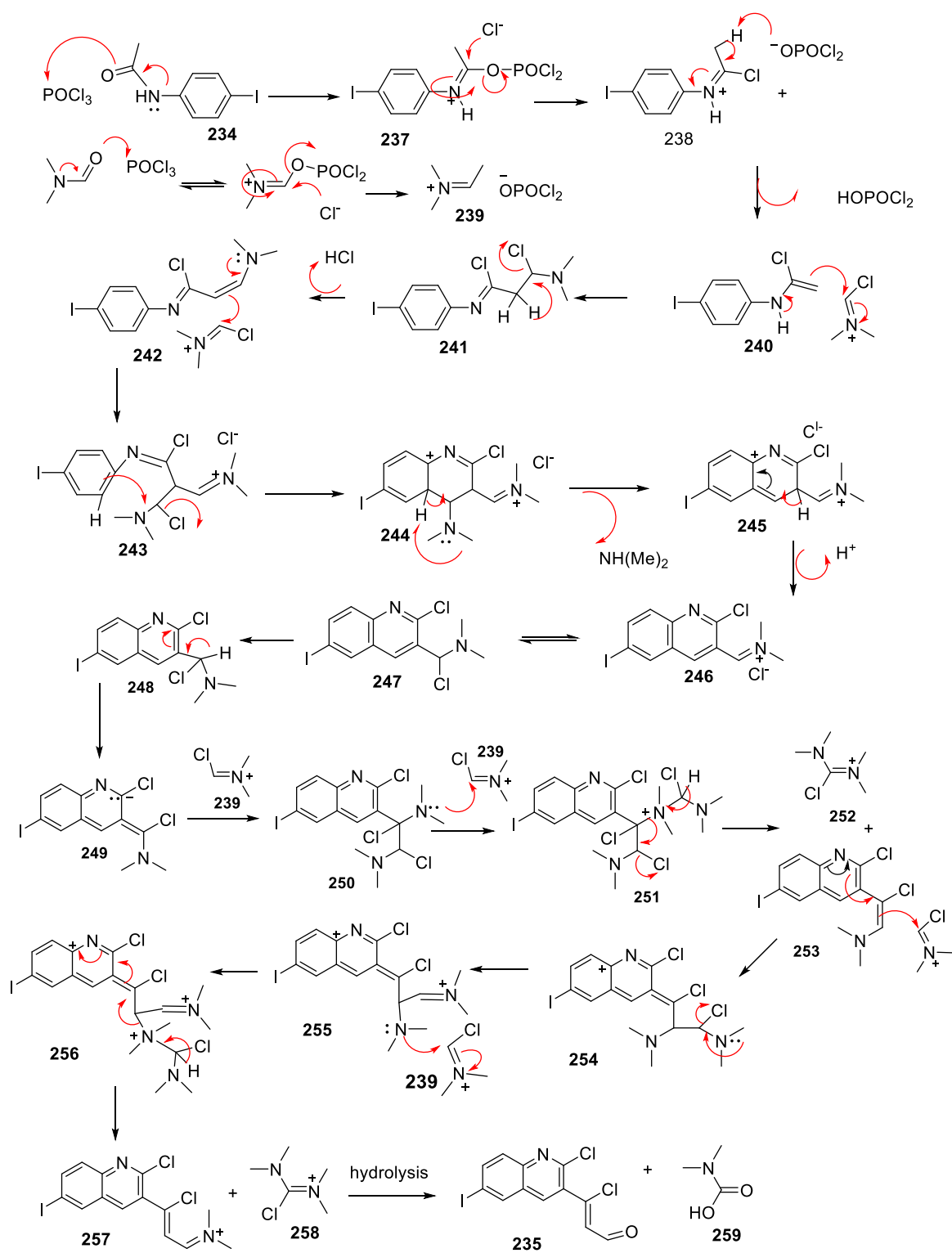
Previously Schiff base **215** was synthesized (Scheme 32) by condensation of **196** with excess ethanol amine. However, its antibacterial activity was found to be poor and in attempt to increase its antibacterial activity, the imine part was removed by hydrolyzing **215** with 20% H_2SO_4 (Scheme 38). The synthesis **235** and **236** (Scheme 38) achieved first by the application Vilsmeier reaction, recrystallization in ethyl acetate followed by substitution of chlorine by various nucleophiles. Vilsmeier-Haack formylation uses dimethylformamide and phosphoryl chloride to furnish Vilsmeier reagent which is a mild electrophilic agent and proceeds only with activated aromatic systems (Meth-Cohn et al. 1981; Thomas and Asokan 2004). Iodine is the least electronegative element of halogens, and we anticipated *p*-iodo acetanilide to provide good yield when it is treated by Vilsmeier reagent. However compound **235** (Scheme 38) was not according to our expectation. Firstly, compound **235** was 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (**235**) rather than the expected 2-chloro-6-iodoquinoline-3-carbaldehyde. Secondly, its amount was only 3.7% of the total yield.



Scheme 38. Synthesis of 2-chloroquinoline-3-carbaldehyde analogs (**232**, **235** and **236**)

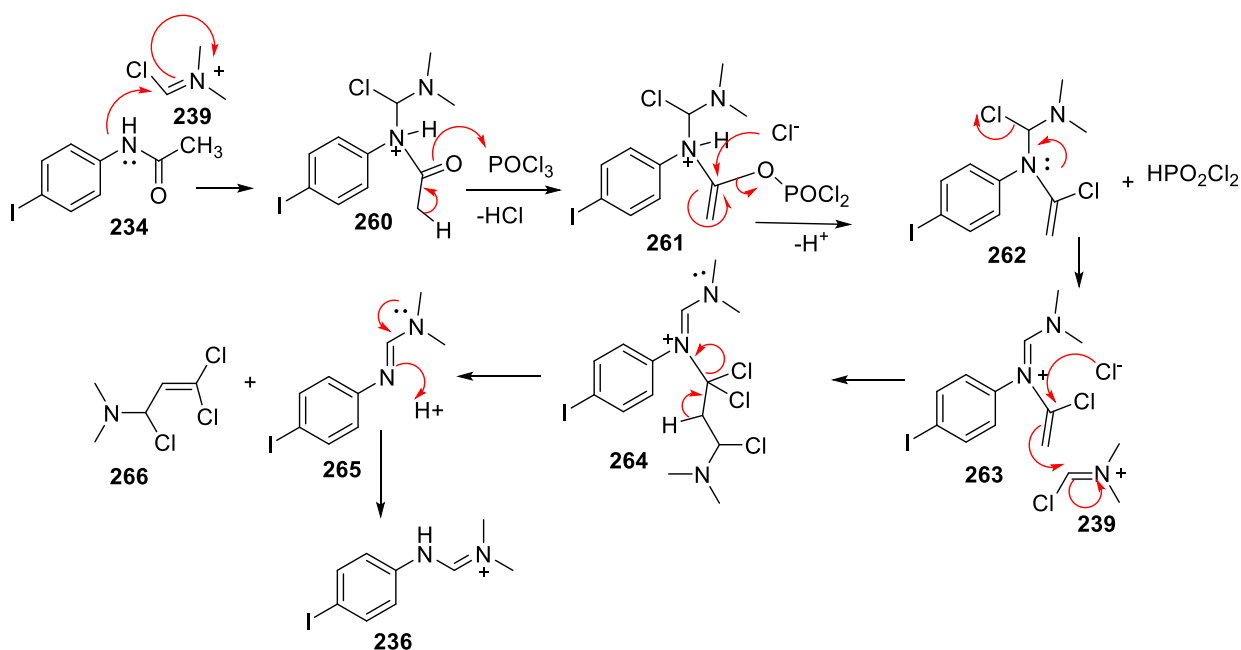
The major product about 96.3% of the yield was N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (**236**) which was confirmed from the spectral data obtained from ¹H and ¹³C NMR and DEPT-135. Presumably, it was formed by attack of the Vilsmeier reagent at nitrogen giving the N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium as major product. Scheme 39 and 40 showed the proposed mechanism for the formation of these two molecules.

Vilsmeier-Haack formylation using dimethylformamide and phosphoryl chloride involves the formation of halomethyleniminium salt **239** named as Vilsmeier reagent is an intermediate. The broad synthetic utility of this halomethyleniminium salt is not restricted to formylation but is also suitable for electrophilic substitution reactions (Thomas and Asokan 2004). Presumably, when excess amount of chloromethyleniminium salt was generated and when the reaction was allowed to take place for longer duration (22hrs) before quenching intermediate **247** with crushed ice, further conjugation through chloromethyleniminium salt addition and elimination of protons afforded compound **235** through several sequential steps. Originally, the precise mechanism of Meth-Cohn synthesis of quinolones was not discussed explicitly (Meth-Cohn et al. 1981). Recently, Hamama *et al* (2018) proposed related mechanism using SOCl₂ in place POCl₃ which provided a good vision on the mechanism of Vilsmeier formulation (Hamama et al. 2018). Dain *et al* (2004) had also proposed related mechanism of Vilsmeier–Haack reactions of carbonyl compounds in the synthesis of substituted pyrones and pyridines (Thomas and Asokan 2004). Here a related mechanism has been suggested for the reaction leading to the formation of compound **235** (Scheme 39).



Scheme 39. Proposed mechanism of formation of 2-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (235)

N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium salt (**236**) may be formed by direct attack of nitrogen of **234** on chloromethyleniminium salt giving an intermediate **260**. The rest of the intermediates were formed by elimination of protons from **260** followed electrophilic addition of POCl_3 affording intermediate **261**, which then underwent addition of Cl^- and elimination of H^+ to provide **261** which further rearranged to **236** (Scheme 40).



Scheme 40. Proposed mechanism of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (**236**)

4.2 Antibacterial activities synthesized compounds

Pathogens are caused both by prokaryotic and eukaryotic organisms (Brack, Weeks, and Museum 2016). Bacteria and viruses are the typical known pathogens microorganisms. Bacteria cause a number of infections that vary from asymptomatic to acute and fatal. Toxins produced by bacteria are responsible for various bacterial diseases (Brack, Weeks, and Museum 2016). Based on the characteristics of their cell wall, bacteria are classified as Gram positive or Gram negative (Doron and Gorbach 2008). Infections including bloodstream infections, pneumonia, wound or surgical site infections, and meningitis in healthcare settings are caused by Gram-negative bacteria while diseases such as anthrax, diphtheria, enterococcal infections, erysipelothriscosis and listeriosis are caused by Gram-positive bacteria (Woodford and

Livermore 2009). Gram-negative bacteria are resistant to multiple drugs and are increasingly resistant to most available antibiotics (Pontefract et al. 2020).

4.2.1 Antibacterial activity of quinoline-3-carbaldehyde derivatives

Quinolines are pharmacologically active compounds used to treat various life threatening diseases (Sreenivasa 1998). In an attempt to find out lead compounds against bacteria, the synthesized compounds were evaluated for their antibacterial activities against various strains of bacterial pathogens. The results obtained are depicted in Table 2 and Figure 11. The data in Table 2 show that all synthesized compounds displayed medium to good activity against two or more bacterial strains. The mean inhibition zones ranged from the lowest (6 mm at 300 $\mu\text{g/mL}$) to the highest (11.3 mm at 500 $\mu\text{g/mL}$). Three of the ten compounds; **200**, **203**, and **208**, showed good activities against *E. coli*, among which **200** and **203** revealed better activities with mean inhibition zone of 11.00 ± 1.33 and 11.33 ± 1.11 mm diameter at 500 $\mu\text{g/mL}$, respectively. Seven of the ten compounds (**196**, **198**, **199**, **200**, **206**, **208**, and **209**) (Scheme 31 and 32) showed antibacterial activity against *B. subtilis*, maximum inhibition zone in the series (7.67 ± 0.44 mm) was observed for compounds **196** and **198**. None of the synthetic compounds demonstrated activity against *S. aureus* except **196**, **197**, **200**, and **207** which showed little activity (7.0 ± 0.67 mm).

Among the synthetic compounds, **196**, **197**, **198**, and **207** displayed comparable activity against *P. aeruginosa*, and the maximum inhibition zone was 10.00 ± 0.44 mm for compound **207** better than that of the positive control (8.33 ± 0.44 mm) at the same concentration of (500 $\mu\text{g/mL}$). Most probably 2-oxo functional group seems responsible for bioactivity of **207** against *P. aeruginosa*. Compounds **196** and **200** displayed moderate to good activity against all tested bacterial species, while compound **205** showed no activity against all tested organisms except *B. subtilis* with inhibition zones of 6.33 ± 0.44 mm and 7.33 ± 0.44 mm at concentrations of 300 and 500 $\mu\text{g/mL}$, respectively. Thus indicating the-3-carbaldehyde and 3-carboxylic acid groups are essential for bioactivity of quinilones. The two compounds even exhibited better activities than ciprofloxacin against *P. aeruginosa*. Compound **208** and **209** have a related structure, the only difference is the substituent at 2-position which is methoxy for **208** and ethoxy for **209**. Thus, it seems that 2-methoxy group is important for activities of **208** especially against *E. coli*.

Table 2. The inhibition zone of quinoline-3-carbaldehyde derivatives in mm (mean $\pm$ SD).

conc ($\mu\text{g/ml}$)	Compounds						Bacteria strains
	196	197	198	199	200	ciprofloxacin	
300	6.33 $\pm$ 0.44	0	0	0	9.0 $\pm$ 0.55	19.0 $\pm$ 0.67	<i>E. coli</i>
500	6.67 $\pm$ 0.89	0	0	0	11.33 $\pm$ 1.11	21.0 $\pm$ 0.55	
300	7.0 $\pm$ 0.67	0	7.0 $\pm$ 0.67	7.0 $\pm$ 0.67	6.67 $\pm$ 0.89	30 $\pm$ 0.67	<i>B. subtilis</i>
500	7.67 $\pm$ 0.44	0	7.67 $\pm$ 0.44	7.33 $\pm$ 0.44	7.0 $\pm$ 0.67	33 $\pm$ 0.67	
300	6.33 $\pm$ 0.44	6.0 $\pm$ 0.00	0	0	6.0 $\pm$ 0.00	9 $\pm$ 0.67	<i>S. aureus</i>
500	7.0 $\pm$ 0.67	6.33 $\pm$ 0.44	6.0 $\pm$ 0.00	0	6.33 $\pm$ 0.44	11 $\pm$ 0.67	
300	7.0 $\pm$ 0.67	7.67 $\pm$ 0.44	8.0 $\pm$ 0.67	6.33 $\pm$ 0.44	7.0 $\pm$ 0.00	7.0 $\pm$ 0.00	<i>P. aeruginosa</i>
500	9.0 $\pm$ 0.67	9.33 $\pm$ 0.44	9.67 $\pm$ 1.11	6.33 $\pm$ 0.44	8.0 $\pm$ 0.67	8.33 $\pm$ 0.44	
conc ($\mu\text{g/ml}$)	compounds						bacteria strain
	203	206	207	208	209	ciprofloxacin	
300	7.33 $\pm$ 0.66	0	0	8.0 $\pm$ 0.67	0	19.0 $\pm$ 0.67	<i>E. coli</i>
500	11.0 $\pm$ 1.33	0	0	9.33 $\pm$ 0.89	0	21.0 $\pm$ 0.55	
300	0	6.67 $\pm$ 0.44	0	6.33 $\pm$ 0.44	6.33 $\pm$ 0.44	30 $\pm$ 0.67	<i>B. subtilis</i>
500	0	7.33 $\pm$ 0.44	0	7.33 $\pm$ 0.44	7.33 $\pm$ 0.44	33 $\pm$ 0.67	
300	0	0	6.0 $\pm$ 0.44	0	0	9 $\pm$ 0.67	<i>S. aureus</i>
500	0	0	6.67 $\pm$ 0.44	0	0	11 $\pm$ 0.67	
300	6.0 $\pm$ 0.00	6.33 $\pm$ 0.44	8.33 $\pm$ 0.44	0	0	7.0 $\pm$ 0.00	<i>P. aeruginosa</i>
500	6.33 $\pm$ 0.44	7.33 $\pm$ 0.44	10.0 $\pm$ 0.44	0	0	8.33 $\pm$ 0.44	

0: no inhibition zone

The antibacterial activity of the synthesized analogs of quinolines were further presented in Figure 13

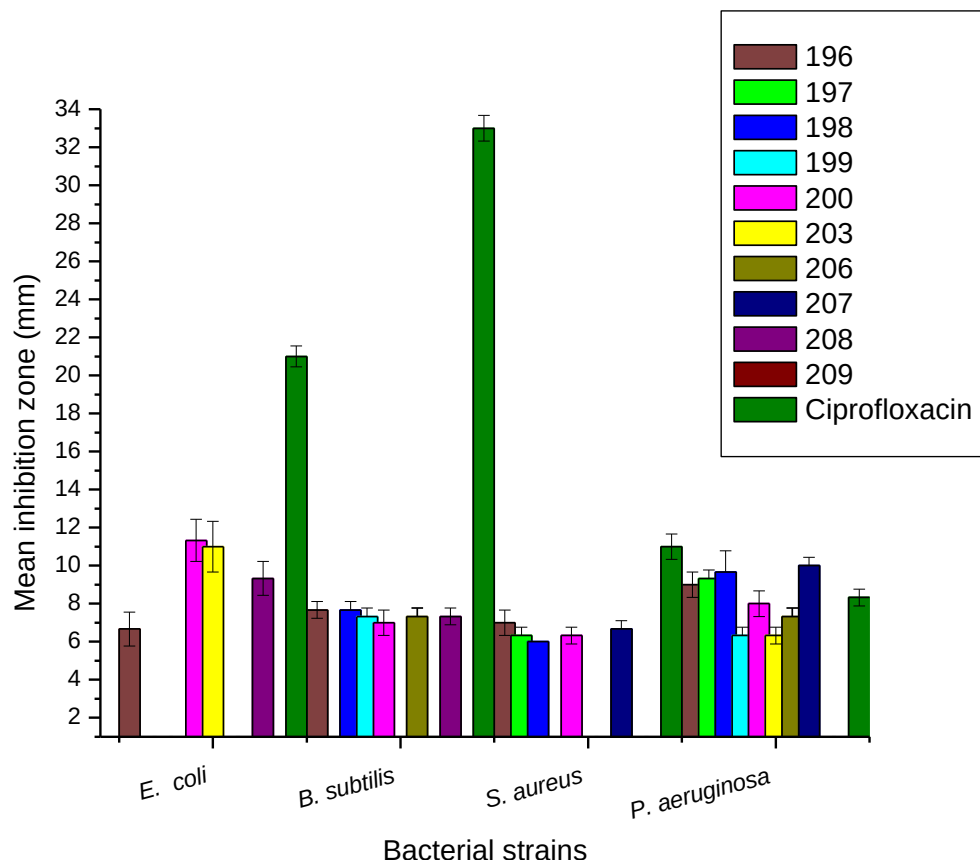


Figure 13. The inhibition zone of the synthetic compounds in mm (mean $\pm$ SD) at 500 μ g/mL.

4.2.2 Antibacterial activities of quinoline-stilbenes and pinacol of quinolines

Quinolines and their derivatives have been associated with various biological activities. Compounds containing quinoline moiety have shown good amoebicidal, bactericidal, fungicidal, and antimalarial activities.(Kaminsky and Meltzer 1968) Expecting similar trends, the antibacterial activities of the newly synthetic compounds were screened against two Gram-negative and two Gram-positive bacteria namely *E. coli* (ATCC, 25922) *S. typhimurium* (ATCC 13311). *S. aureus* (ATCC25923), and *B.subtilis* (ATCC6633) using paper disc diffusion method. The results are given below (Table 5 and Figure 14).

The results in Table 5, revealed that some the compounds exhibited antibacterial activity. The mean inhibition zone for five of the synthetic compounds against *E. coli* varies from 8.0 ± 0.82 to 16.0 ± 0.82 mm while for standard drug (ciprofloxacin) at the same concentration was 18.67 ± 0.47 diameter. Compound **216** (with mean inhibition zone of 12.67 ± 0.82 mm) and **229** (with mean inhibition zone of 16.0 ± 0.82 mm) were with maximum activity against *E. coli*.

Table 3. Antibacterial activities of quinoline-stilbenes and pinacol of quinolines

	Bacterial strains	<i>S. aureus</i>		<i>B. subtilis</i>		<i>E. coli</i>		<i>S. typhimurium</i>	
	Concentration	500µg	250µg	500µg	250µg	500µg	250µg	500µg	250µg
Compounds	211	0	0	0	0	10 ± 0.82	8.67 ± 1.25	8.33 ± 1.72	6.67 ± 0.47
	213	0	0	0	0	0	0	0	0
	214	0	0	0	0	8.0 ± 1.25	8.0 ± 1.25	0	0
	215	0	0	0	0	9.33 ± 1.25	8.0 ± 0.82	0	0
	226	0	0	0	0	12.67 ± 0.82	11.33 ± 1.70	0	0
	217	0	0	0	0	0	0	9.67 ± 1.25	9.67 ± 0.47
	219	0	0	0	0	16.0 ± 0.82	14.67 ± 0.94	0	0
	222	0	0	0	0	0	0	0	0
	224	16.0 ± 1.63	13.67 ± 0.47	0	0	0	0	8.67 ± 0.47	8.33 ± 1.25
	ciprofloxacin	11.67 ± 0.47	11.33 ± 0.67	15.59 ± 0.82	14.67 ± 0.82	18.67 ± 0.47	18.67 ± 0.47	13.56 ± 0.82	11.33 ± 0.67

0: inhibition zone was not observed

Compounds **211**, **217**, and **224** have moderate activities against *S. typhimurium*. Within the series compound **217** with mean inhibition zone of 9.67 ± 0.47 mm diameter was the maximum while 13.56 ± 0.82 mm mean inhibition zone was observed for the standard drug at the same concentration. In addition, **224** showed strongest activity against *S. aureus* with 16.0 ± 1.63 mm mean inhibition zone compared with 11.67 ± 0.47 mm for that of the standard drug. However none of the compounds showed activity against *B. subtilis* (ATCC6633).

Overall, among compounds reported herein, only seven of them, **211**, **214**, **215**, **216**, **217**, **219**, and **224** displayed promising bioactivity. In these synthetic compound, the phenyl nucleus didn't bear any substituents and substituents in the quinoline nucleus was limited to the second

and eight position. As a limitation, quinoline analogs synthesized by application of Vilsmeier-Hack reaction require activated aniline derivatives (activated ortho position relative to amino group) to afford sufficient yields. However, most important bioisosters such as F, Cl, carbonyl, carboxylic acid, sulfonyl group are electron withdrawers and deactivators and cannot be incorporated in the quinoline nucleus.(Meanwell 2013) But the current scaffold overcomes this limitation since the phenyl nucleus can bears various type of functional groups (electron withdrawer or donors) in one or more of the five positions on the phenyl ring. Thus current strategy provides manageable way to optimize the bioactivity through structural modification. Owing to the tolerance of Wittig reaction for various functional groups, phenyl ring can comprise one or more bioisosters which would be used to improve the bioactivity of quinoline-stilbenes. Regarding compound **224**, which was generated by pinacolization of 2-chloro-8-methylquinoline-3-carbaldehyde (**223**), huge improvement in the bacterial activity was observed relative to the monoalcohol (3-methoxy-5-methylnaphthalen-2-yl)methanol of the related reactant. In part one above, we reported (3-methoxy-5-methylnaphthalen-2-yl)methanol had poor antibacterial activity. It exhibited activity only against *E. coli* (with mean inhibition zone of 9.33 ± 0.89 mm diameter) whereas, the correspondent diol (**224**) was active against both *S. aureus* (with mean inhibition zone of 16.0 ± 1.63 mm diameter) and *S. typhimurium* (with mean inhibition zone of 8.67 ± 0.47 mm diameter) respectively (Table 3). Furthermore, the study revealed that an opportunity of developing stronger bioactive molecules by pinacolization of quinoline-3-carbaldehyde derivatives bearing various bioisosteric substituents.

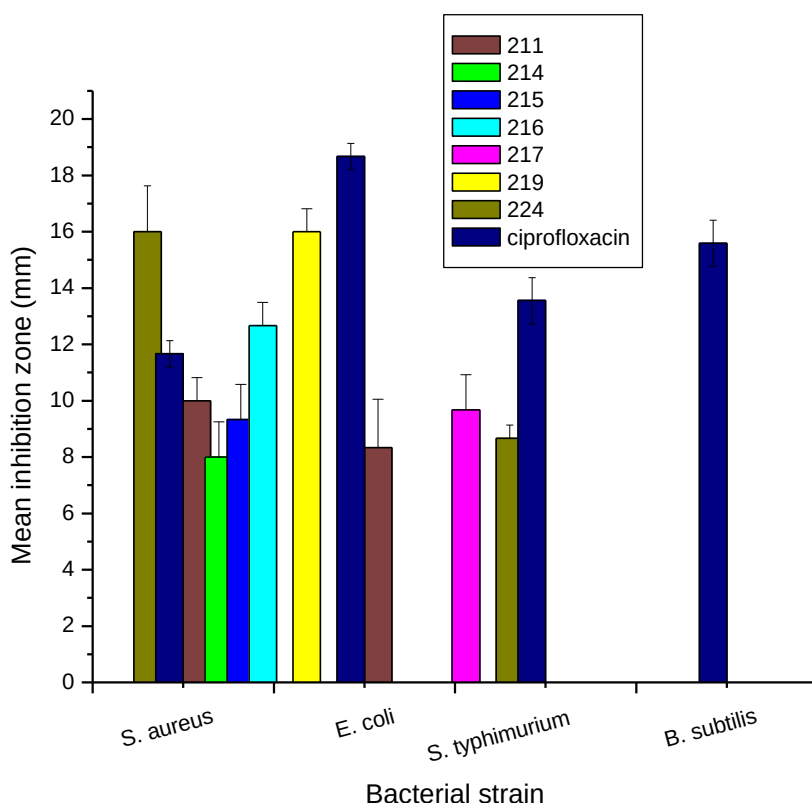


Figure 14. Antibacterial activity of quinoline-stilbenes and pinacol of quinolines at 500 μ g/mL

4.2.3 Antibacterial activities of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs

All of the synthetic compounds were screened for their *in vitro* antibacterial activities using paper disc diffusion methods against two Gram positive *S. aureus* (ATCC25923) and *S. pyogenes* (ATCC 27853)) and two Gram negative *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC2592) bacterial strains (Table 4). Six of the compounds, namely **225**, **226**, **227**, **227**, **232** and **214** showed good activity with mean inhibition zone ranged from 9.7 ± 2.5 to 20.7 ± 1.5 mm compared with ciprofloxacin with a mean inhibition zone range 14.3 ± 0.58 to 22.0 ± 1.0 mm, which was the reference drug in the experiment. However, none of them were stronger than ciprofloxacin. Only compound **227** had equal activity with ciprofloxacin against *E. coli*. Among them, the two none quinoline Schiff base compounds (**230** and **236**) and 2-phenylquinoline-4-carboxylic acid (**62**) didn't show any significant mean inhibition. Compounds **62**, **230**, and **236** showed little activity with mean inhibition zone of 7.0 ± 0 and 9.67 ± 0.58 mm against *E. coli* and *P. aeruginosa*.

Table 4. The antibacterial activities of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs

Compounds	Concentration ($\mu\text{g}/\mu\text{L}$)	Zone of inhibition in mm			
		<i>S. aureus</i>	<i>S. pyogenes</i>	<i>E. coli</i>	<i>P.aeruginosa</i>
62	0.10	0.0 $\pm$ 0.0	0	0	0
	0.20	7.0 $\pm$ 0.0	0	7.0 $\pm$ 0.0	0
225	0.10	11.0 $\pm$ 1.0	11.0 $\pm$ 1.0	12.7 $\pm$ 2.5	15.7 $\pm$ 1.5
	0.20	12.3 $\pm$ 0.58	13.7 $\pm$ 0.58	13.0 $\pm$ 2.0	19.3 $\pm$ 3.1
226	0.10	10.3 $\pm$ 0.58	10.0 $\pm$ 0.0	9.7 $\pm$ 2.5	11.3 $\pm$ 2.3
	0.20	13.0 $\pm$ 1.7	13.3 $\pm$ 2.1	12.7 $\pm$ 1.2	19.7 $\pm$ 1.5
227	0.10	11.0 $\pm$ 3.0	10.3 $\pm$ 1.2	14.3 $\pm$ 1.5	12.0 $\pm$ 2.0
	0.20	14.3 $\pm$ 1.5	12.3 $\pm$ 1.2	14.0 $\pm$ 1.5	20.7 $\pm$ 1.5
228	0.10	11.0 $\pm$ 1.0	11.0 $\pm$ 1.0	13.7 $\pm$ 2.51	14.0 $\pm$ 1.0
	0.20	15.7 $\pm$ 0.58	11.0 $\pm$ 1.7	16.0 $\pm$ 1.7	19.7 $\pm$ 1.5
230	0.10	8.33 $\pm$ 0.58	7.0 $\pm$ 0.0	7.0 $\pm$ 0.58	7.33 $\pm$ 0.58
	0.20	9.67 $\pm$ 0.58	8.33 $\pm$ 0.58	8.67 $\pm$ 0.58	9.67 $\pm$ 0.58
232	0.10	9.7 $\pm$ 2.3	0	9.0 $\pm$ 1.0	0
	0.20	10.7 $\pm$ 2.1	0	9.7 $\pm$ 2.9	9.3 $\pm$ 2.1
235	0.10	12.7 $\pm$ 0.58	8.7 $\pm$ 2.1	13.7 $\pm$ 2.5	13.3 $\pm$ 1.5
	0.20	13.3 $\pm$ 1.5	11.0 $\pm$ 0.0	14.7 $\pm$ 2.1	19.7 $\pm$ 1.5
236	0.10	0	0	0	0
	0.20	0	0	7.7 $\pm$ 0.58	0
Ciprofloxacin	0.10	14.7 $\pm$ 0.58	14.7 $\pm$ 0.58	14.3 $\pm$ 0.58	19.0 $\pm$ 0.0
	0.20	16.33 $\pm$ 0.58	16.0 $\pm$ 0.0	14.3 $\pm$ 2.1	22.0 $\pm$ 1.0
DMSO	0	0	0	0	0

0: inhibition zone was not observed

The results are expressed as mean $\pm$ SD for three experiments (n = 3).

When the maximum mean inhibition zone was considered per bacterial strain, compound **228** exhibited 15.7 $\pm$ 0.58 mm mean inhibition against *S. aureus*, at concentration of 0.2 $\mu\text{g}/\mu\text{L}$ while ciprofloxacin was 16.33 $\pm$ 0.58 mm at the same concentration, compound **225** showed 13.7 $\pm$ 0.58

mm against *S. pyogenes* while ciprofloxacin was 16.0 ± 0.0 mm, compound **228** showed 16.0 ± 1.7 mm against *E. coli* whereas ciprofloxacin was 14.3 ± 0.58 mm, and compound **227** showed 20.7 ± 1.5 mm against *P. aeruginosa* whereas ciprofloxacin was 22.0 ± 1.0 mm at $0.2 \mu\text{g}/\mu\text{L}$ concentration. In general, on average maximum inhibition zone (15.6 mm) was recorded by compound **228** while (17.7 mm) by the control at the conc. of $0.2 \mu\text{g}/\mu\text{L}$. Thus **228** displayed best mean inhibition zone among the synthetic compounds reported herein followed by compound **226**, **227** and **235** consecutively.

Literature report showed moderate activity for the antibacterial activity of 2-phenylquinoline-4-carboxylic acid (**62**) and some of its analogs, however at concentration used here ($0.1 \mu\text{g}/\mu\text{L}$ and $0.2 \mu\text{g}/\mu\text{L}$) **62** didn't show any antibacterial activity (Patel et al. 2017; Wadher et al. 2009) while the [2,3'-biquinoline]-4-carboxylic acid analogs showed much better activity compared to it. The antibacterial activities of the synthetic compounds were elaborated further in Figure 15 and compared with that ciprofloxacin at $0.01 \mu\text{g}/\text{mL}$ concentration. The figure clearly showed that compound **225**, **226**, **228**, and **235** have better activities against all the four bacterial strains.

Compound **232** which was developed by acid hydrolysis of **215** showed little improvement in its antibacterial activity. Compound **215** showed activity only against *E-coli* with mean inhibition zone of 9.33 ± 1.25 mm at concentration of $500 \mu\text{g}/\text{mL}$ while compound **232** showed activity against *E-Coli*, *S. aureu* and *P.aeruginosa* with mean inhibition zone of 8.67 ± 0.58 , 9.67 ± 0.58 , and 9.3 ± 2.1 mm at a concentration of $200 \mu\text{g}/\text{mL}$ respectively. Thus the quinoline-3-carbaldehyde analog showed better activity than its ethanol amine Schiff base analog.

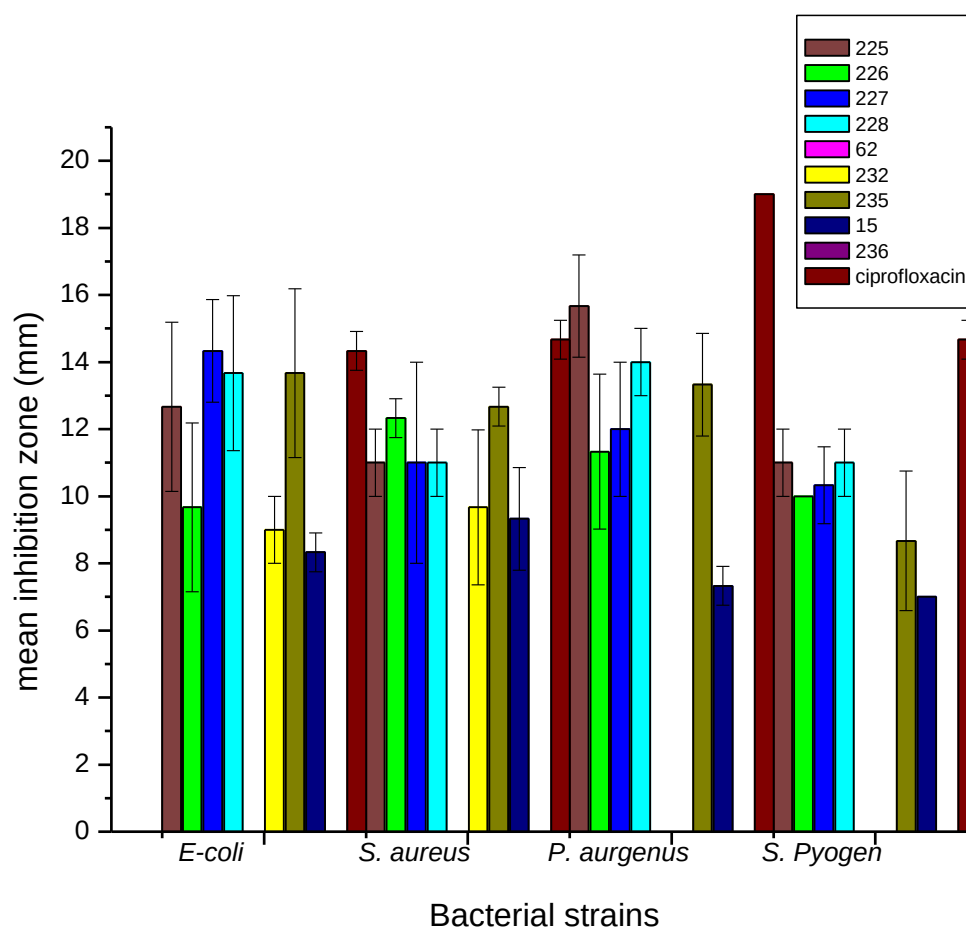


Figure 15. The inhibition zone (mm) of synthetic compounds at 0.1 $\mu\text{g}/\mu\text{L}$. The error bar indicates the standard deviation

4.3 Radical scavenging activities of compounds

DPPH antioxidant assay was developed by Blois (1958) with the view point to determine the antioxidant activity in a like manner by using a stable free radical α, α -diphenyl- β -picrylhydrazyl (DPPH) (Saha et al. 1970). DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolorized in the presence of antioxidants (Saha et al. 1970). DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. Qualitatively, antioxidants decolorize the purple color of DPPH and the intensity of the color notifies the extent of the reaction. Quantitatively the change in absorbance at 517 nm was used to quantify the radical scavenging capacity of substances. The DPPH assay is based on both electron transfer (SET)

and hydrogen atom transfer (HAT) reactions (Liang and Kitts 2014). DPPH assay is relatively an easy, economic and rapid method to evaluate the radical scavenging activity of non-enzymatic antioxidants (Liang and Kitts 2014). Because of these merits, DPPH was used to determine the radical scavenging capacity of the synthetic compounds.

4.3.1 Radical scavenging activity of quinoline-3-carbaldehyde derivatives

Compounds exhibiting antioxidant activity reduced the absorbance at 517 nm, which is due to DPPH radical in addition to their ability to turn the purple colored DPPH to yellow. In this work, the radical scavenging activities of the synthesized compounds were evaluated using DPPH method, and the findings are depicted below (Table 5).

Table 5. Percentage inhibition of quinoline-3-carbaldehyde and its derivatives and ascorbic acid

Compounds	% radical scavenging activity at				IC ₅₀ (μg/ml)
	12.5μg/mL	25μg/mL	50μg/mL	100μg/mL	
196	20.91	22.86	26.77	32.64	217.5
197	14.76	18.95	26.14	31.53	188.8
198	16.71	16.99	23.7	35.44	162.3
199	37.4	45.22	51.93	67.02	45.9
200	22.86	24.82	28.73	33.76	216.9
203	18.11	21.71	23.7	23.98	512.3
206	5.81	6.65	9.45	15.04	381.7
207	1.9	12.24	22.86	23.42	196.3
208	16.7	20.63	25.94	33.2	191.1
209	18.39	23.98	24.54	25.1	493.3
Ascorbic A.	83.23	84.91	85.19	85.47	4.5

The DPPH radical scavenging activity of the synthesized compounds was compared with ascorbic acid which is used as positive control, and the result is presented below (Figure 14).

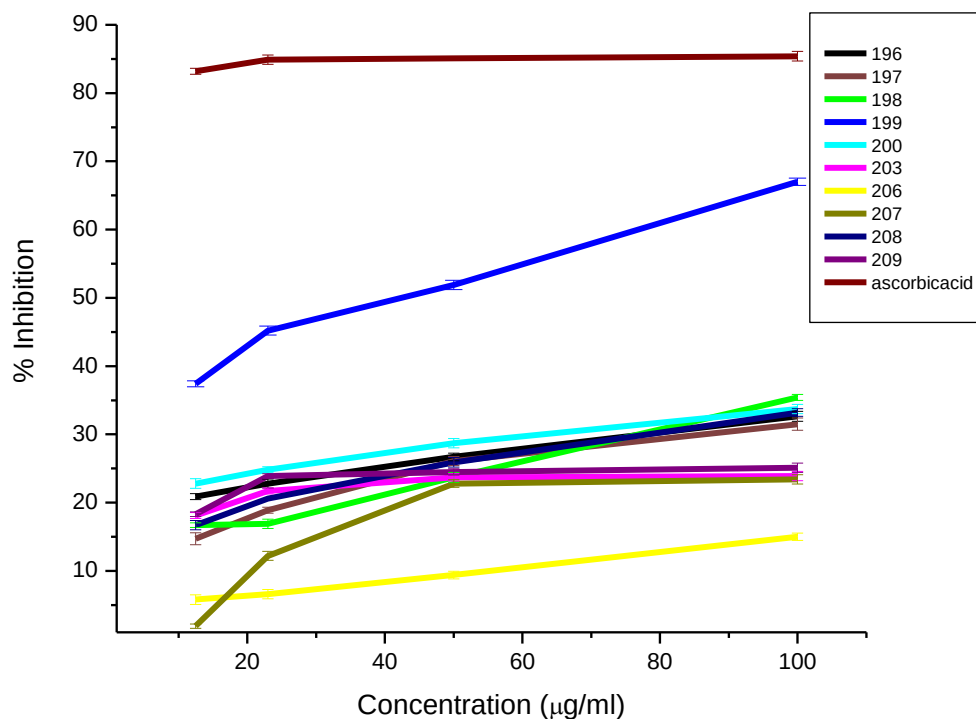
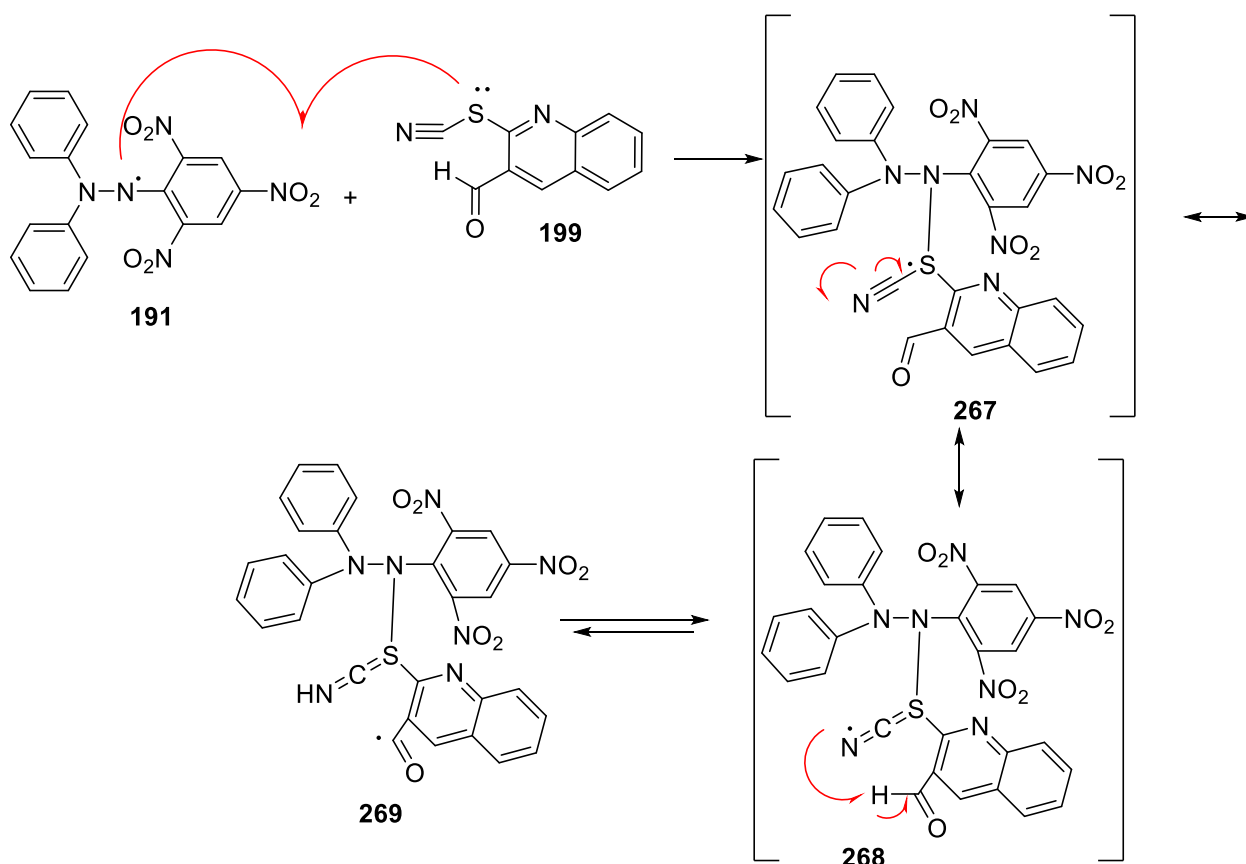


Figure 16. The Percentage inhibition of free radical DPPH by the synthetic compounds

Generally the antioxidant scavenging activities of the synthetic compounds in this batch are low relative to ascorbic acid. Only compound **199** exhibited percentage inhibition greater than half that of ascorbic acid (Table 5). Compound **199**, doesn't have such easily transferable hydrogen; however, sulfur of thiocyanate functional group may donate an electron to lone pair electron on the nitrogen atom to form bond with sulfur (Scheme 41).



Scheme 41. Proposed radical scavenging mechanism of compound **199**

4.3.2 The Radical Scavenging Activities of quinoline-stilbenes and pinacol of quinolines

Most of the synthetic quinoline-stilbenes and their analogs possess moderate antioxidant activity relative to ascorbic acid (Table 6). Compound **211**, **216**, and **222** were only strong just as a half of the ascorbic acid at same concentration and the others were even weaker. However the procedure can be optimized to increase the antioxidant properties by introducing hydroxyl, thiol, and selenium groups on phenyl nucleus as much as required. The antioxidant activity of these synthetic compounds was elaborated and compared in Figure 17 with line diagram,

Table 6. Percent radical scavenging activity and IC₅₀ values of quinoline-stilbenes and pinacol of quinolines

Compounds	% radical scavenging activity at				IC ₅₀ ($\mu\text{g/ml}$)
	12.5 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	
211	32.21 $\pm$ 0.44	35.39 $\pm$ 0.67	41.06 $\pm$ 0.87	50.10 $\pm$ 0.25	43.32
213	40.09 $\pm$ 0.47	42.21 $\pm$ 0.82	43.27 $\pm$ 0.47	45.93 $\pm$ 0.67	33.08
214	33.72 $\pm$ 0.27	42.48 $\pm$ 0.52	43.81 $\pm$ 0.47	43.98 $\pm$ 0.67	41.58
215	33.54 $\pm$ 0.82	36.73 $\pm$ 0.82	38.58 $\pm$ 0.67	40.09 $\pm$ 0.92	37.48
216	20.97 $\pm$ 0.82	34.07 $\pm$ 0.82	36.64 $\pm$ 1.25	36.73 $\pm$ 1.25	33.41
217	27.88 $\pm$ 0.67	31.86 $\pm$ 0.72	38.50 $\pm$ 0.47	41.06 $\pm$ 0.87	35.34
219	31.15 $\pm$ 0.82	35.13 $\pm$ 0.82	45.13 $\pm$ 0.67	55.54 $\pm$ 0.92	42.63
222	37.17 $\pm$ 0.57	39.55 $\pm$ 0.87	48.58 $\pm$ 0.67	52.21 $\pm$ 0.72	45.03
224	33.62 $\pm$ 0.47	36.73 $\pm$ 0.67	42.21 $\pm$ 1.25	42.21 $\pm$ 0.82	39.06
Ascorbic acid	86.9 $\pm$ 0.52	89.99 $\pm$ 0.67	90.03 $\pm$ 0.71	90.33 $\pm$ 0.82	4.50

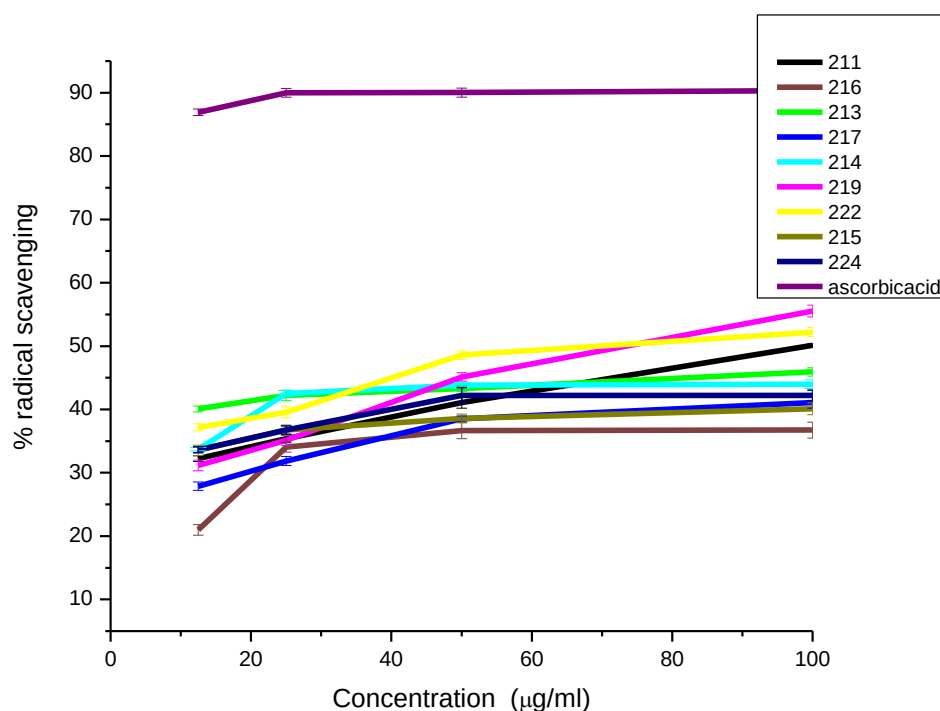


Figure 17. Percent radical scavenging activity of of stilbenes and pinacol of quinolines

The IC₅₀ values of the synthetic compound were calculated (Table 6). As, the lower the IC₅₀, the higher the antioxidant activity of substances. IC₅₀ values vary from 33.41 µg/mL for **216** to 43.32 µg/mL for **211**. The data indicated that the synthesized compounds displayed higher IC₅₀ value compared with ascorbic acid (4.50 µg/mL) used as natural antioxidant. This indicates that the activities shown by the synthetic compounds are moderate as radical scavengers. This is in good agreement with literature reported for closely related compounds (Puskullu et al. 2016).

4.3.3 The radical scavenging activities of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs

The measurements were made after DPPH-sample mixtures were kept at 37°C in dark oven for 30 minutes to attain steady state equilibrium. The results showed that, most of the synthetic compounds to possess very good radical scavenging activity (Table 7). Two of the synthetic compounds, namely **227** and **235** with IC₅₀ values 1.25 and 1.75 µg/mL, respectively are stronger radical scavenger than ascorbic acid (IC₅₀ of value of 4.5 µg/mL). Thus these two compounds were the strongest antioxidant agents. Structurally **227** has acidic proton and electron rich aromatic nucleus and **235** possesses an iodine atom which is labile and has low electronegativity with easy transferable electron and an α,β -conjugated double bond may also be involved in an electron transfer process with DPPH. For, the other three compounds; **226**, **62**, and **236**, the IC₅₀ values are between 15-25 µg/mL showing that, these have also significant antioxidants even though weaker than ascorbic acid. Structurally these compounds contain C=C, carboxylic acid and iodo as a labile functional groups. The remaining two compounds **230** and **232** are moderate in their radical scavenging activities whereas **225**, and **228** displayed weak activity (Table 7). The antioxidant properties of these compounds also shown below along with that of ascorbic acid (Figure 18).

Table 7. The % inhibition of novel [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs

Compounds	concentrations in $\mu\text{g/mL}$					IC_{50} ($\mu\text{g/mL}$)
	25	20	15	10	5	
62	52.48 $\pm$ 1.23	51.34 $\pm$ 0.49	27.52 $\pm$ 1.72	25.81 $\pm$ 0.49	7.81 $\pm$ 0.25	22.40
225	29.22 $\pm$ 1.37	27.38 $\pm$ 0.49	26.67 $\pm$ 1.07	24.96 $\pm$ 0.49	21.98 $\pm$ 2.45	85.4
226	58.78 $\pm$ 2.53	52.20 $\pm$ 0.65	50.64 $\pm$ 1.86	40.36 $\pm$ 1.59	34.75 $\pm$ 2.46	17.2
227	83.26 $\pm$ 1.72	80.85 $\pm$ 0.43	79.86 $\pm$ 0.99	77.72 $\pm$ 1.05	52.27 $\pm$ 2.98	1.25
228	17.64 $\pm$ 1.31	14.61 $\pm$ 0.25	13.48 $\pm$ 0.25	10.35 $\pm$ 0.25	2.55 $\pm$ 0.0	75.1
230	25.25 $\pm$ 0.88	27.23 $\pm$ 0.0	24.82 $\pm$ 0.25	11.20 $\pm$ 0.25	4.40 $\pm$ 0.24	42.75
222	33.48 $\pm$ 1.48	24.54 $\pm$ 0.88	24.68 $\pm$ 0.43	22.13 $\pm$ 1.86	9.36 $\pm$ 1.27	41.8
235	89.50 $\pm$ 0.65	84.78 $\pm$ 0.37	80.30 $\pm$ 0.97	64.52 $\pm$ 1.12	53.41 $\pm$ 0.87	1.75
236	45.53 $\pm$ 1.28	44.40 $\pm$ 0.24	31.34 $\pm$ 0.98	29.32 $\pm$ 1.08	6.52 $\pm$ 0.25	25.0
Ascorbic acid	95.83 $\pm$ 1.19	86.46 $\pm$ 0.54	76.60 $\pm$ 0.53	64.40 $\pm$ 0.62	48.91 $\pm$ 0.49	4.5

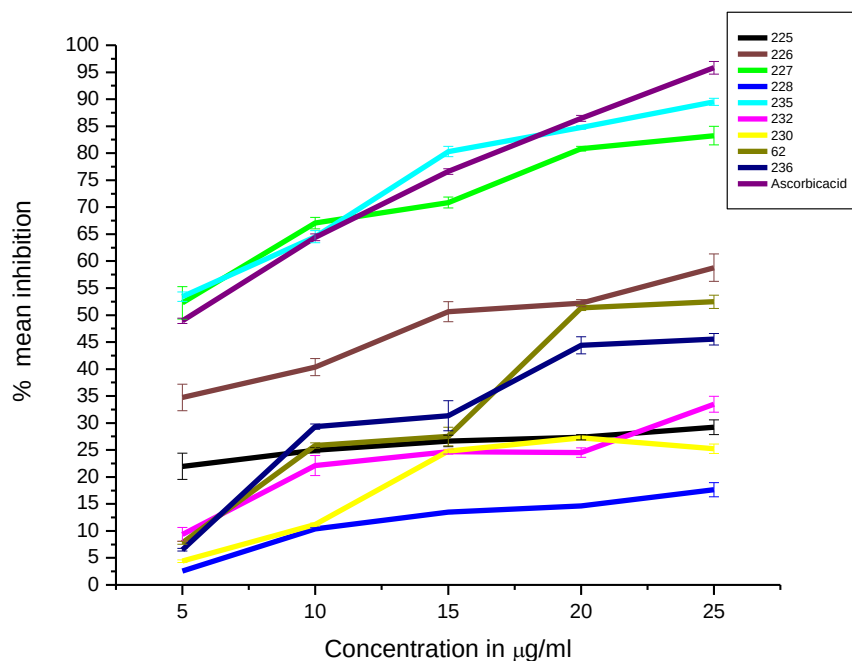


Figure 18. The percent inhibition of the compounds and the standard

4.4 Molecular docking value of compounds (199, 227 and 235) against human

peroxidoxin 5 (PDB ID:1HD2)

Antioxidant compounds play a fundamental role as health protecting factor. Free radicals such as nitric oxide, superoxide and hydroxyl are the oxygen-centered free radicals, and they are also called reactive oxygen species (ROS) (Nayab et al. 2020). These oxygen species are produced in the human body and can become toxic when generated in excess. Mammalian uses various enzymes to maintain the redox homeostasis of cells. Peroxiredoxins (PRDX), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST), as well as reduced glutathione (GSH) some of these enzymes (Declercq *et al.* 2001; Fisher 2011). We have performed an *in silico* molecular docking study to investigate the antioxidant binding pattern of three most active molecules (**199**, **227**, and **235**) against *human peroxiredoxin* (PDB ID: 1HD2) and the result was compared with that of the reference compound (ascorbic acid) (Table 8). The result showed that the binding energies of the complexes between the three compounds and *human peroxiredoxin 5* (Table 8) were found to be in the range of -4.1 to -4.5 kcal/mol. Moreover the binding energies of the compounds were found to be comparable with binding energy of ascorbic acid (Table 8). The results indicated that the three compound interact favorably with the target proteins. From our molecular docking results, it was found that compound **227** was the most potent inhibitor of *human peroxiredoxin 5* interacting favorably with its catalytic site *via* three H-bonding interactions with (Cys-47, Thr-44 and Thr-147); two van der Waals interactions (Pro-40 and Gly-46); and four Alkyl/Pi–Alkyl interactions with (Pro-45, Ile-119, Phe-120 and Arg-127) (Table 8). Compound **199** was interacting with the active site of the enzyme *via* four der Waals interactions with Thr-44, Phe-120, Thr-147, and Leu-149; two H-bonding interaction with Arg-127 and Gly-46 and with Pro-45 *via* Alkyl/Pi–Alkyl interactions. Compound **235** interacts with the enzyme through five Alkyl/Pi–Alkyl interactions with Pro-45, Leu-116, Ile-119, Phe-120 and Leu-149; and two Van-der Walls interactions with Thr-44 and Gly-46 (Table 8). Compound **235** didn't interact with *human peroxidoxin 5* *via* H-bonding. Its strong antioxidant property may be attributed to its interaction to other redox homeostasis enzymes.

Table 8. Molecular docking value of compounds (**199**, **227** and **235**) against *human peroxidoxin* (PDB ID:1HD2)

Compound s	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
199	-4.2	Arg127, Gly-46	Pro-45	Thr-44, Phe-120, Thr-147, Leu-149
227	-4.5	Cys-47, Thr-44, Thr-147	Pro-45, Ile-119, Phe-120, Arg-127	Pro-40, Gly-46
235	-4.1	--	Pro-45, Leu-116, Ile-119, Phe-120, Leu-149	Thr-44, Gly-46
Ascorbic Acid	-4.9	Cys-47, Thr-44, Gly-46, Thr-147	Pro-40, Pro-45, Phe-120, Arg-127, Leu-149	--

4.5 In silico Pharmacokinetics (Drug-likeness) study

Drug discovery and development needs to pass through various stages. Synthesizing or isolation of a lead compound may considered as first step of the multistep process. Then optimization of a lead compound which involves *in vitro* and *in vivo* bio screening is the next time and resource demanding complex multistep process. The *in vivo* lead compound optimization comprises investigating the pharmaceutical, pharmacokinetic and pharmacodynamic interactions of a lead compound (Balani et al. 2005). Each of these steps demands resources including sophisticated laboratories. Thus various *in silico* analysis methods were developed to mitigate the time and resources required for optimization of a lead compound (Tian et al. 2015). Predicting ADME (absorption, distribution, metabolism and excretion) profiles of the bioactive molecules is one of the best filters in the virtual screening of bioactive compounds in order to be an effective drug in early preclinical development. Therefore drug-likeness analysis of the synthetic compounds was computed with SwissADME prediction software to evaluate their drug-like properties. *In silico* analysis tools were employed to evaluate and optimize the novel synthetic compounds developed herein.

4.5.1 *In silico* Pharmacokinetics (Drug-likeness) study of quinoline-stilbenes and pinacol of quinolones

The SwissADME prediction result showed that all the eighteen synthesized (Table 9 and 10) satisfy Lipinski's rule of five with zero violations. The K_p values of all molecules are within the range of (– 3.9 to – 7.38 cm/s) inferring low skin permeability. The predicted logP values revealed that they have optimal lipophilicity (ranging from -1.13 to 3.86). The SwissADME prediction parameters showed that all the compounds have high gastrointestinal (GI) absorption, most of them have blood brain barrier (BBB) permeation (except **215**, **219**, **222**, **224**, and **226**) and no compounds are substrate of permeability glycoprotein (P-gp) (except compound **215**, **224** and **235**). From the structure of compound **236** the negative value of logP is expected. Compound **236** bears a positive charge on one of the nitrogen atoms which contributed for its poor lipophilicity. The same compound is also the substrate of P-glycoprotein. Of course, compound **236** showed poor in vitro and molecular docking activity. Thus these synthesized compounds (Table 9 and 10) are expected to exhibit good intestinal absorption and bioavailability. These prediction results indicate that all the synthesized compounds (Table 9 and 10) can be active pharmacological agents.

Table 9. In-Silico drug-likeness predictions of quinoline-stilbenes and pinacol of quinolones computed by SwissADME.

Compounds	Formula	Mol. Wt. (g/mol)	NHD	NHA	NRB	TPSA (Å ²)	LogP (cLogP)	Skin Permeation Value (log Kp) cm/s	GI absorption	BBB permeant	Pgp substrate	Lipinski's rule of Five Violation
211	C ₁₇ H ₁₂ ClN	265.74	0	1	2	12.89	3.16	-4.07	High	Yes	No	0
213	C ₁₇ H ₁₃ NO	247.29	1	1	2	32.86	2.58	-5.23	High	Yes	No	0
214	C ₁₈ H ₁₅ NO	261.32	0	2	3	22.12	3.36	-4.51	High	Yes	No	0
215	C ₁₄ H ₁₇ N ₃ O ₂	259.3	3	4	6	77.74	2.22	-7.38	High	No	Yes	0
216	C ₁₈ H ₁₄ ClN	279.76	0	1	2	12.89	3.41	-3.9	High	Yes	No	0
217	C ₁₈ H ₁₅ NO	261.32	1	1	2	32.86	2.94	-5.07	High	Yes	No	0
219	C ₂₄ H ₁₉ N ₃ O ₂	381.43	1	3	5	70.74	3.58	-3.99	High	No	No	0
222	C ₂₆ H ₂₂ N ₂ O ₂	394.47	1	3	6	51.22	3.86	-4.55	High	No	No	0
224	C ₂₂ H ₂₀ N ₂ O ₄	376.41	2	6	5	84.7	3.64	-6.42	High	No	Yes	0
Vosarosin	C ₁₈ H ₁₉ N ₅ O ₄ S	401.45	2	9	5	136.1 3	0.963	-8.98	High	No	Yes	0

NHD = Number of Hydrogen donor, NHA = Number of Hydrogen acceptor, NRB = Number of rotatable bonds, and TPSA = total polar surface area

Table 10. In Silico drug-likeness predictions of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs by SwissADME

NHD = Number of Hydrogen donor, NHA = Number of Hydrogen acceptor, NRB = Number of rotatable bonds, and TPSA = total

Compound No	Formula	Mol. Wt. (g/mol)	NH D	NHA	NRB	TPSA (Å ²)	LogP (cLogP)	Skin Permeatio n Value (log Kp) cm/s	GI absorptio n	BBB permeant	Pgp substrat e	Lipinski' s rule of Five Violation
225	C ₁₉ H ₁₁ ClN ₂ O ₂	334.76	1	4	2	63.08	2.34	-5.07	High	Yes	No	0
226	C ₂₁ H ₁₅ ClN ₂ O ₂	362.81	1	4	2	63.08	2.8	-4.72	High	No	No	0
227	C ₂₁ H ₁₆ N ₂ O ₃	344.36	1	5	3	72.31	2.89	-5.33	High	Yes	No	0
228	C ₂₁ H ₁₆ N ₂ O ₃	344.36	1	5	3	72.31	2.82	-5.33	High	Yes	No	0
62	C ₁₆ H ₁₁ NO ₂	249.26	1	3	2	50.19	1.98	-5.42	High	Yes	No	0
230	C ₁₀ H ₁₁ NO ₂	177.2	1	3	2	49.66	1.42	-6.05	High	Yes	No	0
232	C ₁₂ H ₁₂ N ₂ O ₂	216.24	2	3	4	62.22	1.58	-6.31	High	Yes	No	0
235	C ₁₂ H ₆ Cl ₂ INO	377.99	0	2	2	29.96	2.63	-5.69	High	Yes	No	0
236	C ₉ H ₁₂ IN ₂	275.11	1	0	2	15.04	-1.13	-6.66	High	Yes	Yes	0
Vosaroxi n	C ₁₈ H ₁₉ N ₅ O ₄ S	401.45	2	9	5	136.13	0.963	-8.98	High	No	Yes	0

polar surface area

4.6 *In silico* molecular docking study

Bacterial DNA gyrase are vital for the survival of bacteria. Recently, researchers have explored a range of synthetic inhibitors as antibacterial drugs to target *DNA gyrase* (Ghorab et al. 2020; Hailekiros, Kedir, and Endale 2021). DNA gyrase an enzyme belonging to a member of bacterial topoisomerase which controls the topology of DNA (Dorman and Dorman 2016; Ziraldo, Hanke, and Levene 2019). Molecular docking is frequently used in the process of computer aided drug design (CADD). It can be applied in different stages of the drug design process in order to: (1) predict the binding mode of already known ligands; (2) identify novel and potent ligands and (3) as a binding affinity predictive tool (Bartuzi et al. 2017). Therefore, we have carried out the molecular docking analysis for the synthesized compounds to elucidate their binding interactions with *DNA gyrase* and compared with the clinical drug inhibitor (ciprofloxacin)

4.6.1 *In silico* molecular docking study of quinoline-3-carbaldehyde derivatives against *E. coli* DNA gyrase B

In general, antimicrobial agents target the key components of bacterial metabolism to disable the bacteria such as cell-walls, DNA gyrase, DNA-directed RNA polymerase, protein synthesis, and enzymes. DNA gyrase, an enzyme belonging to a member of bacterial topoisomerase, controls the topology of DNA during transcription, replication, and recombination by introducing transient breaks to both DNA strands (Allouche 2012; Dorman and Dorman 2016). In this regard, bacterial DNA gyrase is essential for bacterial survival and therefore necessary to be exploited as an antibacterial drug target (Beck et al. 2015). Therefore, in the present study, the molecular docking analysis of the synthetic compounds was conducted to investigate their binding pattern with DNA gyrase and compared them with standard inhibitor (ciprofloxacin). The synthetic compounds (Table 11) were found to have minimum binding energy ranging from -6.2 to -7.3 kcal/mol (Table 4), with the best result achieved with compound **203** (-7.3 kcal/mol). The binding affinity, H-bond, and residual interaction of the ten compounds and ciprofloxacin were summarized in Table 11.

Table 11. Molecular docking value of quinoline-3-carbaldehyde derivatives

Compounds	Affinity (kcal/mol)	H-bond	Receptor interactions
196	-6.2	Asp-73,	Val-43, Val-71, Ile-78, Ala-47
197	-6.2	Asp-73, Thr-165, Gly-77	Ile-94, Ile-78
198	-6.4	Asp-73, Thr-165, Gly-77	Ile-94, Ile-78, Ala-47
199	-6.3	Asp-73, Asn-46, Thr-165, Gly-77	Val-43, Val-167, Ala-47
200	-6.3	Asn-46, Glu-50, Gly-77	Asp-73, Ala-47, Ile-78, Pro-79, Arg-76, Gly-75, Thr-165
203	-7.3	Arg-76, Asn-46, Gly-77, Thr-165	Asp-73, Ile-78, Ile-94, Pro-79, Glu-50, Gly-75
206	-6.3	--	Ile-78, Val-167, Val-120
207	-6.2	--	Ala-47, Asn-46, Ile-78, Thr-165, Val-167
208	-6.0	Asp-73	Ile-78
209	-6.3	Asp-73, Gly-77	Ala-47, Glu-50, Ile-78, Pro-79
Ciprofloxacin	-6.9	Asp-73, Arg-76, Asn-46	Ala-47, Ile-78, Ile-94, Pro-79, Glu-50, Gly-77

Compared to ciprofloxacin, the synthesized compounds (Table 11) showed similar residual interactions profile with amino acid residues Ile-94, Pro-79 Glu-50, Gly-77, Ile-78, and Ala-47 and H-bond with Asp-73, Arg-76, and Asn-46. Compounds **197**, **198**, and **199** have additional hydrogen bonding interaction with amino acid residue Thr-165. The compounds **196** (Val-43, Val-71), **199** (Val-167), **206** (Val-120 and Val-167), and **207** (Val-167) have shown additional hydrophobic interaction with amino acid residues. The residual amino acid interactions of synthesized ligands (except **206** and **207**) with DNA gyrase (6f86) in this study were well in agreement with the previously reported binding modes that include the crucial interactions between the ligand, Asp-73 (Xiao et al. 2014), and the water molecule. The *in silico* interaction results match the *in vitro* analysis of the synthesized compounds; **196** (−6.2 kcal/mol), **203** (−7.3 kcal/ mol), **200**(−6.3 kcal/mol), and **208** (−6.0 kcal/mol) showed good activities against *E. coli*, among which compounds **200** (−6.3 kcal/mol) and **203** (−7.3 kcal/mol) revealed better activity. The compounds **206** and **207** docking results partially matched the ciprofloxacin interactions with amino acid residues. Based on the *in silico* molecular docking analysis results, compounds **200** and **203** showed comparable residual interactions and docking score of ciprofloxacin. Therefore, compound **203** might have the best antibacterial agents among the compounds reported herein based on molecular docking study. However *in vitro* antibacterial

activity against *E. coli* is zero and contradicts the molecular docking score. The binding affinity, H-bond, and residual interaction of the ten compounds were summarized in Table 11. The 3-dimensional binding interaction of compounds **198**, **203** and ciprofloxacin against *E. coli* gyrase B complex is illustrated in Figures 19–21.

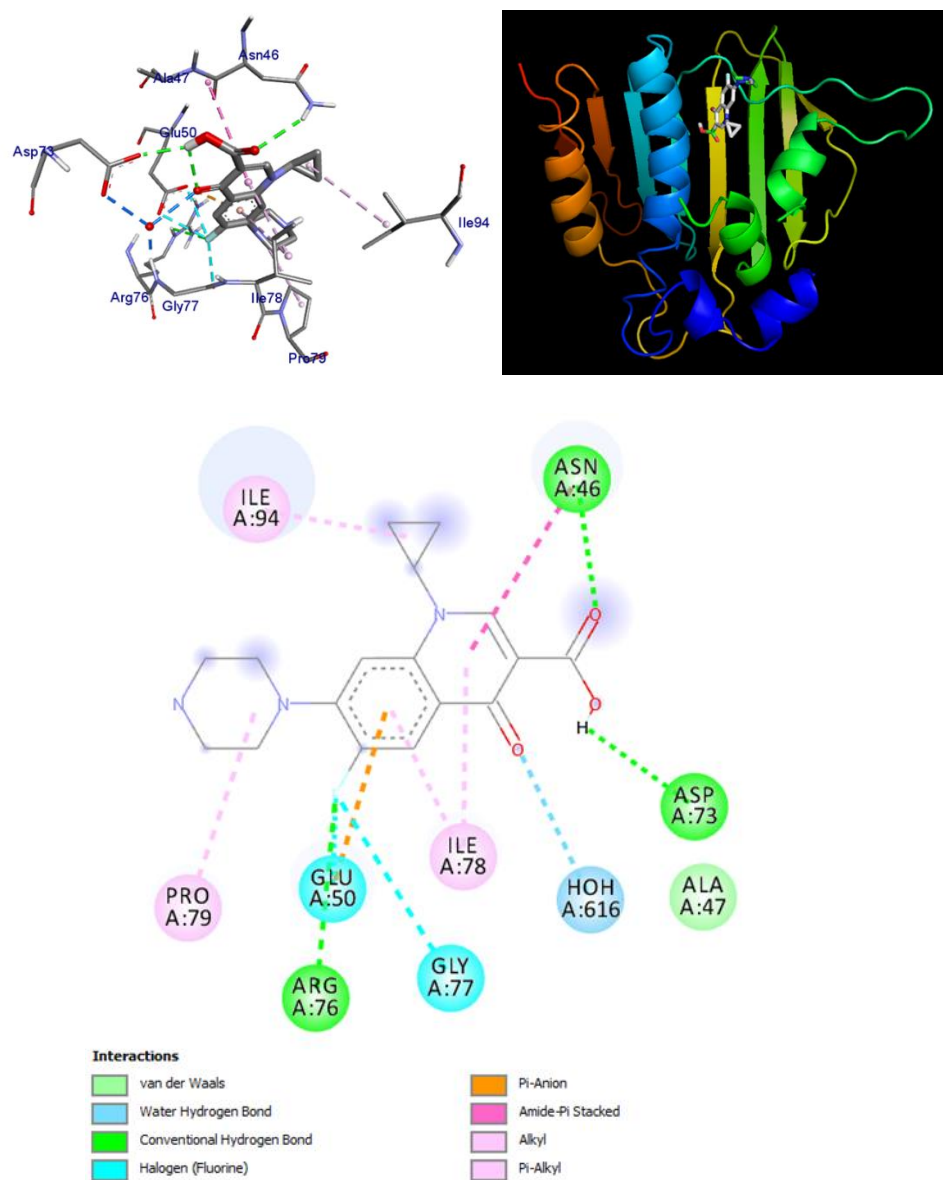


Figure 19. The binding interactions of Ciprofloxacin against E.coli DNA gyraseB (PDB ID: 6F86).

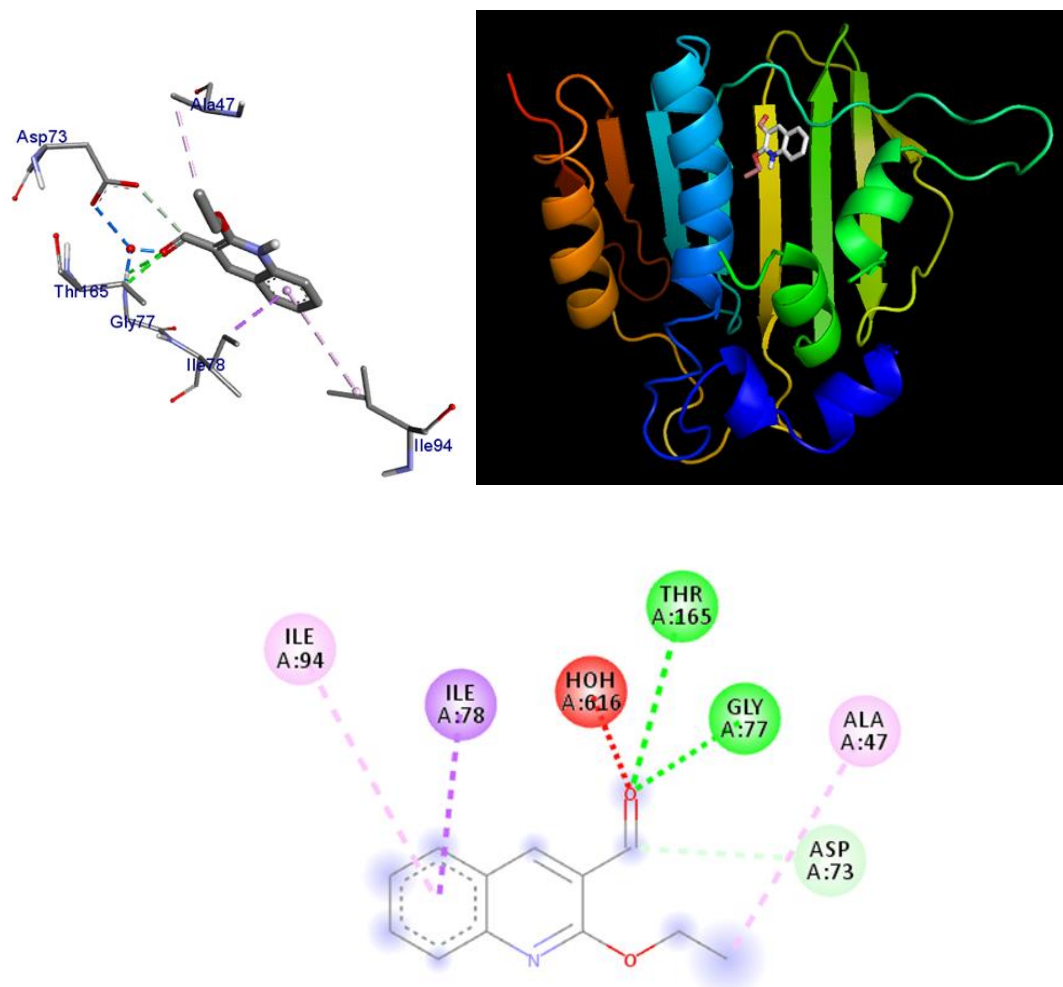


Figure 20. The binding interactions of compound **198** against *E. coli* DNA gyraseB (PDB ID: 6F86)

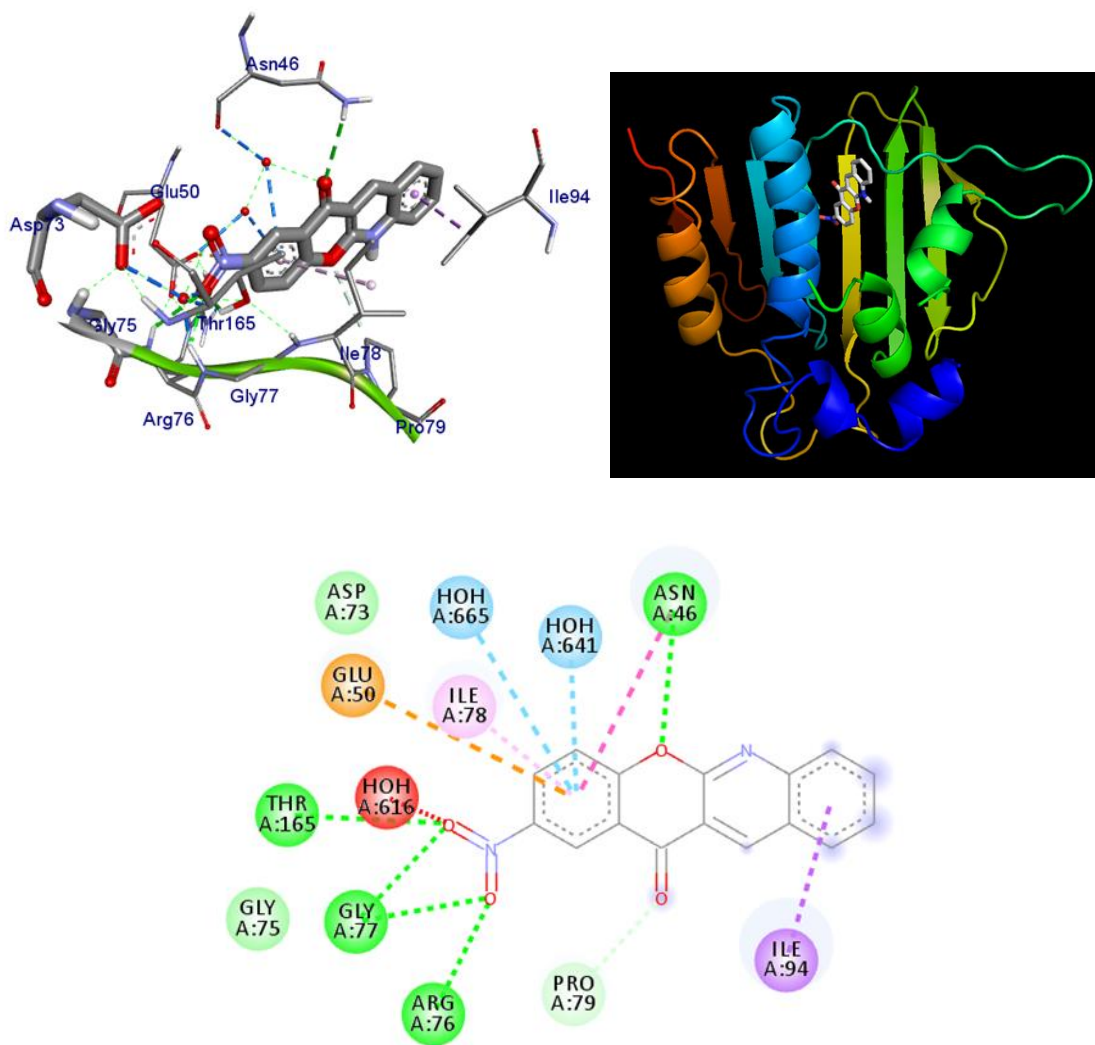


Figure 21. The binding interactions of compound **203** against *E.coli* DNA gyraseB (PDB ID: 6F8)

4.6.2 *In silico* molecular docking study of quinoline-stilbenes and pinacol of quinolones *E. coli* DNA gyrase A

Similar to the previous experiment, the molecular docking study of the synthetic compounds was carried out to examine their binding pattern against *E. coli* DNA gyrase A and compared with standard clinical drug (Ciprofloxacin). The compounds (Table 12) displayed minimum binding energies ranging from -5.6 to -7.1 kcal/mol, with the best result achieved using compounds **216**(-6.7 kcal/mol), **217**(-6.7 kcal/mol), **198**(-6.9 kcal/mol), and **224** (-7.1 kcal/mol). The binding affinity, H-bond and residual interaction of the nine compounds and ciprofloxacin are presented in Table 12. All the compounds displayed interactions within the

binding site of the clinical drug Ciprofloxacin. The synthesized compounds (Table 12) shown residual interactions profile with amino acid residues Ala-633, Val-685, Leu-735, Val-787, Arg-838 and H-bond with Ile-683, Val-737, and Gln-788. Among them, the synthesized compound **224** (– 7.1 kcal/mol) had almost similar hydrogen bonding and hydrophobic interactions with that of ciprofloxacin (– 7.3 kcal/mole). The compounds **215** and **217** have shown hydrogen bond interactions with amino acid residue Leu-735. The compounds **215** and **224** have shown hydrogen bond interaction with amino acid residue Ile-683 similar to Ciprofloxacin. The compound **213** (Val-737), and **216** (Arg-838, **219** (Arg-630, Thr-632) have shown hydrogen bond interaction with different amino acid residues. The *in silico* interaction results are agreeing with in vitro anti-microbial analysis of the synthesized compounds against *E.Coli*. The compounds **216** (– 6.7 kcal/mol), **217** (– 6.7 kcal/mol), **219** (– 6.9 kcal/mol), and **224** (– 7.1 kcal/mol) showed good activities against *E.coli*, among them compound **224** revealed better activity(Seeliger and De Groot 2010). The compound **219**, and **222** do not show any hydrogen bond interaction with any of the amino acid residues within the active site.

The compounds **211**, **213**, **214**, **215**, and **222** docking results were partially matching the clinical drug Ciprofloxacin interactions with amino acid residues. Based on the *in silico* molecular docking analysis result compound **224** (– 7.1 kcal/mol) showed the highest binding affinity compared to ciprofloxacin (– 7.3 kcal/mol). Therefore, based on molecular docking result, compound **224** might be a better anti-bacterial agent than the other synthesized compounds reported herein. However, the *in vitro* assay against *E. coli* is zero and doesn't agree with what has been predicted by molecular docking score. The binding affinity, H-bond and residual interaction of nine compounds presented in this section are summarized in Table 12 and the binding interactions of **219** and **224** against *E.coli* DNA gyrase A were depicted in Figures 22 and 23

Table 12. Molecular docking study of quinoline-stilbenes and pinacol of quinolones against *E.coli* DNA gyrase A (PDB ID 1ZI0)

Ligands	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
211	– 6.1	--	Ile-736, Asp-686, Val-787	Val-685, Val-737, Ile-683, Gly-684, Thr-632, Gln-788
213	– 6.4	Val-737	Ile-736, Arg-739	Val-685, Leu-735, Gln-788
214	– 6.2	--	Ile-736, Asp-686	Val-685, Val-737, Ile-683, Gly-684, Thr-632, Val-787, Gln-788, Leu-735
215	– 5.6	Ile-683, Leu-735	Ile-736	Gly-684, Val-685, Gln-788, Ser-734, Val-787
216	– 6.7	Arg-838	Ile-736, Val-787, gln-837	Val-685, Asp-686, Leu-735, Val-737, Ala-786, Gln-788, Leu-836
217	– 6.7	Leu-735	Val-787	Val-685, Asp-686, Ser-734, Val-737, Ile-683, Gly-684, Arg-838, Leu-836, Gln-837
219	– 6.9	Arg-630, Thr-632	Ala-633	Arg-580, Ile-634, Ile-631, Ile-683, Gly-684, Val-685, Asp-686, Val-733, Ser-734, Leu-735, Ala-786, Leu-836
222	– 6.5	Ile-634, Arg-580	Ala-633, Ile-736, Asp-686	Asp-579, Thr-632, Ile-683, Gly-684, Val-685, Val-737, Leu-735, Gln-788
224	– 7.1	Ile-683	Val-787, Ile-736, Asp-686	Arg-580, Thr-632, Asp-579, Gly-684, Val-685, Leu-735, Val-737, Arg-739, Gln-788, Gln-837, Arg-838
Ciprofloxacin	– 7.3	Ile-683, Val-737, Gln-788,	Ala-633, Val-685, Leu-735, Val-787, Arg-838	Gly-684, Asp-686, Ile-736, Arg-739

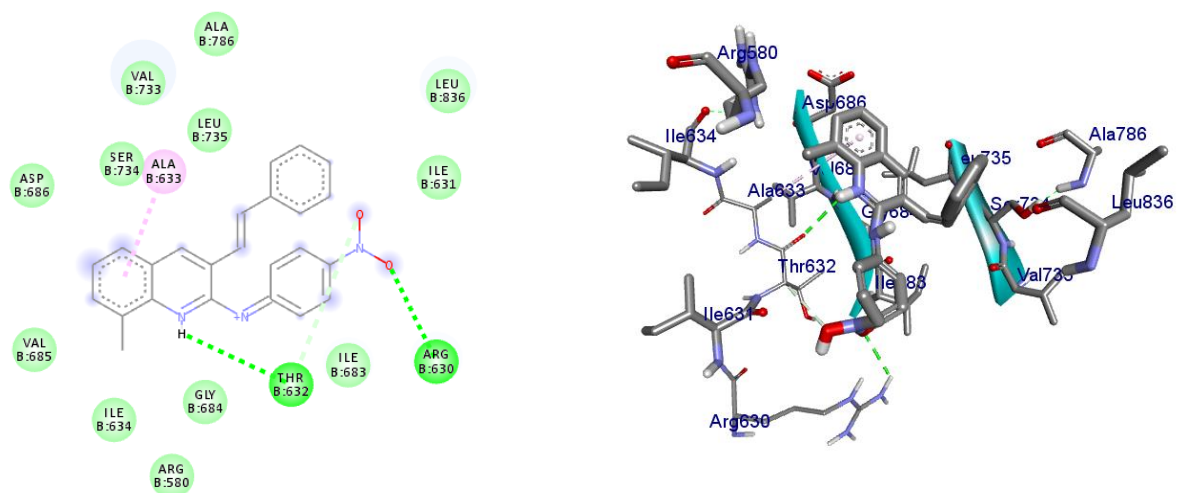


Figure 22. The binding interactions of compound **219** against *E.coli* DNA gyrase A (PDB ID: 1ZI0)

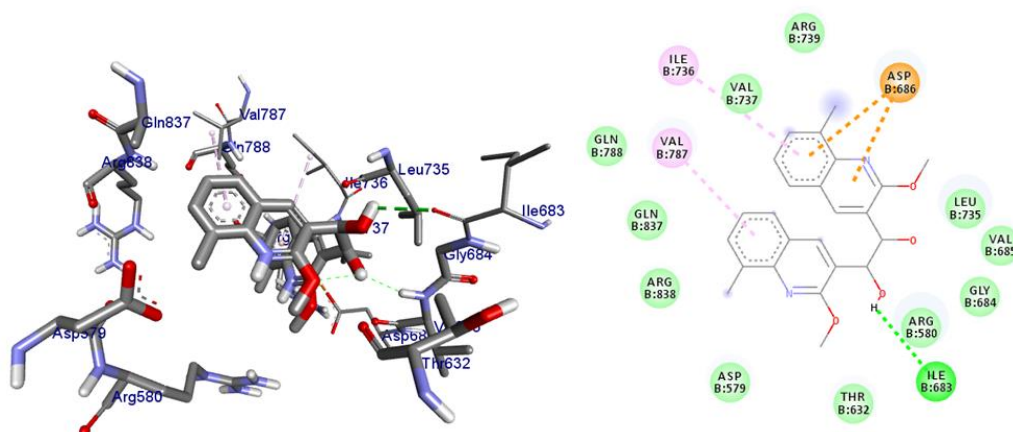


Figure 23. The binding interactions of compound **224** against *E.coli* DNA gyrase A (PDB ID: 1ZI0)

4.6.3 *In silico* molecular docking evaluation of quinoline-stilbenes and pinacol of quinolones against human *Topoisomerase II α* (PDB ID: 4fm9)

Topoisomerase I and *II* have been the key targets in the anti-cancer drug development (Jadhav and Karuppayil 2017). Vertebrate species produce two isoforms of topo II: *topo II α* and *topo II β* . These enzymes display a high degree of amino acid sequence identity (~70%), however, they differ in their protomer molecular masses (170 kDa versus 180 kDa, respectively) and are encoded by separate genes (McClendon and Osheroff 2007). *Topo II α* is overexpressed in proliferating cells and serves as a biomarker for cell proliferation, while expression of *topo II β* is evenly distributed across all cells. Therefore, *topo II α* is considered to be responsible for the

anticancer effects of topo II inhibitors (Skok et al. 2020). In the present investigation, we have studied the molecular docking interactions of the synthesized compounds against human *topoisomerase II α* and compared with the anti-cancer drug (vosoroxin). The synthesized compounds (Table 13) were found to have minimum binding energy ranging from -7.0 to – 8.0 kcal/mol (Table 13), with the best result achieved using compound **224** (– 8.0 kcal/mol), **217** (– 7.9 kcal/mol), **211**, **213** and **216** (– 7.8 kcal/mol), **219** (– 7.7 kcal/mol), **213** and **222**(– 7.5 kcal/mol), **215** (– 7.0 kcal/mol) and Vosaroxin (– 6.9 kcal/mol); The binding affinity, H-bond and residual interaction of the nine compounds and vosaroxin (anti-cancer drug) are summarized in Table 13. Compared to vosoroxin, the synthesized compounds (Table 13) shown similar residual interactions profile with amino acid residues such as Leu-705, Ile-577, Pro-593 Glu-682, Tyr-684, Arg-672, Gln-542 and H-bond with Ser-591, Leu-685, and Leu-592. The compounds also showed additional Van-der Walls interactions residue as compared to the reference drug. The *in-silico* analysis shown that the compound **224** (– 8.0 kcal/mol), **217**(– 7.9 kcal/mol), **211**, **213** and **216** (– 7.8 kcal/mol) revealed better activities. Based on the *in silico* molecular docking analysis results, compounds (Table 13) shown high residual interactions and docking scores than vosoroxin. Hence, compounds **211**, **213**, **216**, **217** and **224** might have better anti-cancer agents than the other compounds reported herein. The binding affinity, H-bond and residual interaction of ten compounds are summarized in Table 13. The *in silico* molecular docking analysis against human *topoisomerase II α* is stronger than the docking against *E.coli DNA gyrase A*.

Table 13. Molecular docking values of quinoline-stilbenes and pinacol of quinolones against human *topoisomerase II α* (PDB ID: 4fm9)

Compounds	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
211	-7.8	Leu-685	Pro-593, Leu-705	Glu-682, Ser-591, Tyr-684, Leu-592, Gln-544, Glu-702, Ser-709, Lys-701
213	-7.8	Leu-685	Leu-705	Arg-672, Tyr-684, Tyr-686, Ser-591, Gln-544, Glu-702, Ser-709, Lys-701
214	-7.5	Leu-685	Pro-593, Leu-705	Arg-672, Tyr-686, Leu-592, Gln-544, Glu-682, Glu-702, Ser-547, Ser-709, Lys-701
215	-7.0	Tyr-590, Lys-550, Asp-543, Leu-685	Leu-705, Pro-593, Leu-592, Glu-702,	Thr-540, Asp-541, Ser-547, Gln-544, Ser-709, Ser-591, Tyr-686, Gln-542
216	-7.8	Leu-685	Pro-593, Leu-705	Tyr-686, Glu-702, Ser-547, Ser-709, Gln-544, Tyr-590, Lys-701, Tyr-684
217	-7.9	Leu-592, Gln-544	Pro-593, Leu-705, Tyr-686, Leu-685	Ser-591, Glu-702, Ser-547, Gln-542
219	-7.7	Ser-547	Pro-593, Leu-705, Leu-592, Ile-577, Gln-542,	Asp-543, Tyr-590, Gln-544, Glu-702, Leu-685, Tyr-684, Asp-683, Glu-682
222	-7.5	--	Pro-593, Leu-592, Gln-542, Leu-705. Ile-577	Ser-547, Asp-543, Gln-544, Leu-685, Tyr-684, Asp-683, Glu-596
204	-8.0	Arg-672, Gln-544, Gln-542	Pro-593, Leu-592, Glu-682, Ile-577, Gln-544, Leu-705	Asp-543, Tyr-590, Tyr-686, Ser-591, Leu-685, Tyr-684, Glu-596, jAsp-831
Vosaroxin	-6.9	Ser-591, Leu-685, Leu-592	Leu-705, Ile-577, Pro-593	Glu-682, Tyr-684, Arg-672, Gln-542

The 3-dimensional binding interaction of **211**, **217**, **224** and vosaroxin against human *topoisomerase II α* is illustrated in Figures 24-27

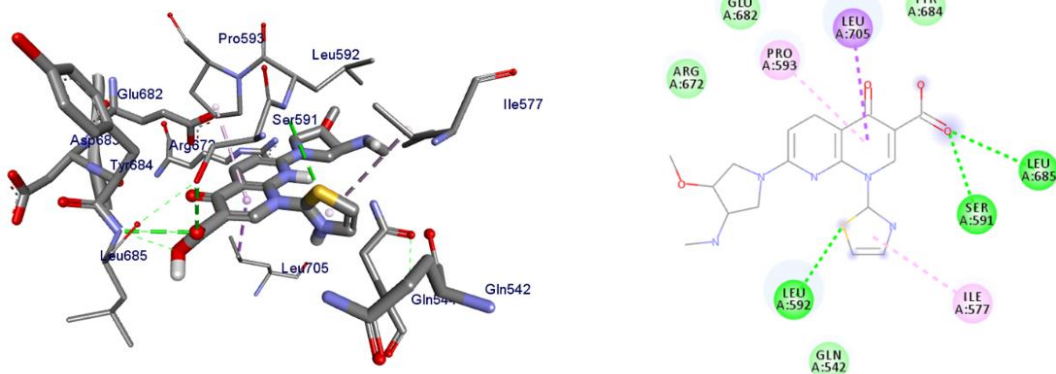


Figure 24. The binding interactions of vosaroxin against Human *Topoisomerase II α* (PDB ID: 4fm9).

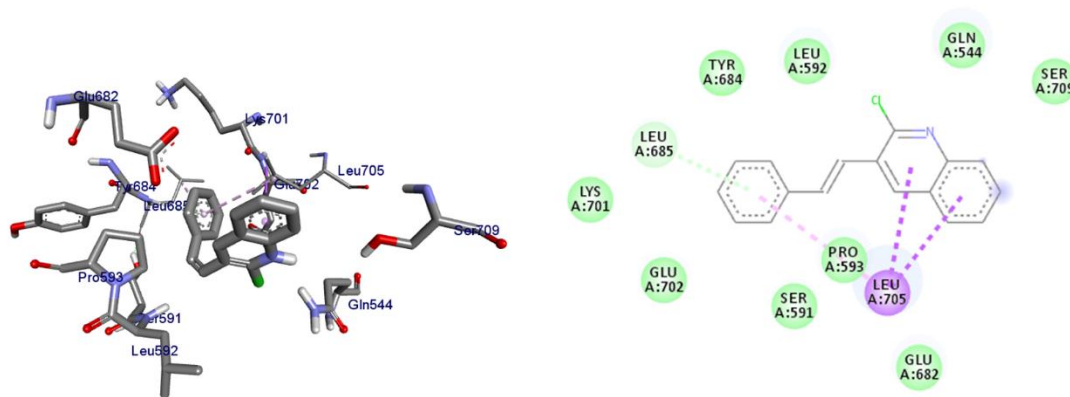


Figure 25. The binding interactions of **211** against Human *Topoisomerase II α* (PDB ID: 4fm9).

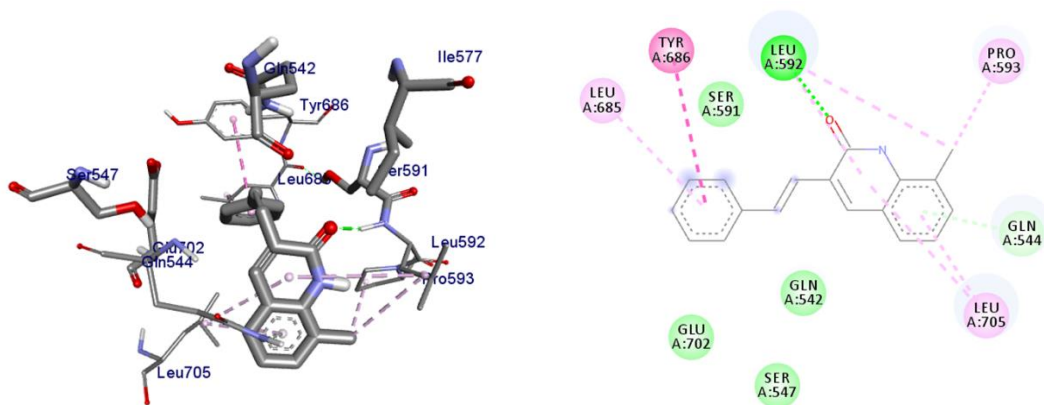


Figure 26. The binding interactions of **217** against Human *Topoisomerase II α* (PDB ID: 4fm9).

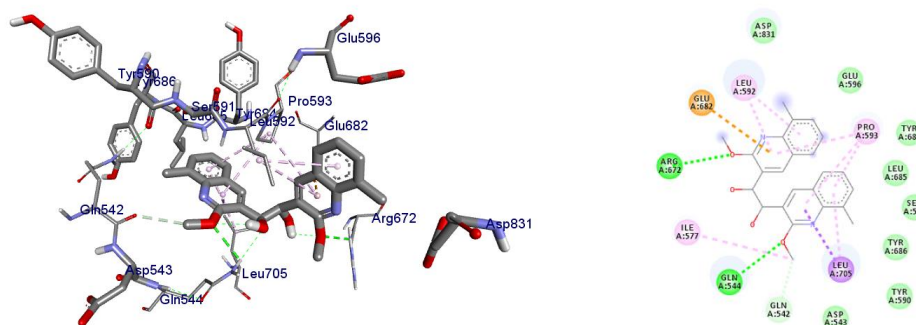


Figure 27. The binding interactions of **224** against Human *Topoisomerase IIα* (PDB ID: 4fm9).

4.6.4 *In silico* Molecular docking study of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against *E-coli* DNA gyrase A

Bacterial DNA gyrase are vital for the survival of bacteria. Recently, researchers have explored a range of synthetic inhibitors as antibacterial drugs to target *DNA gyrase* (Ghorab et al. 2020; Hailekiros, Kedir, and Endale 2021). Therefore, we have carried out the molecular docking analysis for the synthesized compounds to evaluate their binding interactions with *DNA gyrase A* and compared with the clinical drug inhibitor (ciprofloxacin). The synthesized compounds (Table 14) were found to have maximum binding energy values ranging from -5.4 to -7.9 kcal/mol (Table 14), with the best score achieved for compound **228** (-7.9 kcal/mol). The binding affinity, H-bond and residual interaction of nine compounds and ciprofloxacin were summarized in Table 14. Compared to ciprofloxacin, compounds (Table 14) shown similar residual interactions with amino acid residues Glu-50, Gly-77, Ile-78, Asn-46, Ile-94, and Ala-47 and H-bond with Asp-73, Arg-76, and Thr-165. Compounds **225**, **226**, and **227** have additional hydrogen bonding interaction with amino acid residue Pro-79. The compounds **225**(Asn-46, Glu-50), **226** (Asn-46), **62** (Asn-46, Gly-77), and **232** (Gly-77) have shown additional hydrogen bonding interactions with amino acid residues. The compound **228** recapitulate the residual amino acid interactions of ciprofloxacin against *DNA gyrase A* (6f86). In this study, the residual interactions of the compounds (Table 14) were well in agreement with the previously reported binding modes that includes the essential interactions between the ligand, Asp-73, and the water molecule. The molecular docking analyses results are in good agreement with *in vitro* analysis of the synthesized compounds, **225** (-6.9 kcal/mol), **226** (-6.8 kcal/mol), **227** (-6.9 kcal/mol), **228** (-7.9 kcal/mol), **62** (-7.2 kcal/mol) and **232** (-6.8 kcal/mol) activities against *E. coli*. The *in-silico* analysis shown that the compound **228** (-7.9 kcal/mol)

and **62** (– 7.2 kcal/mol) revealed better activity. The compounds **230**(– 5.4 kcal/mol, **235**(– 6.1 kcal/mol), and **236** (– 5.4 kcal/mol)) docking results were partially matching the ciprofloxacin interactions with amino acid residues. Based on the *in silico* molecular docking study, compounds **228** and **62** shown comparable residual interactions and docking scores with reference compound (ciprofloxacin). Therefore, compound **226** might have better anti-bacterial agents than the other compounds reported herein. But the *in vitro* antibacterial assay result for **62** is very low and it is in sharply contradiction with the molecular docking value.

The molecular docking data of the synthesized compounds were also carried out against *S. aureus* topoisomerase IV (PDB ID 2INR) to evaluate their binding interactions with topoisomerase IV in case of Gram positive bacteria and comparable with the data obtained for drug inhibitor (ciprofloxacin). The synthesized compounds (Table 15) here also found to have maximum binding energy ranging from – 4.5 to – 5.9 kcal/mol (Table 15), with the best result achieved using compound **228** (– 5.9 kcal/mol) and (– 4.9 kcal/mol) for ciprofloxacin. The same three synthetic compounds namely; **225**, **227**, and **228** displayed the maximum binding affinity with topoisomerase IV of *S. aureus*. Thus, compounds **225**, **227** and **228** exhibited good antibacterial activities against both gram negative and gram positive bacteria both in *in vitro* and molecular docking evaluations (Table 15) and further optimization of these compounds can be used to develop effective broad spectrum antibacterial activity lead compounds. The 3-dimensional binding interaction of nine compounds and ciprofloxacin against *E. coli* gyrase A complex is illustrated in Figures 28–36.

Table 14. Molecular docking value of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against *E.coli* DNA gyrase A (PDB ID: 6F86)

Compound s	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
62	-7.2	Gly-77, Thr-165, Asn-46	Ala-47, Val-43, Val-167, Ile-78	Gly-75
225	-6.9	Glu-50, Pro-79, Asn-46	Ile-94, Ile-78	Asp-73, Asp-49, Gly-77
226	-6.8	Pro-79, Asn-46	Ile-78, Ile-94, Glu-50	Asp-73, Asp-49, Gly-77, Thr-165
227	-6.9	Thr-165	Asn-46, Ile-78, Pro-79, Ile-94, Glu-50	Asp-73, Ala-47, Arg-76, Gly-77
228	-7.9	Asp-73, Thr-165, Pro-79, Glu-50	Ala-47, Val-167, Ile-78, Ile-94	Val-43
230	-5.4	Val-43	Asp-73, Glu-50, Ile-78	Gly-77, Asn-46, Ala-47, Val-167
232	-6.8	Asp-73, Thr-165, Gly-77	Asn-46, Ile-78, Ile-94	Val-43, Ala-47, Arg-76, Pro-79, Gly-75, Glu-50
235	-6.1	--	Pro-79, Ile-78, Arg-76, Glu-50	Asp-73, Asn-46, Ala-47, Gly-77, Thr-165
236	-5.4	Val-43	Asp-73, Glu-50, Ile-78	Gly-77, Asn-46, Ala-47, Val-167
ciprofloxacin	-7.2	Asp-73, Arg-76, Thr-165	Glu-50, Gly-77, Ile-78, Asn-46	Ala-47

Table 15. Molecular docking values of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against *S. aureus* topoisomerase IV (PDB ID 2INR)

Compounds	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
62	-5.7	Ser-141, Asn-360, Thr-139	Phe-142	Val-140
225	-5.9	Glu-356	--	Arg-35, Ser-349, His-353, Asn-352, Asp-335, Ile-355, Ala-359
226	-5.8	Ser-141	Glu-138, Asn-360	Phe-142, Thr-139, Val-140
227	-5.9	Ser-141, Asn-360	Glu-138, Thr-139	Glu-356, Val-140, Phe-142
228	-5.9	Ser-141, Asn-360, Thr-139	Glu-138	Glu-356, Val-140
230	-4.7	Val-140	Phe-142, Asn-360	Ser-141, Thr-139
232	-4.5	Glu-138	--	Ser-141, Phe-142, Val-140, Asn-360, Thr-139
235	-4.8	Asn-352	Arg-35, Glu-356, Ile-355, Ser-349, Asp-335	His-353
236	-5.4	Val-140	Glu-138	Thr-139, Phe-142
Ciprofloxacin	-4.9	Glu-138, Ser-141, Asn-360	Thr-139, Glu-356, Arg-35	Met-154

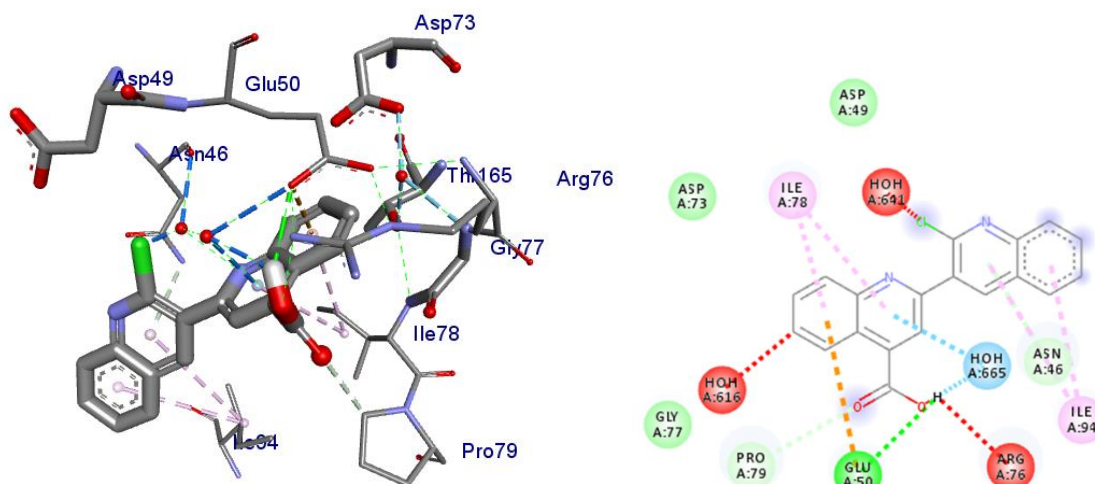


Figure 28. The binding interactions of **225** against *E. coli* DNA gyrase A (PDB ID: 6F86).

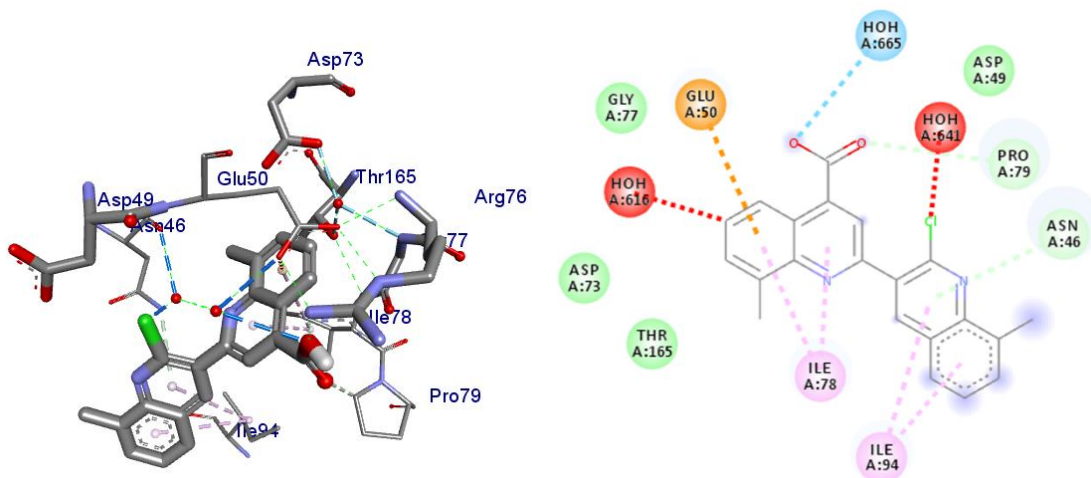


Figure 29. The binding interactions of **226** against *E. coli* DNA gyrase A (PDB ID: 6F86).

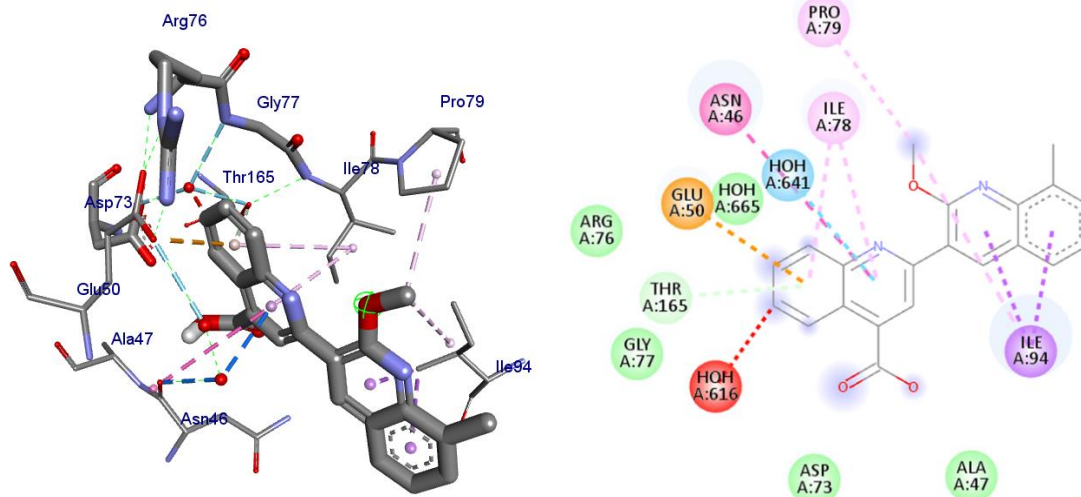


Figure 30. The binding interactions of **227** against *E.coli* DNA gyrase A (PDB ID: 6F86)

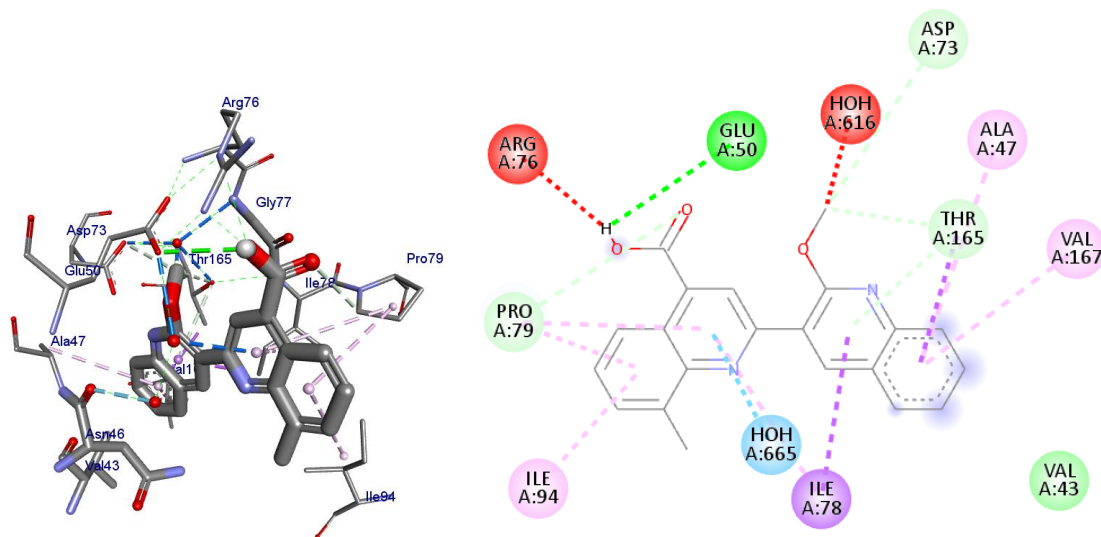


Figure 31. The binding interactions of **228** against *E.coli* DNA gyrase A (PDB ID: 6F86).

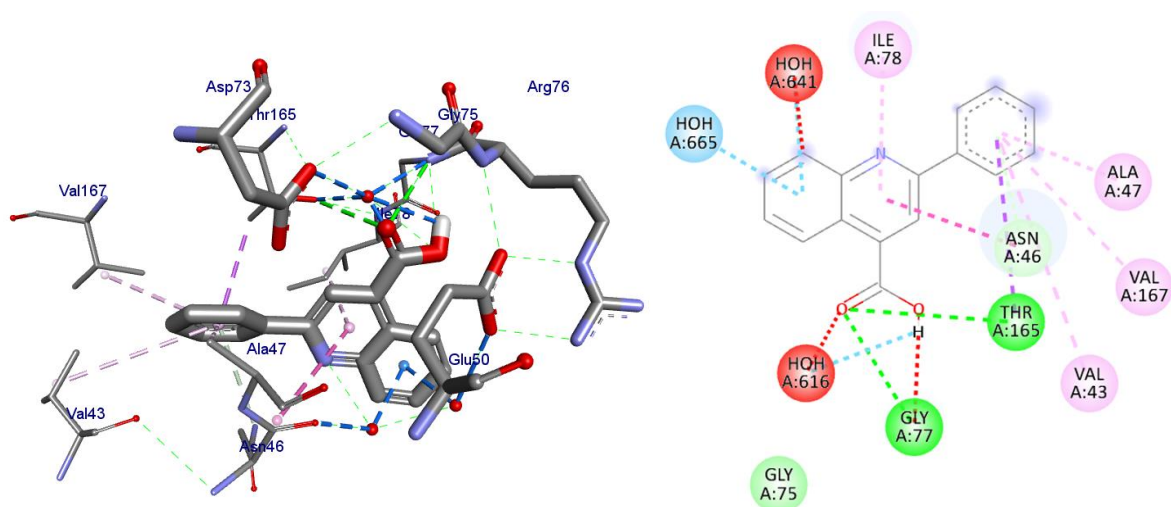


Figure 32. The binding interactions of **62** against *E.coli* DNA gyrase A (PDB ID: 6F86).

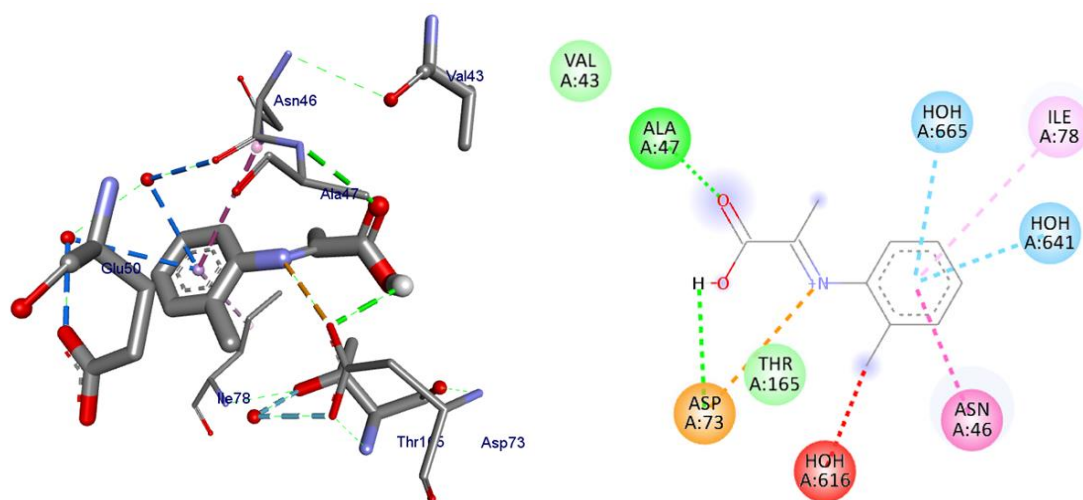


Figure 33. The binding interactions of **230** against *E.Coli* DNA gyrase A (PDB ID: 6F86)

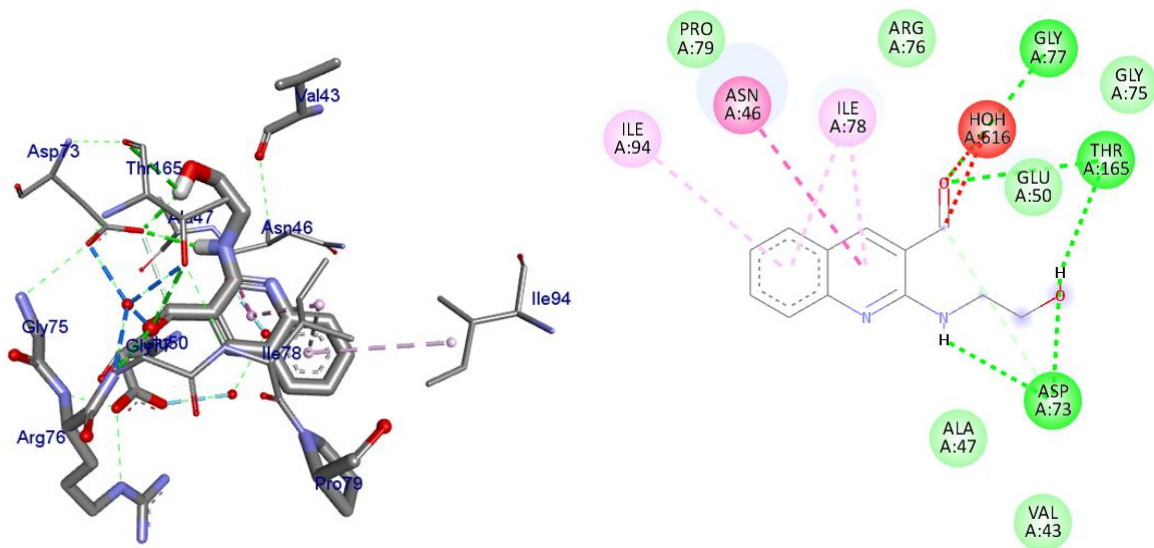


Figure 34. The binding interactions of **232** against *E.coli* DNA gyrase A (PDB ID: 6F86)

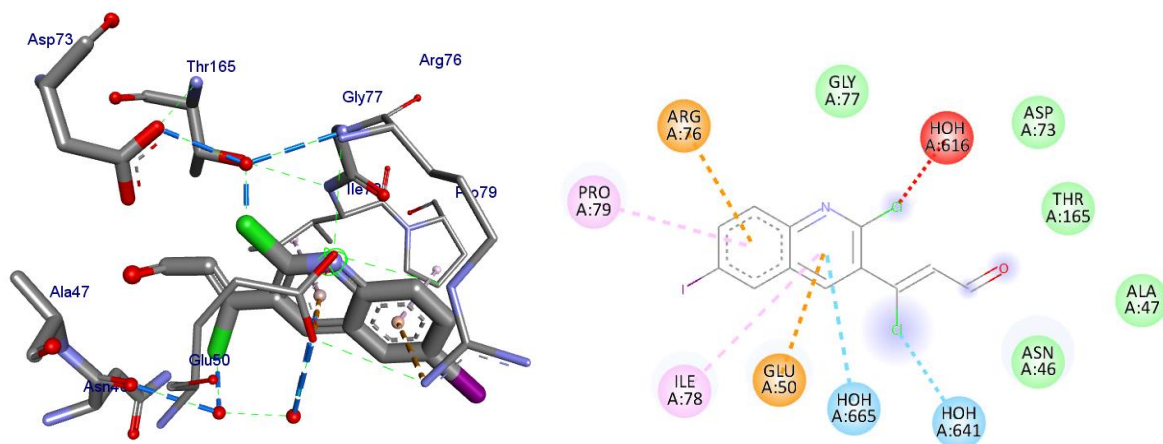


Figure 35. The binding interactions of **235** against *E.coli* DNA gyrase A (PDB ID: 6F86).

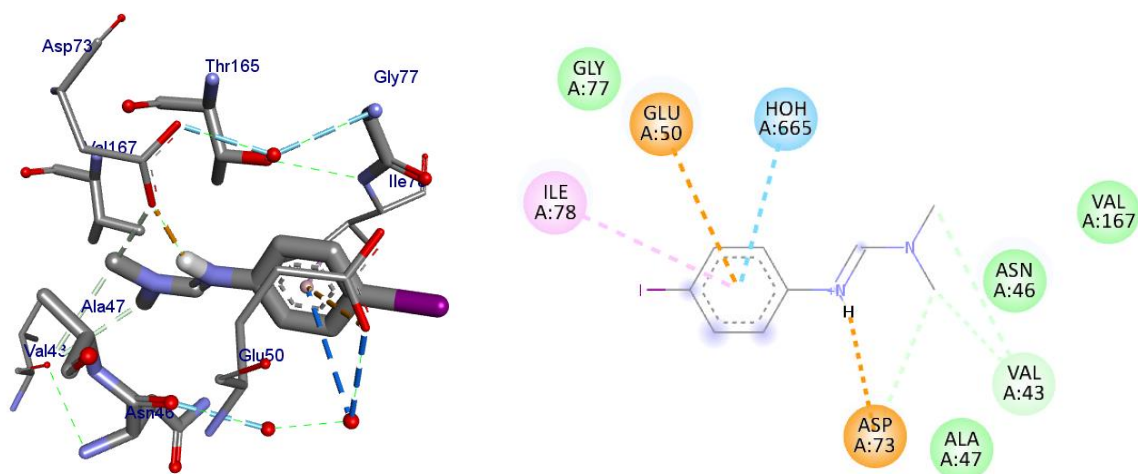


Figure 36. The binding interactions of **236** against *E.coli* DNA gyrase A (PDB ID: 6F86).

4.6.5 Molecular docking study of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against human *topoisomerase II α*

We have also investigated the binding interactions of these compounds (Table 16) against human *topoisomerase II α* and compared with the anti-cancer drug (vosoroxin). The synthesized compounds (Table 16) were found to have minimum binding energy ranging from – 5.6 to – 8.8 kcal/mol (Table 16), with the highest scores were obtained for compound **225**(– 8.6 kcal/mol), **226** (– 8.8 kcal/mol), and **228** (– 8.3 kcal/mol). The binding affinity, H-bond and residual interactions of the nine compounds and vosoroxin (anti-cancer drug) are summarized in Table 16. Compared to vosoroxin, the compounds (Table 16) shown similar residual interaction profiles with amino acid residues Leu-705, Ile-577, Pro-593, Glu-682, Tyr-684, Arg-672, Gln-542 and H-bond with Ser-591, Leu-685, and Leu-592. Compounds **225**, **226**, and **227** have additional hydrogen bonding interaction with amino acid residue Pro-79. The compounds **225** (Asn-46, Glu-50), **226**(Asn-46), **62** (Asn-46, Gly-77), and **232**(Gly-77) have shown additional hydrogen bonding interaction with amino acid residues. The synthesized compounds (except **235** and **236**) shown similar residual amino acid interactions of vosoroxin against human *topoisomerase II α* (4fm9). In this study, the residual interactions of compounds (Table 16) were well in agreement with the previously reported binding modes that includes the essential hydrogen interactions between the ligand and the amino acid residues (Ser-591, and Leu-685). The *in-silico* analysis revealed that the compounds **225** (– 8.6 kcal/mol), **226** (– 8.8 kcal/mol), and **62** (– 8.3 kcal/mol) to possess better activity. The compounds **235**(– 5.6

kcal/mol), and **236** (– 6.6 kcal/mol) docking results were partially matching the vosoroxin interactions with amino acid residues. Based on the in silico molecular docking analysis results, compounds **225**, **226**, and **228** shown high residual interactions and docking score than vosoroxin. Therefore, compounds **225**, **226**, and **228** might have better anti-cancer agents than the other compounds reported herein. The binding affinity, H-bond and residual interaction of ten compounds were summarized below (Table 16).

Thus, in the future it will be important to undergo in *vitro* and in *vivo* anticancer evaluation for the three compounds (**225**, **226**, and **228**) which showed highest docking analyses scores. These compounds may also serve as a lead to plan further anticancer compounds based on similar scaffold.

Table 16. Molecular docking values of [2,3'-biquinoline]-4-carboxylic acid and additional quinoline-3-carbaldehyde analogs against Human *Topoisomerase II α* (PDB ID: 4fm9)

Compounds	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
62	-7.6	Ser-591, Leu-685	Pro-593, Leu-705	Tyr-686, Glu-702, Ser-547, Asp-543, Glu-682
225	-8.6	Leu-685	Leu-592, Pro-593, Leu-705	Glu-682, Tyr-684, Ser-591, Tyr-686, Gln-542, Glu-702, Ser-547, Asp-543
226	-8.8	Ser-591, Leu-685	Pro-593, Leu-592, Leu-705	Arg-672, Tyr-684, Tyr-686, Glu-702, Gln-542
227	-7.7	Leu-685, Gln-544	Ser-709, Leu-592, Pro-593, Leu-705, Glu-682	Arg-672, Asp-543, Glu-702, Tyr-686, Ser-591, Tyr-590, Tyr-684
228	-8.3	Ser-591, Leu-685, Gln-544	Leu-592, Pro-593, Leu-705, Glu-682, Tyr-684, Ser-709	Tyr-686, Glu-702, Gln-542, Asp-543
230	-7.6	Ser-547, Glu-702, Lys-550, Gln-542	Leu-705, Leu-685, Tyr-686	Ser-591, Tyr-590, Tyr-684, Lys-701, Gln-544, Gly-546, Asp-543, Asp-541
232	-7.1	Ser-547, Lys-550	Leu-705, Tyr-590, Gln-544, Asp-543, Tyr-686, Gln-542	Ser-709, Glu-702, Asp-541, Leu-592
235	-5.6	Ser-547, Asp-543	Leu-685, Leu-705, Glu-702, Lys-701	Ser-591, Tyr-590, Lys-550, Gln-542, Tyr-686, Asp-683, Tyr-684
236	-6.6	Lys-550	Leu-705, Glu-702, Gln-542, Leu-685	Tyr-684, Lys-701, Tyr-686, Asp-543, Ser-547, Ser-591, Leu-592
Vosaroxin	-6.9	Ser-591, Leu-685, Leu-592	Leu-705, Ile-577, Pro-593	Glu-682, Tyr-684, Arg-672, Gln-542

The 3-dimensional binding interaction of compounds against human *topoisomerase II α* is illustrated in Figures 37–44

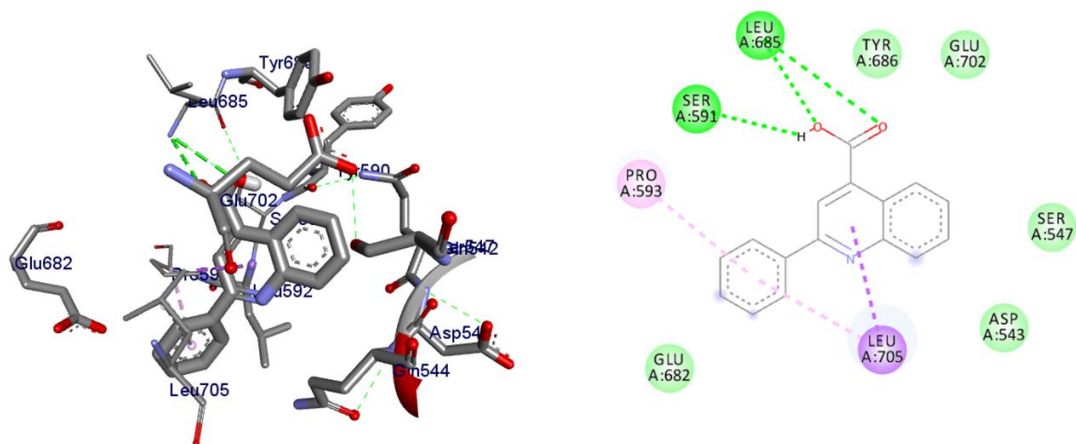


Figure 37. The binding interactions of **62** against Human *Topoisomerase II α* (PDB ID: 4fm9).

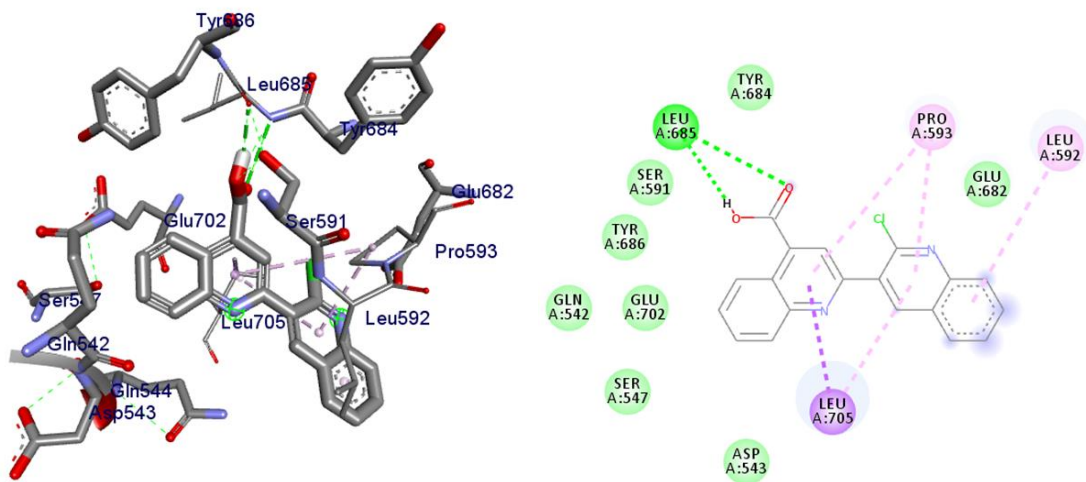


Figure 38. The binding interactions of **225** against Human *Topoisomerase II α* (PDB ID: 4fm9)

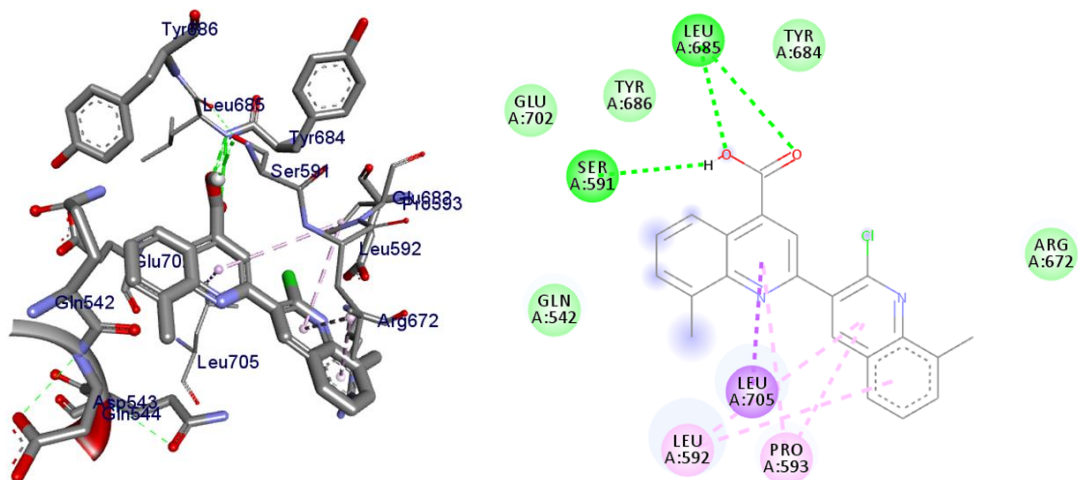


Figure 39. The binding interactions of **226** against Human *Topoisomerase IIα* (PDB ID: 4fm9)

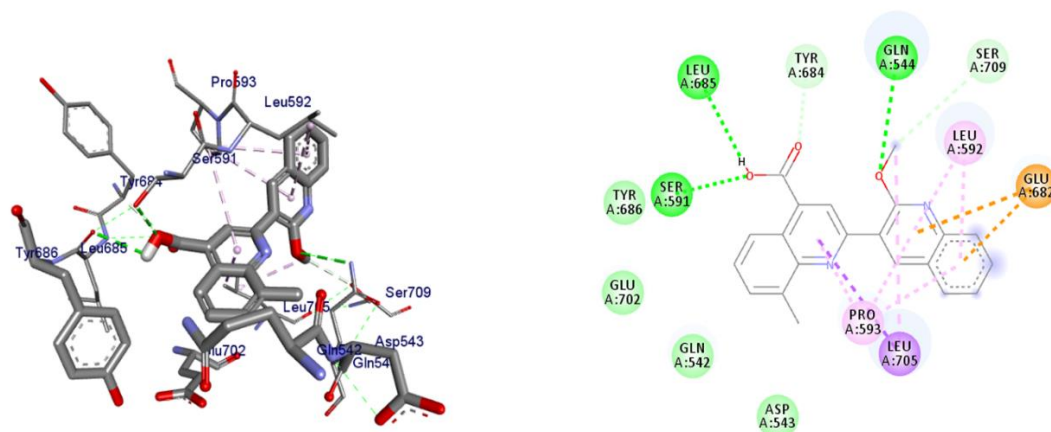


Figure 40. The binding interactions of **227** against Human *Topoisomerase IIα* (PDB ID: 4fm9).

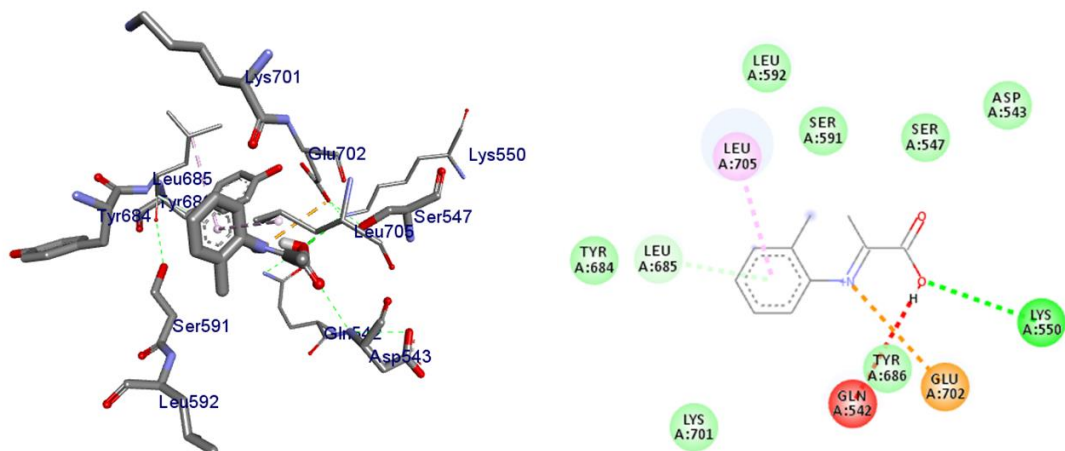


Figure 41. The binding interactions of **230** against Human *Topoisomerase IIα* (PDB ID: 4fm9).

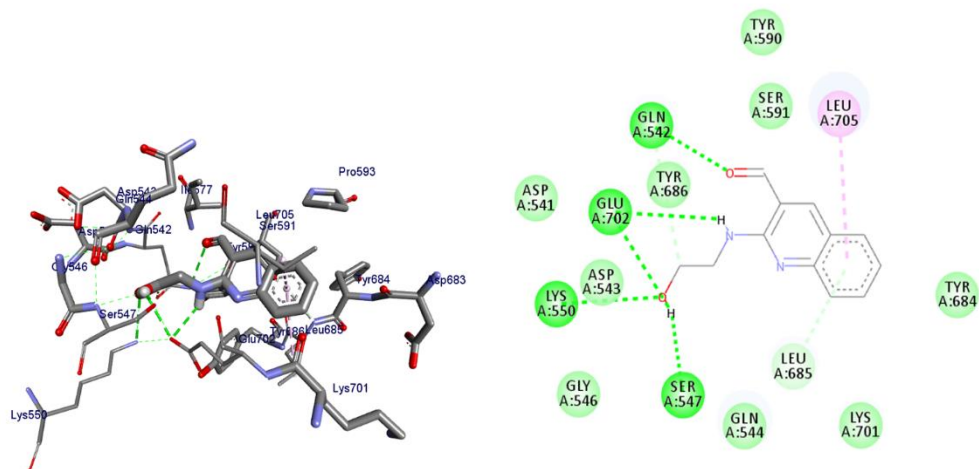


Figure 42. The binding interactions of **232** against Human *Topoisomerase IIα* (PDB ID: 4fm9).

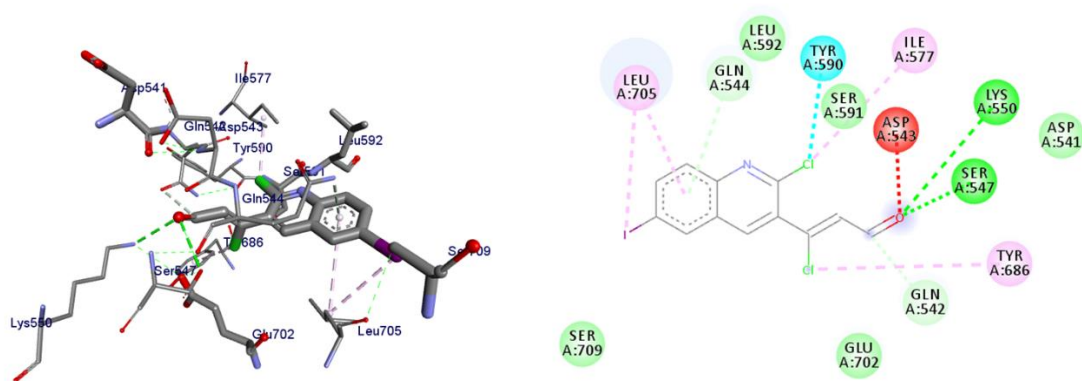


Figure 43. The binding interactions of **235** against Human *Topoisomerase IIα* (PDB ID: 4fm9)

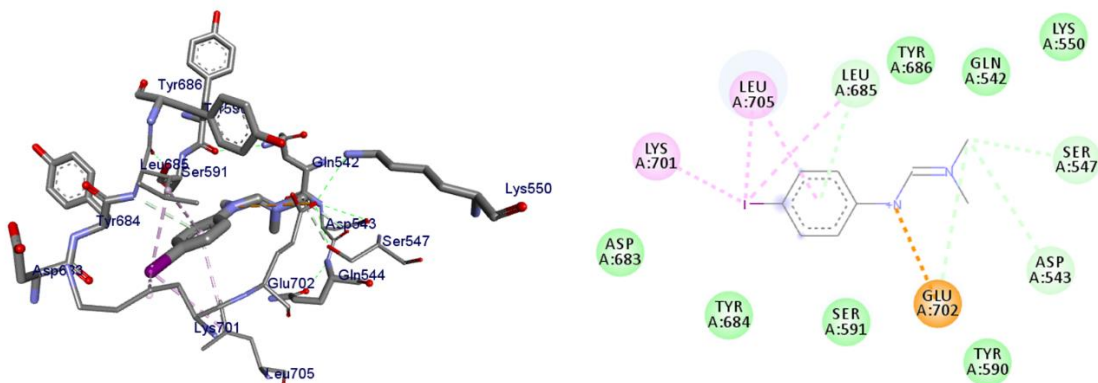


Figure 44. The binding interactions of **236** against Human *Topoisomerase IIα* (PDB ID: 4fm9).

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this dissertation, 26 novel quinoline analogs and 2 Schiff bases were synthesized by the employing of Vilsmeier-Haack, Wittig, Doebner, nucleophilic substitution, oxidation, reduction and hydrolysis reactions. The *in vitro* antibacterial activity of the synthetic compounds was evaluated by paper disc diffusion method against three Gram negative and three Gram positive bacterial strains. Compound **172** (9.0 ± 0.67 mm) against *P. aeruginosa*, **183** (8.33 ± 0.44 mm) against *P. aeruginosa*, **190** (10.0 ± 0.8 mm), **198** (16.0 ± 0.82 mm) both against *E. Coli*, **203** (16.0 ± 1.63 mm) against *S. aureus* and 8.67 ± 0.47 mm against *S. typhimurium*, **204** and **205** (13.7 ± 0.58 mm) against *P. pyogenes*, **206** (20.7 ± 1.5 mm) against *P. aeruginosa* and **207** (15.7 ± 0.58 mm against *S. aureus*, 16.0 ± 1.7 mm against *E. coli*, 19.7 ± 1.5 mm against *P. aeruginosa*) were among those that showed higher antibacterial mean inhibition zone compared to ciprofloxacin (whose mean inhibition range 16.33 ± 0.58 mm to 22.0 ± 1.0 mm on above four strains). *In-silico* drug-likeness predictions and molecular docking evaluations were with a good agreement the result of *in vitro* antibacterial activities of some of compounds (**225**, **226**, **227**, and **228**) and disagree with few of them (**203**). The radical scavenging properties of the compounds were also evaluated by DPPH assay, and most of them showed moderate antioxidant activities. Three of them, **199** (IC_{50} $45.9 \mu\text{g/mL}$), **227** (IC_{50} $1.25 \mu\text{g/mL}$), and **235** (IC_{50} $1.75 \mu\text{g/mL}$), exhibited the strongest antioxidant activity relative the reference (ascorbic acid, with IC_{50} $4.5 \mu\text{g/mL}$). Molecular docking scores of the three compounds (**199**, **227** and **235**) and that of the ascorbic against human *peroxiredoxin 5* varies from -4.1 to -4.9 kcal/mol supporting the *in vitro* assay result further. The molecular docking score of **227** (-4.5 kcal/mol) is much closer to ascorbic acid (-4.9 kcal/mol) than **199** and **235** and the acidic proton on compound **227** might be involved in H-bond interactions within the active site of the enzyme. Eighteen (18) of the synthetic compounds were docked against *E-coli* DNA gyrase, *S. aureus* topoisomerase IV, and human topoisomerase II α in order to investigate their anticancer activities. Some of them showed strong binding affinity for the enzyme than the reference drug (vosaroxin), of which compounds **190**, **192**, **195**, **196**, **203**, **204**, **205**, and **207** showed high binding energy values. These suggest the compounds can possess better anticancer properties.

5.1. Recommendations

The lack of chemicals, laboratory apparatus, equipment, and budget have limited the scope and variety of the synthesis as well as the evaluation of their biological activities. However, the *in vitro* antibacterial and *in-silico* molecular docking evaluation results revealed that some of the synthetic compounds have strong antibacterial property. Thus, we recommend the followings:

1. The bioactivity of quinoline-stilbene hybrids can be further improved by incorporating the necessary bioisosteric groups around the new scaffold. Thus, it appears possible to incorporate various biosteric groups on phenyl ring of quinoline-stilbenes which could improve their bioactivity further as long as the necessary bioisosters are obtainable.
2. Both *in vitro* and *in vivo* anticancer assay are not currently conducted in Ethiopia. Compounds **190**, **192**, **195**, **196**, **203**, **204**, **205**, and **207** that showed minimum affinities in molecular docking analyses against human *topoisomerase II α* deserves further *in vitro* and *in vivo* anticancer assay in case opportunity is available in the future.
3. The evaluation of biological activities need to be expanded in varieties and quality. Currently *in vitro* antibacterial assay is the only method used to conduct antibacterial activities. However, in the future it will be essential to determine the minimum inhibitory concentration (MIC) and conducting *in vivo* assays for those compounds that showed better mean inhibition zones.
4. However, besides bacterial infection, fungus infection also impose serious threat to human health.

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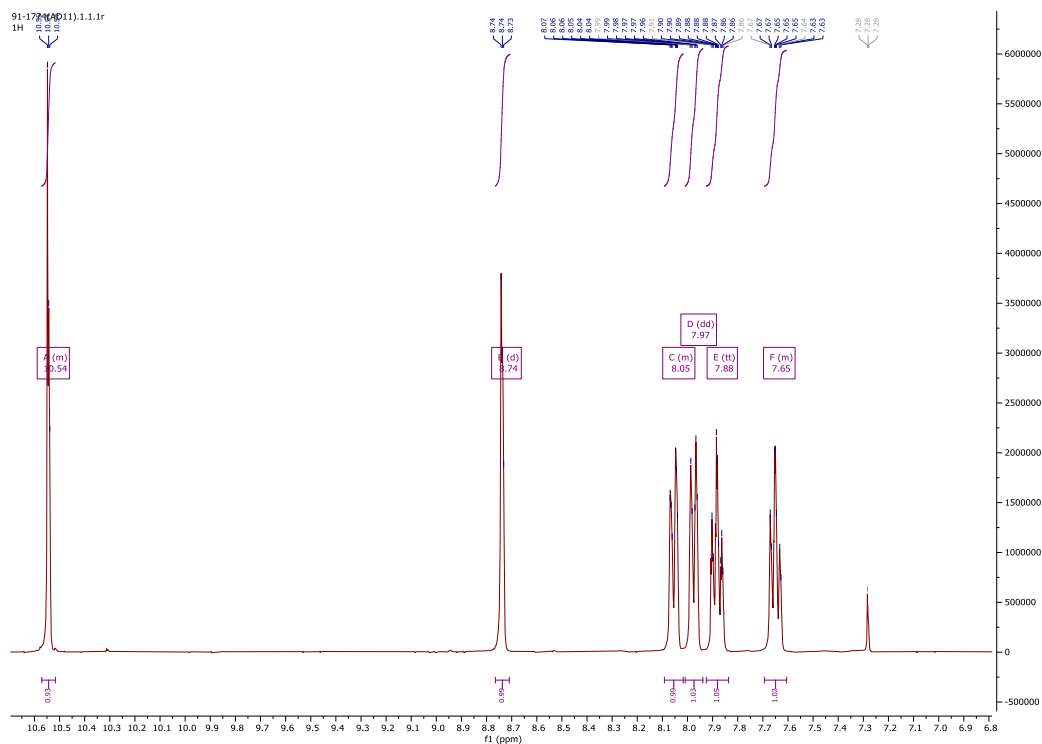
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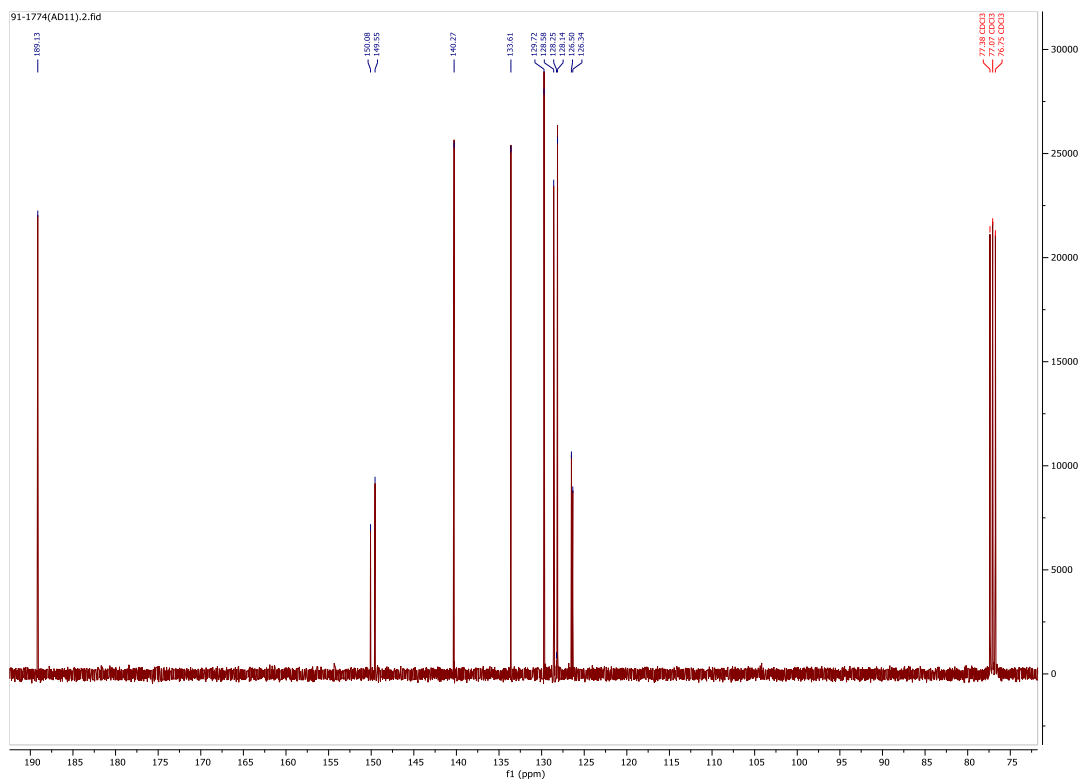
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APPENDIXES

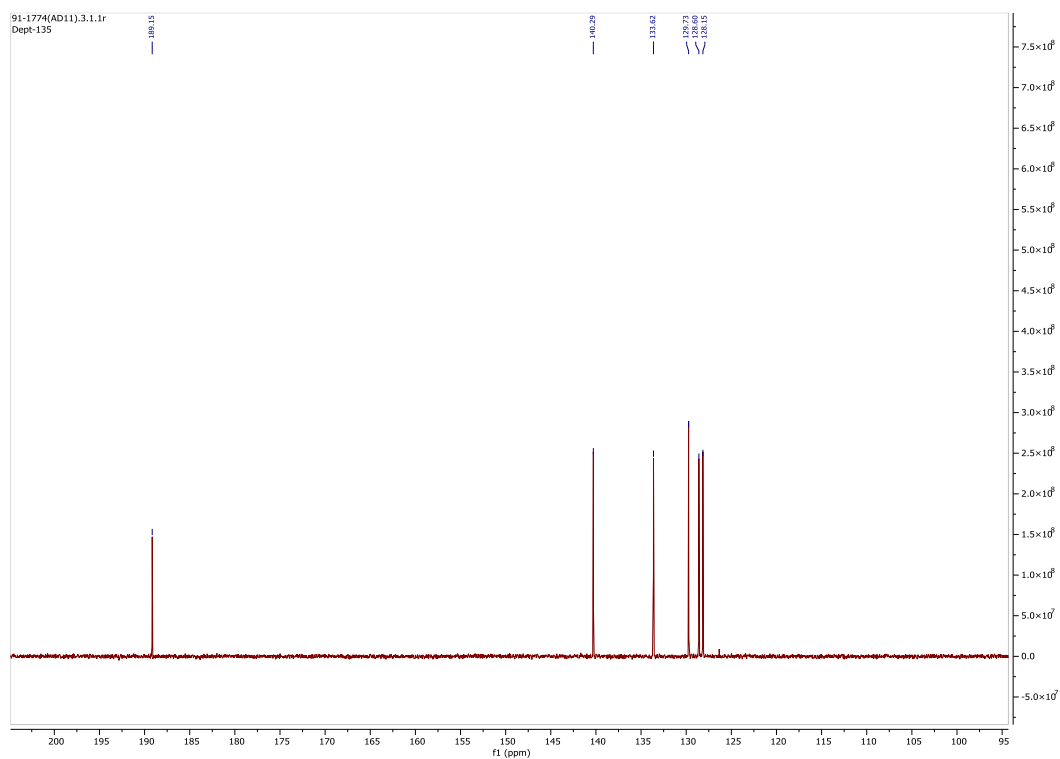
Appendix 1. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-Chloroquinoline-3-carbaldehyde (**196**)



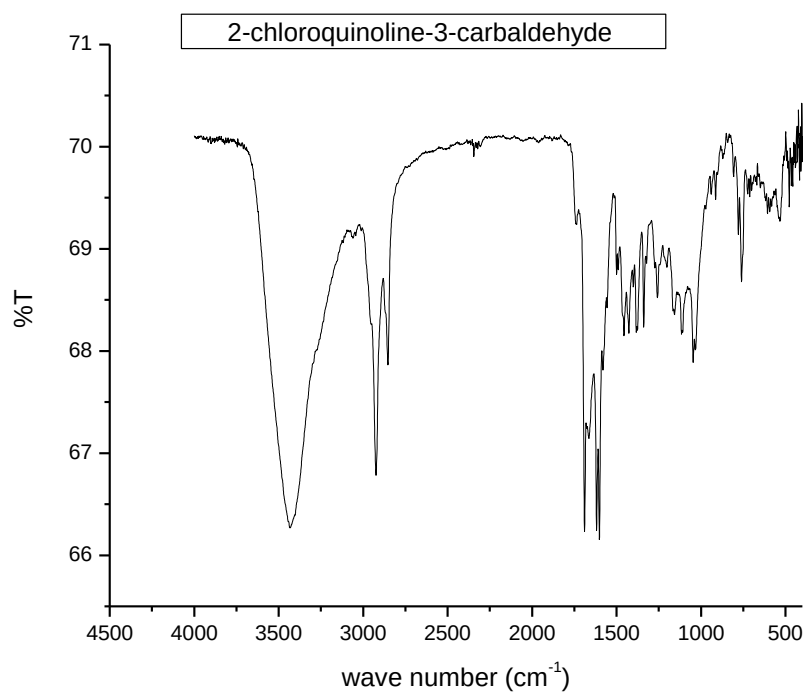
Appendix 2. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Chloroquinoline-3-carbaldehyde (**196**)



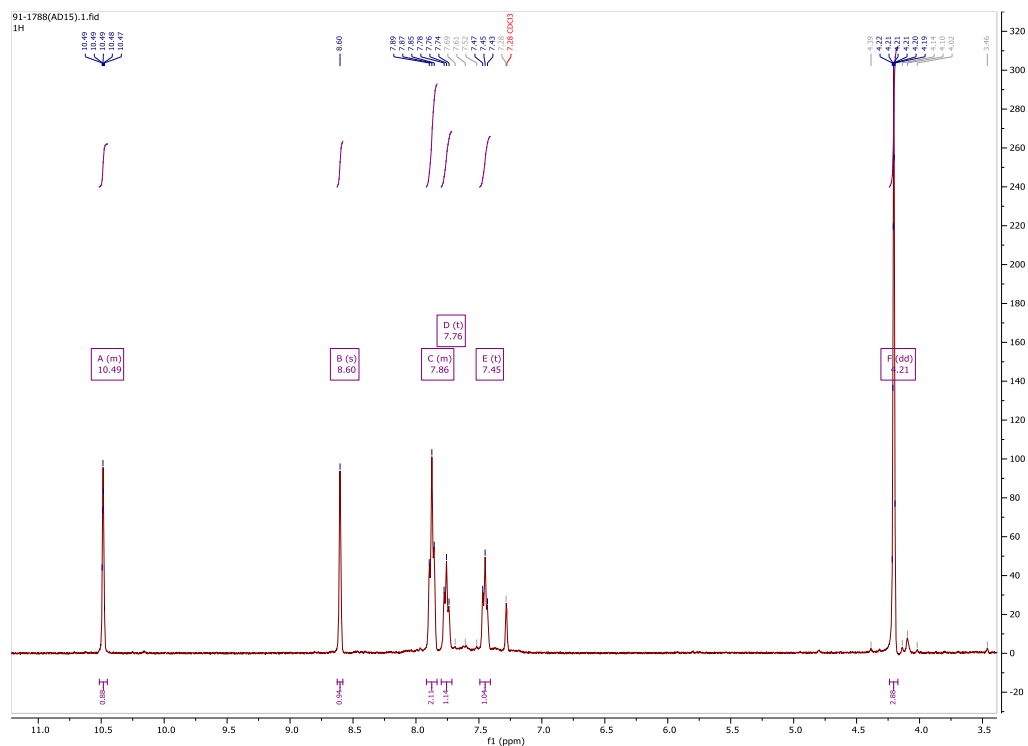
Appendix 3. The DEPT (100 MHz, CDCl₃) spectrum of 2-Chloroquinoline-3-carbaldehyde (**196**)



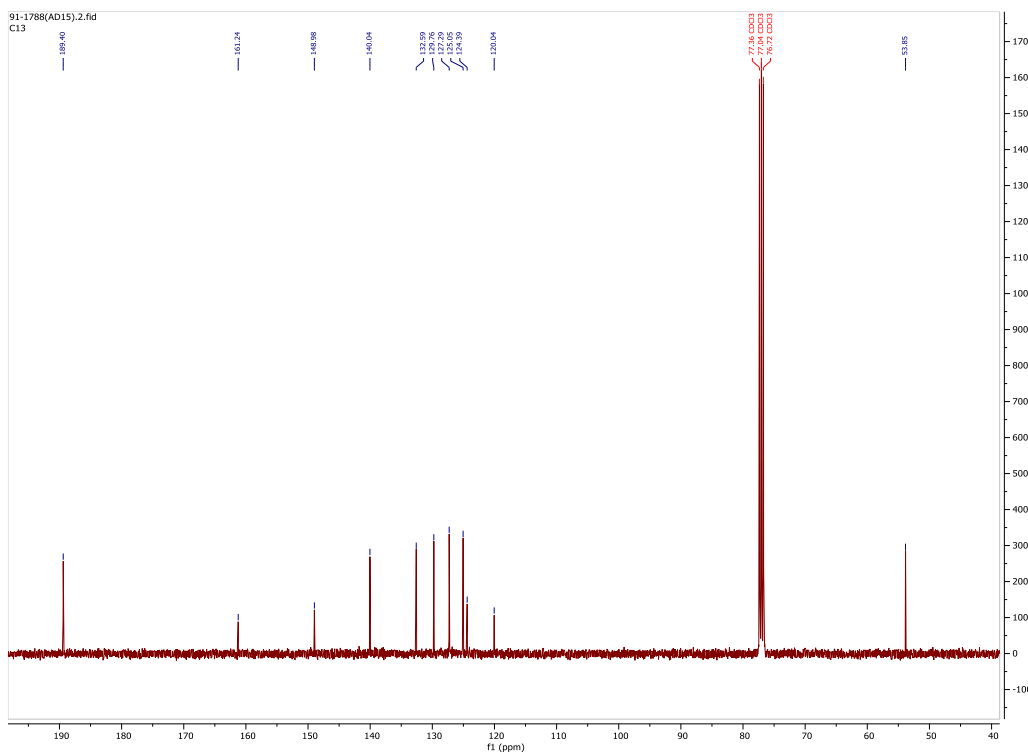
Appendix 4. The IR spectrum of 2-chloroquinoline-3-carbaldehyde (**196**)



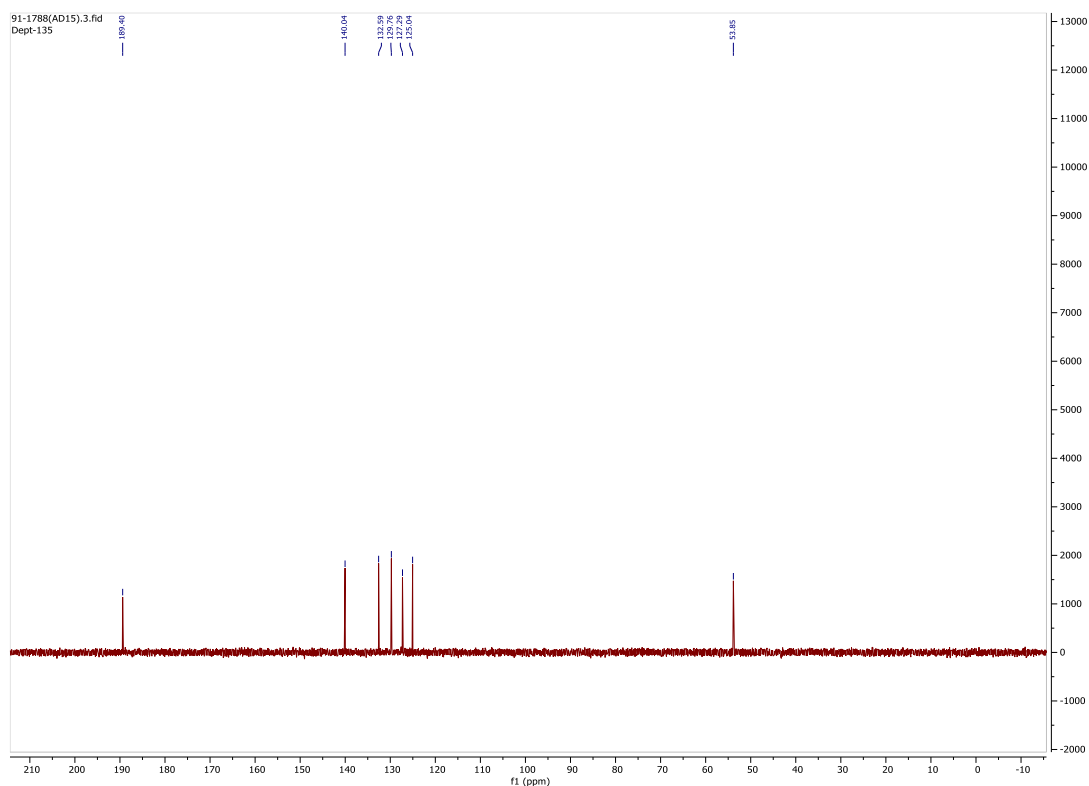
Appendix 5. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-methoxyquinoline-3-carbaldehyde (**197**)



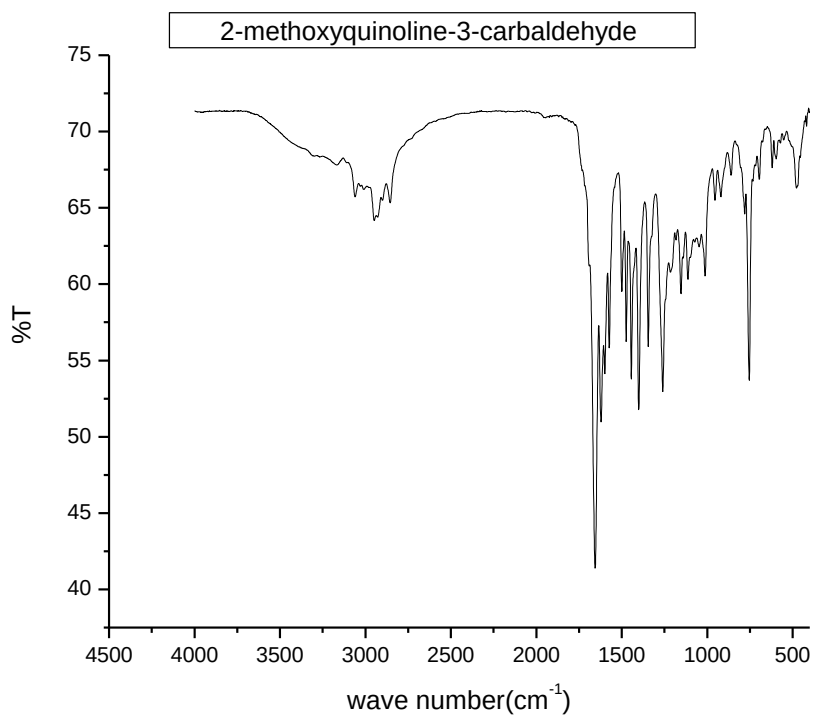
Appendix 6. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-methoxyquinoline-3-carbaldehyde (**197**)



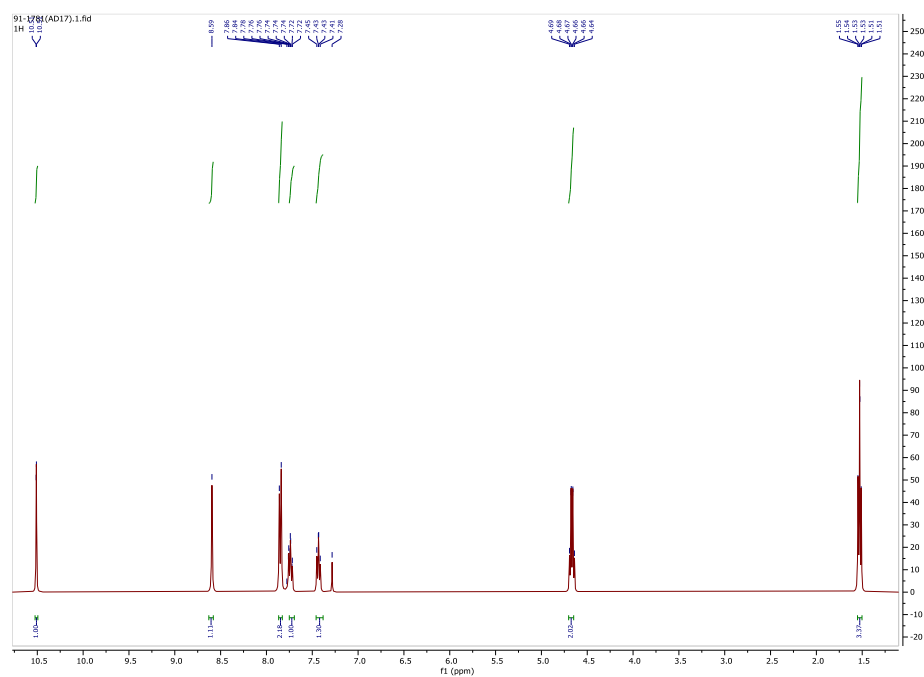
Appendix 7. The DEPT (100 MHz, CDCl₃) spectrum of 2-methoxyquinoline-3-carbaldehyde (**197**)



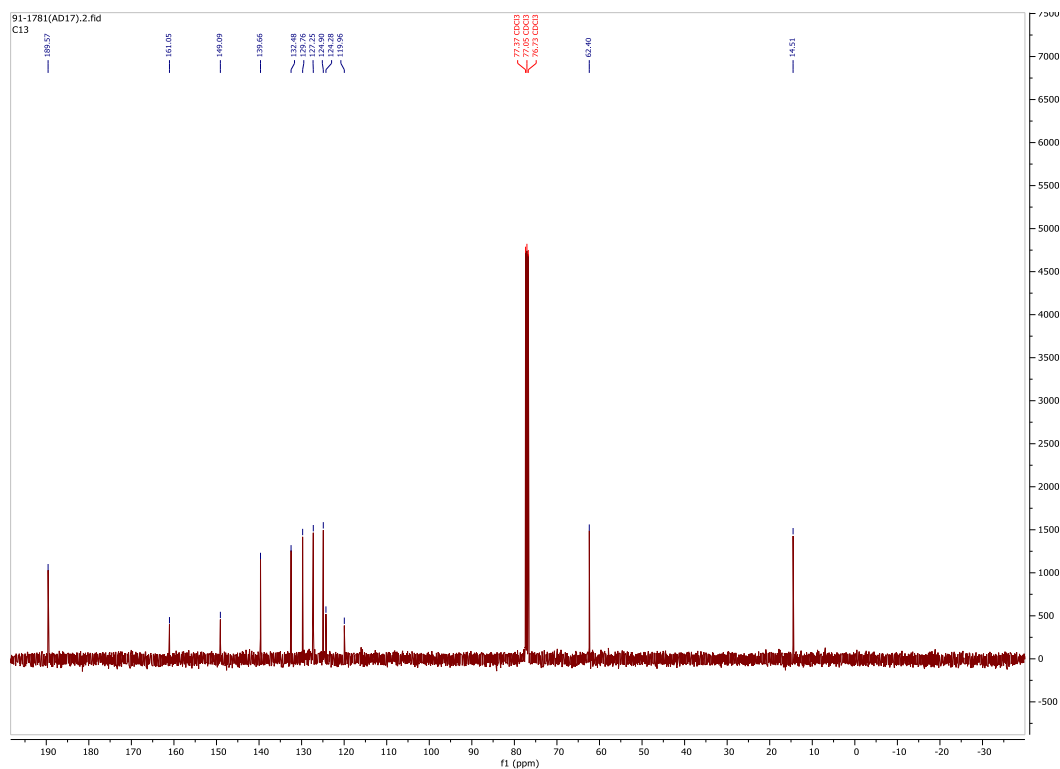
Appendix 8. The IR spectrum of 2-methoxyquinoline-3-carbaldehyde (**197**)



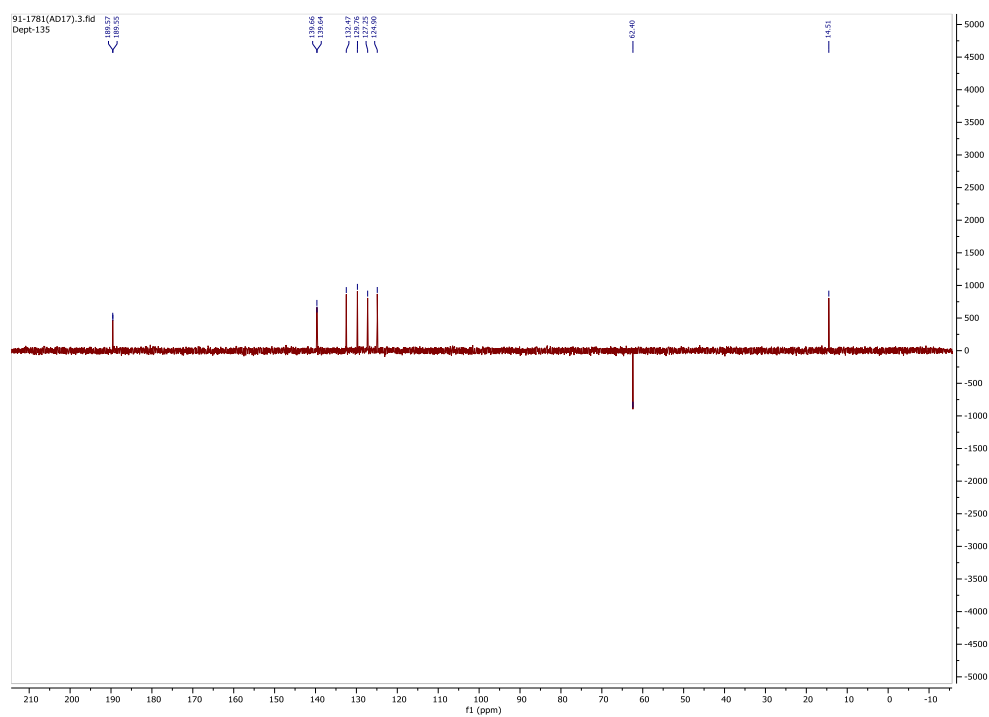
Appendix 9. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-Ethoxyquinoline-3-carbaldehyde (**198**)



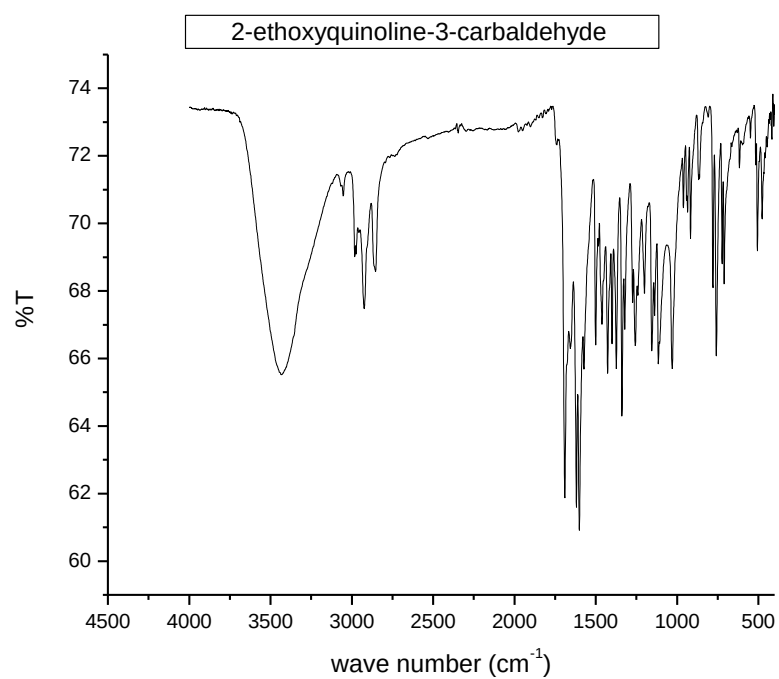
Appendix 10. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Ethoxyquinoline-3-carbaldehyde (**198**)



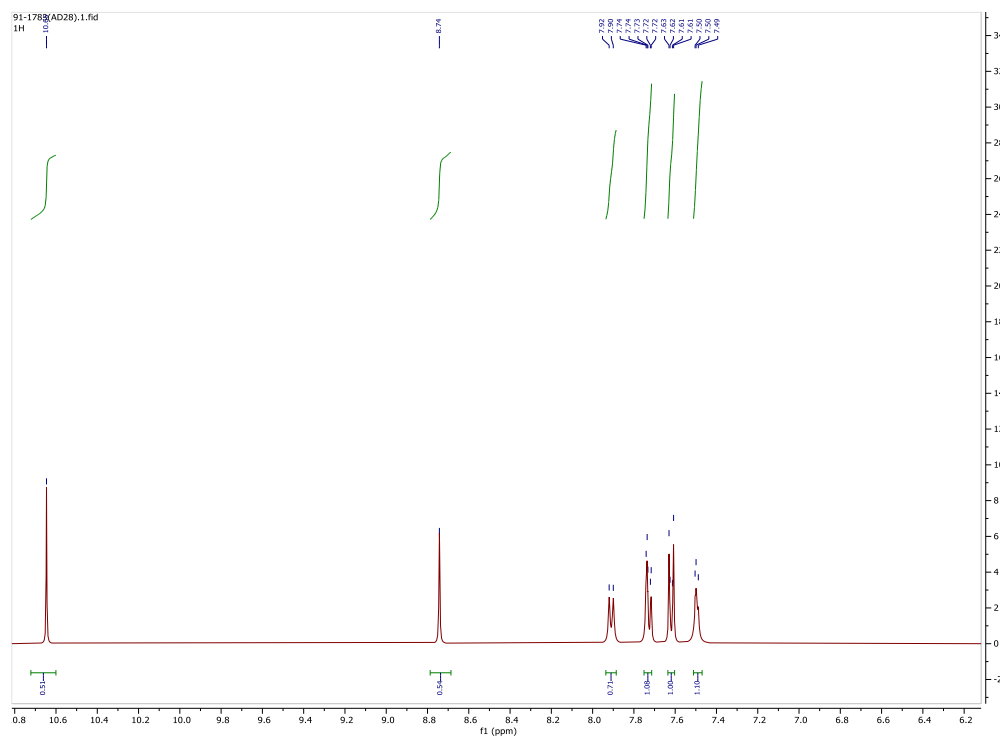
Appendix 11. DEPT (100 MHz, CDCl₃) spectrum of 2-Ethoxyquinoline-3-carbaldehyde (**198**)



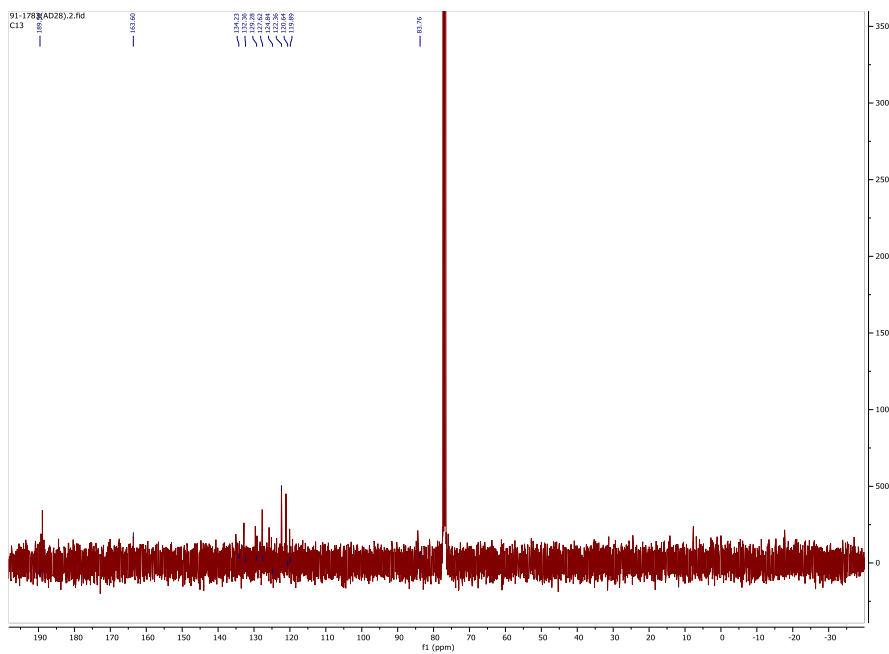
Appendix 12. The FTIR spectrum of 2-Ethoxyquinoline-3-carbaldehyde (**198**)



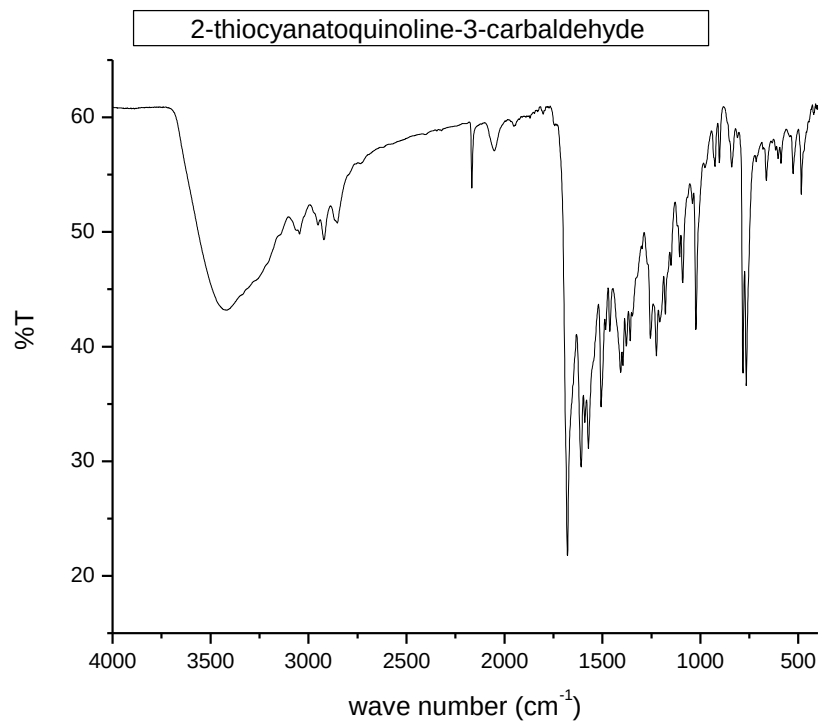
Appendix 13. The ^1H NMR (400 MHz, CDCl_3) spectrum of 2-thiocyanatoquinoline-3-carbaldehyde (199)



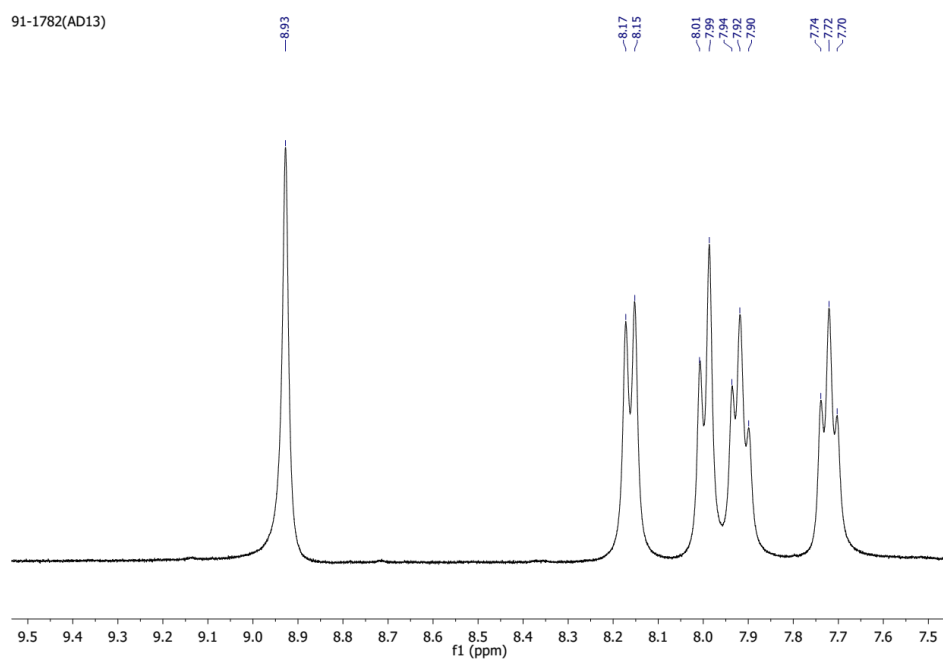
Appendix 14. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-thiocyanatoquinoline-3-carbaldehyde (199)



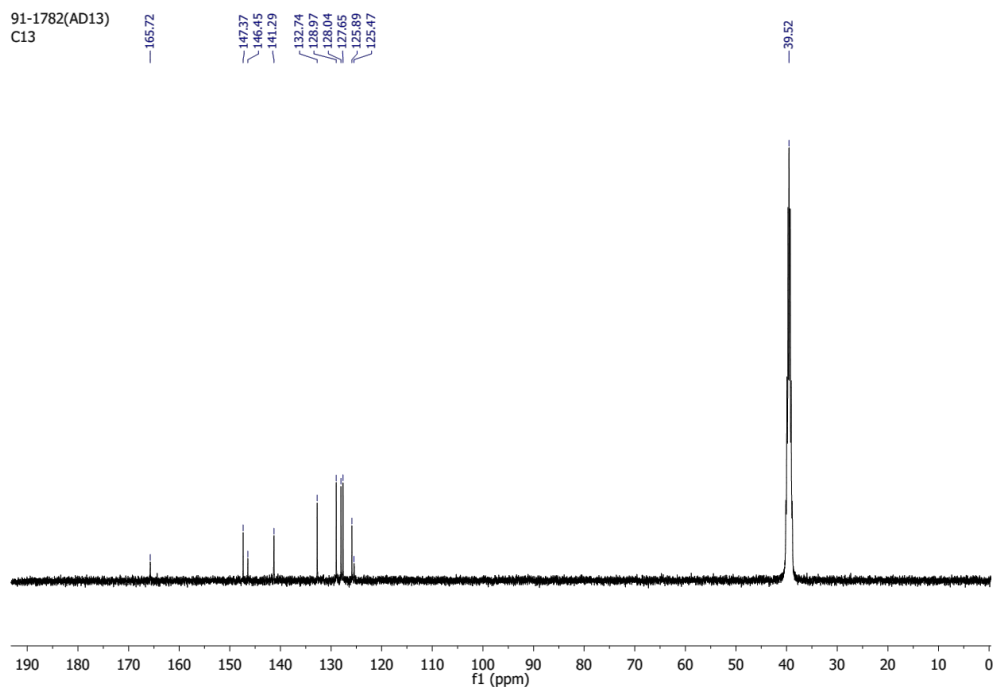
Appendix 15. The IR spectrum of 2-thiocyanatoquinoline-3-carbaldehyde (**199**)



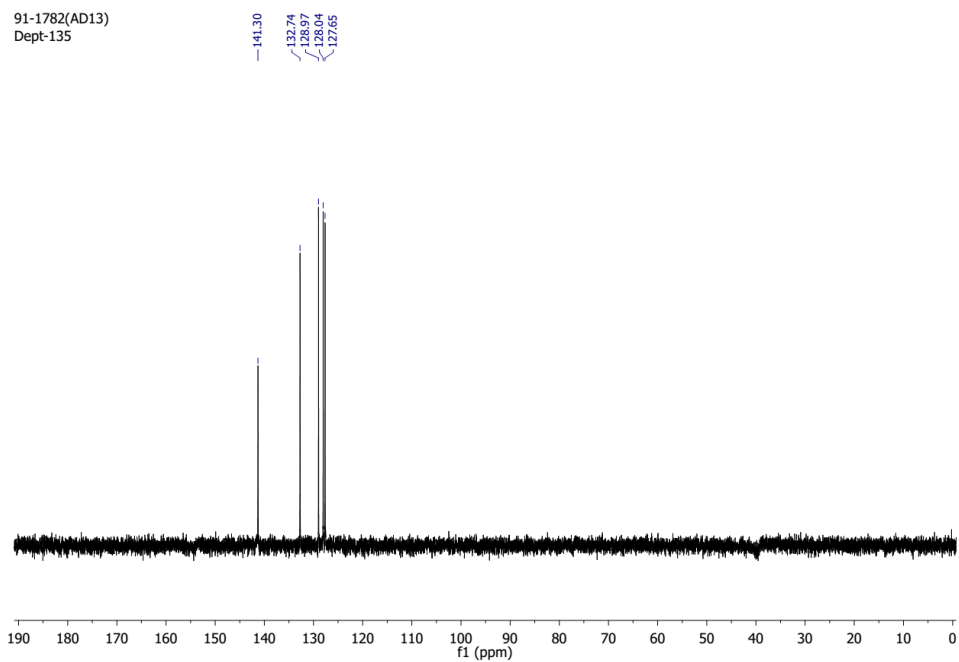
Appendix 16. The ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of 2-Chloroquinoline-3-carboxylic Acid (**200**)



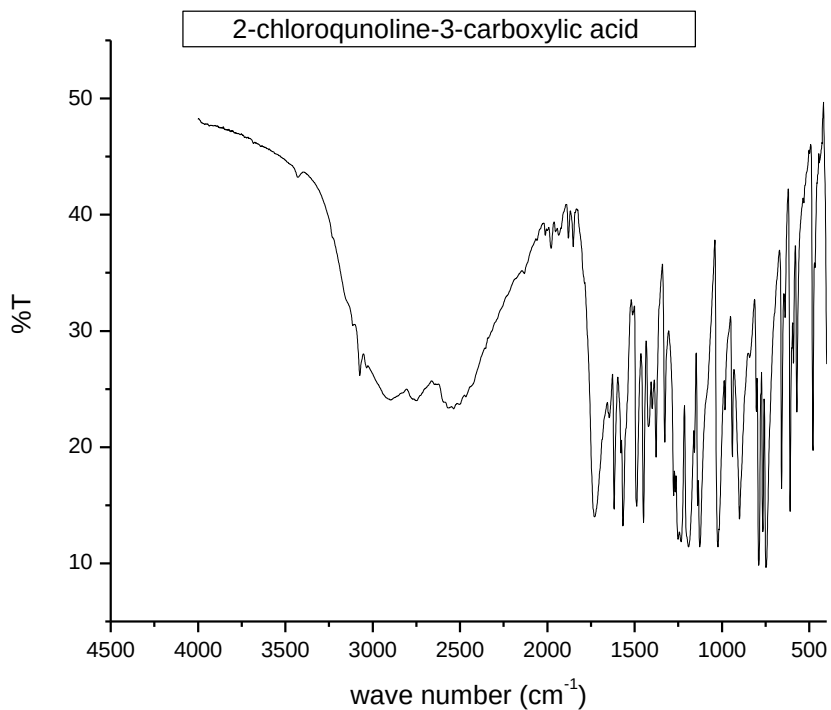
Appendix 17. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 2-Chloroquinoline-3-carboxylic Acid (200)



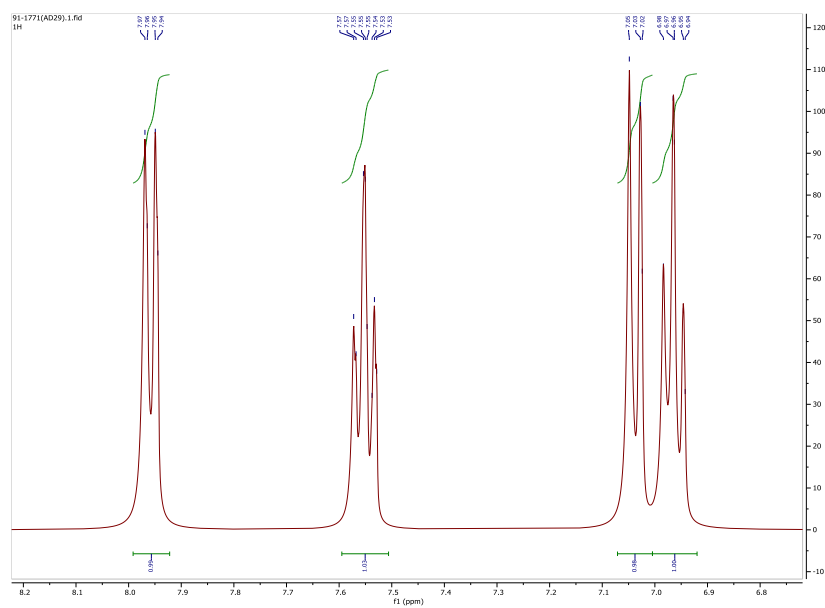
Appendix 18. The DEPT-135 (100 MHz, DMSO-d_6) spectrum of 2-Chloroquinoline-3-carboxylic Acid (200)



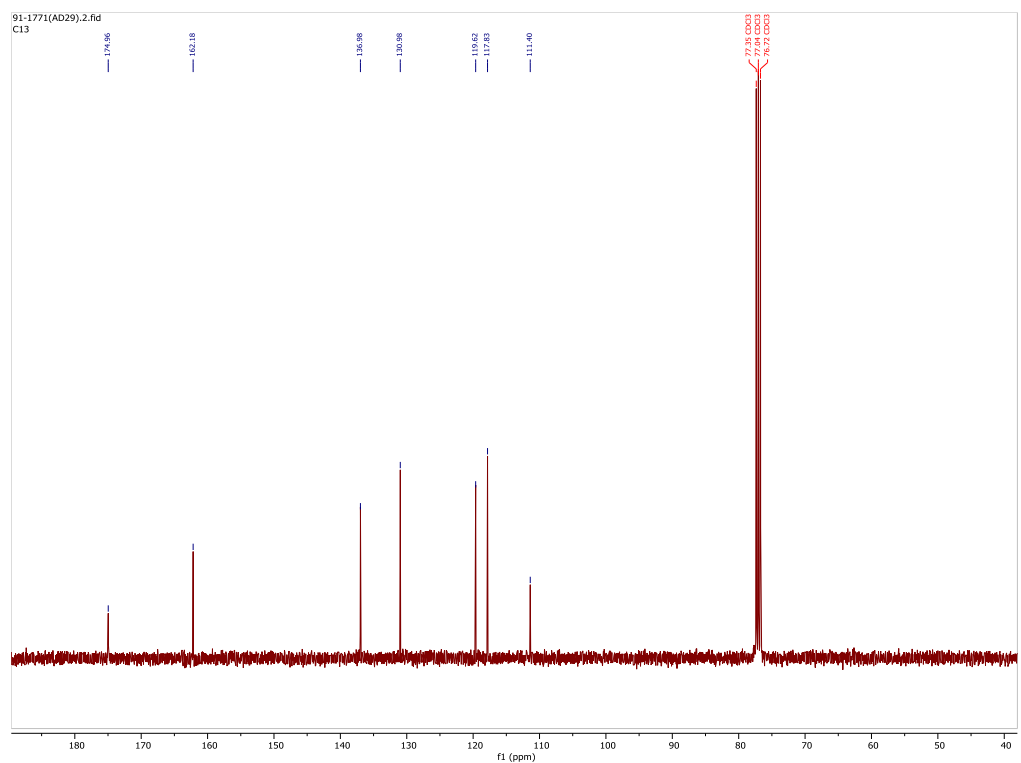
Appendix 19. The IR spectrum of 2-chloroquinoline-3-carboxylic acid (**200**)



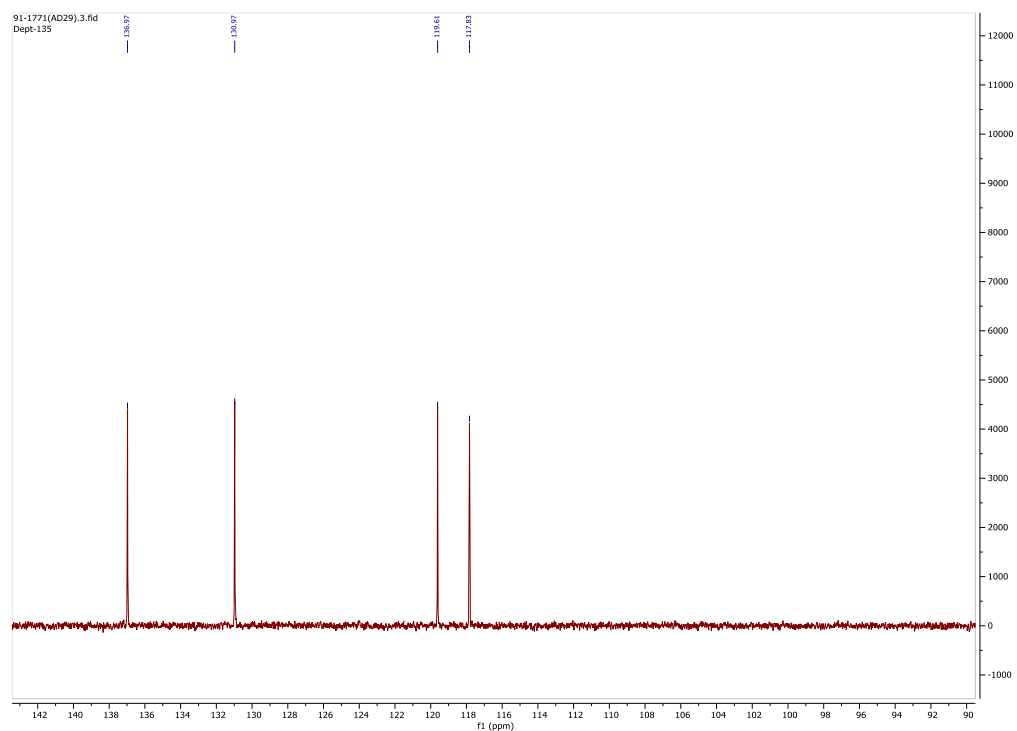
Appendix 20. The ^1H NMR (400 MHz, CDCl_3) of spectrum 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (**203**)



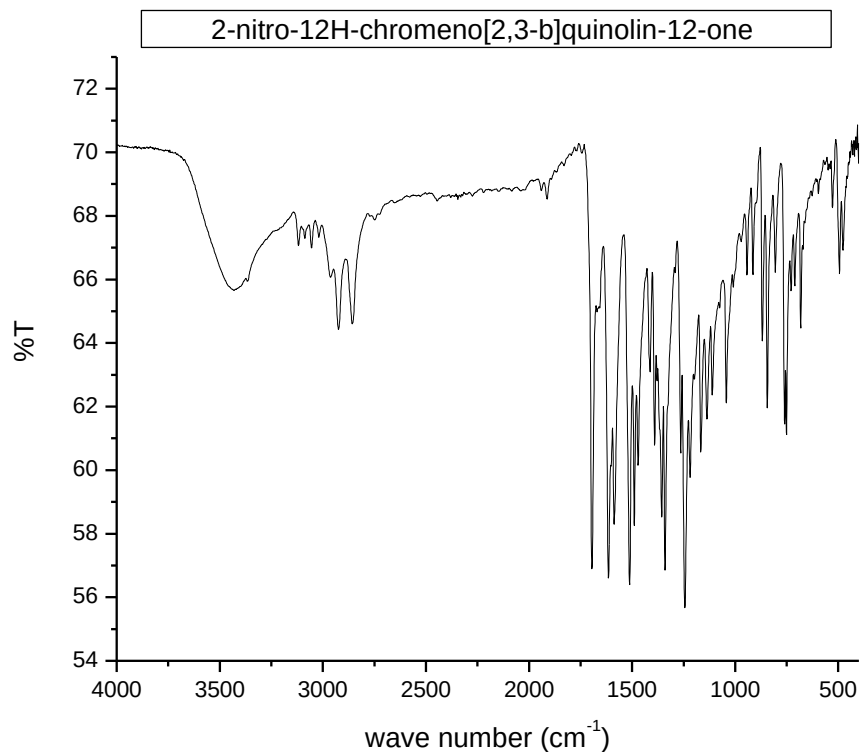
Appendix 21. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (**203**)



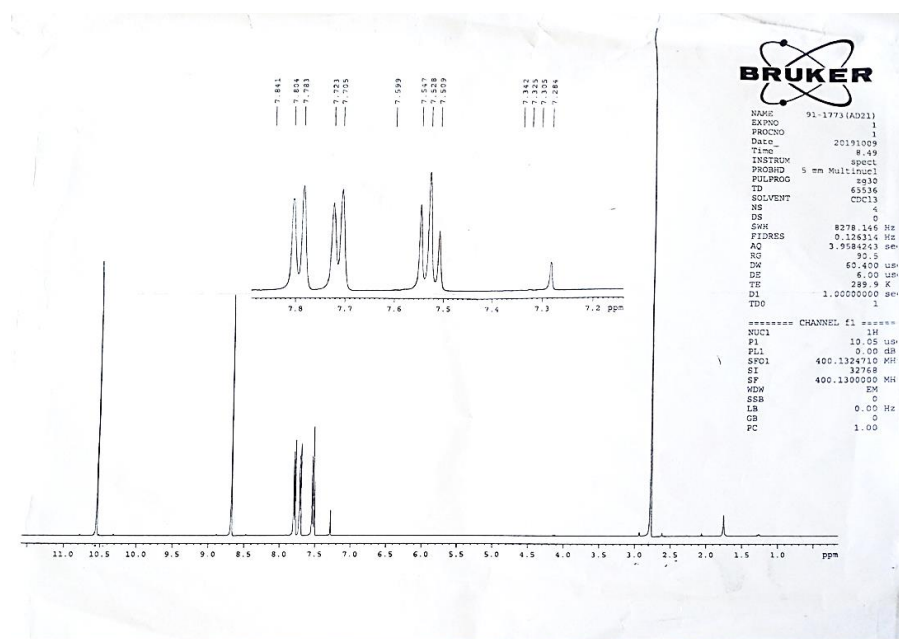
Appendix 22. The DEPT-135 NMR (100 MHz, CDCl_3) spectrum of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (**203**)



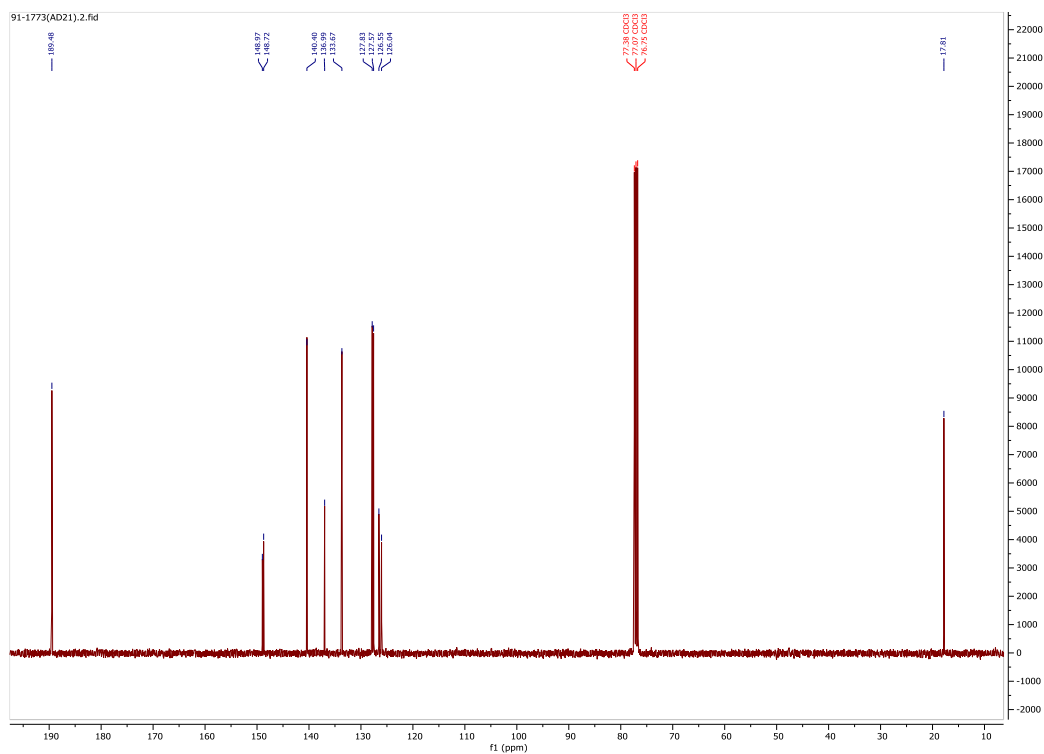
Appendix 23. IR spectrum of 2-Nitro-12H-chromeno[2,3-b]quinolin-12-one (203)



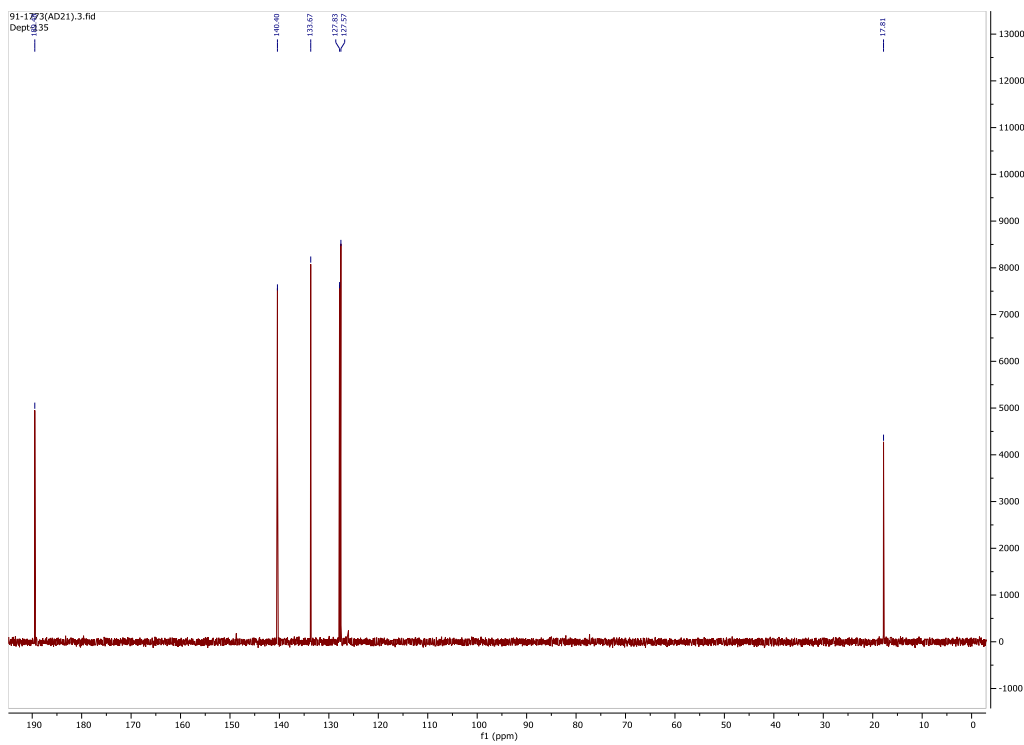
Appendix 24. The ¹H NMR (400 MHz, CDCl₃) spectrum of 2-Chloro-8-methylquinoline-3-carbaldehyde (206)



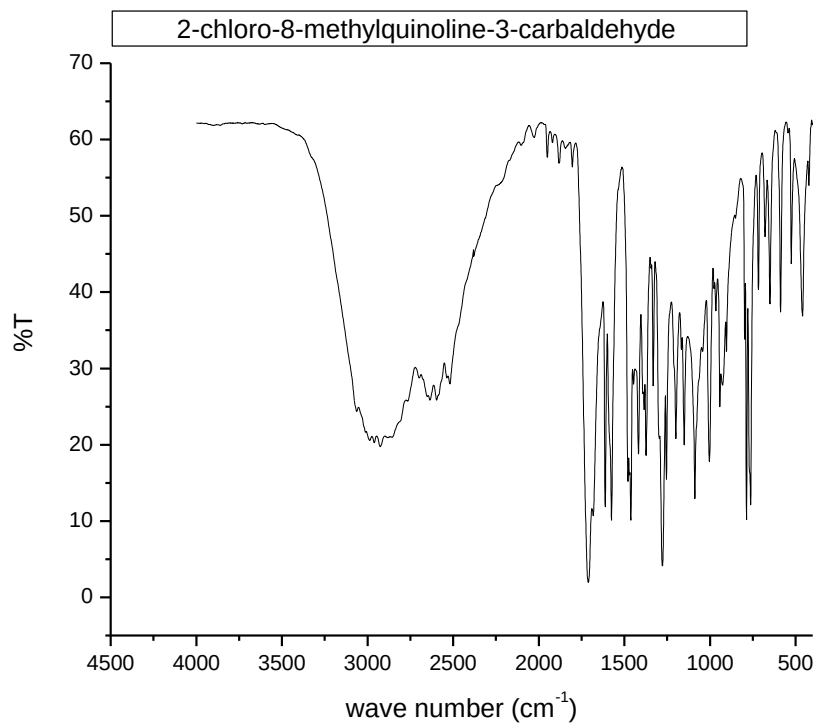
Appendix 25. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 2-Chloro-8-methylquinoline-3-carbaldehyde (**206**)



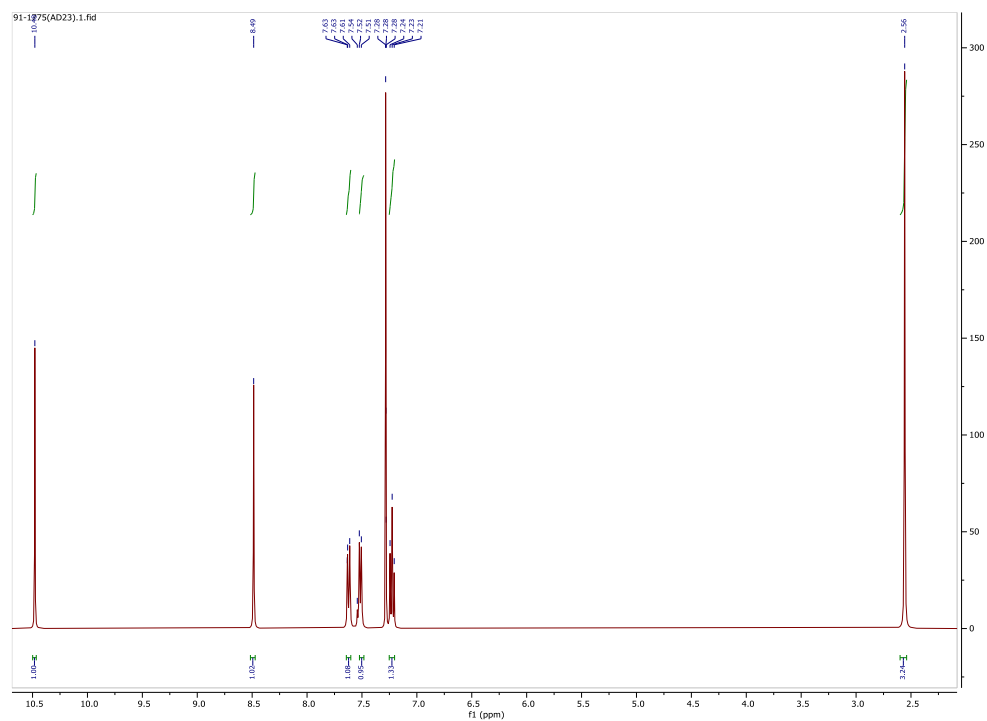
Appendix 26. The DEPT-135 (100 MHz, CDCl_3) spectrum of 2-chloro-8-methylquinoline-3-carbaldehyde (**206**)

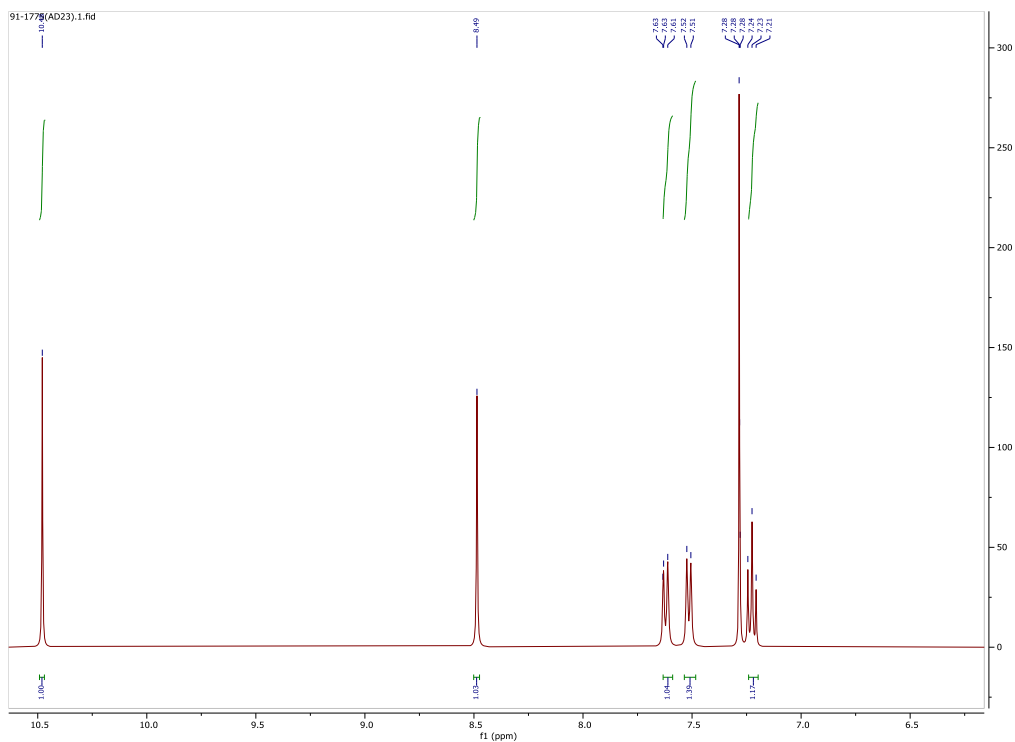


Appendix 27. The IR spectrum of 2-Chloro-8-methylquinoline-3-carbaldehyde (**206**)

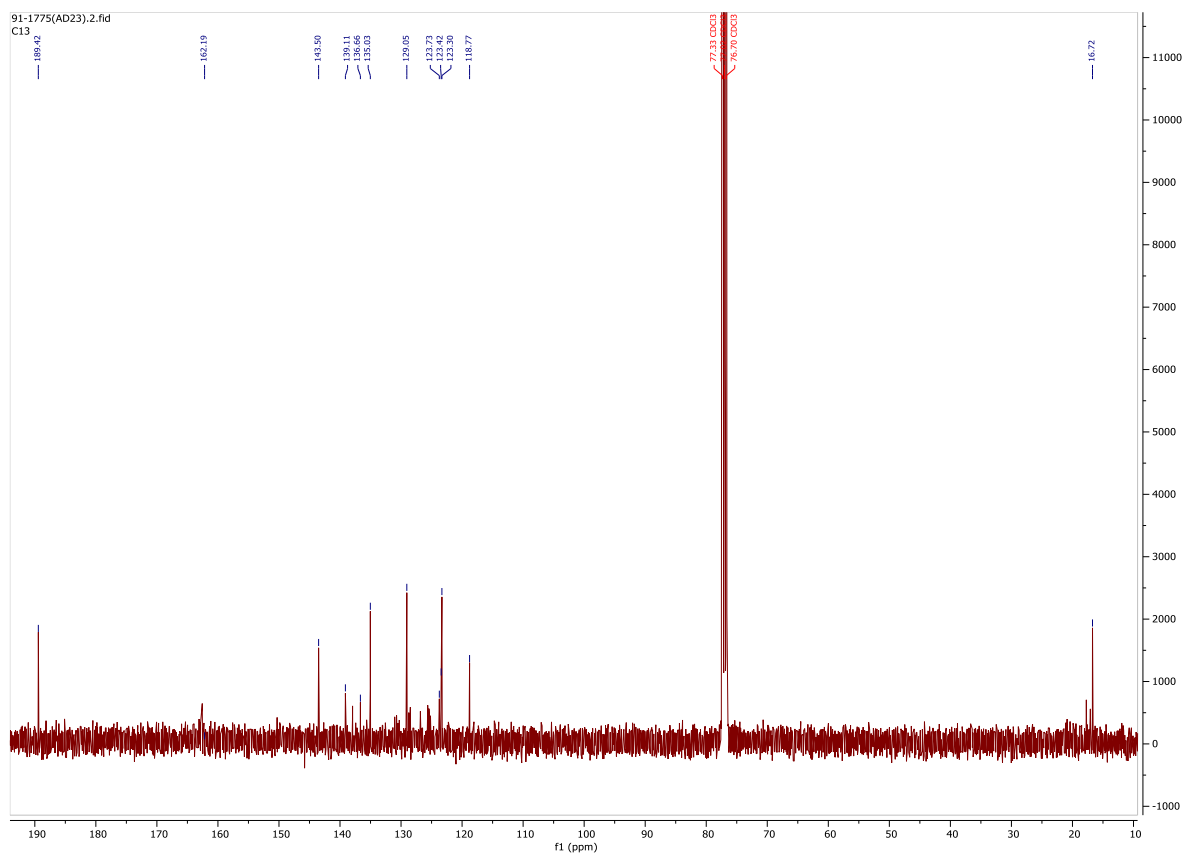


Appendix 28. The ¹H NMR (400 MHz, CDCl₃) spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (**207**)

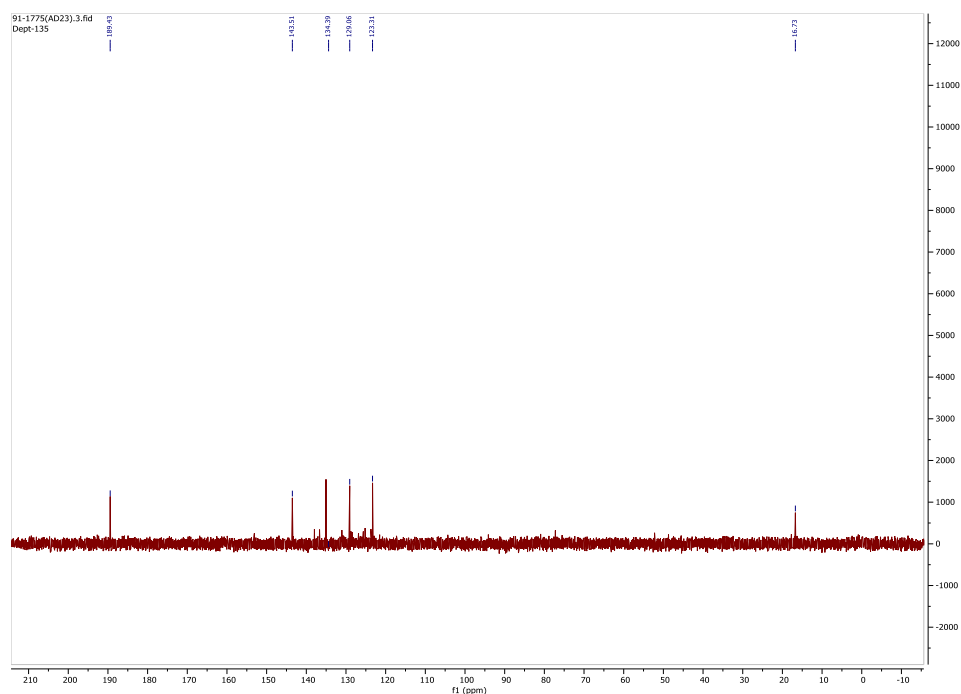




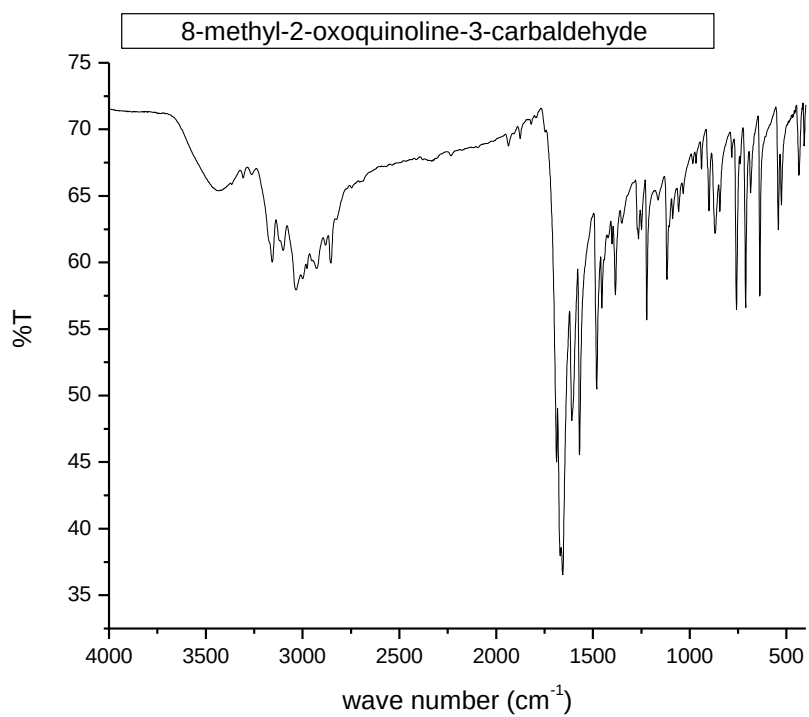
Appendix 29. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (**207**)



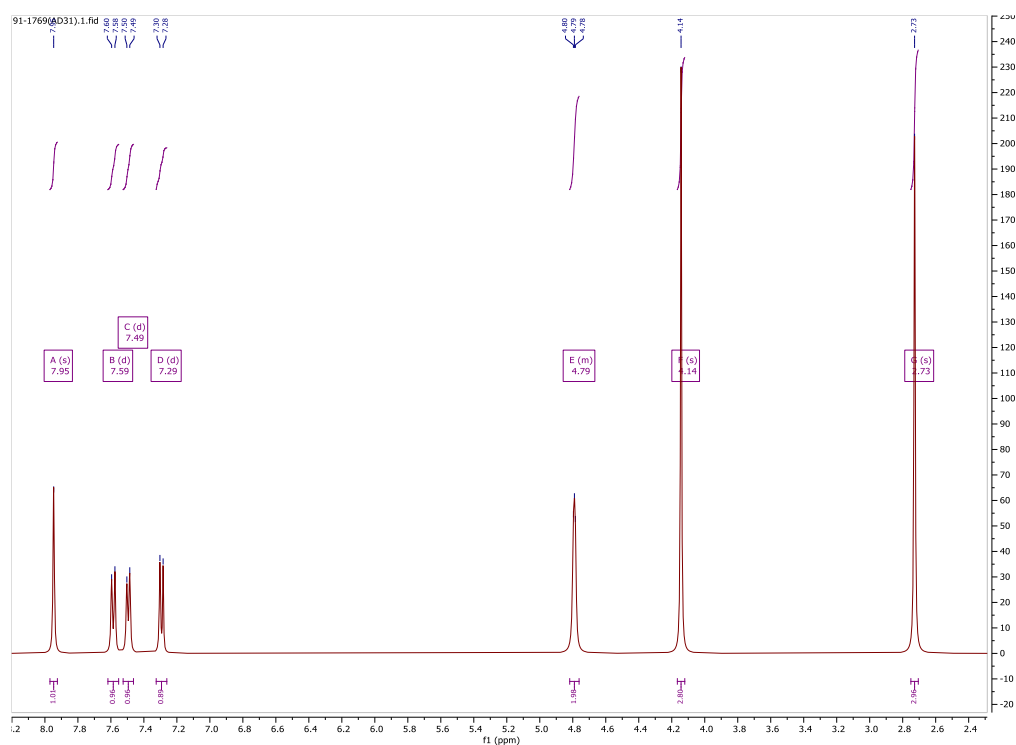
Appendix 30. The DEPT-135 (100 MHz, CDCl₃) spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (**207**)



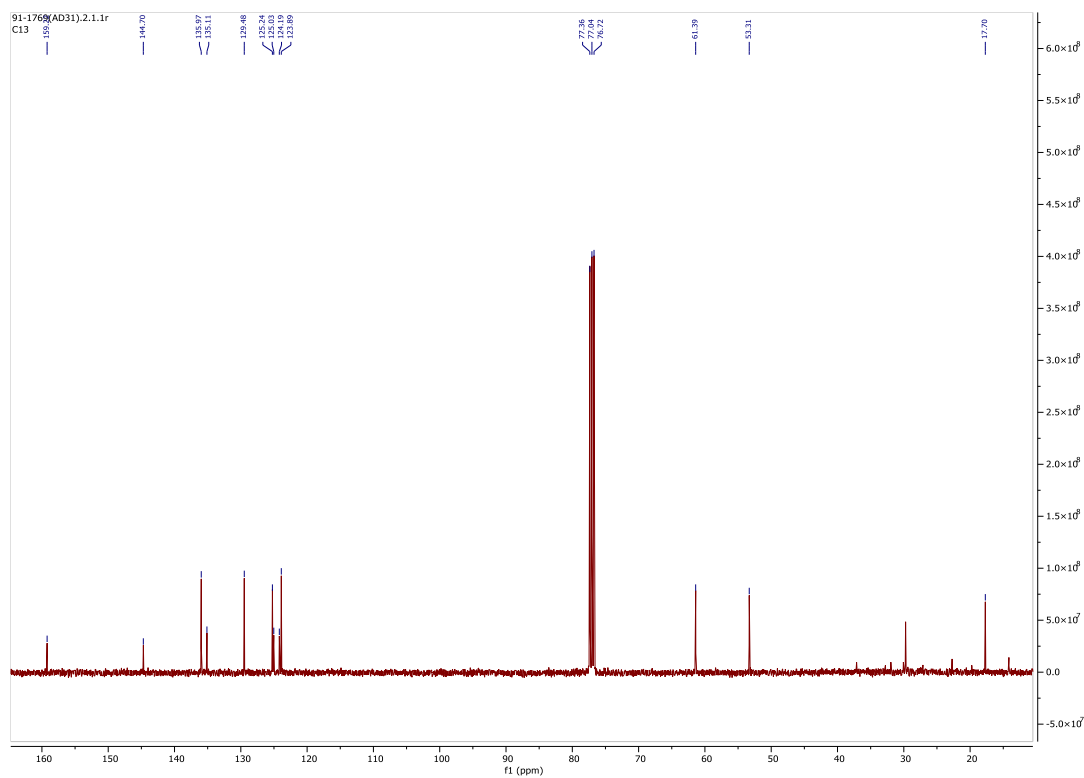
Appendix 31. The IR spectrum of 8-Methyl-2-oxoquinoline-3-carbaldehyde (**207**)



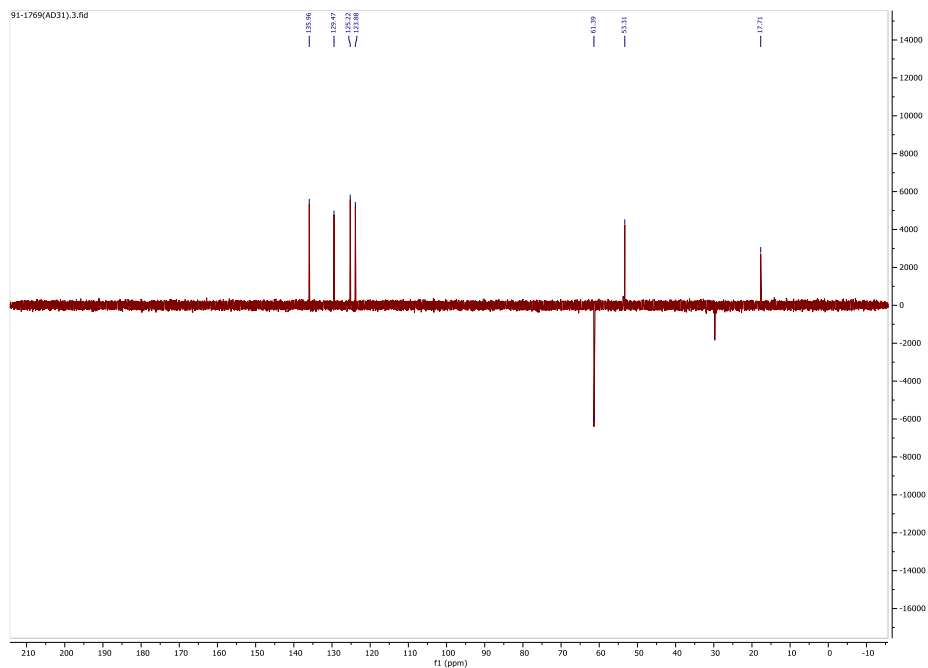
Appendix 32. The ^1H NMR (400 MHz, CDCl_3) spectrum of (2-methoxy-8-methylquinolin-3-yl)methanol (**208**)



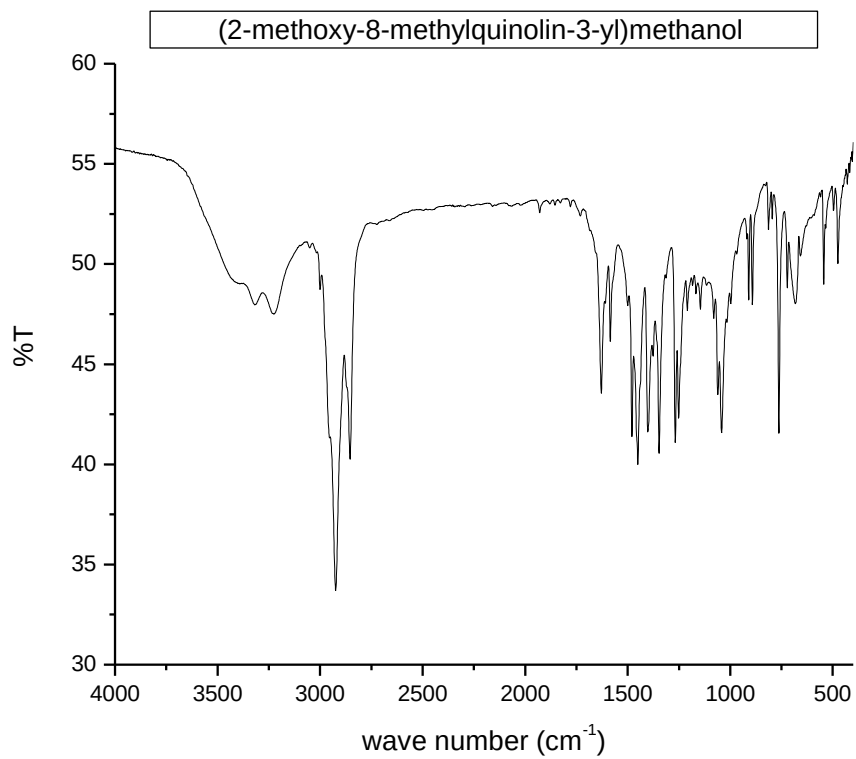
Appendix 33. The ^{13}C NMR (100 MHz, CDCl_3) spectrum of (2-methoxy-8-methylquinolin-3-yl)methanol (**208**)



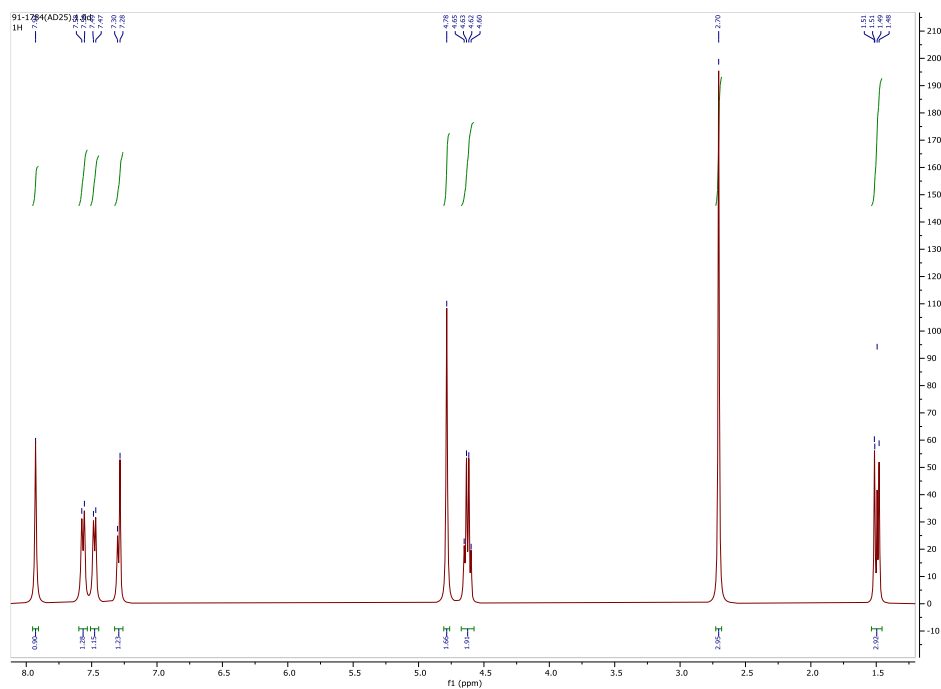
Appendix 34. The DEPT-135 (100 MHz, CDCl₃) spectrum of (2-methoxy-8-methylquinolin-3-yl) methanol (**208**)



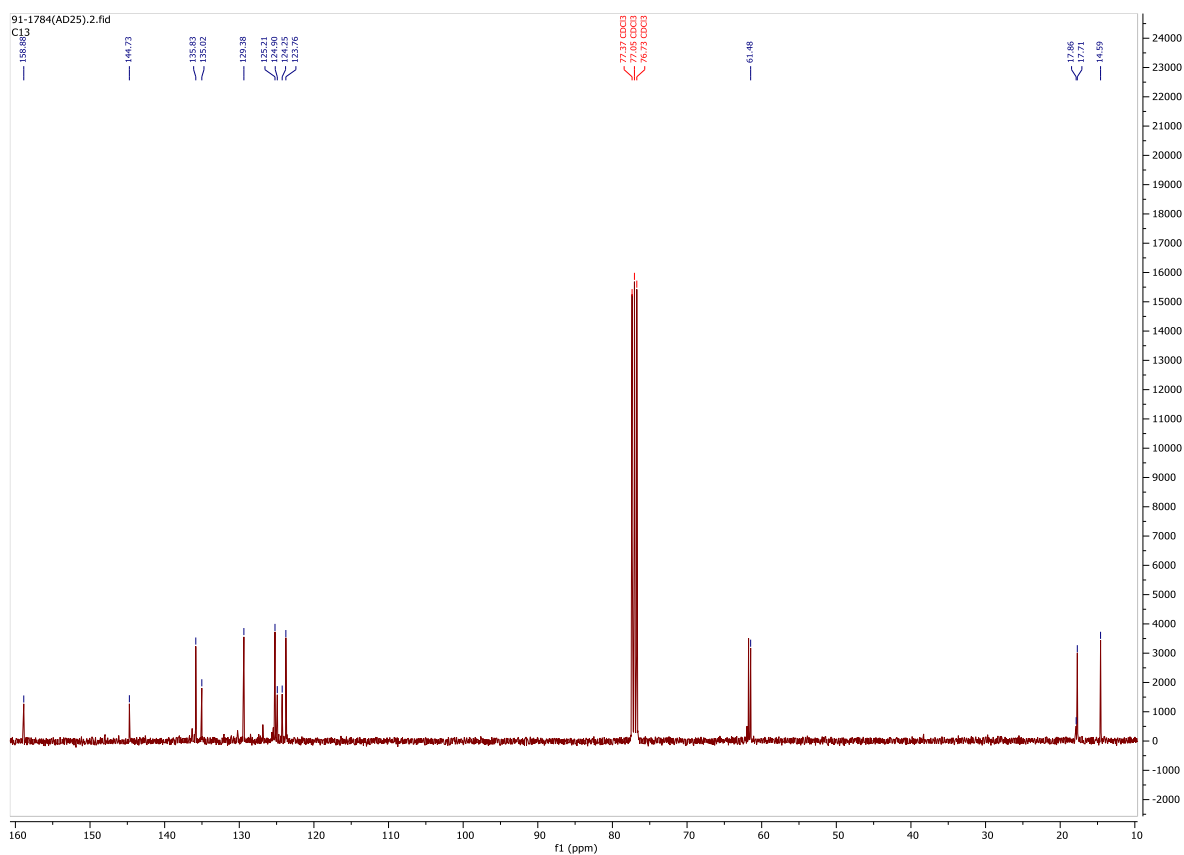
Appendix 35. The IR spectrum of (2-methoxy-8-methylquinolin-3-yl) methanol (**208**)



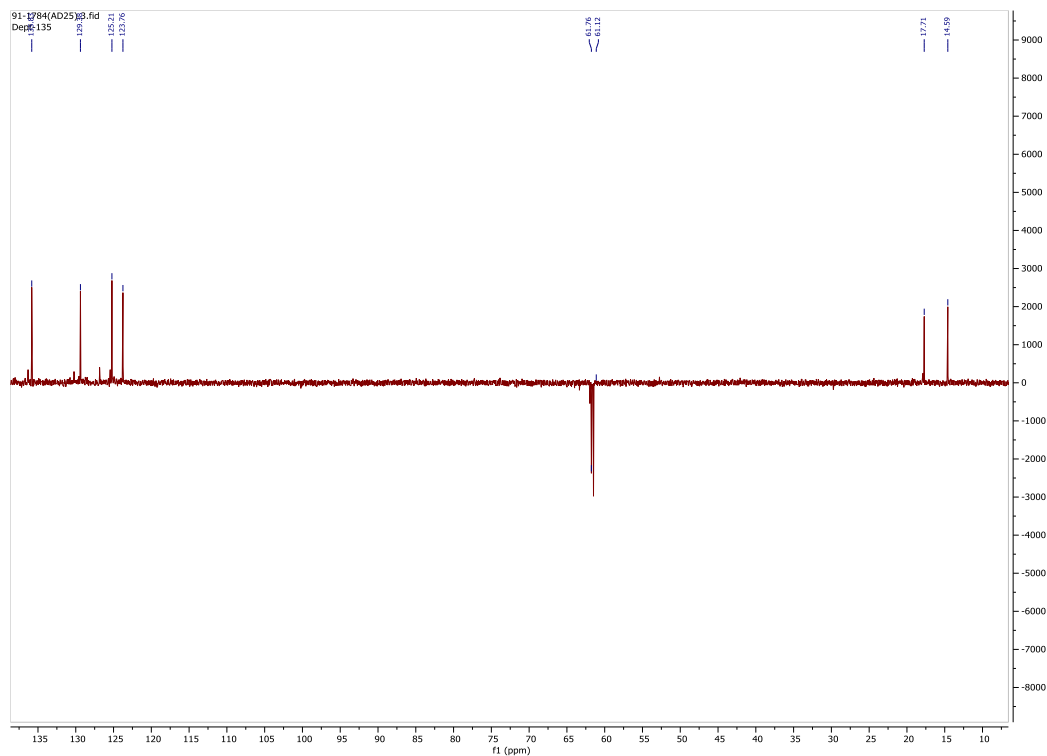
Appendix 36. The ^1H NMR(400 MHz, CDCl_3) spectrum of (2-Ethoxy-8-methylquinolin-3-yl)methanol (**209**)



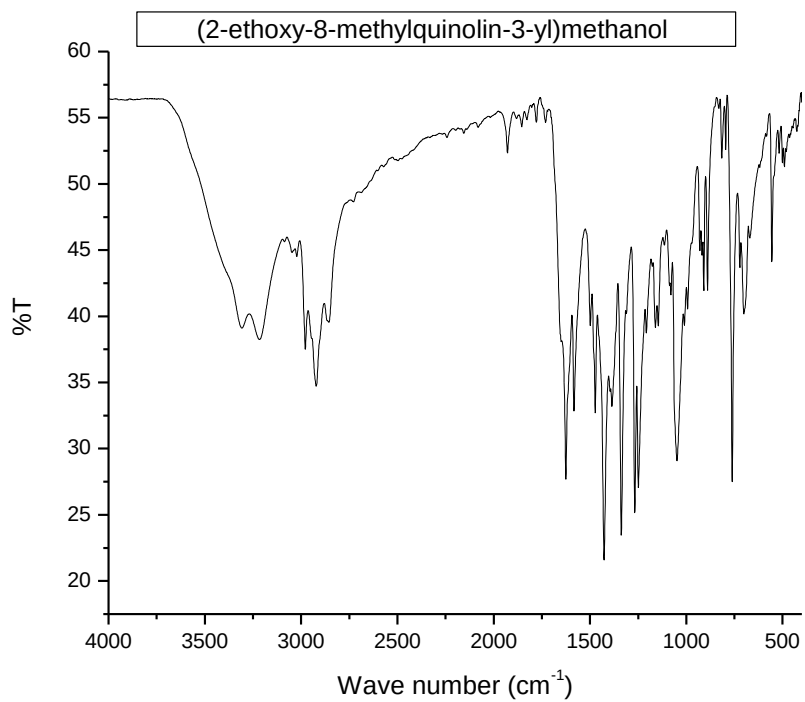
Appendix 37. The ^{13}C NMR (100 MHz, CDCl_3) of (2-Ethoxy-8-methylquinolin-3-yl)methanol (**209**)



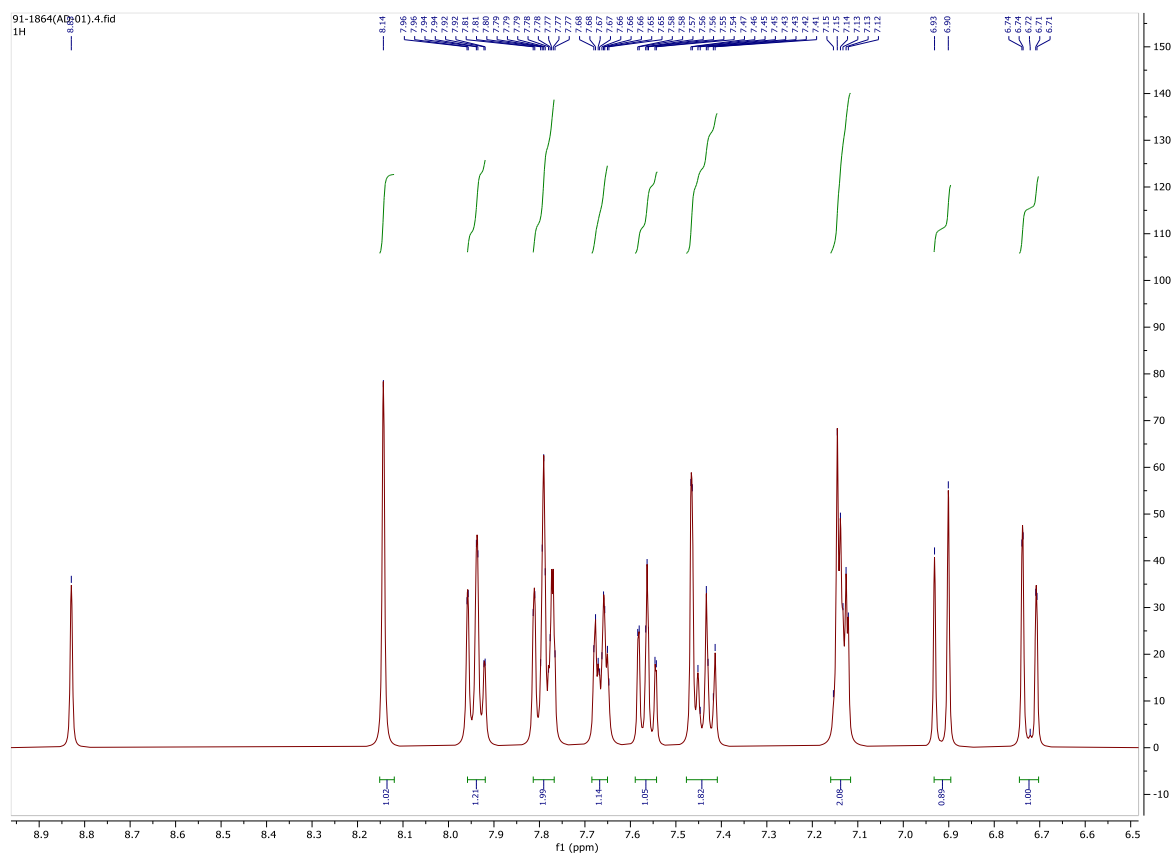
Appendix 38. The DEPT-135(100 MHz, CDCl₃) spectrum of (2-Ethoxy-8-methylquinolin-3-yl)methanol (**209**)



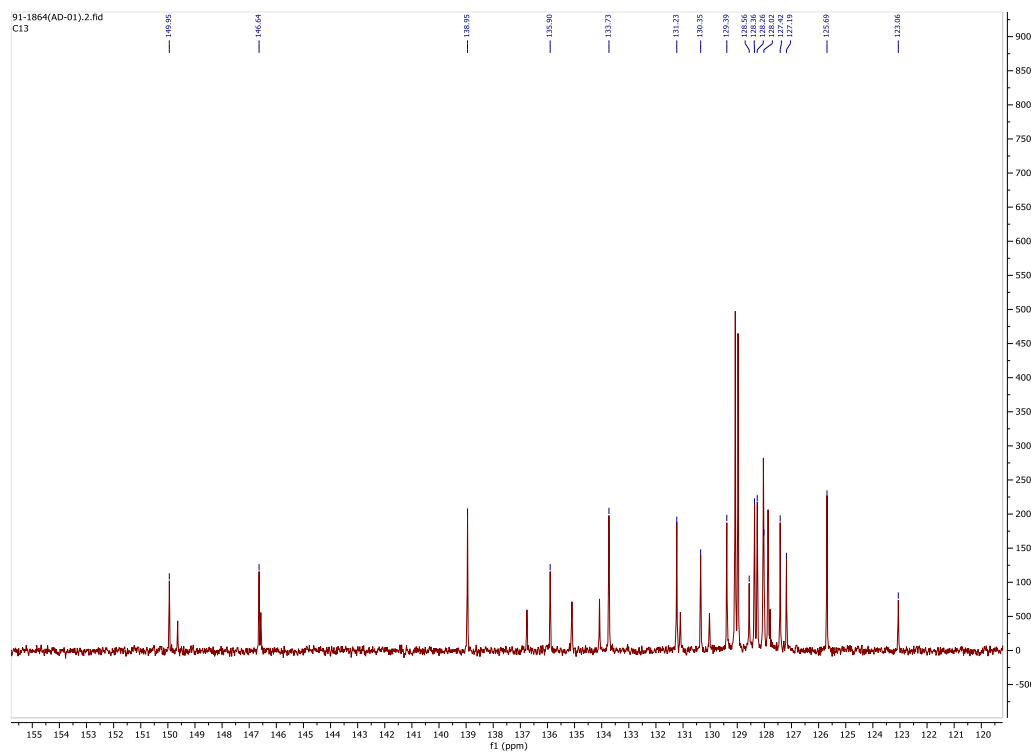
Appendix 39. The IR spectrum of (2-Ethoxy-8-methylquinolin-3-yl)methanol (**209**)



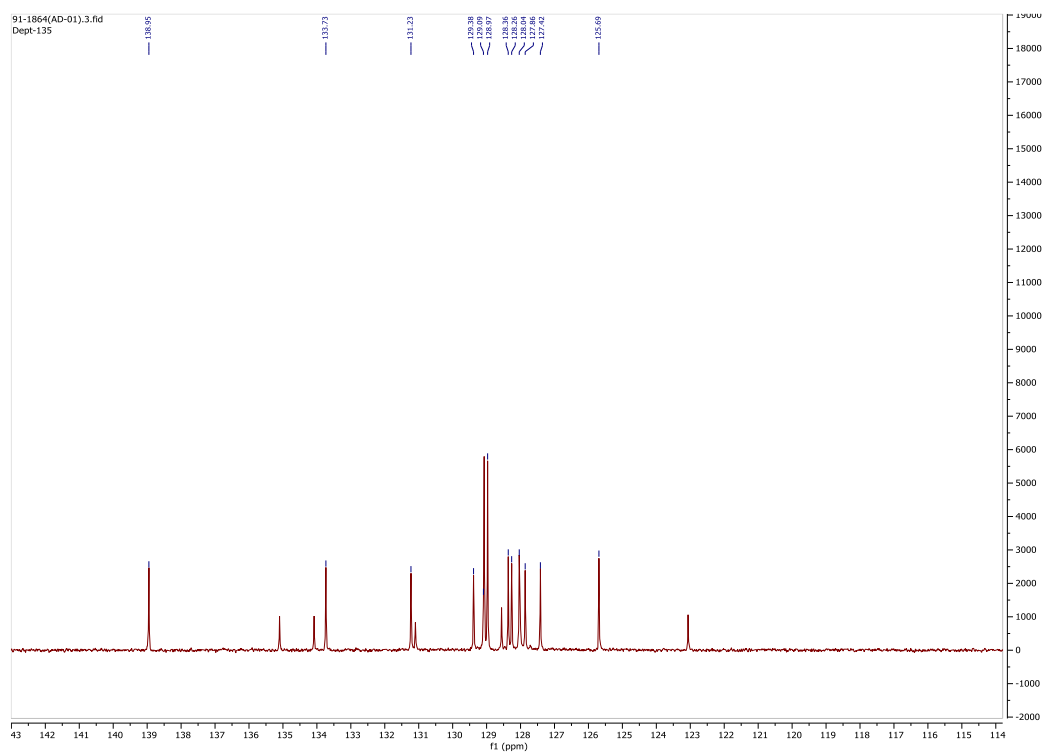
Appendix 40. ^1H NMR (400 MHz, DMSO- d_6) spectrum of (Z)-2-chloro-3-styrylquinoline (**211**)



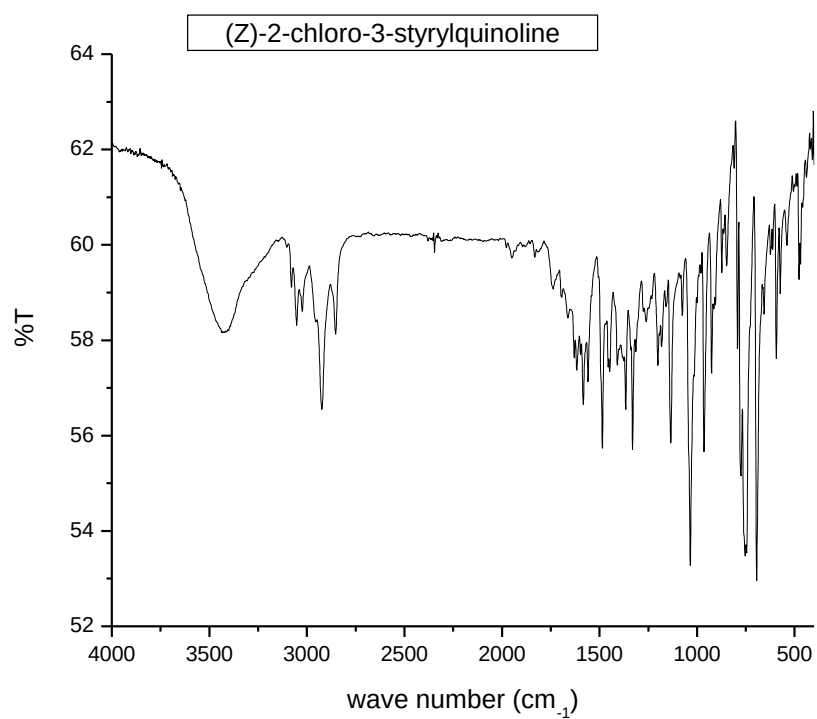
Appendix 41. ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of (Z)-2-chloro-3-styrylquinoline (**211**)



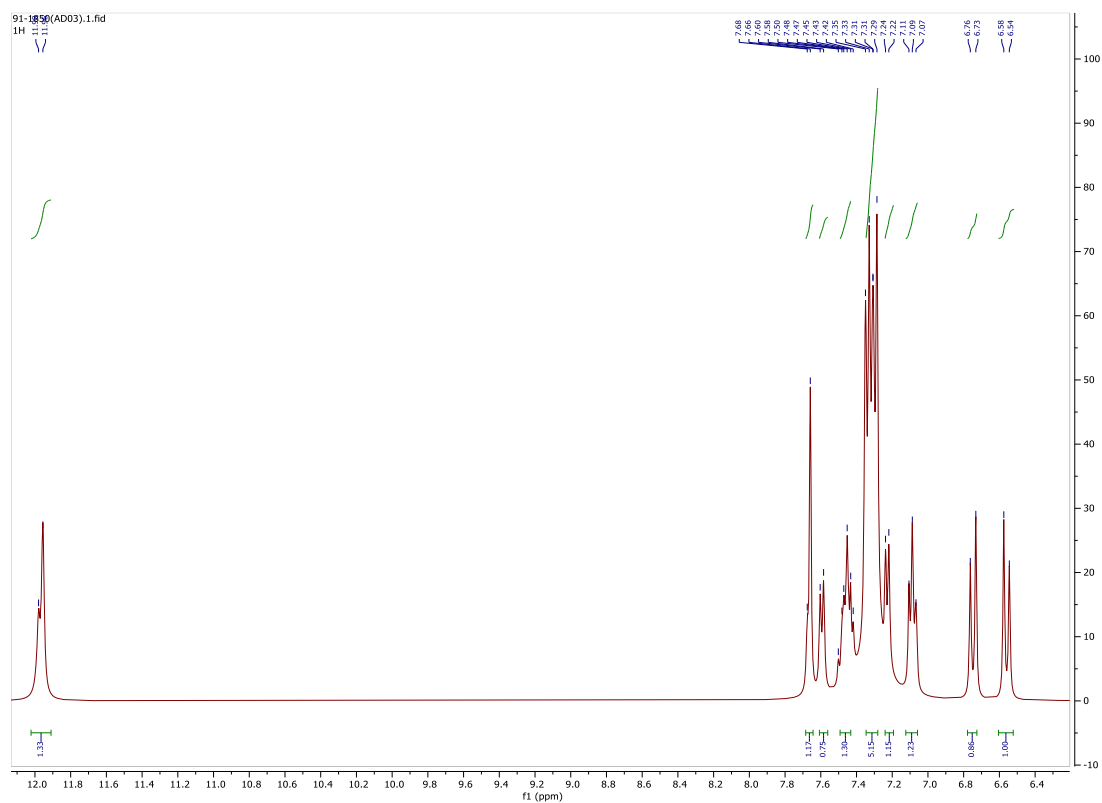
Appendix 42. The DEPT-135(100 MHz, DMSO-d₆) spectrum of (Z)-2-chloro-3-styrylquinoline (**211**)



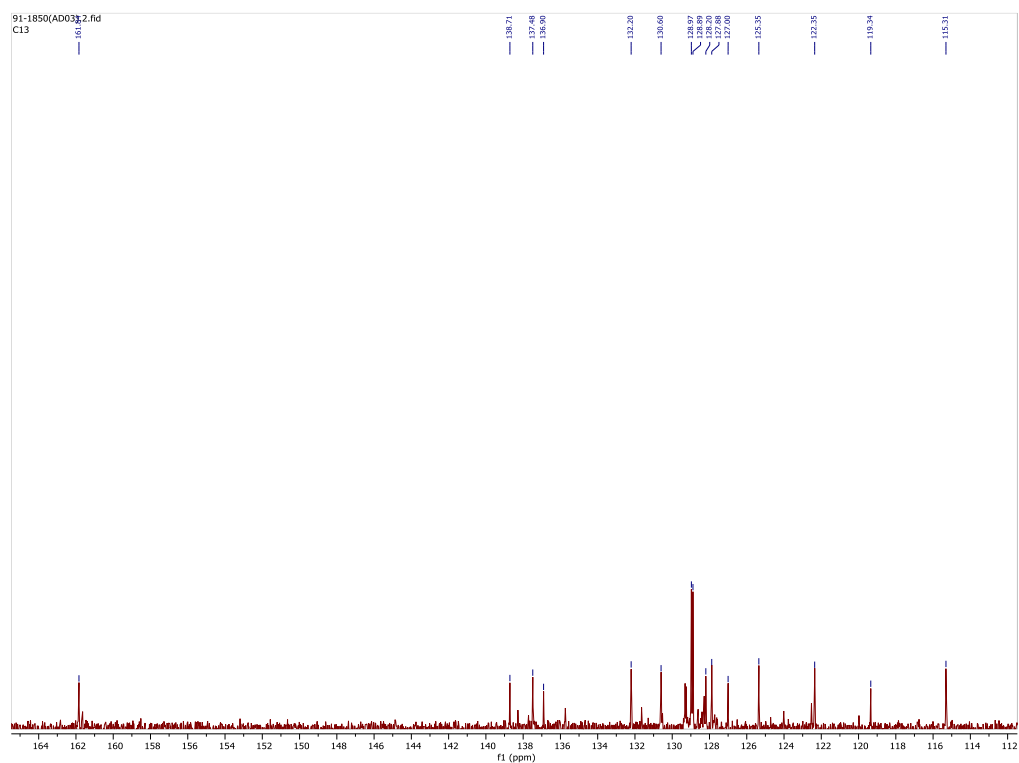
Appendix 43. The IR spectrum of (Z)-2-chloro-3-styrylquinoline (**211**)



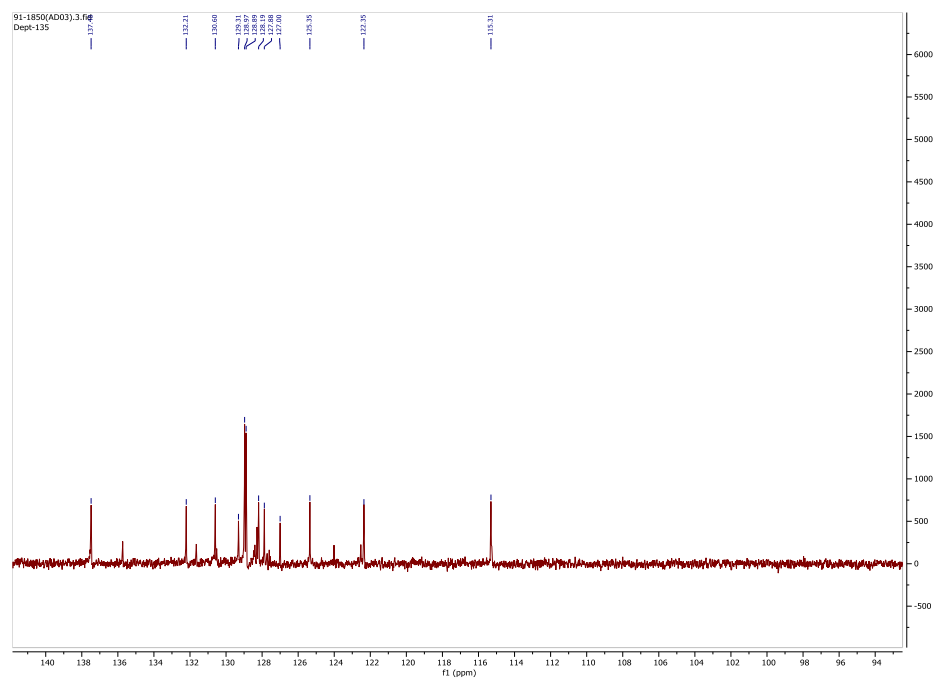
Appendix 44. ^1H NMR (400 MHz, DMSO-d_6) spectrum of (Z)-3-styrylquinolin-2(1H)-one (**213**)



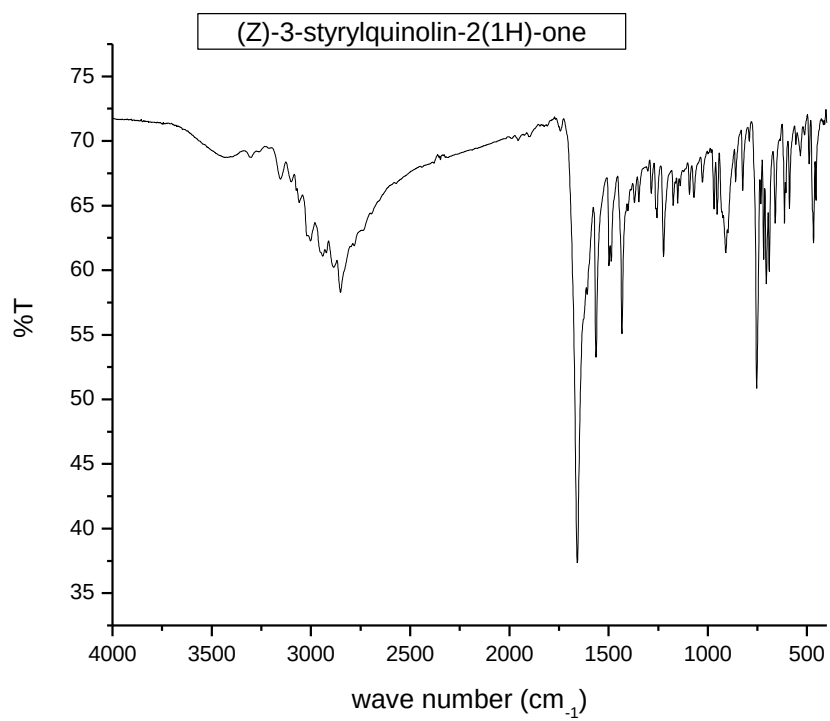
Appendix 45. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of (Z)-3-styrylquinolin-2(1H)-one (**213**)



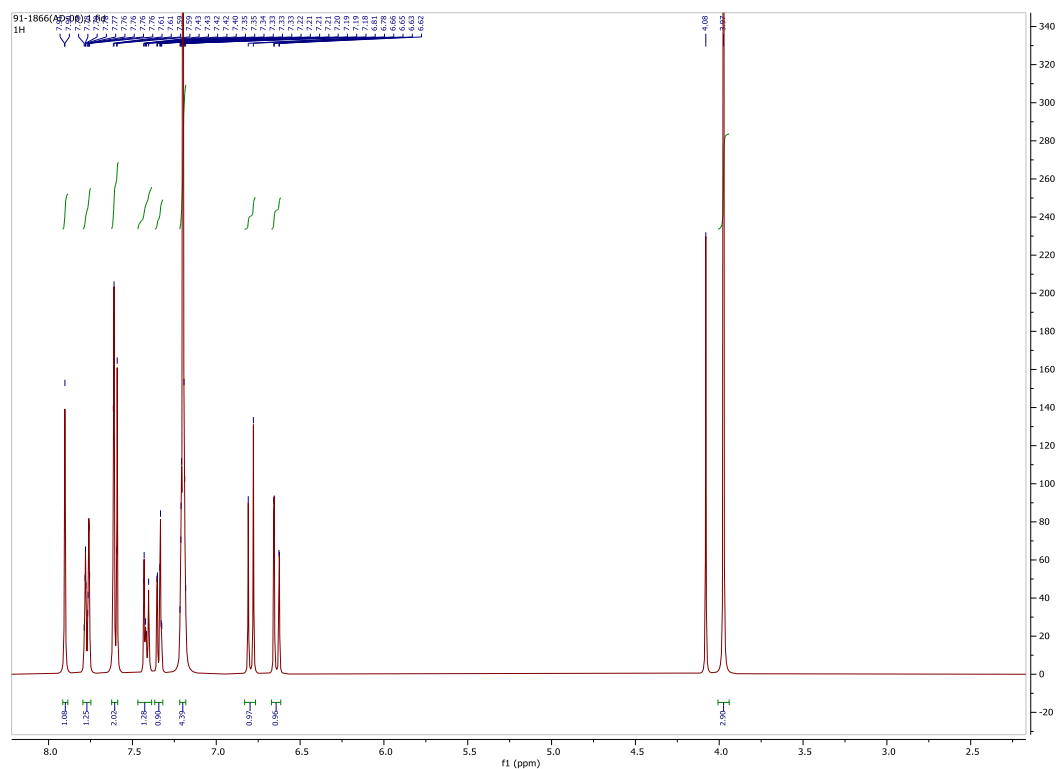
Appendix 46. The DEPT-135 (100 MHz, DMSO-d₆) spectrum of (Z)-3-styrylquinolin-2(1H)-one (213)



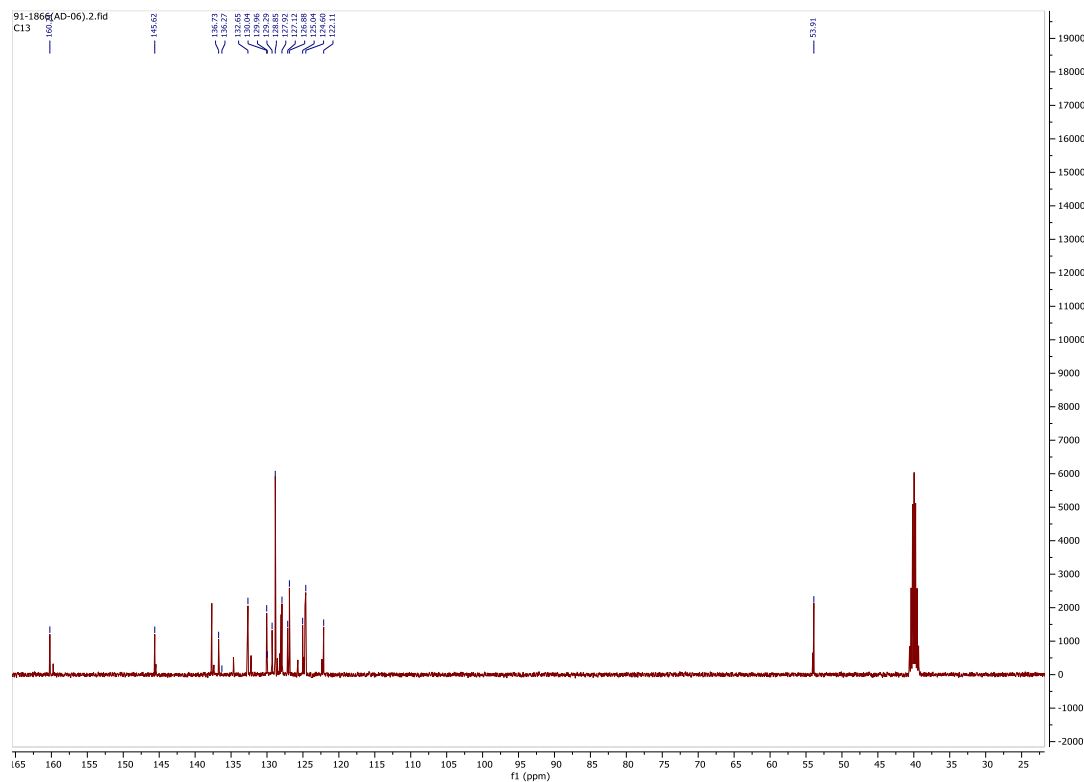
Appendix 47. The IR spectrum of (Z)-3-styrylquinolin-2(1H)-one (213)



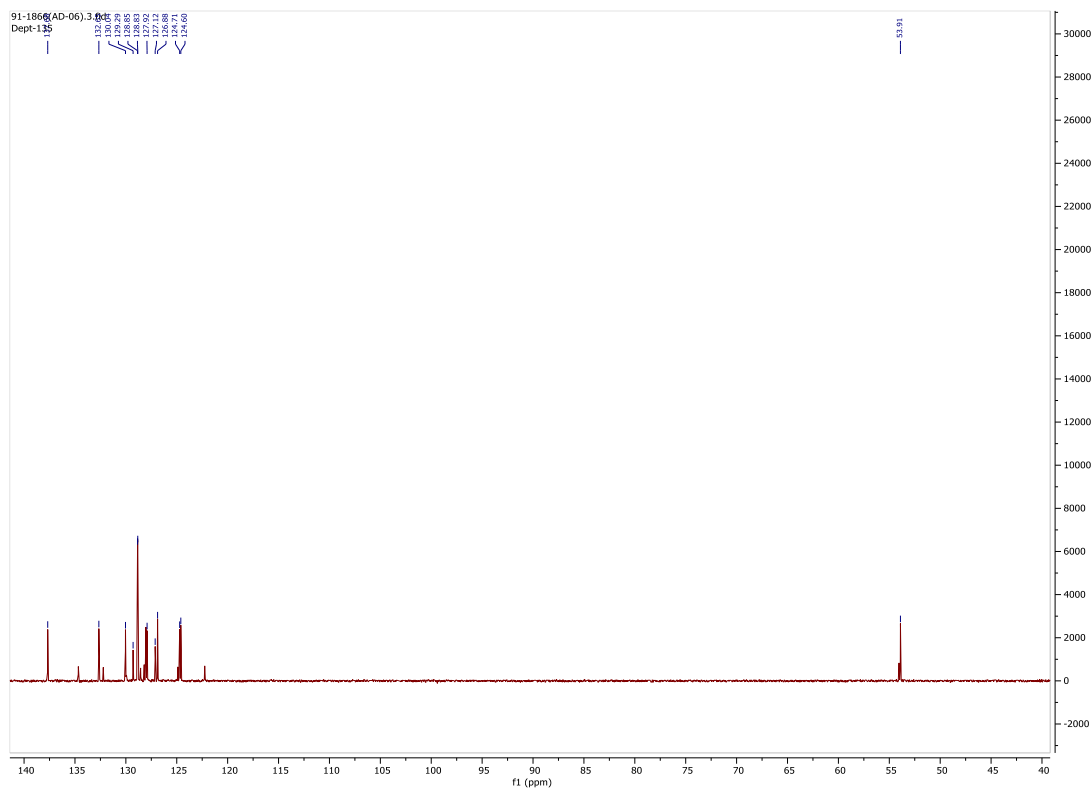
Appendix 48. ^1H NMR (400 MHz, CDCl_3) spectrum of (Z)-2-methoxy-3-styrylquinoline (**214**)



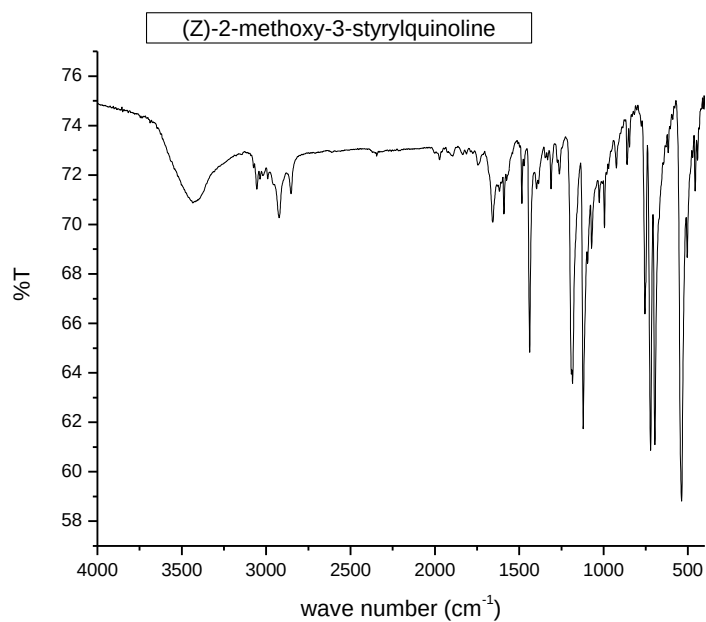
Appendix 49. ^{13}C NMR (100 MHz, CDCl_3) spectrum of (Z)-2-methoxy-3-styrylquinoline (**214**)



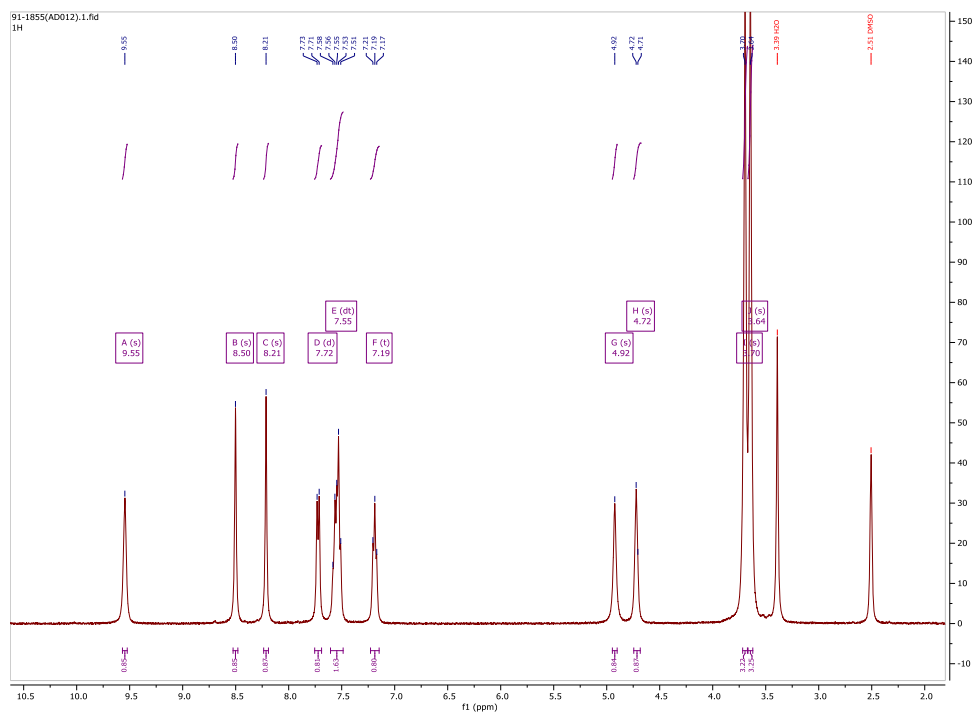
Appendix 50. The DEPT-135 (100 MHz, CDCl₃) spectrum of (Z)-2-methoxy-3-styrylquinoline (**214**)



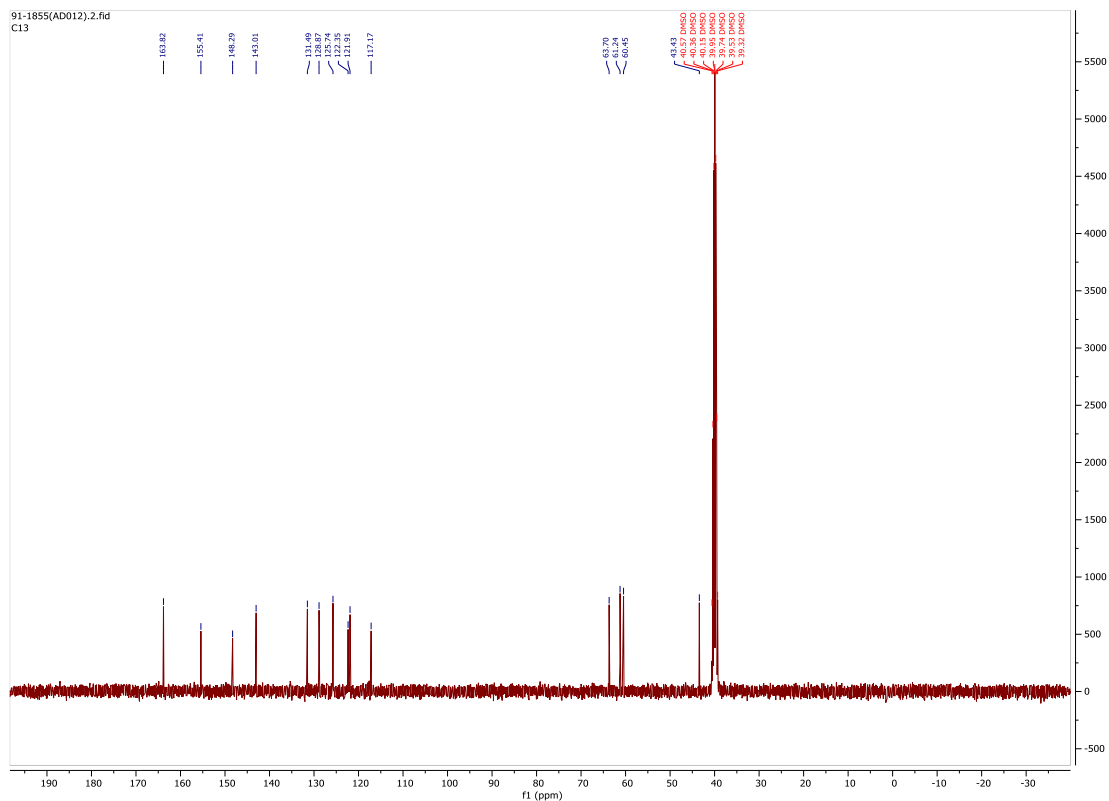
Appendix 51. The IR spectrum of (Z)-2-methoxy-3-styrylquinoline (**214**)



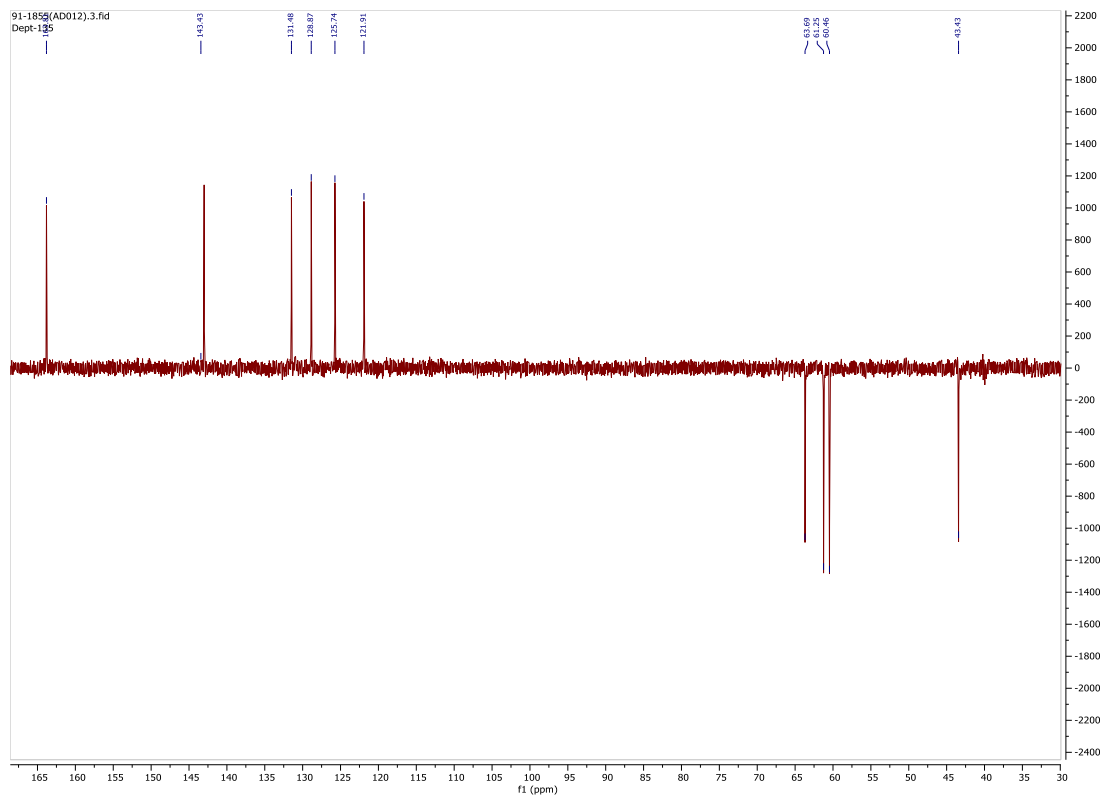
Appendix 52. ^1H NMR (400 MHz, DMSO-d_6) spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215)



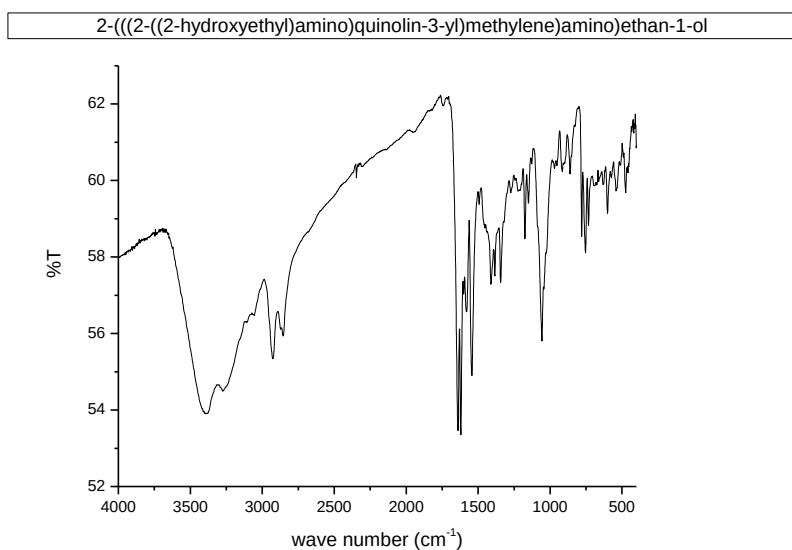
Appendix 53. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (215)



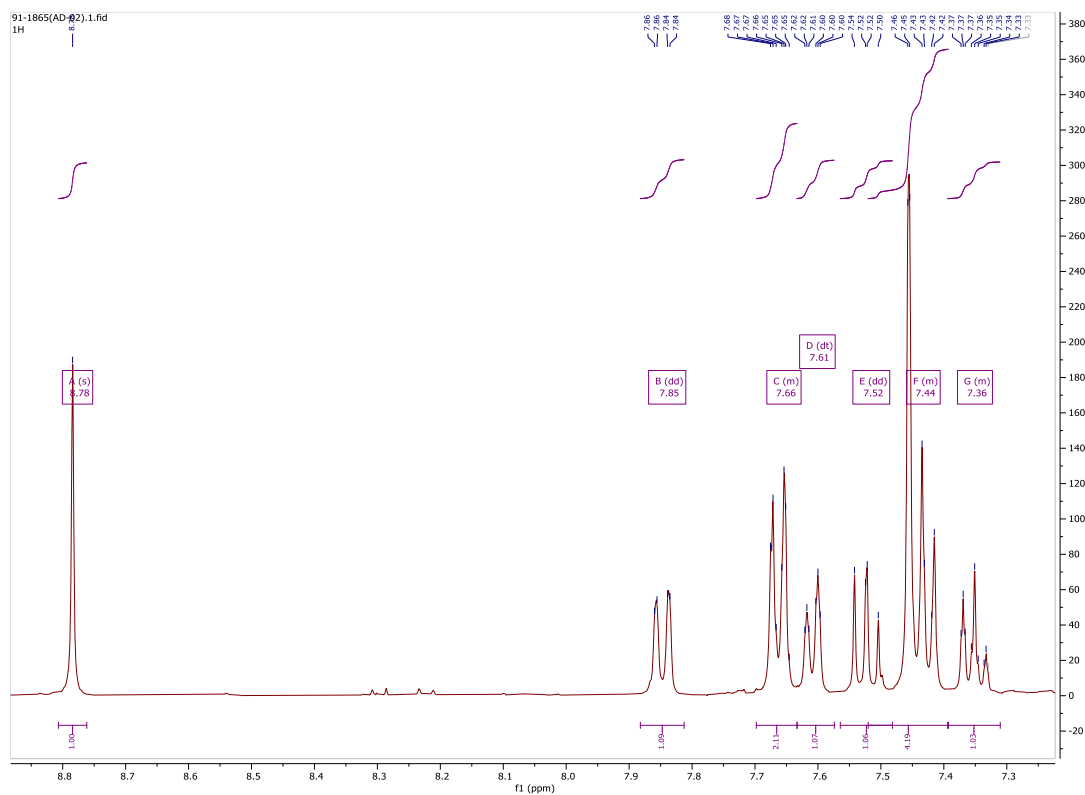
Appendix 54. The DEPT-135 (100 MHz, DMSO-d₆) spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (**215**)



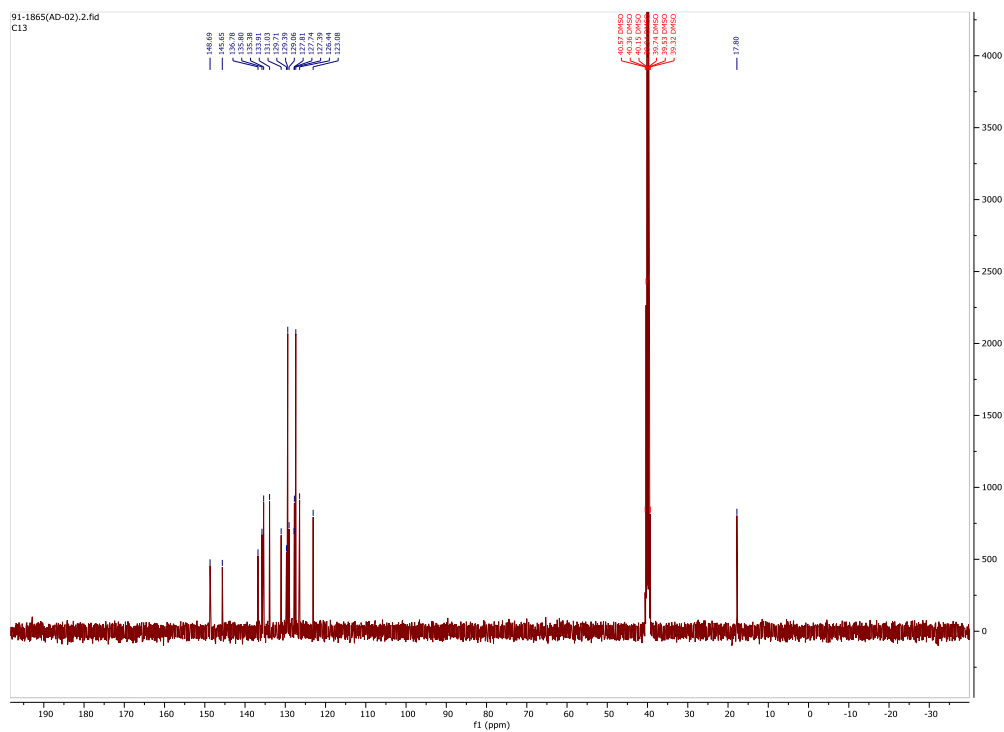
Appendix 55. The IR spectrum of 2-(((2-((2-hydroxyethyl)amino)quinolin-3-yl)methylene)amino)ethan-1-ol (**215**)



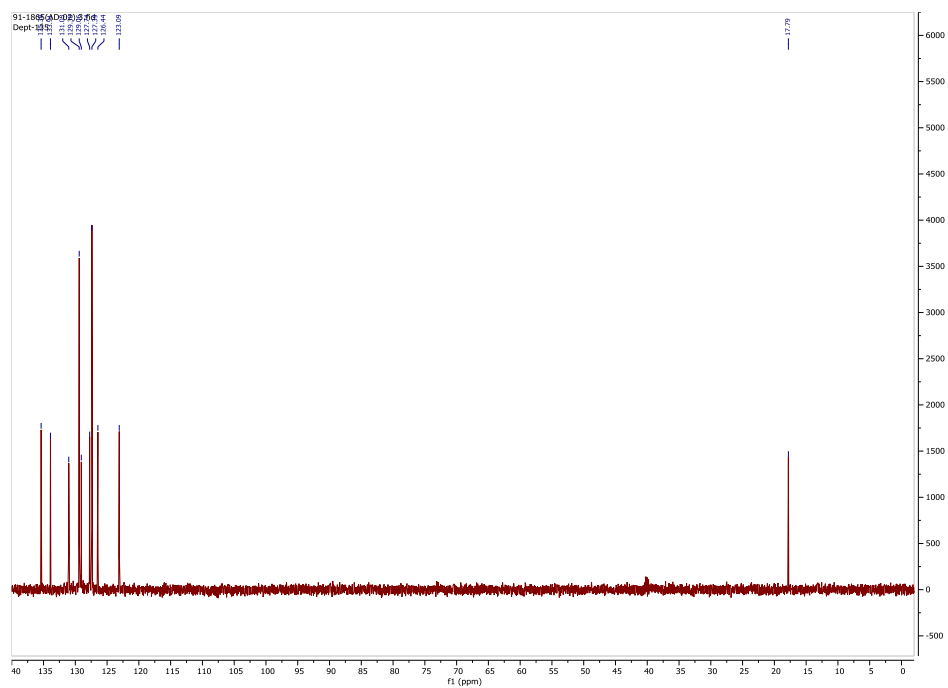
Appendix 56. ^1H NMR (400 MHz, DMSO-d_6) spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (216)



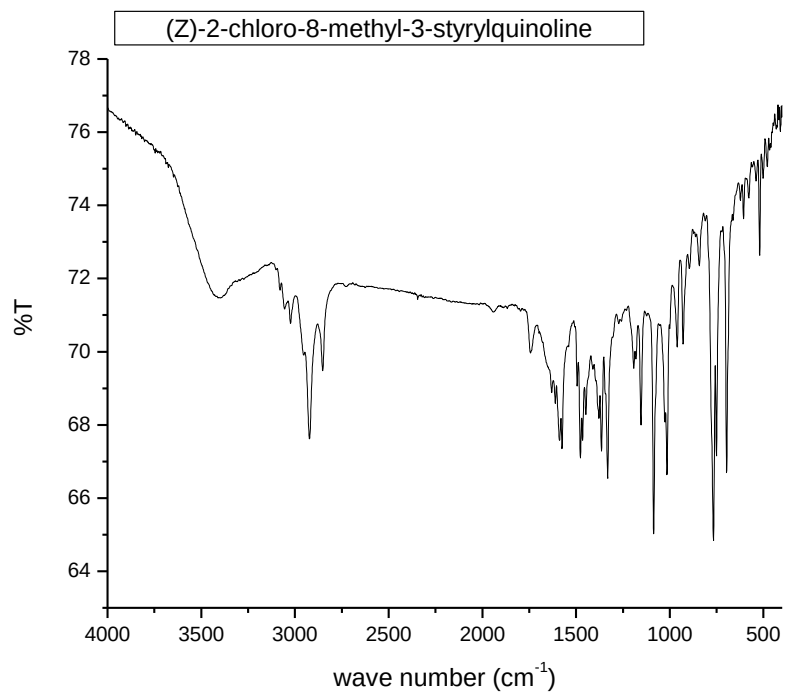
Appendix 57. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (216)



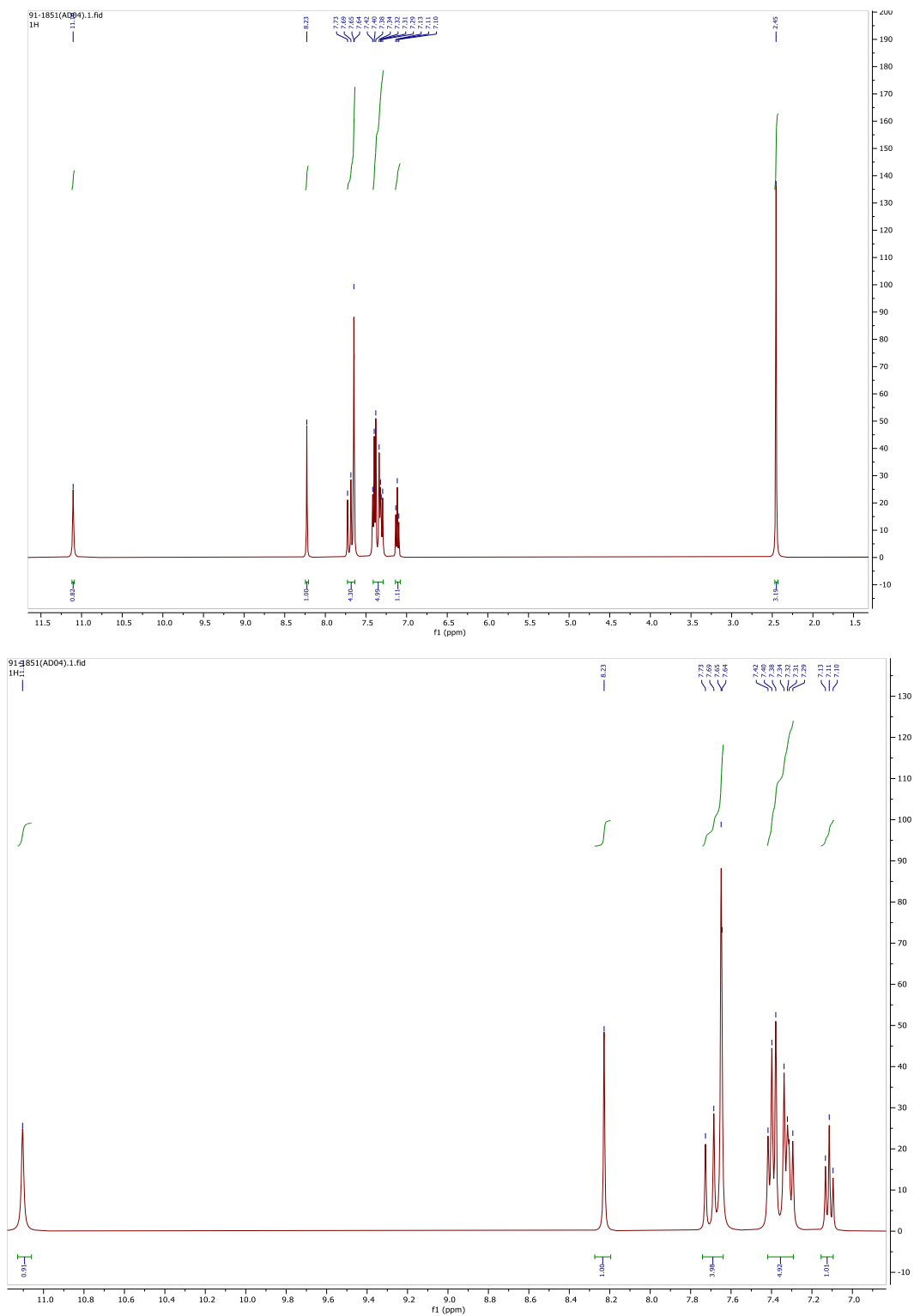
Appendix 58. The DEPT-135 (100 MHz, DMSO- d_6) spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (**216**)



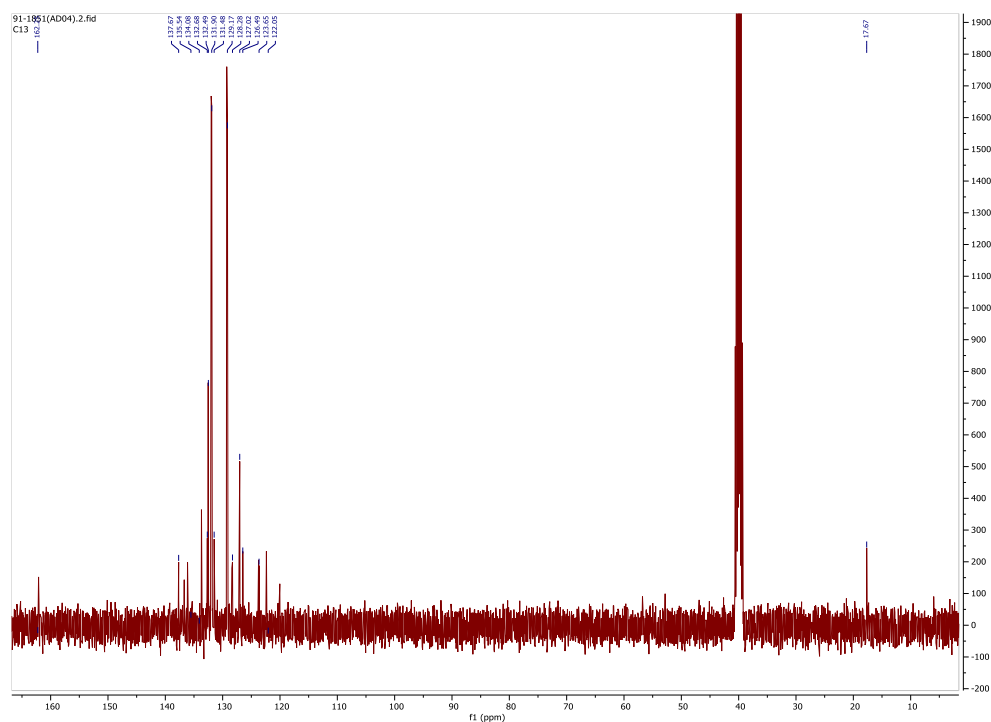
Appendix 59. The IR spectrum of (Z)-2-chloro-8-methyl-3-styrylquinoline (**216**)



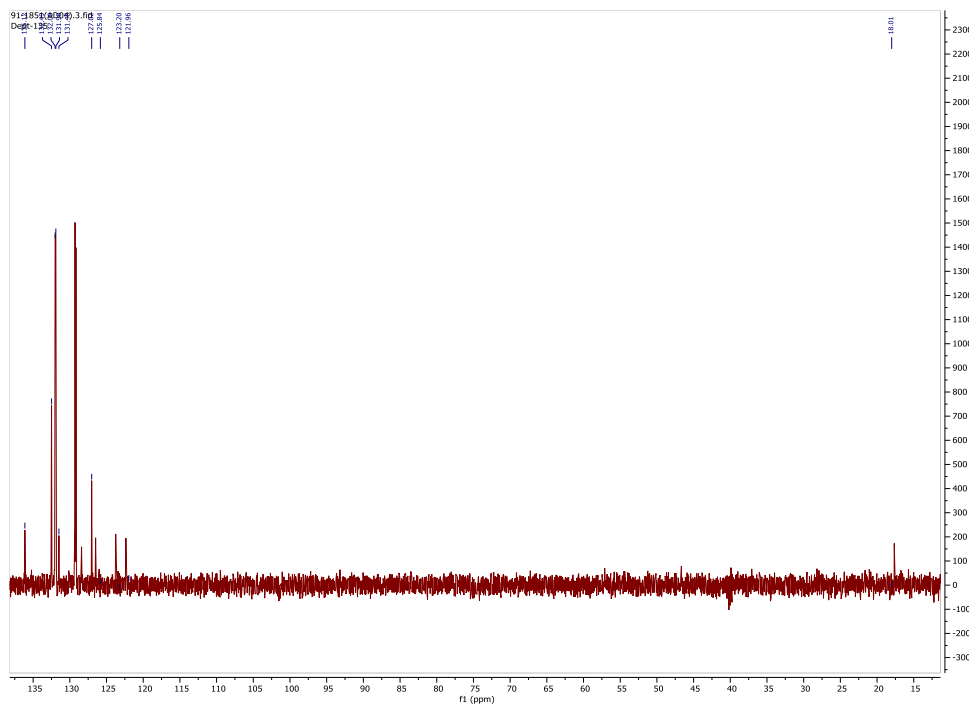
Appendix 60. ^1H NMR (400 MHz, DMSO- d_6) spectrum of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)



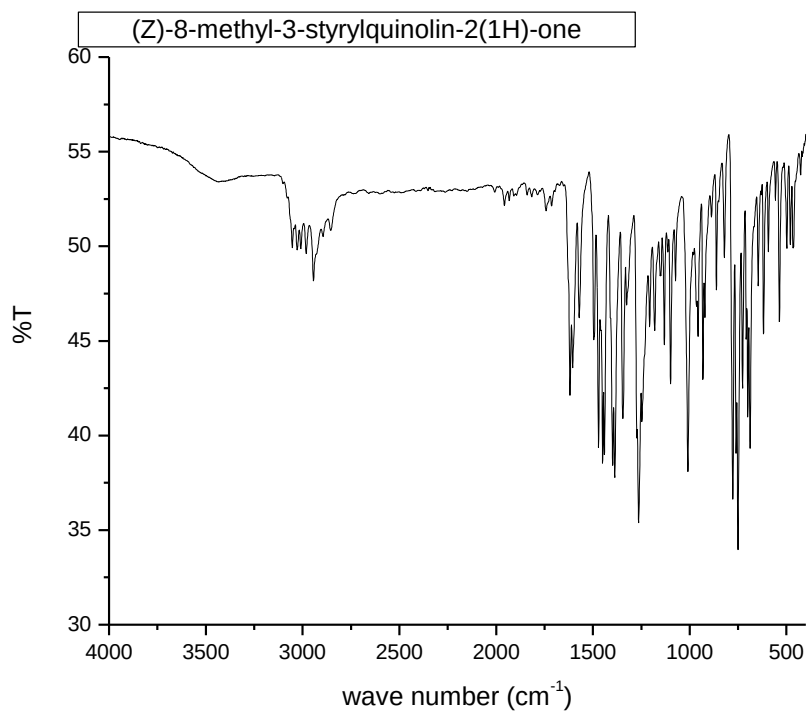
Appendix 61. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)



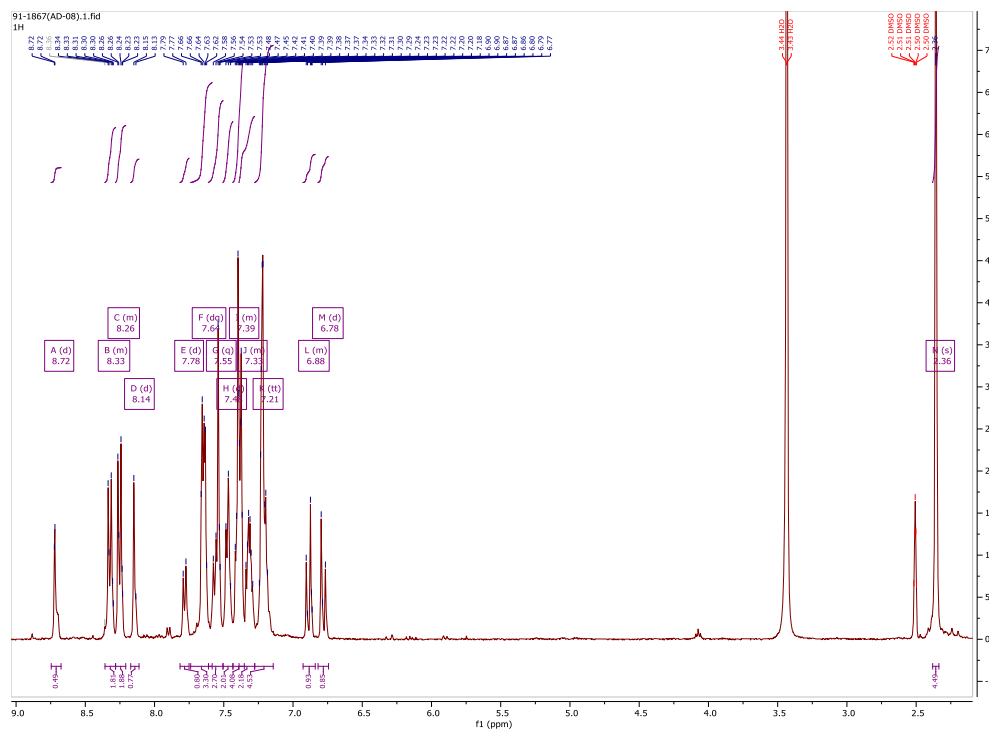
Appendix 62. The DEPT-135 (100 MHz, DMSO-d_6) spectrum of (Z)-8-methyl-3-styrylquinolin-2(1H)-one (217)

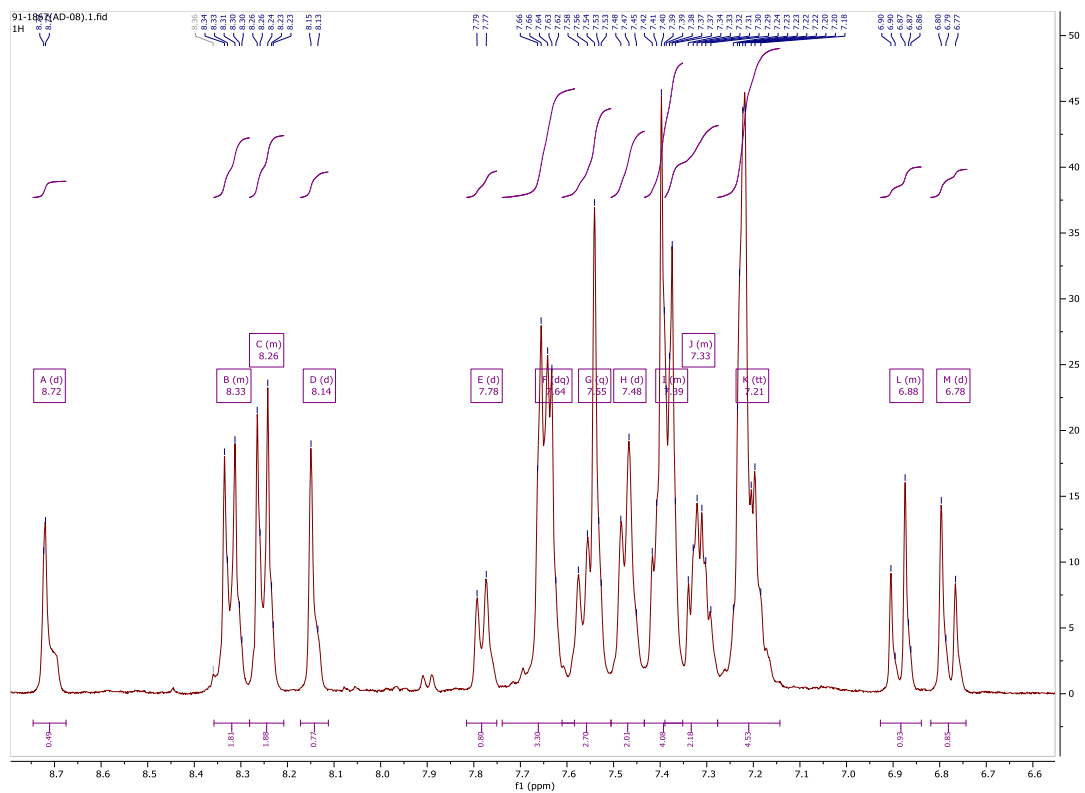


Appendix 63. The IR spectrum (Z)-8-methyl-3-styrylquinolin-2(1H)-one (**217**)

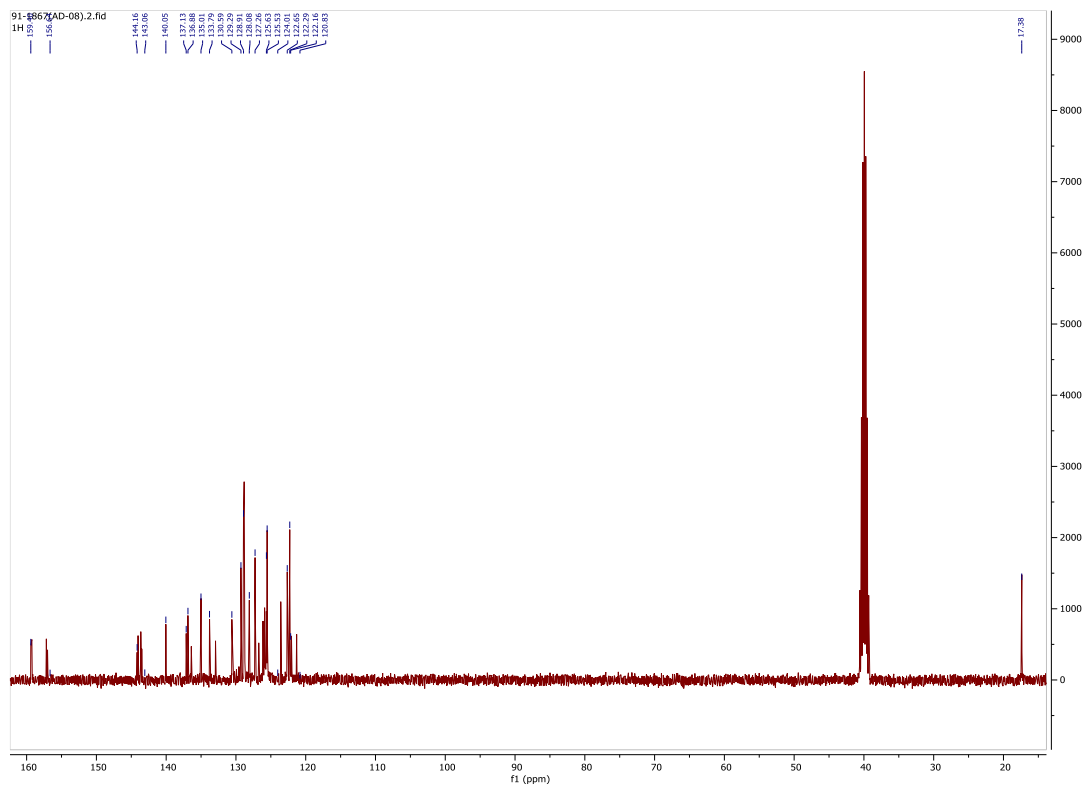


Appendix 64. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (**219**)

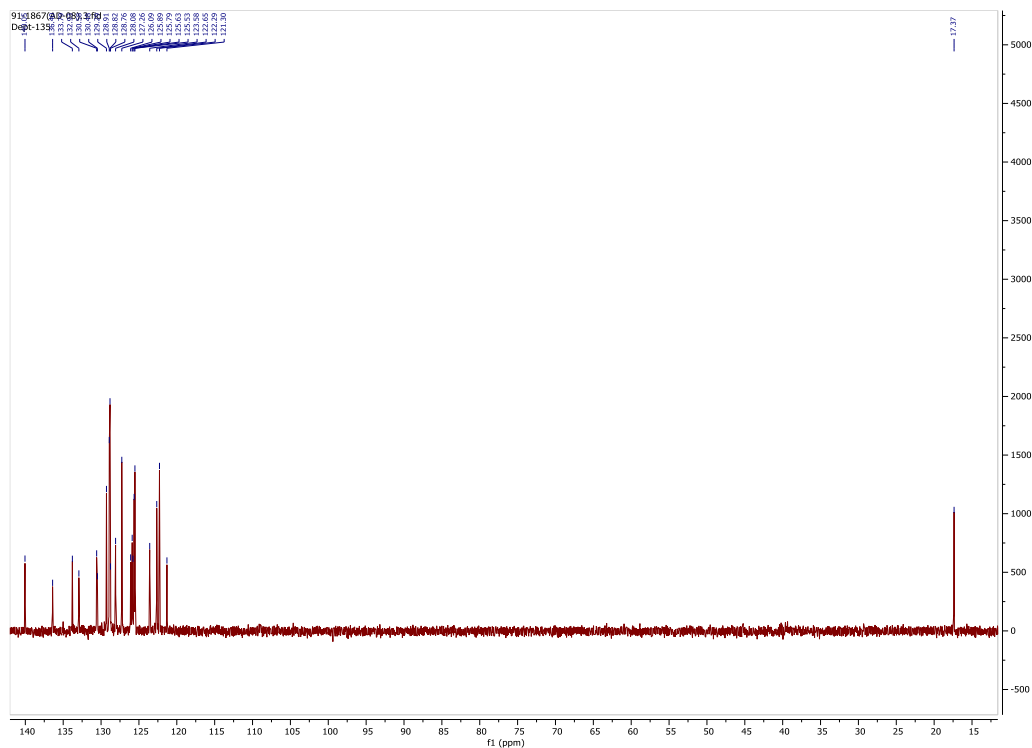




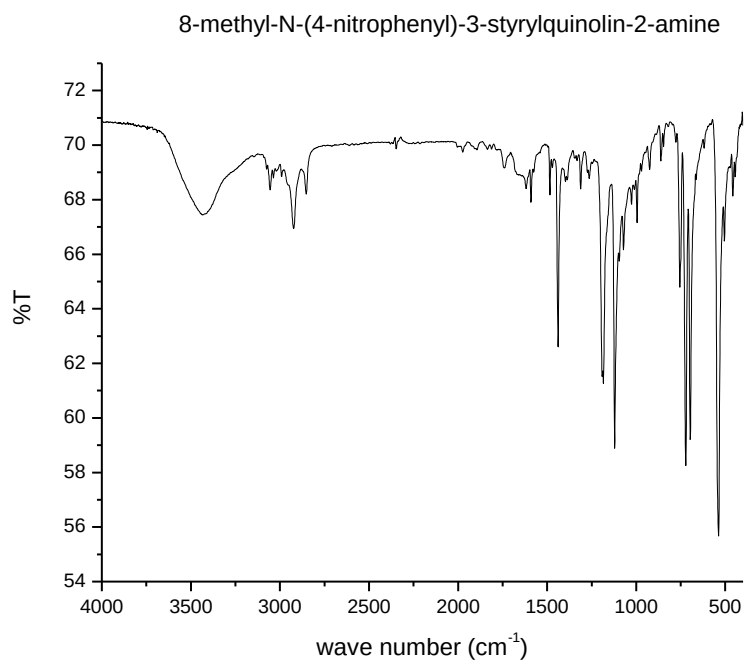
Appendix 65. ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (**219**)



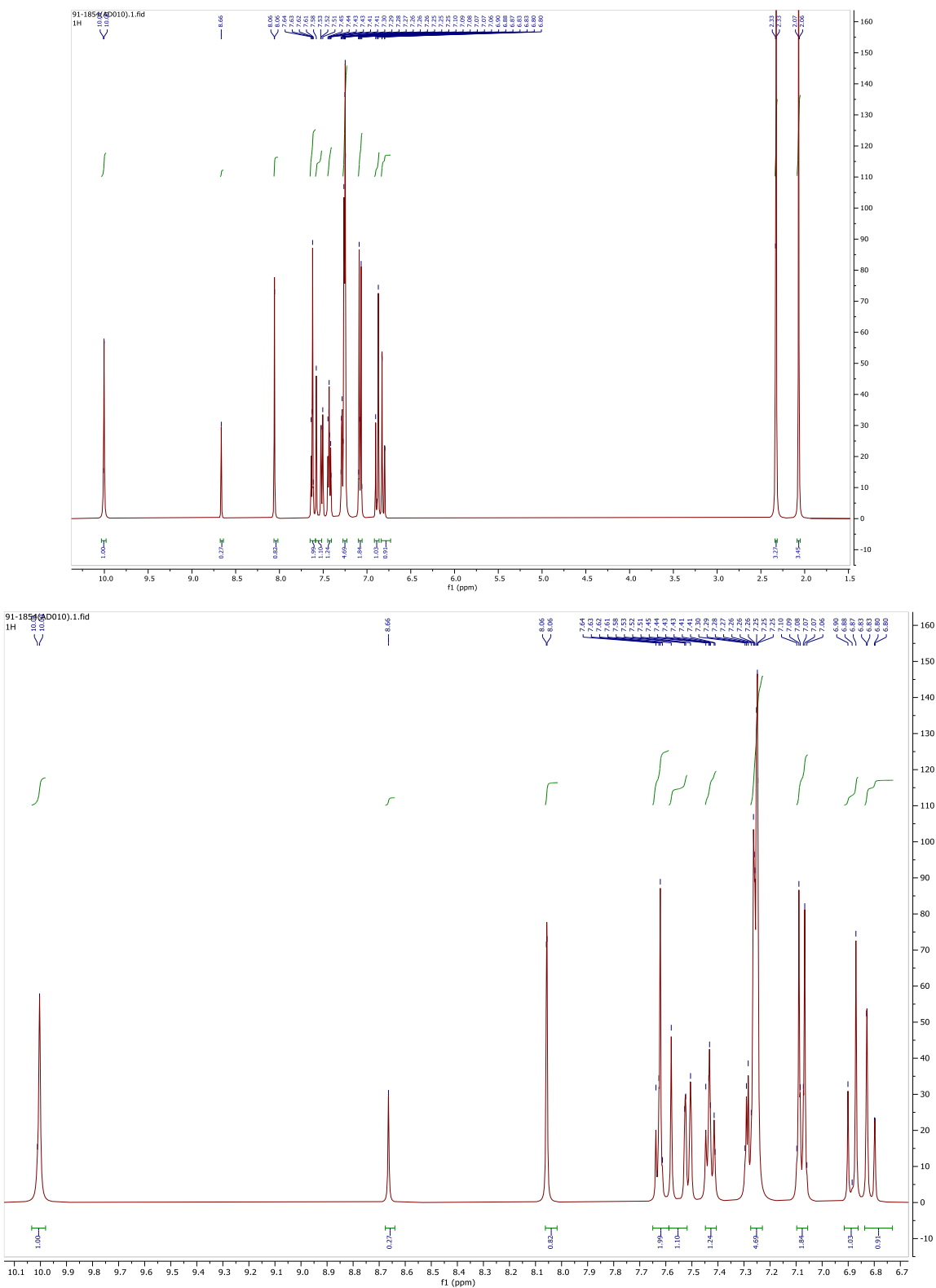
Appendix 66. The DEPT-135 (100 MHz, DMSO-d₆) spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (**219**)



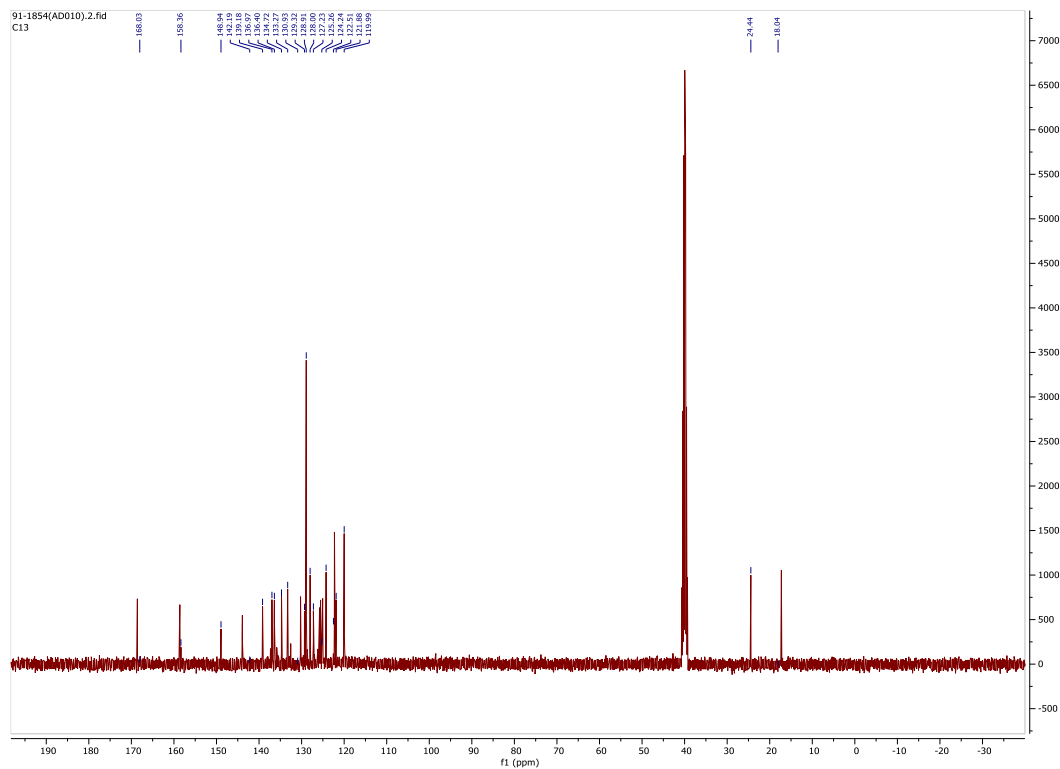
Appendix 67. The IR spectrum of 8-methyl-N-(4-nitrophenyl)-3-styrylquinolin-2-amine (**219**)



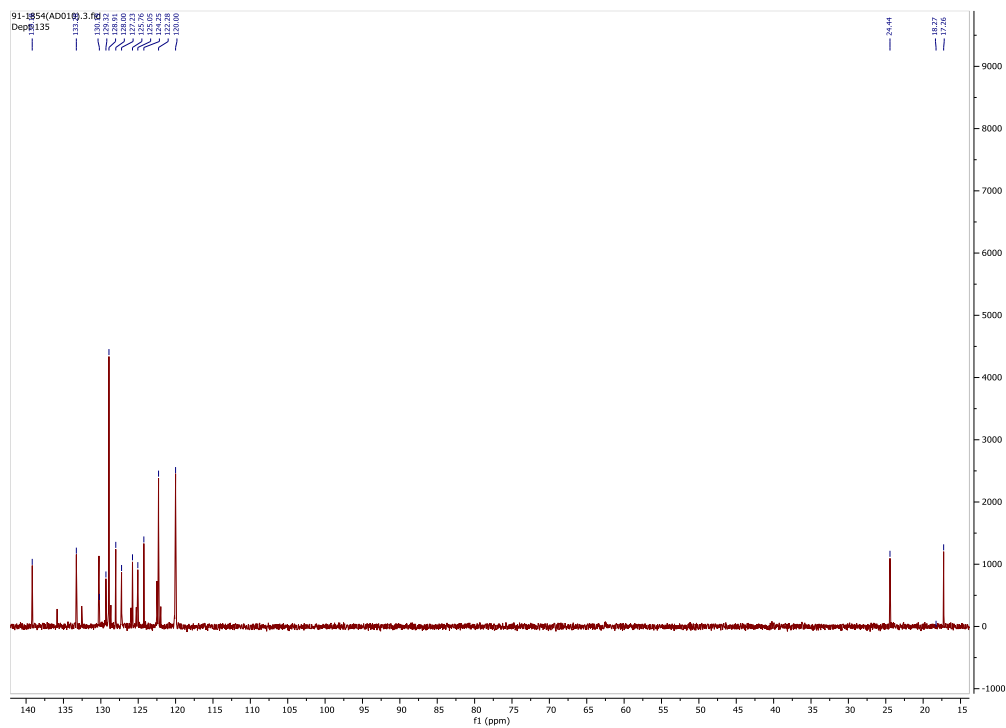
Appendix 68. ¹H NMR (400 MHz, DMSO-d₆) spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (**222**)

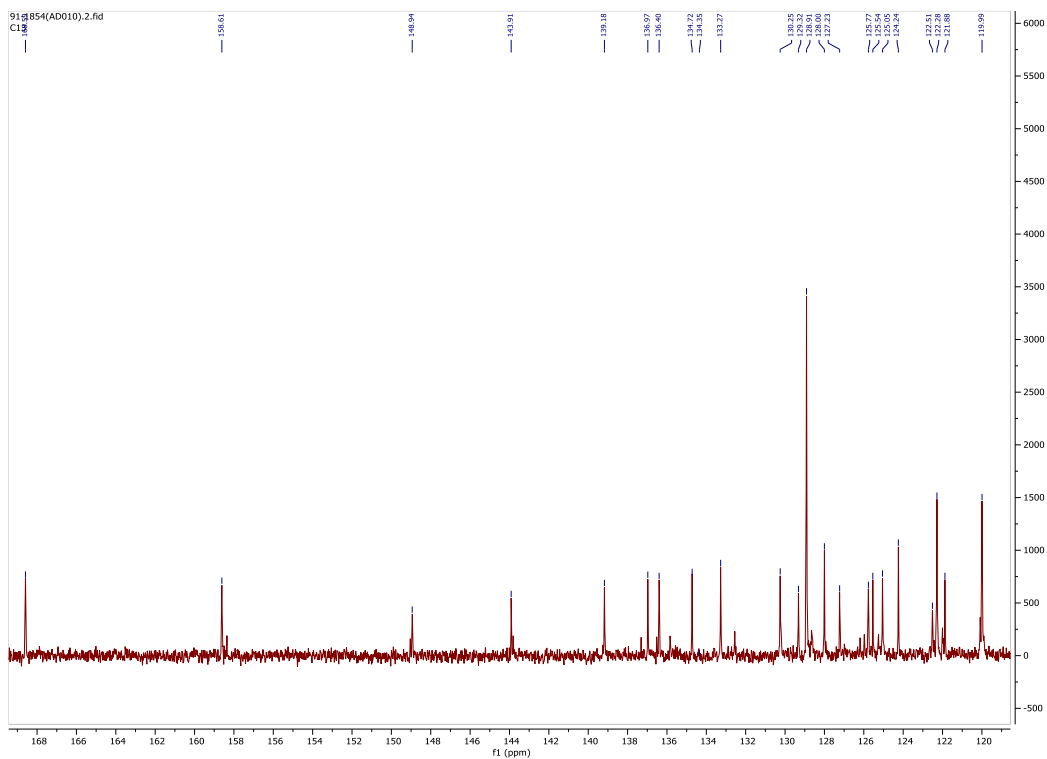


Appendix 69. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222)

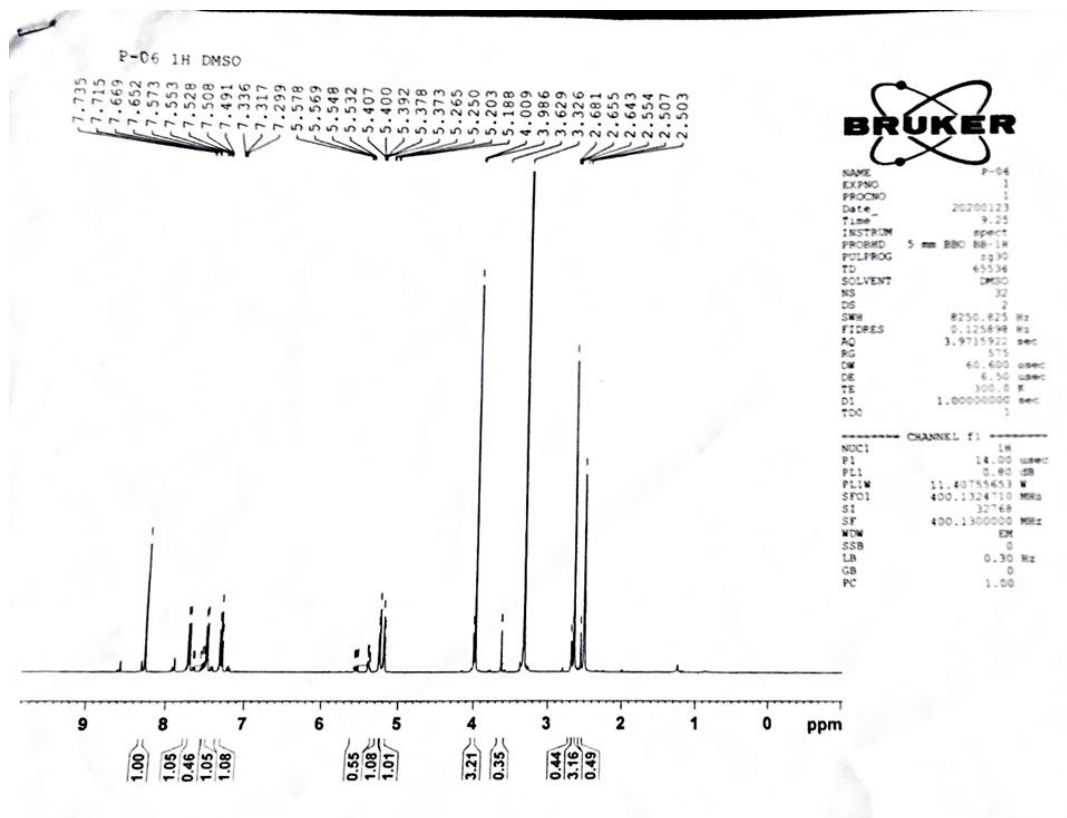


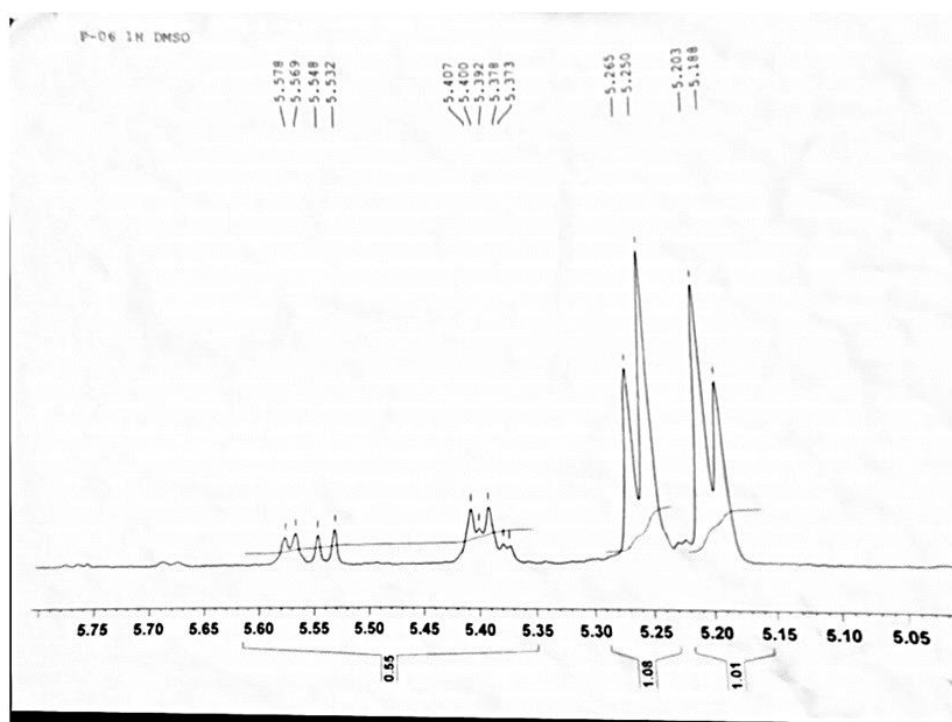
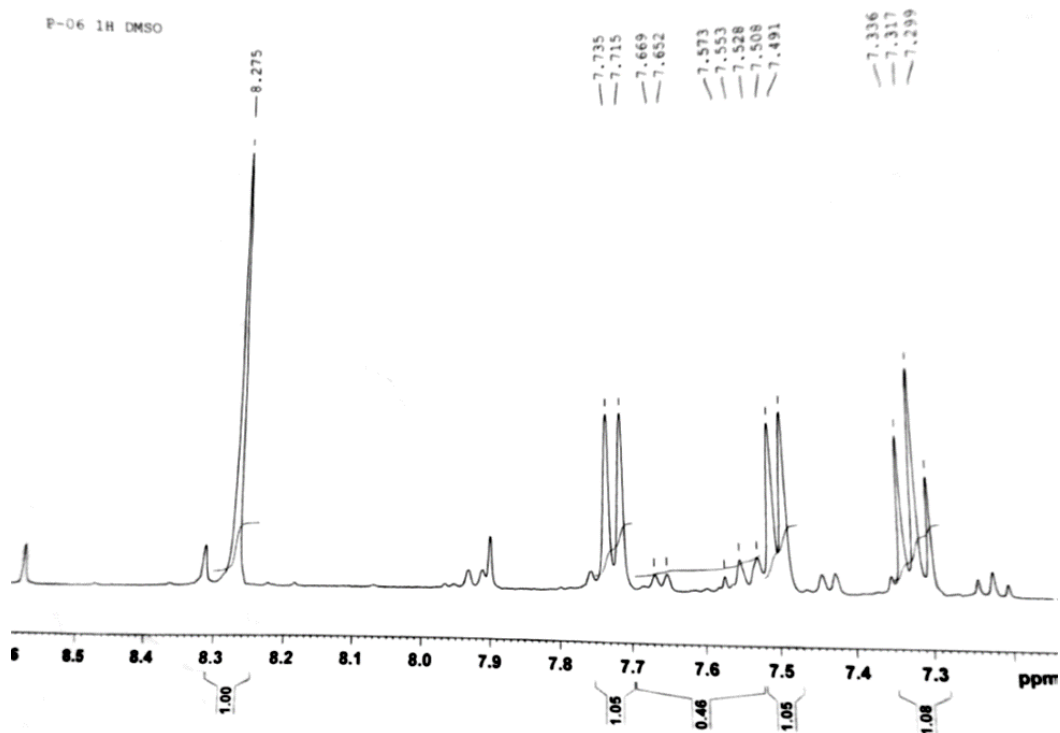
Appendix 70. The DEPT-135 (100 MHz, DMSO-d_6) spectrum of (Z)-N-(4-((8-methyl-3-styrylquinolin-2-yl)methyl)phenyl)acetamide (222)

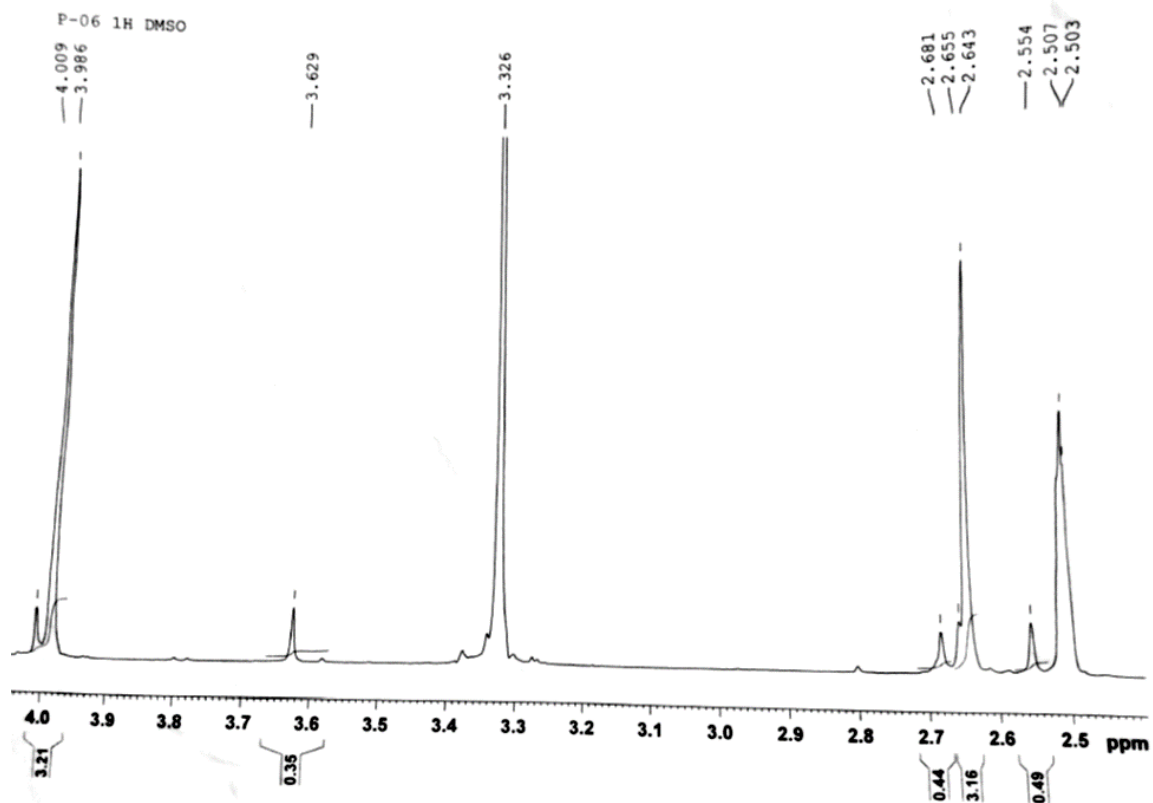




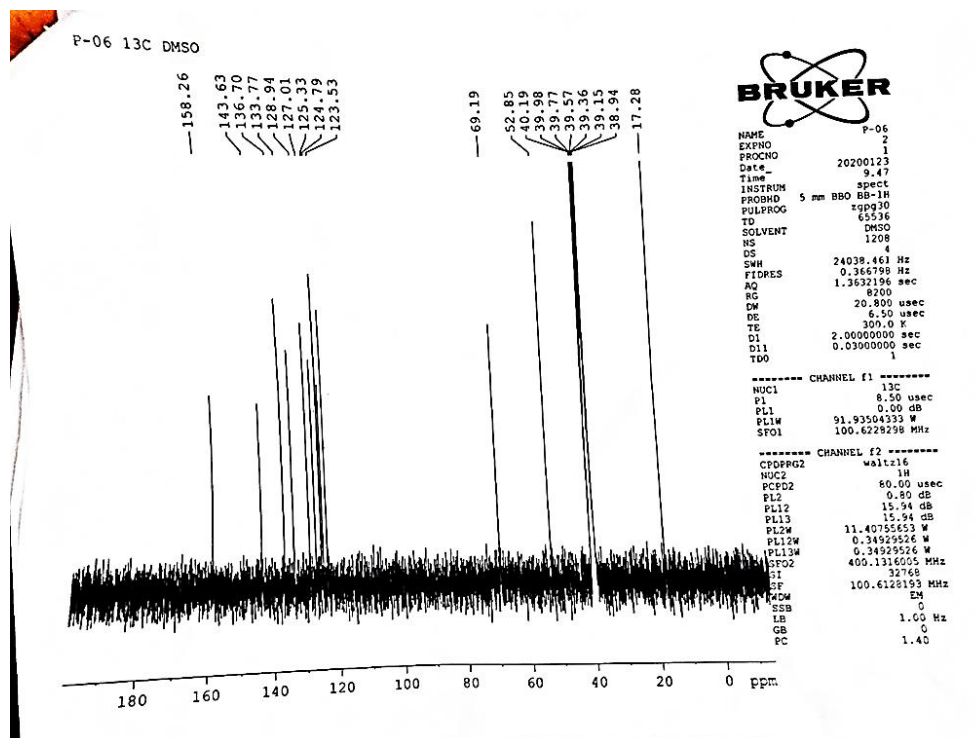
Appendix 72. ^1H NMR (400 MHz, DMSO-d_6) spectrum of Methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (**224**)



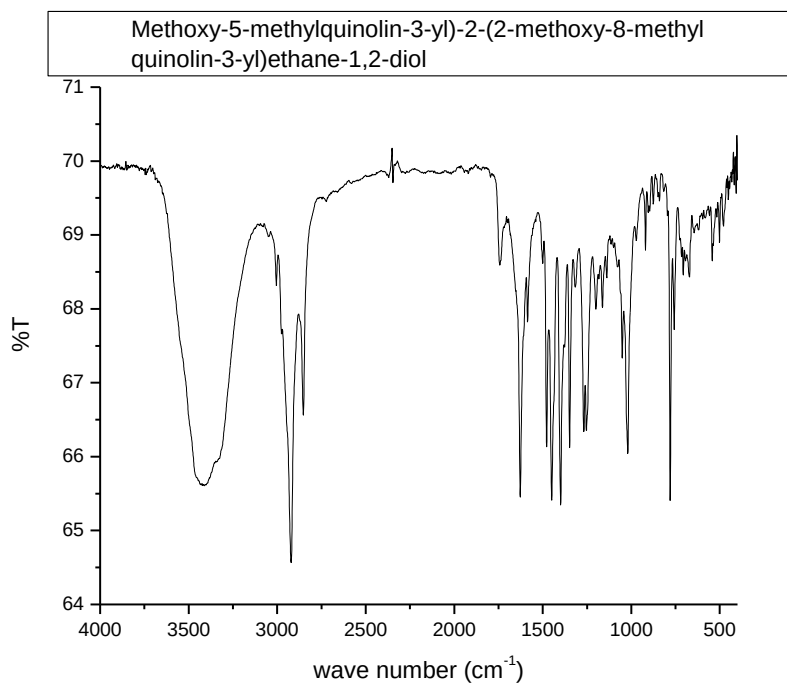




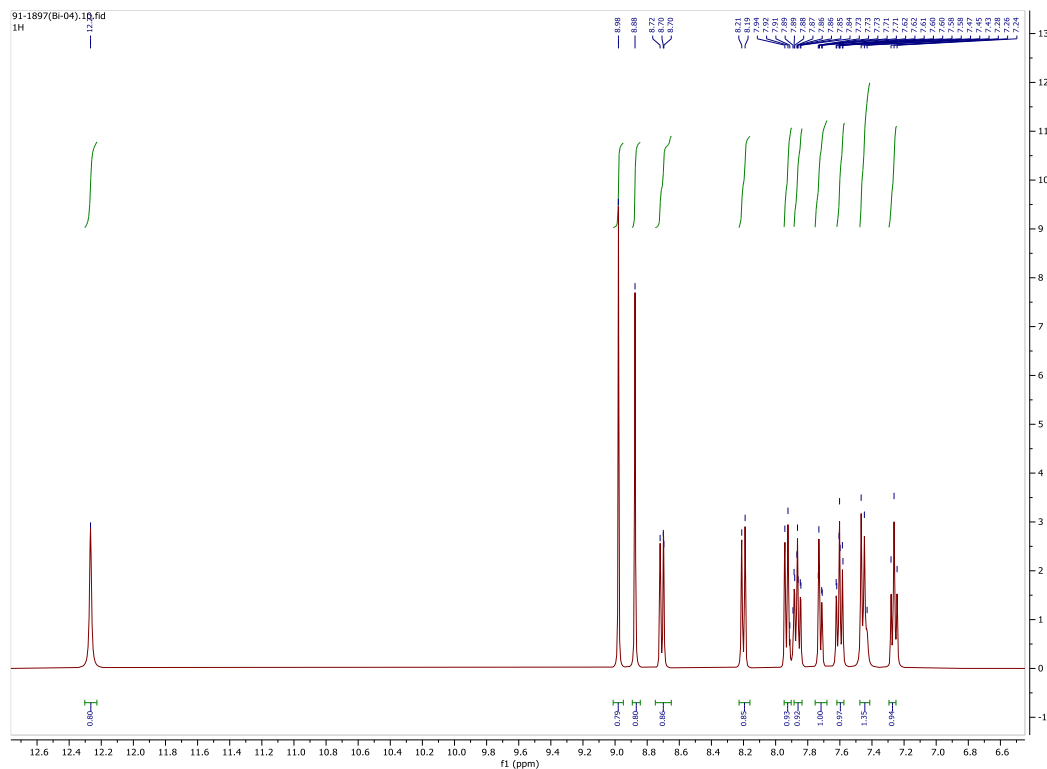
Appendix 73. ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of Methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224)



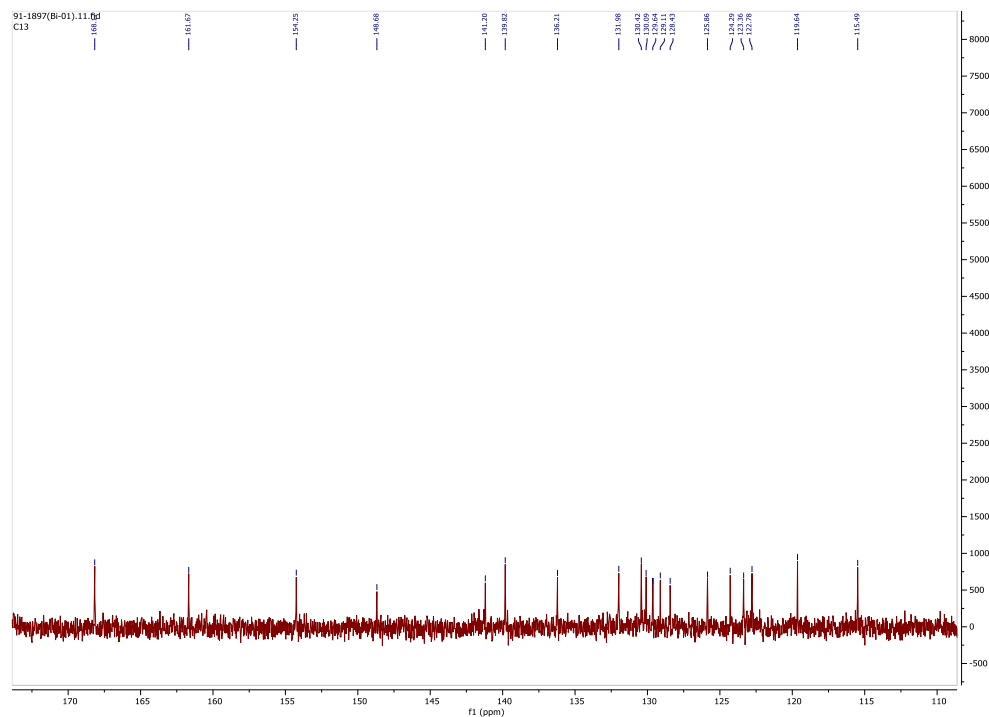
Appendix 74. The IR spectrum of Methoxy-5-methylquinolin-3-yl)-2-(2-methoxy-8-methylquinolin-3-yl)ethane-1,2-diol (224)



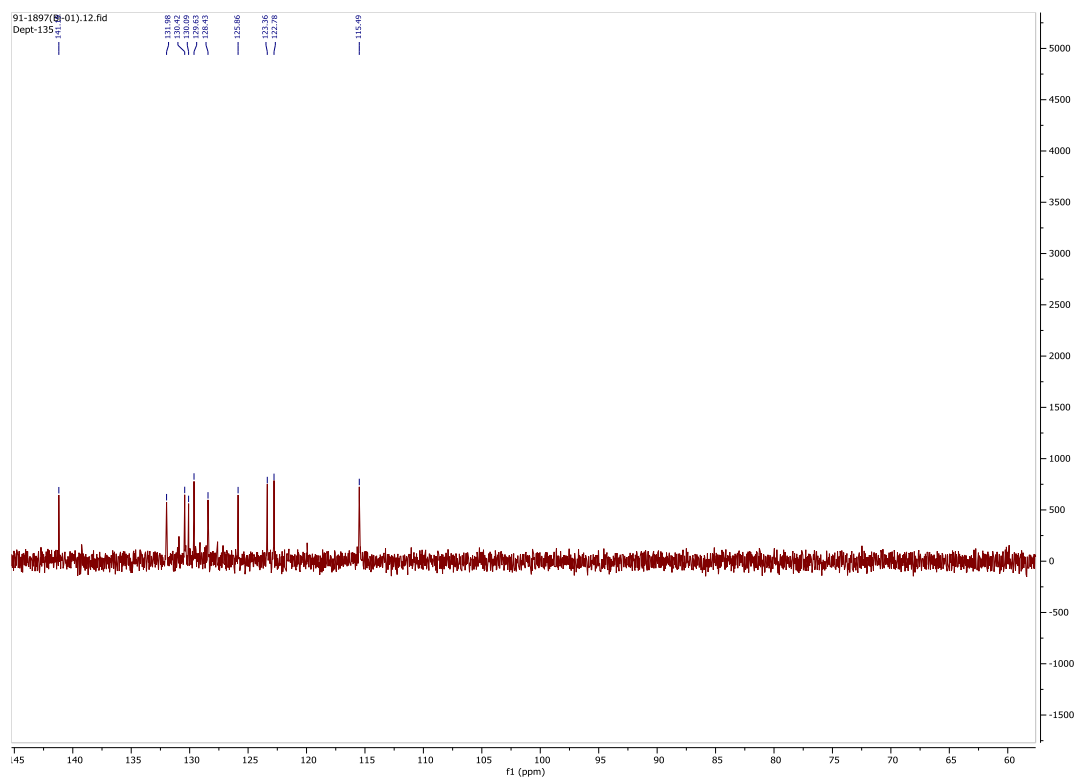
Appendix 75. The ^1H NMR (400 MHz, DMSO- d_6) spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (**225**)



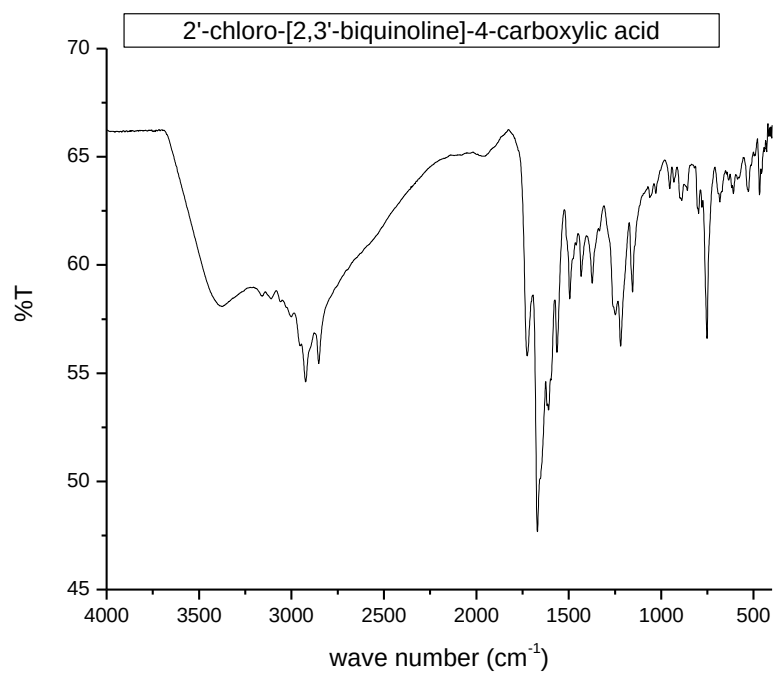
Appendix 76. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (**225**)



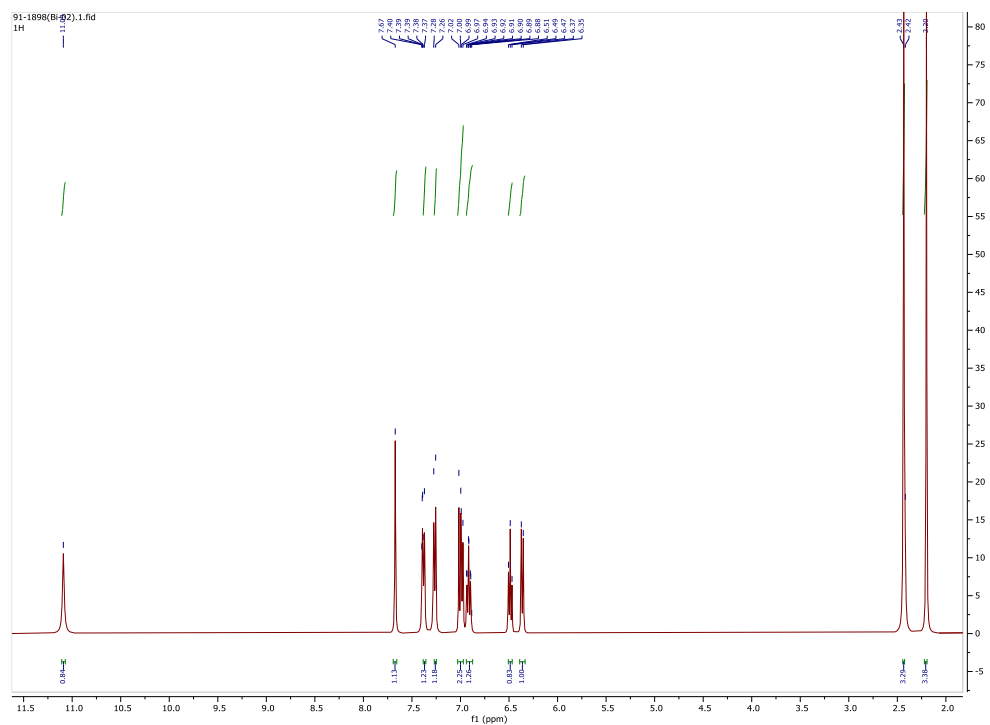
Appendix 77. The DEPT (400 MHz, DMSO-d₆) spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225)



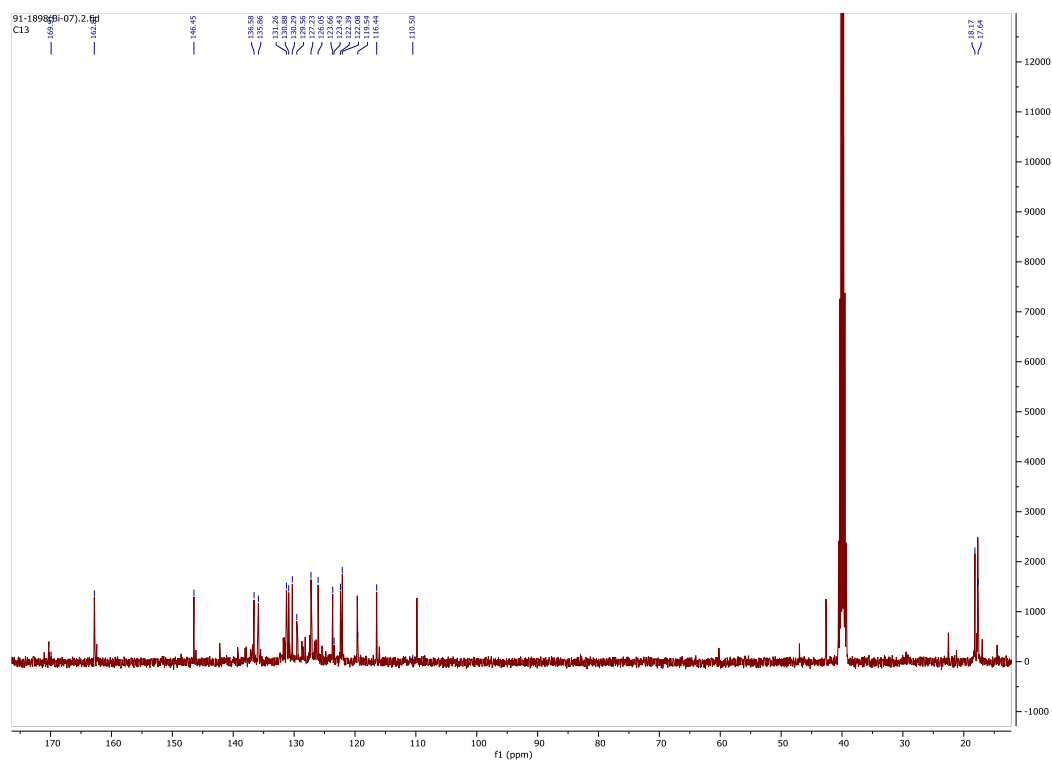
Appendix 78. The IR spectrum of 2'-chloro-[2,3'-biquinoline]-4-carboxylic acid (225)



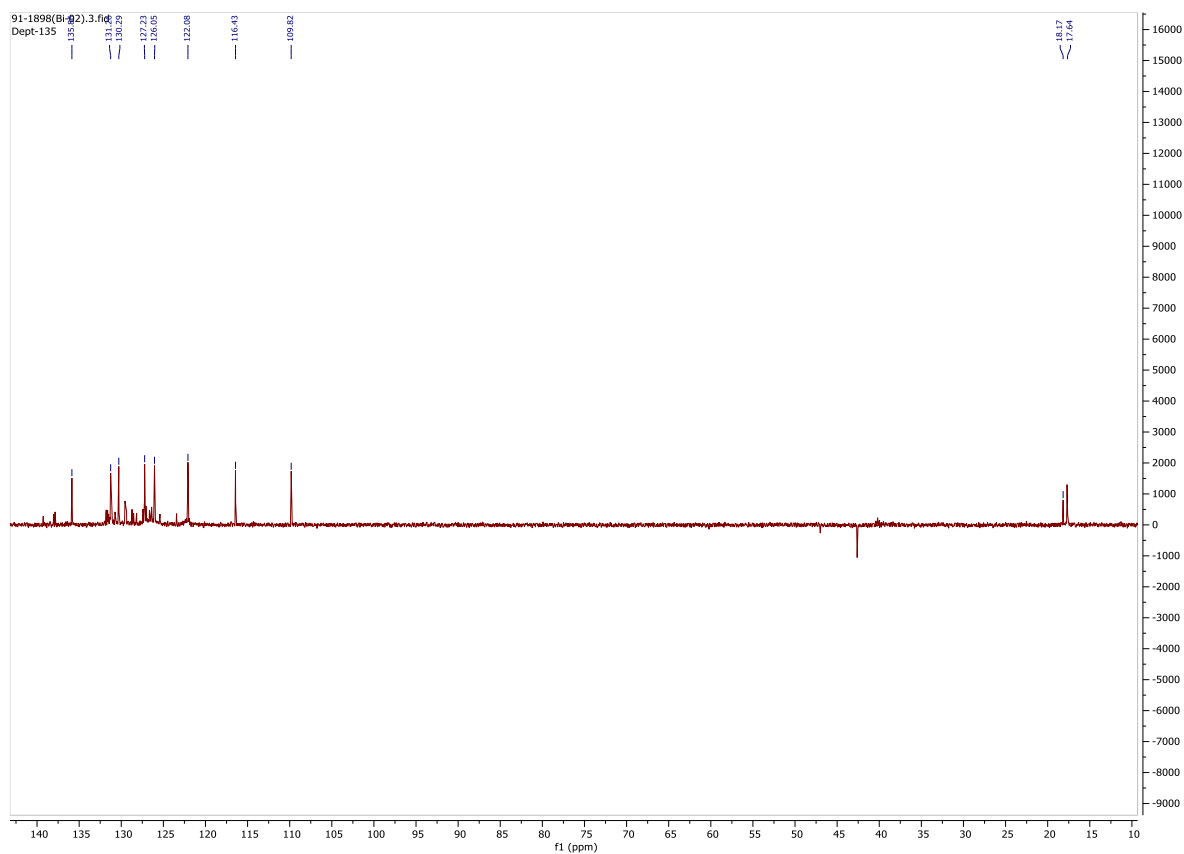
Appendix 79. The ¹H NMR (400 MHz, DMSO-d₆) spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**226**)



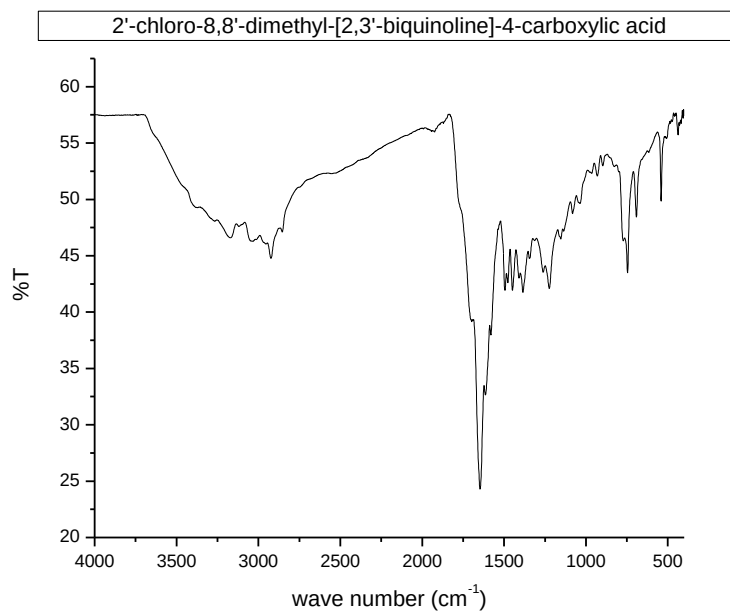
Appendix 80. The ¹³C NMR (100 MHz, DMSO-d₆) spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**226**)



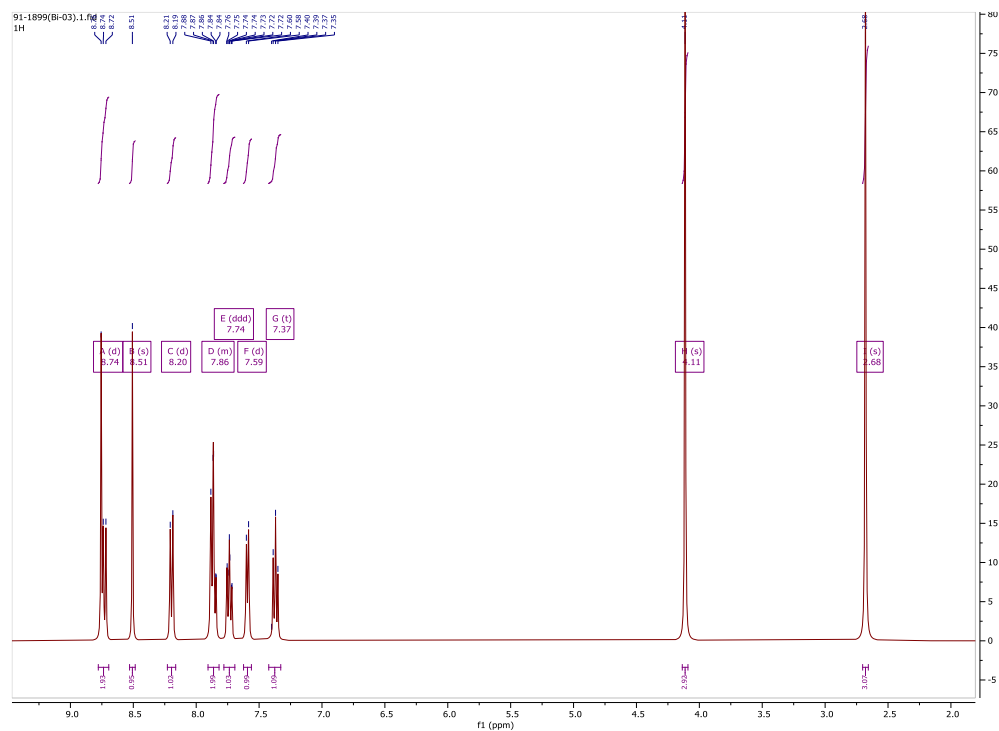
Appendix 81. The DEPT (100 MHz, DMSO-d₆) spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**226**)



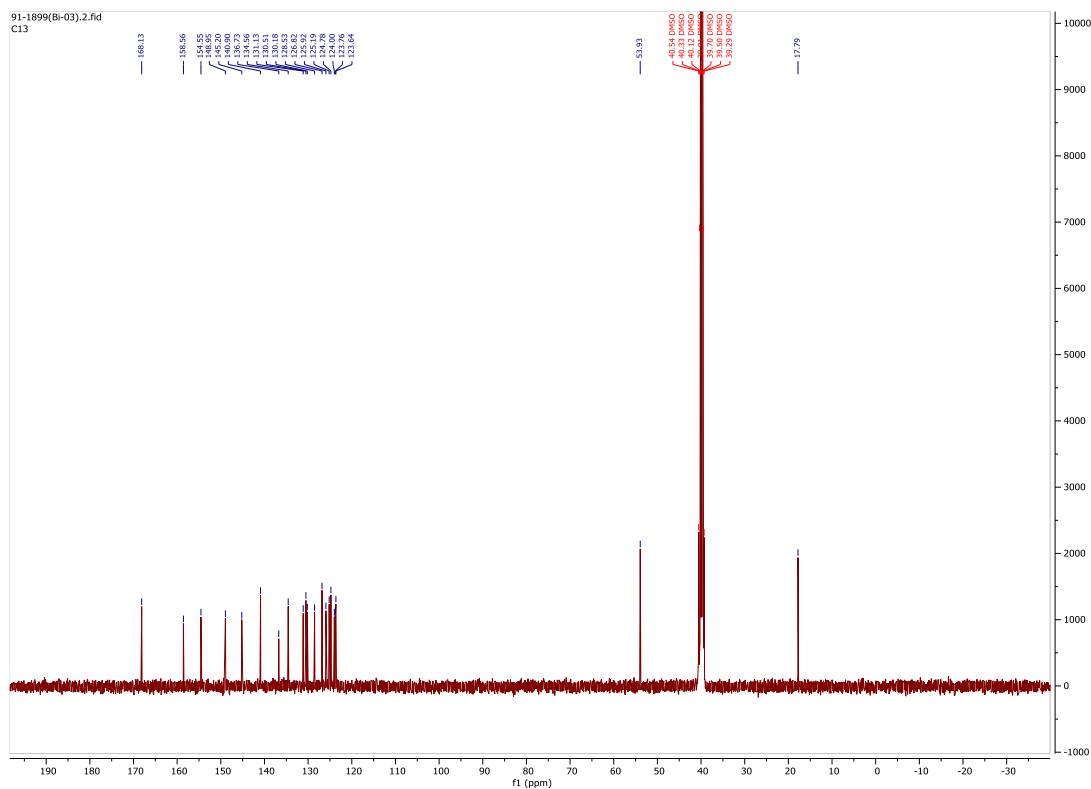
Appendix 82. The IR spectrum of 2'-chloro-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**226**)



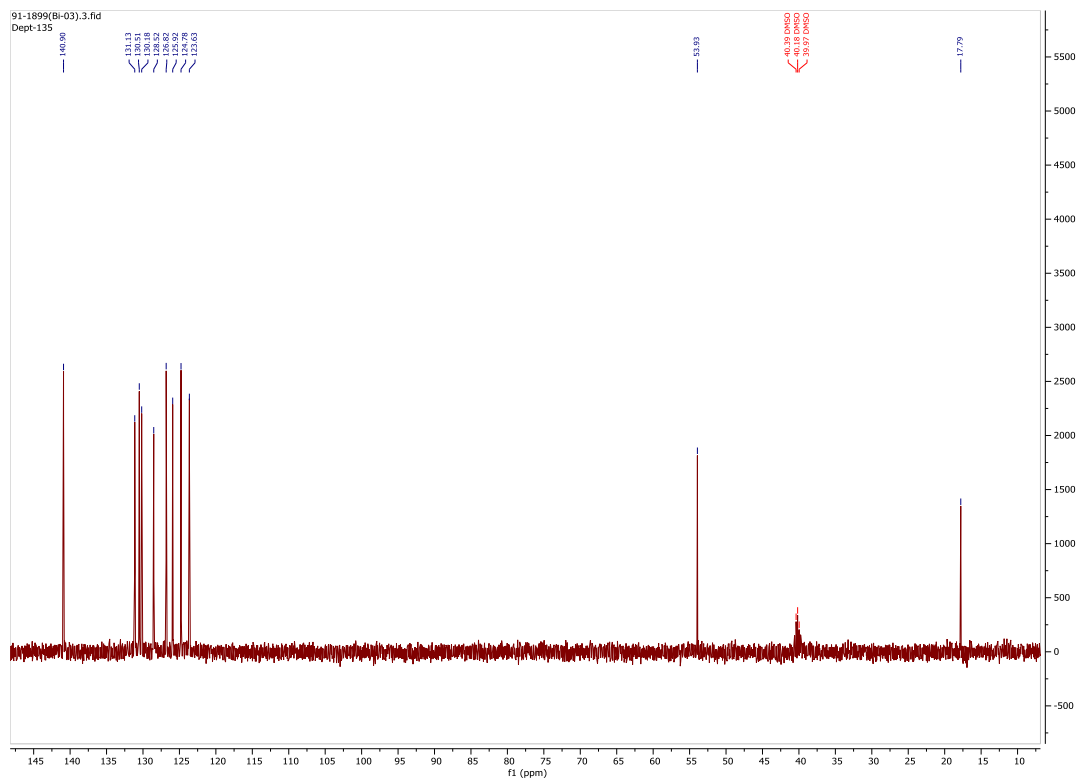
Appendix 83. The ^1H NMR (400 MHz, DMSO-d_6) spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (**227**)



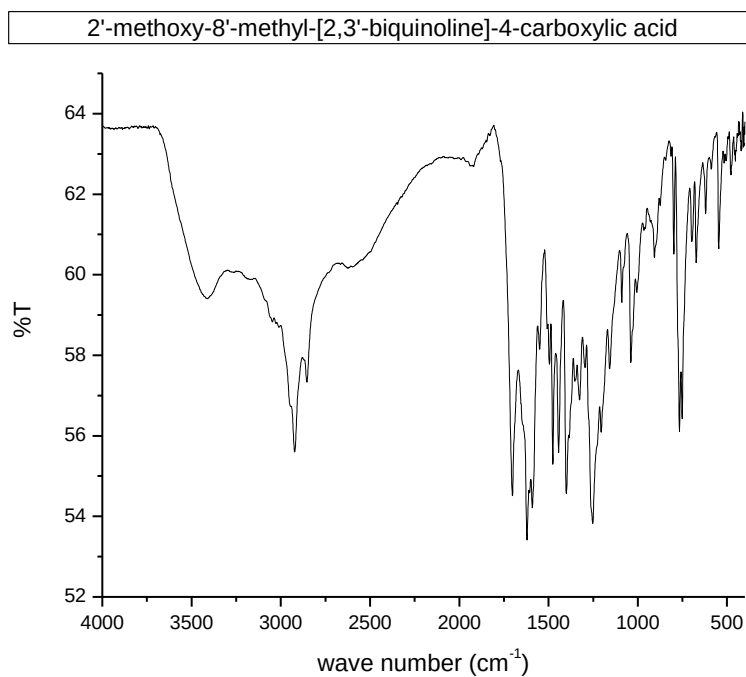
Appendix 84. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (**227**)



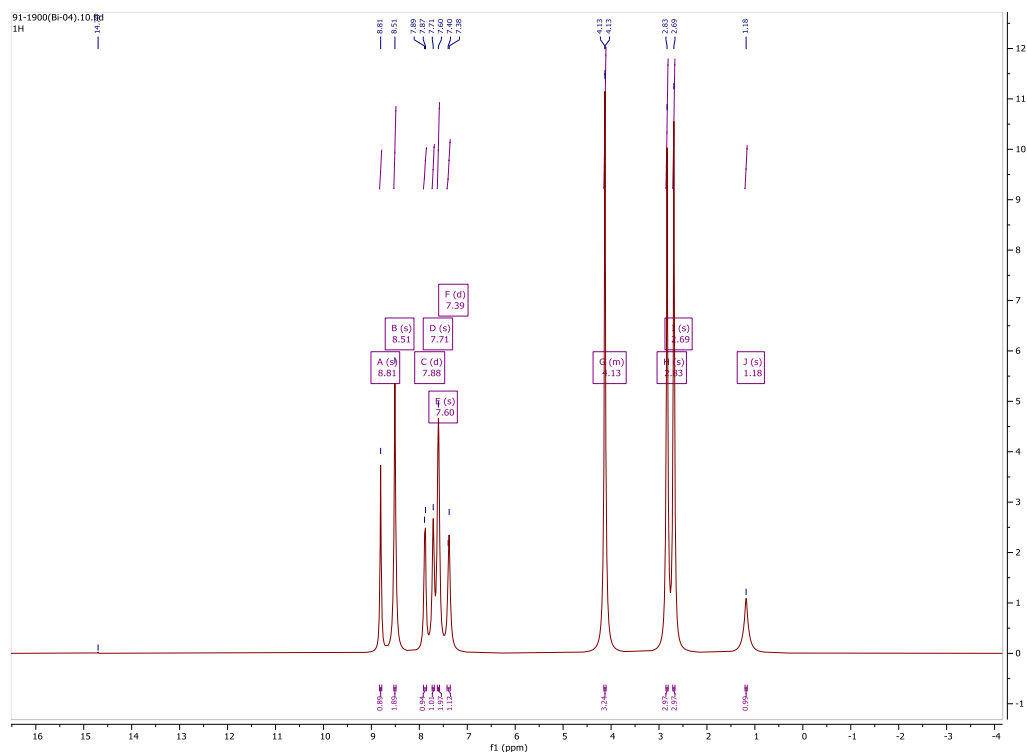
Appendix 85. The DEPT-135 (100 MHz, DMSO-d₆) spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (**227**)



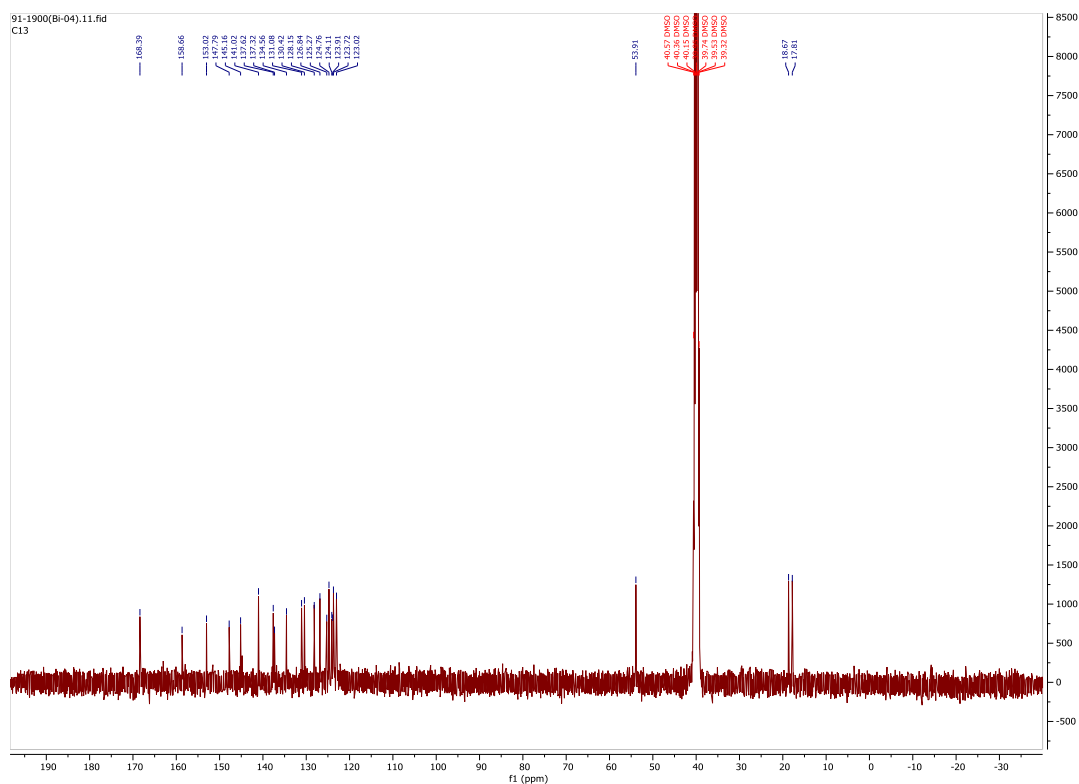
Appendix 86. The IR spectrum of 2'-methoxy-8'-methyl-[2,3'-biquinoline]-4-carboxylic acid (**227**)



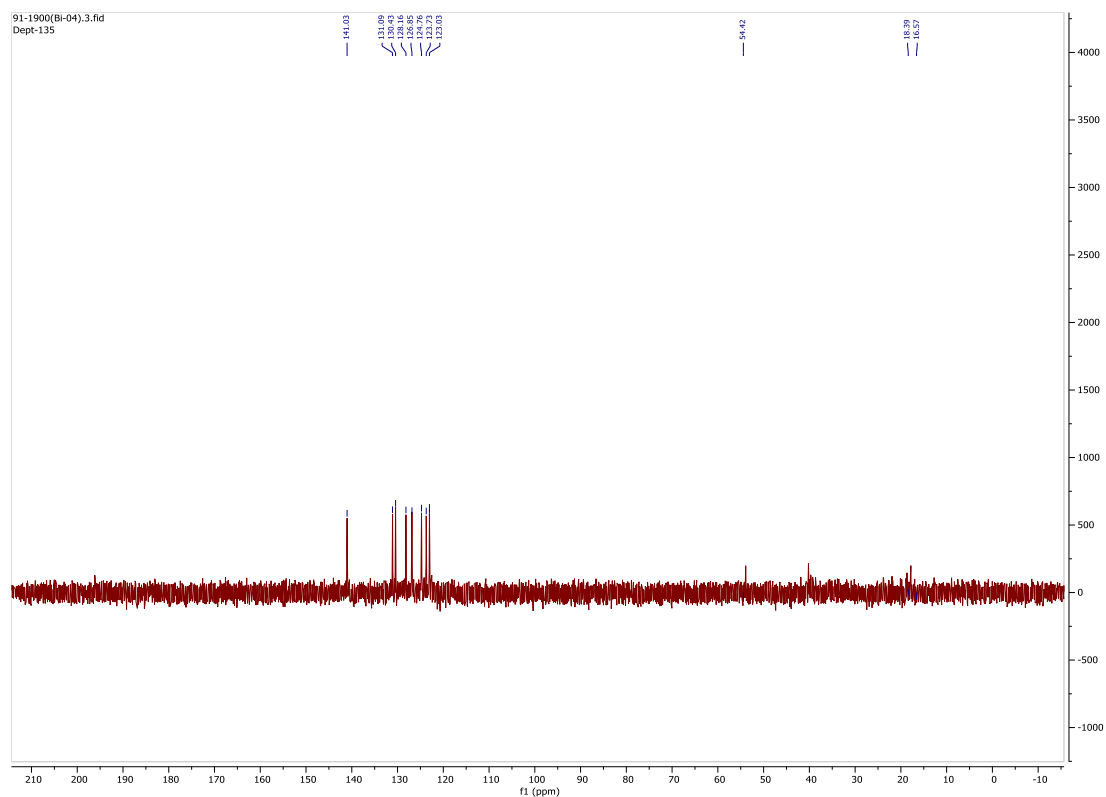
Appendix 87. The ^1H NMR(400 MHz, DMSO-d_6) of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**228**)



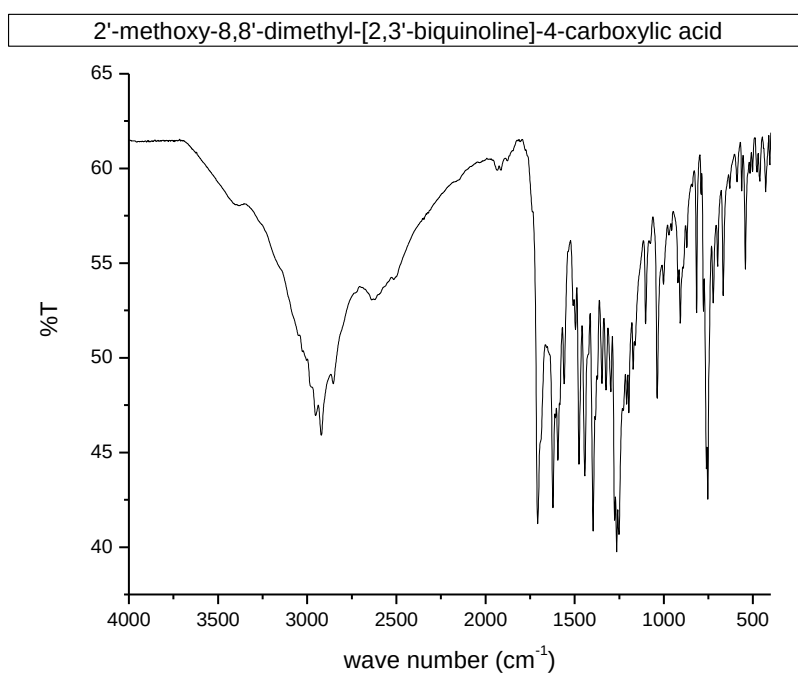
Appendix 88. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**228**)



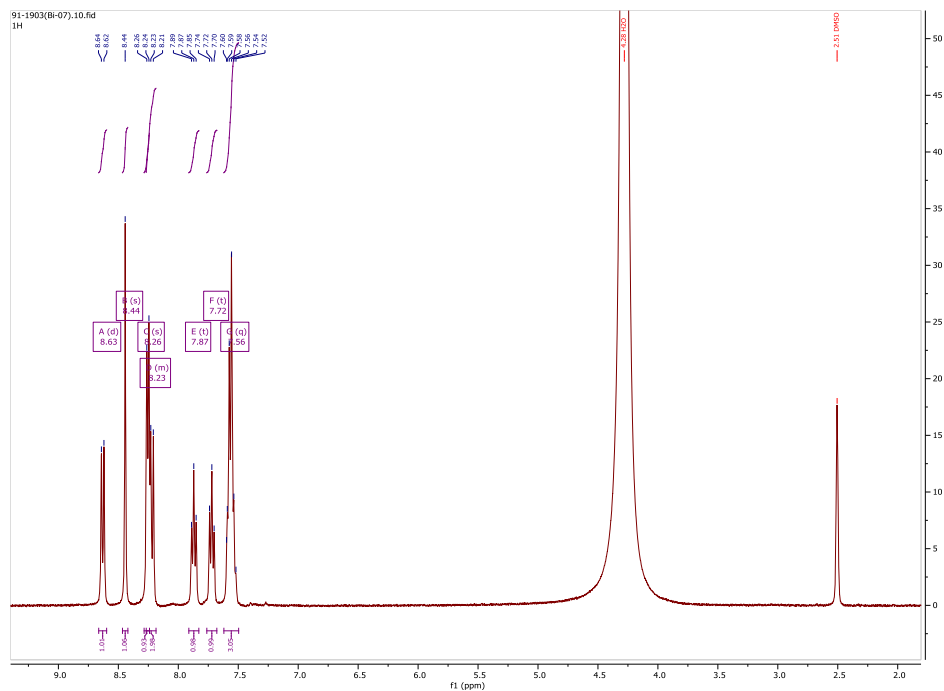
Appendix 89. The DEPT-135 (100 MHz, DMSO-d₆) spectrum of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**228**)



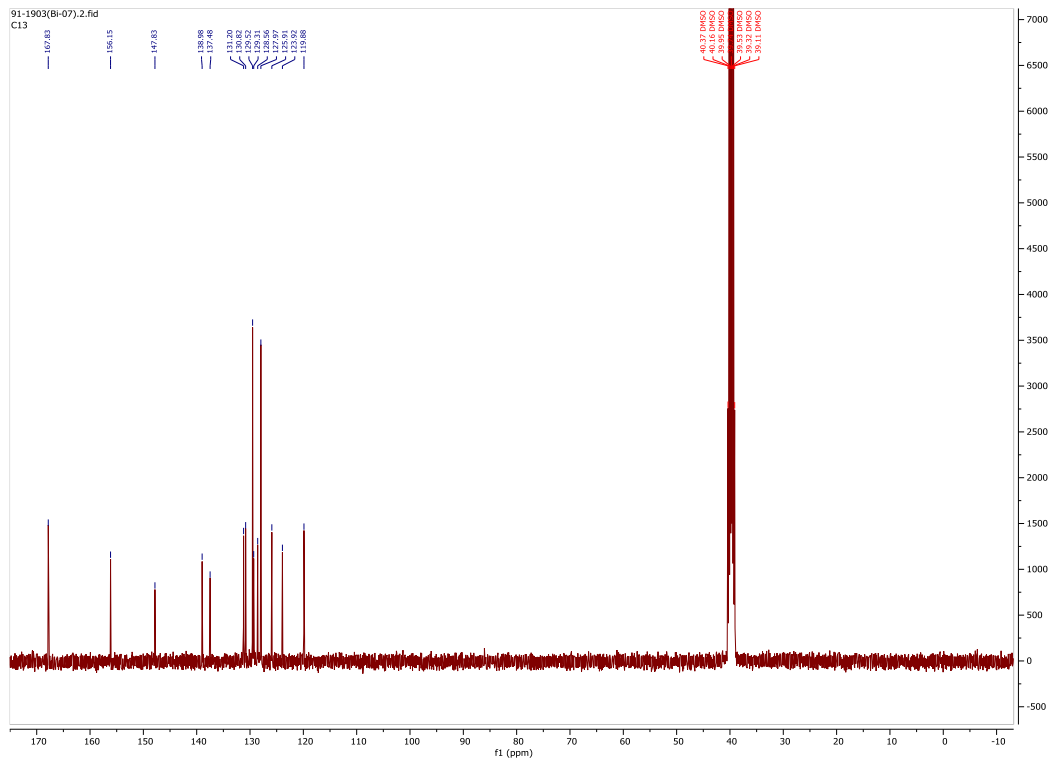
Appendix 90. The IR spectrum of 2'-methoxy-8,8'-dimethyl-[2,3'-biquinoline]-4-carboxylic acid (**228**)



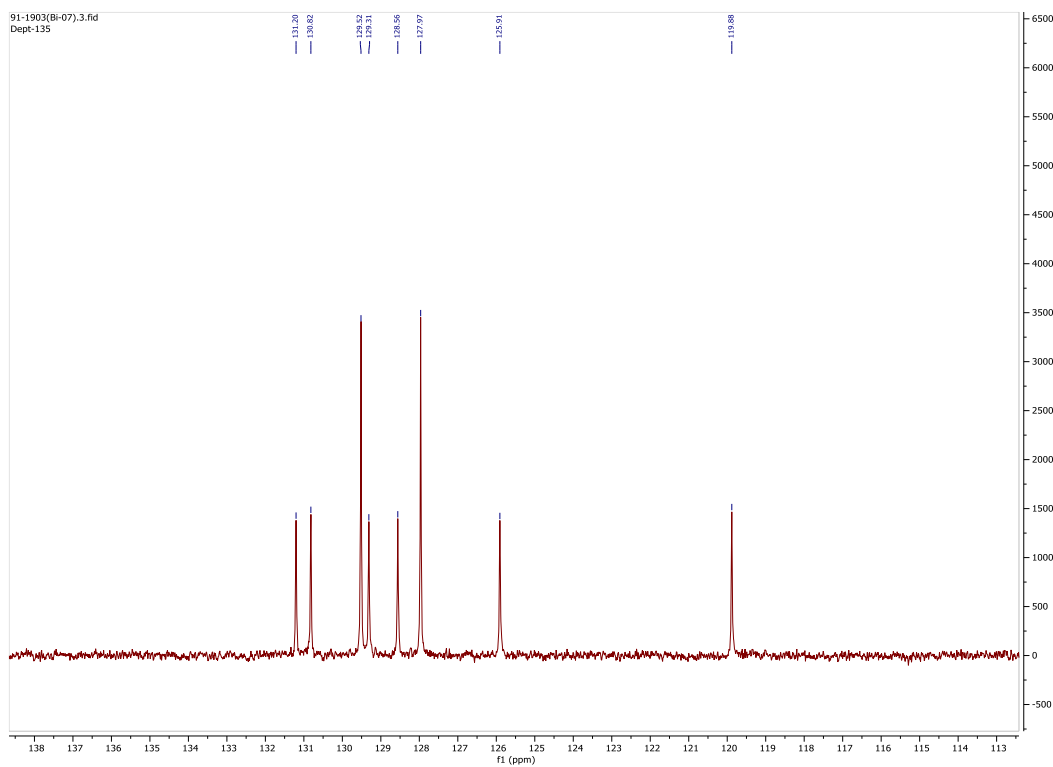
Appendix 91. The ^1H NMR spectrum (400 MHz, DMSO-d_6) of 2-phenylquinoline-4-carboxylic acid (62)



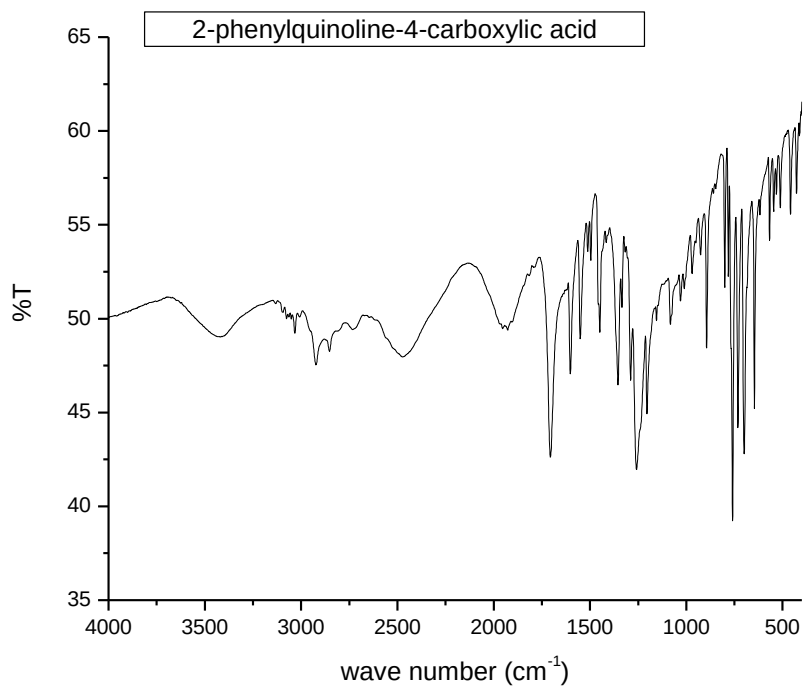
Appendix 92. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 2-phenylquinoline-4-carboxylic acid (62)



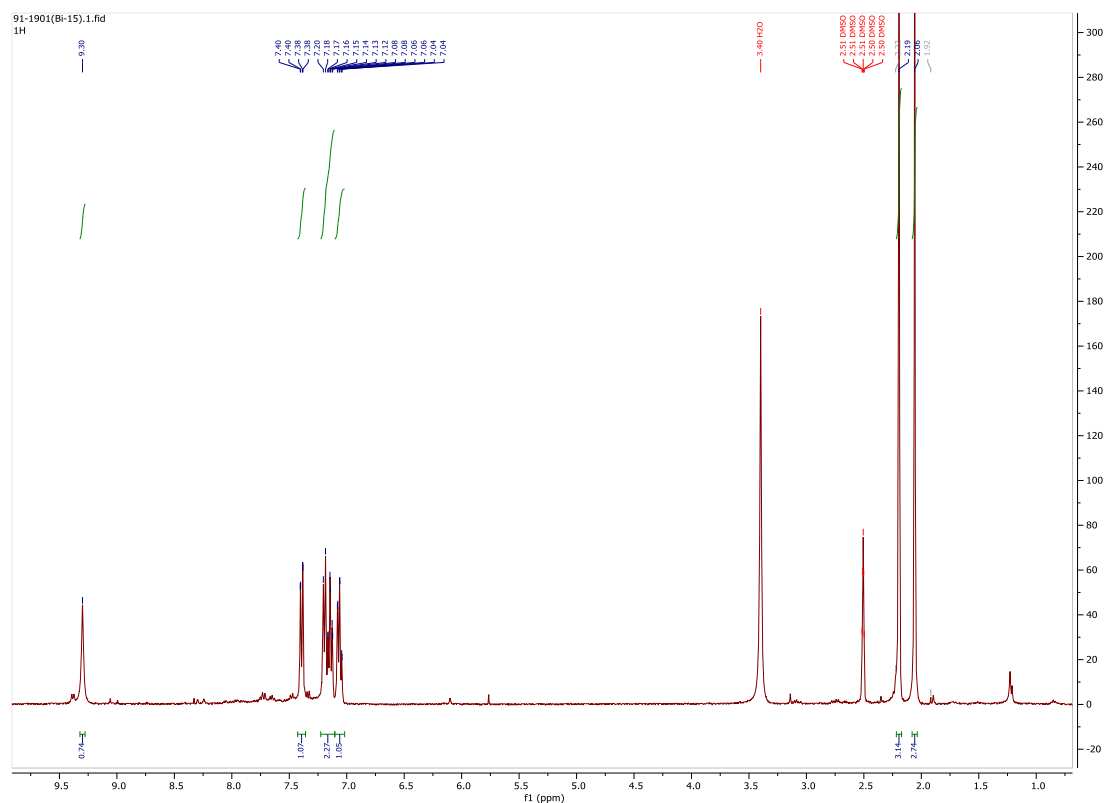
Appendix 93. The DEPT-135 (100 MHz, DMSO-d₆) spectrum of 2-phenylquinoline-4-carboxylic acid (62)



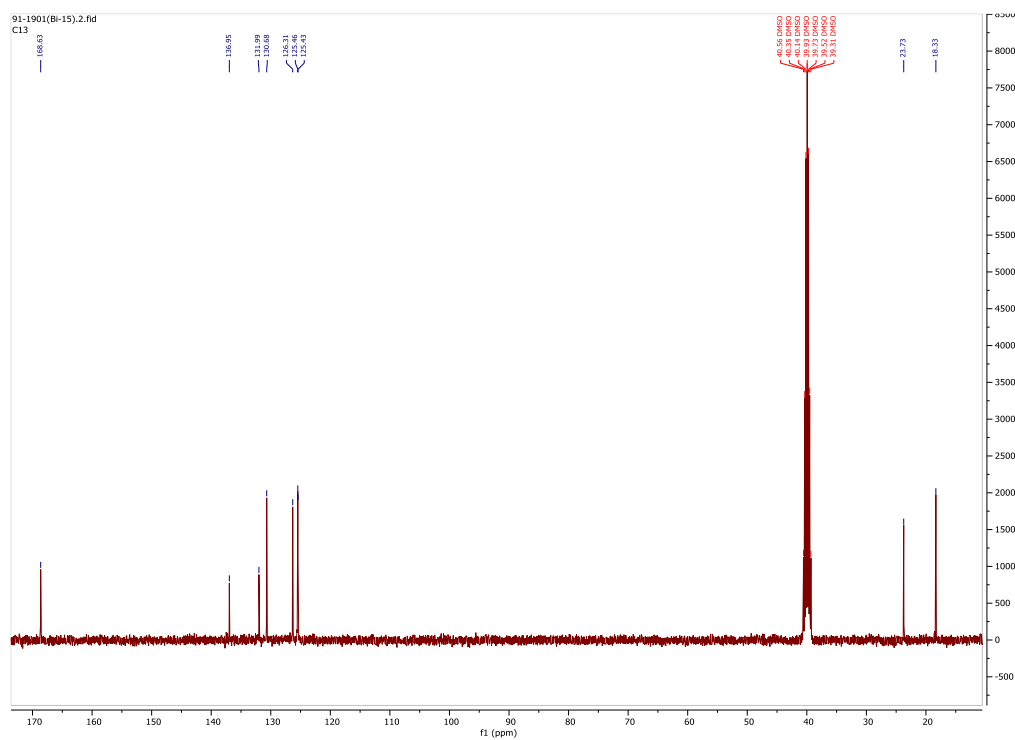
Appendix 94. The IR spectrum of 2-phenylquinoline-4-carboxylic acid (62)



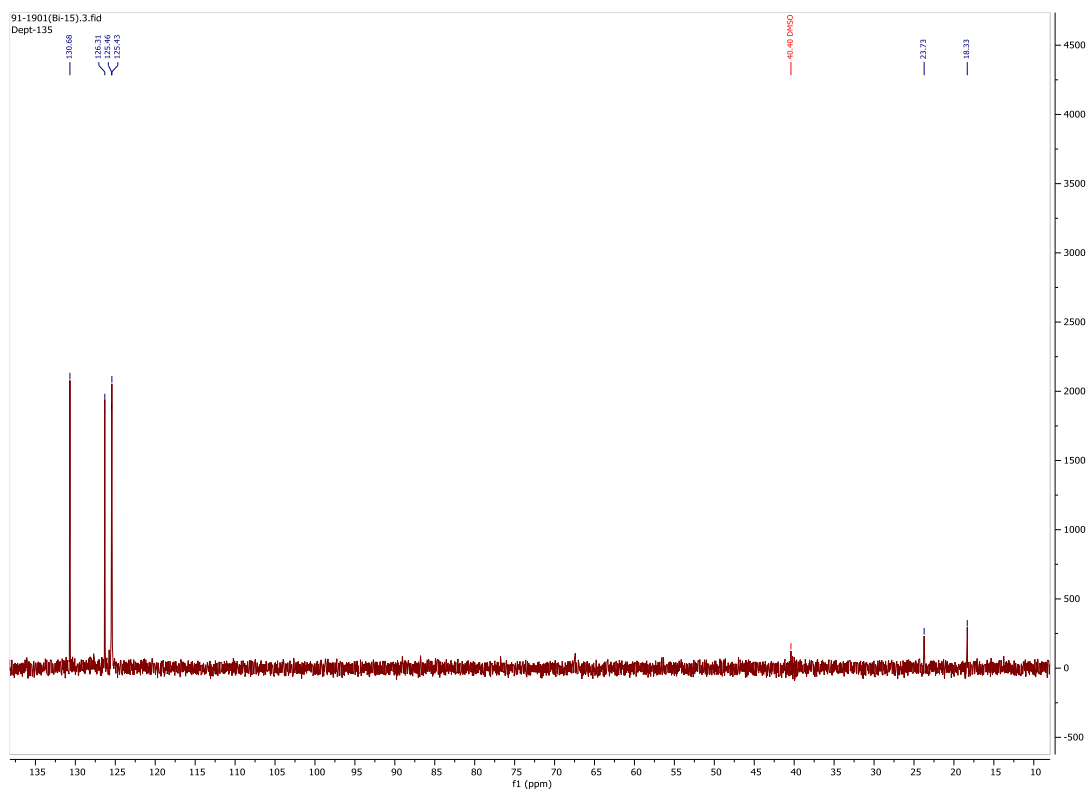
Appendix 95. The ^1H NMR (400 MHz, DMSO- d_6) spectrum of 2-(o-tolylimino)propanoic acid (**230**)



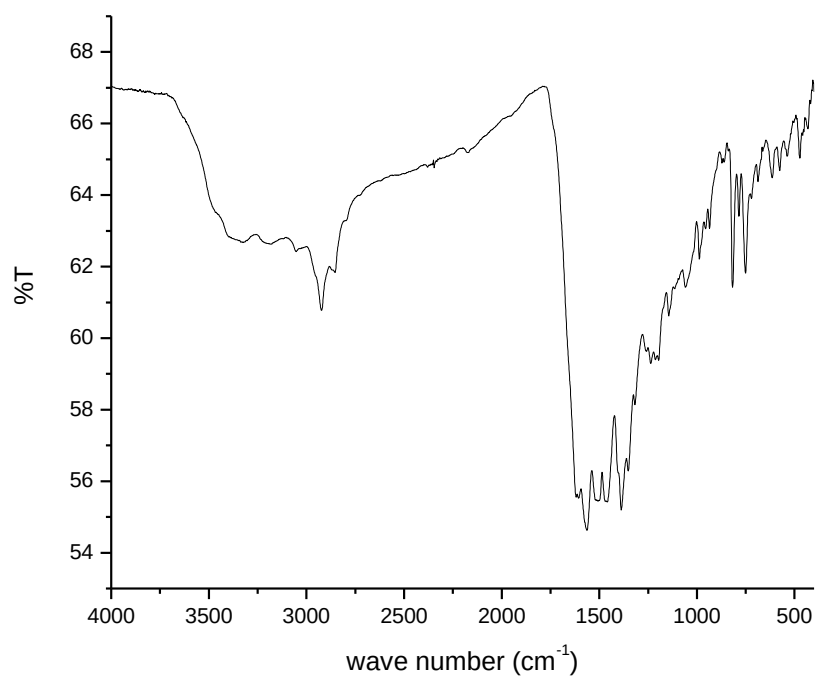
Appendix 96. The ^{13}C NMR (100 MHz, DMSO- d_6) spectrum of 2-(o-tolylimino)propanoic acid (**230**)



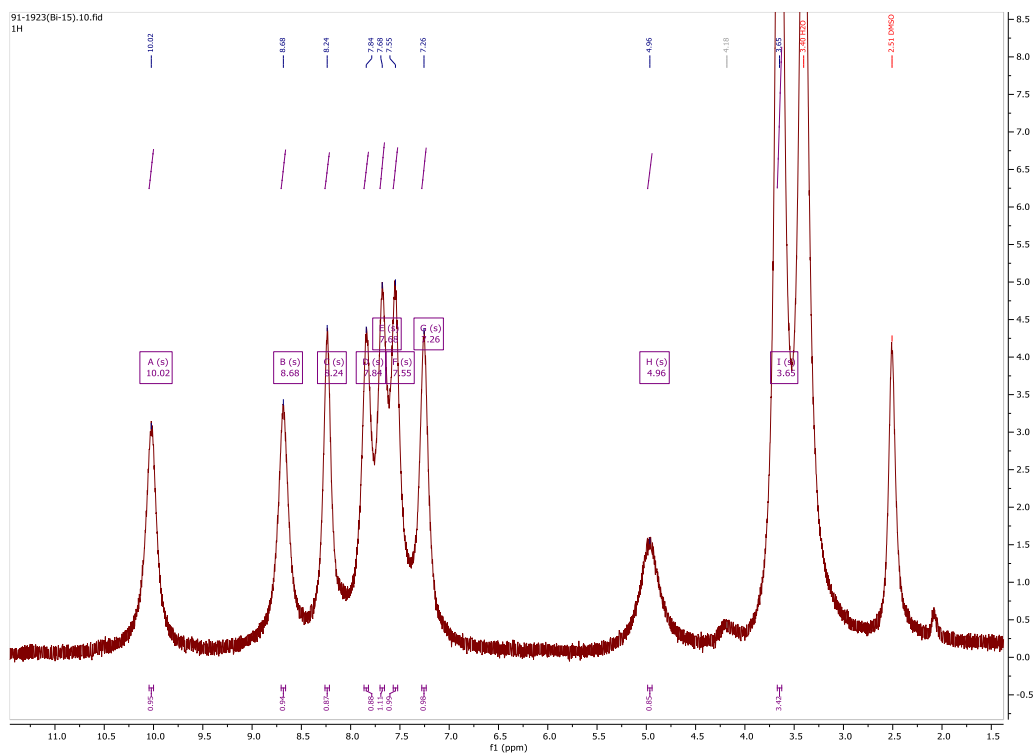
Appendix 97. The Dept-135 NMR (100 MHz, DMSO-d₆) spectrum of 2-(o-tolylimino)propanoic acid (230)



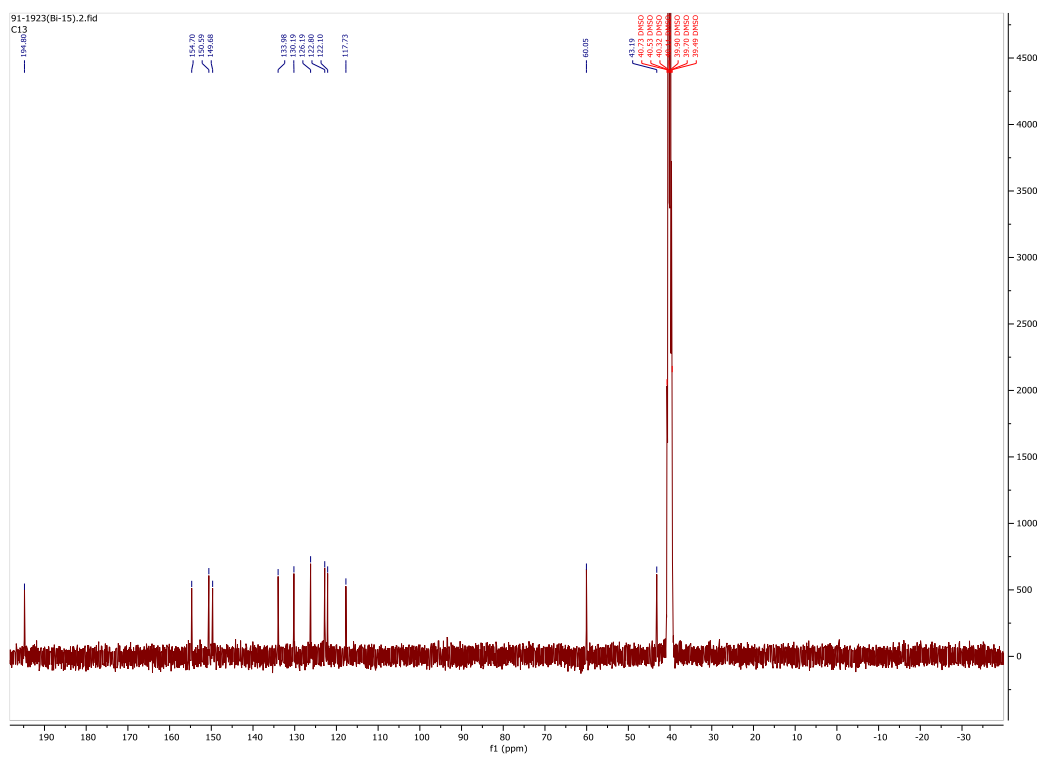
Appendix 98. The FTIR spectrum of 2-(o-tolylimino)propanoic acid (230)



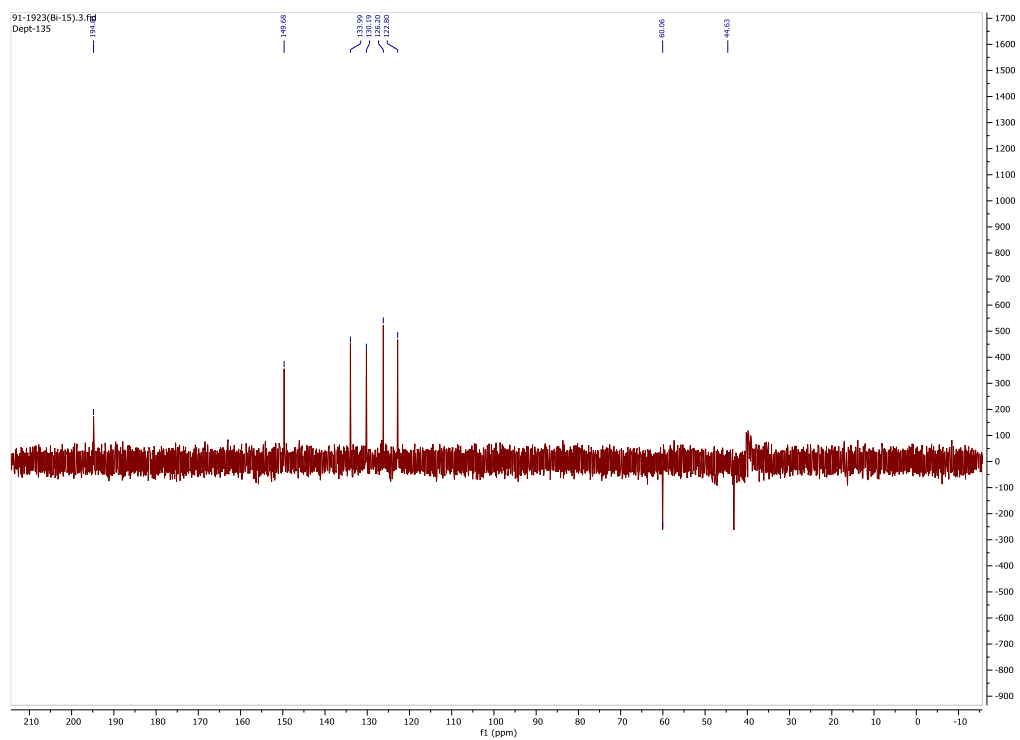
Appendix 99. The ^1H NMR (400 MHz, DMSO-d_6) spectrum of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (**232**)



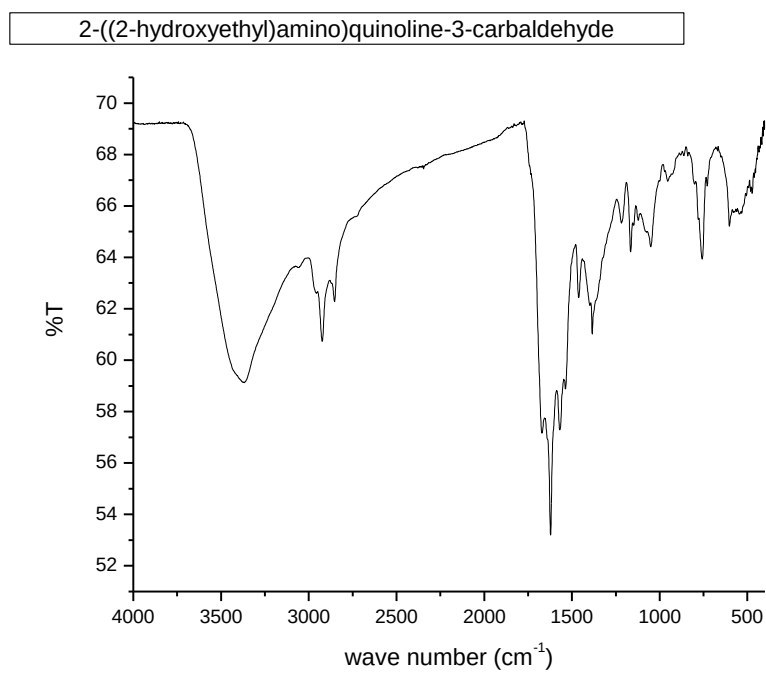
Appendix 100. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (**232**)



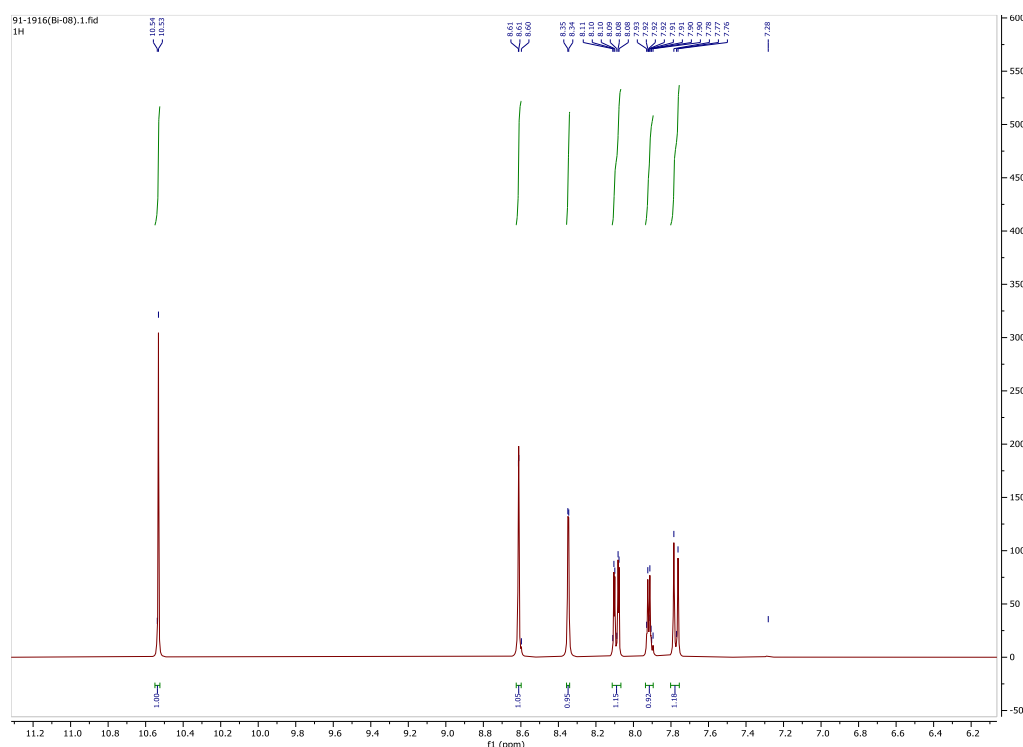
Appendix 101. The DEPT-135 spectrum of (100 MHz, DMSO- d_6) 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (**232**)



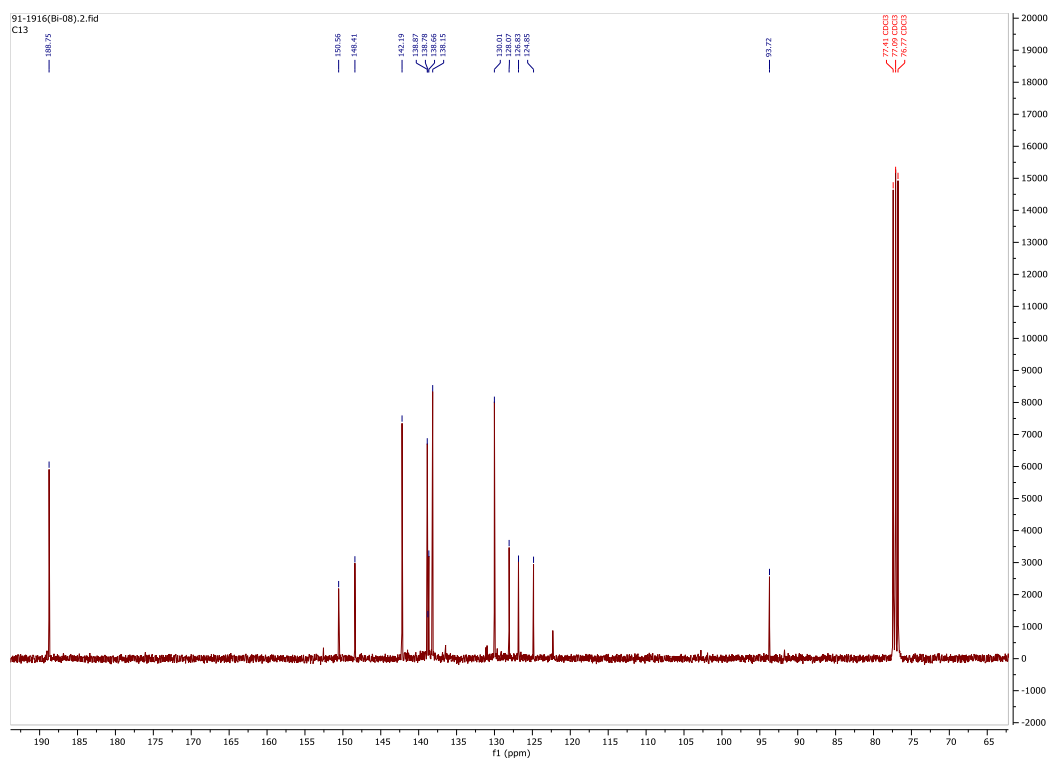
Appendix 102. The IR spectrum of 2-((2-hydroxyethyl)amino)quinoline-3-carbaldehyde (**232**)



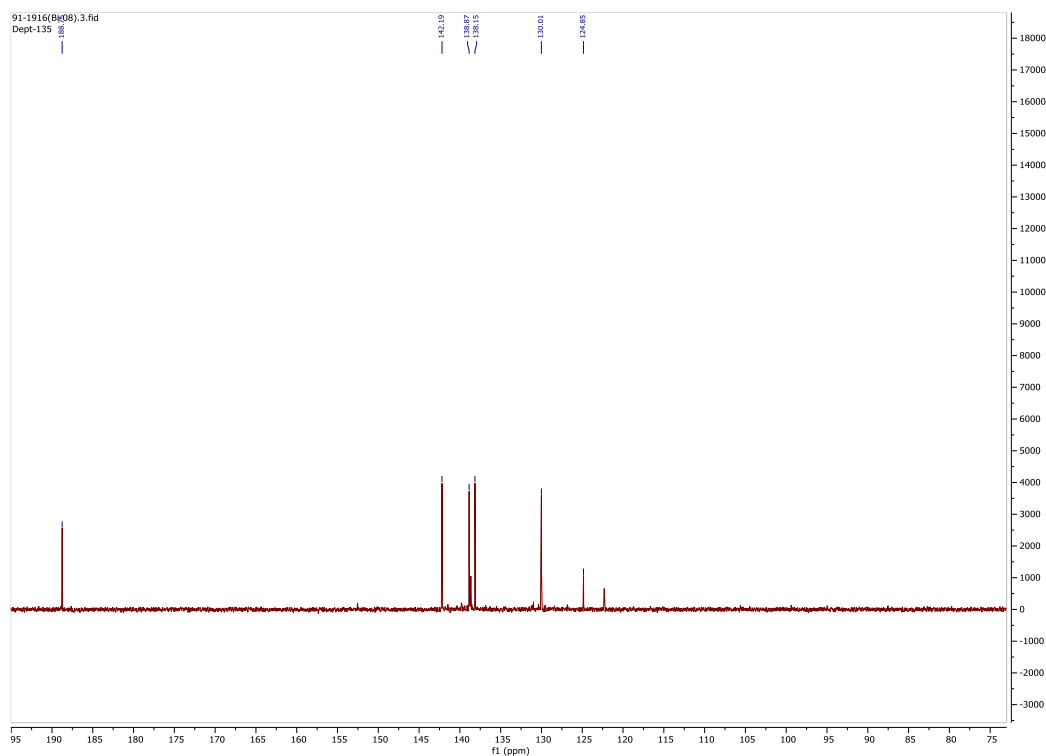
Appendix 103. The ^1H NMR (400 MHz, CDCl_3) spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (**235**)



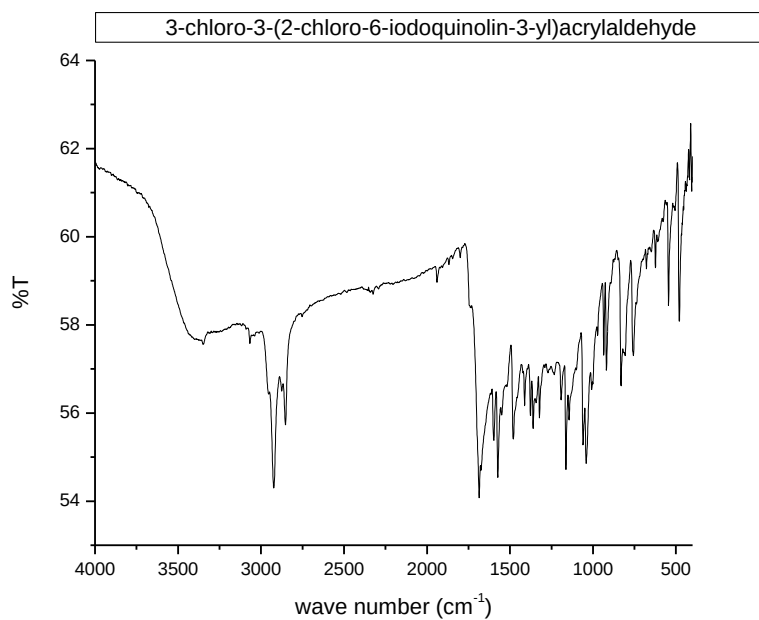
Appendix 104. The ^{13}C NMR (400 MHz, CDCl_3) spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (**235**)



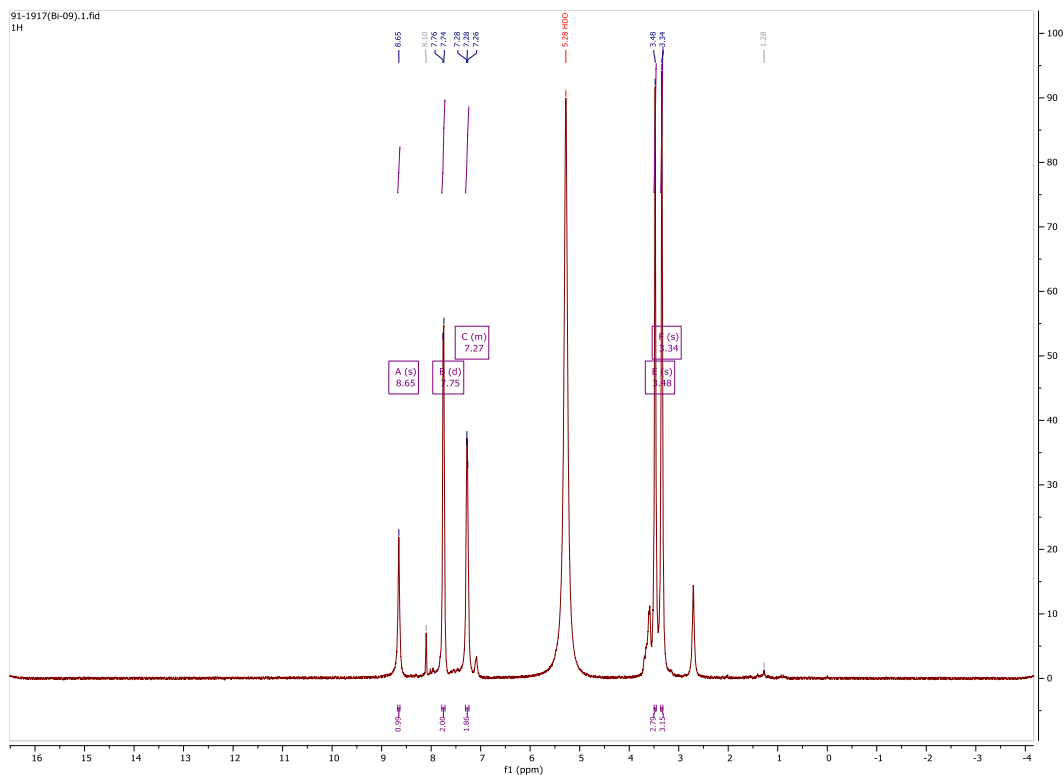
Appendix 105. The DEPT-135 (100 MHz, CDCl₃) spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (**235**)



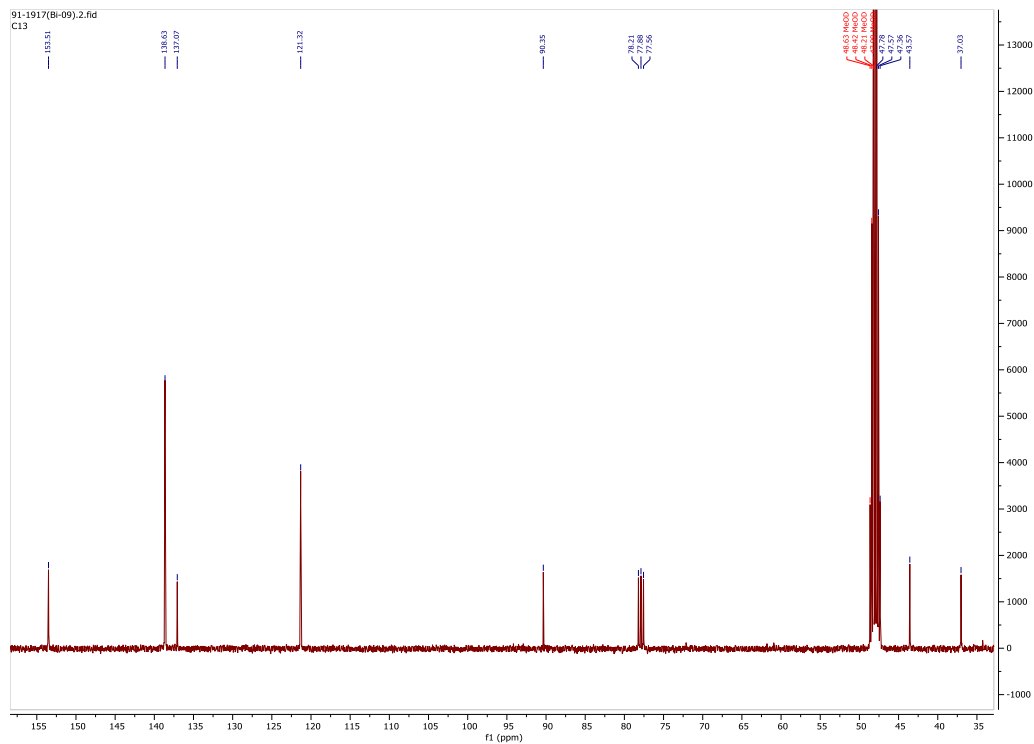
Appendix 106. The IR spectrum of 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde (**235**)



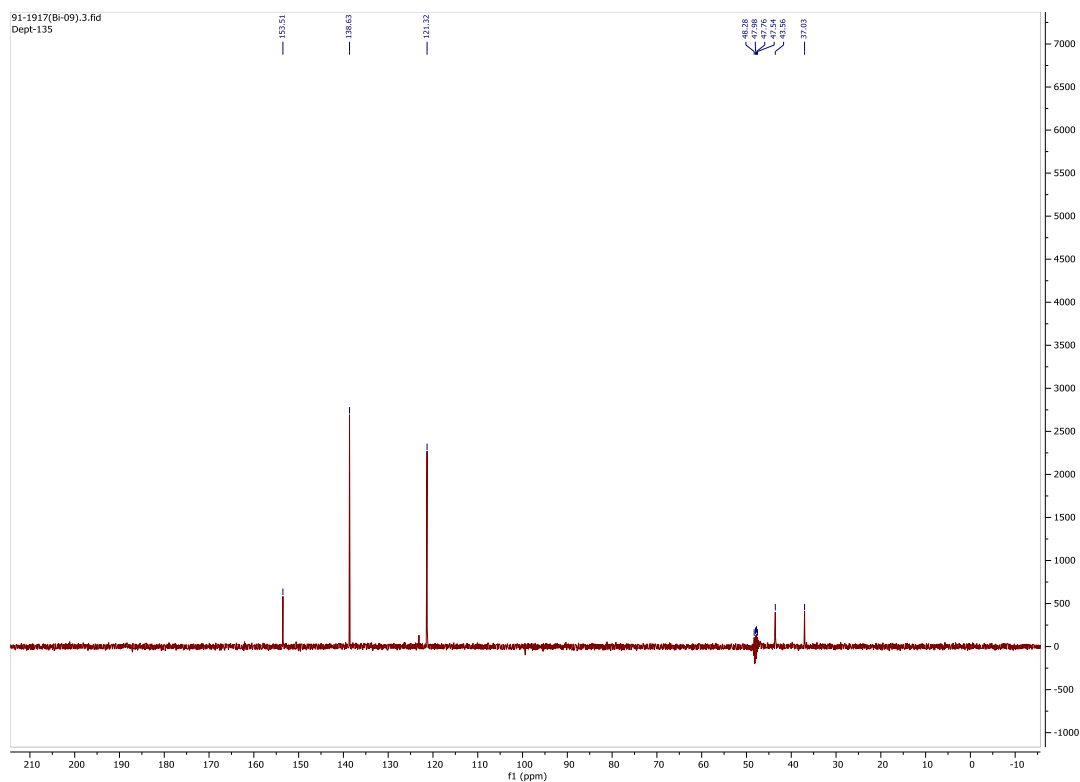
Appendix 107. The ^1H NMR (400 MHz, CDCl_3 and CD_3OH) of *N*-(((4-iodophenyl)amino)methylene)-*N*-methylmethanaminium (**236**)



Appendix 108. The ^{13}C NMR (400 MHz, CDCl_3 and CD_3OH) spectrum of *N*-(((4-iodophenyl)amino)methylene)-*N*-methylmethanaminium (**236**)



Appendix 109. The DEPT-135 (100 MHz, $CDCl_3$ and CD_3OH) of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (**236**)



+69*

Appendix 110. The IR spectrum of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (**236**)

