

**Synthesis, Characterization and Biological Studies of Cr(III), Cu(II) and
Zn(II) Complexes of Metformin and Novel Oxo ligands: A Combined
Experimental and Computational Study**



Mamaru Bitew Alem

A PhD Dissertation Submitted to the Department of Applied Chemistry

School of Applied Natural Sciences

**Presented in Partial Fulfillment for the Requirements of the Degree of Doctor
of Philosophy in Applied Chemistry (Bioinorganic Chemistry)**

Office of Postgraduate Studies

Adama Science and Technology University

Adama, Ethiopia

July, 2023

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Declaration

I hereby declare that this dissertation titled “**Synthesis, Characterization and Biological Studies of Cr(III), Cu(II) and Zn(II) Complexes of Metformin and Novel Oxo ligands: A Combined Experimental and Computational Study**” is my own original work conducted at the Applied Chemistry Department, Adama Science and Technology University, under the supervision of Professor Tegene Desalegn and Dr. Taye Beyene Demissie. It has never been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this dissertation have been duly acknowledged through appropriate list of references.

Recommendation

We, the supervisors of this dissertation, hereby certify that we have read and revised the dissertation entitled “**Synthesis, Characterization and Biological Studies of Cr(III), Cu(II) and Zn(II) Complexes of Metformin and Novel Oxo ligands: A Combined Experimental and Computational Study**” prepared under our guidance by **Mamaru Bitew Alem** and submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Chemistry (**Bioinorganic Chemistry**). Therefore, we recommend the final submission of the dissertation to the department.

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Approval page

We hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled “**Synthesis, Characterization and Biological Studies of Cr(III), Cu(II) and Zn(II) Complexes of Metformin and Novel Oxo ligands: A Combined Experimental and Computational Study**” by **Mamaru Bitew Alem**.

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We, the undersigned, members of the Board of Examiners of the dissertation open defense by **Mamaru Bitew Alem** have read and evaluated the dissertation entitled “**Synthesis, Characterization and Biological Studies of Cr(III), Cu(II) and Zn(II) Complexes of Metformin and Novel Oxo ligands: A Combined Experimental and Computational Study**” and examined the candidate during open defense. This is, therefore, to certify that the dissertation is accepted for partial fulfillment of the requirement of the degree of Doctor of Philosophy in Applied Chemistry (**Bioinorganic Chemistry**).

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Contents	page
Declaration	i
Recommendation.....	ii
Approval page	iii
Acknowledgement.....	v
List of Figure	xi
List of tables	xiv
List of schemes	xv
List of appendixes	xvi
List of Abbreviations.....	xviii
ABSTRACT	xix
1. INTRODUCTION.....	1
1.1 Statement of the problem	9
1.2 Hypothesis	11
1.3 Objectives of the study	11
1.3.1 General objectives	11
1.3.2 Specific Objectives.....	12
1.4 Significance of the study	12
1.5 Delimitation of the study	13
2. LITERATURE REVIEW	14
2.1 Therapeutic potentials of metal complexes.....	14
2.2 Therapeutic potentials of Cr(III), Cu(II) and Zn(II) metal complexes.....	15
2.3 Cancer, antimicrobial resistance and oxidative stress	18
2.3.1 Cancer	18
2.3.2 Antimicrobial resistance	20
2.3.3 Oxidative Stress	20
2.4 Synthesis and characterization of mixed-ligand Cr(III), Cu(II) and Zn(II) metal complexes	22
2.4.1 Synthesis and biological activity of metformin-based metal complexes.....	22
2.4.2 Synthesis and biological activity quinoline based metal complexes	25
2.4.3 Synthesis and biological activity 1,10-phenanthroline-based metal complexes.....	28

2.4.4 Synthesis and biological activity of chrysin-based metal complexes	34
2.5 Methods for antioxidant activity evaluation	38
2.5.1 Antioxidant activity testing methods	39
2.6 Methods for cytotoxic activity evaluation	40
2.7 Methods for antibacterial activity evaluation	40
2.8 Screening of molecules for their drug-likeness	41
2.9 Overview of webserver for molecular screening	43
2.9.1 SwissADME	43
2.9.2 ADMETLab	44
2.9.3 Pro Tox-II	45
3 MATERIALS AND METHODS	46
3.1 Materials	46
3.2 Computational Screening of a bidentate flavonoid ligand	46
3.2.1 Ligand preparation	46
3.2.2 Physicochemical property, drug likeness, and pharmacokinetic predictions	47
3.2.3 Toxicity prediction	48
3.2.4 Quantum chemical calculations for the ligand screening	48
3.2.5 Molecular docking study	51
3.3 Synthesis of Cr(III), Cu(II) and Zn(II) mixed ligand complexes of chrysin and 1,10-phenanthroline	51
3.4 Synthesis of Cr(III), Cu(II) and Zn(II) mixed ligand complexes of chrysin and metformin	52
3.5 Synthesis of 1,10-phenanthroline based Cu(II) and Zn(II) mixed ligand complexes	53
3.6 Characterization of ligands and their complexes	54
3.6.1 Characterization of Ligands	54
3.7 Characterization of the synthesized metal complexes	54
3.7.1 Thin layer chromatography and melting point analysis	54
3.7.2 UV-Vis spectroscopy analysis	55
3.7.3 Molar conductance analysis	55
3.7.4 Fourier-transform infrared spectroscopy (FTIR) analysis	55
3.7.5 Thermogravimetric analysis	55

3.7.6 High-resolution mass spectrometry analysis	56
3.7.7 Scanning electron microscopy and energy dispersive spectroscopy (SEM-EDS) analysis.....	56
3.7.8 X-ray diffraction analysis	57
3.8 Biological Activity	57
3.8.2 Antibacterial activity analysis.....	58
3.8.3 Antioxidant activity using DPPH (1-1-diphenyl 2-picryl hydrazyl) assay.....	59
3.9 Computational studies	60
3.9.1 Density functional theory and time-dependent DFT calculations	60
3.9.2 Molecular docking studies	61
3.9.3 Pharmacokinetic Prediction and BOILED Egg Model.....	61
4. RESULTS AND DISCUSSION	63
4.1 Analysis of physicochemical properties and drug-likeness.....	63
4.2 Synthesis and Characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of 1,10-phenanthroline and chrysin.....	80
4.2.1 Physicochemical properties	81
4.3 Characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of 1,10-phenanthroline and chrysin (54 – 56).....	82
4.3.1 FTIR analysis of the Cr(III), Cu(II) and Zn(II) complexes of chrysin and 1,10-phenanthroline	82
4.3.2 UV-Vis/TD-DFT analysis of the ligands and complexes (54 – 56)	85
4.3.3 Mass spectrometric analysis of the as-synthesized complexes.....	88
4.3.4 SEM-EDX analysis of the complexes (54 – 56).....	91
4.3.5 Thermogravimetric analysis of chrysin and 1,10-phenanthroline based mixed ligand complexes	92
4.3.6 Powder x-ray diffraction analysis of chrysin and 1,10-phenanthroline complexes (54 – 56).....	94
4.3.7 Molar conductance of the complexes	96
4.4 Synthesis and characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of metformin and chrysin	97
4.4.1 Physicochemical properties of chrysin and metformin complexes of Cr(III), Cu(II) and Zn(II)	98

4.5 Characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of metformin and chrysin	99
4.5.1 FTIR analysis	99
4.5.2 UV-Vis/TD-DFT analysis of the complexes of metformin coordinated chrysin based complexes (57 – 59).....	101
4.5.3 Mass spectrometric analysis of mixed ligand complexes (57 – 59)	104
4.5.4 SEM-EDX analysis of the complexes (57 – 59).....	106
4.5.5 Thermogravimetric Analysis	107
4.5.6 Powder X-ray diffraction analysis of chrysin and metformin mixed ligand complexes.....	110
4.5.7 Molar conductance of chrysin and metformin complexes of Cr(III), Cu(II) and Zn(II).....	111
4.6 Synthesis and characterization of Cu(II) and Zn(II) mixed drug complexes (60 – 62)	112
4.6.1 Physicochemical properties mixed-drug complexes (60 – 62)	113
4.7 Characterization of 1,10-phenanthroline based Cu(II) and Zn(II) mixed drug complexes	114
4.7.1 FTIR Analysis of the complexes (60 – 62)	114
4.7.2 UV-Vis/TD-DFT analysis of the complexes (60 – 62).....	117
4.7.3 Mass Spectrometric Analysis.....	119
4.7.4 Thermogravimetric Analysis (60 – 62).....	121
4.7.5 Powder X-ray Diffraction analysis of Cu(II) complexes (60 - 61).....	123
4.8 Biological activity of the synthesized complexes	124
4.8.1 Biological activity of chrysin and 1,10-phenanthroline based Cr(III), Cu(II) and Zn(II) complexes (54 – 56)	124
4.8.1.1 Cytotoxicity activity of complexes (54 - 56)	124
4.8.1.2 Antibacterial Activity	127
4.8.1.3 Antioxidant activity	130
4.8.1.4 Quantum chemical descriptors analysis of the complexes	131
4.8.2 Biological activity of chrysin and metformin Cr(III), Cu(II) and Zn(II) complexes (57 - 59).....	138
4.8.2.1 Cytotoxicity study of the complexes (57 - 59)	138

4.8.2.2 Antibacterial activity of metformin and chrysin heteroleptic complexes (57 - 59).....	142
4.8.2.3 Antioxidant activity of the complexes (57 – 59).....	144
4.8.2.4 Quantum Chemical descriptor analysis of the complexes (57 - 59).....	144
4.8.2.5 Frontier molecular orbitals analysis of the complexes (57 – 59).....	145
4.8.2.6 Molecular docking study of the complexes (57 – 59) against estrogen receptor alpha (ER α ; PDB: 5GS4)	146
4.8.2.7 Molecular docking against <i>S. aureus</i> (PDB: 2w9h)	148
4.8.3 Biological activity of mixed drug metal Cu(II) and Zn(II) complexes (60 - 61)	152
4.8.3.1 <i>In vitro</i> cytotoxicity studies (60 - 61)	152
4.8.3.2 Antibacterial activity of the complexes	153
4.8.3.3 Antioxidant activity evaluation.....	155
4.8.3.4 Quantum Chemical Analysis of complexes (60 – 62)	156
4.8.3.5 ADMET profile and BOILED Egg map evaluation	158
4.8.3.6 Molecular docking analysis	160
5.1 CONCLUSIONS	163
5.2 RECOMMENDATIONS	164
6.0 REFERENCES	165
APPENDIX	185
List of publications.....	223

List of Figure

Figure 1. 3D and 2D-structural representation of metformin.....	22
Figure 2. Reported structures of metformin containing Cu(II) complexes adopted from (Ramadan et al., 2020)	23
Figure 3. Metformin and glycine amino acid heteroleptic complexes of Cr(III) and Zn(II)(Aljazzar, 2021).	24
Figure 4. Structures of quinolone (9), nalidixic acid (10) and fluoroquinolones (11)	25
Figure 5. 3D (a) and 2D(b)-structural representation of ciprofloxacin	26
Figure 6. Structures of some ciprofloxacin based Cu(II) complexes (Yu et al., 2008) (Lawal et al., 2020)(Y. Wang et al., 2010	27
Figure 7. 3D (a) and 2D (b)-structural representation of 1,10-phenanthroline	29
Figure 8. Reported structures of 1,10-phenanthroline based Co (II) and Ru(III) complexes (Abebe et al., 2020; Abebe & Hailemariam, 2016; Atakilt et al., 2018; Tamiru et al., 2019).....	31
Figure 9. 1,10-phenanthroline based Zn(II)-heteroleptic complexes (Chiniforoshan et al., 2015; Das et al., 2006; dos Santos et al., 2015).....	33
Figure 10. Chemical structure of flavonoid (2-phenyl-chromone) constructed using Chemcraft (a) and Chem Draw ultra 12.0.2 (b).	36
Figure 11. Structure and potential chelation sites of flavanols (a) and chrysin (b).....	36
Figure 12. Reported structures Cu(II) and Zn(II) complexes of chrysin (Gençkal, 2020; E Halevas et al., 2021; Lin et al., 2017).....	38
Figure 13. Structure of DPPH	39
Figure 14. Five years of publication trend in ScienceDirect website using SwissADME and ADME keywords.....	44
Figure 15. Schematic representation of ligand screening flowchart	48
Figure 16. The structures of the flavonoid derivatives considered for the ligand screening. The numbers (36 to 53) stand for 36 = Apigenin, 37 = Baicalein, 38 = Butein, 39 = Chrysin, 40 = Ellagic acid, 41 = Eriodyctiol, 42 = Fisetin, 43 = Kaempferol, 44 = Quercetin, 45 = Genistein, 46 = Isorhamnetin, 47 = Luteolin, 48 = Morin, 49 = Naringenin, 50 = Wogonin, 51 = Myricetin, 52 = acarbose and 53 = metformin.	50
Figure 17. Schematic representation of the research design for ligand screening, synthesis, characterization and biological activity.....	53
Figure 18. A representative working page of SwissADME and its BOILED-Egg model skeleton	62
Figure 19. 2D (upper panel) and 3D (upper panel) representations of the binding modes of compounds Apigenin to Human α -amylase (PDB ID: 4W93).	76
Figure 20. 2D (upper panel) 3D (lower panel) representations of the binding modes of chrysin (39) to Human α -amylase (PDB ID: 4W93).....	78
Figure 21. 2D (Upper panel) and 3D (Lower panel) representations of the binding modes of Metformin (53) to Human α -amylase (PDB ID: 4W93).....	79
Figure 22. FTIR Spectra of the functional group regions (A) and fingerprint region (B) of the ligands (1,10-phen (a), Cry(b)) and their metal complexes (54 (c), 55 (d)and 56 (e))	84
Figure 23. UV-Vis and TD-DFT calculated absorption spectrum of 1,10-phenanthroline (a) and chrysin (b).....	86
Figure 24. UV-Vis (Black) and TD-DFT(red) absorption spectra of complex (54)	87
Figure 25. Uv-Vis (Black) and TD-DFT(red) absorption spectra of heteroleptic complex (55)	87
Figure 26. UV-Vis (a) and TD-DFT (b) absorption spectra of heteroleptic complex 56.....	88

Figure 27. Mass spectra of Cr(III) complex (54)	88
Figure 28. Mass spectra of Cu(II) complex (55)	89
Figure 29. Mass Spectra of Zn(II) complex (56).....	90
Figure 30. EDX spectra of 54 (a), 55 (b) and 56 (b) and the SEM images of 54 (d), 55 (e) and 56 (f)	91
Figure 31. Thermogravimetric(TGA/DTA) presentation of Cr(III) complex (54)	92
Figure 32. Thermogravimetric (TGA/DTA) presentation of complexes 55 (a) and 56 (b)	93
Figure 33. X-ray diffraction patterns for 54 (a), 55 (b) and 56 (c) mixed ligand complex of chrysin and 1,10-phenanthroline.	95
Figure 34. FTIR spectra of metformin, chrysin and 57 complex	100
Figure 35. FTIR spectra of metformin, chrysin and 58 complex	100
Figure 36. FTIR spectra of metformin, chrysin and 59 complex	101
Figure 37. The UV-Vis and TD-DFT spectrum of complex 57(a) and its alternative structure (b)	102
Figure 38. UV-Vis and TD-DFT spectrum of complex 58 (a) and 59 (b)	104
Figure 39. Mass spectra of chrysin and metformin-based complex Cr(III) complex (57).....	105
Figure 40. EDX analysis of metformin-chrysin mixed ligand metal complexes 57 (a) 58 (b) and 59(c) and their respective SEM images of 57 (d) 58 (e) and 59 (f)	107
Figure 41. Thermogravimetric analysis of Cr(III) complex (57)	108
Figure 42. Thermogravimetric analysis of chrysin and metformin complexes 58 (a) and 59 (b)	109
Figure 43. X-ray diffraction patterns for Cr(III) (57(a)), Cu(II)(58(b)) and Zn(II)(59(c)) mixed ligand complexes.	111
Figure 44. FTIR spectra of the synthesized metal complexes 60 (a) and 61 (b) and the ligands	115
Figure 45. UV-Vis spectra of the synthesized mixed ligand metal Cu(II) complex (60)	117
Figure 46. UV-Vis spectra of mixed ligand metal complex (61).....	118
Figure 47. UV-Vis spectra of mixed ligand metal Zn(II) complex (62)	119
Figure 48. Mass spectrometric analysis of complexes 60 (a) and 61 (b)	120
Figure 49. TGA (blue) and DTA (red) curves for complexes 60 (a) and 61(b)	123
Figure 50. X-ray diffraction pattern of complex 60 (a) and 61 (b) in the powder form.....	124
Figure 51. Cytotoxicity of chrysin based 1,10-phenanthroline complexes (54 - 56) and the positive control (Cisplatin) against MCF-7 cell lines	125
Figure 52. Photomicrographs of the cellular morphology of untreated MCF-7 (a) and the changes induced by complex 54 (b), 55 (c) 56 (d) and cisplatin (e) at 50 μ M.....	127
Figure 53. Antibacterial activity of 1,10-phenanthroline and chrysin mixed ligand complexes against E. coli(a), S. aureus (b), P. aeruginosa (c) and S. pyogenes (d).....	128
Figure 54. Minimum inhibition zone for the antibacterial activity of 1,10-phenanthroline and chrysin mixed ligand complexes of Cr(III), Cu(II) and Zn(II) (54 – 56).....	129
Figure 55. Antioxidant activity of the synthesized metal complexes (54 -56) against DPPH radical	130
Figure 56. Graphical presentations of quantum chemical descriptors	132
Figure 57. Optimized geometry, HOMO – LUMO and eigen values of 55 (left) and 56 (right) metal complexes	134
Figure 58. The binding interactions of 56 against estrogen receptor alpha (ER α ; PDB: 5GS4)	135

Figure 59. The binding interactions of 55 against <i>S. aureus</i> (Dihydrofolate reductase) (PDB: 2w9h).....	138
Figure 60. Cytotoxicity of chrysin based metformin complexes (57 - 59) and the positive control (Cisplatin) against MCF-7 cell lines	140
Figure 61. Photomicrographs of the cellular morphology of untreated MCF-7 (a) and the changes induced by complex 57 (b), 58 (c), 59 (d) and cisplatin (e) at 100 μ M.....	141
Figure 62. Antibacterial activity of metformin-based complexes (57 – 59)	142
Figure 63. Zone of inhibitions of complexes against <i>E. coli</i> (a), <i>S. aureus</i> (b), <i>P. aeruginosa</i> (c) and <i>S. pyogenes</i> (d)	143
Figure 64. The antioxidant activity of the synthesized metal complexes (57 - 59) against DPPH radical	144
Figure 65. The frontier molecular orbitals and associated band gap energies of complexes (57 - 59).....	146
Figure 66. The binding interactions of 58 against estrogen receptor alpha (ER α ; PDB: 5GS4)	148
Figure 67. The binding interactions of 59 against <i>S. aureus</i> (PDB: 2w9h).....	150
Figure 68. The binding interactions of ciprofloxacin against <i>S. aureus</i> (PDB: 2w9h)	151
Figure 69. Cell viability of MCF-7cancer cell line against complexes 60 and 61 and cisplatin.....	153
Figure 70. Mean inhibition zone diameter of mixed-drug metal complexes 60 and 61 and ciprofloxacin in mm at 25 and 50 μ g/mL.....	154
Figure 71. Graphical presentation of the antioxidant activity of complexes (60 - 62).....	155
Figure 72. HOMO – LUMO distribution and band gap energy of the synthesized metal complexes	158
Figure 73. BOILEDegg model of the ligands (1,10-phenanthroline, Ciprofloxacin, and metformin), 60, 61 and Cisplatin.....	159
Figure 74. The (a) 3D and (b) 2D presentations of the binding interactions of complexes 60 and 61 against estrogen receptor alpha (ER α ; PDB: 5GS4)	162

List of tables

Table 1. The physicochemical property of the flavonoids and the two control drugs	64
Table 2. Comparison of solubility, partition coefficient, water solubility, and pKa of flavonoids predicted using different methods	66
Table 3. Absorption, Distribution and Excretion parameters of the flavonoids and the two controls	68
Table 4. The interaction of target Flavonoids with cytochromes P450 isoforms predicted using SwissADME	70
Table 5. Pro Tox II predicted Organ toxicity, toxicological end points and acute toxicity	71
Table 6. Toxicity profile of flavonoids and the two controls predicted using different methods	73
Table 7. Quantum chemical descriptors of the flavonoid derivatives calculated using DFT/B3LYP hybrid functional was used together with 6–311++G(d,p) basis set	74
Table 8. Molecular docking value of flavonoids against Human α -amylase (PDB ID: 4W93) .	77
Table 9. Physicochemical properties of complexes (54 - 56)	82
Table 10. Temperature range values for decomposition and corresponding weight loss values	94
Table 11. Physicochemical properties of metformin-based metal complexes	99
Table 12. Temperature range values for decomposition and corresponding weight loss values	109
Table 13. Physicochemical properties of the synthesized mixed drug metal complexes.....	114
Table 14. Vibrational frequencies (experimental/calculated) of selected functional groups of the ligands and their metal complexes	116
Table 15. Temperature range values for decomposition and corresponding weight loss values	121
Table 16. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against estrogen receptor alpha (ER α ; PDB: 5GS4)	136
Table 17. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against S. aureus (PDB: 2w9h).....	137
Table 18. Quantum chemical descriptors of metal complexes (57 – 59)	145
Table 19. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against estrogen receptor alpha (ER α ; PDB: 5GS4)	147
Table 20. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against S. aureus (PDB: 2w9h).....	149
Table 21. Quantum chemical descriptor analysis of the ligands and the synthesized metal complexes	156
Table 22. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against estrogen receptor alpha (ER α ; PDB:5GS4)	160

List of schemes

Scheme 1. Schematic presentation of Cu (II) complex derived from benzimidazole-o-vanillin Schiff base and 1,10-phenanthroline	55
Scheme 2. Schematic representations of 1,10-phenanthroline-chrysin mixed ligand metal complexes (54 - 56)	81
Scheme 3. Schematic representations of metformin-chrysin mixed ligand metal complexes	98
Scheme 4. Proposed structure of Cr(III) complex (57) in solid and solution forms	112
Scheme 5. The design of the coordination spheres of the synthesized metal complexes	113

List of appendixes

Appendix 1. 2D and 3D representations of the binding modes of compounds Biacalieu (37) and Butien (38) to Human α -amylase.....	185
Appendix 2. 2D and 3D representations of the binding modes of Ellagic acid (40) and Eriodyctiol (41).	186
Appendix 3. 2D and 3D representations of the binding modes of Fisetin (42) and Kaempferol (43).	187
Appendix 4. 2D and 3D representations of the binding modes of Quercetin (44) and Genistein (45).	188
Appendix 5. 2D and 3D representations of the binding modes of Isorhamnetin (46) and Luteolin (47).	189
Appendix 6. 2D and 3D representations of the binding modes of Morin (48) and Naringenin (49)	190
Appendix 7. 2D and 3D representations of the binding modes of Wogonin (50) and Myricetin (51)	191
Appendix 8. Calculated FTIR spectra of chrysin and its complexes (54 – 56).....	192
Appendix 9. Calculated FTIR spectra of 1,10-phenanthroline	192
Appendix 10. Mass spectrometric analysis of complex 54	193
Appendix 11. Mass-spectrometric analysis of complex 55.....	193
Appendix 12. Mass spectrometric analysis of complex 56.....	194
Appendix 13. Calculated FTIR spectra of the ligands (metformin and chrysin) and their complexes (57 – 59)	194
Appendix 14. Mass spectrometric analysis of complex 58.....	195
Appendix 15. Mass spectrometric analysis of complex 59.....	196
Appendix 16. FTIR spectra of the ligands and complex 62.....	197
.....	197
Appendix 17. Overlayed FTIR spectra of Cu(II) (60) and Zn(II) (62) complexes of metformin and 1,10-phenanthroline.....	197
Appendix 18. FTIR Spectra of ciprofloxacin hydrogen chloride (Cip)	198
Appendix 19. FT-IR spectra of metformin hydrogen chloride (a) and 1,10-phenanthroline monohydrate (b)	198
Appendix 20. Calculated FTIR spectra of ciprofloxacin (cipro) and complexes (60 – 62)	199
Appendix 21. TD-DFT calculated spectra of ciprofloxacin hydrogen chloride.....	199
Appendix 22. TD-DFT calculated spectra of ciprofloxacin hydrogen chloride.....	200
Appendix 23. Mass spectrometric analysis of complex 62	201
Appendix 24. TG and DTG curves for complex 62	201
Appendix 25. Antibacterial activity of 1,10-phenanthroline and chrysin mixed ligand complexes	202
(MIZ and SD values are given)	202
Appendix 26. Concentration Vs absorbance plot for the antioxidant activity of chrysin-based metal complexes (54 - 56)	202
Appendix 27. Quantum chemical descriptors of the ligands and the complexes (54 – 56)	203
Appendix 28. HOMO - LUMO distribution of chrysin (bottom), complex 54 (center) and 1,10-phenanthroline (top).	203
Appendix 29. The binding interactions of 54 against estrogen receptor alpha (ER α ; PDB: 5GS4)	204

Appendix 30. The binding interactions of 55 against estrogen receptor alpha (Er α ; PDB: 5GS4)	205
Appendix 31. The binding interactions of cisplatin against estrogen receptor alpha (Er α ; PDB:	206
Appendix 32. The binding interactions of 54 against <i>S. aureus</i> (PDB: 2w9h)	206
Appendix 33. The binding interactions of 56 against <i>S. aureus</i> (PDB: 2w9h)	207
Appendix 34. Antibacterial activity of metformin and chrysin mixed ligand complexes (57 – 59) (SD values are given)	208
Appendix 35. The binding interactions of 57 against estrogen receptor alpha (ER α ; PDB: 5GS4)	209
Appendix 36. The binding interactions of 57 against estrogen receptor alpha (ER α ; PDB: 5GS4)	210
Appendix 37. The binding interactions of 59 against estrogen receptor alpha (Er α ; PDB: 5GS4)	211
Appendix 38. The binding interactions of 57 against <i>S. aureus</i> (PDB: 2w9h)	212
Appendix 39. The binding interactions of 58 against <i>S. aureus</i> (PDB: 2w9h)	213
Appendix 40. HOMO-LUMO distribution of ciprofolxacin	214
Appendix 41. Morphological changes of MCF-7 cell lines induced by: (top) with PBS, and (bottom) with 12.5 μ M complex 61	214
Appendix 42. Mean inhibition zone of the synthesized mixed ligand metal complexes	215
Appendix 43. Physicochemical and drug-likeness screening of the ligands, cisplatin and complexes (60 – 61)	215

List of Abbreviations

α -GI	α -glucosidase inhibitory
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
AMR	Antimicrobial resistance
BOILED-Egg	Brain Or IntestinaL EstimateD permeation method
DPPH	1-1-diphenyl 2-picryl hydrazyl
B3LYP	Becke, 3-parameter, Lee–Yang–Parr
DFT	Density Functional Theory
DNA	Deoxyribose nucleic acid
FLs	Free ligands
FMOs	Frontier molecular orbitals
GIT	Gastrointestinal tract
HBAs	Hydrogen-bond acceptors
HBDs	Hydrogen-bond donors
iLOGP	implicit n-octanol/water partition coefficient
LRF	Lipinski 's rule of five
MEP	Molecular electrostatic potential
ROS/RNS	Reactive oxygen/nitrogen species
FTIR	Fourier Transform Infrared
SDD	Swiss Drug Design
SOD	Superoxide dismutase
TGA	Thermal gravimetric analysis
TLC	Thin layer chromatography
TMCs	Transition metal complexes
TPSA	Topological polar surface area
vNN	Variable Nearest neighbor
WHO	World Health Organization

ABSTRACT

The synthesis and use of transition metal complexes for medical and health related applications has gained momentum in recent years. Transition metal complexes offer a rich platform for use as therapeutic agents ranging from antimicrobial and anti-inflammatory to anti-proliferative and enzyme inhibitory activities. Their unique electronic and stereochemical properties: molecular geometries, ligand exchange, redox, catalytic, and photophysical reactions are among the major reasons for the convergence of attention towards the compounds' both therapeutic and diagnostic roles. Even though promising results are reported and different metal complexes are under clinical trial, the incidence and death associated to oxidative stress, microbial infection, antimicrobial resistance and cancer are rapidly increasing. These urged us to synthesize metal complexes using novel ligand screening tools and drug repositioning strategies. In this work, Cr(III), Cu(II) and Zn(II) heteroleptic complexes of metformin and oxo-ligands were synthesized and subjected to cytotoxicity, antibacterial and antioxidant activity evaluations. A naturally available ligand chrysin was employed after a series of *in-silico* aided screening protocols which showed its drug-like molecular nature and potential for complexation with metals. Metformin and ciprofloxacin drugs as well as 1,10-phenanthroline were also used as ligands for the synthesis of the metal complexes. The synthesized metal complexes were characterized using UV-Vis, FTIR, MS, SEM-EDX, TGA/DTA, XRD and molar conductance measurements. In order to support and well describe the experimental results DFT, TD-DFT, *In-silico* ADMET and molecular docking computational tools were employed. All the synthesized complexes were confirmed to possess polycrystalline natures with average crystallite sizes ranging from 18 – 36 nm. Furthermore, mixed ligand complexes of wide range crystalline materials with percent crystalline index ranging from 22 – 94% were synthesized. Biologically, *in-vitro* cytotoxic activity against human breast adenocarcinoma cancer cell line (MCF-7), antibacterial activity against two gram-negative (*E. coli* and *P. aeruginosa*) and two gram-positive (*S. aureus* and *S. pyogenes*) bacterial strains, and antioxidant activity against DPPH radical were evaluated. The statistical analysis (ANOVA) for significance difference ($p < 0.05$) showed that variations in metal center and concentration have significance effect on the bacterial activity of the metal complexes. Promising results from the *in-vitro* cytotoxic activity with IC_{50} value ranging 3.72 – 44.88 μ M compared to the positive control cisplatin (18.62 μ M) were found. Among the synthesized complexes three Cu(II) complexes that consists of 1,10-phenanthroline and chrysin (**55**), 1,10-phenanthroline and metformin (**60**) and,

1,10-phenanthroline and ciprofloxacin (**61**) were found to show unique cytotoxicity profile with IC₅₀ values of 3.72, 4.29 and 7.58 μ M, respectively. A Cr(III) complex (**54**) of 1,10-phenanthroline and chrysin was found to be the fourth cytotoxic complex against MCF-7 cell lines with IC₅₀ 8.08 μ M. The overall *in vitro* results analysis showed that **54 (Cr(III))**, **55(Cu(II))**, **60(Cu(II))** and **61(Cu(II))** metal complexes have better cytotoxicity compared to the standard drug cisplatin. The biological significance of the two Cu(II) complexes (**60**) and (**61**) was confirmed by the BOILED-egg model and band gap energy for their respective minimal neurotoxicity and increased global softness. Moreover, the antibacterial activity of the complexes showed that all cultured bacteria were susceptible to the synthesized metal complexes (**54 – 61**) with inhibition diameter zone ranging from 8.00 mm – 17.33 mm and percent activity index ranging from 33 to 86%. In addition, the newly synthesized complexes experienced the innate antioxidant nature of the metal centers for scavenging the DPPH free radical (up to 81% at 400 ppm). The biological activity and quantum chemical descriptor findings showed the structure-activity and ligand type dependence of the complexes. Computationally, the biological significance of the synthesized metal complexes was inferred from the small HOMO-LUMO band gap energy, large binding affinity, small inhibition constant and inability to permeate the blood brain barrier.

Keywords: Transition metal complexes, chrysin, metformin, ciprofloxacin, 1,10-phenanthroline, cytotoxicity, antibacterial, antioxidant, MCF-7 cell line, molecular docking, quantum chemical descriptors

1. INTRODUCTION

Systematic studies of natural and synthetic organic molecules for exploring their applications have been the core of researches over past few years. There are huge reports on the effectiveness and efficacy of these organic molecules for treatment of various diseases. The research for alternative biologically active compounds has also taken another dimension in the current study. As prospective replacements for biologically active organic molecules which lacks versatile electronic and structural features (change in oxidation state, ligand exchange and multi-targeting) (Anthony et al., 2020), and possess negative side effects, metal complexes with biological activity are becoming more important in medicine (Schattschneider, Kettenmann, Hinojosa, Heinrich, & Kulak, 2019). The use of metals for the therapeutic purpose is a very old concept. The broad portfolio of new metal-based therapies progressing through clinical trials demonstrates the potential for new metal-containing compounds in the management of diseases. As a result transition metal complexes (TMCs) have a wide range of potential medicinal applications (Noffke, Habtemariam, Pizarro, & Sadler, 2012). They offer a rich platform for being used as therapeutic agents (Kilpin & Dyson, 2013) ranging from antimicrobial and anti-inflammatory to anti-proliferative and enzyme inhibitory activities (Loginova et al., 2020). As a result, interest in new metal-based therapeutic agents is growing in the bioinorganic chemistry era due to the emergence of clinical trials with various mechanisms of actions that could potentially bring new chemotherapeutic approaches to the management of diseases (Boros, Dyson, & Gasser, 2020; Damena, Zeleke, Desalegn, B. Demissie, & Eswaramoorthy, 2022; Kacsir et al.; Olelewe & Awuah, 2023; Scalese, Kostenkova, Crans, & Gambino, 2022). Numerous metal-based promising chemotherapeutic agents have been reported after the discovery of cisplatin ($\text{cis-}[\text{PtCl}_2(\text{NH}_3)_2]$) for general oncology in 1978 (Karges, Stokes, & Cohen, 2021) and salvarsan (3-amino-4-hydroxyphenyl-As(III) compounds) for syphilis treatment (Anthony et al., 2020). The opportunity that gives TMCs immense considerations in therapeutics application is the dependence of their biological activity by the type of the metal ion, oxidation state, type of metal and ligand, number of ligands and isomers (John, Dasan, Joseyphus, & Joe, 2019). Hence, the synthesis and use of transition metal complexes for medical and health related applications has gained momentum in recent years.

TMCs have unique electronic and stereochemical properties: molecular geometries, ligand exchange, redox, catalytic, and photophysical reactions are among the major reasons for the convergence of attention towards the compounds' both therapeutic and diagnostic roles (Amarsy, Papot, & Gasser, 2022; Karges et al., 2021; Liang et al., 2021). These give them the potential to interact with biomolecules in unique ways and by distinct mechanisms of action (Liang et al., 2021). As part of metal based therapeutic agents design and discovery, several metal-organic compounds such as silver, gold, platinum, vanadium, ruthenium, chromium, copper, zinc, manganese etc. using ligands which have already biological activity have been widely explored and have shown different biological activity (Anthony et al., 2020; Noffke et al., 2012). Hence, they are a promising approach to the discovery of new drugs with different mechanisms of action (Archibald, 2009; Schattschneider et al., 2019). Unlike organic compounds, metal-organic complexes have geometrical diversity due to the range of coordination numbers (two to ten) which is highly important in drug design (Anthony et al., 2020). Also, the presence of high electronegativity difference between the atoms (metals and ligands), and relatively weak bonds between the atoms forming the metal-organic complexes allow the interaction of the desired complexes with biological targets (Noffke et al., 2012). Specifically, the enzyme inhibition activity of metal complexes suggested to follow either of the three proposed mechanisms: in the first type of mechanism, complexes that contain a non-coordinating metal center and active ligand where the ligands are the biologically active component and are responsible for protein binding/enzyme inhibition. In this type of complexes, the metal may hinder or enhances the biological activity through redox events. In the second approach, active metal center complexes, the ligands are usually not bioactive, but are present to stabilize or protect the reactive metal center. The third type of the complexes are active metal and active ligand complexes where both the metal and the ligands are biologically active and are mutually responsible for the biological activity of the complex (Kilpin & Dyson, 2013). Moreover, metal complexes are suggested to follow exceptional modes of actions such as ligand exchange, reactive oxygen generation, redox activation and catalytic generation of toxic species or depletion of essential substrates over their organic counterparts (Frei et al., 2020). Hence, research interests in metal based pharmaceuticals have shown appreciable promise in the development of different compounds having biological activity as new insulin sensitivity-enhancing agents (Koleša-Dobravc et al., 2018), anti-oxidant (Atabey-Ozdemir, Demirkiran, Yildiz, Tekin, & Coban, 2017; Sulpizio, Müller, Zhang, Brecker, & Rompel, 2016),

anti-microbial (Abebe et al., 2020; Abebe & Hailemariam, 2016), anti-tumor (Krasnovskaya et al., 2020), DNA binding (Porkodi & Raman, 2018), and cytotoxicity (Bravo-Gómez, García-Ramos, Gracia-Mora, & Ruiz-Azuara, 2009; Buchtík, Trávníček, & Vančo, 2012; Johnstone, Suntharalingam, & Lippard, 2016; Reina, Hernández-Ayala, Bravo-Gómez, Gómez, & Ruiz-Azuara, 2021). As a result, several metal-based complexes have been designed and have reached stages of human clinical trials (Claudel, Schwarte, & Fromm, 2020).

Chromium in its +3 oxidation state is among the ubiquitous metals considered to be biologically relevant (Vincent, 2004), both in animal feeding and human nutrition in its trivalent state (Jie Dong, Ning Ning Wang, et al., 2018). It is believed to be involved in the metabolism of carbohydrates, lipids, and proteins mainly by increasing the efficiency of insulin (Kralovec et al., 2009; Pechova & Pavlata, 2007; Vincent, 2004). After the first suggestion that Cr(III) compounds could act as a nutritional enhancement to glucose metabolism (Pechova & Pavlata, 2007) by Schwarz and Mertz, there has been a search for different Cr(III) supplements with Cr(III)-picolinate being the oldest. Moreover, *in vitro* and *in vivo* findings have shown that trivalent chromium has biological benefits such as enhancing the antioxidant enzyme defense system, facilitating insulin signaling or insulin sensitivity (Anderson et al., 1997; Hao et al., 2011), suppressing free radical formation and inhibiting the protein glycosylation (Jie Dong, Ning Ning Wang, et al., 2018), decreasing serum glucose (Pandey, Jain, & Mathur, 2012), lowering blood lipids (Anderson et al., 1997), reducing cholesterol and triglyceride levels in the blood (C. Wang, Chen, Pan, Gao, & Chen, 2017), lowering glycated hemoglobin (El-Megharbel, 2015), and inhibiting α -glucosidase activity (Jie Dong, Ning Ning Wang, et al., 2018). However, the bioavailability and efficacy of Cr(III) are found to be dependent on the ligands that chelate to chromium ion (Peng & Yang, 2015; Ulas et al., 2015). This is because the specific ligand environment around Cr(III) could affect the biological activity and bioavailability of chromium (Syed Ahmad et al., 2012; Peng & Yang, 2015).

Copper(II) and zinc(II) are endogenous metal ions having enhanced antimicrobial and antioxidant activities with promising features as metallodrug (Sulpizio et al., 2016). Metal complexes of Cu(II) and Zn(II) may be subjected for the design and synthesis of such possibilities having such biological activities. Copper-containing coordination compounds attract wide attention due to the

redox activity and biogenicity of copper ions, providing multiple pathways of biological activity. The use of huge classes of natural and synthetic ligands for the synthesis and application of their coordination compounds as bioactive agents have been reported. Among reported works with flavonoid ligands, the higher antioxidant, anti-inflammatory and tumor cell cytotoxicity activities of Naringin–Cu (II) complex, $[\text{Cu}(\text{Naringin})(\text{CH}_3\text{OH})_2]^+$ (Vinayagam & Xu, 2015), a cytotoxicity and DNA binding studies of Cu(II) and Zn(II) complexes of flavonoids (Quercitrin, Myricitrin and Rutin) which exhibit an intercalative mode of DNA binding, nuclease and DNase activity were reported (Atabey-Ozdemir et al., 2017). Moreover, the higher antioxidant activity/free radical scavenging activity of Zn(II)- Butien (66%) than that of Butien (19%) has been reported elsewhere (Sulpizio et al., 2016), revealing enhanced biological activity of metal complexes than the ligands alone. Zn(II) is the only first series transition element with one oxidation state, vital for healthy functioning of many of the body systems, contained in nearly 300 enzymes and involved in the metabolism of protein, carbohydrate, lipid, and energy. Also, it is an important regulatory metal ion in cell metabolism, proliferation, differentiation and apoptosis (Al-Fartusie & Mohssan, 2017; Dutta & Mukta, 2012; Stefanidou, Maravelias, Dona, & Spiliopoulou, 2006; Tapiero & Tew, 2003). The involvement of Zn(II) in homeostasis, immune responses, oxidative stress, apoptosis and ageing is associated with the presence of the metal in proteins such as metallothionein and superoxide dismutase (Al-Fartusie & Mohssan, 2017; Stefanidou et al., 2006; Tapiero & Tew, 2003). The ability of Zn(II) to stabilize the cell membrane structure, its presence in the structure of the SOD and its ability to maintain the metallothionein tissue concentrations makes Zn(II) a powerful antioxidant metal (Stefanidou et al., 2006). Since the discovery of the first Zn(II)-based antimicrobial, pyrrithione zinc ($\text{C}_{10}\text{H}_8\text{N}_2\text{O}_2\text{S}_2\text{Zn}$) in 1930 which was used to treat microbial infections of skin and hair, different types of zinc coordination compounds with radioprotective (Bratu et al., 2014), tumor photosensitizers (Roguin, Chiarante, Vior, & Marino, 2019), antidiabetic (Koleša-Dobravc et al., 2018), anticonvulsant (d'Angelo et al., 2008), anti-inflammatory (Deb, Lakshman, Ghosh, Jana, & Paine, 2020), antimicrobial (Damen, Alem, et al., 2022a), antioxidant (Bratu et al., 2014; Damen, Alem, et al., 2022a; Laguta, 2012; Sulpizio et al., 2016), antitumor (Porchia, Pellei, Del Bello, & Santini, 2020), cytotoxicity and DNA-binding (Atabey-Ozdemir et al., 2017; dos Santos, Ely, Dumas, & Moura, 2015), anti-Alzheimer's disease (Prati et al., 2016; Zafar et al., 2021), and in other neglected diseases (Ong, Roy, Andrews, & Gasser, 2018) have been reported. It is important to note that the success rate of metal-containing

compounds is remarkable compared to purely organic compounds for their biological activity (Frei et al., 2020). Accordingly, the pharmacological and toxicological properties of most drugs and biologically active ligands owns enhancement when administered in the form of metallic complexes (Liang et al., 2021; Refat, 2007; Rizzotto, 2012; Samsonowicz & Regulska, 2017). Research reports indicated that the type of the ligand is vital to the desired biological activity of the metal complex (Karges et al., 2021; Kilpin & Dyson, 2013; Liang et al., 2021; Renfrew, 2014).

Flavonoids are polyphenolic plant driven chemical compounds with ideal coordination sites to the metal ions with wide spectrum of pharmacological properties such as antidiabetic (AL-Ishaq, Abotaleb, Kubatka, Kajo, & Büsselberg, 2019; X. Zhang et al., 2015), antiviral, antibacterial (Dai et al., 2019; Y. Xie, Yang, Tang, Chen, & Ren, 2015), anti-inflammatory (He et al., 2018), cardioprotective (Testai, Martelli, Cristofaro, Breschi, & Calderone, 2013), anticancer (Alper et al., 2019), antiaging (Guo, Zhang, Guan, & Zhou, 2019), neuroprotective/antioxidant (Alper et al., 2019; Ayaz et al., 2019; Krych & Gebicka, 2013), antiproliferative (Ragab, Yahya, El-Naa, & Arafa, 2014) and antiestrogenic (Collins-Burow et al., 2012; Collins-Burow, Burow, Duong, & McLachlan, 2000). Chrysin (5,7-dihydroxyflavone) is among the biologically active bidentate flavonoid family ligands. The metal complex of which are reported for their anticancer (Halevas et al., 2020; Nandanwar & Kim, 2019; Spoerlein, Mahal, Schmidt, & Schobert, 2013), inhibiting xanthine oxidase (Lin, Zeng, Zhang, Liao, & Gong, 2017), antioxidant (Gençkal, 2020; Halevas, Mavroidi, & Pelecanou, 2021; Halevas, Mavroidi, Pelecanou, & Hatzidimitriou, 2021), cytotoxic (E Halevas et al., 2021), and antibacterial activities. Also, chrysin is reported as solubility enhancer in solid dispersions (S. H. Lee, Lee, Song, & Han, 2019).

Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) is a broad-spectrum antimicrobial drug used for the treatment of different infections: urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community acquired pneumonia, acute bronchitis and sinusitis (Cuprys et al., 2018; M. N. Patel, Patel, & Joshi, 2013; Saha et al., 2004). The presence of oxygen donor atoms on the 4-oxo and adjacent carboxyl groups gives it the potency to chelate with metal ions (Efthimiadou, Psomas, Sanakis, Katsaros, & Karaliota, 2007).

Metformin hydrochloride (N, N-dimethyl biguanide) is an oral hypoglycemic agent commonly prescribed for the treatment of type II diabetes mellitus (Mahmoud, Ammar, & Sallam, 2017). Also, it has analgesic, antimalarial and antimetabolite therapeutic values. It has two amino groups with the ability to chelate metals (Vasanth, Shekhar, & PV, 2018; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020). Enhancement in the biological activity of metformin-metal complexes has been reported in previous works (Aljazzar, 2021; Dong, Liu, Liang, & Yang, 2018; El-Megharbel, 2015).

1,10-phenanthroline is a classic and one of the most frequently used chelating agent in coordination chemistry, with ideally placed nitrogen atoms, rigid planar structure, and a ligand yielding metal complexes with interesting biological and physiological properties (Abebe & Hailemariam, 2016; Bayeh, Abebe, Thomas, & Linert, 2019; Nunes et al., 2020). It has been reported that the toxicity of 1,10-phenanthroline significantly changes when coordinated to metal ions (Abebe et al., 2020).

The synthesis and biological activity of mixed drug metal complexes employing 1,10-phenanthroline, metformin and quinolones have attracted researchers in recent years due to enhancements in the biological activity after coordination to metal ions (Abebe et al., 2020; Abebe & Hailemariam, 2016; Bayeh et al., 2019; Lawal et al., 2020; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020; Yu, Tang, Yi, Liu, & Li, 2008). On the other hand, the incidence of diseases like microbial infection, oxidative stress and cancer are increasing. Hence, there is a great demand to search for more potent alternative drug sources to alleviate the problem.

Cancer is the leading cause of death worldwide with female breast cancer being the most diagnosed cancer (2.26 million cases) (Ferlay et al., 2021). Chemotherapy is among the extensively applied methods in the fight against breast cancer. The discovery of antiproliferative effects of cisplatin and other platinum-based chemotherapeutic drugs has seen tremendous research on the transition metal-based coordination compounds for their antitumor activities (Buchtík et al., 2012). Cisplatin, oxaliplatin, carboplatin, nedaplatin, heptaplatin and lobaplatin are square planar platinum-based chemotherapeutic drugs approved for human cancer treatment worldwide (Mitra, 2016). However, toxic adverse effects associated with these drugs such as hepatotoxicity, myelosuppression, nausea, neuropathy, nephrotoxicity, ototoxicity, and acquired/inherent drug resistances due to

repeated doses are the main concerns among the various drawbacks of platinum-based chemotherapeutic agents. To overcome the aforementioned drawbacks, many attempts have been made to prepare metal complexes with improved cytotoxic effects against cancer cell lines and decreased negative side effects compared to the platinum-based chemotherapeutic agents (Buchtík et al., 2012). The use of essential metals as central atoms in the coordination sphere of a metal based therapeutic agent has been suggested as one of the possible approaches proposed to decrease metal-related toxicity. Cu(II) complexes have been used as representatives of these endogenous metals because copper is one of the most important trace elements involved in several enzymatic and protein functions in metabolism. Promising activities of copper-based coordination compounds for target-specific next-generation anticancer therapeutics have been reported (Azam, Raza, & Sumrra, 2018); for example Cu(II) complexes of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone (O,O donor) and other nitrogen-containing (N,N donor) ligands such as 2,2'-bipyridine and 1,10-phenanthroline derivatives showed promising cytotoxicity against human breast cancer cell lines (MCF-7) (Buchtík et al., 2012; Johnstone et al., 2016). 1,10-phenanthroline and 2,2'-bipyridine based Cu(II) complexes showed antitumor activity better than cisplatin in both *in-vitro* and *in-vivo* tests (Correia et al., 2015; Nunes et al., 2020; Ruiz-Azuara & E Bravo-Gomez, 2010).

Casiopeinas® are a class of mixed chelate, cationic copper complexes which have well known antineoplastic properties. Recent reports indicated that a group of the Casiopeinas that consists of 1,10-phenanthroline and 2,2'-bipyridine (bipy) Cu(II) square planar complexes have demonstrated antiproliferative and antineoplastic activity in *in vitro* and *in vivo* experiments (Bravo-Gómez et al., 2009; Reina et al., 2021). Inspired by the promising results of Casiopeinas®, we designed the synthesis of Cu(II) based mixed drug metal complexes of N-N-N-N and N-N-O-O donor type ligands with promising cytotoxic activity results in *in vitro* tests. On the other hand, bacterial infections are one of the main causes of death, especially in developing countries (Okeke et al., 2005). Despite the development of broad-spectrum antibiotics over the past 70 years, these life-threatening bacteria continue to cause a high rate of mortality and morbidity. This is the result of the increased antimicrobial resistant bacteria (Kebede, Yihunie, Abebe, Addis Tegegne, & Belayneh, 2022). Leading causes of illness and death such as gastrointestinal, respiratory, sexually transmitted diseases, and nosocomial infections, and the emergence and rapid spread of resistance has seriously jeopardized the management of all these illnesses (Okeke et al., 2005).

Antimicrobial resistance (AMR) refers to the microbe's capacity to fend off the effects of drugs that once effectively treated it. Widespread antibiotic usage is what causes AMR, which is a global issue that may easily cross international borders and spread between continents and poses a severe threat to public health (Moges et al., 2014). It is suggested that the extensive use and misuse of antimicrobials for the last decades have been associated with the explosion of antimicrobial resistance (Christaki, Marcou, & Tofarides, 2020). What gives AMR more apprehension worldwide is, medical procedures such as organ transplantation, cancer chemotherapy, diabetes management and major surgery become very high risk without effective antimicrobials for prevention and treatment of infections (Organization, 2014). On the other hand, the forecasts of the economic costs for AMR are similar to those of a 2 °C rise in global average surface temperature, above preindustrial levels (Roope et al., 2019). Africa in general and east Africa in particular is a vulnerable region to AMR with a high load of infectious diseases (Ayukekbong, Ntemgwa, & Atabe, 2017). A study reported that the burden of AMR is comparable to the combined effect of influenza, tuberculosis and HIV/AIDS. It is also reported that the impact is highest in infants and the elderly. Disturbingly, the study also reported that 39% of all AMR were caused by bacteria that are resistant to last-line antibiotics, making them very difficult or impossible to treat (Cassini et al., 2019). As per the report of World Health Organization (WHO), new resistance mechanisms are emerging and spreading globally, threatening the ability of scientists in the field to treat common infectious diseases, resulting in prolonged illness, disability, and death (Organization, 2014). Besides the existing resistance to antibiotics, the development of new antibiotics is deteriorating at the same time (Nathan, 2020). On the other hand, poor solubility, stability, and side effects are leading to inefficiency to the currently available antibacterial drugs. WHO and numerous other groups and researchers agree that the spread of AMR is an urgent issue requiring a global, coordinated action plan to address. Hence, these combined issues urged to explore new innovative strategies, new means of dealing with bacterial disease (Eleraky, Allam, Hassan, & Omar, 2020). Metal complexes of oxo, nitro and thio-based ligands are among the promising candidates for future metal based antimicrobial agents (Frei et al., 2020).

Oxidative stress is an increase in the steady-state levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Also, it is a decreased antioxidant defense mechanism, and occurs

when the amount of ROS and RNS is more than the defensive system can remove (Wei et al., 2009). As a result, the antioxidant competence is challenged with various problems and which in turn makes the body system unable to maintain redox homeostasis. This condition is, at least in part, associated with the occurrence of several chronic diseases (Görlach et al., 2015). It is suggested to be the main cause for several chronic diseases: adiposity, diabetes and diabetic complications, arthritis, neurodegenerative diseases, cancer, chronic kidney disease, cardiovascular diseases, endothelial cell dysfunction, aging and atherosclerosis (Alfadda & Sallam, 2012; Liguori et al., 2018; Wei et al., 2009). On the other hand, research findings reported that ROS/RNS generation could be induced by hyperglycemia (diabetes mellitus) systematically in multiple organs which in turn causes depression of antioxidant capacity (Mule & Singh, 2018; Nazarian-Samani, Sewell, Lorigooini, & Rafieian-Kopaei, 2018; Sun et al., 2020). In this work, we employed ligands, design and synthesize different metal complexes that could act as anticancer/cytotoxic, antioxidant and antimicrobial.

In a condition where short time and small budget with high certainty of a drug development is required, computational pharmacology is a fast-growing research field focusing on the development of techniques for employing software and databases to generate and analyze molecular, biological and medical data from diverse sources (Ekins, Mestres, & Testa, 2007; Zhou et al., 2020). The methods have been used since the starting of a drug discovery to screen and identify new lead compounds available in molecular libraries (Daina, Michielin, & Zoete, 2017; Jie Dong, Ning-Ning Wang, et al., 2018; Schyman, Liu, Desai, & Wallqvist, 2017) enabling parallel optimization of compound efficacy, drug-like properties and thereby playing a role in improving the quality of drug candidates. In this work, different computational pharmacology tools have been employed to screen a ligand, and predict the bioactivity of the ligands and the metal complexes.

1.1 Statement of the problem

Antimicrobial resistance and cancer are among the top ten health threat and second leading cause of death, respectively, worldwide. Microbial infection, oxidative stress and cancer are closely linked as cancer would be induced by oxidative stress and cancer care is highly affected by antimicrobial resistance. In fact, recently, antibiotics misuse has led to the evolution of superbugs that are resistant to almost all antibiotics. According to WHO report, it is estimated that 700,000

people die each year worldwide due to bacterial infections, and this number is expected to rise to 10 million by 2050 unless efforts are made to curb the bacterial drug resistance or develop new antibiotics. As a result, it is becoming an urgent priority for policy-makers. Similarly, a meta-analysis study on “resistance of *Staphylococcus aureus* to antimicrobial agents in Ethiopia” reported that the level of antimicrobial resistance to almost all of antimicrobial agents: penicillin, cephalosporins, tetracyclines, chloramphenicol, methicillin, vancomycin and sulphonamides ranges from 43 - 77% the highest being amoxicillin . A similar research and a three years’ retrospective study by Alemayehu and his coworkers on “the burden of antimicrobial resistance at tertiary care hospital, southern Ethiopia” reported a remarkable resistance to most of the antibiotics mainly, penicillin (81.8%) and cotrimoxazole (81.1%), for gram-positive bacteria and ampicillin (92.5%), tetracycline (85%) and cotrimoxazole (93.1%), for gram negative bacteria. Besides, it was reported that *S. aureus* and *K. pneumoniae* were the most recurrent isolate from gram-positive and gram-negative bacterial strains, respectively.

On the other hand, cancer continues to be the second cause of death next to heart worldwide, accounting for an estimated 10 million deaths in 2020. In the same period, there were approximately 19.5 million new cancer cases and 685,000 deaths with female breast cancer being the most diagnosed cancer (2.26 million cases). Research findings suggested the involvement of oxidative stress in carcinogenesis due to its tendency to cause injury to cells and induces gene mutation.

Hence, the search for alternative treatment methods or drugs for the multidrug resistant bacterial strains needs special concerted attention. As part of strategies to search for solution to the existing problems, chemotherapy employing metal complexes as alternative therapeutic agents has been for the last decades. In this regard, different researchers reported the antibacterial, antioxidant and cytotoxic or anticancer activities of metal complexes. However, metal complexes that employ chrysin with N, N-donor ligands metformin and 1,10-phenanthroline have never been explored to the best of the researchers’ knowledge.

Flavonoids are polyphenolic plant driven chemical compounds with ideal coordination sites to metal ions and possess wide spectrum of pharmacological properties. However, the synthesis and

biological studies of heteroleptic flavonoid based metal complexes that passes through *in silico* aided ligand screening process have never been reported to best of the researchers' knowledge.

The very wide existence of infectious disease, the alarmingly increasing prevalence of AMR and the increased interest towards essential metal based coordination compounds for cancer treatment are among the major reasons that motivated us to synthesize novel Cr(III), Cu(II) and Zn(II)-metal-based therapeutic agents. Hence, the metal complexes of a flavonoid were designed by employing one or more ligands that bring heterolepticity so as to bring a significant enhancement to the desired biological activity of a given metal complex. On the other hand, the study considered employing a currently working drug molecules metformin and ciprofloxacin with other bioactive oxo and nitro ligands.

1.2 Hypothesis

The biological activity of oxo and nitro ligand are found to be enhanced up on complexation with metal ions. Hence, we hypothesize that Cr(III), Zn(II) and Cu(II) complexes of chrysin and nitro ligands (metformin and 1,10-phenanthroline), and mixed drug Cu(II) and Zn(II) complexes would show better anti-oxidant, antimicrobial and anticancer/cytotoxic activity than the free ligands alone. More importantly, metformin has been serving as a first line drug for patients with type II diabetes and considered as low-cost antioxidant. In addition, chrysin is a phytochemical reported to possess wide spectrum of biological role. These properties of the utilized ligands would help us reduce the toxicity concerns. We believe that the bioactivity of ligands and the metal complexes would show differences on the bases of their structure. Moreover, *in vitro* anti-oxidant, antimicrobial and cytotoxic bioactive flavonoids will show differences in terms of toxicity, drug likeness, and pharmacokinetic parameters. Hence, preliminary *in-silico* screening of the requested flavonoid-based ligands was suggested to be important.

1.3 Objectives of the study

1.3.1 General objectives

The main objective of this work was to synthesize and assess the biological activity of Cr(III), Cu(II) and Zn(II) complexes of metformin and O, O and N, N-donor ligands.

1.3.2 Specific Objectives

The specific objectives of the dissertation were:

- To screen and employ a bidentate ligand among structurally similar and biologically active flavonoid derivatives using different computational approaches
- To synthesize Cr(III), Cu(II), Zn(II) heteroleptic complexes of 1,10-phenanthroline and chrysin
- To synthesize Cr(III), Cu(II), Zn(II) heteroleptic complexes of metformin and chrysin
- To synthesize mixed drug Cu(II) heteroleptic complexes of 1,10-phenanthroline with metformin
- To synthesize mixed drug Cu(II) heteroleptic complexes of 1,10-phenanthroline and ciprofloxacin
- To synthesize mixed drug Zn(II) heteroleptic complexes of 1,10-phenanthroline and metformin
- To characterize the synthesized heteroleptic metal complexes using UV-Vis, FTIR, TGA/DTA, MS, XRD and SEM-EDX
- To study the *in vitro* cytotoxicity of the synthesized metal complexes against breast cancer cell lines (MCF-7) and compare with a standard, cisplatin
- To study the *in vitro* antimicrobial activity of the synthesized metal complexes and compare with the standard ciprofloxacin
- To evaluate the *in vitro* antioxidant activities of synthesized metal complexes against DPPH free radical and compare with the standard ascorbic acid
- To determinate the *in-silico* toxicity, pharmacokinetics and drug likeness of the ligands.
- To study the quantum chemical, vibrational and absorption properties of the ligands and metal complexes
- To carry out molecular docking study of the synthesized metal complexes against selected bacterial strains and breast cancer proteins

1.4 Significance of the study

Oxidative stress, antimicrobial resistance and cancer are interrelated and continue to be the burden of all age group worldwide. Professionals in the field of pharmacy, chemistry and biology have been working to provide wide-spectra metal therapeutics. In this work, we have reported a ligand screening protocol using webserver and computational tools (DFT and molecular docking) which

can change the usual ligand selection practice among inorganic chemists. More importantly, heteroleptic metal complexes with promising cytotoxicity, antibacterial and antioxidant activity are reported. The reported metal complexes have diverse architectural and geometrical features to be introduced as new complexes in to coordination chemistry and stoichiometry. Hence, the output of the work will give directions to chemical biologists, pharmacists, biologists, chemists and environmentalists. In this work, DFT and DT-DFT computational methods were used to shed light on the experimental findings. Hence, the association of quantum chemical parameters and *in vitro* antibacterial/antioxidant activities are well presented. Accordingly, the reported findings could be used in the teaching materials of graduate and postgraduate programs. Overall, industries and researchers in the same or related fields are immediate beneficiaries of the work.

1.5 Delimitation of the study

In this work, we have synthesized metal complexes of selected oxo/nitro ligands and metformin in heteroleptic fashion using three different metal ions (Cr(III), Cu(II) and Zn(II)). There are other biologically important metals (Fe, Mo and W) and it would be good in terms of data, structure, stoichiometry and bioactivity, if we were able to include more metals. However, due to time bound and budget restriction of the program, we synthesized metal complexes of only three metals. Moreover, the synthesized metal complexes were subjected for cytotoxicity, antimicrobial and antioxidant evaluations. On the basis of the results obtained from the tested activities, it would be a great opportunity to include the antidiabetic and other biological activities. However, due to the absence of the instruments and reagents, this work was limited to cytotoxicity, antimicrobial and antioxidant evaluations.

2. LITERATURE REVIEW

2.1 Therapeutic potentials of metal complexes

A number of inorganic elements play important roles in the biochemical processes, therapy and diagnosis (Mukherjee & Sadler, 2007; Ronconi & Sadler, 2007). This is due to the fact that they exist in coordination to a biomolecule or appropriate ligand which gives them unique structural and chemical properties compared to purely organic compounds (Karges et al., 2021). Many organic compounds used as medicine are evidenced not to have a purely organic mode of action; some are metal-activated or bio-transformed by metal ions, others have a direct or indirect effect on metal ion metabolism, confirming the essential of a metal ion presence in a biologically active molecule design (Ronconi & Sadler, 2007). As part of research interest in medicinal coordination chemistry, the number and type of ligand are essential for the activity of a metal complex. Hence, employing multifunctional ligands for metal-based therapeutic agents offer many exciting possibilities, including targeting specific tissues, membrane receptors, or endogenous molecules, and can play an integral role in modulating the potential toxicity of a metallodrug to achieve success in areas of diagnosis and therapy (Ronconi & Sadler, 2007). Copper, zinc, iron and manganese are incorporated into metalloenzymes and play catalytic roles facilitating numerous biochemical reactions vital for life (Saeed Ahmad, Isab, Ali, & Al-Arfaj, 2006).

Coordination chemistry has grown with rich diversity that provides exciting prospects for the design of novel therapeutic agents with unique mechanisms of action (Ronconi & Sadler, 2007). Control of side-effects and specific targets (tissues, organs, or cells) for the desired activity are key in the design of biologically active metal complex. Hence, understanding the specific role of a given metal changes the usual term to classify metal as toxic and non-toxic. This is due to the fact that toxicity strongly depends on concentration, some of the elements that are classified as toxic are found to be therapeutically effective within narrow concentration ranges, and biologically-essential elements can become toxic at high doses (Ronconi & Sadler, 2007). As part of research interest in medicinal coordination chemistry, the number and type of ligand are essential for the activity of a metal complex (Mukherjee & Sadler, 2007). Hence, employing multifunctional ligands for metal-based therapeutic agents offer many exciting possibilities, including targeting specific tissues, membrane receptors, or endogenous molecules, and can play an integral role in

modulating the potential toxicity of a metallodrug to achieve success in areas of diagnosis and therapy (Ronconi & Sadler, 2007).

Based on their unique electronic properties, reactivity, and stereochemical features, metal-containing compounds exhibit the ability to interact with biomolecules and biological systems through mechanisms of action often not readily accessible to organic molecules. Their excellent structural versatility, three-dimensionality, ligand exchange reactions, and redox, catalytic, and photophysical activities all make them attractive to exploit in coordination complexes for use in medicine and biotechnology. Importantly, the ability of metal complexes to promote new mechanisms of action may lead to the discovery of new treatments and therapies. These intriguing properties allow metal complexes to complement the existing pharmaceutical landscape, which is predominantly composed of organic molecules (Claudel et al., 2020; Efthimiadou et al., 2007; Farrell, 2003; Karges et al., 2021; Loginova et al., 2020; Rizzotto, 2012; C. X. Zhang & Lippard, 2003). Taking advantage of these, various metal-containing compounds have been clinically approved for a wide range of therapeutic applications. Increasing efforts not only to identify bioactive metal complexes, but also to fully understand their mechanisms of action are critical to their progress and greater acceptance in the medical community. Further research into therapeutic applications of metal complexes reveals new compounds with increased potency.

2.2 Therapeutic potentials of Cr(III), Cu(II) and Zn(II) metal complexes

Transition metal complexes (TMC) have been explored for their healing potential since the beginning of 20th century. TMCs have been widely used for chemical and physical sciences due to their unique electronic and stereochemical properties and hence they offer a rich platform for being used as therapeutic agents (Amarsy et al., 2022; Karges et al., 2021; Liang et al., 2021). Unique properties of transition metal complexes: molecular geometries, ligand exchange, redox, catalytic, and photophysical reactions, gives them the potential to interact and react with biomolecules in unique ways and by distinct mechanisms of action (Liang et al., 2021; Renfrew, 2014).

Trace elements play key roles in many physiological processes, in particular immunity and metabolism. *In vitro* and *in vivo* studies have witnessed that complexes or coordination compounds of metals like Cr, Mn, Mo, Cu, Co, Zn, W and V have significant role in the treatment of several

illnesses such as type II diabetes (T2D), oxidative stress, microbial infections, and cancer etc. (Pandey et al., 2012).

Therapeutic use and applications of metal complexes are of rising clinical and commercial significance, being in the front line of choices for the treatment of various disorders (Azam et al., 2018; Farrell, 2003). The search and synthesis of metal-drug complexes using drug molecules as a ligand is growing, and considerable attention is being paid to metallo-drugs (Lawal et al., 2020; Saha et al., 2004; Y. Wang et al., 2010)(M. N. Patel, Patel, & Joshi, 2013)(Abbas, Kamel, & Khamais, 2019; Dunn & Peters, 1995; Pernicova & Korbonits, 2014; Vasantha et al., 2018; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020). It is well known that metal complexes of oxo, nitro and thio-based ligands have considerable biological activities: antibacterial, anti-oxidant, antifungal, antiviral, anticancer, etc. Typically, complex compounds of drugs and drug-based Schiff bases have been synthesized to increase the bioactivity (Al-Qadsy et al., 2020; Azam et al., 2018; Refaat & El-Din, 2018). This type of compound can act in two different ways: (i) the metal–organic complex exhibits the biological activity over a biological target, and (ii) the complex act as a carrier to transport the organic molecule to the active site and produce the therapeutic activity. In both cases, the synergic action of the components improves the biological activity while minimizing the side effects (Villamizar-Delgado, Porras-Osorio, Piñeros, et al., 2020). On the other hand, the synthesis of metal complexes of bioactive molecules using ligands of plant origin are showing potential interest in metal based therapeutic agents in free radical scavenging, antimicrobial, and antidiabetic activity than the organic molecule alone (Abebe et al., 2020; Bratu et al., 2014; Laguta, 2012).

Copper(II) and zinc(II) metals are endogenous metals having enhanced antimicrobial and antioxidant bioactivities with promising features as metallo-drugs (Sulpizio et al., 2016). The higher antioxidant, anti-inflammatory and tumor cell cytotoxicity activities of Naringin–Cu (II) complex, $[\text{Cu}(\text{Naringin})(\text{CH}_3\text{OH})_2]^+$ (Vinayagam & Xu, 2015), cytotoxicity and DNA binding study of Cu(II) and Zn(II) complexes of flavonoids (Quercitrin, Myricitrin and Rutin) (Atabey-Ozdemir et al., 2017) with intercalative mode of DNA binding, nuclease and DNase mechanism of actions, higher antioxidant activity of Zn(II)(Butien) (66%) than that of Butien (19%)(Sulpizio et al., 2016), and others have been reported. This inferred the enhanced biological activity of metal

complexes relative to the free ligands alone. Moreover, Cu(II) coordination compounds possess several biological activities: antivirals, fungicides, pesticides which corresponds to the chelating ability and positive redox potential of the metal (Bitu, Hossain, Zahid, Zakaria, & Kudrat-E-Zahan, 2019). Research works reported the ability of Cu(II) to induce cytotoxicity on quinolones (Yu et al., 2008), cytotoxicity of Cu(II) chelates (Liberta & West, 1992; Minkel, Chan-Stier, & Petering, 1976; Rajendiran et al., 2007), and the antimicrobial activity of a distorted square planar Cu(II) complex derived from the Schiff base of metformin and 2-pyridine carbaldehyde (J. Gao, 2007). The great biochemical action of Cu(II) is suggested to be due to either binding to nucleic acids via its coordination to ligands or binding to albumins and proteins (Lawal et al., 2020). Zn(II) is a biologically significant element involved in DNA synthesis and repair. It plays a significant role in the transcription factor function and translation of the genetic message. Cell survival and tissues protection are highly depended on the presence of Zn, in particular it is critical for cellular proliferation, differentiation, apoptosis, immunity and reproduction (Pellei, Del Bello, Porchia, & Santini, 2021). As a Lewis acid, the most common binding site is nitrogen of histidine, followed by sulfur of cysteine, oxygen of aspartate/glutamate, but also carbonyl oxygen of peptide bond, carbonyl oxygen of asparagine/glutamine, and hydroxyl oxygen of tyrosine (Pellei et al., 2021).

Chromium (Cr) is ubiquitous metal considered to be biologically relevant (Vincent, 2004), both in animal feeding and human nutrition in its trivalent state (C. Wang et al., 2018a). It is involved in the metabolism of carbohydrates, lipids, and proteins mainly by increasing the efficiency of insulin, although fortunately Cr(III)-deficiency is difficult to achieve (Kralovec et al., 2009; Pechova & Pavlata, 2007; Vincent, 2004). After the first suggestion that a biological Cr(III) compound could act as a nutritional enhancement to glucose metabolism was traced in 1959 (Pechova & Pavlata, 2007) by Schwarz and Mertz, different Cr(III) supplements have been searched with Cr(III)-picolinate being the oldest one (Pandey et al., 2012). In 1977 the role of Cr in human nutrition is reported on a patient with impaired glucose tolerance (Pechova & Pavlata, 2007). The bioavailability and efficacy of Cr(III) towards diabetes is found to depend on the ligands chelating to Cr (Peng & Yang, 2015; Ulas et al., 2015). *In vitro* and *in vivo* findings have shown that trivalent Cr(III) provides biological benefits: enhancing the antioxidant enzyme defense system, facilitate insulin signaling/insulin sensitivity (Anderson et al., 1997; Hao et al., 2011), suppress free radical formation and inhibit the protein glycosylation (C. Wang et al., 2018a),

decreasing serum glucose, lowering blood lipids (Anderson et al., 1997), reducing cholesterol and triglyceride levels in the blood (C. Wang et al., 2017), lowering glycated hemoglobin (El-Megharbel, 2015), and α -glucosidase inhibitory activity (C. Wang et al., 2018a). The synthesis and strong antioxidant activity of the Cr(III)-quercetin complex, $[\text{Cr}(\text{quercetin})_2\text{ClH}_2\text{O}]\cdot 4\text{H}_2\text{O}$, Cr(III)-morin, $[\text{Cr}(\text{morin})(\text{H}_2\text{O})_2]\text{Cl}\cdot \text{H}_2\text{O}$ and Cr(III)-rutin, $[\text{Cr}_2(\text{rutin})(\text{H}_2\text{O})_8]\text{Cl}_4$ than the free flavonoids were reported (Chen, Sun, Liang, & Song, 2009). However, its biological activity is largely found to depend on the ligand molecule coordinated to it. And hence, the specific ligand environment around Cr(III) could affect the biological activity and bioavailability of Cr(III) (F. Li et al., 2012; Peng & Yang, 2015). Hence, developing novel chromium complexes with less toxic organic ligands is of great interest.

2.3 Cancer, antimicrobial resistance and oxidative stress

2.3.1 Cancer

Cancer is the speedy creation of anomalous cells that grow beyond their usual boundaries and become the second leading cause of death (Piñeros, Mery, Soerjomataram, Bray, & Steliarova-Foucher, 2021). Breast cancer is the third most frequent cancer, major health burden and one of the most common malignant diseases in women worldwide (Ban & Godellas, 2014; Rojas & Stuckey, 2016). Among the attempts made to treat breast cancer, chemotherapy has been extensively studied next to surgery and radiation. Platinum based metallodrugs are among the major chemotherapeutic drugs employed to the treatment of cancer (Johnstone, Park, & Lippard, 2014). Cisplatin, oxaliplatin and carboplatin, nedaplatin, heptaplatin and lobaplatin are square planar platinum-based chemotherapeutic drugs used worldwide (Buchtík et al., 2012; Johnstone et al., 2016). They have been used and are effective for the treatment of numerous human cancers. These anticancer agents have inspired the development of new metal-based anticancer therapeutic agents. However, the efficacy of current systemic and targeted therapies, safety, toxicity and the development of resistance are concerning limitations related to platinum based anticancer agents (Popolin et al., 2017; Prouse, 2010; Rivera & Gomez, 2010). Drug resistance, allergic reactions, decreased immunity, severe kidney problems, gastrointestinal disorders, and hearing loss are some of the reported limitation of using cisplatin (Ciarimboli, 2014). Moreover, the neurotoxicity, nephrotoxicity and emetogenesis of platinum based anticancer agents are reported (Buchtík et al., 2012; C. X. Zhang & Lippard, 2003). The toxicity profile together with the development of resistance urged the need to prepare other transition metal-based complexes with concomitant

cytotoxicity enhancement towards human cancer cell lines and decreased negative side-effects (Buchtík et al., 2012).

The use of essential metals as a central metal in the coordination sphere of a metal therapeutic agents is suggested to be a possible approaches to decrease metal related toxicity (Buchtík et al., 2012). Copper(II) complexes as representatives of these essential metals attracted the focus of many researchers (Atabey-Ozdemir et al., 2017; Krasnovskaya et al., 2020; Lawal et al., 2020; Nunes et al., 2020; Rajendiran et al., 2007), because copper is one of the most important trace elements next to iron and zinc and involved in the function of several redox active metalloenzymes (Buchtík et al., 2012). Promising activities of copper-based coordination compounds for target-specific next-generation anticancer and non-steroidal anti-inflammatory therapeutics have been reported (Azam et al., 2018). Cu(II) complexes of 2-phenyl-3-hydroxy-4(1H)-quinolinone (O,O donor) and other nitrogen containing (N,N donor) ligands such as 2,2'-bipyridine and 1,10-Phenanthroline derivatives were synthesized and evaluated against human breast cancer cell lines (MCF) in which over all promising result for cytotoxicity were reported (Buchtík et al., 2012). 1,10-phenanthroline and 2,2'-bipyridine based Cu(II) complexes are reported to show antitumor activity *in-vitro* and *in-vivo* experiments (Correia et al., 2015; Nunes et al., 2020; Ruiz-Azuara & E Bravo-Gomez, 2010) better than that of cisplatin (Correia et al., 2015). Casiopeínas are a group of 1,10-Phenanthroline and 2,2'-bipyridine (bipy) based Cu(II) square planar complexes with N-N-O-O type coordination (Bravo-Gómez et al., 2009). Remarkably, a bipyridine analog and N-N-O-O type square planar Cu(II) complex, [Cu(4,4'-dimethyl-2,2' bipyridine)(acetylacetonate)]NO₃ is in clinical trials from the group (Reina, Contreras, De Paz, Ruiz-Azuara, & Ayala, 2022). It is also reported that the organic motifs, framework, and the donor atom set played a crucial role for a copper-based complex to exhibit anticancer and anti-inflammatory nature (Azam et al., 2018). More importantly, the possible mechanisms for anticancer activities of Cu(II) complexes: DNA interaction, mitochondrial impairment, proteasome inhibition, and ROS genera have been proposed (Nunes et al., 2020). The promising findings and the search for new Cu-based metal therapeutics that would be used as chemotherapy for breast cancer initiated us to design and synthesize a Cu(II) based mixed drug metal complexes of N-N-N-N and N-N-O-O donor type ligands.

2.3.2 Antimicrobial resistance

The widespread use, overuse, and misuse of antibiotics for the last decades have been associated with the explosion of antimicrobial resistance (AMR) (Buchy et al., 2020; Prestinaci, Pezzotti, & Pantosti, 2015). It is increasingly a serious public health threat, and becoming worldwide problem (Christaki et al., 2020; Moges et al., 2014). Hence, the wide availability of effective antibiotics is under threat, endangering modern health care (Roope et al., 2019): organ transplantation, cancer chemotherapy, diabetes management and major surgery (Organization, 2014). It is reported that an estimated 700,000 people have died each year worldwide. As a result, it is becoming an urgent priority for policy-makers (Willyard, 2017). The burden of AMR is comparable to the combined effect of influenza, tuberculosis and HIV/AIDS. Disturbingly, a study finding showed that 39% of all AMR were caused by bacteria that are resistant to last-line antibiotics, making them impossible to treat (Lusti-Narasimhan, Pessoa-Silva, & Temmerman, 2013). There is a rise in resistance to antibiotics, and at the same time the development of new antibiotics is deteriorating (Nathan, 2020). On the other hand, poor solubility, stability, and side effects are leading to inefficiency to the currently available antibacterial drugs (Eleraky et al., 2020). These combined issues urged us to explore innovative strategies of dealing with bacterial disease. The success rate of metal-containing compounds compared to purely organic compounds as antibacterial and antifungal activity is becoming remarkable (Frei et al., 2020). The pharmacological and toxicological properties of most drugs own enhancement when administered in the form of metallic complexes (Refat, 2007). The synthesis and biological activity of mixed drug metal complexes employing 1,10- phenanthroline, metformin and quinolones have been attracted researchers in recent years due to enhancements in the biological activity after coordination to metal ions (Abebe et al., 2020; Abebe & Hailemariam, 2016; Bayeh et al., 2019; Lawal et al., 2020; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020; Yu et al., 2008).

2.3.3 Oxidative Stress

Free radicals, reactive oxygen/nitrogen species (ROS/RNS) are responsible to induce different chronic diseases including diabetes and cancer. They are generated either from external factors or as byproducts of biochemical reactions (Refaat & El-Din, 2018). The formation of ROS/RNS are scavenged by molecules which are antioxidant in nature and inhibit the formation of ROS (Neha, Haider, Pathak, & Yar, 2019). Human beings have three isoforms of a protein, superoxide dismutase (Zu/Zn-SOD, M-SOD and extracellular) which are natural antioxidants. They catalyzes

the disproportionation of superoxide anion ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2), which is then detoxified to oxygen and water by catalase or glutathione peroxidase (Asakura & Kitahora, 2018). The role of exogenic phytochemicals/ligands and their metal complexes as antioxidant have been implicated. The higher anti-oxidant activity of Cu and Zn complexes of oxo-ligands, isoliquiritigenin and butein relative to the free ligands were reported (Sulpizio et al., 2016). Encouraging antioxidant and antifungal activities of Co, Ni and Cu-based complexes of oxo, nitro and thio-ligands were reported (Asakura & Kitahora, 2018). Similarly, a review work by (Samsonowicz & Regulska, 2017) reported that Cr(III)-quercetin complex has high electron donating and thereby high oxidant activity than quercetin alone. Also, more effective radical scavenging activity of Cr(III)-, Fe(II)-, Cu(II)-, Zn(II)- and V(IV)-rutin complexes than free ligand are reported which may be due to the additional superoxide dismutating centers due to the introduction of metal center (Samsonowicz & Regulska, 2017).

Increased ROS/RNS and decreased antioxidant potential has been inferred to associates with the incidence of T2D (Liguori et al., 2018). In this regard, dietary antioxidants are thought to have potential capacities to prevent ROS induced diseases. Because, antioxidant reduces the occurrence of different disorders like: aging, cancer, diabetes, inflammation, liver disease, cardiovascular disease, cataract and nephrotoxicity and neurodegenerative disorders (Görlach et al., 2015). As a strategy to combat the chronic and prevalent disease, the design of metal-based antidiabetic agents from ligands that have anti-oxidant activity will be promising approach. The anti-oxidant properties of metal complexes of oxo-ligands such as flavonoid based complexes of Cr(III), Zn(II) and Cu(II) are reported (Chen et al., 2009; Panhwar & Memon, 2014b; Porkodi & Raman, 2018; Sulpizio et al., 2016), and better anti-oxidant activity of the metal complexes were reported. Studies revealed the very strong association between diabetes and oxidative stress (Görlach et al., 2015; He et al., 2018; Liguori et al., 2018). On the other hand, molecules that have anti-oxidant activity are found to show anti-diabetic activity. Hence, working with molecules that have anti-oxidant activity will be beneficial to synthesize potential antidiabetic agents. In this work, we aim to evaluate the antioxidant activities of heteroleptic nitro- and oxo-ligands based complexes of the above-mentioned metal ions. On the other hand, the synthesis and biological activities of mixed drug metal complexes employing 1,10-phenanthroline, metformin and ciprofloxacin have been attracted researchers in recent years due to enhancements in the biological activities after

coordination to metal ions (Abebe et al., 2020; Abebe & Hailemariam, 2016; Bayeh et al., 2019; Lawal et al., 2020; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020; Yu et al., 2008).

2.4 Synthesis and characterization of mixed-ligand Cr(III), Cu(II) and Zn(II) metal complexes

2.4.1 Synthesis and biological activity of metformin-based metal complexes

Metformin (N, N-dimethyl biguanide (Figure 1)), originally extracted from *Galega officinalis* in the 1920s, is an oral hypoglycemic agent in clinical use for over half a century commonly prescribed for the treatment of type II diabetes mellitus (Aljazzar, 2021; Mahmoud et al., 2017). It has also acclaimed therapeutic values as analgesic, antimalarial and antimetabolite for organisms that inhibit the metabolism of folic acid (Bailey, 2017). Chemically, it is a hydrophilic base that exists as a cationic species at physiological pH (Kim, 2021). Pharmaceutically, it exists in the form of metformin hydrogen chloride which is freely water soluble, and soluble in acetone, chloroform and ether (Abbas et al., 2019). Metformin (Figure 1) is a good candidate for metallo-drug coordination that can bind to metal ions in bidentate coordination mode through its two amino groups (Dunn & Peters, 1995; Pernicova & Korbonits, 2014; Vasantha et al., 2018; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020) as shown in Figure 2 and Figure 3.

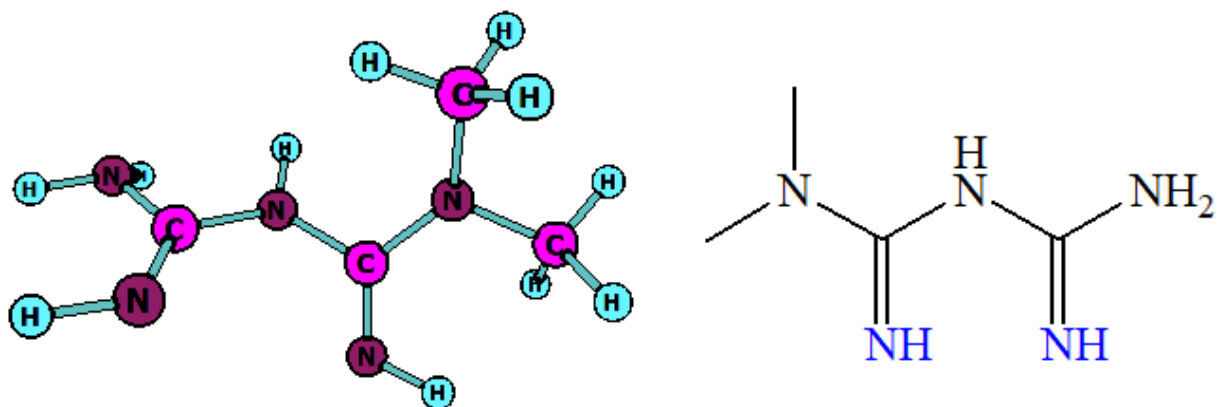


Figure 1. 3D and 2D-structural representation of metformin

A metal-drug of Co(II) and Cu(II) geometrically octahedral coordination compounds were synthesized using mixed ligand mefenamic acid and metformin (**1**). The UV-Vis spectra of the ligands (metformin and mefenamic acid) were found to be unaffected (220 nm, $\pi \rightarrow \pi$ transition) upon coordination to Cu(II) and Co(II) metal centers. The reported metal complexes are found to be octahedral in geometry. Biologically, the Co(II) analog showed enhanced antibacterial activity

against gram positive bacteria (Abbas et al., 2019). Ramadan and his coworkers reported the synthesis of mixed ligand Cu(II) complexes consisting of a homoleptic 2:1 ratio of metformin to Cu(II) (**2**), and heteroleptic 1:1:1 ratios employing metformin with L-glycine (**3**) or iminodiacetic acid (**4**) (Figure 2). Three d→d electronic transitions: $dxz, dyz \rightarrow dx^2-y^2$, $dxy \rightarrow dx^2-y^2$ and $dz^2 \rightarrow dx^2-y^2$ are usually experienced for square-pyramidal Cu(II). These transitions are observed at 425 nm ($dxz, dyz \rightarrow dx^2-y^2$) and broad peaks from 550 to 700 nm which are associated to superimposed $dxy \rightarrow dx^2-y^2$ and $dz^2 \rightarrow dx^2-y^2$ transitions (Ramadan et al., 2020). The reported compounds (**2-4**) presented in Figure 2 showed excellent catalytic performance toward the air conversion of 3,5-di-tertbutylcatechol to 3,5-di-tertbutylquinone which is biologically significant (Ramadan et al., 2020). Moreover, the crystal system and space group of **2**, **3**, and **4** were reported to be triclinic, orthorhombic and monoclinic with P-1, P 2₁ 2₁ 2₁, and P121/c1 space groups respectively for complexes **2**, **3**, and **4**.

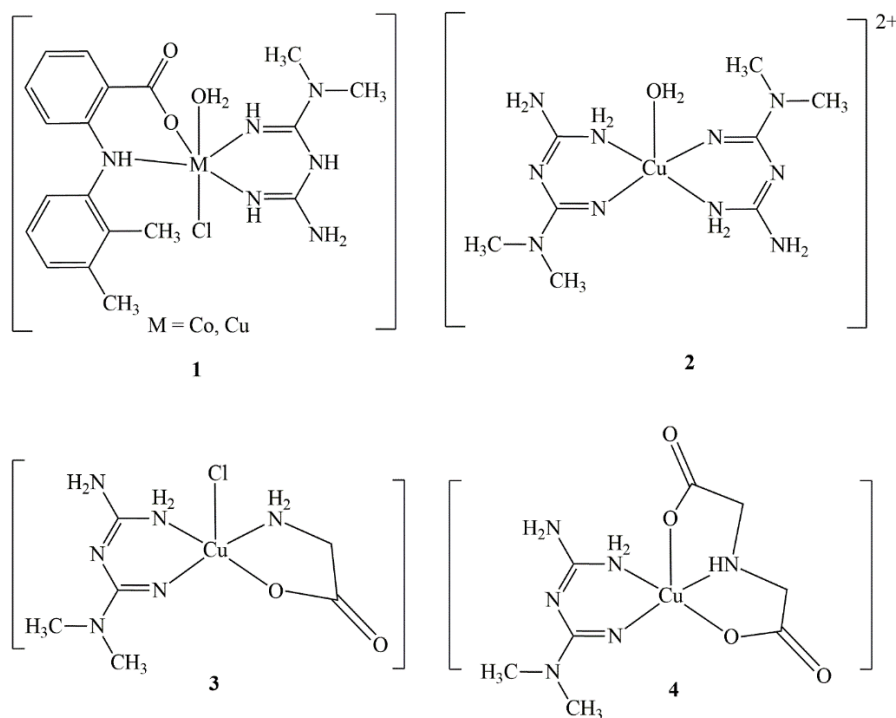


Figure 2. Reported structures of metformin containing Cu(II) complexes adopted from (Ramadan et al., 2020)

Very recently, metformin based antihyperglycemic Co(II), Ni(II), Cu(II) and Zn(II) coordination compounds were synthesized. The single crystals structures were reported as [Co(C₄H₁₁N₅)₃]Cl₂

.2H₂O, [Ni(C₄H₁₁N₅)(C₄H₁₀N₅)]Cl.H₂O, [Cu(C₄H₁₁N₅)₂]Cl₂.H₂O and [Zn(C₄H₁₂N₅)Cl₃] (Villamizar-Delgado, Porras-Osorio, Piñeros, et al., 2020). A spectral characterization study by Aljazzar reported the synthesis of metformin-based zinc (5) and chromium (6) heteroleptic complexes with tetrahedral and octahedral geometries, respectively. The crystal system of the complex (5) was reported to be monoclinic with P2₁/c space group. Moreover, the study reported that the coordination of metformin to the metal centers via N atom of imino (–C=NH) groups (Aljazzar, 2021). *In-vivo* anti-diabetic potentials of metformin based homoleptic octahedral Cr(III) complexes (7 and 8) synthesized from methanolic solutions of metformin and CrCl₃. 6H₂O in 1:2 and 1:3 metal to ligand ratios, respectively were studied for compound 7 and 8. The reported compounds showed enhanced blood sugar lowering capacity than metformin alone. The XRD and SEM analysis of complex 7 was reported to be amorphous structure of the complex and uniform nature of the complex with variant grain sizes and shapes.

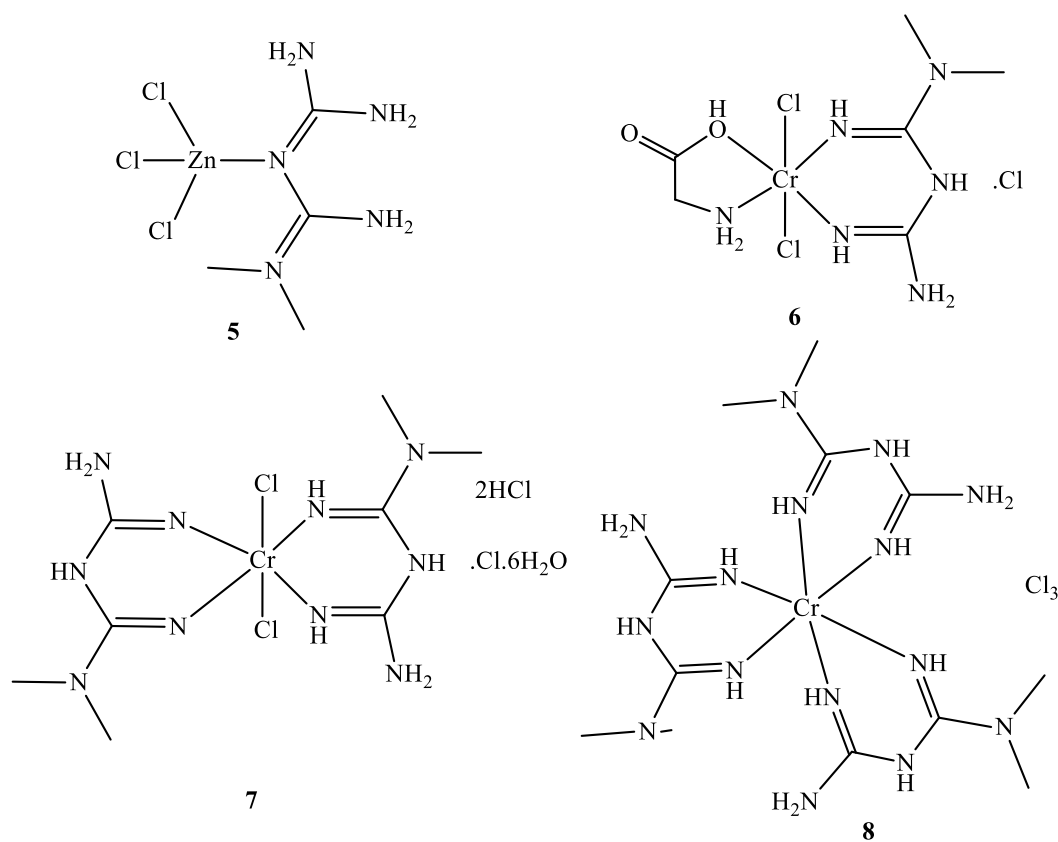


Figure 3. Metformin and glycine amino acid heteroleptic complexes of Cr(III) and Zn(II)(Aljazzar, 2021).

2.4.2 Synthesis and biological activity of quinoline based metal complexes

Quinolones (**9**) are a group of synthetic antibacterial agents structurally related to the first analog nalidixic acid (**10**), which are very active against aerobic Gram-negative microorganisms but less active against Gram-positive microorganisms (Da Silva, De Almeida, De Souza, & Couri, 2003; M. N. Patel et al., 2013). Pharmacologically, they are used to treat urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, sexually transmitted diseases, prostatitis, acute bronchitis and sinusitis. Fluoroquinolones (**11**) are antibacterial drugs which are representatives of quinolones with 4-oxo-1,4-dihydroquinoline skeleton. They have been reported to possess activity against wide range of Gram-positive and Gram-negative bacteria (Patel, Chhasatia, & Gandhi, 2009; Refat, 2007). It has been also reported that the mechanism of action of quinolones results from targeting *topoisomerase IV* and targeting *DNA gyrase*, respectively for gram-positive and gram-negative bacteria. Hence, they are commonly used to treat infections: urinary tract and respiratory infections (Efthimiadou et al., 2007; Lawal et al., 2020; Saha et al., 2004).

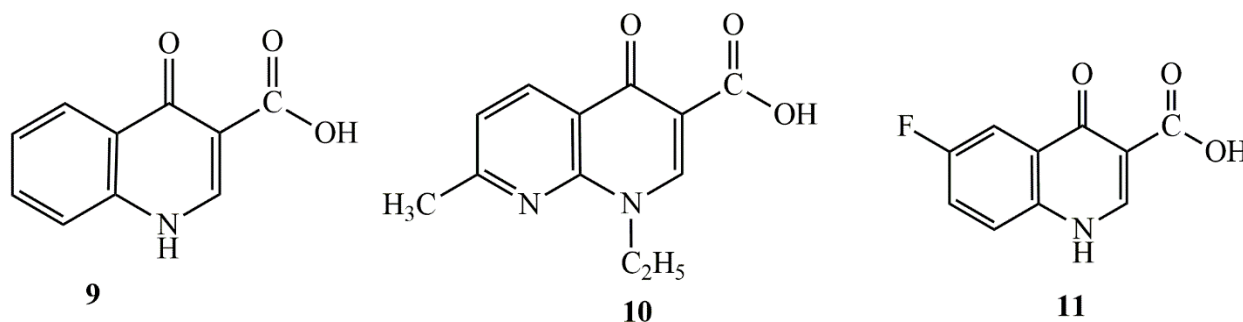


Figure 4. Structures of quinolone (**9**), nalidixic acid (**10**) and fluoroquinolones (**11**)

Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) (Figure 5) is a family of fluoroquinolones which is a broad-spectrum antimicrobial drug used for the treatment different infections: urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community acquired pneumonia, acute bronchitis and sinusitis (Cuprys et al., 2018; M. N. Patel et al., 2013; Saha et al., 2004).

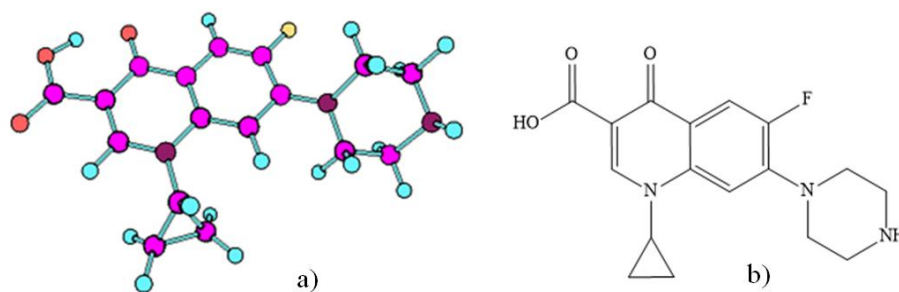


Figure 5. 3D (a) and 2D(b)-structural representation of ciprofloxacin

The presence of oxygen donor atoms on the 4-oxo and adjacent carboxyl groups (Figure 5) gives ciprofloxacin the potency to chelate with metal ions (Efthimiadou et al., 2007). Modified pharmacological and toxicological properties are possessed by metal-drug complexes than the drugs alone. As a result the coordination chemistry, crystal structure and antibacterial activities of homoleptic and heteroleptic metal complexes of ciprofloxacin with metal ions: Cu(II) (M. N. Patel et al., 2013) (Yu et al., 2008) (M. N. Patel et al., 2013) (Y. Wang et al., 2010) (Lawal et al., 2020), Co(II) (Patel et al., 2009) (Yu, Tang, Yi, & Liu, 2009), Ni (II) (Yu, Chen, Liang, Zhou, & Fun, 2006), Mg(II) (Yu et al., 2006), Fe(III) (Yu et al., 2009) have been reported, being Cu(II) complexes the most dominantly studied. Some of the reported structures are presented in Figure 6.

The hydrothermal synthesis Cu(II) complex (**12**) from aqueous solution of $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ metal salt precursor and ciprofloxacin in ethanol at 110°C , its antibacterial activities, and crystal structure has been reported. The antibacterial activity of the complex was reported to be stronger than ciprofloxacin against *Pseudomonas aeruginosa* but similar activities against *Staphylococcus aureus*, *E. coli* and fungal strain, *Candida albicans* (Yu et al., 2008). Its structural analogs for which one of the ciprofloxacin substituted by N, N or N, O donor ligand are reported (**13-15**). Complex **14** (**Cu1 -Cu7**) was prepared from methanolic solution of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, the ligand and methanolic solution of ciprofloxacin in the presence of CH_3ONa in 1:1:1 ratio by adjusting the pH at 6.8. The structure of the complexes were confirmed to be distorted square pyramidal as observed in the UV-Vis spectrum at 600 nm which is associated to the $d \rightarrow d$ transitions of the d^9 system of the metal center. The antibacterial activity of the Cu(II) complexes against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*); and three Gram-negative (*Serratia marcescens*, *Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains revealed that complexes **Cu1** -

Cu4 were more potent against the tested microorganisms than complexes **Cu5** - **Cu7** and the free ciprofloxacin.

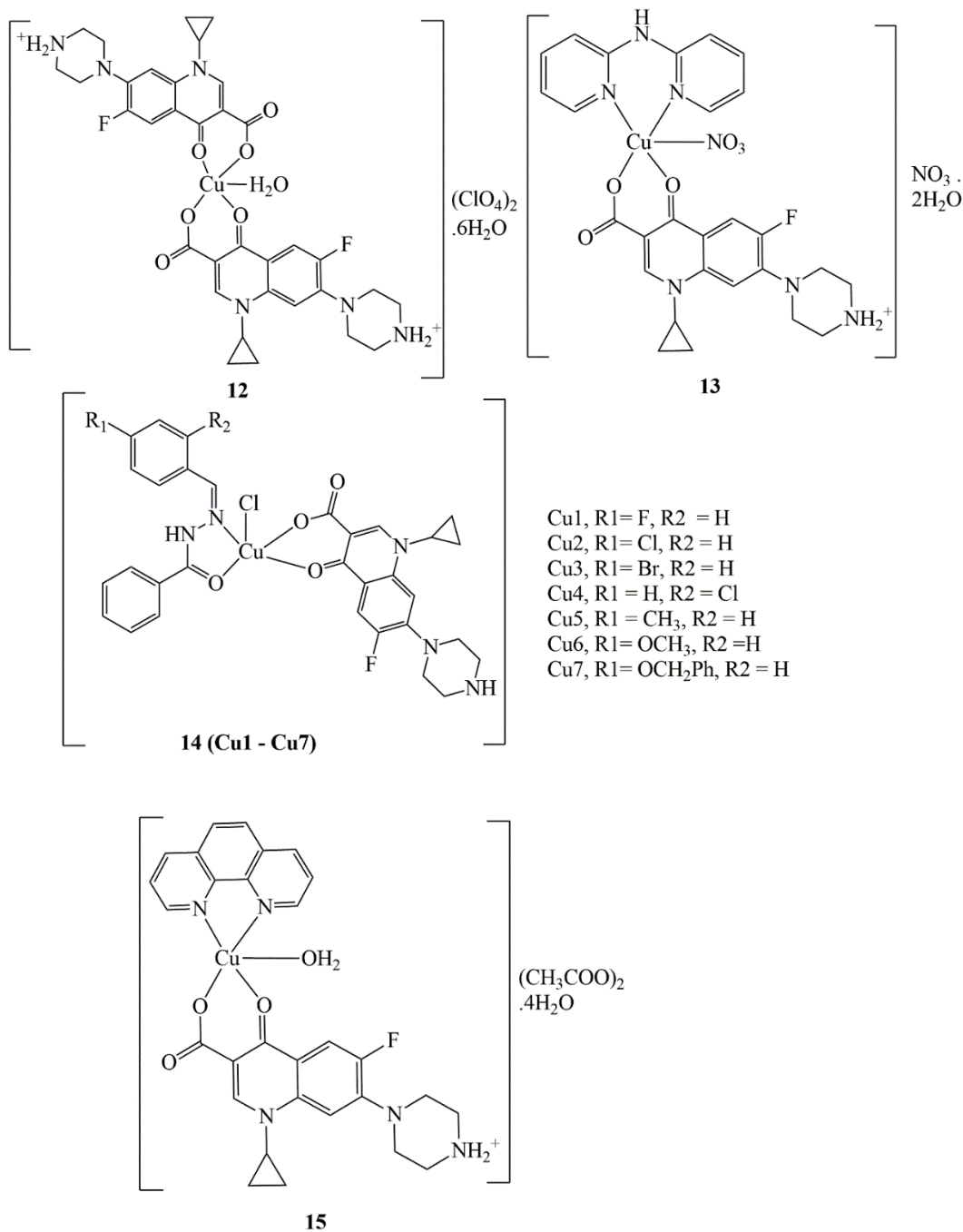


Figure 6. Structures of some ciprofloxacin based Cu(II) complexes (Yu et al., 2008) (Lawal et al., 2020)(Y. Wang et al., 2010)

The reported enhancement would be best described in the light of Overtone's concept (Anjaneyulu & Rao, 1986) and chelation theory (Rosu et al., 2010). The antimicrobial, DNA binding and

superoxide dismutase activities of the reported complexes were found to increase with the increase in the electronegativity of the substituted group on neutral bidentate N, O donor ligand (**14**, Figure 6). In another study, antibacterial activity of Cu(II) complex (**13**) of mixed ligand synthesized from the quinolone derivative drug ciprofloxacin together with the N-donor ligand 2,2'-bipyridylamine has been reported (Lawal et al., 2020). The complex had a distorted square-pyramidal structure. A green crystal compound (**13**) was prepared from the reaction of aqueous solution of 20 ml $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, methanolic solutions of 2,2'-bipyridylamine, aqueous solution of ciprofloxacin in the presence of 4 mmol NaOH in 1:1:2 mole ratio respectively for $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$: 2,2'-bipyridylamine: ciprofloxacin by adjusting the pH of the solution to 7.5. The antibacterial activity evaluation (agar disk diffusion method) for three gram-positive strains: *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus megaterium*; and two gram-negative strains: *Salmonella typhi* and *Escherichia coli* showed that complex showed better antimicrobial effects at all concentrations (10 and 20 mg/mL) against gram positive bacterial strains (*Staphylococcus aureus* and *Bacillus megaterium*) and gram-negative *Salmonella typhi* compared to the parent ciprofloxacin ligand. This could be due to the metal complex's ability to inhibit enzyme activity as a result of increased lipophilic following chelation, giving the metal complex to cross the cell membrane along with the coordinated ligand (ciprofloxacin) thereby inhibit the growth of the bacteria (Lawal et al., 2020; M. N. Patel et al., 2013). A mixture of hydro-methanolic solution of ciprofloxacin, methanolic solution of 1,10-phenanthroline and solid $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 1:1:1 ratio (acetic acid adjusted pH of 5-6) at room temperature and slow evaporation resulted complex **15**. The as-synthesized complex was found to be biologically active with higher DNA-binding ability and higher antibacterial activity against *S. aureus* and *K. pneumoniae* than ciprofloxacin alone (Y. Wang et al., 2010).

2.4.3 Synthesis and biological activity of 1,10-phenanthroline-based metal complexes

1,10-phenanthroline (Figure 7) is a classic bidentate ligand and one of the most frequently used chelating agent in coordination chemistry, with ideally placed nitrogen atoms, rigid planar structure, and a ligand yielding metal complexes with interesting biological and physiological properties (Abebe & Hailemariam, 2016; Bayeh et al., 2019; Nunes et al., 2020). It is a heterocyclic, hydrophobic, conjugated heteroaromatic system, bioactive molecule, a versatile building block organic compound and its coordination compounds yields interesting antimicrobial

properties *in vitro* against a broad-spectrum of bacterial and fungal pathogens. It has been reported that 1,10-phen and its derivatives have been widely used class of ligands in bioinorganic chemistry. Metal complexes of 1,10-phenanthroline are stable, shown potential value as fluorescent probes and characteristic features for DNA binding (Nunes et al., 2020; Pal, Alam, Paul, & Sheikh, 2019; Viganor, Howe, McCarron, McCann, & Devereux, 2017). A research finding showed that the toxicity of 1,10-phenanthroline significantly changes when coordinated to metal ions (Abebe et al., 2020).

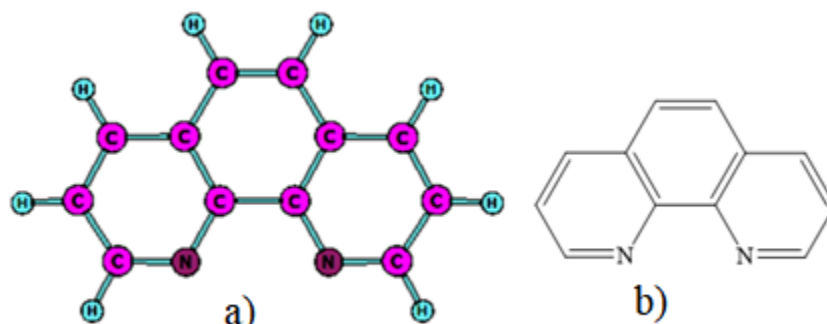


Figure 7. 3D (a) and 2D (b)-structural representation of 1,10-phenanthroline

Different types of 1,10-phenanthroline-based metal complexes have been reported for their promising antibacterial activities. Atakilt Abebe and his group have synthesized 1,10-phenanthroline based heteroleptic complexes (**16** - **24**) in which **16** and **23** were used as precursor complexes. Adenine containing mixed ligand mono and binuclear Co(II) complexes (**17** and **19**) (Abebe et al., 2020) in which 1,3-diaminopropane used as a spacer were reported to be soluble in DMSO, acetonitrile and water; and insoluble in all non-polar solvents. The antibacterial activity evaluation of **17** and **19** against Gram-negative: *Escherichia coli* and *Salmonella typhus*; and Gram-positive: *Staphylococcus aureus* and *Staphylococcus epidermis* showed enhanced antibacterial activity of **19** relative to the precursor complexes **16** and **17**.

A dark-brown octahedral Co(II) complex (**18**) was synthesized from an aqueous solution of sodium cytoside and **16** in 1:1 ratio, and the product was separated using dichloromethane and recrystallized from methanol (Atakilt, Bayissa, Sendek, & Kibret, 2018). The reported compound was evaluated against three Gram-positive bacteria (*Staphylococcus aureus*, *Methicillin resistant Staphylococcus aureus*, *Streptococcus pneumoniae*) and three strains of Gram-negative bacteria

(*Escherichia coli* and *Klebsiella pneumonia*). It was demonstrated that the reported complex (**18**) was antibacterial against to all the tested strains in which the free ligand cytosine showed no activity. The observed increase in antibacterial activity could be explained on the basis of Overtone's concept and Tweedy's chelation theory (Tweedy, 1964). According to Overtone's concept of cell permeability, lipid membranes surrounding cells only support the passage of lipid-soluble substances, so lipid solubility is believed to be an important factor controlling antimicrobial activity. In chelation, the polarity of the metal ion is further reduced due to ligand orbital overlap and partial sharing with the positively charged donor group of the metal ion. Furthermore, it increases the delocalization of π -electrons throughout the chelate ring, increasing the lipophilicity of the complex. This increased lipophilicity increases the penetration of the complex into lipid membranes and blocks the metal-binding sites of microbial enzymes (Imran, Iqbal, Iqbal, & Ijaz, 2007; Raman, Muthuraj, Ravichandran, & Kulandaisamy, 2003; Rizzotto, 2012). These metal complexes also disrupt cellular respiratory processes, thereby blocking protein synthesis and limiting further growth of the organism. The difference in the activity of the metal complexes against different organism found to depend on either the impermeability of the cell membranes or difference in ribosomes of microbial cells (Adhikary, Banerjee, Chakraborty, & Ianelli, 2013) (Dharmaraj, Viswanathamurthi, & Natarajan, 2001).

The synthesis, characterization and antibacterial potentials of mono and binuclear Co(II) centered octahedral complexes (**20 - 22**) employing acetamide and adenine co-ligands, and **16** as a precursor complexes were reported (Tamiru, Abebe, Abebe, & Liyew, 2019). The antimicrobial activity reports showed that the binuclear (**22**) complex showed higher antibacterial activity against two Gram-positive: *Staphylococcus aureus* and *Streptococcus pyogenes*, and two Gram-negative: *Escherichia coli* and *Klebsiella pneumoniae* bacterial strains. It is suggested that the bis intercalation of the two planar phenanthroline ring systems together with the expected flexibility and lipophilicity would play the role for enhanced antibacterial activity of the binuclear complex. The same group of authors reported Ru(III) centered 1,10-phenanthroline based complexes, **23** and **24** in which a chloride ion is substituted by guanine for **24** (Abebe & Hailemariam, 2016).

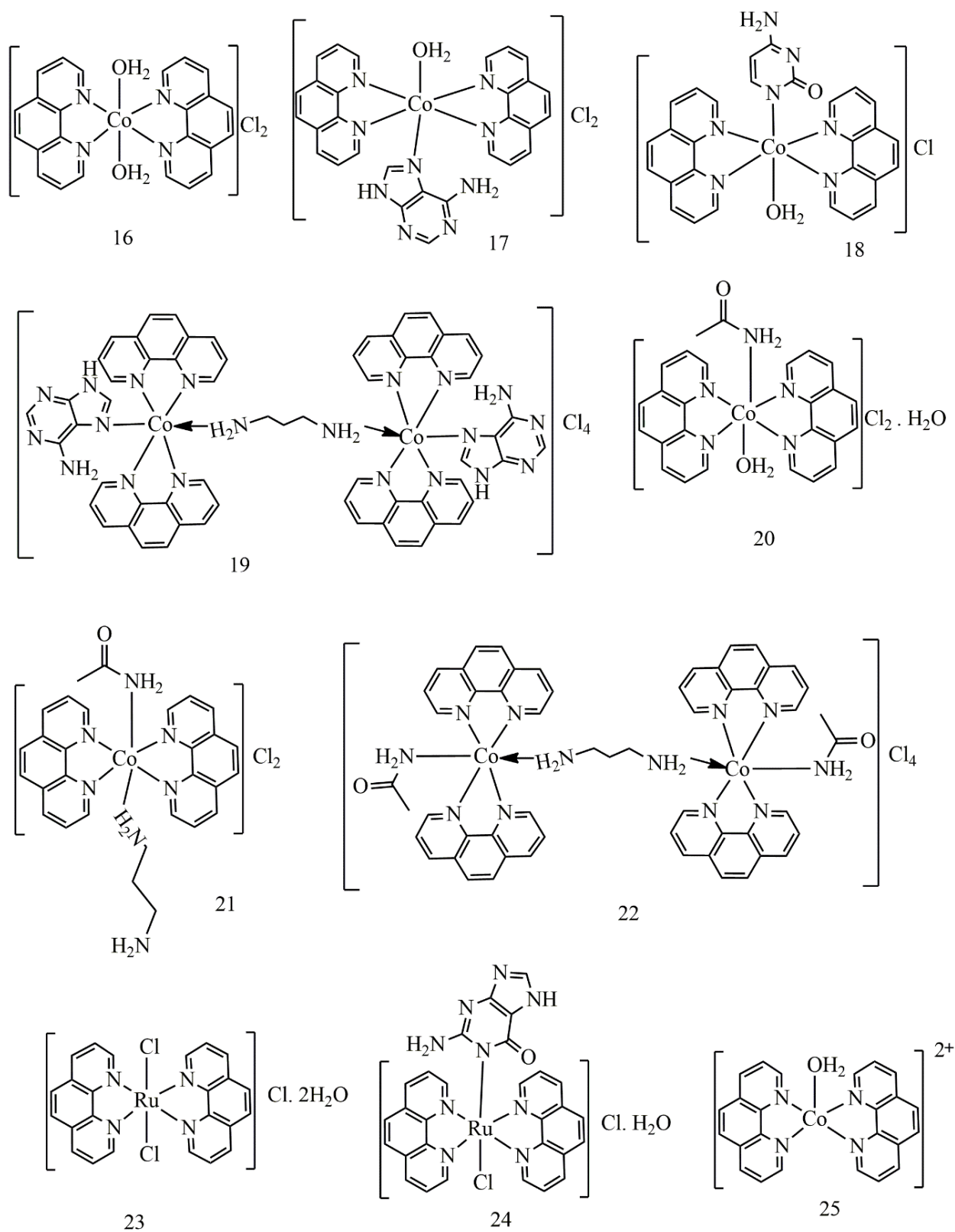
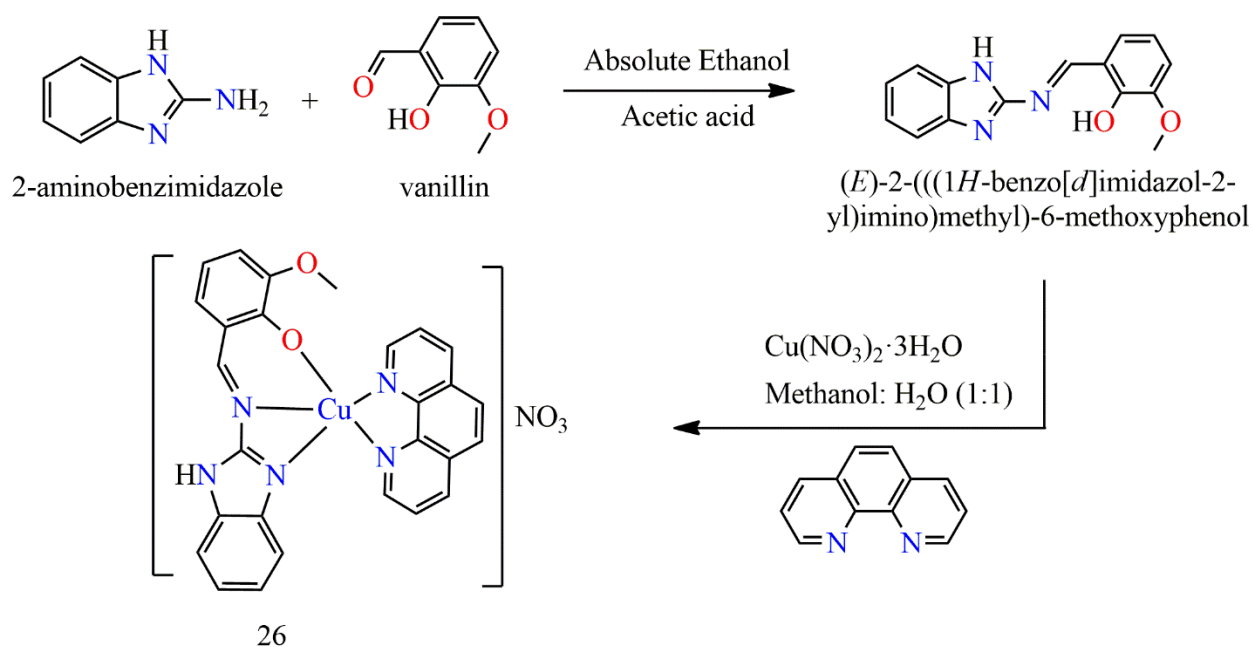


Figure 8. Reported structures of 1,10-phenanthroline based Co (II) and Ru(III) complexes (Abebe et al., 2020; Abebe & Hailemariam, 2016; Atakilt et al., 2018; Tamiru et al., 2019)

The reported Ru(III) centered complexes were subjected for antibacterial study against two Gram-positive: *S. aureus* (ATCC 25923) and methicillin resistant *S. aureus* (clinical isolate)), and two Gram-negative: (*E. coli* (ATCC255922) and *K. pneumoniae* (ATCC986605)) bacteria. The reported results demonstrated that the metal complexes promising antibacterial activity which may be due to the reduced polarity of the metal ions up on coordination. The synthesis, characterization and stability in cell incubation media of **25** which is structurally analogous to **16** were reported (Nunes et al., 2020). Promising cytotoxic activity against the A2780 cells as a function of incubation time with IC₅₀ value 20.9 μ M at 3h were reported. The authors reported that the stability of Cu(I)-ligand affects the cytotoxicity. The reported activity infers that changing the metal center of a coordination sphere might brough a different biological activity, showing structure activity roles.

The synthesis of a cytotoxic 1,10-phenanthroline based Cu(II) heteroleptic complex against MCF-7 cell lines are presented in Scheme 1 (Hussain et al., 2019). The cytotoxicity of the reported complex was compared with the Schiff base ligand (Scheme 1) and 1,10-phenanthroline, and enhanced cytotoxicity against MCF-7 cell line were found for the metal complex. The higher cytotoxic activity of complex **26** was suggested to be due to the stronger DNA binding ability of 1,10-phenanthroline resulted from its deeper insertion between the DNA base pairs, resulting cell death.

Zinc is an essential trace element distributed in the entire body from 2 to 4 g (Fujimoto, Yasui, & Yoshikawa, 2013), and human plasma normally contain about 15 μ M of Zn(II) (Vijayaraghavan, Pillai, & Subramanian, 2012). It is a Lewis acid and its complex participate in numerous biochemical processes: synthesis, storage, and secretion of insulin (Ranasinghe, Pigera, Galappatthy, Katulanda, & Constantine, 2015); β -cell function, insulin action, glucose homeostasis (Koleša-Dobravc et al., 2018); α -glucosidase inhibitory (α -GI) effect and insulin-mimetic activity (Murakami, Yasui, & Yoshikawa, 2012); antiepileptic (dos Santos et al., 2015), DNA-binding (Zhu et al., 2019) (Jadeja, Chhatrola, & Gupta, 2013), antibacterial (Chiniforoshan et al., 2015).



Scheme 1. Schematic presentation of Cu (II) complex derived from benzimidazole-o-vanillin Schiff base and 1,10-phenanthroline (Hussain et al., 2019).

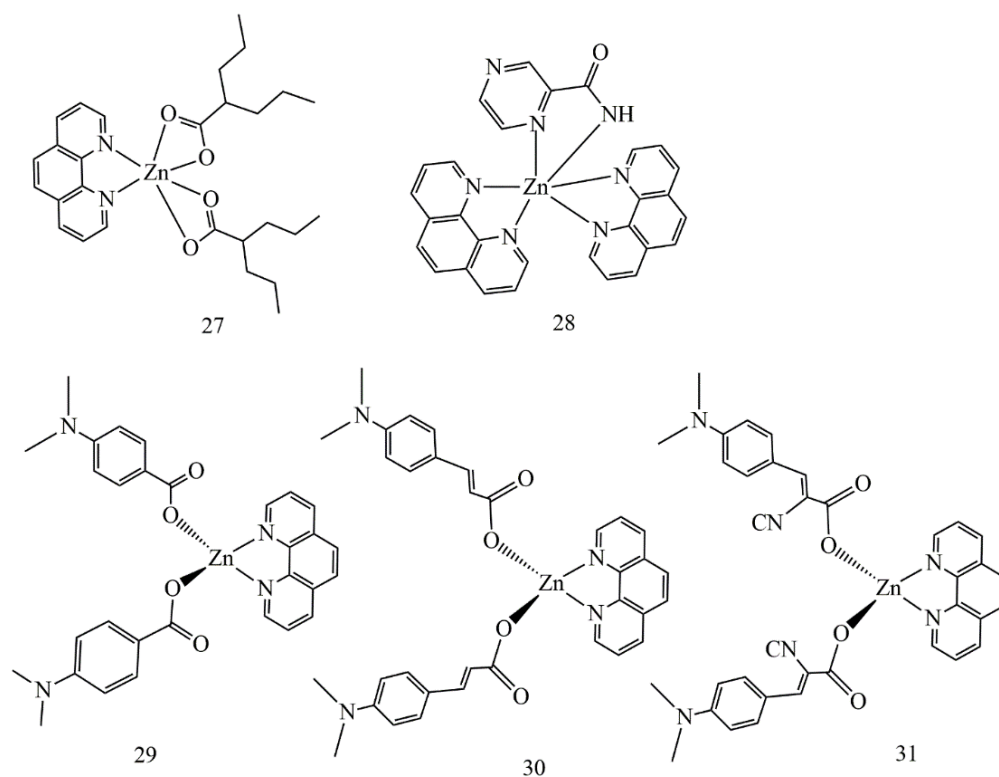


Figure 9. 1,10-phenanthroline based Zn(II)-heteroleptic complexes (Chiniforoshan et al., 2015; Das et al., 2006; dos Santos et al., 2015)

The synthesis of a distorted octahedral zinc organo-complex (**27**) using an old drug used in seizure, bipolar disorder and migraine headaches therapy called valproic acid, and 1,10-phenanthroline, and its toxicity evaluation by the brine shrimp model, were reported (dos Santos et al., 2015). The reported results demonstrated that synthesized Zn(II) complex ($IC_{50} = 78 \mu\text{g/m}$) showed higher toxicity against *Artemia salina* than its structural analog where 2,2'-bipyride ($IC_{50} = 143 \mu\text{g/m}$) is substituted for 1,10-phenanthroline. Whereas, a pyrazinamide based Zn(II)-1,10-phenanthroline complex, **28** were screened for antimicrobial activity against *Escherichia coli* ($26.0 \pm 2.00 \text{ mm}$), and *Bacillus turingiensis* ($29.1 \pm 1.90 \text{ mm}$) by the disk diffusion method (Chiniforoshan et al., 2015).

Apart from the biological applications of Zn(II) complexes showed hyperpolarizability for non-linear optical materials, inferring wide range of applications of the metal complexes. In the search of 2D-non-linear optical materials with high hyperpolarizability 1,10 phenanthroline based Zn(II) metal complexes employing 4-dimethylaminobenzoic acid (**29**), 4-dimethylamino cinnamic acid (**30**) and 4-dimethylamino-a-cyanocinnamic acid (**31**) ligands were reported (Chiniforoshan et al., 2015; Das et al., 2006; dos Santos et al., 2015) from $\text{Zn}((\text{O}_2\text{C}_2\text{H}_3)_2)_2 \cdot 2\text{H}_2\text{O}$ precursor (Figure 9). The reported complexes demonstrated large enhancement of hyperpolarizability relative to the 1D dipolar ligands (Das et al., 2006). It was reported that the hyperpolarizabilities of the complexes showed strong increment from **29** to **31** (β (10^{-30} esu), 49.0 – 107).

2.4.4 Synthesis and biological activity of chrysin-based metal complexes

Bioactive chemical compounds obtained from medicinal plants have provided numerous clinically used medications. Flavonoids are polyphenolic phytochemicals that have proven a wide variety of biological activities with their high tendency to chelate metal ions (Balunas & Kinghorn, 2005; Ikeda, Novak, Maria, Velosa, & Pereira, 2015; J. Zhang et al., 2010). They are a group of low molecular weight substances based on 2-phenyl-chromone nucleus (Figure 10) (C. Wang et al., 2018b). Chemically, they are polyphenolic compounds possessing fifteen carbon atoms; two benzene rings joined by a linear three-carbon chain (Singh, Arif, Khan, & Sharma, 2014) that denote the most abundant natural anti-oxidant (Pietta, 2000; J. Zhang et al., 2010). They are widely distributed in plant kingdom and exhibit characteristic pharmacological properties including antidiabetic (AL-Ishaq et al., 2019; X. Zhang et al., 2015), antiviral, antibacterial (Dai et al., 2019; Y. Xie et al., 2015), anti-inflammatory (He et al., 2018), cardioprotective (Testai et al., 2013),

anticancer (Alper et al., 2019), antiaging (Guo et al., 2019), neuroprotective/antioxidant (Alper et al., 2019; Ayaz et al., 2019; Krych & Gebicka, 2013), antiproliferative (Ragab et al., 2014) and antiestrogenic/estrogenic (Collins-Burow et al., 2012; Collins-Burow et al., 2000). Moreover, previous studies indicated that metal complexes of flavonoids have better anti-oxidant activity than the free ligands alone. This is due to that fact that attainment of extra resonating site and better stability of flavonoid metal complexes radical than free flavonoids would possess (Jabeen et al., 2017; Martinez-Gonzalez, Díaz-Sánchez, de La Rosa, Bustos-Jaimes, & Alvarez-Parrilla, 2019).

The interest of using natural flavonoid based antioxidants as therapeutic tools is resulted from diabetic physiopathology associated with oxidative stress (El Omari et al., 2019). The mechanisms of action for flavonoids to serve as antioxidant emanates from their structure (presence of hydroxyl groups in ortho position and additional functional groups like carbonyls) and it would be due to their tendency to suppress reactive oxygen species formation scavenge reactive oxygen species and up regulate or protect antioxidant defenses (Pietta, 2000). Literature reports indicate that the antioxidant activity of flavonoids increases up on complexation with metals as confirmed by their superoxide dismutase mimicking activity (Alfadda & Sallam, 2012; Khater, Ravishankar, Greco, & Osborn, 2019) and DPPH radical scavenging activity (Alexiou et al., 2017; Chen et al., 2009).

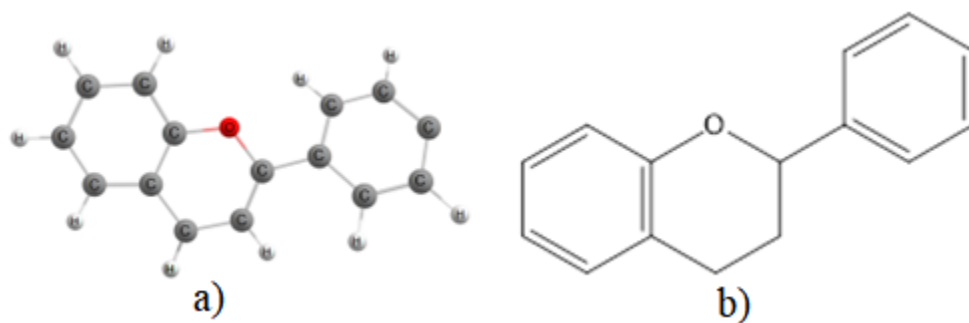
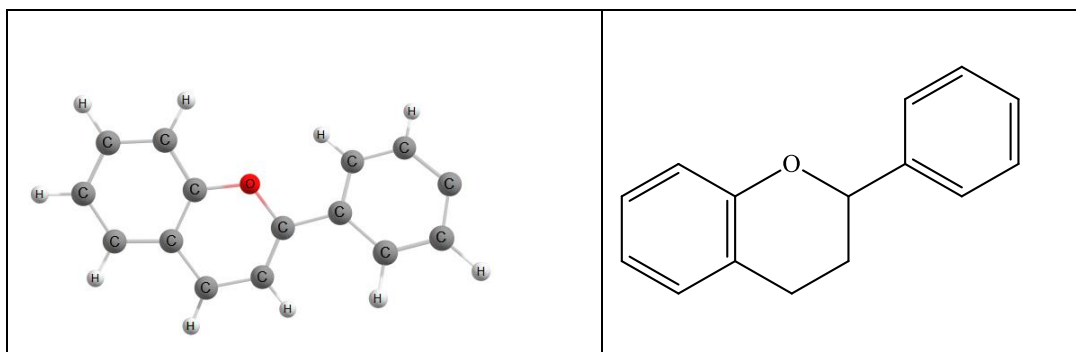


Figure 10. Chemical structure of flavonoid (2-phenyl-chromone) constructed using Chemcraft (a) and Chem Draw ultra 12.0.2 (b).

Flavonoids are extensively studied in searching for drug molecule due to their wide-ranging biological activities such as antimicrobial, antioxidant, antitumor, neuroprotective and cardioprotective properties (Alexiou, Silva, Romoff, & Ferreira, 2016; Khater et al., 2019; R. Pereira et al., 2007). The potential chelation sites of flavonoids/flavanols are given in Figure 11. Their ability to chelate with metal ions/atoms provided them new approach for drug discovery programs, with better pharmacological activities and clinical profiles than the parent flavonoids (Khater et al., 2019).

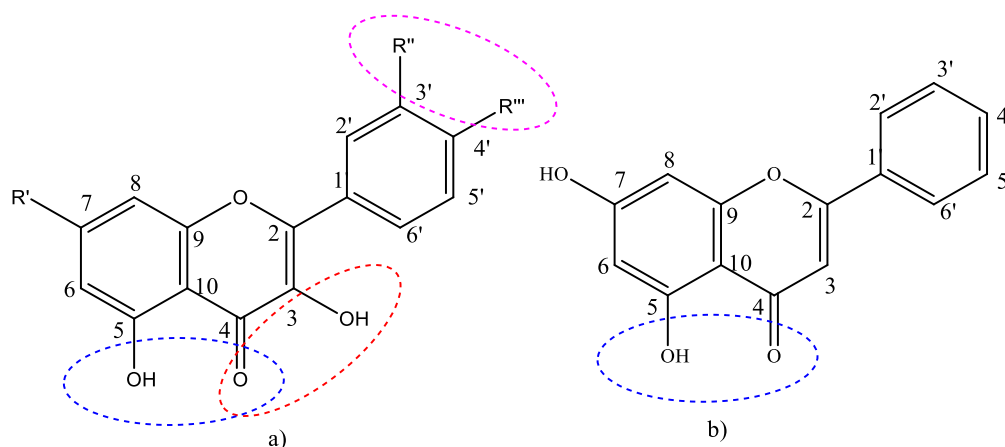


Figure 11. Structure and potential chelation sites of flavanols (a) and chrysin (b)

In ongoing efforts to investigate new generation of natural product based metallodrugs, flavonoid-metal complexes find the potential to be active candidates for prevention and therapy (Gençkal, 2020; Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021) (E Halevas et al., 2021; Spoerlein et al., 2013). Accordingly, several flavonoid based homo- and heteroleptic metal complexes with promising biological activities are reported. It has been indicated that Quercetin(Q)-Cr (III) complex, $[\text{Cr}(\text{Q})_2\text{ClH}_2\text{O}]\cdot 4\text{H}_2\text{O}$ (Chen et al., 2009); a trinuclear chromium (III) complex, $[\text{Cr}_3\text{O}(\text{CH}_3\text{CO}_2)_6(\text{Naringenin})(\text{H}_2\text{O})_2]$ (Alexiou et al., 2017); Cr (III)-rutin complex, $[\text{Cr}_2(\text{rutin})(\text{H}_2\text{O})_8]\text{Cl}_4$ (Panhwar & Memon, 2014b); $[\text{Cr}(3,7\text{-dihydroxyflavone})_3]$ (Alexiou et al., 2016); Cr(III)-morin, $[\text{Cr}(\text{morin})(\text{H}_2\text{O})_2]\text{Cl}\cdot\text{H}_2\text{O}$ (Chen et al., 2009) and $[\text{Cu}(\text{Naringin})(\text{CH}_3\text{OH})_2]^+$ (Vinayagam & Xu, 2015) are heteroleptic metal complexes found to show enhanced antioxidant activity. Among the reported complexes of Cu(II) square planer geometries with N,N,O,O-type donor mixed ligand had promising biological activities and some of them have entered stage III clinical trial (Correia et al., 2015; Nunes et al., 2020; Ruiz-Azuara

& E Bravo-Gomez, 2010). 1,10-phenanthroline and 2,2'-bipyridine N,N donor and rigid planar chelators are among the most commonly used ligands to create the required 3D metal complex structure with enhanced biological activities. Reported structures of Cu(II) and Zn(II) centered chrysin based homo- and heteroleptic complexes are presented in Figure 12. A homoleptic Cu(II)-chrysin complex (**32**) was synthesized from the reaction of $\text{Cu}(\text{O}_2\text{C}_2\text{H}_3)_2 \cdot \text{H}_2\text{O}$ and chrysin in 1:2 metal ligand ration in the presence of NaOH which is half the molarity of chrysin. The synthesized complex, $[\text{Cu}(\text{chrysin})_2\text{H}_2\text{O}]$ (**32**) possessing octahedral geometry was subjected for xanthine oxidase inhibition test. The results showed that the IC_{50} values of $0.82 \pm 0.034 \mu\text{M}$, $1.26 \pm 0.04 \mu\text{M}$, $2.93 \pm 0.02 \mu\text{M}$, respectively for **32**, chrysin and the standard allopurinol, demonstrating the inhibition ability of complex towards xanthine oxidase was superior relative to the ligand and the standard (Lin et al., 2017). The obtained xanthine oxidase inhibition was supported with molecular docking study against xanthine oxidase (bovine milk, PDB ID: 1FIQ). The molecular docking study revealed that compound **32** followed an inhibition mechanism of a bridge plugged the channel and surrounding space.

Compounds **33** and **34** were prepared from the reaction of chrysin, $\text{Cu}(\text{ClO}_4) \cdot 6\text{H}_2\text{O}$ and co-ligands 2,2-pyridine and substituted 1,10-phenanthroline (Figure 12) (Gençkal, 2020). The synthesized complexes were found to exhibit much higher antioxidant (**34** >> **33**) capabilities than that of chrysin which could be associated to the easier hydrogen atom transfer after complexation. A similar structural analog to **33** with metal center Zn(II), a complex **35** was reported for a research interest raised from the search for novel metallodrug with improved pharmacological properties (Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021). The complex has shown enhanced radical scavenging potential in a DPPH assay compared to free chrysin and the standard ascorbic acid. These findings indicated the promising anti-oxidant activity of Cu(II) and Zn(II) centered chrysin based heteroleptic complexes.

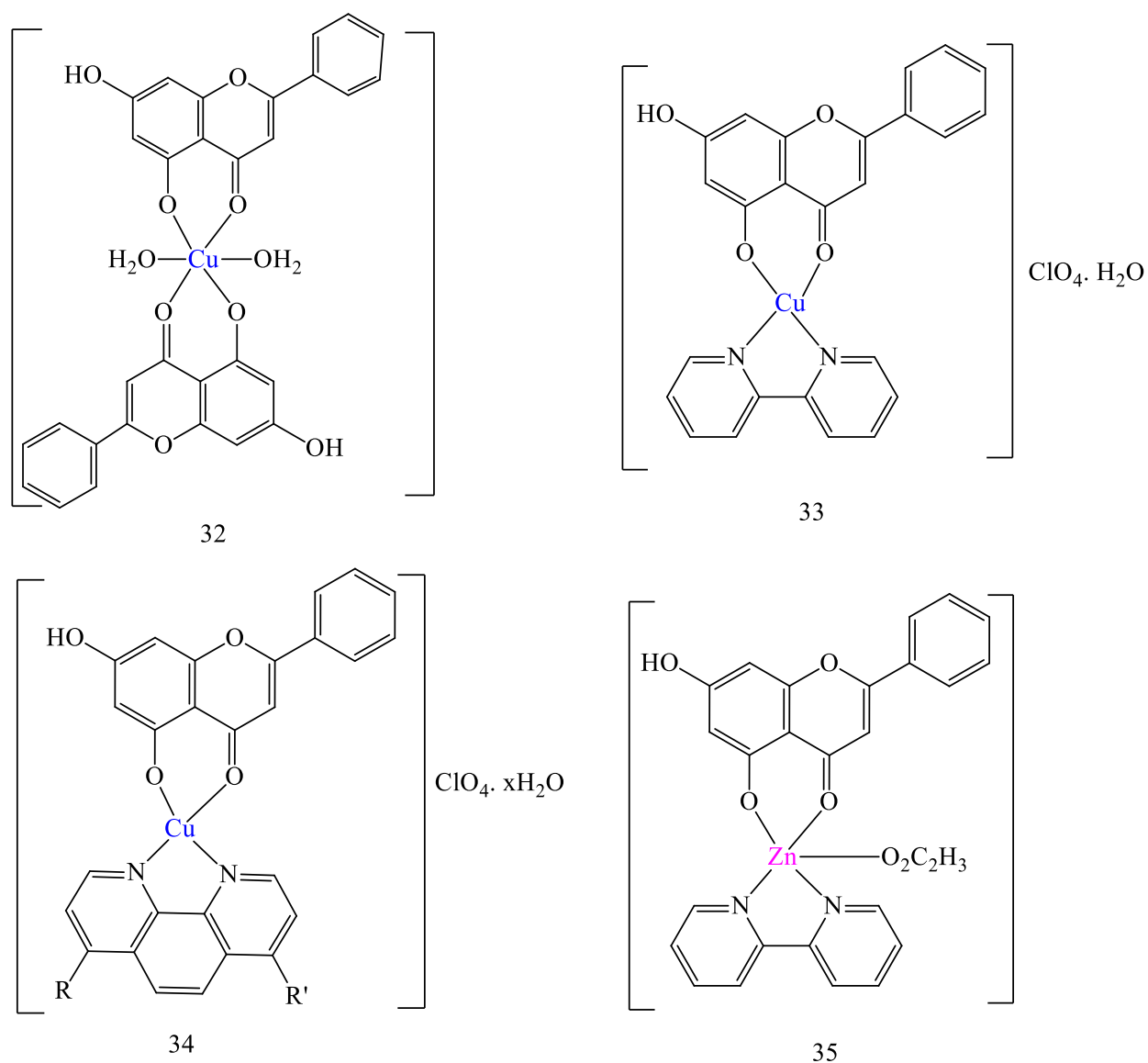


Figure 12. Reported structures Cu(II) and Zn(II) complexes of chrysin (Gençkal, 2020; E Halevas et al., 2021; Lin et al., 2017)

2.5 Methods for antioxidant activity evaluation

Oxidative stress is an imbalance in the prooxidant-antioxidant equilibrium in normal cells caused by reduced concentration of antioxidants and increased in the number of oxygen, nitrogen or carbon-based reactive species (Gulcin, 2020; Munteanu & Apetrei, 2021). The concept was introduced as the dissolution of the prooxidant-antioxidant equilibrium by Sies H, a German physiologist (Sies, 1997). It happens when protective enzymes: superoxide dismutase (SOD), catalase and peroxidase are overwhelmed (Antolovich, Prenzler, Patsalides, McDonald, &

Robards, 2002). Whereas, antioxidant activity is a biological system's ability to readily detoxify ROS/RNS and to repair the resulting damage (Gulcin, 2020). Mechanistically, antioxidants act by inhibiting the generation of ROS/RNS, reducing capacity, metal chelating capacity, and hydrogen peroxide decomposition activity. They also act by inhibiting the initiation and propagation steps of ROS/RNS, leading to delaying the oxidation process and there by termination of the reaction (Antolovich et al., 2002; Gulcin, 2020; Pisoschi & Negulescu, 2011).

2.5.1 Antioxidant activity testing methods

Antioxidant capacity concept was first initiated from chemistry and advanced to biology, medicine, epidemiology and nutrition (Floegel et al., 2011). Different antioxidant activity protocols have been developed to evaluate and examine the antioxidant activity of potential antioxidants and hence showed remarkable progress in the past few decades (Munteanu & Apetrei, 2021). These protocols differ in their chemistry and in the way end points are measured (Huang, Ou, Prior, & chemistry, 2005; Munteanu & Apetrei, 2021).

2.5.1.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

DPPH (Figure 13) is one of a few stable and commercially available organic nitrogen radicals due to the delocalization of the spare electron on the whole molecule (Pisoschi & Negulescu, 2011). It has a UV-vis absorption maximum at 515 nm. It involves preparation of a solution of DPPH in methanol (3.9 mL, 25 mg/L) and follow the reaction progress at 515 nm after 30 minutes reaction of time with the 0.1 mL sample volume (Huang et al., 2005).

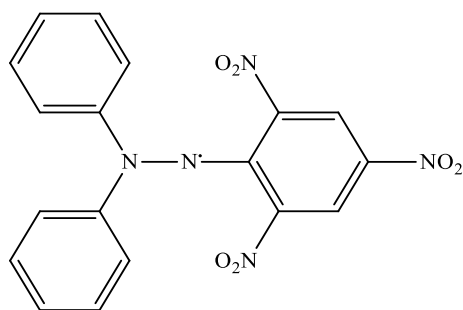


Figure 13. Structure of DPPH

The DPPH assay is technically simple, but some disadvantages limit its applications. Besides the mechanistic difference from the HAT reaction that normally occurs between antioxidants and peroxy radicals, DPPH is long-lived nitrogen radical, which bears no similarity to the highly

reactive and transient peroxy radicals involved in lipid peroxidation. Antioxidants that react quickly with peroxy radicals may react slowly or may even be inert to DPPH. In recent years, this method was modified to methanolic solution of DPPH (5×10^{-5} mol/L), 40 minutes of reaction times with the samples and absorbance reading at 517 nm (Eleftherios Halevas, Barbara Mavroidi, & Maria Pelecanou, 2021)

2.6 Methods for cytotoxic activity evaluation

Cytotoxicity is the toxicity caused by the action of chemotherapeutic agents on living cells, and cytotoxicity assays measure the ability of a chemotherapeutic agents to cause cell damage or cell death to a target cell. Cytotoxicity evaluation methods involves the use of dyes: tetrazolium, trypan blue, alamar blue, neutral red, and coomassie blue. This procedure distinguishes different cells by color based on the ratio of color uptake in both live and dead cells (F. Li et al., 2012). The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (methyl thiazolyl tetrazolium) also called as MTT assay is a colorimetric cytotoxicity assay for rapid assessment of cell proliferation and measurement of metabolism or function of the cells used. It is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells (W. Li, Zhou, & Xu, 2015) (Srivastava et al., 2018). Living cells contain NAD(P)H-dependent oxidoreductase enzymes that reduce methyl thiazolyl tetrazolium (MTT) to purple formazan crystals. Formazan crystals are soluble in DMSO and the resulting-colored solution can be quantified by measuring absorbance at 570 nm. Briefly, it involves the incubation of plate cells at approximately 104 cells/well in 96 well plates for 24 hr at 37 °C, aspirate old media (add 100 µL of treatment media to all wells and incubate for a further 24 hr), addition of 100 µL of working MTT solution diluted from 50 – 100 mg/mL stock solution to each well to achieve 0.5 mg/mL. After 4hr carefully aspirate MTT solution from each well and dissolve the formazan precipitate by addition of 100 µL DMSO and finally read absorbance at 570nm in plate reader and blank (no cells))

2.7 Methods for antibacterial activity evaluation

Antibacterial susceptibility testing can be used for drug discovery, disease epidemiology and prediction. Agar disk-diffusion method is the official method for routine antimicrobial susceptibility testing in many clinical microbiology laboratories (Jorgensen & Turnidge, 2015). It offers many advantages over other methods due to its simplicity, low cost, the ability to test

enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided. Moreover, it is reported to show good correlation between the *in vitro* data and the *in vivo* evolution. These all contributed to the common use of the method to screen plant extracts, essential oils and other therapeutic agents (Kreger, Craven, & McCabe, 1980; Nijs et al., 2003). In this method, agar plates are inoculated with a standardized inoculum of the test microorganism, paper discs of 6 mm containing the test compound at a desired concentration are placed on the agar surface, the petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured in mm and reported as minimum zone of inhibition (Balouiri, Sadiki, & Ibnsouda, 2016). Disk diffusion is the most common method employed for antibacterial activity testing. However, it is impossible to distinguish bactericidal and bacteriostatic effects using this method because bacterial growth inhibition does not mean the bacterial death. Moreover, the method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium (Nijs et al., 2003).

2.8 Screening of molecules for their drug-likeness

Knowing drug-like molecules/ligands is the main goal of all knowledge-based approach to drug discovery. There have been numerous attempts to forecast drug similarity. Lipinski 's rule of five (LRF) emerged from these studies as a guide for providing medicinal chemists with a basic means of predicting potential problems with solubility and permeability (Ghose, Herbertz, Salvino, & Mallamo, 2006). LRF is a rule of thumb introduced by Christopher A. Lipinski in 1997 for assessing drug ability or for determining whether a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans. The rule states that an orally active drug should not violate more than one of the following criteria: less than 5 hydrogen-bond donors (HBDs), less than 10 hydrogen-bond acceptors (HBAs), a molecular mass less than 500 Da and log P not greater than 5 (Lipinski, Lombardo, Dominy, & Feeney, 1997). Besides these, the topological polar surface area of a molecule is known to affect the pharmacological effect of a molecule. A study reported that molecules with a TPSA value of 140 Å² and above would be poorly absorbed (< 10 percent fractional absorption), while those molecules with a TPSA value less than 70 or 60 Å² would be well absorbed (> 90 per cent)(Clark, 1999). These physicochemical properties are known

to affect the pharmacokinetics properties of a drug molecule such as absorption, distribution, metabolism, and excretion. Drug absorption pharmacokinetics refers to the mechanism of how drugs migrate through the body's organs to enter the systemic circulation. Whereas, drug distribution is the transportation of drugs from one tissue to another tissue in an affinity dependent manner (Shin, Kang, & No, 2017). Poor aqueous solubility and poor permeability of a drug are the most common factors affecting drug absorption from the gastrointestinal tract (GIT). When delivering an active ingredient orally, it must first dissolve in gastrointestinal fluids before it can then permeate the membranes of the gastrointestinal tract to reach systemic circulation. Therefore, a drug with poor aqueous solubility will display dissolution rate-limited absorption (Devadasu, Deb, Maheshwari, Sharma, & Tekade, 2018). The most important pharmacokinetic parameter that has strong association with absorption is lipophilicity which is a key physicochemical property that plays a crucial role in determining ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiles and the overall suitability of drug candidates (Arnott & Planey, 2012).

Development and design of a drug consist of discovering and characterizing new targets, synthesizing new lead molecules, screening new lead molecules for biological activities in vitro and/or in vivo, and physicochemical characterization of leads (Arnott & Planey, 2012). These makes the area resource and capital intensive that necessitates multi-disciplinary knowledge, high cost, advanced approaches, complex and high-risk process (Chordia & Kumar, 2018; Xue, Li, Xie, & Wang, 2018). The design and development of drug molecules requires earlier assessment of absorption, distribution, metabolism and excretion (ADME) at a stage where multiple compounds are considered, but access to the physical samples is limited (Arnott & Planey, 2012; Daina et al., 2017). Due to limited pharmacokinetics and toxicity profile of the drug-like molecules under investigation, only 11% of the drugs succeed to enter clinical development stage to reach the market place (Jie Dong, Ning-Ning Wang, et al., 2018; Ghose et al., 2006). As a result, the pharmaceutical industry remains under immense pressure to counter the high rate of attrition in drug development that triggered an increased interest of computer aided toxicity and pharmacokinetic prediction studies (Waring et al., 2015). Computer aided methods save time, human and material resources in the search of new drug-like molecules in contrast to experimental synthesis, characterization and biological analysis works (Azminah et al., 2019; Plewczynski, Łaźniewski, Grotthuss, Rychlewski, & Ginalska, 2011).

Pharmacokinetics and toxicity profile of the drug under investigation are the major determinants in the course of successful drug development (Ahmed & Alkali, 2019; Moroy, Martiny, Vayer, Villoutreix, & Miteva, 2012). The available experimental (*in-vivo/in-vitro*) techniques to evaluate pharmacokinetic, toxicity and drug-likeness are resource intensive protocols that demand the use of animals and different chemicals. As such *in silico* approaches have been used as valid alternatives to the experimental procedures for prediction of pharmacokinetics and toxicity of phytochemicals isolated from plants (Krüger, Maltarollo, Wrenger, & Kronenberger, 2019). To date, many freely available online tools have been developed in that direction. Web-based platforms: ADMETlab, Pro Tox-II and SwissADME have been developed to predict ADMET properties (Banerjee, Eckert, Schrey, & Preissner, 2018; Daina et al., 2017; Jie Dong, Ning-Ning Wang, et al., 2018).

2.9 Overview of webservers for molecular screening

2.9.1 SwissADME

SwissADME is integrated into the Swiss Drug Design (SDD) work space, developed by the molecular modeling group of the Swiss Institute of Bioinformatics. It consists of plat-forms: ligand-based virtual screening, biotarget prediction, molecular docking, and molecular mechanics. The web tool provides fast and robust predictions of physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry usability, including in-house expert methods such as BOILED-Egg, iLOGP and bioavailability. The SwissADME web tools were presented at <http://www.swissadme.ch> and a user friendly webtool. Besides the unique access to competent methods (such as iLOGP or BOILED-Egg), SwissADME strengths are by no means comprehensive: various input methods, calculations for multiple molecules and the ability to view, save and share results for each single molecule or via global, intuitive and interactive graphs compared to other ADME and pharmacokinetic web-tools pk-CSM and admetSAR (Daina et al., 2017).

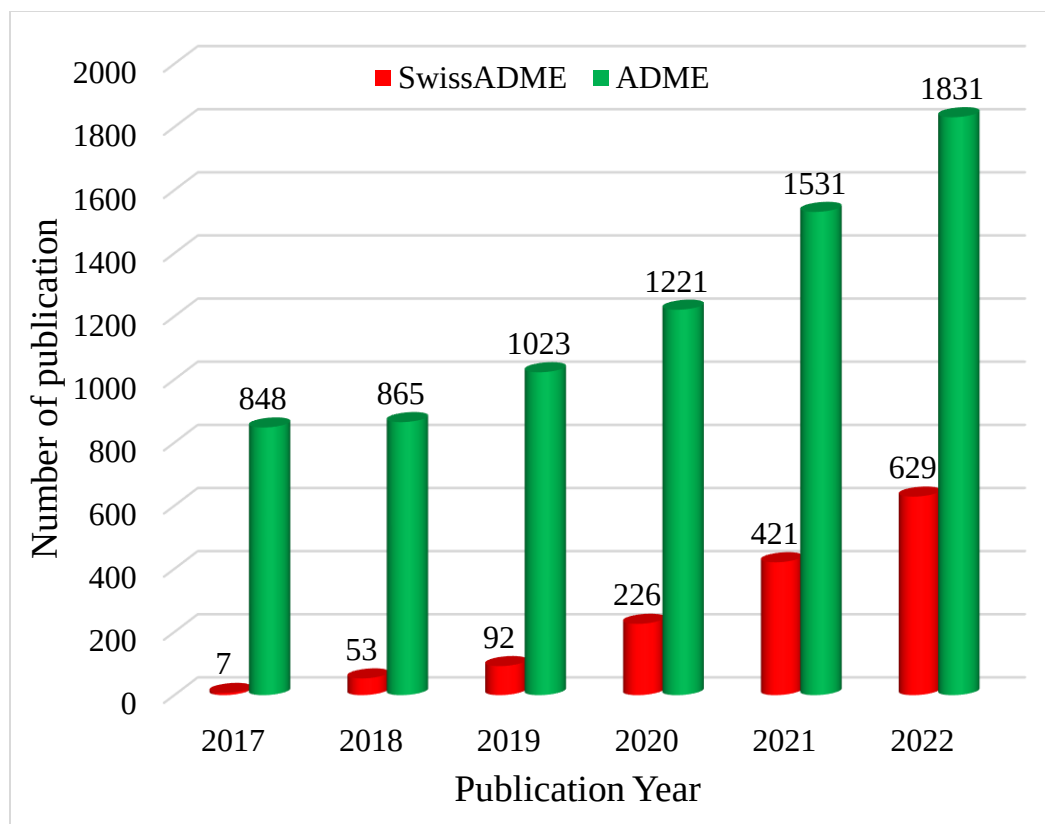


Figure 14. Five years of publication trend in ScienceDirect website using SwissADME and ADME keywords

Research reports showed that publication trends using SwissADME web tool and ADME profiling are increasing for the last five years as clearly presented in Figure 14. Research works uncover the significance of SwissADME in the design and development of novel anticancer, antitubercular and antimicrobial agents (Bakchi et al., 2022).

2.9.2 ADMETlab

ADMETlab was released by the team of Dong *et al.*, in 2018 to facilitate the ADMET evaluation. The server is a publicly available with spontaneous interface and free access. It was developed with ADMET database and Web platform main components which share a common running environment. The main modules designed to suitably assess ADMET properties in ADMETlab were drug-likeness evaluation, ADMET prediction (31 endpoints assessment), systematic ADMET evaluation for single chemical and similarity searching based on ADMET database with 288,967 entries (Jie Dong, Ning-Ning Wang, et al., 2018). As part of frequent efforts to provide the scientific community with a complete, precise and effective online platform for ADMET

related evaluation of parameters of compounds webserver was updated to its version 2.0 which supports two single-molecule evaluation and batch screening computational modes, allowing for the calculation of 88 ADMET related parameters. Also, it is designed to include 17 physicochemical properties, 13 medicinal chemistry properties, 23 ADME properties, 27 toxicity endpoints and 8 toxicophore rules. It is believed to have greater capacity to assist medicinal chemists in accelerating the drug research and development process. The updated version has resolved the number of properties predicted; physicochemical properties 7 to 17, medicinal chemistry 6 to 13, ADME 21 to 23, toxicity 7 to 27, toxicophore rule 0 to 8, and computation time from more than 2h to 82 seconds (Xiong et al., 2021).

2.9.3 Pro Tox-II

Pro Tox-II is a toxicity prediction web tool that incorporates molecular similarity, pharmacophores, fragment propensities and machine-learning models for the prediction of various toxicity endpoints. It employs a total of 33 models and the platform is divided into a five different classifications such as six acute toxicity; one organ toxicity; four toxicological endpoints; twelve toxicological pathways and fifteen toxicity targets (Banerjee, Dehnbostel, & Preissner, 2018; Banerjee, Eckert, et al., 2018; Drwal, Banerjee, Dunkel, Wettig, & Preissner, 2014). One of the toxicity parameters predicted using Pro tox-II is the median lethal dose (LD_{50}) at which 50% of test subjects die upon exposure to a compound value in mg/kg body weight. Toxicity classes are defined according to the globally harmonized system of classification of labeling of chemicals. LD_{50} values are given in mg/kg: Class I: fatal if swallowed ($LD_{50} \leq 5$); Class II: fatal if swallowed ($5 < LD_{50} \leq 50$); Class III: toxic if swallowed ($50 < LD_{50} \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class VI: non-toxic ($LD_{50} > 5000$). In this work, the toxicity classes of the flavonoids used for ligand screening were categorized according to the classification of labelling of chemicals of Pro-Tox II (Banerjee, Eckert, et al., 2018).

3 MATERIALS AND METHODS

3.1 Materials

All the chemicals and reagents used in this work were of analytical grade and used without any treatment. 1,10-phenanthroline (BDH chemical Ltd., Poole, England), Chrysin (Sigma Aldrich), metal salts: chromium chloride hexahydrate ($\text{CrCl}_6 \cdot 6\text{H}_2\text{O}$), $\text{Cu}(\text{O}_2\text{C}_2\text{H}_3) \cdot \text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{Zn}(\text{O}_2\text{C}_2\text{H}_3) \cdot 2\text{H}_2\text{O}$ were obtained from Loba Chemie PVT Ltd. Triethylamine, NaHCO_3 , Mueller-Hinton agar, Methanol, Ethanol, HCl, DMSO, Ethyl acetate and Dichloromethane (DCM) and Diethyl ether were purchased from (Alpha Chemika). Metformin hydrogen chloride (Met.HCl) and ciprofloxacin hydrogen chloride (Cip.HCl) were obtained from Cadila Pharmaceuticals PLC, Ethiopia. For biological activity tests, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) positive controls were purchased from Sigma-Aldrich.

Software packages and Web Servers: Several software packages were used in order to characterize free ligands (FLs) and their metal complexes in terms of their electronic structure. Chemoffice 2012 (Cambridge soft corp., Cambridge, MA, U.S.A.) were used to sketch structures used in this work. Gauss View 6.0 (Gaussian, Inc., 340 Quinipic St Bldg 40, Wallingford, CT, 06492, USA) and Chemcraft 8.0 were used to build molecules, set up input files, visualize vibrational frequencies and absorption spectra, and visualize frontier molecular orbitals (FMOs) (Dennington, Keith, & Millam, 2009). Geometry optimizations and frequency calculations were performed using Gaussian® 16 software package (Frisch, 2016). SwissADME web tool together with its BOILED Egg model (Daina et al., 2017) and ADMETlab (Jie Dong, Ning-Ning Wang, et al., 2018) were employed to predict pharmacokinetic parameters and drug-likeness of flavonoid molecules, and screen ADMET properties of ligands, respectively. ProTox-II webserver (Banerjee, Eckert, et al., 2018) and variable nearest neighbor (vNN) method (Schyman et al., 2017) were used to predict the toxicity profile of flavonoids screened for their bidentate chelating nature.

3.2 Computational Screening of a bidentate flavonoid ligand

3.2.1 Ligand preparation

Anti-diabetic bioactive flavonoid derivatives (Figure 16) were retrieved from literatures (A. S. Pereira et al., 2019; Salehi et al., 2019). Chemoffice (12.0) was employed to draw the two-dimensional structure of selected flavonoids and controls (Metformin and acarbose), and saved in

cdx format. The chemical structures of the target compounds were submitted to SwissADME tool and converted to their canonical simplified molecular input line entry system (SMILE). Also, canonical simplified molecular input line was copied and submitted to the canonical smiles section of ProTox-II virtual lab, vNN and ADMETlab. The overall procedure for a biocompatible ligand screening is represented in Figure 15.

3.2.2 Physicochemical property, drug likeness, and pharmacokinetic predictions

Pharmaceutically significant descriptors and physically relevant properties of the target flavonoids (Figure 16) were predicted (Ojha, Gahlot, Tiwari, Pathak, & Kakkar, 2011; Tiwari et al., 2009). The physicochemical properties (molar refractivity, topological polar surface area, number of hydrogen bond donors/number of hydrogen bond acceptors), lipophilicity ($\log P_{O/w}$), pharmacokinetics properties (GI absorption, BBB permeation, P-gp substrate, cytochrome-P enzymes inhibition, skin permeation ($\log K_p$)) which are critical parameters for prediction of the absorption and distribution of drugs within the body (Pathak et al., 2017), and drug likeness (Lipinski's rule of five) were predicted via SwissADME (Daina et al., 2017). The percent absorption (% Abs) of the ligands were calculated using the reported formula $\% \text{ Abs} = 109 - 0.345 \text{TPSA}$ (Remko, 2009). The ADMETlab (Jie Dong, Ning-Ning Wang, et al., 2018) web server was used to predict physicochemical properties ($\log S$, $\log D$, $\log P$); *Absorption*: CaCO_3 Permeability (CaCO_2), P-gp inhibitor/substrate (P-gp), Human Intestinal Absorption (HIA), 20% bioavailability (F20%), and 30% bioavailability (F30%)); *Distribution*: Plasma Protein Binding (PPB), Blood Brain Barrier (BBB) and Volume Distribution (VD) and *Excretion*: Half Life ($T_{1/2}$) and Clearance (Cl). The properties of CaCO_2 , VD, PPB, Cl, $T_{1/2}$ were expressed numerically, whereas the rest of the pharmacokinetic parameters were expressed categorically (Jie Dong, Ning-Ning Wang, et al., 2018). Metabolism related parameter, human liver microsomal (HLM) assay was predicted using variable nearest neighbor (vNN) method (Schyman et al., 2017). Besides, water solubility, $\log P$ and $\log S$ values of the flavonoid derivatives were retrieved from drug bank (Wishart et al., 2018), which are predicted using ALOGPS 2.1 and ChemAxon. The molecular descriptors and properties generated from all webtool/webservers were considered to screen the best candidate from the sixteen flavonoids (Pathak et al., 2017).

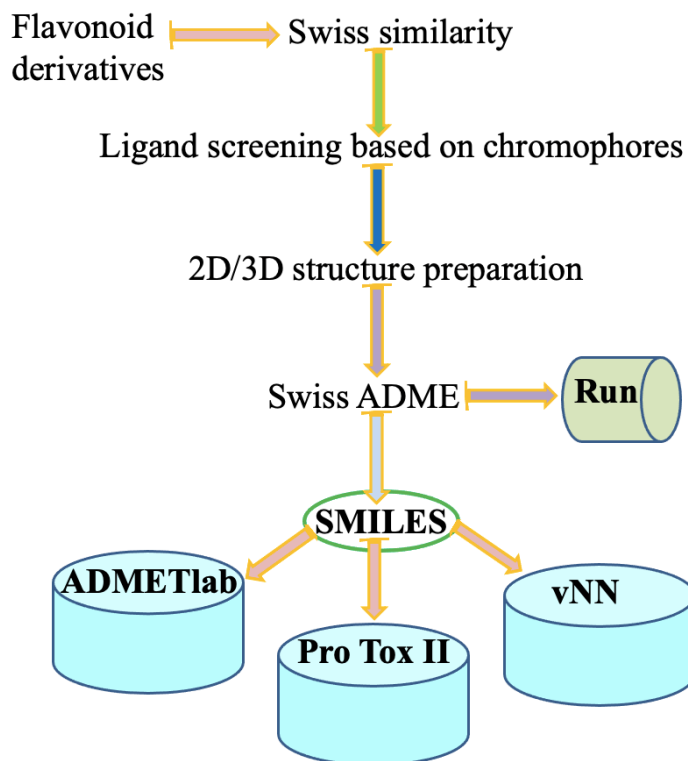


Figure 15. Schematic representation of ligand screening flowchart

3.2.3 Toxicity prediction

A total of 19 parameters were predicted to study the toxicity profile of the sixteen flavonoids and the two controls. The toxicological endpoints (Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity) and the level of toxicity (LD_{50} , mg/Kg) of the studied flavonoids were determined using ProTox-II server (Banerjee, Eckert, et al., 2018; Drwal et al., 2014). Toxicity profiles - the hERG potassium channel inhibition (cardiotoxicity), H-HT (Human Hepatotoxicity), AMES (Ames Mutagenicity) and SkinSen (Skin sensitization) - were predicted using ADMETlab (Jie Dong, Ning-Ning Wang, et al., 2018). Drug induced liver injury (DILI), mitochondrial membrane potential (MMP) toxicity and cytotoxicity parameters were predicted using the vNN method (Schyman et al., 2017).

3.2.4 Quantum chemical calculations for the ligand screening

Geometry optimizations and frequency calculations of the structures (Figure 16) were performed using the Gaussian 09 (M. J. Frisch et al., 2009) program package and the results were visualized using GaussView 06 software. Geometries of the compounds for the density functional theory (DFT) calculations in which the DFT/B3LYP (Beck, 1993; C. Lee, Yang, & Parr, 1988; Stephens,

Devlin, Chabalowski, & Frisch, 1994) hybrid functional was used together with 6–311++G(d, p) basis set (Krishnan, Binkley, Seeger, & Pople, 1980). Such an approach has been shown to give reliable results for related organic molecules (Demissie, Ruud, & Hansen, 2015; Fjellaksel et al., 2018; Gezhagn, Temam, & Lelisho, 2021). The optimized geometries were confirmed to be real minima without any imaginary vibrational frequency by performing vibrational frequency calculations at the same level of theory. Quantum chemical descriptor set was chosen to obtain pertinent electronic and structural features (Ojha et al., 2011). The frontier molecular orbitals (FMOs), energy gap ($\Delta E = E_{LUMO} - E_{HOMO}$), electronegativity ($\chi = -\frac{1}{2} (E_{HOMO} + E_{LUMO})$), electronic chemical potential ($\mu = \frac{1}{2} (E_{HOMO} + E_{LUMO}) = -\chi$), global chemical hardness ($\eta = \frac{1}{2} (E_{LUMO} - E_{HOMO})$), global softness ($\sigma = 1/2\eta$), global electrophilicity index ($\omega = \mu^2/2\eta$), and nucleophilicity index ($Nu = 1/\omega$), and dipole moment were also calculated and analyzed at the same level of theory (Barakat et al., 2015; Ismael, Abdel-Mawgoud, Rabia, & Abdou, 2020).

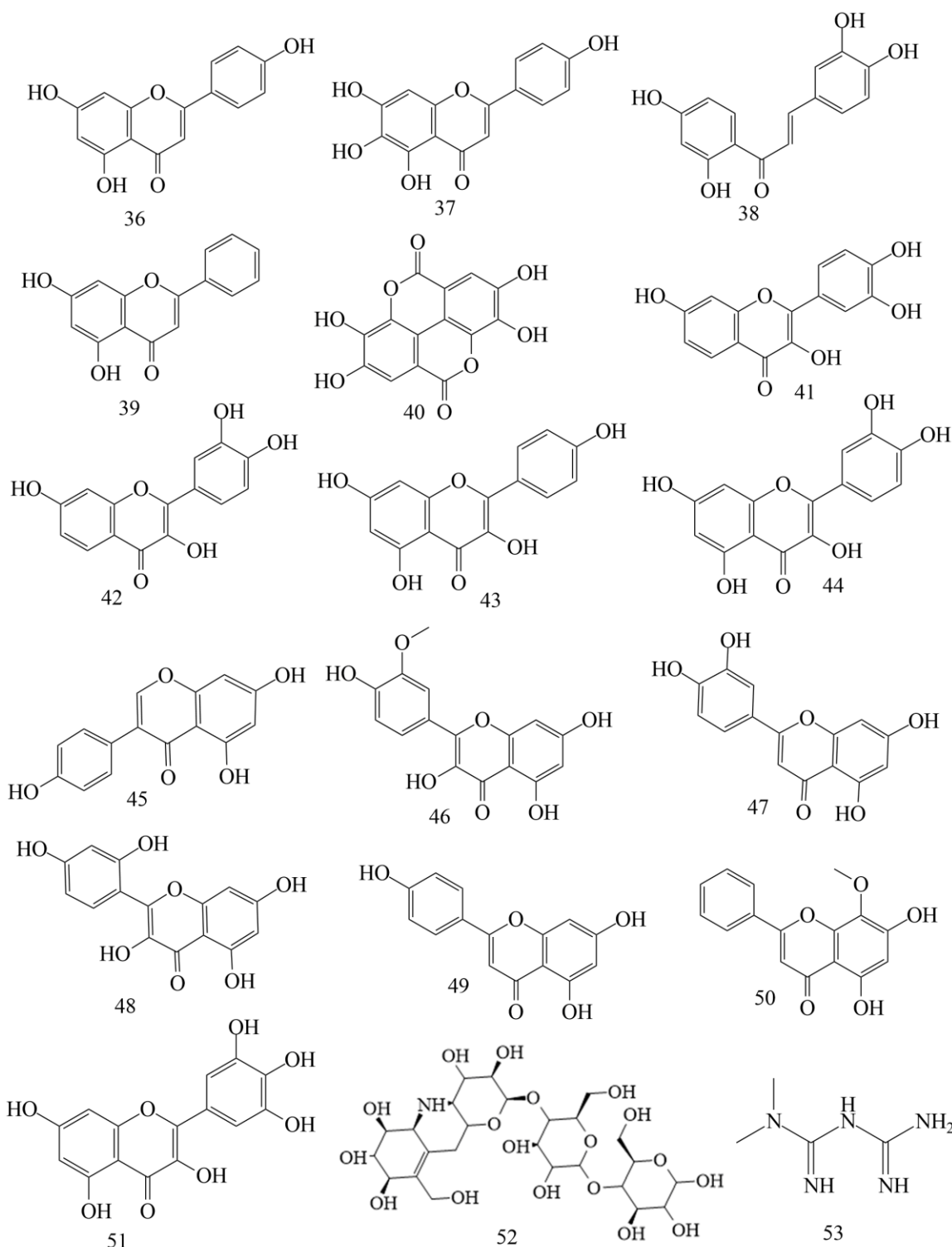


Figure 16. The structures of the flavonoid derivatives considered for the ligand screening. The numbers (36 to 53) stand for 36 = Apigenin, 37 = Baicalein, 38 = Butein, 39 = Chrysin, 40 = Ellagic acid, 41 = Eriodyctiol, 42 = Fisetin, 43 = Kaempferol, 44 = Quercetin, 45 = Genistein, 46 = Isorhamnetin, 47 = Luteolin, 48 = Morin, 49 = Naringenin, 50 = Wogonin, 51 = Myricetin, 52 = acarbose and 53 = metformin.

3.2.5 Molecular docking study

The molecular docking study of the target flavonoids (Figure 16) was made by following the reported method (Allouche, 2011). The 3D structure of human pancreatic α -amylase was obtained from Protein Data Bank (Williams et al., 2015) (PDB ID: 4W93). Auto Dock Vina 4.2 (MGL tools 1.5.7) with previously reported protocol used to dock the protein Human pancreatic α -amylase and the flavonoid compounds [35-39]. Briefly, the PDB file for ER α was downloaded from the Protein Data Bank. The co-crystallized substrate and water molecules were removed from the receptor using the MGL 1.5.6 software. After cleaning the protein, only polar hydrogens were added together with the Kollman charges. The grid box was constructed using 120, 120, and 120 pointing in the x, y, and z dimensions, respectively, with a grid point spacing of 0.375 Å. The center grid box was set at -12.055, -10.491, and 5.964 Å for the x, y, and z centers, respectively. The total number of 9 genetic algorithms was as a default, which generated nine different conformations for each of the molecules (Ördög & Grolmusz, 2008). The conformers with the lowest binding free energies were used for the visualization of the interactions between the active amino acids and the molecule using the Discovery Studio software.

3.3 Synthesis of Cr(III), Cu(II) and Zn(II) mixed ligand complexes of chrysin and 1,10-phenanthroline

In this work, *in silico* aided ligand screening protocol was followed to synthesize mixed ligand metal complexes as clearly presented in Figure 17. Heteroleptic Cr(III) complex (**54**) of 1,10-phenanthroline and an oxo-ligand, chrysin, (5,7-dihydroxy-2-phenyl-4H-chromen-4-one) were prepared according to the method reported by Abebe A., et al., (Abebe & Hailemariam, 2016) with minor modifications. In brief, 1 mmol of chrysin (0.254 g) was added to a mixture of 20 mL of methanol: ethanol solution (1:3 ratio) in the presence of a deprotonating agent, 1 mmol NaOH and stirred for 20 minutes resulting a clear yellow solution. To the clear yellow solution, 1 mmol of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (0.266 g) was added slowly and refluxed at 90 °C in an oil bath. The resulting light green solution was refluxed for 1 h and 20 'min', then 1 mmol of methanolic solution of 1,10-phenanthroline was added slowly. Then 1 drop of triethylamine was added to the reaction mixture to maintain the pH of the solution alkaline and the reflux was continued for additional 6 hours and 40 minutes. The progress of the reaction was monitored by TLC. The resulting brown solution was filtered; cooled, small amount of diethyl ether was added to the filtrate, allowed to stand overnight.

The resulting jelly like brown precipitate was left to dry slowly at room temperature. The presence and absence of chloride ion in the ionization sphere was determined using AgNO_3 test.

Heteroleptic metal complexes Cu(II) (**55**) and Zn(II) (**56**) were synthesized employing chrysin with a co-ligand 1,10-phenanthroline according to the reported method of Lin S et al., (Lin et al., 2017) with little modifications. For **55**, 1 mmol of chrysin (0.254 g) was added to a mixture of 20 mL of methanol: ethanol solution (1:3 ratio) in the presence of a deprotonating agent, 1 mmol NaOH and stirred for 20 'min' resulting a clear yellow solution. To the clear yellow solution, 1 mmol $\text{Cu}(\text{O}_2\text{C}_2\text{H}_3)\cdot\text{H}_2\text{O}$ (0.199 g) was added slowly. The resulting blue-green solution was stirred for 20 minutes, then 1 mmol of methanolic solution of 1,10-phenanthroline (0.198 g) was added slowly followed by addition of 140 μL (1 mmol) triethylamine dropwise. The resulting green brown solution was refluxed at 60 $^\circ\text{C}$ for 6 h. The progress of the reaction was monitored with TLC. Finally, diethyl ether was added to the obtained green-brown solution, which was then stored in refrigerator at 4 $^\circ\text{C}$ for two weeks. Two weeks later green brown powders were collected from the filtrate. The same procedure was employed for the synthesis of Zn(II) mixed ligand complex **56** using 1 mmol of $\text{Zn}(\text{O}_2\text{C}_2\text{H}_3)\cdot 2\text{H}_2\text{O}$ (0.219 g), 8 h of reflux and a yellow-orange powder was obtained and characterized.

3.4 Synthesis of Cr(III), Cu(II) and Zn(II) mixed ligand complexes of chrysin and metformin

Heteroleptic metal complex Cr(III) (**57**) consisting of a metformin and oxo-ligand, chrysin were prepared according to the reported method of (Lin et al., 2017) with minor modifications. To the clear hot methanolic solution of metformin, 1 mmol of $\text{CrCl}_3\cdot 6\text{H}_2\text{O}$ (0.266 g) was added slowly and stirred resulting a fade-green solution. 1mmol of chrysin (0.254 g) in mixture of methanol: ethanol (1:3) was added to the reaction mixture in the presence of a deprotonating agent, 1mmol NaOH. Triethylamine (1 mmol, 140 μL) used to maintain the pH of the solution alkaline and the reflux was continued for extra 8 h. The resulting green-brown solution was filtered, treated with diethyl ether, and allowed to stand for two weeks for slow removal of solvent and drying. A light green powder was obtained from slow evaporation at room temperature. Similar to complex 54, a AgNO_3 test was used to check the presence and absence of ionizable chloride ion.

The Cu(II) complex (**58**) chrysin and a co-ligand metformin was synthesized according to the reported method of (Lin et al., 2017) with minor modifications. Briefly, to 1mmol of chrysin (0.254

g) in hot methanol: ethanol solution (1:3 ratio) in the presence of a deprotonating agent, NaOH (1mmol), 1 mmol of $\text{Cu}(\text{O}_2\text{C}_2\text{H}_3)_2 \cdot \text{H}_2\text{O}$ (0.199 g) was added slowly. The resulting yellow-green solution was refluxed and stirred for 50 ‘min’, then 1 mmol of methanolic solution of metformin (0.165 g) was added slowly followed by addition of 1mmol of triethylamine (140 μL) and the reaction mixture was refluxed for extra 8 h. The progress of the reaction was monitored with TLC. The reaction mixture was filtered and treated with diethyl ether, stored in refrigerator at 4 $^\circ\text{C}$ for two weeks. Two weeks later, gray-brown powders were collected using slow evaporation. Complex **59** of a Zn(II) metal center with the same structural analogs were obtained using a similar procedure after 6 h reflux, yielding yellow-orange powder with visible grain size. The schematic representation of the ligand screening, synthesis, characterization and biological activity of the metal complexes reported in this work are presented in Figure 17.

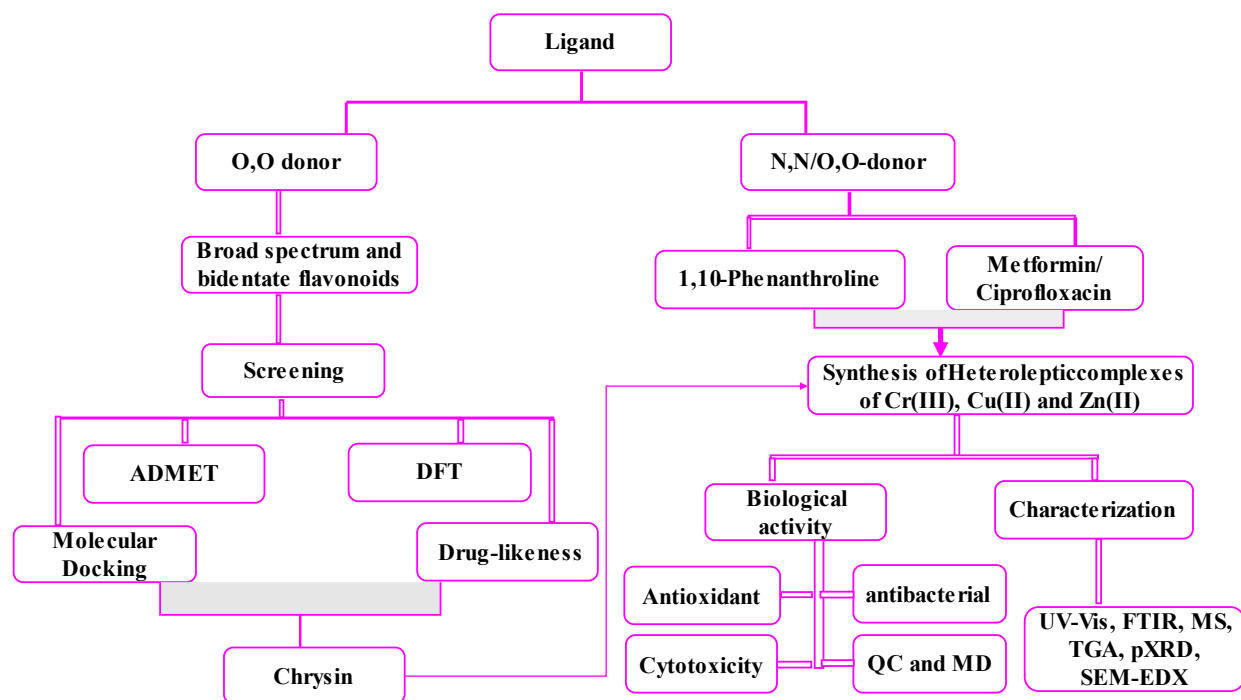


Figure 17. Schematic representation of the research design for ligand screening, synthesis, characterization and biological activity.

3.5 Synthesis of 1,10-phenanthroline based Cu(II) and Zn(II) mixed ligand complexes

The synthesis and biological activity evaluations of Cu(II) (**60** and **61**) and Zn(II) (**62**) metal mixed ligand complexes containing metformin (1,1-dimethyldibiguanide) and ciprofloxacin, and 1,10-phenanthroline monohydrate have been conducted. Complex **60**, **61** and **62** were prepared by

adapting reported procedures (Vasanth et al., 2018) with minor modifications. Briefly, a methanolic solution of 1,10-phenanthroline monohydrate (0.198 g, 1mmol) was added to each of the round bottom flask that contain a hydro methanolic solution (5:1 methanol-water) of $\text{Cu}(\text{CO}_2\text{CH}_3)_2 \cdot \text{H}_2\text{O}$ and ZnCl_2 (5:1 methanol-water) being stirred magnetically for 30 'min' at room temperature. A hydro-methanolic solution of metformin hydrogen chloride (0.166 g, 1 mmol) for compounds **60** and **62**, and ciprofloxacin for compound **61** which are dissolved in the presence of NaHCO_3 (1 mmol) were added slowly from a dropping funnel under continuous stirring. A deep blue colored solution for compound **60**, green solution for compound **61** and white solution for compound **62** (protonated metformin of **60**) were refluxed for 3 h each resulting dark brown, green and white powder, respectively. The reaction progress was monitored using TLC. Each of the obtained precipitates were filtered and repeatedly washed with ice cold methanol and dried over CaCl_2 .

3.6 Characterization of ligands and their complexes

3.6.1 Characterization of Ligands

In this work two sets of ligands were used. The first one was chrysin 5,7-dihydroxy-2-phenyl-4H-chromen-4-one) which was employed based on the *in silico* ADMET, DFT and molecular docking screening protocols. The other set of ligands are ligands used for their medicinal value (metformin (1,1-dimethyldibiguanide) and ciprofloxacin), and 1,10-phenanthroline monohydrate which was used for its planarity and chelating effect. These ligands were subjected for Fourier-transform infrared spectroscopy (FT-IR) and Ultraviolet–visible spectroscopy (UV-Vis) analysis both experimental and DFT/TD-DFT calculations. The associated wavelength and vibrational frequencies were compared with their metal complexes.

3.7 Characterization of the synthesized metal complexes

Different characterization instruments were used to characterize the synthesized metal complexes reported in this work.

3.7.1 Thin layer chromatography and melting point analysis

Thin layer chromatography is the most convenient and economic technique used mainly for monitoring the progress of a reaction and obtain qualitative information on reaction completeness. It employs mixture of solvents of different ratios to confirm the disappearance of the reactants and appearance of the product (Hemmateenejad, Akhond, Mohammadpour, & Mobaraki, 2012). In this dissertation work, thin layer chromatography (MERCK Silica gel 60 F254) together with

UV Cabinet (UV-Vis lamp at 254 and 365 nm) was used to monitor the progress of the chemical reactions. A digital mobile camera was used to detect TLC spot pictures. The melting points of the complexes were determined using capillary tubes (Thiele tube) and melting point apparatus.

3.7.2 UV-Vis spectroscopy analysis

The electronic spectra of the ligands and their metal complexes were recorded using UV-Vis spectrophotometer (SM-1600 Spectrophotometer) from 200 - 800 nm range at room temperature. Both the ligands (chrysin, 1,10-phenanthroline, metformin and ciprofloxacin) and the synthesized metal complexes were dissolved in DMSO. The spectrometer was switched on and allowed to scan the operating conditions for 30 min and set zero reading for the solvent (DMSO). A quartz cell of 1.0 cm thickness was used to hold the dilute solutions of 1.0×10^{-4} M. Finally, the absorbance and wavelength (nm) of the readings was subjected for plotting using Origin 2018 and the changes made to the characteristic absorption lambda maximum ($\lambda_{\max}$) of the ligands were used to characterize the synthesized metal complexes.

3.7.3 Molar conductance analysis

The molar conductance of the metal complexes was recorded at room temperature using electrical conductometer (AD8000). The complexes were prepared in 1.0×10^{-3} M in DMSO. The reading was done for in triplicate. The same molarity and temperature conditions were used to measure the electrical conductivity of the complexes.

3.7.4 Fourier-transform infrared spectroscopy (FTIR) analysis

In order to analyze the changes made after complexation, both ligands and the synthesized metal complexes were subjected for FTIR analysis. Potassium bromide (KBr) tablets of 0.8 g were pressed with 1 mg of the test samples. The spectrum of the ligands and their complexes were obtained on the middle region of the infrared ($4000 - 400 \text{ cm}^{-1}$) using a FTIR instrument (Perkin-Elmer BX spectrometer, Shimadzu Corporation, Japan). The wavenumber (cm^{-1}) and percent transmittance data of the test samples was subjected for Origin 18 for further analysis.

3.7.5 Thermogravimetric analysis

The thermogravimetric/differential thermal analysis of the synthesized complexes was conducted using a thermal analyzer (TGA/DTA, DTG-60H SHIMADZU). Briefly, 8 mg of the test sample was loaded into a high-temperature crucible which is suspended in a computer-controlled furnace and electro-balance with microgram sensitivity. Sample mass and furnace temperature were

continuously recorded over the course of the run to record the thermal behavior of the synthesized complexes from 25 – 800 °C with a heating rate of 10 °C/min under nitrogen-atmosphere (20 mL/min). Finally, the temperature, TGA, and DTA data were obtained. The TGA data were treated to calculate the mass, weight loss percent, weight percent of the samples. A multi-curve graph was plotted using Origin 2018 and used to assign the associated fragments and thermal stability of the complexes.

3.7.6 High-resolution mass spectrometry analysis

The high resolution mass spectra of the synthesized Cr(III), Cu(II) and Zn(II) metal complexes were obtained with a Waters-LCT-Premier mass spectrometer. Briefly, a high-resolution mass spectrum (Waters-LCT-Premier mass spectrometer) that operates in 2 ng/μL of sample concentration, 2500 V capillary voltage, a desolvation temperature of 250 °C using nitrogen gas at 250 L/hr, electrospray positive ionization mode, Bruker APEX II CCD area detector diffractometer, with graphite monochromated Mo K α radiation (50 kV, 30 mA) and temperature of measurement at 173(2) K coupled with APEX 2 data collection software were employed. The teste sample was dissolved in methanol to a concentration of 2 ng/μL and introduced by direct infusion. The collection method involved 4-scans of width 0.5 and 512×512bit data frames. The data reduction was achieved by means of the program SAINT+ and face indexed absorption corrections were made using XPRE.

3.7.7 Scanning electron microscopy and energy dispersive spectroscopy (SEM-EDS) analysis

The morphology and elemental composition of the synthesized metal complexes was determined using scanning electron microscopy (JSM-6500F system JEOL, Tokyo, Japan) coupled with energy dispersive x-ray spectroscopy (EDS). Briefly, 50 mg of the powders for each of the samples were mounted on a copper stub with carbon tape. All the samples were analyzed at 9.7 mm working distance with accelerating voltage of 15.0 kV and magnification ranging from 350 x to 1200 x. The irradiated samples resulted the emission of x-rays that characteristic to the elements present in the synthesized metal complexes. The presence, absence and abundance of the atoms expected to present in the synthesized complexes were confirmed by sung the X-ray intensity of the characteristic elements which is directly proportional to the concentration of the elements in the metal complexes.

3.7.8 X-ray diffraction analysis

X-ray diffraction was used to identify the crystalline and polycrystalline nature of the synthesized metal complexes, and identify the crystalline system of the synthesized complexes. In this work, an x-ray diffraction (XRD-7000, Shimadzu Co., Japan) that record peak at diffraction angle (2θ) ranging from 10 – 80 degrees were used. The X-ray wavelength of $\text{CuK}\alpha$ ($\lambda = 1.5406 \text{ \AA}$) radiation that operated at 40kV and 30 mA was employed (Holzwarth & Gibson, 2011). The obtained diffraction angles and associated peak intensities were used to identify the phase of the synthesized metal complexes. Moreover, the average crystallite size of the complexes were calculated using the Debye–Scherrer equation (Holzwarth & Gibson, 2011). Hence, the phase identification of the synthesized complexes was accomplished by comparing the experimental XRD pattern with a reference pattern obtained from crystallography open database (COD) for inorganic materials. The comparison between the experimental XRD pattern and the reference was conducted using QUALX2.0 (Altomare et al., 2015). Accordingly, determinations of the crystal system of the complexes, the space group of the complexes, the inter-planar spacing (d), the Miller indices (hkl) and the lattice parameters (a , b , c , α , β and γ) of the synthesized complexes were conducted using the same protocol.

3.8 Biological Activity

3.8.1 Cytotoxicity: Cells and cell maintenance

MCF-7 breast cancer cells previously stored in liquid nitrogen at -180°C , were cultured at 37°C in a humidified 5% CO_2 atmosphere. The cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine, 50 IU/mL penicillin, and 50 $\mu\text{g/mL}$ streptomycin (Koobotse, Schmidt, Holly, & Perks, 2020).

3.8.1.1 Cytotoxicity Assay

Cytotoxicity of the synthesized complexes (**54** - **62**) was evaluated using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay as per manufacturer instructions (Roche). Preliminary test was conducted to screen metal complexes for the desired activity. Following the preliminary screening, standard protocol for MTT assay was employed. Briefly, MCF-7 breast cancer cells were seeded in a clear, flat-bottom 96-well plate at a density of 5×10^3 cells well^{-1} in 100 μL of growth medium. The cells were allowed to attach for 24 h. The synthesized compounds were dissolved in phosphate-buffered saline (abbreviated PBS, $\text{pH} \sim 7.4$) and serially diluted with

growth media to prepare different concentrations ranging from 25 to 0.78 μ M. Spent growth media was discarded and the cells were then treated with the compounds diluted with growth media for 24 h at 37.5 °C. Control cells were treated with a mixed solvent (PBS) at concentrations matching those of the compounds following the same procedure. Cisplatin was included as a positive control. After the 24 h incubation period, 10 μ L of MTT labelling reagent (final concentration of 0.5 mg/mL) was added to each well and incubated at 37.5 °C for 4 h in a humidified 5% CO₂ atmosphere. DMSO was added to each well to dissolve formazan crystals. Finally, optical density was measured at 570 nm using a Multiskan™ FC Microplate photometer (ThermoFisher Scientific). A bright-field optical images were taken using inverted type fluorescence microscope (Olympus IX51, Japan) equipped with cooled CCD camera (QICAM 12-bit Mono Fast 1394, QImaging Ltd., Canada) to perform the image of treated and untreated MCF-7 cells. All the experiments were conducted in triplicate.

3.8.2 Antibacterial activity analysis

The antibacterial activity evaluation of the free ligands and all the synthesized mixed ligand transition complexes were conducted by adapting reported procedure (Lemilemu, Bitew, Demissie, Eswaramoorthy, & Endale, 2021) with minor modifications. The media was prepared by dissolving 38 g of Mueller Hinton agar (MHA) medium in 1000 mL of distilled water and autoclaved at 121 °C for 15 ‘min’. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and allowed to solidify under sterile condition at room temperature. The bacterial cultures were inoculated into the nutrient broth (inoculation medium) and incubated overnight at 37 °C. Inoculated medium containing a 24 h grown culture was added aseptically to the nutrient medium and mixed systematically to get an even distribution. The solution was poured to approximately 20 mL of sterile MHA in sterile culture plates and allowed to attain room temperature. Sterile agar-disc diffusion previously soaked in a known concentration of the synthesized metal complexes and standard drugs was prepared in DMSO using nutrient agar tubes and carefully placed at the center of the labeled seeded plate. Mueller–Hinton sterile agar plates were seeded with indicator bacterial strains (1.3×10^8 cfu/mL) and allowed to stay at 37 °C for 3 h. Sterile filter paper disks with a diameter of 6 mm were placed over these plates. Finally, the plates were incubated at 37 °C for 24 h. The mean inhibition zones were measured with a ruler and compared with the positive control (a disk containing ciprofloxacin) of the same concentration

in millimeter. DMSO was used as negative control during the whole test. The mean inhibition zone (MIZ) was expressed as mean value $\pm$ standard deviation.

Clinical isolates were obtained from Adama Public Health Research and Referral Laboratory Center, Ethiopia. Gram negative strains *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853), and Gram-positive strains *Staphylococcus aureus* (ATCC25923) and *Streptococcus pyogenes* (ATCC19615) were considered. Different concentrations were prepared from the synthesized compounds by dissolving 5 mg of each compound in 5 mL DMSO to make 1 mg/mL of standard solution. The standard solution was serially diluted to furnish the test solution (25 μ g/mL and 50 μ g/mL) sample for each synthesized metal complexes. The concentrations of each sample were incorporated into sterile blank paper discs and dried at 37 °C (Piotto et al., 2017).

In vitro antibacterial activities of the synthesized metal complexes: chrysin-based mixed ligand complexes; **54 - 56**, metformin-based complexes; **57 – 59** and mixed drug complexes, **60 - 62** were conducted in triplicate. Known concentration of the synthesized metal complexes and a positive control, ciprofloxacin was prepared in DMSO. The mean inhibition zones were measured with a ruler in mm. DMSO was used as a negative control.

Statistically, all the antibacterial activity data were analyzed using IBM SPSS (Version 25). Univariate analysis of variance and PostHoc multiple comparison for the observed means (assuming LSD and Tukey equal variances) among metal complexes were conducted. The significant difference among the antibacterial activity of the complexes were conducted by considering a data with P values less than 0.05 statistically significant.

3.8.3 Antioxidant activity using DPPH (1-1-diphenyl 2-picryl hydrazyl) assay

The DPPH scavenging activity study was conducted following previously reported method (KWON, Apostolidis, & Shetty, 2008). Solution of DPPH in methanol (400 ppm) was prepared and 1.0 mL of this solution was added to 3 mL of each ligand and metal complex solutions at different concentrations (25 – 400 ppm) and the reaction mixture was vortexed for 10 s and allowed to stand at room temperature for 30 min in a dark. Similar concentration of the standard, ascorbic acid (AA) was used and measured. The absorbance of the resulting mixture and the control solution was recorded at 517 nm. The 50 % radical scavenging activity (IC₅₀) of the test compounds

(ligands and metal complexes) was computed from the regression equation of the concentration versus the radical scavenging activity of each test compound. The percentage of DPPH radical scavenging activity was calculated using equation 1.

$$\% \text{ DPPH radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100\% \text{ --- (1)}$$

where A_c is absorbance of the control and A_s is the absorbance of a test sample

3.9 Computational studies

3.9.1 Density functional theory and time-dependent DFT calculations

Density functional theory (DFT) calculations employing B3LYP-GD3 (Beck, 1993; C. Lee et al., 1988; Stephens et al., 1994) hybrid functional together with 6-311++G(d, p) basis set (Krishnan et al., 1980) for atoms (H, C, N, F, O and Cl) of chelating agents were used to study the quantum chemical properties of the synthesized metal complexes. Los Alamos National Laboratory 2-Double-zeta (LanL2DZ) pseudopotentials were applied for the metal atom (Cr(III), Cu(II) and Zn(II)) (Hay & Wadt, 1985) to account for relativistic effects. The non-bonding interactions during the calculations were corrected using Grimme's dispersion correction (Grimme, Furche, & Ahlrichs, 2002). This is because such a combination of functional and basis sets has been used and gave results that are in a good agreement with the experiment in previous studies (Demissie & Hansen, 2016; Demissie et al., 2015; Lemilemu et al., 2021). The vibrational frequency calculations were performed at the same level of theory to confirm the optimized geometries were real minima without any imaginary vibrational frequency. The frontier molecular orbitals (FMOs): highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbitals (LUMO), and quantum chemical descriptors: energy gap ($\Delta E = E_{LUMO} - E_{HOMO}$), electronegativity ($\chi = -\frac{1}{2}(E_{HOMO} + E_{LUMO})$), electronic chemical potential ($\mu = \frac{1}{2}(E_{HOMO} + E_{LUMO}) = -\chi$), global chemical hardness ($\eta = \frac{1}{2}(E_{LUMO} - E_{HOMO})$), global softness ($\sigma = 1/2\eta$), global electrophilicity index ($\omega = \mu^2/2\eta$), and nucleophilicity index ($Nu = 1/\omega$), were calculated and analyzed at the same level of theory (Barakat et al., 2015; Ismael et al., 2020). Such quantum chemical descriptors are pertinent in DFT and are used to establish how the structure, stability and reactivity of compounds relate with their biological activities (Pratiwi, Ibrahim, & Tjahjono, 2020).

3.9.2 Molecular docking studies

The 2D and 3D interaction of the synthesized complexes with the binding sites of estrogen receptor alpha (ER α ; PDB:5GS4) (M. Xie et al., 2017) and the protein of *S. aureus* (dihydrofolate reductase: PDB: 2w9h)) were studied using molecular docking. The geometries of the metal complexes were optimized using the B3LYP-GD3/6-311++G(d,p)/LanL2DZ method prior to docking and converted to PDB files using the Gaussview software. The molecular docking studies of the compounds (**54** - **62**) and the positive control (cisplatin) were performed using the AutoDock 4.2.6 software (Allouche, 2011). The same protocols to previous works were used (Bitew et al., 2021; Damena, Alem, et al., 2022a; Lemilemu et al., 2021). Briefly, the PDB file for ER α was downloaded from the Protein Data Bank with a resolution of 2.4 Å (M. Xie et al., 2017). The co-crystallized substrate and water molecules were removed from the receptor using the MGL 1.5.6 software. After cleaning the protein, only polar hydrogens were added together with the Kollman charges. Non-polar hydrogen atoms were merged and Gasteiger partial atomic charges were assigned to the molecules. Standard docking parameters for all the light and metal atoms were used. The grid box was constructed using 120, 120, and 120 pointing in the x, y, and z dimensions, respectively, with a grid point spacing of 0.375 Å. The center grid box was set at -12.055, -10.491, and 5.964 Å for the x, y, and z centers, respectively. Lamarckian genetic algorithm (LGA) program (Morris et al., 1998) with an adaptive whole method search in the AutoDock was selected and set at 100, which generated one hundred different conformations for each of the molecules (Damena, Alem, et al., 2022a, 2022b). The conformers with the lowest binding free energies were used for the visualization of the interactions between the active amino acids and the molecules using the Discovery Studio software.

3.9.3 Pharmacokinetic Prediction and BOILED Egg Model

The synthesized metal complexes (**60** and **61**) whose molecular weights fit to the model and the ligands were subjected for physicochemical and pharmacokinetic (ADME) evaluation using SwissADME (Daina et al., 2017). Cisplatin was used as a positive control. The protocol to predict physicochemical properties, pharmacokinetic and drug likeness evaluation, and BOILED egg model (Figure 18) of the metal complexes and the ligands were conducted according to previous work (Bitew et al., 2021; Damena, Alem, et al., 2022a; Lemilemu et al., 2021).

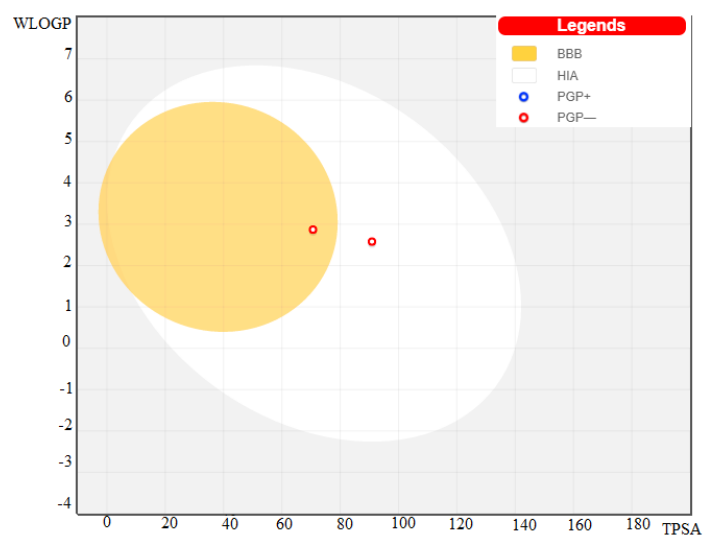
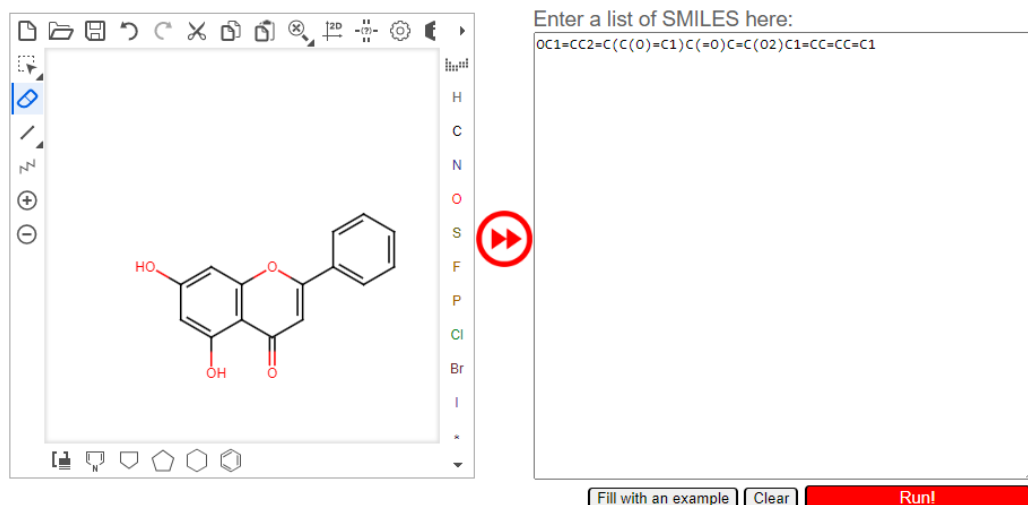


Figure 18. A representative working page of SwissADME and its BOILED-Egg model skeleton

4. RESULTS AND DISCUSSION

In this work, a bidentate naturally available ligand was screened using *in-silico* ADMET screening tools for further metal complexation. Mixed-ligand transition metal complexes of Cr(III), Cu(II), and Zn(II) using metformin, chrysin and 1,10-phenanthroline ligands were successfully synthesized and characterized. The structures of the complexes were supported with DFT and TD-DFT calculations. Finally, the biological applications of the as-synthesized metal complexes against breast cancer (MCF-7) cell line, gram-negative and gram-positive bacteria, and DPPH radical were evaluated. In this chapter, details of the result and discussion are presented.

4.1 Analysis of physicochemical properties and drug-likeness

In this study sixteen flavonoids listed in Figure 16 were screened for drug-likeness properties following standard computational procedure, and the result revealed that fifteen of them do not violate Lipinski's rule of five (Lipinski et al., 1997) for oral availability, demonstrating that the compounds have drug-like molecular nature (Table 1) except myricetin (**51**) holding six NHBDs (> 5). It is important to note that Lipinski's rule of five is essential for rational drug design and it has been suggested that the low permeability or poor absorption for a given compounds results when it violates one of Lipinski's rule of five (Pathak et al., 2017). Molecular weight, LogP and number of hydrogen bond acceptors (NHBAs) of all flavonoids are within the recognized values of less than 500, 3 and 10, respectively.

Research findings demonstrated that the molecules with a total polar surface area (TPSA) of $140 \text{ \AA}^2$ and above would be poorly absorbed ($< 10\%$ fractional absorption), while those with a TPSA of $60 \text{ \AA}^2$ would be well absorbed ($> 90\%$) (Clark, 1999). The TPSA and percent absorption (%Abn) of the flavonoid derivatives showed the preference of apigenin (**36**), baicalein (**38**), chrysin (**39**), genistein (**45**), and wogonin (**50**) with better and comparable percent absorption to the control metformin (**53**). It is found that the %Abn and TPSA values follow the order chrysin (**39**) $>$ wogonin (**50**) $>$ metformin (**53**) $>$ genistein (**45**) = apigenin (**36**) = baicalein (**37**). The results also showed an inverse relationship between TPSA and %Abs, and direct relationship between %Abs and MR. The analysis of the %Abs of the sixteen studied flavonoids and the two controls for their percent absorption (Table 1) showed that chrysin (**39**) have the highest percent absorption (84.6%) than all the other flavonoids and the two controls, whereas metformin (**53**) displayed better percent absorption (78.3%) than acarbose. These results are in good agreement with a previous report on

pharmacokinetic properties of apigenin (**36**), eriodyctiol (**41**), fisetin (**42**), kaempferol (**43**) and quercetin (**44**) via molinspiration and pkCSM servers (Vijayakumar, Ramesh, Joji, & Kannan, 2020).

Table 1. The physicochemical property of the flavonoids and the two control drugs

	Physicochemical Property								
Flavonoid	MW	NHBAs	NHBDs	TPSA	%Abs	MR	log S	LogD	LogP
36	270.24	5	3	90.9	77.64	73.99	-3.595	0.487	2.577
37	270.24	5	3	90.90	77.64	73.99	-3.578	0.583	2.577
38	272.25	5	4	97.99	75.19	74.34	-3.427	0.266	2.405
39	254.24	4	2	70.67	84.62	71.97	-3.709	0.847	2.871
40	302.19	8	4	141.34	60.24	75.31	-3.076	-0.740	1.313
41	288.25	6	4	107.22	72.01	73.59	-3.534	0.311	2.216
42	286.24	6	4	111.13	70.66	76.01	-3.417	0.284	2.282
43	286.24	6	4	111.13	70.66	76.01	-3.427	0.273	2.282
44	302.24	7	5	131.36	63.68	78.03	-3.534	0.142	1.988
45	270.24	5	3	90.90	77.64	73.99	-3.632	0.563	2.577
46	286.24	6	4	111.13	70.66	76.01	-3.427	0.273	2.282
47	286.24	6	4	111.13	70.66	76.01	-3.502	0.302	2.282
48	302.24	7	5	131.36	63.68	78.04	-3.282	0.113	1.988
49	286.24	6	4	111.13	70.66	76.01	-3.427	0.273	2.282
50	284.26	5	2	79.90	81.43	78.46	-3.696	0.927	2.88
51	318.24	8	6	151.59	56.70	80.06	-3.220	-0.068	1.694
52	645.6	19	14	321.17	-1.80	136.69	-1.368	2.543	-8.565
53	129.16	2	4	88.99	78.30	36.93	-0.656	-0.192	-1.034

The calculated result of Log S showed that chrysin (**39**) (-3.709) is the least soluble molecule and ellagic acid (**40**) (-3.076) has the highest solubility. The relatively high solubility of ellagic acid (**40**) might be due to the presence of four hydroxyl and two ketone pharmacophores (Tiwari et al.,

2009) as compared to the other flavonoids studied in this work. The computed results for the distribution coefficient ($\log D_{7.4}$) of the flavonoids (Table 1) showed that they have high solubility with low permeability by passive transcellular diffusion (Kubinyi, Mannhold, Krogsgaard, & Timmerman, 1993). It is predicted that acarbose (**52**) has moderate solubility, moderate permeability and low metabolism with $\log D_{7.4}$ value of 2.543 and very low lipophilicity of -8.565.

The distribution coefficients ($\log P$) of the flavonoids (**36** and **38**) range between 1.313 and 2.871. The results of the $\log P$ values showed that they have optimal lipophilicity (Optimal: $0 < \log P < 3$). The $\log P$ results for the two controls, acarbose (**52**) and metformin (**53**), are less than zero, -1.034 and -8.565, respectively, suggesting that the two compounds have poor membrane permeability. The $\log P$ of apigenin (**36**) (3.07) and chrysin (**39**) (3.44) were found to be greater than three as predicted in ALOGPS (Table 2) which might be due to the small number of hydroxyl groups present in both compounds. The water solubility (mg/mL) of the flavonoids considered in this work was retrieved from the drug bank (predicted using ALOGPS) and tabulated in Table 2. It is predicted that ellagic acid (**40**) has high solubility (0.823 mg/mL), whereas chrysin (**39**) was found to have low solubility (0.105 mg/mL). These results are in agreement with those predicted using ADMETlab as well as with the experimental findings suggesting that chrysin (**39**) has low aqueous solubility and high absorption or absorption enhancer role (S. H. Lee et al., 2019).

The pK_a values recorded range from 5.54 to 7.91 indicating that the studied flavonoids fall under the category of neutral organic acids, in good agreement with experimental pK_a of apigenin (**36**), ellagic acid (**40**) and quercetin (**44**) (7.12, 6.69, and 7.17, respectively). The relatively higher pK_a of naringenin (**49**) (7.91), apigenin (**36**) (6.63), chrysin (**39**) (6.58), luteolin (**47**) (6.57) and genistein (**45**) (6.55) may cause difficulty of deprotonation, suggesting that the ligands have stronger coordination ability with metals (Lu et al., 2014). On the other hand, the lipophilicity ($\log P_{o/w}$) study showed that wogonin (**50**) (2.880), chrysin (**39**) (2.871), genistein (**45**) (2.557), apigenin (**36**) and **37** (2.577) are highly lipophilic than the other molecules, whereas quercetin (**44**) and morin (**48**) (1.988) were found to be less lipophilic ligands next to ellagic acid (**40**) (1.313) (Table 2). The presence of one methoxy group in the benzoyl part of wogonin (**50**) makes it more lipophilic than chrysin (**39**). It has also been found that the structural resemblance in benzoyl or cinnamoyl part of the flavonoid derivatives play a role in their physicochemical properties. The calculated $\log P_{o/w}$ of the flavonoid derivatives considered in this work is found to range between

1.313 and 2.880. Our predicted results are in good agreement with the previously reported $P_{o/w}$ values of 1.92, 2.27, 3.11 and 1.82 for apigenin (**36**), eriodictiol (**41**), fisetin (**42**) and kaempferol (**43**), respectively (Daina et al., 2017). Although further clinical study is required, the predicted results suggest that oral and intestinal absorptions are possible.

Table 2. Comparison of solubility, partition coefficient, water solubility, and pKa of flavonoids predicted using different methods

Molecule	Log S		Water solubility	Partition coefficient (Log P)			pKa
	Method			Method			ChemAxon
	ALOGPS	ADMETlab	Pred. (mg/mL)	ChemAxon	ALOGPS	ADMETlab	
36	-3.4	-3.595	0.118	2.71	3.07	2.577	6.63
37	-	-3.578	-	-	-	2.577	-
38	-	-3.427	-	-	-	2.405	-
39	-3.4	-3.709	0.105	3.01	3.44	2.871	6.58
40	-2.6	-3.076	0.823	2.32	1.59	1.313	5.54
41	-	-3.534	-	-	-	2.216	-
42	-3.3	-3.417	0.151	1.81	2.03	2.282	6.32
43	-3.2	-3.427	0.178	2.46	1.99	2.282	6.44
44	-3.1	-3.534	0.261	2.16	1.81	1.988	6.44
45	-3.3	-3.632	0.123	3.08	3.04	2.577	6.55
46		-3.427	-	-	-	2.282	-
47	-3.3	-3.502	0.138	2.4	2.73	2.282	6.57
48		-3.282	-	-	-	1.988	-
49	-3.1	-3.427	0.214	2.84	2.47	2.282	7.91
50		-3.696	-	-	-	2.880	-
51	-3	-3.22	0.301	1.85	1.66	1.694	6.43

4.1.1 Pharmacokinetics properties

Absorption

To evaluate the absorption property of the flavonoids, we predicted Caco-2-Permeability (Papp), *P-glycoprotein* (*P-gp*), substrate or inhibitor (*P-gp*inh/*P-gp*sub), Human intestinal absorption (HIA), 20% bioavailability (F_{20}), and 30% bioavailability (F_{30}) as presented in Table 3. It was

found that the values of Caco-2-Permeability were in the range from -4.732 to -6.63 cm/s, suggesting that they have low to moderate permeability. The high negative Caco-2-Permeability (cm/s) of myricetin (**51**) (-6.63 cm/s), morin (**48**) (-6.206 cm/s), kaempferol (**43**) (-6.163 cm/s) and ellagic acid (**40**) (-6.001 cm/s) is mainly due to the presence of large number of NHBDs and NHBAs in their structures which have the potential to render their permeability. The two controls, acarbose (**17**) and metformin (**53**) are predicted to have Caco-2 permeability values of -5.803 cm/s and -7.128 cm/s, respectively. The predicted HIA data of the sixteen flavonoids and the two control drugs are represented categorically as 0 and 1 (Table 3) which showed that apigenin (**36**), chrysin (**39**), fisetin (**42**), genistein (**45**), wogonin (**50**), and metformin (**53**) have high HIA ($\geq 30\%$) whereas acarbose (**52**) and the rest six flavonoids were predicted to have HIA below 30% (Table 3). Flavonoids baicalein (**37**), chrysin (**39**), genistein (**45**), wogonin (**50**) and the control, metformin (**53**) predicted to show bioavailability greater than 30%, whereas the rest of the flavonoids are predicted to show bioavailability between 20 to 30%. Acarbose (**52**) is known for its extremely low bioavailability and less than 2% of absorption as active drug (Wishart et al., 2018). The results indicated low bioavailability ($< 20\%$, Table 3) and extremely low percent absorption for acarbose (-1.8 %, Table 1).

Distribution

Plasma Protein Binding (PPB), Blood Brain Barrier (BBB) permeability, and Volume of distribution (VD) were parameters considered to study the distribution of drug candidates (Table 3). The PPB of apigenin (**36**), eriodictiol (**40**), fisetin (**42**), kaempferol (**43**) and quercetin (**44**) are predicted to be above 90% to bind to common blood proteins significantly, indicating that the pharmacologic effect of these compounds is low. The control drugs, acarbose (**52**) and metformin (**53**) were found to weakly bind to common blood proteins. This could be one of the reasons that make acarbose as a working drug, while it has very low percent absorption (-1.8 %). Baicalein (**37**), chrysin (**39**), ellagic acid (**40**), eriodictiol (**41**), wogonin (**50**) and metformin (**53**) were predicted to permeate BBB using ADMETlab. However, our prediction using SwissADME webtool showed that only chrysin (**39**) can permeate BBB. On contrary to previous report on ellagic acid (**40**) studied using pkCSM server (Al-Nour, Ibrahim, & Elsaman, 2019), our study showed positive result predicted by ADMETlab for ellagic acid (**40**) to permeate BBB. The volume of distribution (VD) in L/Kg were predicted to be all negative, indicating higher distribution of the flavonoids derivatives and the two control drugs in plasma than in tissues, confined to plasma

proteins. The hydrophilic character of the flavonoids contributed to low values of VD. The more negative value of VD for quercetin (-1.56) and less negative value for wogonin (-0.32) followed by chrysin (-0.35) among flavonoids were also presented in Table3. The high VD values of wogonin (50) and chrysin (39) - relative to the other flavonoids showed the consistency of absorption and distribution parameters.

Table 3. Absorption, Distribution and Excretion parameters of the flavonoids and the two controls

Flavonoid	Absorption						Distribution			Excretion	
	Caco-2	Pgp inh	Pgp sub	HIA	F20	F30	PPB	BBB	VD	T1/2	CL
36	-4.985	0	0	1	1	0	90.03	0	-0.58	1.358	1.90
37	-5.120	1	0	0	1	1	85.90	1	-0.69	1.259	1.92
38	-5.204	0	0	0	1	0	86.74	0	-0.92	1.102	1.66
39	-4.973	0	0	1	1	1	89.57	1	-0.35	1.667	1.98
40	-6.001	0	0	0	0	0	65.67	1	-0.99	1.109	1.43
41	-5.302	0	0	0	1	0	92.20	1	-0.91	0.634	1.89
42	-5.126	0	0	1	1	0	91.06	0	-0.95	0.586	1.90
43	-5.075	0	0	0	1	0	90.85	0	-0.98	0.696	1.90
44	-6.168	0	0	0	1	0	94.99	0	-1.56	0.200	2.00
45	-4.999	0	0	1	1	1	90.537	0	-0.58	1.299	1.941
46	-5.075	0	0	0	1	0	90.847	0	-0.97	0.696	1.941
47	-5.123	0	0	0	1	0	91.796	0	-0.93	0.745	1.919
48	-6.206	0	0	0	1	0	87.95	0	-1.46	0.714	2.002
49	-5.075	0	0	0	1	0	90.847	0	-0.97	0.696	1.941
50	-4.732	1	0	1	1	1	86.612	1	-0.32	1.706	1.894
51	-6.630	0	0	0	1	0	76.595	0	-1.39	0.940	1.709
52	-7.128	0	0	0	0	0	21.14	0	-1.22	1.320	0.50
53	-5.803	0	0	1	1	1	23.65	1	-0.19	1.806	0.90

Caco-2 = CaCO₃ permeability, Pgp inh/Pgp sub = permeability glycoprotein inhibitor/substrate, HIA = human intestinal absorption, F20 = 20% bioavailability, F30 = 30% bioavailability, PPB = plasma protein binding , BBB = blood brain barrier , VD = volume distribution, T_{1/2} = half-life and Cl = clearance

4.1.2 Metabolism and excretion

The skin permeability (Kp) values obtained for all the flavonoid derivatives considered in this study are in the range of -7.4 to -5.35 cm/s (Table 4) inferring low skin permeability (Daina et al., 2017). It is found that log Kp increases (more negative) with the increase in molecular size (Table 8). The results indicated that eriodyctiol (**41**) is the only flavonoid to act as drug transporter of P-glycoprotein substrate from target flavonoids considered (Table 4). This may lead to pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites (Hollenberg, 2002). In contrary, it is the only molecule that can act as CYP1A2 inhibitor (Table 4). Its interaction with p-gp together with its carcinogenicity will lead eriodyctiol (**41**) to be excluded from the list of bidentate ligands for metal complexation. Except ellagic acid (**40**), the rest of the flavonoid derivatives considered in this study were predicted to interact with CYP3A, making them very suitable molecules as CYP3A4 probes (Table 4). CYP3A enzymes are important determinants in the therapeutic efficacy and toxicity of numerous drugs. The interactions at the CYP3A level are often the cause of pronounced drug-drug interactions (van Waterschoot & Schinkel, 2011). Baicalein (**37**), chrysin (**39**), fisetin (**42**), kaempferol (**43**), quercetin (**44**), genistein (**45**), isorhamnetin (**46**), luteolin (**47**), morin (**48**), naringenin (**49**) and wogonin (**50**) are predicted as D6 inhibitors. Previous study by May & Schindler, (May & Schindler, 2016) showed that the interaction of antidiabetic drugs with other drugs in CYP3A4 and CYP2D6 inhibition mechanism has an additive effect for antidiabetic drugs (May & Schindler, 2016). These results suggest that the flavonoids studied in this work potentially have antidiabetic effect in agreement with previous reports (A. S. Pereira et al., 2019; Salehi et al., 2019). In this study, only butein (**38**) and wogonin (**50**) are predicted to interact with 2C9. Previous report indicated that the inhibition and induction of the CYP enzymes are probably the common causes for most drug interactions (Hollenberg, 2002). Hence, the predicted carcinogenicity and immunotoxicity (Table 5) together with its interaction with 2C9 would lead butein as a flavonoid derivative with high safety risk. In general, our predicted results could be used as important inputs for further experimental studies.

Table 4. The interaction of target Flavonoids with cytochromes P450 isoforms predicted using SwissADME

Molecule	GI abn	BBB prn	p-gp substrate	CYP 1A2 In	CYP 2C19 In	CYP 2C9 In	CYP 2D6 In	CYP 3A4 In	log Kp (cm/s)	HLM	MMP
36	High	No	No	Yes	No	No	No	Yes	-5.8	√	√
37	High	No	No	Yes	No	No	Yes	Yes	-5.7	Ø	√
38	High	No	No	Yes	No	Yes	No	Yes	-5.96	Ø	√
39	High	Yes	No	Yes	No	No	Yes	Yes	-5.35	Ø	√
40	High	No	No	Yes	No	No	No	No	-7.36	Ø	X
41	High	No	Yes	No	No	No	No	Yes	-6.62	Ø	Ø
42	High	No	No	Yes	No	No	Yes	Yes	-6.65	Ø	√
43	High	No	No	Yes	No	No	Yes	Yes	-6.7	Ø	√
44	High	No	No	Yes	No	No	Yes	Yes	-7.05	Ø	√
45	High	No	No	Yes	No	No	Yes	Yes	-6.05	Ø	√
46	High	No	No	Yes	No	No	Yes	Yes	-6.7	Ø	√
47	High	No	No	Yes	No	No	Yes	Yes	-6.25	Ø	√
48	High	No	No	Yes	No	No	Yes	Yes	-7.05	Ø	√
49	High	No	No	Yes	No	No	Yes	Yes	-6.7	Ø	√
50	High	No	No	Yes	No	Yes	Yes	Yes	-5.56	Ø	√
51	Low	No	No	Yes	No	No	No	Yes	-7.4	Ø	√
52	Low	No	Yes	No	No	No	No	No	-16.29	Ø	X
53	High	No	No	No	No	No	No	No	-7.84	Ø	Ø

logKp skin permeation, abn = absorption, prn= permission, In= Inhibitor, GI= gastrointestinal, BBB= blood brain barrier, In=inhibitor. Symbols X, √ and Ø represents inactive, active and no high confidence prediction available, respectively.

The human liver microsomal (HLM) stability and mitochondrial membrane potential (MMP) parameters of the flavonoid derivatives predicted via the vNN method (Table 4) showed that most of the flavonoid derivatives showed mitochondrial toxicity. Ellagic acid (**40**) and acarbose (**52**) were predicted to be inactive for MMP. Apigenin (**36**) was active for HLM prediction, and could

be metabolized rapidly by the liver that will reduce its effective therapeutic concentration in the body (Schyman et al., 2017).

4.1.3 Toxicity profile/toxicological endpoints

Herein, the organ toxicity (hepatotoxicity) and toxicological endpoints (carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity) of the sixteen flavonoid derivatives were predicted using Pro Tox II webserver. The predicted descriptors are presented in Table 5.

Table 5. Pro Tox II predicted Organ toxicity, toxicological end points and acute toxicity

Flavonoid	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	LD ₅₀ (mg/Kg)	Acute Toxicity class
36	inactive	inactive	inactive	inactive	inactive	2000	4
37	inactive	Active	inactive	active	inactive	3919	5
38	inactive	Active	Active	inactive	inactive	1000	4
39	inactive	inactive	inactive	inactive	inactive	2500	5
40	inactive	Active	inactive	inactive	inactive	2991	4
41	inactive	Active	inactive	inactive	inactive	2000	4
42	inactive	Active	inactive	inactive	inactive	159	3
43	inactive	inactive	inactive	inactive	inactive	3919	5
44	inactive	Active	inactive	active	inactive	159	3
45	inactive	inactive	inactive	inactive	inactive	2500	5
46	inactive	inactive	inactive	inactive	inactive	3919	5
47	inactive	Active	inactive	active	inactive	3919	5
48	inactive	inactive	Active	inactive	inactive	3919	5
49	inactive	inactive	inactive	inactive	inactive	3919	5
50	inactive	inactive	inactive	inactive	inactive	3919	5
51	inactive	Active	inactive	active	inactive	159	3
53	Inactive	Inactive	Inactive	inactive	Inactive	1300	4

LD₅₀ = 50% lethal dose (mg/Kg), Class 1 = fatal if swallowed (LD₅₀ ≤ 5), Class 2: fatal if swallowed (5 < LD₅₀ ≤ 50), Class 3: toxic if swallowed (50 < LD₅₀ ≤ 300), Class 4: harmful if swallowed (300 < LD₅₀ ≤ 2000) Class 5: may be harmful if swallowed (2000 < LD₅₀ ≤ 5000) Class 6: non-toxic (LD₅₀ > 5000)

Absence of toxicity is one of the major factors for selecting a compound as a therapeutic candidate (Sadeghi, Moradi, Madanchi, & Johari, 2021). The results show that all of the compounds are not hepatotoxic and cytotoxic. Of the considered flavonoid derivatives, baicalein (37), myricetin (51), luteolin (47) and quercetin (44) and butein (38) show two toxicological endpoints; carcinogenicity and mutagenicity, and carcinogenicity and immunotoxicity, respectively. On the other hand, ellagic acid (40), eriodictiol (41), fisetin (42) and morin (50) show one toxicological endpoint (carcinogenicity). Apigenin (36), chrysin (39), kaempferol (43), wogonin (50), naringenin (49), isorhamnetin (46), and genistein (45) are free from any of the predicted toxicological endpoints. Acute toxicity predictions indicate that the flavonoids considered are not fetal; however, they can be categorized as harmful toxic classes.

The median lethal dose (LD₅₀) values were found to be in the range from 159 -3919 mg/Kg (Table 5). According to the globally harmonized system of classification of labeling of chemicals (as described in Pro Tox II), quercetin (44), fisetin (42), and myricetin (51) are predicted as toxic (Class III), whereas apigenin (36), butein (38), ellagic acid (40) and eriodictiol (41) are harmful (Class IV) and the rest were categorized as “may be harmful” (Class V) toxicity classes. The results show that most of the drug-like compounds have the tendency to have carcinogenic activities than any toxicological endpoints.

Besides the ADMETlab descriptors, vNN descriptors such as hERG Blockers (hERG), human hepatotoxicity (H-HT), Ames Mutagenicity (AMES), Skin sensitization (SkinSen) were used to predict the toxicity of the flavonoid derivatives and the two controls. The results are presented in Table 6. The numbers 1 and 0 in the category section (Table 6) stand for positive and negative, respectively, for the predicted toxicities. Except apigenin (36), baicalein (37) and genistein (45), the rest of the flavonoid derivatives and the two control drugs were not predicted to be human ether-a-go-go related gene (hERG) blockers. However, most of them were predicted to show H-HT and mutagenicity (Table 6)

The vNN predicted drug induced liver injury (DILI) and cytotoxicity results (Table 6) showed that most of the flavonoid derivatives are not cytotoxic which is in line with results from Pro Tox II prediction. Apigenin (36) and acarbose (52) were predicted with inactive and active, respectively, to induce liver injury. The results also indicate the tendency of acarbose (52) to cause DILI (Table 6), which is in agreement with previous report on the risk of hepatotoxicity of acarbose (52) (L.

Zhang et al., 2016) and the probability of acarbose-induced hepatotoxicity (Hsiao, Liao, Cheng, & Wu, 2006).

Table 6. Toxicity profile of flavonoids and the two controls predicted using different methods

Flavonoids considered	Toxicity					
	ADMETlab					vNN
	hERG	H-HT	AMES	SkinSen	DILI	Cytotoxicity
36	1	1	0	0	X	X
37	1	1	1	0	Ø	X
38	0	1	0	0	Ø	Ø
39	0	0	0	0	Ø	X
40	0	1	0	0	Ø	Ø
41	0	0	1	0	Ø	Ø
42	0	1	1	0	Ø	X
43	0	1	1	0	Ø	X
44	0	1	1	0	Ø	X
45	1	1	0	0	Ø	Ø
46	0	1	1	0	Ø	X
47	0	1	1	0	Ø	X
48	0	0	1	0	Ø	X
49	0	1	1	0	Ø	X
50	0	1	0	0	Ø	X
51	0	0	1	0	Ø	X
52	0	0	0	0	√	Ø
53	0	1	0	1	Ø	Ø

The symbols X, √ and Ø represents inactive, active and no high confidence prediction available, respectively. hERG = cardiotoxicity, H-HT= human hepatotoxicity, AMES = Ames Mutagenicity and SkinSen = Skin sensitization

4.1.4 Density functional theory analysis of the ligands

The frontier molecular orbital analysis (HOMO-LUMO) was performed to predict the reactivity of the flavonoid derivatives. Density functional theory-based quantum chemical descriptors (DFTB-QCDs) help to identify the associations between chemical structures and properties, and play an essential role in chemistry, environment, pharmacology, and health science research. The quantum chemical descriptors such as E_{HOMO} and E_{LUMO} play an important role in predicting the energy gap of molecules and determining the anti-oxidant, reactivity, hardness and softness of molecules (Damena, Alem, et al., 2022a, 2022b). DFT chemical descriptors: electronegativity (χ),

electronic chemical potential (μ), global chemical hardness (η), global softness (σ), global electrophilicity index (ω) and nucleophilicity index (Nu) were derived from the energy gap ($E_g = E_{LUMO} - E_{HOMO}$). The results are presented in Table 7.

Table 7. Quantum chemical descriptors of the flavonoid derivatives calculated using DFT/B3LYP hybrid functional was used together with 6–311++G(d,p) basis set

FLs	E_g (eV)	χ	μ	η	σ	ω	Nu	Dipole Moment (D)
36	4.190	0.155	-0.155	0.077	6.494	0.156	6.393	6.679
37	4.114	0.158	-0.158	0.076	6.615	0.165	6.043	7.272
38	2.838	0.180	-0.180	0.052	9.587	0.309	3.234	4.336
39	2.428	0.129	-0.129	0.045	11.207	0.187	5.333	8.293
40	4.339	0.172	-0.172	0.080	6.272	0.185	5.413	1.976
41	4.142	0.156	-0.156	0.076	6.569	0.160	6.232	5.520
42	3.764	0.157	-0.157	0.069	7.230	0.178	5.616	5.143
43	3.867	0.154	-0.154	0.071	7.036	0.166	6.011	6.342
44	3.794	0.157	-0.157	0.070	7.173	0.178	5.627	6.225
45	7.982	0.063	-0.063	0.147	3.409	0.013	74.683	8.927
46	3.867	0.154	-0.154	0.071	7.037	0.166	6.013	6.339
47	4.082	0.157	-0.157	0.075	6.666	0.165	6.065	7.019
48	3.787	0.159	-0.159	0.070	7.185	0.181	5.539	4.287
49	3.869	0.154	-0.154	0.071	7.034	0.166	6.024	6.269
50	3.956	0.159	-0.159	0.073	6.879	0.173	5.765	8.622
51	3.812	0.156	-0.156	0.070	7.138	0.174	5.754	6.364
53	14.281	0.047	-0.047	0.262	1.905	0.004	237.574	5.795

E_g (eV) = band gap energy, χ = electronegativity, μ = chemical potential, η = hardness, σ = softness, ω = electrophilicity index and Nu = nucleophilicity index

The calculated energy gap between HOMO and LUMO for the studied flavonoid derivatives were in the range from 2.428 eV (chrysin (**39**)) to 7.982 eV (genistein (**45**)), whereas metformin (**53**) has the largest band gap. The high HOMO energy (-4.734 eV), the small energy gap (2.428 eV), small chemical hardness (0.045 eV), and large softness (11.207 eV) calculated for chrysin (**39**) molecule makes the molecule a good candidate as a chelating agent.

On the other hand, the high dipole moment (8.293 Debye) value found for chrysin (**39**) makes the molecule a preferred ligand for biological activity (antimicrobial). Among the sixteen flavonoid derivatives, the highest HOMO-LUMO energy gap, hardness, and high nucleophilicity were predicted for genistein (**45**) (Table 7). Based on the quantum chemical reactivity descriptors, the isoflavone class of flavonoids, genistein (**45**) has the highest chemical hardness (0.147 eV) and the lowest electrophilicity (0.013 eV). Whereas the highest electrophilicity (0.309 eV) and the lowest chemical hardness (0.052 eV), next to chrysin (**39**) (0.045 eV), was predicted for the chalcone family, butein (**38**). The electrophilicity, electronegativity and softness quantum chemical descriptors for apigenin (**36**)/genistein (**45**) were found to be 0.077/0.147 eV, 6.494/3.409 eV and 0.156/0.013 eV, respectively, indicating good chelating nature of apigenin (**36**) over genistein (**45**).

4.1.5 Molecular docking evaluation of the ligands

The enzyme α -Amylase has been a pivotal target in anti-diabetic drug development. In the present work, we studied the interactions between the Human α -amylase (PDB ID: 4w93) protein against the flavonoids and compared the results against the clinical drug metformin. The 2D and 3D representations of apigenin (**36**)-, chrysin (**39**)- and the control metformin (**53**)-protein interactions are given from Figure 19 to Figure 21, and the rest of flavonoids are given in supplementary materials (Appendix 1 - 7). The binding affinity, H-bond and residual interaction of flavonoids and metformin (**53**) (anti-diabetic drug) were summarized in Table 8. Compared to metformin (**53**), the flavonoid compounds shown significant interaction within the active site of the protein with the key amino acids Asp 197, Glu 233, Asp 197, Glu 233, Trp 59, Tyr 62, His 101, Leu 162, Arg 195, His 299 and Leu 165. The tested flavonoids showed a binding energy ranging from -7.5 to -8.3 kcal/mol. All the investigated compounds showed strong binding score compared to the clinical drug metformin (**53**) with -4.5 kcal/mol. The top seven flavonoids with low binding energies were apigenin (**36**), baicalein (**37**), chrysin (**39**), ellagic acid (**40**), kaempferol (**43**), quercetin (**44**), and luteolin (**47**).

In a separate previous work it has been reported that compound quercetin (**44**) has low binding energy (-8.0 kcal/mol) against α -glucosidase (Hyun, Eom, & Kim, 2014), inferring the inhibitory potential of the compound towards α -amylase and α -glucosidase.

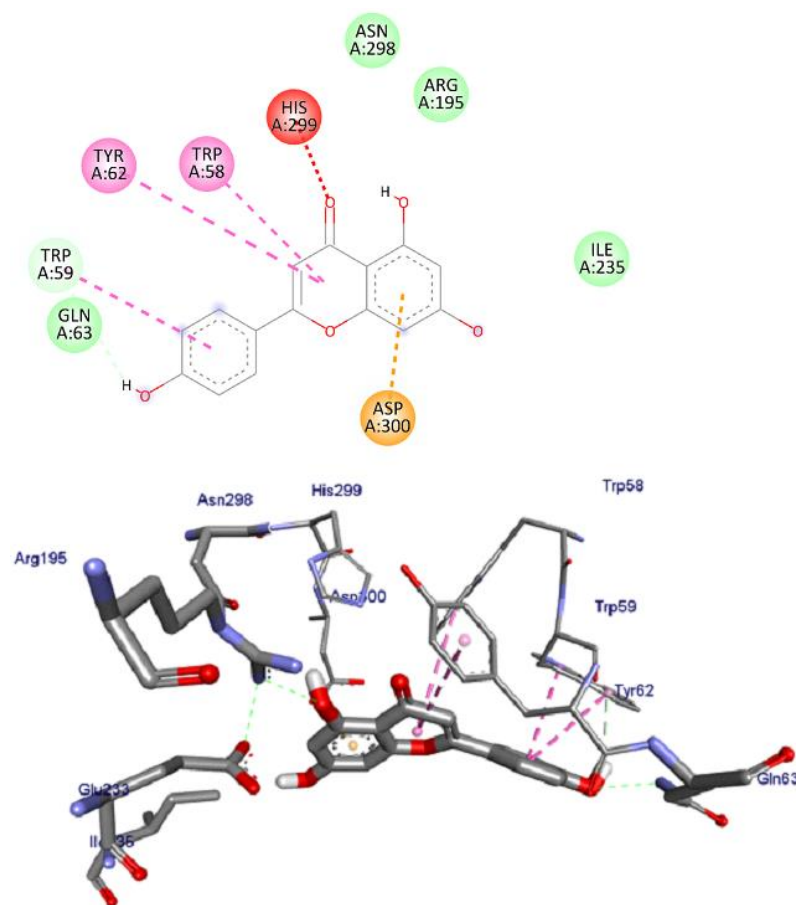


Figure 19. 2D (upper panel) and 3D (upper panel) representations of the binding modes of compounds Apigenin to Human α -amylase (PDB ID: 4W93).

Overall, the *in-silico* docking analysis indicated that apigenin (**36**) interacts with the α -amylase residues of Tyr 62, Trp 58, His 299, Asp 300, Asn 298, Arg 195 and Ile 235 with binding energy -8.3 kcal/mol, chrysin (**39**) interacts with the α -amylase residues of Trp 58, Trp 59, Tyr 62, His 299 and Gln 63 with binding energy -8.1 kcal/mol, kaempferol (**43**) interacts with the α -amylase residues of Trp 58, Trp 59, Tyr 62, His 299, Glu 233, Asp 197, Arg 195, Ala 198, and Leu 165 with binding energy -8.1 kcal/mol, quercetin (**44**) (-8.1 kcal/mol), and luteolin (**47**) interacts with the α -amylase residues of Trp 59, Tyr 62, Arg 195, Trp 58, His 299, Glu 233, Ala 198, and Leu 165 -8.3 kcal/mol are better anti-diabetic agents compared to other compounds reported in this study.

Table 8. Molecular docking value of flavonoids against Human α -amylase (PDB ID: 4W93)

Compound	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
36	- 8.3	Gln 63	Tyr 62, Trp 58, His 299, Asp 300	Asn 298, Arg 195, Ile 235
37	-8.1	Asp 197, Glu 233	Trp 59, Tyr 62, Hoh 773, Hoh 789	Leu 165, His 101, Leu 162, Ala 198, Arg 195, Asp 300, His 299
38	-7.6	Gln 63	Trp 50 Tyr 62, Trp 58	Thr 163, Leu 165, His 299, Arg 195, Asn 298, Glu 233, Asp 300
39	-8.2	Arg 195, Asp 300	Trp 59, Trp 58, Tyr 62, His 299	Gln 63
40	-8.0	Glu 233	Trp58, Trp 59	His 305, Asp 300, Arg 195 Asp 197, His 101, Tyr 62, Leu 165, Gln 63
41	-7.8	Gln 63, Tyr 62 Glu 233	Trp 59, Tyr 62	Leu 165, Trp 58, His 299, Asp 197, Arg 195, Ala 198
42	-7.8	---	Tyr 62, Trp 59	Trp 58, His 299, Arg, 195, Asp 197, Ala 198, His 101, Gln 63
43	-8.1	Gln 63, Tyr 62	Trp 59, Tyr 62	Trp 58, His 299, Glu 233, Asp 197, Arg 195, Ala 198 Leu 165
44	-8.1	Gln 63, Tyr 62, Glu 233, Asp 197	Trp 59, Tyr 62	Leu 165, Ala 198, Arg 195, Trp 58, His 299
45	-7.7	Gln 63, Asp 197	Trp 59	Ala 198, Glu 233
46	-7.6	Gln 63, Asp 197	Trp 59, Tyr 62, Asp 197, Glu 233, Arg 195	Leu 165, His 101, Trp 58, His 299
47	-8.3	Gln 63	Trp 59, Tyr 62, Arg 195	Trp 58, His 299, Glu 233, Ala 198, Leu 165
48	-7.5	Gln 63	Tyr 62, Trp 59, Asp 300	Asp 197, Arg 195 His 299, Trp 58
49	-7.6	Gln 63, Arg 195, Glu 233	Trp 59, Tyr 62	Trp 58, His 299, Leu 165
50	-7.8	Arg 195 Asp 300	Trp 59 Tyr 62, His 299, Leu 162	Gln 63, Asp 197, Glu 233, Asn 298, ILE 235
51	-8.0	His 299, Gln 63, Asp 197	Trp 59, Asp 197, Tyr 62	Gln 63, His 101, Arg 195
53	-4.5	Asp 197, Glu 233	Asp 197, Glu 233	Trp 59, Tyr 62, Leu 165, His 101, Leu 162, Arg 195, His 299

The lower binding energies predicted for compounds apigenin (**36**), chrysin (**39**), kaempferol (**43**), quercetin (**44**), luteolin (**47**) relative to the control metformin (**53**) also indicated that they fit well in the binding pocket of the human α -amylase forming a stable inhibitor-protein complex.

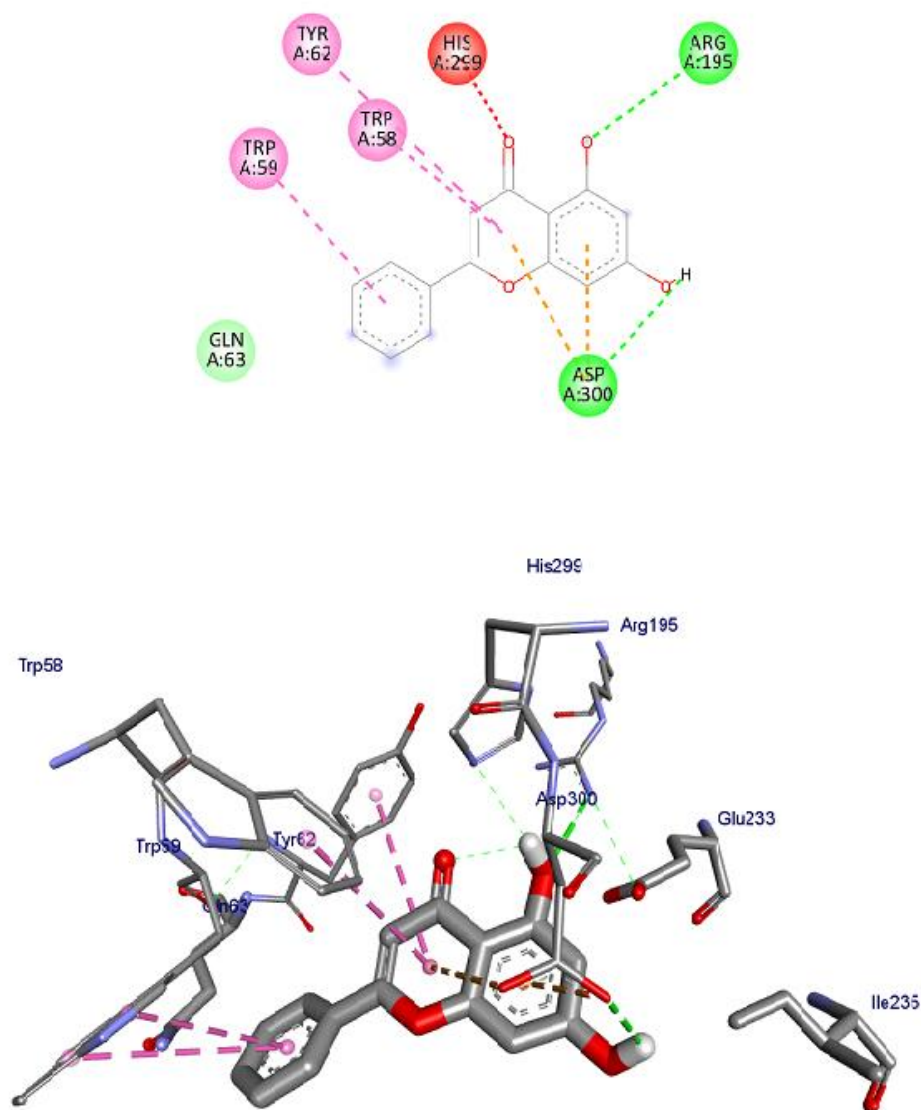


Figure 20. 2D (upper panel) 3D (lower panel) representations of the binding modes of chrysin (**39**) to Human α -amylase (PDB ID: 4W93)

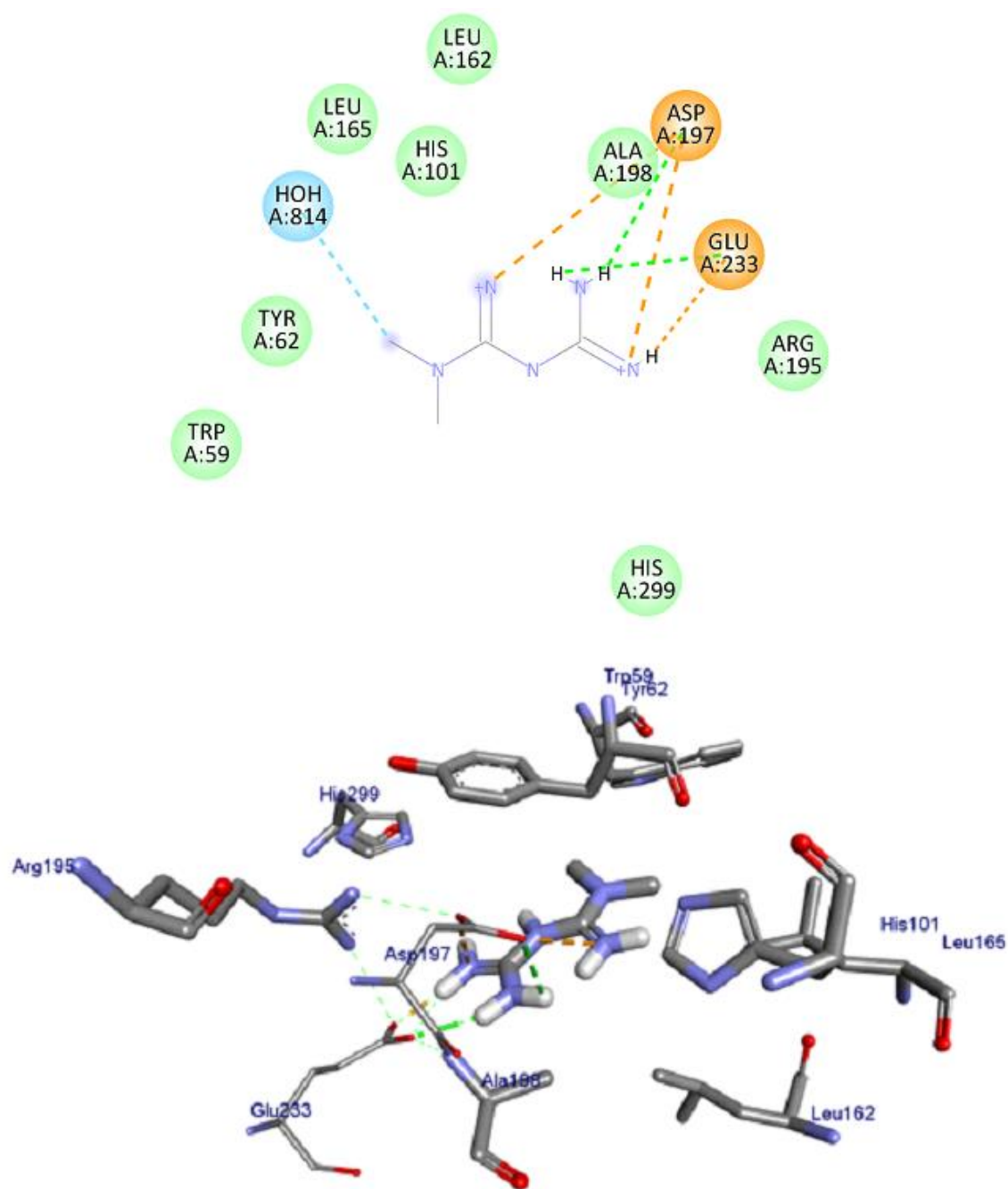
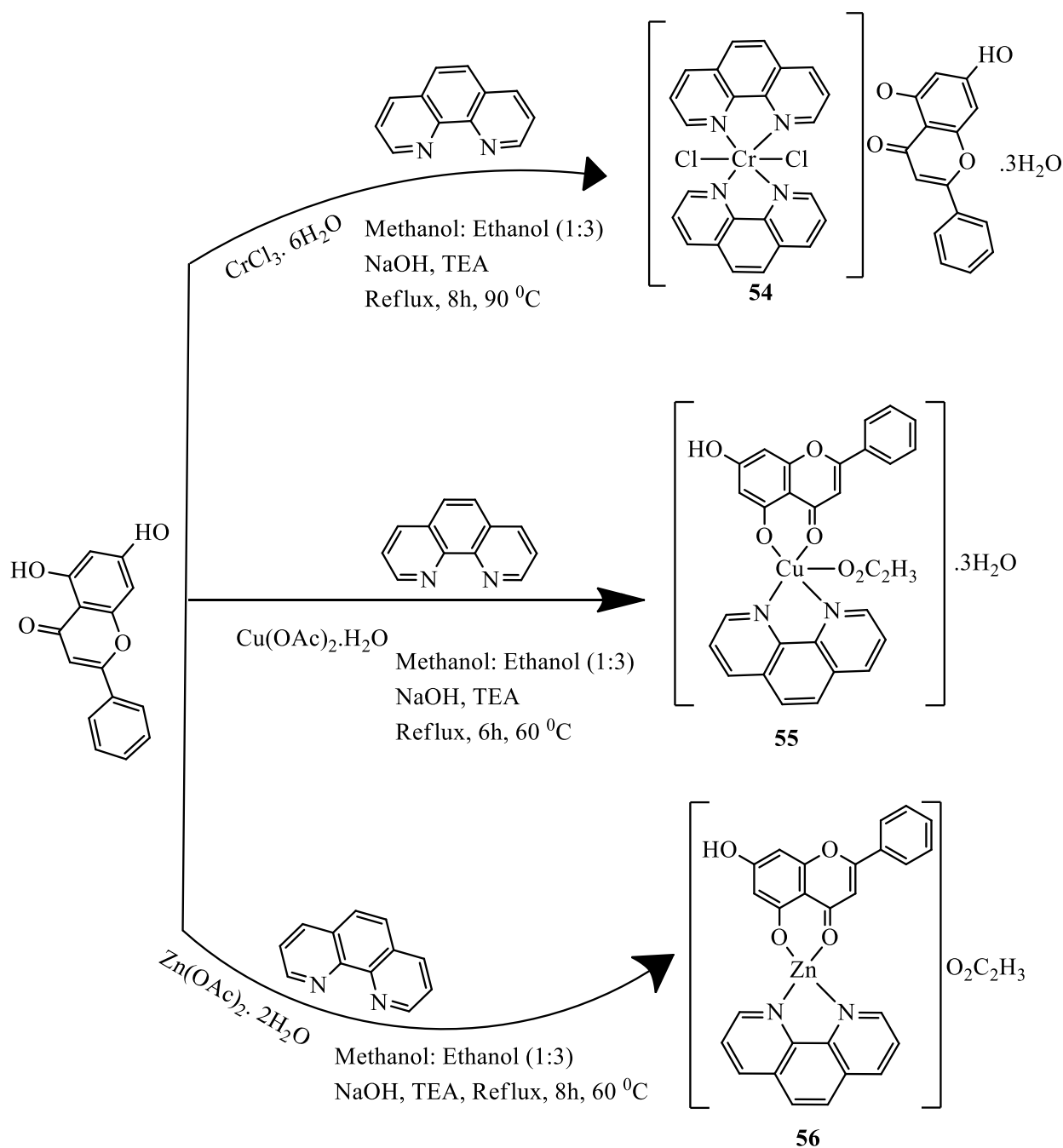


Figure 21. 2D (Upper panel) and 3D (Lower panel) representations of the binding modes of Metformin (53) to Human α -amylase (PDB ID: 4W93)

4.2 Synthesis and Characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of 1,10-phenanthroline and chrysin

The heteroleptic metal complexes (**54** - **56**) were synthesized as described in Scheme 2. The reported metal complexes were synthesized in 1:1 metal to ligands ratio for **55** and **56** complexes. The complex **54**, was synthesized in 1:1:2 ratio of metal salt: chrysin:1,10-phenanthroline ligands resulted a differently structured hybrid organic-inorganic salt of Cr(III) complex. The physicochemical properties of the synthesized metal complexes are presented in Table 9.



Scheme 2. Schematic representations of 1,10-phenanthroline-chrysin mixed ligand metal complexes (**54** - **56**)

4.2.1 Physicochemical properties

The synthesized metal complexes (**54** - **56**) were subjected for solubility test using solvents of different polarity. Complex **54** (Cr(III)) was found to be soluble in polar aprotic solvents: DMSO and DMF (dimethylformamide); slightly soluble in polar solvents: water, methanol, ethanol; but

insoluble in solvents like dichloromethane, ethyl acetate, chloroform and hexane. However, **55** (Cu(II)) and **56**(Zn(II)) were soluble in only DMF and DMSO solvents. The percentage yield, melting point, color and state of the synthesized metal complexes are presented in Table 9.

Table 9. Physicochemical properties of complexes (**54** - **56**)

Compound	Color	State	Mass (%) Yield)	M.pt (Decom)(°C)	Conductivity ($\Omega^{-1}\text{mol}^{-1}\text{cm}^2$, 25 °C)
54 (Cr(III))	Brown	Powder	0.584 g (84)	200-202	53.90
55 (Cu(II))	Green- brown	Powder	0.303 g (50)	>205	27.56
56 (Zn(II))	Light- yellow	Powder	0.113g (20)	>318	51.80

4.3 Characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of 1,10-phenanthroline and chrysin (**54** – **56**)

4.3.1 FTIR analysis of the Cr(III), Cu(II) and Zn(II) complexes of chrysin and 1,10-phenanthroline

The FTIR spectra for both ligands (chrysin and 1,10-phenanthroline) and the metal complexes (**54** (Cr(III)), **55** (Cu(II)) and **56** (Zn(II))) are presented in Figure 22 and Appendix 8 - 9. It has been reported that the free chrysin showed characteristics vibrations at 3428 cm^{-1} , 3060 cm^{-1} and 1652 cm^{-1} , respectively for $\nu_{(\text{O-H})}$, $\nu_{(\text{C-H})}$ and $\nu_{(\text{C=O})}$ stretching vibrations (E Halevas et al., 2021). In this work, characteristic vibrational peaks (experimental/B3LYP calculated) of the free chrysin were observed at $3430/3698$, $2944/3123$ and $1648/1608\text{ cm}^{-1}$ which could be associated to the $\nu_{(\text{O-H})}$, $\nu_{(\text{C-H})}$ and $\nu_{(\text{C=O})}$ stretching vibrations, respectively, in agreement to the previous reports (E Halevas et al., 2021; Sundaraganesan, Mariappan, & Manoharan, 2012). The large vibrational frequency difference between experimental $\nu_{(\text{O-H})}$ and B3LYP calculated $\nu_{(\text{O-H})}$ might be due to the combination of electron correlation effects and basis set deficiencies (Karabacak, Cinar, Kurt, Sudha, & Sundaraganesan, 2012). The decrease in the $\nu_{(\text{O-H})}$ peak intensity of the free chrysin from 52% transmittance to 94% and 95%, respectively for **55** and **56** complexes (Figure 22 A) confirms the coordination of chrysin to the metal centers in a monoprotinated fashion. The relatively large

intensity at 3430 cm^{-1} of complex **54** (87% transmittance) compared to **55** and **56** is a confirmation that the bonding in chrysin is different for complex **54**, in the ionization sphere.

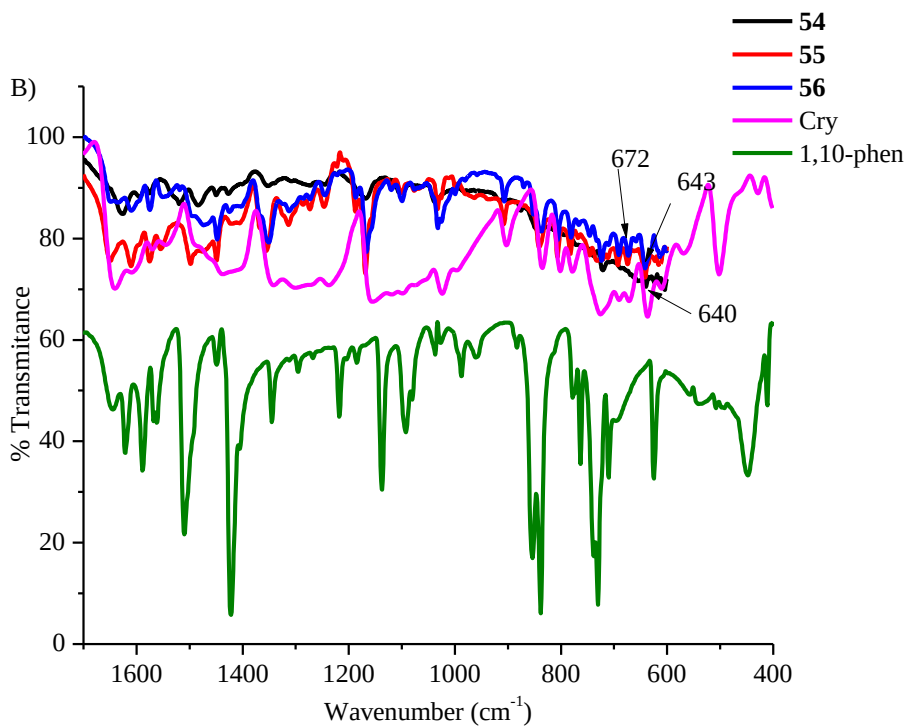
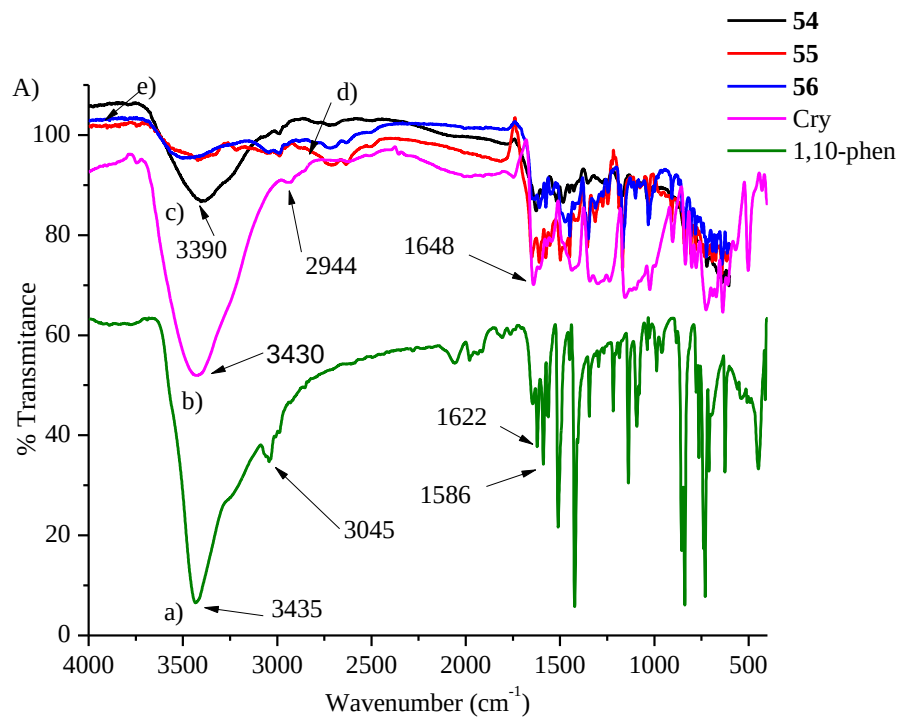


Figure 22. FTIR Spectra of the functional group regions (A) and fingerprint region (B) of the ligands (1,10-phen (a), Cry(b)) and their metal complexes (54 (c), 55 (d) and 56 (e))

Moreover, the vibrational frequency of $\nu_{\text{C=O}}$ shifts to a higher frequency of 1648 and 1656 cm^{-1} for **55** and **56**, respectively, suggesting that the metals bind chrysin via the oxygen atom of the carbonyl group of a flavonoid ring which is consistent with previously reported works (Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021). In the experimental/computational FTIR analysis, the stretching vibrational bands of $\nu_{\text{C=C}}$ (1622/1592 cm^{-1}) and $\nu_{\text{C=N}}$ (1586/1550 cm^{-1}) functional groups for 1,10-phenanthroline monohydrate ligand were shifted to lower frequencies 1606/1608 cm^{-1} and 1555/1512 cm^{-1} for **55**, 1614/ 1611 cm^{-1} and 1546/1528 cm^{-1} for **56** with decreased intensity, respectively. These shifts appeared at 1630/1608 and 1521/1429 cm^{-1} for complex **54**. The stretching vibrational bands, $\nu_{\text{O-H}}$ (3435 cm^{-1}) associated to water of crystallization of 1,10-phenanthroline diminished following the metal-ligand coordination. The decrease in their intensity confirmed the formation of rigid and symmetric structure due to complexation with the metal center (Abebe et al., 2020). In addition to the observed vibration frequency shifts, the formation of metal-ligand bonds was confirmed by the appearance of new vibrational bands (experimental/calculated) at 674/734 and 643/586 cm^{-1} for **55** and 672/724 cm^{-1} and 640/565 cm^{-1} for **56** (Figure 22) which were due to the formation of M-O and M-N bonds, respectively. The vibrational band for Cr-N bonds were found to appear at 640/720 cm^{-1} .

4.3.2 UV-Vis/TD-DFT analysis of the ligands and complexes (**54** – **56**)

The UV-Vis absorption spectra of the ligands chrysin and 1,10-phenanthroline in DMSO together with their TD-DFT calculated results are presented in Figure 23. The characteristic absorption spectra of chrysin in DMSO were reported to appear at 270 nm (intense) and 330 nm (less intense) which are associated to benzoyl system and cinnamoyl system respectively (Lin et al., 2017). In this work, the UV-Vis /TD-DFT calculated absorption spectrum of chrysin in DMSO was found to appear at 267/261 nm and 320/325 nm, respectively, corresponding to $\pi \rightarrow \pi^*$ transitions of benzoyl system and cinnamoyl system of the molecule which are agreement to the previously reported works (Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021; Sundaraganesan et al., 2012). The free 1,10-phenanthroline absorption spectra (experimental/TD-DFT) were found at 228/226 nm and 263/258 nm which corresponds with $\pi \rightarrow \pi^*(\text{C=C})$ and $n \rightarrow \pi^*(\text{C=N})$ transitions, respectively (Alem et al., 2022; Tamiru et al., 2019).

In the electronic spectra of 1,10-phenanthroline and chrysin a bathochromic shift to a longer wavelength 277 nm, 276 nm and 272 nm, were observed with slight decrease in absorption for

complexes **54**, **55** and **56**, respectively (Figure 24 - Figure 26). Whereas, the broad peak of chrysin that appeared at 320/325 nm was found to diminish which might be due to the extension of conjugated system with metal complexation. A new stronger absorption peaks that appeared at 413 nm (24213 cm^{-1}) and 391 (25575 cm^{-1}), respectively for **54** and **55** resulted from the formation of complexes between chrysin, 1,10-phenanthroline and Cr(III) and Cu(II) metal centers (Dehghan & Khoshkam, 2012). The coordination of Cr(III) with chrysin caused the red shift of 9 nm and 93 nm, respectively, for benzoyl and cinnamoyl part of chrysin electronic absorptions. This indicates the chelation might have brought enhanced conjugation to the cinnamoyl system of chrysin than to the benzoyl system. Thus, we reasonably concluded that the coordination of chrysin was via its carbonyl oxygen (Panhwar & Memon, 2014a).

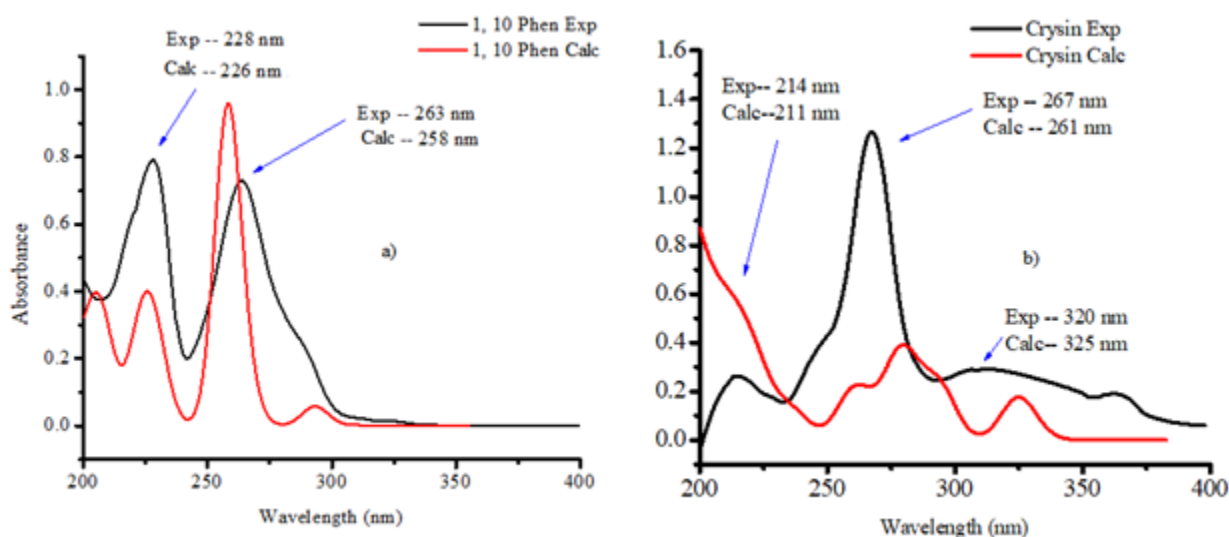


Figure 23. UV-Vis and TD-DFT calculated absorption spectrum of 1,10-phenanthroline (a) and chrysin (b)

The absence of a peak above 400 nm (25000 cm^{-1}) for complex **56** could be due to absence of ligand-to metal charge transfer bands in Zn(II) metal centered complexes (Figure 26). However, the resulting changes in the electronic structure of the ligands up on complexation of Zn(II) metal center as confirmed by the observed spectral pattern of the complex **56** were clear indicative of the involvement of the ligand in formation of coordination compound with metal center .

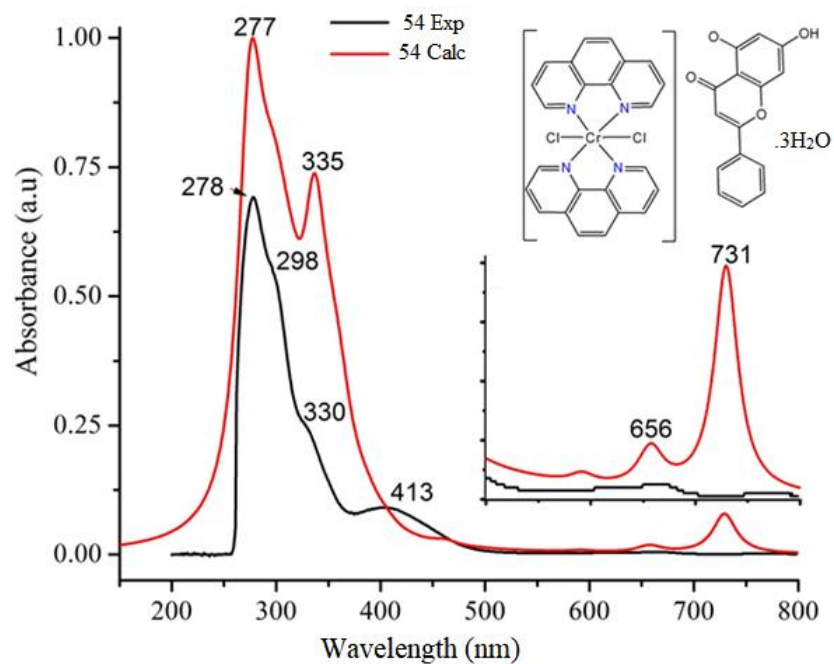


Figure 24. UV-Vis (Black) and TD-DFT(red) absorption spectra of complex (**54**)

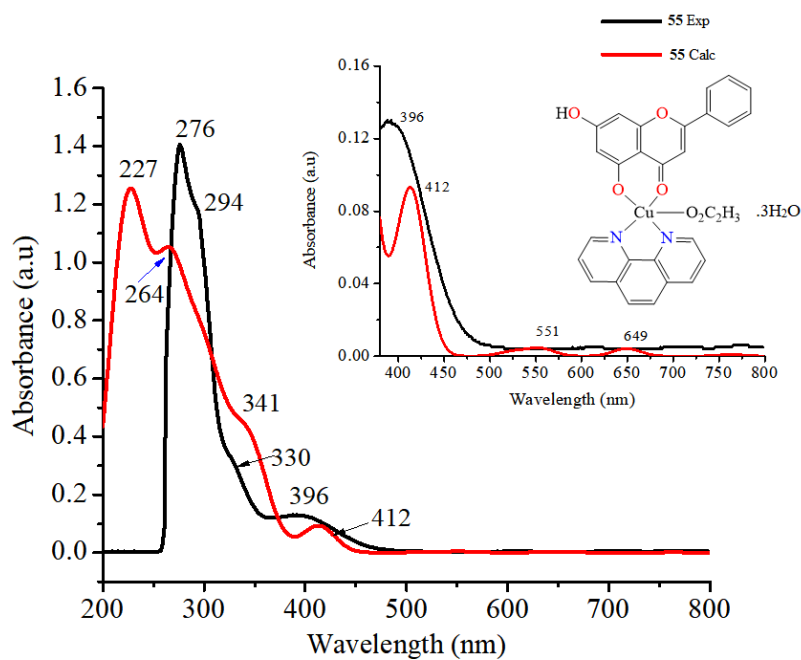


Figure 25. Uv-Vis (Black) and TD-DFT(red) absorption spectra of heteroleptic complex (**55**)

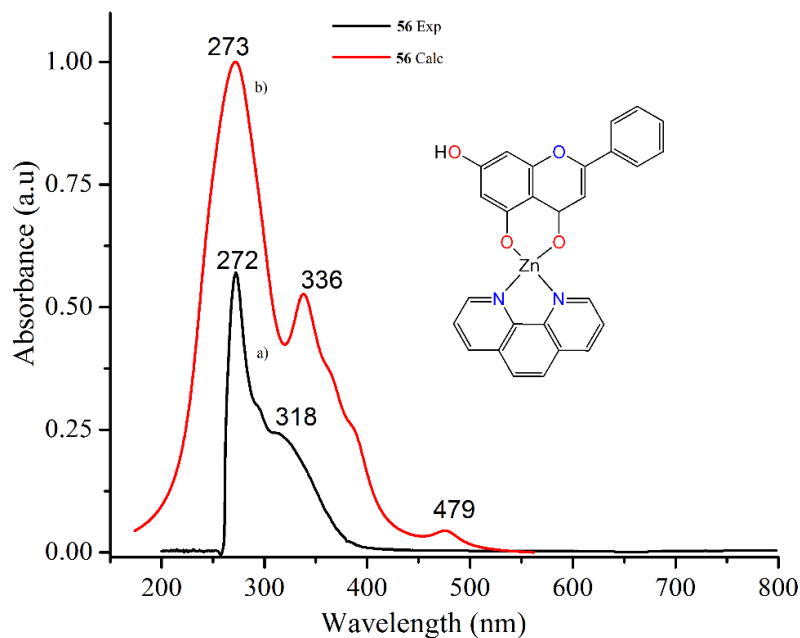


Figure 26. UV-Vis (a) and TD-DFT (b) absorption spectra of heteroleptic complex **56**

4.3.3 Mass spectrometric analysis of the as-synthesized complexes

The high-resolution mass spectrometer were used for mass fragmentation analysis, and the obtained results are presented from Figure 27 – 29 and Appendix (10 - 12).

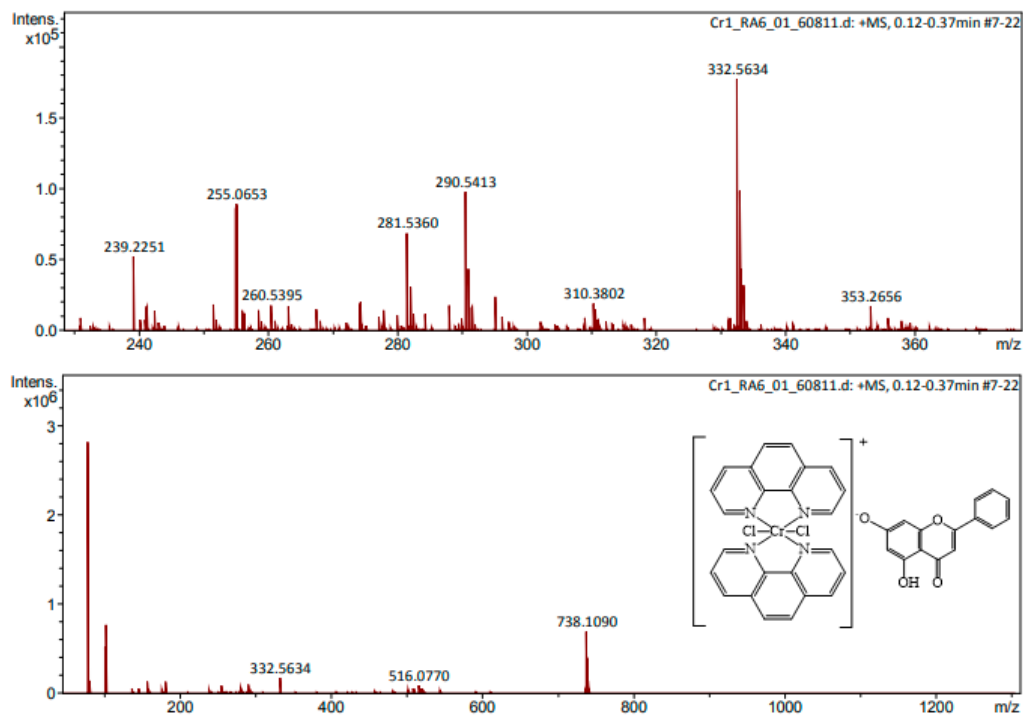


Figure 27. Mass spectra of Cr(III) complex (**54**)

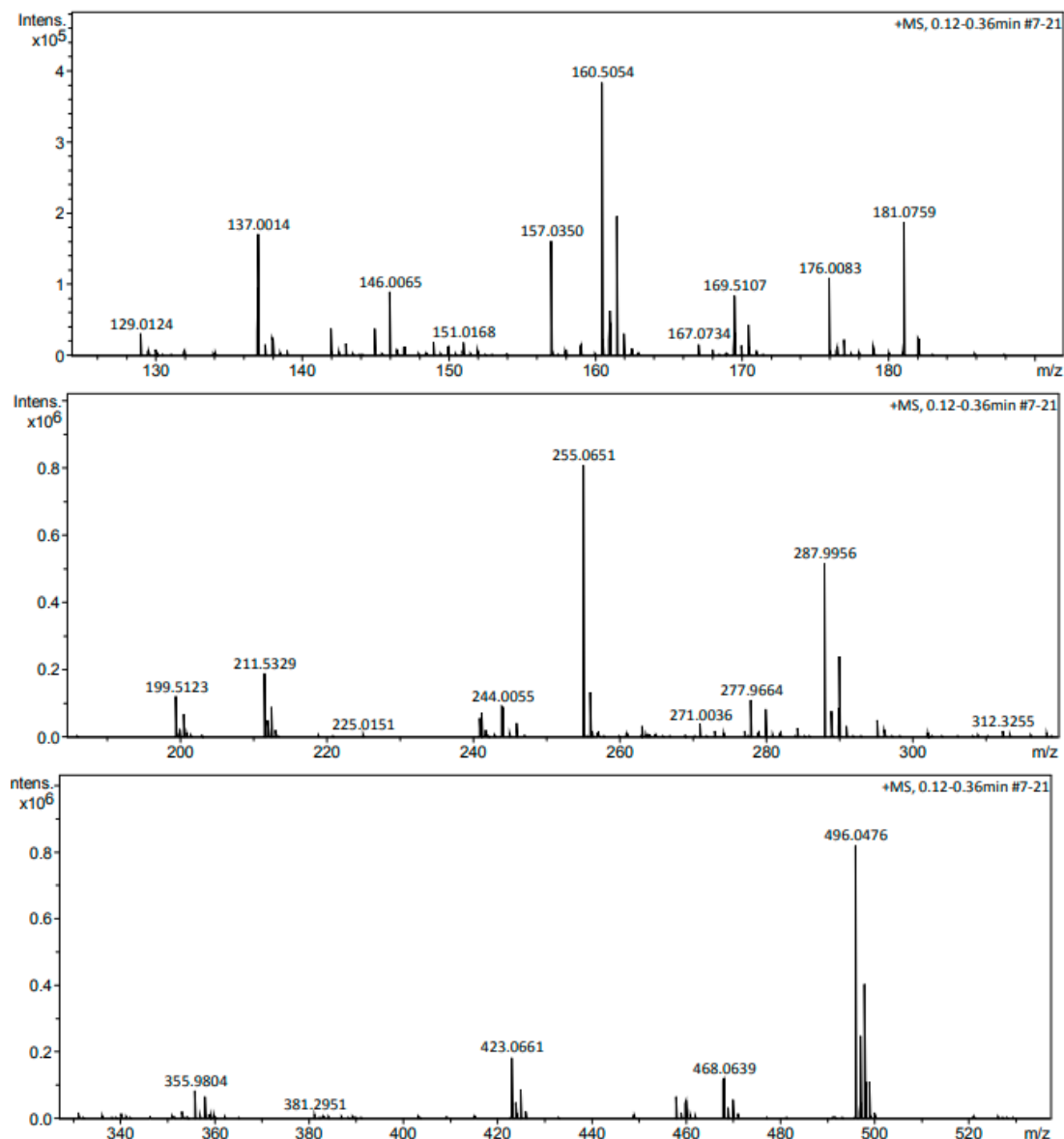


Figure 28. Mass spectra of Cu(II) complex (55)

The mass spectrum of complex **54** exhibited a peak at $m/z = 738.109$ to 741.114 (calc = 735.070), corresponding to the complex cation $[C_{39}H_{25}Cl_2CrN_4O_4]^+$, a base peak at $m/z = 516.077$ (calc = 514.040) correlated to the coordination sphere and methanol ($M^+ + CH_3OH$), whereas a peak at $m/z = 482.015$ (calc = 482.020) corresponds to the chemical entities in the coordination sphere, the Cr-metal center, two 1,10-phenanthroline and two chlorides $[M = C_{24}H_{16}Cl_2CrN_4]^+$ and a m/z value of 255.0653 (calc = 254.060) corresponding to the ligand in the ionization sphere of the complex which is a chrysin fragment $[C_{15}H_{10}O_4]$. The presence of $m+4$ peaks (peaks observed around $m/z = 482.015$ and 516.077) in the mass spectrum confirms the coordination of two

chlorides to the Cr-metal center. In complex **55** (Figure 28 and Appendix 11) the molecular ion peak observed in the mass spectrum at $m/z = 612.959$ (calc = 609.090) can be attributed to the complex cation $[\text{C}_{29}\text{H}_{27}\text{CuN}_2\text{O}_9]^+$, a peak at $m/z = 572.266$ (calc = 573.040) corresponds to the coordination sphere, acetate ion and a water molecule ($\text{M}^+ + \text{H}_2\text{O} + \text{C}_2\text{H}_3\text{O}_2$) $[\text{C}_{29}\text{H}_{22}\text{CuN}_2\text{O}_7]^+$, a peak at $m/z = 496.048$ (calc = 496.050) corresponds to the chemical species in the coordination sphere ($\text{M} = [\text{C}_{27}\text{H}_{17}\text{CuN}_2\text{O}_4]^+$).

The fragments for the coordinating ligands chrysin and 1,10-phenanthroline in complex **55** are found to appear as peaks at 255.065 and 181.076, respectively. Whereas, in complex **56** (Figure 29 and Appendix 12) a molecular ion peak at $m/z = 497.046$ (calc = 497.050) corresponds to a Zn-centered coordination sphere cation $[\text{C}_{27}\text{H}_{17}\text{N}_2\text{O}_4\text{Zn}]^+$. Fragments associated to the coordinating ligands are observed similar to that of **55**. The absence of a coordinating acetate ion in complex **56** suggests it might have been lost due to the operating cone voltage as reported in literature (Lawal et al., 2020).

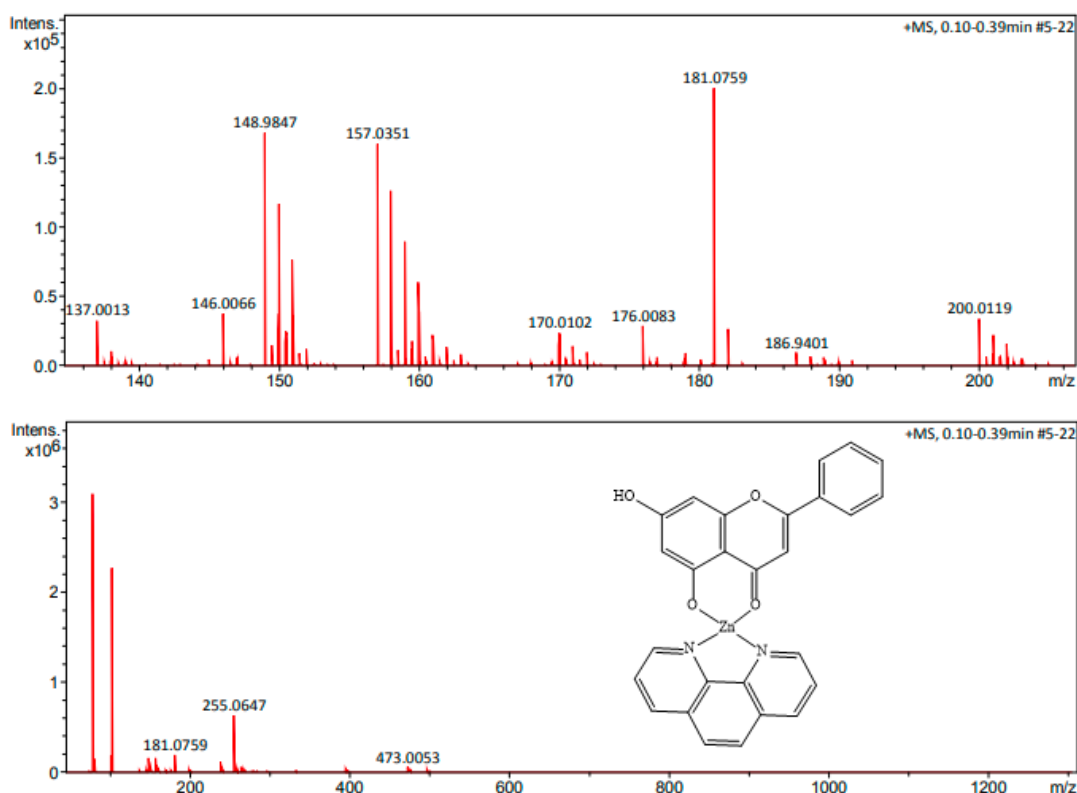


Figure 29. Mass Spectra of Zn(II) complex (**56**)

4.3.4 SEM-EDX analysis of the complexes (54 – 56)

The elemental composition of the reported complexes was obtained from energy dispersive X-ray (EDX) analysis (Figure 30 (a – c)). The morphology of the synthesized Cr(III)(**54**), Cu(II)(**55**) and Zn(II)(**56**) complexes were studied using SEM. The SEM micrographs (Figure 30 (d – f)) disclose that the complexes are in the form of random aggregates, agglomerated particles, do not yield smooth surfaces and the presence of non-uniform sized small grains in line with the polycrystalline phase of the reported complexes confirmed by the XRD patterns (*vide infra*). A relatively more uniform surface provided by complex **54** might be due to its composition difference from complexes **55** and **56**.

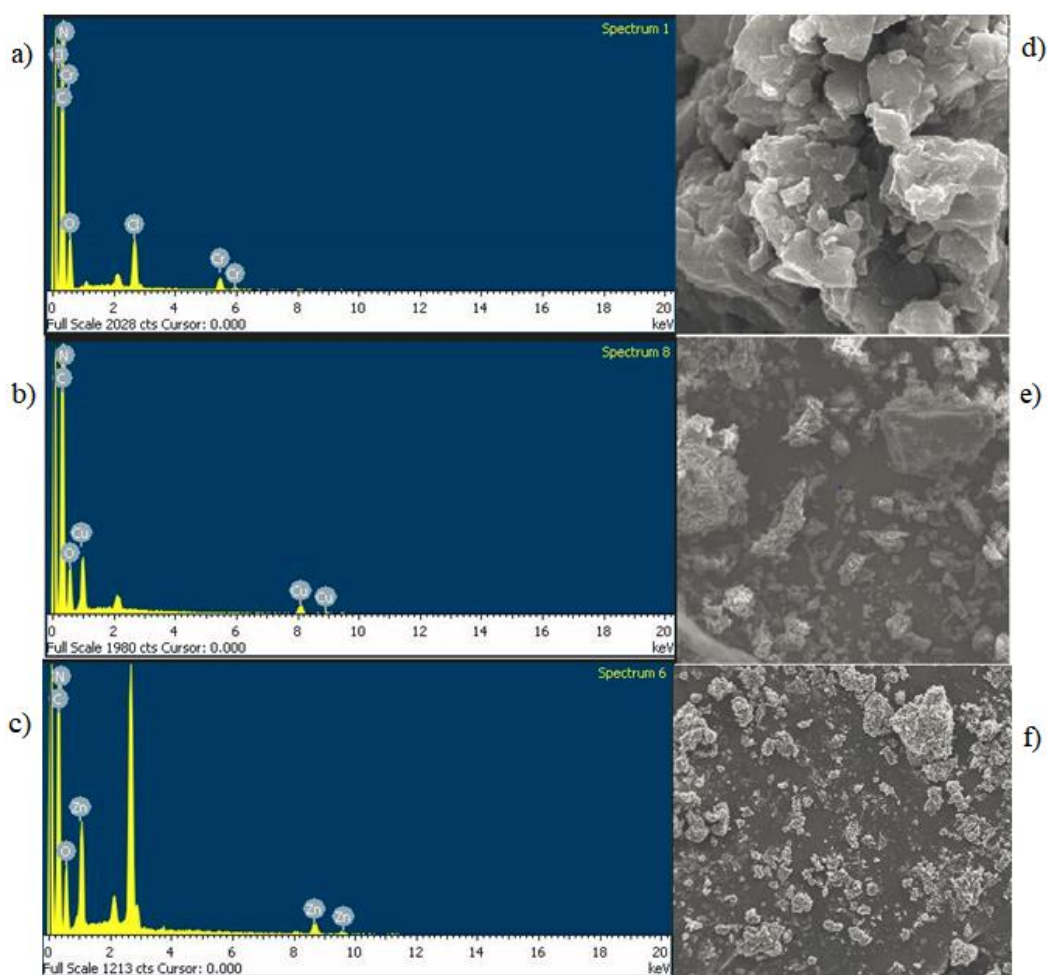


Figure 30. EDX spectra of **54** (a), **55** (b) and **56** (b) and the SEM images of **54** (d), **55** (e) and **56** (f)

The EDX spectrum of complex **54** (Figure 30 a) shows characteristic signals of carbon, nitrogen, oxygen, chlorine and chromium which are the atoms present in the ligands and the metal center, confirming the existence of the complex in CHCrNOCl chemical composition. The EDX spectrum of **55** (Figure 30 b) shows characteristic signals of carbon, nitrogen, oxygen and copper, confirming the chemical composition of the complex to be CHCuNO . Whereas, the EDX spectrum of complex **56** (Figure 30 c) found to show characteristic signals associated with carbon, nitrogen, oxygen and zinc, confirming the formation of a complex compound with composition CHZnNO in agreement to reported in previous works (Damena, Alem, et al., 2022a). The hydrogen atoms of the complexes are not observed on the EDX spectrum. This is due to the fact that lighter elements downwards boron do not have characteristic X-rays or too low to be detected by EDX (Heslop-Harrison, 1990).

4.3.5 Thermogravimetric analysis of chrysin and 1,10-phenanthroline based mixed ligand complexes

The TGA/DTG thermographs assignments of the synthesized complexes (**54** - **46**) are presented in Table 10. The TGA analysis of the complexes was recorded between 25 and 800 °C. Complex **54** (Figure 31) thermally decomposed into two main degradation steps and thermograph showed that: i) From 51.2 – 213.2 °C ($\text{DTG}_{\text{max}} = 126$ °C), the weight loss was 7.21%, corresponding to the release of water molecules, which could be related with the calculated value (7.20%) and ii) From 295.2 – 590.4 °C ($\text{DTG max} = 465$ °C), loss due to $\text{C}_{15}\text{H}_8\text{O}_3 + 2\text{Cl}$ groups with the total weight loss being 41.63% (calculated value: 41.60%) (Ndong et al., 2020).

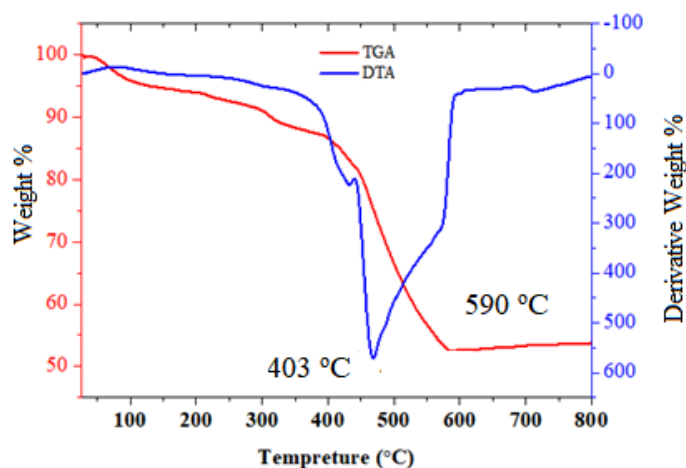


Figure 31. Thermogravimetric (TGA/DTA) presentation of Cr(III) complex (**54**)

Complex **55** (Figure 32) thermally decomposed into three main degradation steps and thermograph showed that: i) From 100 – 211 °C ($DTG_{max} = 162$ °C), the weight loss was 8.81%, corresponding to loss due to three lattice water molecules, which is in accordance with the calculated value (8.84%). ii) From 215 – 484°C ($DTG_{max} = 403$ °C), release of the $C_{12}H_8N_2$ organic moiety, with the total weight loss being 29.47 % (calculated value: 29.49 %) and the final step showed weight loss 43.53% (calculated value: 43.87%) from temperature 490 – 752°C corresponding to the decomposition of release of the CHO_2 and $C_{15}H_{11}O_2$ organic moieties. From the step total weight loss was 81.81 % (calculated: 82.20 %) with the final residue of copper oxide (Abbas et al., 2019; Abd El-Halim, Mohamed, & Khalil, 2017).

Complex **56** (Figure 32) was found to thermally decompose in three main degradation steps and thermograph showed that: i) From 100 – 428 °C ($DTG_{max} = 285$ °C), the weight loss is 3.40%, corresponding to the release of three lattice water molecules, which is in accordance with the calculated value (3.41%); ii) From 429 – 560 °C ($DTG_{max} = 474$ °C), loss due to C_3H_3N groups and with the total weight loss being 10.64% (calculated value: 10.77%); and the final step showed from 565 – 788°C, the weight loss was 51.94 % (calculated value: 52.23%), corresponding to the decomposition of $C_3H_4N_2$ and $C_{15}H_9O_4$ organic moieties (Abbas et al., 2019; Abd El-Halim et al., 2017).

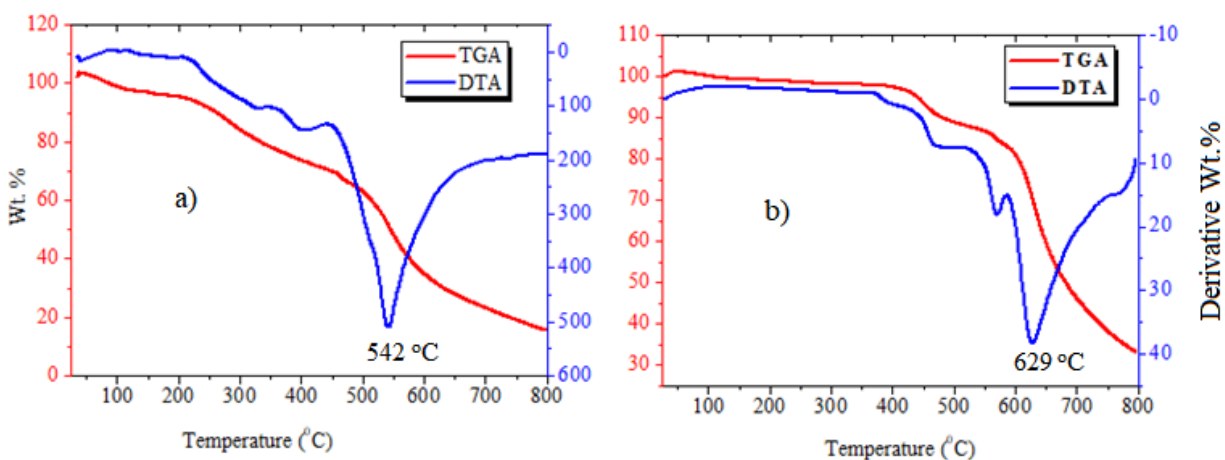


Figure 32. Thermogravimetric (TGA/DTA) presentation of complexes **55** (a) and **56** (b)

Table 10. Temperature range values for decomposition and corresponding weight loss values

Cpds	Decomposition Temp. (°C)	Mass loss (%)		Interpretation
		Obsd.	Calcd.	
54 (Cr(III))	51– 213	7.21	7.19	loss due to water molecules
	295 – 590	41.63	41.60	loss due to C ₁₅ H ₈ O ₃ and 2Cl groups
55 (Cu(II))	100 – 211	8.81	8.84	loss due to three lattice water molecules
	215 – 484	29.47	29.49	release of the C ₁₂ H ₈ N ₂ organic moiety
	490 – 752	43.53	43.87	release of the CHO ₂ and C ₁₅ H ₁₁ O ₂ organic
56 (Zn(II))	100 – 428	3.40	3.41	loss due to the hydroxyl group
	429 – 560	10.64	10.77	loss due to C ₃ H ₃ O groups
	565 – 788	51.94	52.23	loss due to C ₃ H ₄ N ₂ and C ₁₅ H ₉ O ₄

4.3.6 Powder x-ray diffraction analysis of chrysin and 1,10-phenanthroline complexes (54 – 56)

The XRD patterns of the mixed ligand complexes containing chrysin and 1,10-phenanthroline ligands (**54** – **56**) were obtained for the structural characterization (Figure 33). The XRD patterns of **54**, **55** and **56** mixed ligand complexes showed different diffraction peaks with different intensities in the range $2\theta = 5^\circ - 80^\circ$ indicative of the polycrystalline nature of the synthesized complexes (**54** – **56**). Qualx2.0 computer program (Altomare et al., 2015) was used in indexing the diffraction peaks (hkl) and determining the lattice parameters (a , b , c , α , β and γ). The XRD patterns of complex **54** shows diffraction peaks which are characteristic peaks for the monoclinic crystal system with P 1 21/c 1 space group and lattice parameters 7.430 Å, 8.700 Å, 10.668 Å, 90°, 105.08°, 90° for a , b , c , α , β and γ , respectively. Qualx2.0 computer program (Altomare et al., 2015) was used in indexing the diffraction peaks (hkl) and determining the lattice parameters (a , b , c , α , β and γ). The 100% crystal system similarity the synthesized complex (**54**) with 00-100-1338 card number from the crystallography open database (COD) was used for comparison (Merkys et al., 2023; Vaitkus, Merkys, & Gražulis, 2021). A similar crystal system to this work (CrN₄Cl₂ type coordination) was reported in a previous study for an octahedral complex of NiO₄N₂ type (El-Sonbati et al., 2021).

The crystal system of complex **55** was found to be orthorhombic ($\alpha = \beta = \gamma = 90^\circ$) with COD number 00-721-3579. Its space group was found to be $P2_1 2_1 2_1$ and the lattice parameters 14.574 Å, 17.358 Å, 20.413 Å for a, b and c, respectively. Its structural analog, Zn(II) centered complex **56** was found to show similar crystal system, orthorhombic ($\alpha = \beta = \gamma = 90^\circ$) with $Pnma$ space group (COD = 00-701-3841) and lattice parameters 22.361 Å, 10.350 Å, 7.826 Å for a, b, c, respectively. The observed space group difference within the same crystal system might be associated to the difference in geometry of the complexes.

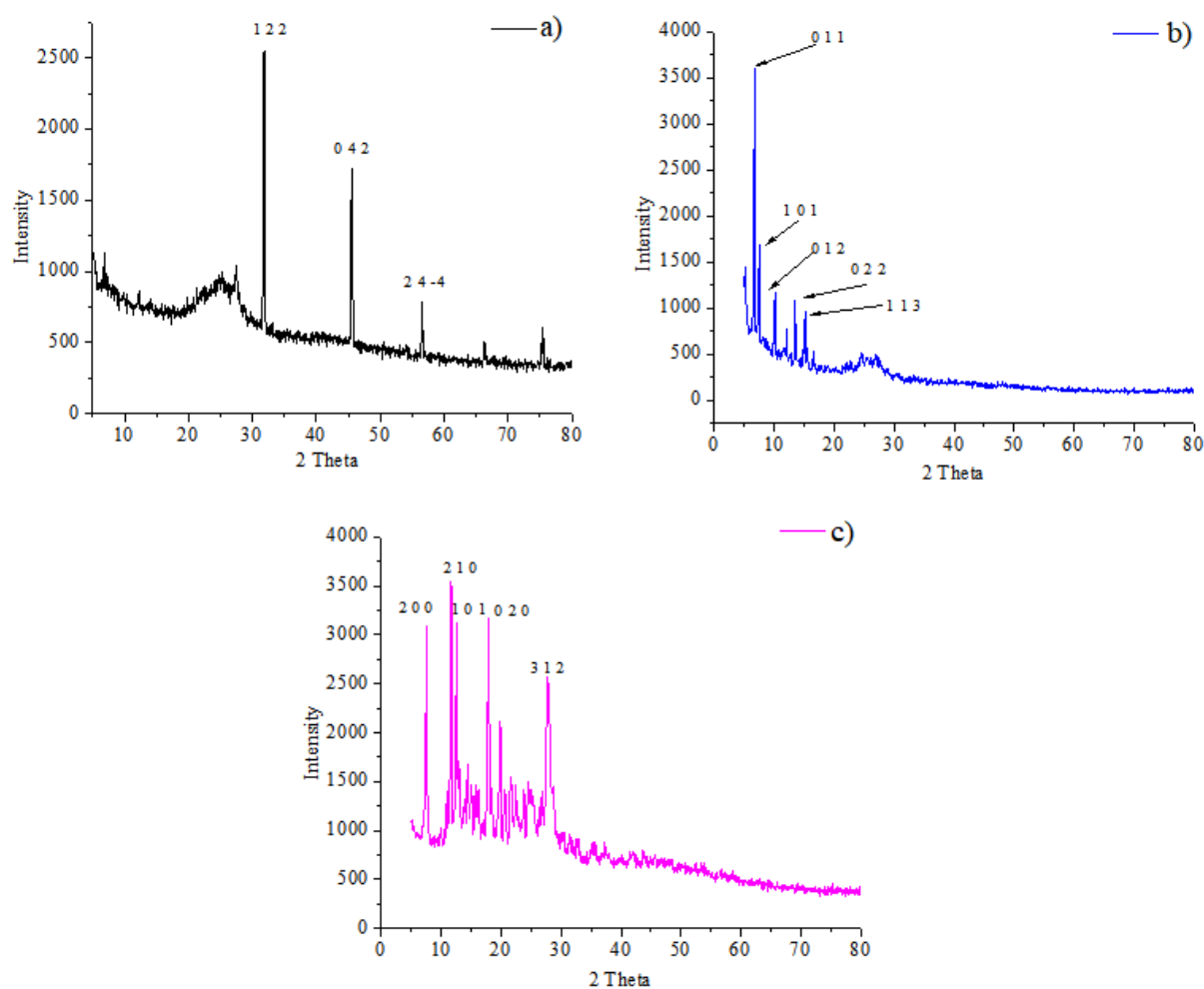


Figure 33. X-ray diffraction patterns for **54** (a), **55** (b) and **56** (c) mixed ligand complex of chrysin and 1,10-phenanthroline.

Moreover, the average crystallite size of the complexes was calculated according to the Debye–Scherrer equation (Holzwarth & Gibson, 2011). It was found that the average crystallite size of the complexes was found to be in the range of nanoparticle size ranging from 18 to 21 nm. The

synthesized complexes were found to follow the order of **56** (18 nm) < **55** (20 nm) < **54** (21 nm) for their average crystallite size. The results showed the synthesis of different metal centered chrysin and 1,10-phenanthroline metal complexes with small average crystallite size difference.

The percent crystalline index of the complexes was calculated using equation 2.

$$\text{Crystalline Index (CI)} = \frac{A_c}{A_c + A_a} \times 100\% \text{ --- (2)}$$

where A_c is the area of crystalline, A_a is the area of the of amorphous part of the materials.

Accordingly, the synthesized mixed ligand complexes were found to show 66.510, 27.208 and 40.914% crystallinity, respectively for **54**, **55** and **56**. The Cu(II) centered complex (**55**) was found to be the least crystalline material followed by its Zn(II) analog (**56**). Then, the number of dislocation lines per unit area of the crystal, dislocation density (δ^*) of the complexes were calculated from its relation to the average crystalline size (D) of the complexes (El-Sonbati et al., 2021; El-Sonbati, Diab, Eldesoky, Morgan, & Salem, 2019) using equation 3.

$$\text{Dislocation density } (\delta^*) = \frac{1}{D^2} \text{ --- (3)}$$

The calculated dislocation density values were found to be 2.324×10^{-3} , 2.559×10^{-3} and $3.069 \times 10^{-3} \text{ nm}^{-2}$ for complexes **54**, **55** and **56**, respectively, confirming a crystalline material has a relatively less dislocation density and less irregularity within the structure. In another previous research work by El-Sonbati and coworkers, dislocation density values ranging from 3.00×10^{-4} – $2.10 \times 10^{-3} \text{ nm}^{-2}$ for Cu(II), Ni(II), Mn(II) and $\text{UO}_2(\text{II})$ mixed ligand polycrystalline complexes were reported (El-Sonbati et al., 2021).

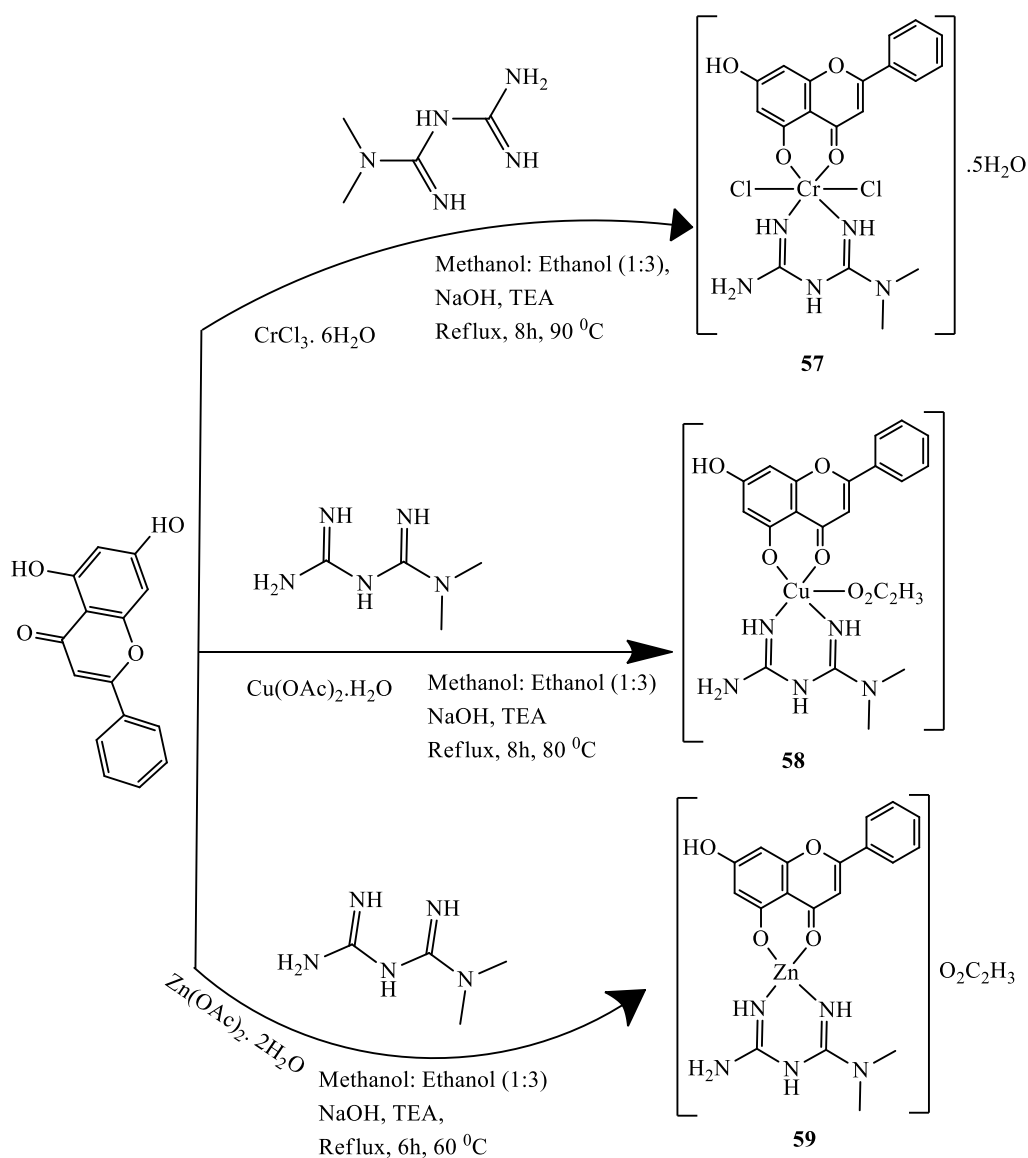
4.3.7 Molar conductance of the complexes

Molar conductance analysis of the complexes was conducted to ascertain electrolytic and non-electrolytic nature of the as-synthesized metal complexes. The molar conductance values were found to be 53.90, 27.56 and $51.80 \Omega^{-1} \text{ mol}^{-1} \text{ cm}^2$, for Cr(III) (**54**), (Cu(II) (**55**) and Zn (II) (**56**) respectively. The results obtained revealed the electrolytic (ionic) nature of **54** and **56**, and non-electrolytic nature of **55** complex (Ali, Wani, & Saleem, 2013). In order to confirm the anionic species in the ionization sphere of complex **54**, a chloride test was performed and the negative result obtained, confirmed the presence of anionic organic species rather than chloride. It is known that the geometry of metal complexes can be predicted from the molar conductance data in

appropriate solvent. In this work, the structures of the synthesized metal complexes were determined by combining the molar conductance with other spectroscopic and spectrometric techniques. Hence, the molecular geometries of complexes were proposed to be octahedral geometry with molecular formula $[\text{Cr}(\text{C}_{12}\text{H}_8\text{N}_2)_2\text{Cl}_2](\text{C}_{15}\text{H}_9\text{O}_4)$ for complex **54**, square pyramidal structure with molecular formula $[\text{Cu}(\text{C}_{12}\text{H}_8\text{N}_2)(\text{C}_{15}\text{H}_9\text{O}_4)(\text{C}_2\text{H}_3\text{O}_2)].3\text{H}_2\text{O}$ for **55** and tetrahedral structure with molecular formula $[\text{Zn}(\text{C}_{12}\text{H}_8\text{N}_2)(\text{C}_{15}\text{H}_9\text{O}_4)]\text{C}_2\text{H}_3\text{O}_2$ for complex **56**.

4.4 Synthesis and characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of metformin and chrysin

The synthesized metal complexes were synthesized in 1:1 metal to ligands ratio as described in Scheme 3.



Scheme 3. Schematic representations of metformin-chrysin mixed ligand metal complexes

4.4.1 Physicochemical properties of chrysin and metformin complexes of Cr(III), Cu(II) and Zn(II)

The synthesized metal complexes (57 – 59) were subjected for solubility test using solvents of varying polarity. Complex Cr(III) (57) was found to be soluble in polar aprotic solvents: DMSO and DMF (dimethylformamide); slightly soluble in polar solvents: water, methanol, ethanol; but insoluble in ethyl acetate, dichloromethane and hexane solvents. It was subjected to chloride test using AgNO_3 and gives negative result for the expected white precipitate, inferring the absence of chloride ion in the ionization sphere. The complexes Cu(II) (58) and Zn(II) (59) were soluble only

in DMF and DMSO solvents. The melting point values recorded showed that the complexes decompose at temperatures greater than 180 °C (Table 11). The percentage yield, melting point, color and state of the synthesized metal complexes are presented in Table 11.

Table 11. Physicochemical properties of metformin-based metal complexes

Compound	Color	State	Mass (% Yield)	M.pt (°C)	Conductivity ($\Omega^{-1}\text{mol}^{-1}\text{cm}^2$, 25 °C)
57 (Cr(III))	Light-green	Powder	0.484g (81.344)	> 220	104.40
58 (Cu(II))	Gray-brown	Powder	0.215g (42.490)	> 180	34.80
59 (Zn(II))	Light-yellow	Powder	0.344g (67.850)	> 210	126.60

4.5 Characterization of heteroleptic Cr(III), Cu(II) and Zn(II) complexes of metformin and chrysin

4.5.1 FTIR analysis

The FTIR spectra of the synthesized complexes are presented from Figure 34 – 36 and Appendix 13. Infrared-spectroscopic spectral analysis reported that metformin can be characterized for its N–H stretching vibration, asymmetrical and symmetrical C–N vibrations, NH₂ deformation, respectively from 3100 – 3490, 1583 –1626, and 1470 –1540 cm⁻¹ in addition to other stretching and bending vibrations (Nakamoto, 2009; Villamizar-Delgado, Porras-Osorio, Pineros, et al., 2020). In this work, these vibrational frequencies were found to appear at 3429, 1637 and 1483–1409 cm⁻¹. The participation of the nitrogen atom in the coordination of the metal center of complex **57** was evidenced from the changes in the intensity and vibrational frequency of the free metformin as shown in Figure 36. Moreover, the characteristic C=O vibrational peak that appeared at 1644 cm⁻¹ was found to red shift to 1655 cm⁻¹ in the metal complex. The presence of peaks at 750 and 609 cm⁻¹ confirmed the formation of Cr–O and Cr–N=C bonds, respectively (Abbas et al., 2019).

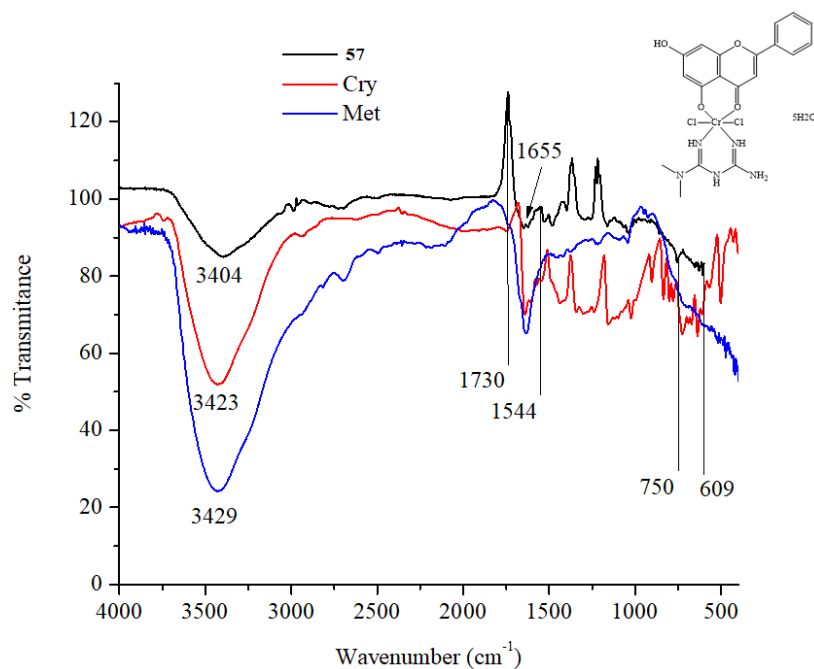


Figure 34. FTIR spectra of metformin, chrysin and **57** complex

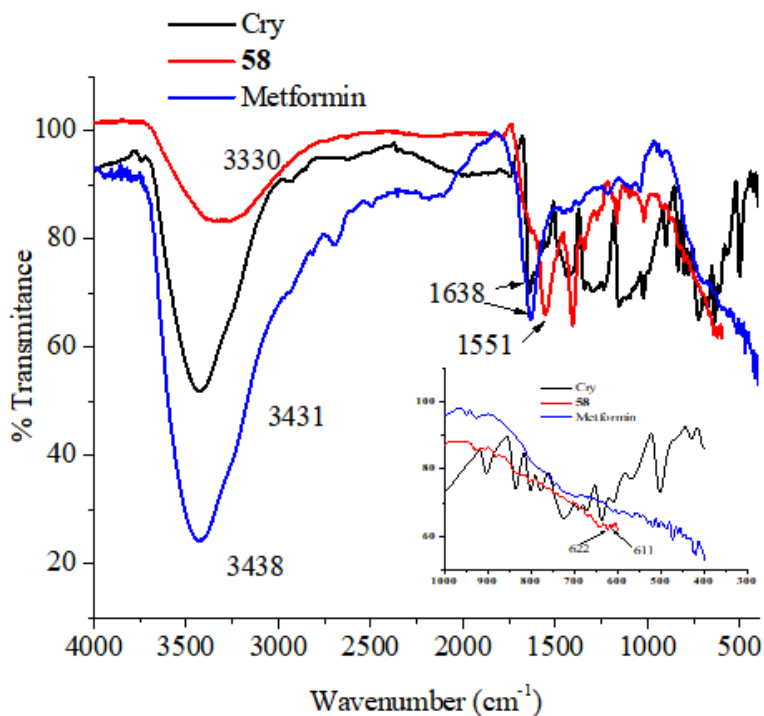


Figure 35. FTIR spectra of metformin, chrysin and **58** complex

In complex **58** (Figure 35), the N–H stretching vibration, C–N vibrations, NH₂ deformation peaks appeared at 3330, 1555 and 1409 cm⁻¹, respectively. Similarly, these vibrational frequencies were

recorded at 3395, 1566 and 1444 cm^{-1} for complex **59**. Whereas, the M-O and M-N vibrational frequencies were observed at 622 and 611 cm^{-1} and 641 and 612 cm^{-1} for **58** and **59** complexes, respectively, confirming the presence of similar coordination environment to the metal centers.

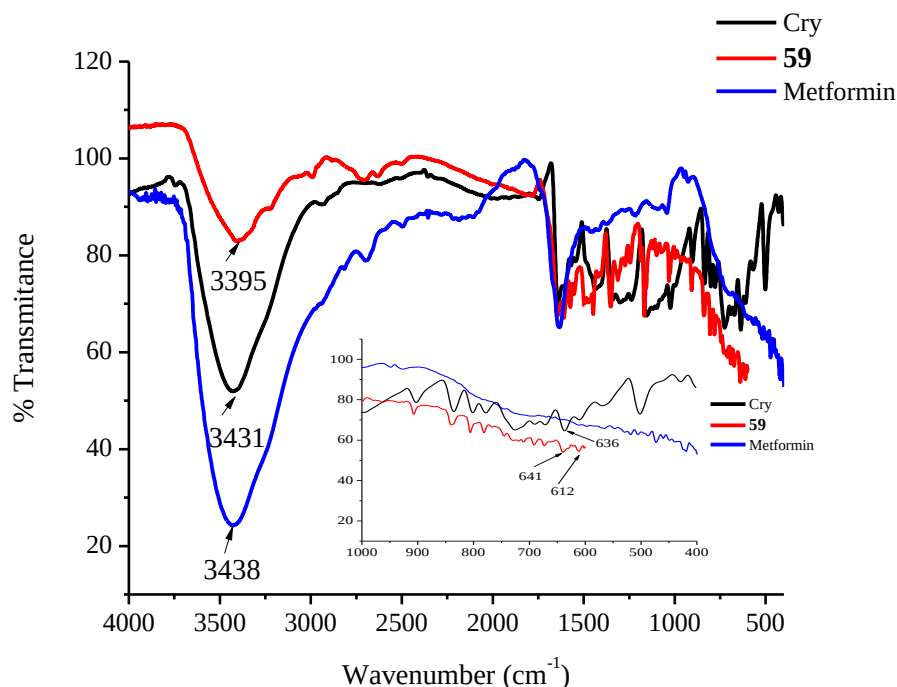


Figure 36. FTIR spectra of metformin, chrysin and **59** complex

4.5.2 UV-Vis/TD-DFT analysis of the complexes of metformin coordinated chrysin based complexes (**57** – **59**)

The UV-Vis spectra of chrysin (experimental /calculated) and associated electronic transitions are discussed (*vide-supra*). Whereas, in the UV-Vis spectra of metformin, the electronic transition was observed at 240/ 236 nm. Following the reaction with Cr(III), a bathochromic shifts to the benzoyl and cinnamoyl bands of chrysin were observed as confirmed by the new peaks appeared at 294/305 nm and 330 nm. Whereas, the absorption peak of metformin was totally diminished, confirming the coordination of the ligand to the metal center. A new stronger absorption peak appeared at 402 nm (426 nm) corresponding to intra-ligand charge transfer within the chrysin structure as clearly observed in the TD-DFT calculated HOMO-LUMO distribution.

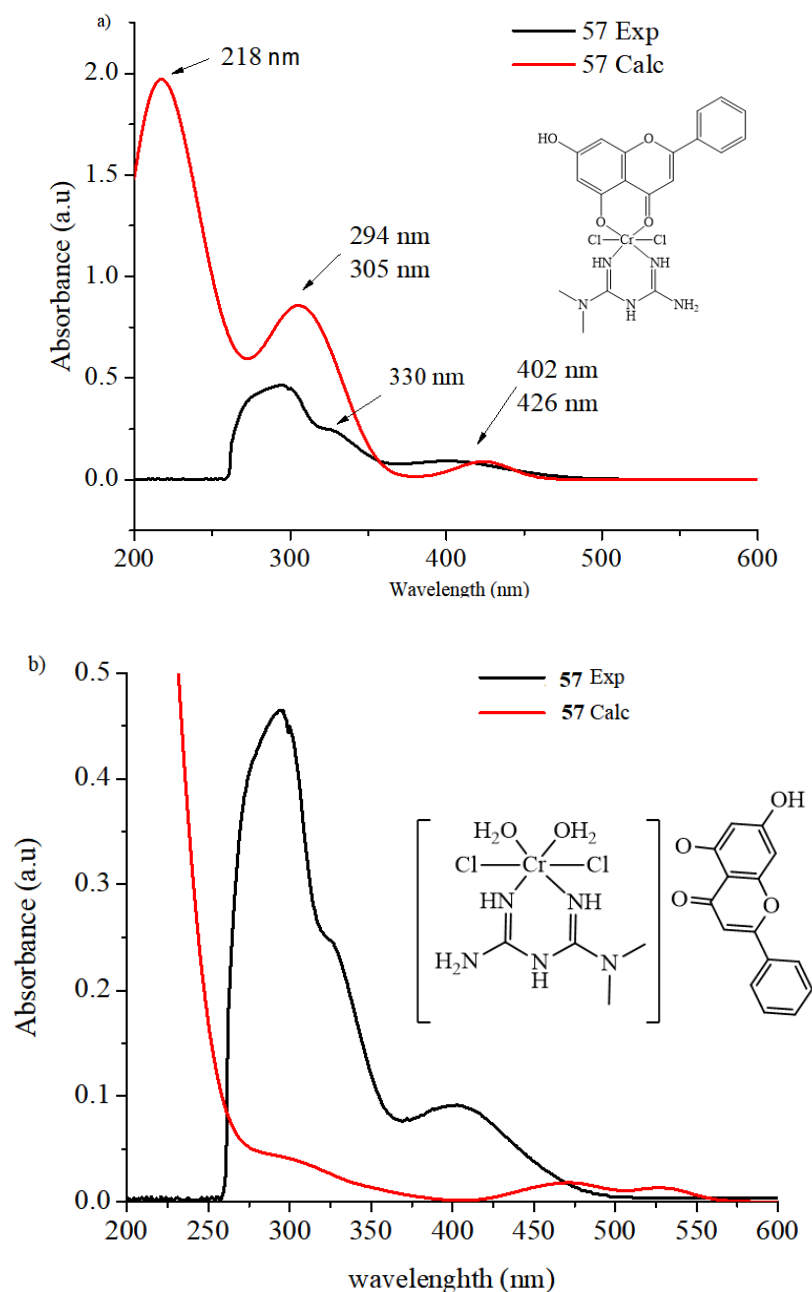


Figure 37. The UV-Vis and TD-DFT spectrum of complex **57**(a) and its alternative structure (b)

From molar conductance measurement, the conductivity of complex **57** was found to be in the conductivity range ($> 50 \Omega^{-1}\text{mol}^{-1}\text{cm}^2$). In order to confirm the observed conductivity, we designed cationic metal complex and run its TD-DFT calculation to see the similarity/difference in the electronic transition (Figure 37 b). However, the TD-DFT spectra (Figure 37) was found to be totally far from the experimental one, confirming the second complex (Figure 37 (b)) is not the possible structure.

The spectral bands of chrysin were reported to appear to be intense at 270 nm (benzoyl system) and less intense 330 nm (benzoyl system) in DMSO (Lin et al., 2017). In this work, the UV-Vis /TD-DFT calculated absorption spectrum of chrysin in DMSO was found to appear at 267/261 nm and 320/325 nm, for $\pi \rightarrow \pi^*$ transitions of benzoyl system and cinnamoyl system of the molecule, respectively which are inline to the previously reported works (Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021; Sundaraganesan et al., 2012). The bathochromic and hypochromic shifts in the characteristic absorption spectra of chrysin observed in complexes **58** and **59** are presented in Figure 38. In complex **58**, the characteristic peak for the benzoyl system of chrysin that appeared at 267/261 was found to undergo bathochromic shift and appeared at 291/301 nm ($\Delta\lambda_{\text{max}} = 24$ nm), whereas a peak associated to the cinnamoyl system of chrysin (320/325 nm, $\pi \rightarrow \pi^*$) and that of metformin was totally contracted, confirming the coordination of the ligands to the Cu(II) center. New broad and less intense peaks that appeared at 403/414 nm would be charge transfer between the two ligands (Ligand-Ligand charge transfer) or metal to ligand charge transfer (MLCT) which again confirms the presence of new electronic phenomenon as a result of metal-ligand coordination.

In complex **59**, the benzoyl system was found to appear at 272/297 nm ($\Delta\lambda_{\text{max}} = 5$ nm) and hypsochromic shift to the cinnamoyl system of chrysin appeared at 316 nm ($\Delta\lambda_{\text{max}} = 4$ nm). The absence of any peak above 400 nm would be a confirmation for the absence of charge transfer in Zn(II) centered complex.

In this class of complexes (**57** - **59**), a new peak at 218, 210 and 215 nm, respectively for **57**, **58** and **59** were observed in TD-DFT calculation. These peaks could be associated to the coordination of metformin detected in the computational study.

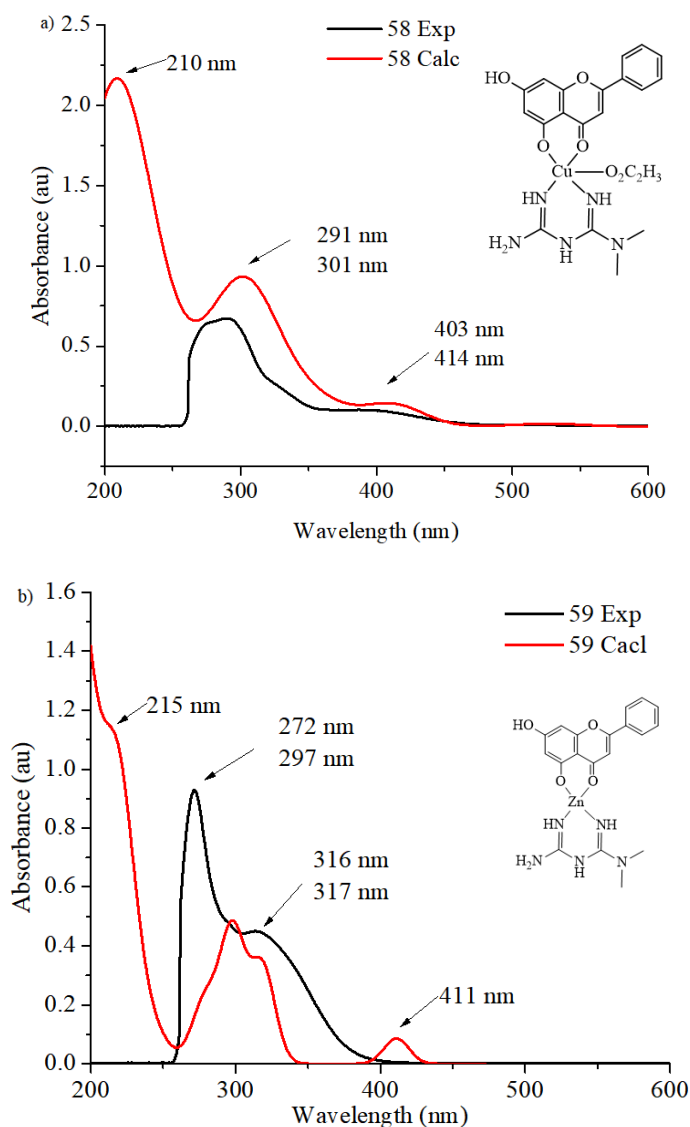


Figure 38. UV-Vis and TD-DFT spectrum of complex **58** (a) and **59** (b)

4.5.3 Mass spectrometric analysis of mixed ligand complexes (**57** – **59**)

The high-resolution mass spectral data of the synthesized complexes (**57** -**59**) were used to study the fragmentation patterns of the complexes. The obtained spectrometric results are presented in Figure 39 and Appendix 14 – 15. Complex **57** was found to exhibit a molecular ion peak at $m/z = 595.000$ (calc = 594.080), attributed to the complex $[C_{19}H_{30}Cl_2CrN_5O_9]$, a peak at $m/z = 576.049$ (calc = 576.070) and a base peak at $m/z = 558.039$ (calc = 558.060) are associated to fragments with formula $[C_{19}H_{28}Cl_2CrN_5O_8]$ and $[C_{19}H_{26}Cl_2CrN_5O_7]$, respectively, in which a water molecule was dissected successively. Two base peaks which appeared at $m/z = 255.065$ (calc = 253.050) and $m/z = 130.109$ (calc = 129.100) could be associated to the ligands chrysin ($C_{15}H_9O_4$) and

metformin ($C_4H_9N_5$), respectively, confirming the coordination of the aforementioned ligands ensuring the feasibility of the designed complexes (Scheme 3 and Figure 39).

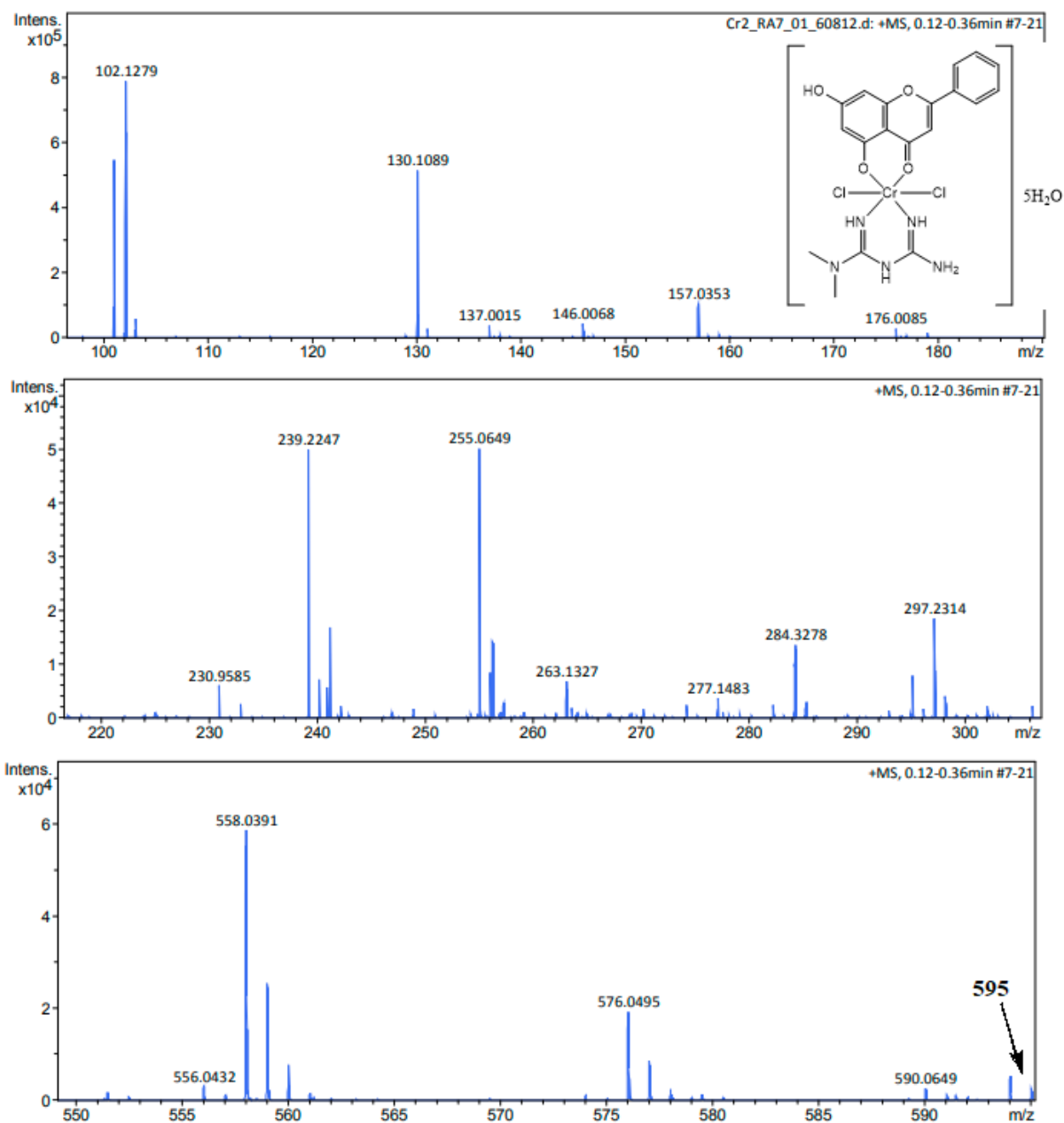


Figure 39. Mass spectra of chrysin and metformin-based complex Cr(III) complex (57)

Moreover, the presence of $m+4$ type peaks to the m/z peaks confirmed the coordination of two chlorides to the Cr (III) center. In complex **58**, the molecular ion peak was not observed due to the increased intensity of the remaining peaks. A peak that appeared at $m/z = 393.992$ (calc =

395.030) which can be attributed to the complex cation $[C_{14}H_{13}CuN_5O_5]^+$, another peak that support the proposed mass fragmentation (Appendix 14) was appeared at $m/z = 353.265$ (calc = 351.040) which corresponds to $C_{13}H_{14}CuN_5O_3$ fragment, a peak at $m/z = 321.131$ (calc = 323.990) corresponds to the chemical fragment $C_{13}H_9CuN_4O_4$. The fragments for the coordinating ligands, chrysin and metformin were found to appear as intense peaks at 255.065 (calc = 253.05) and 130.109 (calc = 129.100), respectively. Similarly, in complex **59** a molecular ion peak at the expected $m/z \sim 503$ was not observed due to intensity differences between the base peaks and the rest of peaks. Whereas, fragments obtained the synthesized complex were found at $m/z = 473.005$ (calc = 471.050) which corresponds to a fragment, $C_{20}H_{17}N_5O_5Zn$ that confirms the presence of parts of chrysin, acetate ion and metformin. Moreover, the presence of the coordinated ligands are confirmed for their characteristic mass spectra appeared at $m/z = 255.065$ (calc = 253.05) and 130.109 (calc = 129.100), respectively, for chrysin and metformin (Appendix 15).

4.5.4 SEM-EDX analysis of the complexes (57 – 59)

. The morphology of the synthesized Cr(III)(**57**), Cu(II)(**58**) and Zn(II)(**59**) complexes were studied using SEM. The SEM micrographs (Figure 40 (d – f)) reveals that the complexes are in the form of uniformly aggregated grains. Complex **57** (d) and **58** (e) do not yield smooth surfaces but showed the presence of uniformly sized small grains in line with their polycrystalline phase. A relatively more uniform and smooth surface was shown by a Zn(II) complex (**59**) which might be due to its high percent crystallinity (shown below). In the EDX spectrum, the Cr(III) complex (**57**) shows signals correspond to chlorine, carbon, oxygen, nitrogen, and chromium, confirming the synthesis of the Cr(III) complex with $CHCrNOCl$ chemical composition. Similarly, the EDX spectrum of the Cu(II) (**58**) and Zn(II)(**59**) complexes showed characteristic signals corresponding to carbon, oxygen, nitrogen together with the respective metals. Hence, the successful synthesis of the designed complexes has been confirmed by the EDX analysis.

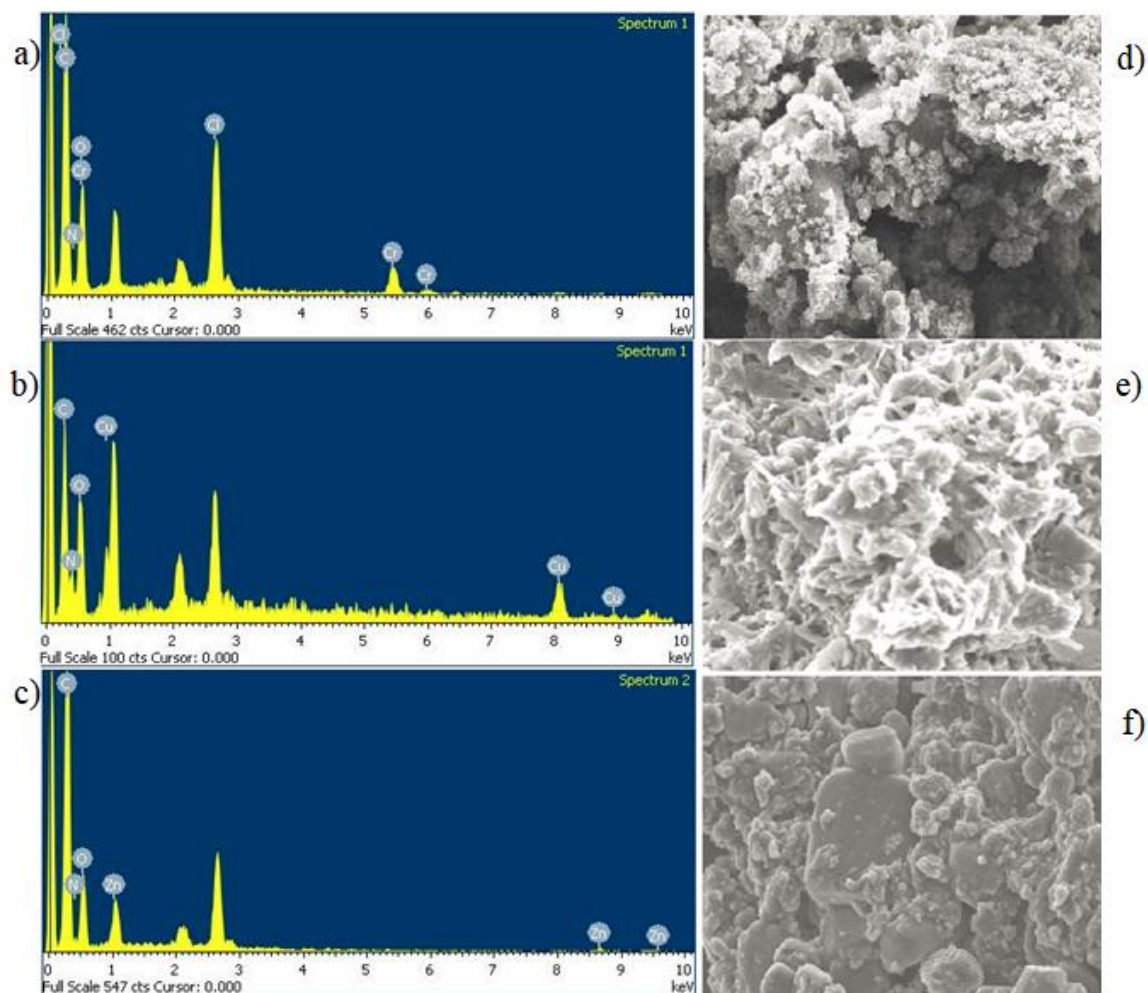


Figure 40. EDX analysis of metformin-chrysin mixed ligand metal complexes **57** (a) **58** (b) and **59**(c) and their respective SEM images of **57** (d) **58** (e) and **59** (f)

4.5.5 Thermogravimetric Analysis

The TGA/DTA analyses of the synthesized metformin-chrysin ligand-based complexes are presented in Figure 41, Figure 42 and Table 12. Complex **57** was thermally decomposed in three main degradation steps and the thermograph showed that: i) From 55.56 – 267.41 °C ($DTG_{max} = 243$ °C), the weight loss was 9.11%, corresponding to the release of water molecules, which is in good accordance with the calculated value (9.10%); ii) from 275.57 – 411.07 °C ($DTG_{max} = 400$ °C) associated to the decomposition of two-chlorine moiety, with the total weight loss being 11.76% (calculated value: 11.73%) and the final step showed decomposition from 420 – 524 °C, ($DTG_{max} = 441$ °C) and a weight loss of 24.42% (calculated value: 24.40%), corresponding to

the decomposition of $C_4H_{11}N_5$ and OH organic moiety with the final residue of chromium oxide (Ndong et al., 2020).

The thermal degradation pattern of complex **58** proceeded in four main degradation steps and the thermograph data revealed that: *i*) From 55 – 207 °C ($DTG_{max} = 103$ °C), the weight loss is 24.65%, corresponding to the release of $3H_2O$, $C_2H_3O_2$ and OH chemical entities, which is in good accordance with the calculated value (24.60%). *ii*) From 209 – 326 °C ($DTG_{max} = 311$ °C), loss due to $C_{13}H_7O$ moiety, with the total weight loss being 32.34% (calculated value 32.31%). *iii*) From 335-449 °C, the weight loss is 10.12% (calculated value: 10.01%), corresponding to the decomposition of CH_3N_3 organic moiety. *iv*) From 500 – 557 °C, the weight loss is 15.25% (calculated value: 15.20%), corresponding to the decomposition of $C_5H_8N_2$ organic moiety and the finally total weight loss is 82.36% (calculated: 82.20%) with the final residue of related to copper oxide (CuO) (Abd El-Halim et al., 2017; Naglah et al., 2021).

In a similar fashion, the TGA and DTA analysis of complex **59**, showed that it thermally decomposed in two main degradation steps: *i*) From 205 – 493 °C, the weight loss is 25.6%, corresponding to the release of $C_4H_9N_5$ molecules, which is in good accordance with the calculated value (25.4%) and *ii*) From 501 – 758 °C, loss due to $C_{15}H_{11}O_2$ groups and, with the total weight loss being 43.81% (calculated value: 43.60%). and the finally the both steps showed total weight loss of 69.41 with residue is related to zinc oxide in line with reported studies (Abd El-Halim et al., 2017; Naglah et al., 2021).

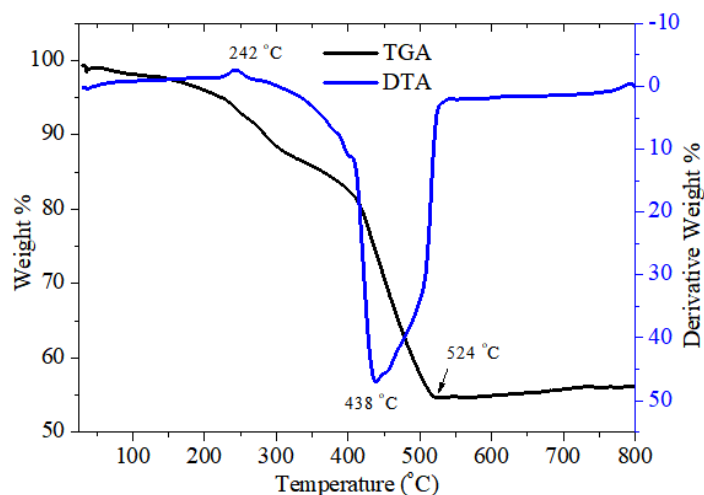


Figure 41. Thermogravimetric analysis of Cr(III) complex (**57**)

Table 12. Temperature range values for decomposition and corresponding weight loss values

	Decomposition Temp. (°C)	Mass loss (%)		Interpretation
		Obsd.	Calcd.	
57 (Cr(III))	55 – 267	9.11	9.8	loss due to water molecules
	275 – 411	11.76	11.74	release of the chlorine moiety
	420 – 524	24.42	24.40	decomposition of C ₄ H ₁₂ N ₅ O organic moiety
58 (Cu(II))	55 – 207	24.65	24.60	loss due to 3H ₂ O, C ₂ H ₃ O ₂ and OH chemical entities
	209 – 326	32.34	32.31	loss due to C ₁₃ H ₇ O moiety
	335 – 449	10.12	10.01	loss due to the decomposition of CH ₃ N ₃ moiety
	500 – 557	15.25	15.20	decomposition of C ₅ H ₈ N ₂ moiety
59 (Zn(II))	205 – 493	25.6	25.4	the release of C ₄ H ₉ N ₅ molecules
	501 – 758	43.81	43.60	loss due to C ₁₅ H ₁₁ O ₂ groups

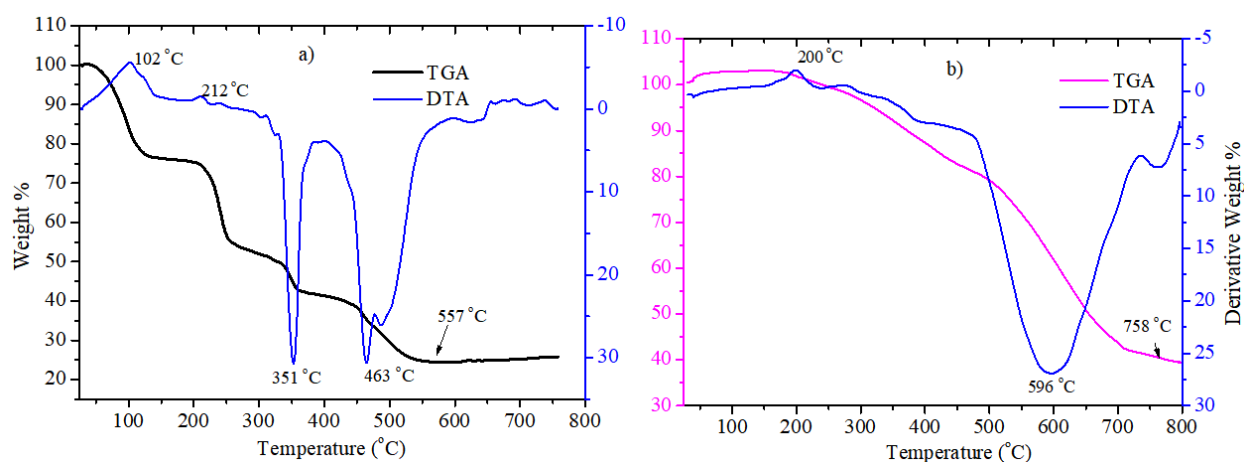


Figure 42. Thermogravimetric analysis of chrysin and metformin complexes **58** (a) and **59** (b)

4.5.6 Powder X-ray diffraction analysis of chrysin and metformin mixed ligand complexes

The powder diffraction (XRD) patterns of metformin and chrysin containing mixed ligand metal complexes (**57** – **59**) were obtained for structural characterization. The XRD patterns of the synthesized complexes showed diffraction peaks which indicate the polycrystalline phases with different intensities in the range $2\theta = 5^\circ - 80^\circ$ (Figure 43). In this regard, complex **57** was found to follow a monoclinic crystal system (COD = 00-451-1884) and P 1 21/c 1 space group with lattice parameters 7.314 Å, 7.631 Å, 20.569 Å, 90° , 99.462° and 90° , respectively for a, b, c, α , β and γ . Complex **58** was found to be a monoclinic crystal system (COD = 00-702-2172) with C 1 2/c 1 space group and lattice parameters 22.6900 Å, 13.916 Å, 20.317 Å, 90° , 90° , and 98.975° , respectively for a, b, c, α , β and γ . Complex **59** was found to be a triclinic crystal system (COD = 00-600-0487) and P -1 space group with lattice parameters, 7.966 Å, 9.082 Å, 11.832 Å, 81.421° , 71.301° , 74.998° , for a, b, c, α , β and γ , respectively.

The average crystalline size of the complexes were calculated according to Debye–Scherrer equation (Holzwarth & Gibson, 2011). It is found that the average crystalline size of the complexes was found to be in the range of nanoparticle with calculated values of 20, 19 and 18 nm, respectively for complexes **57**, **58** and **59**. The percent crystalline index of the complexes was calculated to exist as 31, 23 and 94% crystallinity for **57**, **58** and **59**, respectively, inferring the synthesis of diverse crystalline metal complexes. The number of dislocation lines per unit area of the crystal, dislocation density (δ^*) of the complexes were calculated from its relation to the average crystalline size (D) of the complexes according to previously reported works (El-Sonbati et al., 2021; El-Sonbati et al., 2019). Accordingly, the dislocation density values of complexes **57**, **58** and **59** were calculated to be 2.603×10^{-3} , 2.989×10^{-3} , and $3.083 \times 10^{-3} \text{ nm}^{-2}$ respectively, confirming the as-synthesized crystalline metal complexes have relatively less dislocation density and less irregularity within the structure. El-Sonbati and coworkers reported similar values of dislocation density ranging from $3.00 \times 10^{-4} - 2.10 \times 10^{-3} \text{ nm}^{-2}$ for Cu(II), Ni(II), Mn(II) and $\text{UO}_2(\text{II})$ mixed ligand polycrystalline complexes (El-Sonbati et al., 2021).

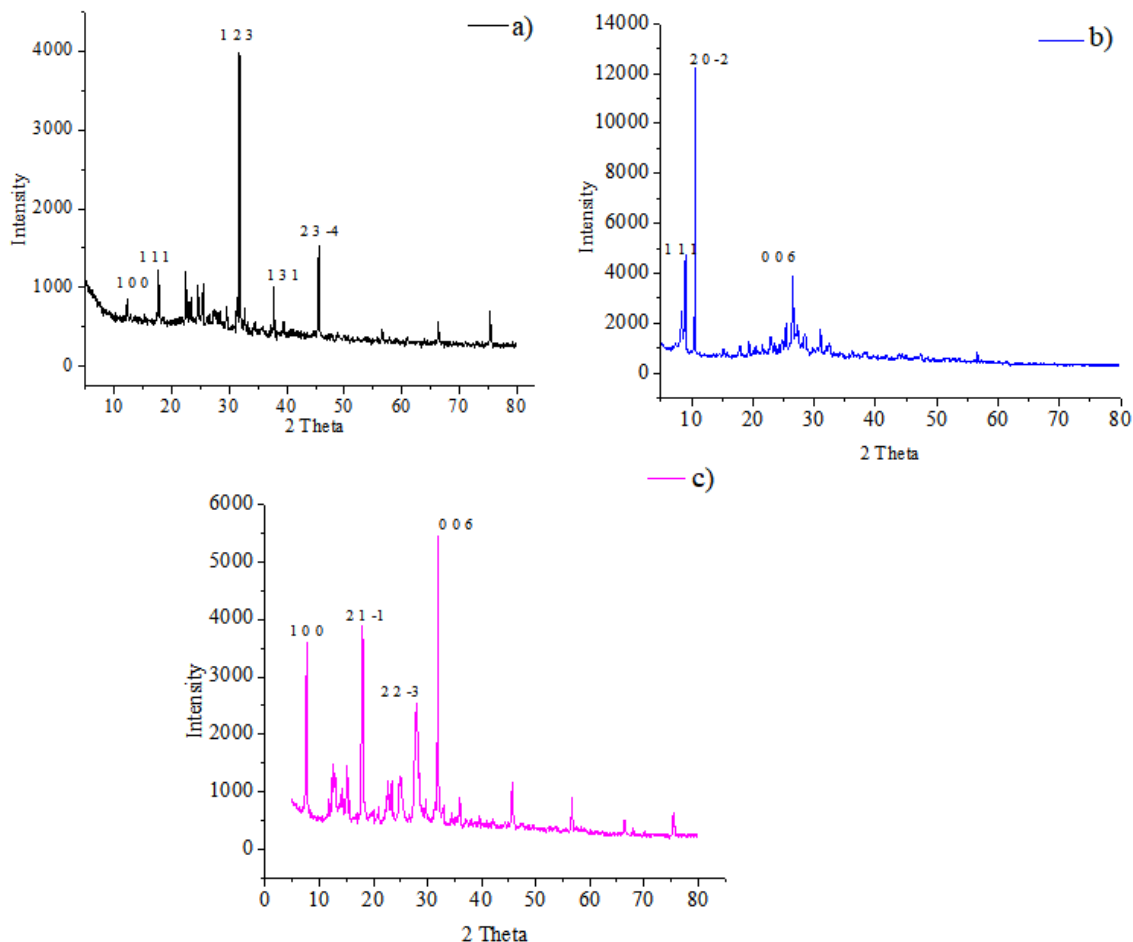
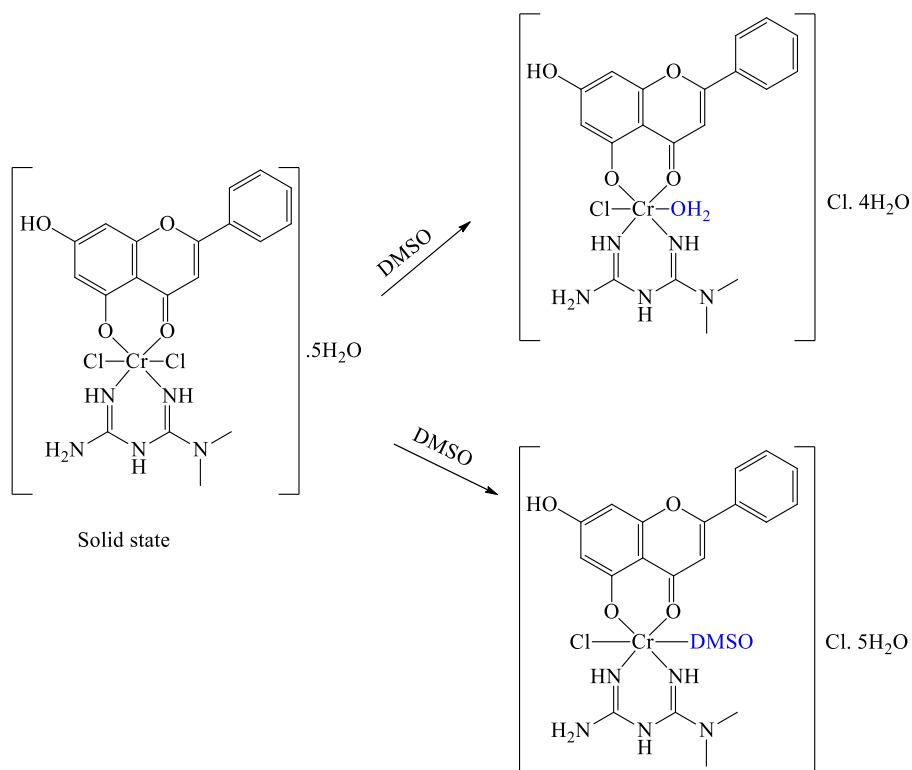


Figure 43. X-ray diffraction patterns for Cr(III) (**57**(a)), Cu(II)(**58**(b)) and Zn(II)(**59**(c)) mixed ligand complexes.

4.5.7 Molar conductance of chrysin and metformin complexes of Cr(III), Cu(II) and Zn(II)

The electrolytic and non-electrolytic nature of the synthesized metal complexes (**57** - **59**) was determined using a molar conductance measurement at 1.0×10^{-3} M solution of the complexes in DMSO. The molar conductance was found to be 104.40, 34.80 and $126.60 \Omega^{-1} \text{ mol}^{-1} \text{ cm}^2$, for complexes **57**, **58** and **59**, respectively. The results indicated that the 1:1 coordination sphere cation and anion ratio with electrolytic (ionic) nature for complex **59**, and non-electrolytic nature of complex **58** (Ali et al., 2013). Complex **57** followed a different conductivity mechanism that exists when a ligand in a coordination sphere is substituted by a water molecule in the water of crystallization or a solvent molecule itself (Ali et al., 2013). On the other hand, the special electronic structure, presence of impurities in the crystal structures and extended ligand π -systems were suggested to be the origins of molar conductivity in neutral complexes (Kobayashi et al.,

1999). In order to confirm the presence of a chloride ion in the ionization sphere of complex **57**, a chloride test was performed and gives a negative result, confirming the absence of a chloride ion supporting the observed electrical conductivity.



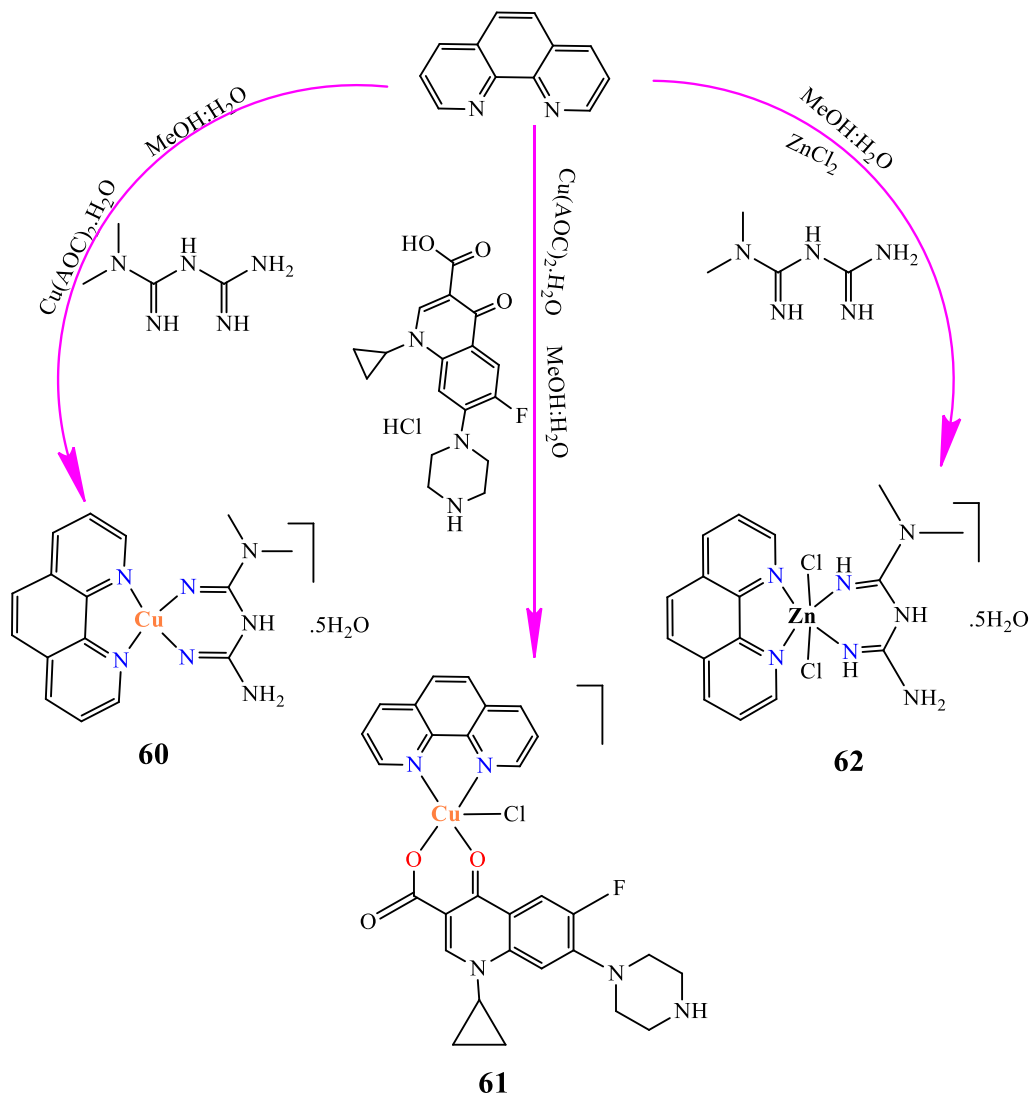
Scheme 4. Proposed structure of Cr(III) complex (**57**) in solid and solution forms

The geometry of metal complexes can be determined from the molar conductance data in appropriate solvent. Hence, the possible structures of the synthesized metal complexes were proposed by combining molar conductance measurements with other spectroscopic (UV-Vis, FTIR), spectrometric techniques (MS) and electroscopic (SEM-EDX) and XRD techniques. Hence, the structures were proposed to be octahedral geometry with molecular formula $[\text{Cr}(\text{C}_{12}\text{H}_8\text{N}_2)(\text{C}_{15}\text{H}_9\text{O}_4)\text{Cl}_2] \cdot 5\text{H}_2\text{O}$ for complex **57**, square pyramidal $[\text{Cu}(\text{C}_4\text{H}_{11}\text{N}_5)(\text{C}_{15}\text{H}_9\text{O}_4)(\text{C}_2\text{H}_3\text{O}_2)]$ for complex **58** and tetrahedral $[\text{Zn}(\text{C}_4\text{H}_{11}\text{N}_5)(\text{C}_{15}\text{H}_9\text{O}_4)]\text{C}_2\text{H}_3\text{O}_2$ for complex **59**.

4.6 Synthesis and characterization of Cu(II) and Zn(II) mixed drug complexes (**60** – **62**)

In this part of the study, complexes of Cu(II) and Zn(II) containing drugs metformin and ciprofloxacin as well as 1,10-phenanthroline were synthesized in a very good (> 70 %) yield in their powder form (Table 13). The schematic representation of the synthesis and the obtained

physicochemical properties of the synthesized metal complexes are given in Scheme 5 and Table 13.



Scheme 5. The design of the coordination spheres of the synthesized metal complexes

4.6.1 Physicochemical properties mixed-drug complexes (60 – 62)

Dimethyl sulfoxide (DMSO) was a solvent used to test the conductivity of the two complexes (**60** and **61**). The molar conductivity results showed that 96 ± 0.340 and $32 \pm 0.047 \text{ } \Omega^{-1}\text{mol}^{-1}\text{cm}^2$ for **60** and **61**, respectively, confirming the non-electrolytic nature of the synthesized complexes which would be due to the absence of ions in the ionization sphere of the synthesized metal complexes. The relatively high conductivity value of complex **60** could be due to the replacement of anionic ligand, metformin by the solvent DMSO which will create a 1:1 type cation – anion ratio resulting increased conductivity.

Table 13. Physicochemical properties of the synthesized mixed drug metal complexes

Compounds	Color	Physical state	Yield (%)	Melting point ($^{\circ}\text{C}$)	Conductivity ($\Omega^{-1}\text{mol}^{-1}\text{cm}^2$)
60 (Cu(II))	Brown	Powder	0.359 g (78.38)	190 – 194	96 ± 0.340
61 (Cu(II))	Green	Powder	0.486 g (79.80)	Decomposes >206	32 ± 0.047
62 (Zn(II))	White	Powder	0.391 g (73.08)	186 – 188	67 ± 0.374

4.7 Characterization of 1,10-phenanthroline based Cu(II) and Zn(II) mixed drug complexes

4.7.1 FTIR Analysis of the complexes (60 – 62)

The FTIR spectra of the ligands and the synthesized metal complexes are presented in Figure 44, Table 14 and Appendix 16 – 17. It has been reported that ciprofloxacin shows characteristic peaks in the functional group region between 3500 and 3450 cm^{-1} for $\nu(\text{O-H})$, from 3000 to 2950 cm^{-1} for $\nu(\text{C-H})$, 1750 to 1700 cm^{-1} for $\nu(\text{C=O}, \text{carbonyl})$ and peaks from 1650 to 1600 cm^{-1} for $\nu(\text{C=O}, \text{quinolone})$ (Sahoo, Chakraborti, & Mishra, 2011). The bands at the 1450 to 1400 cm^{-1} represented $\nu(\text{C-O})$ and the peaks at 1300 to 1250 cm^{-1} suggested bending vibration of $\nu(\text{O-H})$ group which indicated the presence of carboxylic acid. In addition, a strong absorption peak between 1050 and 1000 cm^{-1} was assigned to the C-F group (M. N. Patel et al., 2013). Upon coordination to Cu(II) metal center, the characteristic vibrational bands for carboxylic carbon $\nu(\text{C=O})$ 1704 cm^{-1} and quinolone carbon ($\nu\text{C=O}$, 1608 cm^{-1}) shifted to 1640 cm^{-1} and 1579 cm^{-1} , respectively, confirming the coordination of ciprofloxacin via its carboxylic and quinolone oxygen (Figure 44 b). Moreover, stretching (symmetric and asymmetric) vibrational bands associated to $\nu(\text{COO})_{\text{asy}}$ and $\nu(\text{COO})_{\text{sy}}$ stretches were found to appear at 1515 cm^{-1} and 1301 cm^{-1} for ciprofloxacin coordinated Cu(II) complex in the presence of 1,10-phenanthroline ligand.

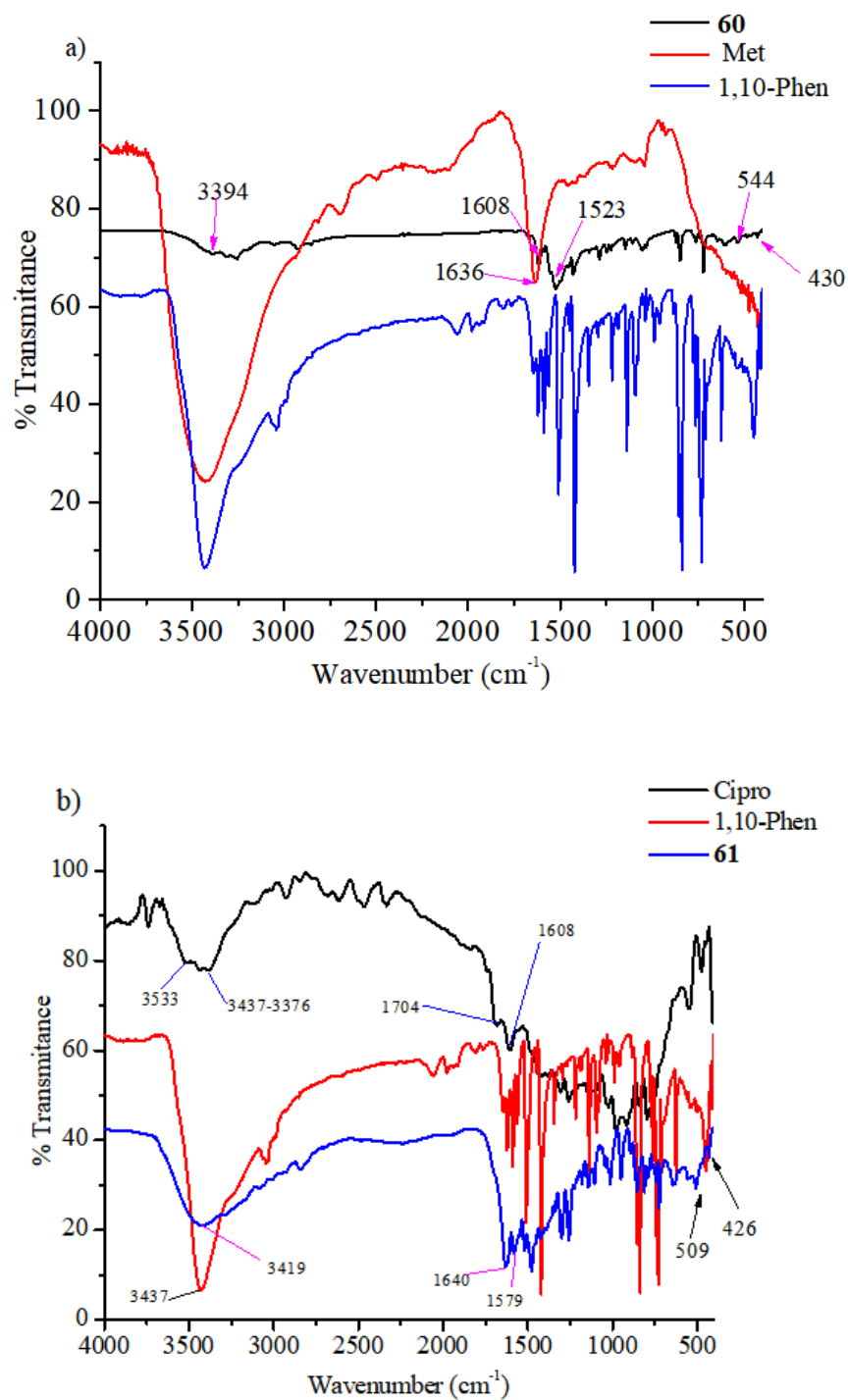


Figure 44. FTIR spectra of the synthesized metal complexes **60** (a) and **61** (b) and the ligands

It has been observed that the FTIR peaks which appeared from 1615 to 1430 cm^{-1} in complex **60** was attributed to the coordination of 1,10-phenanthroline to the metal center through its C=N group. In this work, DFT calculation was used to assist the type of metal-nitrogen vibration.

Experimental/calculated vibrational frequencies at 544/492 cm^{-1} , and 430/ 416 cm^{-1} in complex **60** are associated to metal Cu-N vibrations of the metformin and 1,10-phenanthroline, respectively, confirming the coordination of the ligands (1,10-phenanthroline and metformin) to the metal center Cu(II). Moreover, spectra overlap between the Cu(II) centered complexes (**60**) and its structural analog Zn(II) complex (**62**) showed the similar ligand composition between the complexes (Appendix 17). The difference between the asymmetric and symmetric vibrational bands of carboxylic oxygen atoms is useful to determine the coordination mode of ciprofloxacin. In complex **61**, the difference is found to be 214 cm^{-1} , inferring the monodentate coordination of COO donor group to the Cu(II) metal center (Lawal et al., 2020; Patel et al., 2009).

Table 14. Vibrational frequencies (experimental/calculated) of selected functional groups of the ligands and their metal complexes

Compound	Selected vibrational frequencies (cm^{-1})							
	$\nu(\text{OH})$	$\nu(\text{C}=\text{O})$	$\nu(\text{N}=\text{C})^{\text{a}}$	$\nu(\text{N}-\text{H})$	$\nu(\text{NH}_2)$	$\nu(\text{N}=\text{C})^{\text{b}}$	M-O	M-N
Cip	3520/ -	1710/1694 1608/1529 ^c	-	3444/3406	-	-	-	-
Met	-	-	1647/1643	3430/3520	3423/3569	-	-	-
1,10-phen	3437	-	-	-	-	1587/1549	-	-
60	-	-	1626/1680	3394/3510	3316/3529	1523/1503	-	544/492, 430/416
61	-	1640/1561	-	3419/3442	-	1615/1579	509/452	422/423
62	-	-	1672/1648	3394/3536	3322/3595	1523/1575	-	536/463, 426/416

a- Metformin N=C bond, b- 1,10-phenanthroline N=C bond, c- quinolone C=O

The stretching vibrational bands of 1,10-phenanthroline monohydrate ligand for $\nu\text{C}=\text{C}$ (1622 cm^{-1}) and $\nu\text{C}=\text{C}$ (1586 cm^{-1}) functional groups are shifted to 1615 cm^{-1} (w) and 1515 cm^{-1} (w) with decrease in intensity, respectively. These indicates that the C=C and C=N bond order decreased the ligand coordination to the Cu(II) metal center. On the other hand, the decrease in their intensity indicates the formation of rigid and symmetric structure to the Cu(II) metal center (Abebe et al.,

2020). More importantly, the appearance of bands (experimental/calculated) at 509/452 cm^{-1} and 422/423 cm^{-1} could be associated with the formation of Cu-O and Cu-N bonds, respectively.

4.7.2 UV-Vis/TD-DFT analysis of the complexes (60 – 62)

The absorption spectra of the ligands and the synthesized metal complexes (1.0×10^{-5} M solution in DMSO) were measured using UV-Vis spectrophotometer. The results obtained from UV-Vis spectrophotometer and TD-DFT calculations are presented in Figure 45 and Appendix 21 – 22. The absorption spectra of metformin was observed at 234 nm (SI) and in complex **60** this band has either shifted to 226 nm or appeared as overlapped peaks together with 1,10-phenanthroline absorption peaks as shown in the HOMO-LUMO electron distribution (Appendix 28).

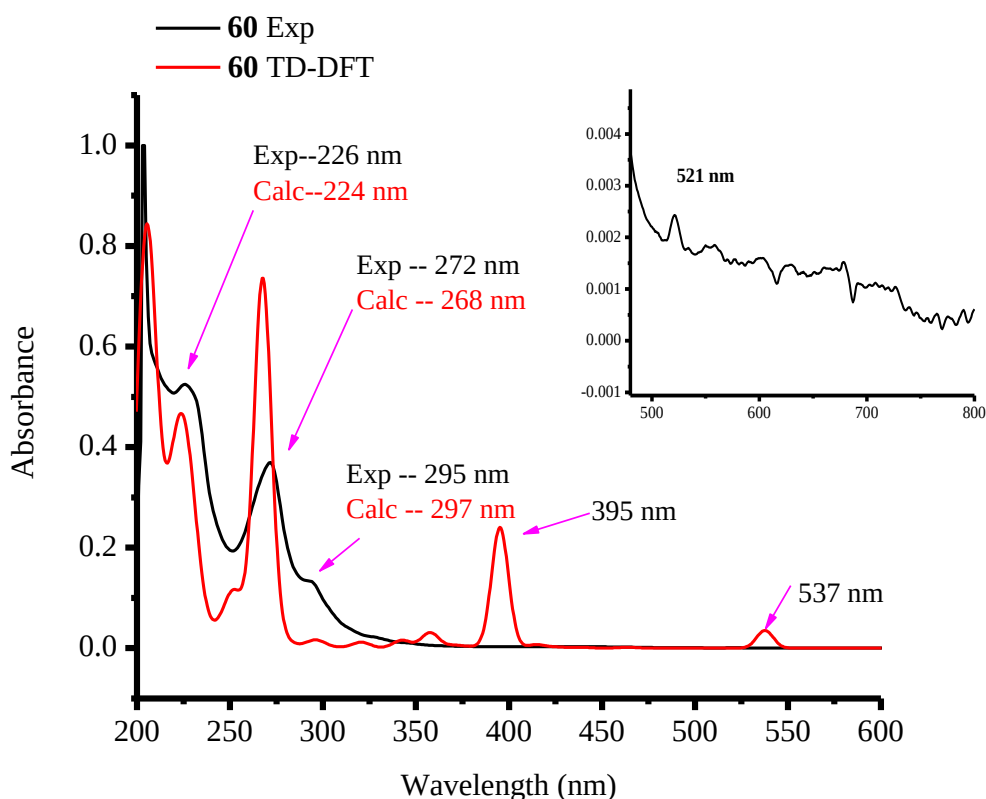


Figure 45. UV-Vis spectra of the synthesized mixed ligand metal Cu(II) complex (**60**)

In the UV-Vis spectra of 1,10-Phenanthroline free ligand (experimental/TD-DFT) the absorption peaks at 228/226 nm and 263/258 nm corresponds with $n \rightarrow \pi^*$ (C=N) and $\pi \rightarrow \pi^*$ (C=C) transitions, respectively (Tamiru et al., 2019). The peak at 263/258 nm is red shifted to 272/268 nm for the complex **60**. A new peak (experimental/TD-DFT) appeared at 295/297 nm would be associated

with the $\pi \rightarrow \pi^*$ intraligand transitions between 1,10-phenanthroline and metformin electrons, confirming the formation of the required metal nitrogen (M-N) bond. A peak that confirms the presence of charge transfer was appeared at 395 nm in the TD-DFT calculation. The presence of a less intense peak that appeared at 521 nm (Figure 45, shown in inset) was further confirmed by TD-DFT at 537 nm, confirming the presence of one $d \rightarrow d$ electronic transition.

In complex **61**, three absorption bands (experimental/TD-DFT) were observed in the UV region of the spectra at 224/225 ($n \rightarrow \pi^*$) and 274/271 and 326/312 nm ($\pi \rightarrow \pi^*$) transition of the ligand's base peaks, whereas a new peak at 348/351 nm appeared upon coordination to the metal center which could be associated to $\pi \rightarrow \pi^*$ electronic transitions due to the formation of metal-oxygen/nitrogen bond (Figure 46). A broad and less intense band observed in the experimental/TD-DFT results that appeared at 645/645 nm could be due to $d \rightarrow d$ ($d_{z^2} \rightarrow d_{x^2-y^2}$) (${}^2B_{1g} \rightarrow {}^2A_{1g}$) electronic transitions, confirming the formation of a square pyramidal coordination compound (Hazra, Dolai, Pandey, Dey, & Patra, 2014).

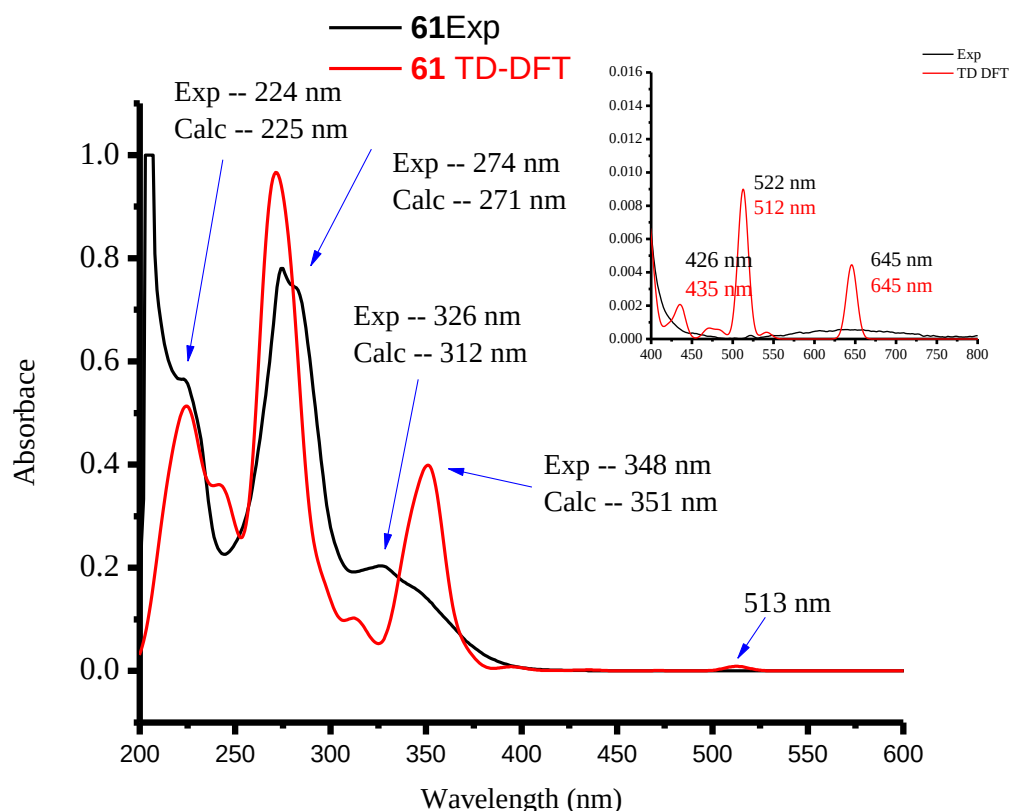


Figure 46. UV-Vis spectra of mixed ligand metal complex (**61**)

Similarly, absorption peaks (experimental/TD-DFT) at 230/225 nm and 266/265 nm were observed in the UV-Vis /TD-DFT spectra of complex **62** (Figure 47). It has been also observed that the absorption peaks for 1,10-phenanthroline and metformin overlapped to 230/225 nm. Whereas, the $\pi \rightarrow \pi^*(C=C)$ electronic transition looks unaffected upon coordination to Zn(II) metal center. A new peak that appeared at 293/297 nm would be due $\pi \rightarrow \pi^*$ intra-ligand transition resulted to the formation of metal-nitrogen bond. The absence of a peak above 400 nm was observed both in the experimental and TD-DFT calculation, confirming the absence of $d \rightarrow d$ electronic transitions (Figure 47, in set).

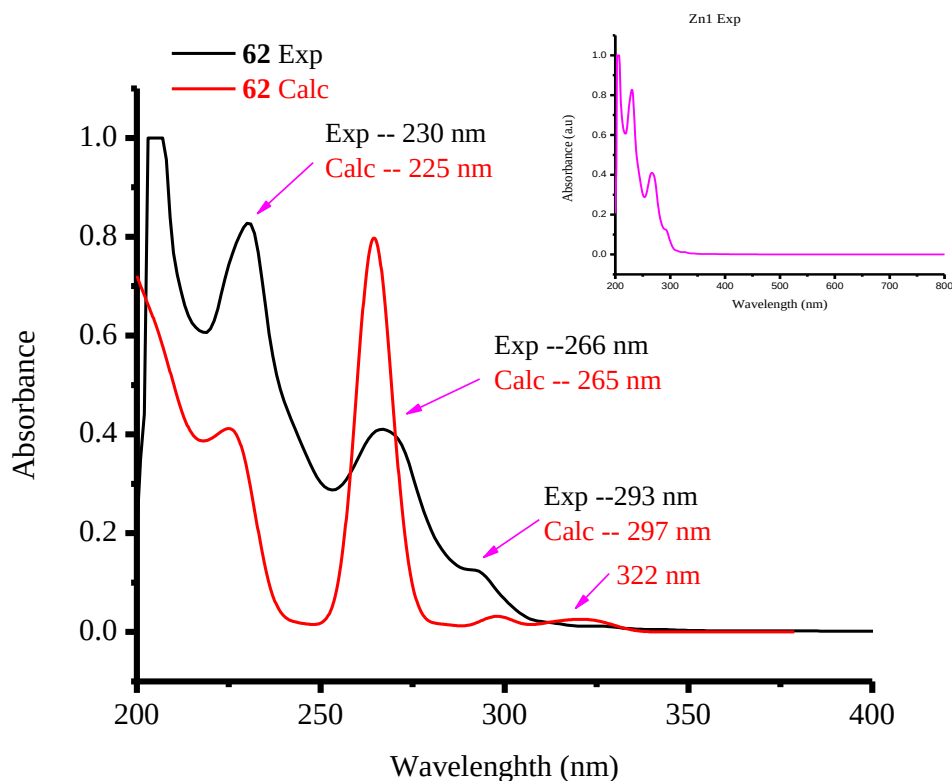


Figure 47. UV-Vis spectra of mixed ligand metal Zn(II) complex (**62**)

4.7.3 Mass Spectrometric Analysis

The mass spectra of the synthesized metal complexes (**60 – 62**) are presented in Figure 48 and Appendix 23. The mass spectrum of complex **60** shows a molecular ion peak at m/z 458.034 (calc = 459.130) corresponding to $[C_{16}H_{26}CuN_7O_5]^+$ (M.Wt of 459.970), a peak at m/z 423.064 (423.110) attributed to $[C_{16}H_{22}CuN_7O_3]$ fragment, a m/z value of 287.993 (287.040) for $[C_{13}H_{12}CuN_4]$ fragment and base peaks at m/z values of 181.073 (calc = 180.210) and 130.103 (calc = 129.160) corresponding to the constituent ligands, $(C_{12}H_8N_2)$ and $(C_4H_{11}N_5)$ fragments,

respectively. The mass spectrum of **61** shows a molecular ion peak at m/z 612.962 (calc = 609.540) corresponding to $[\text{C}_{29}\text{H}_{19}\text{Cl}_2\text{CuFN}_5\text{O}_3]^+$ (M.Wt of 610.120), a peak at m/z 573.124 (calc = 573.120), could be attributed to the two ligands, ciprofloxacin and 1,10-phenanthroline, and the metal ion $[\text{C}_{29}\text{H}_{25}\text{CuFN}_5\text{O}_3]^+$, inferring the proper coordination of the ligands to the Cu(II)-metal center.

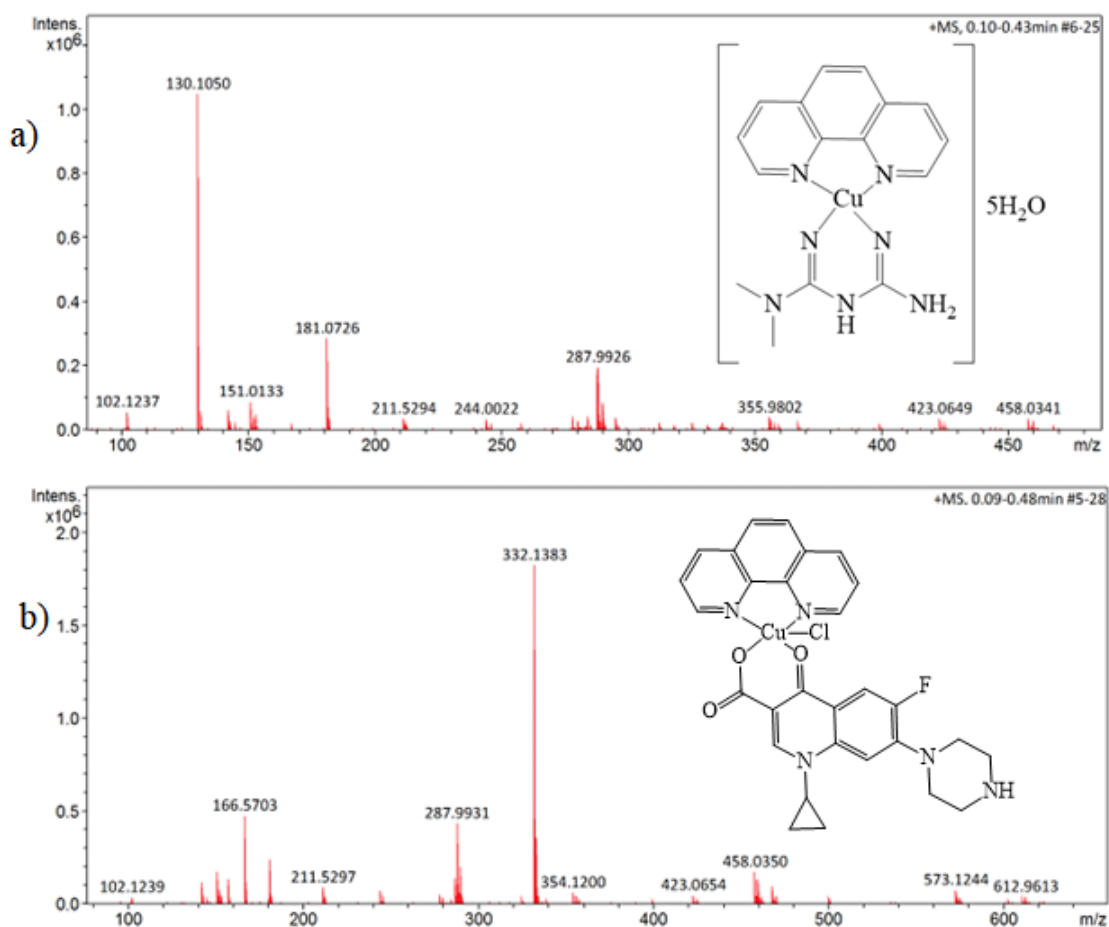


Figure 48. Mass spectrometric analysis of complexes **60** (a) and **61** (b)

Similarly, in complex **62** a peak observed at m/z = 537.052 (calc = 537.10) can be attributed to the complex $[\text{C}_{16}\text{H}_{29}\text{Cl}_2\text{N}_7\text{O}_5\text{Zn}]$ (M.Wt = 537.76) and peaks at m/z = 469.064 (469.090) and 446.041 (445.030), respectively corresponds to $[\text{C}_{16}\text{H}_{27}\text{Cl}_2\text{N}_7\text{Zn}]$ and $[\text{C}_{16}\text{H}_{27}\text{ClN}_7\text{Zn}]$ molecular fragments. Moreover, the presence of peaks that are m/z plus four ($m/z+4$) confirmed the presence of the two chlorine isotopes. Peaks that appeared at m/z = 130.1053 and 181.0730 attributed to the mass of the ligands, metformin and 1,10-phenanthroline, respectively. The mass spectra of the reported complex are presented in Appendix 23.

4.7.4 Thermogravimetric analysis of mixed drug complexes (60 – 62)

The TGA and DTG thermographs assignments of the synthesized complexes are presented in Figure 49 and Table 21. The TGA analysis of complex **60** was recorded between 25 and 800 °C, and it was found to thermally decompose in four main degradation steps and the thermograph showed that: i) from 100 – 200 °C ($DTA_{max} = 180$ °C), the weight loss was 11.68%, corresponding to the removal of three lattice water molecules, which is in accordance with the calculated value (11.74%).

Table 15. Temperature range values for decomposition and corresponding weight loss values

Cpds	Decomposition Temp. (°C)	Mass loss (%)		Interpretation
		Obsd.	Calcd.	
60 (Cu(II))	100 – 200	11.68	11.74	loss due to three lattice water molecules
	230 – 340	32.34	32.41	lost its two coordinated water molecules and metformin moiety ($2H_2O + C_4H_9N_4$) groups
	345– 570	11.44	11.52	release of the C_3H_3N organic moiety
	580 – 750	30.42	30.62	release of the $C_9H_5N_2$ organic moiety
61 (Cu(II))	90 – 225	13.94	13.96	the release of $C_4H_9N_2$ molecules
	230 – 310	8.81	8.93	lost its two chloride and fluoride molecules moiety (Cl + F)
	320 – 550	25.22	25.30	release of the $C_{11}H_8N$ organic moiety
	560 – 700	20.81	20.84	release of the C_9H_5N organic moiety
62 (Zn(II))	120 – 245	16.72	16.80	release of five lattice water molecules
	260 – 400	54.04	54.13	lost its two-chloride ion, metformin and phenanthroline moiety (C_6H_4N , 2Cl and $C_4H_9N_5$) groups
	415 – 625	16.77	16.79	release of the C_6H_4 organic moiety

ii) From 230 – 340 °C (DTG max = 300 °C), the complex lost its two coordinated water molecules and metformin moiety ($2\text{H}_2\text{O} + \text{C}_4\text{H}_9\text{N}_4$) groups, with the total weight loss being 32.34% (calculated value: 32.41%), *iii*) from 345 – 570 °C, the weight loss is 11.44% (calculated value: 11.52%), corresponding to the decomposition of phenanthroline and release of the $\text{C}_3\text{H}_3\text{N}$ organic moiety and the final step showed weight loss 30.42% (calculated value: 30.62%) from temperature 580 – 750 °C corresponding to the decomposition of phenanthroline and release of the $\text{C}_9\text{H}_5\text{N}_2$ organic moiety, and the final residue is related to copper metal with 14.12% (calculated: 13.71%) (Naglah et al., 2021). The overall weight loss was found to be 85.88% (calculated: 86.29%) (Abd El-Halim et al., 2017; Naglah et al., 2021).

The TGA/DTA analysis of complex **61** (MF = $\text{C}_{29}\text{H}_{25}\text{ClCuFN}_5\text{O}_3$) showed that it thermally decomposed in four main degradation steps and the thermograph showed that: *i*) from 90 – 225 °C (DTA_{max} = 200 °C), the weight loss is 13.94%, corresponding to the release of $\text{C}_4\text{H}_9\text{N}_2$ fragment, which is in accordance with the calculated value (13.96%), *ii*) from 230 – 310 °C (DTG max = 270 °C), complex **61** lost its chloride and fluoride moiety (Cl + F) groups, with the total weight loss being 8.81% (calculated value: 8.93%), *iii*) from 320 – 550 °C, the weight loss was 25.22% (calculated value: 25.30%), corresponding to the decomposition of ciprofloxacin and release of the $\text{C}_{11}\text{H}_8\text{N}$ organic moiety and the final step, *iv*) showed weight loss 20.81% (calculated value: 20.84%) from temperature 560 – 700 °C corresponding to the decomposition of phenanthroline and release of the $\text{C}_9\text{H}_5\text{N}$ organic moiety. The step total weight loss is 68.78% (calculated: 69.03) with the final residue related to copper oxide and remaining ligand release ($\text{CuO} + \text{C}_2\text{H}_2\text{O} + \text{C}_3\text{H}_3\text{N}$) with 31.22% (calculated: 31.09%) (Abd El-Halim et al., 2017; Naglah et al., 2021).

Complex **62** (MF = $\text{C}_{16}\text{H}_{29}\text{Cl}_2\text{N}_7\text{O}_5\text{Zn}$) thermally decomposed into three main degradation steps (Table 20 and Appendix 38) and from thermograph it can be described as follows: *i*) From 120 – 245 °C (DTG_{max} = 200 °C), the weight loss is 16.72%, corresponding to the release of five lattice water molecules, which is in accordance with the calculated value (16.80%). *ii*) From 260 – 400 °C (DTG max = 370 °C), due to the loss of two chloride ions, metformin and phenanthroline moiety ($\text{C}_6\text{H}_4\text{N}$, 2Cl and $\text{C}_4\text{H}_9\text{N}_5$), with the total weight loss being 54.04% (calculated value: 54.13%), *iii*) From 415 – 625 °C, the weight loss is 16.77% (calculated value: 16.79%), corresponding to the decomposition of phenanthroline and release of the C_6H_4 organic moiety and

from the step total weight loss is 87.53% (calculated: 87.72%) with the final residue of zinc metal as the final thermal residue with 12.47% (calculated value:12.47%) (Naglah et al., 2021).

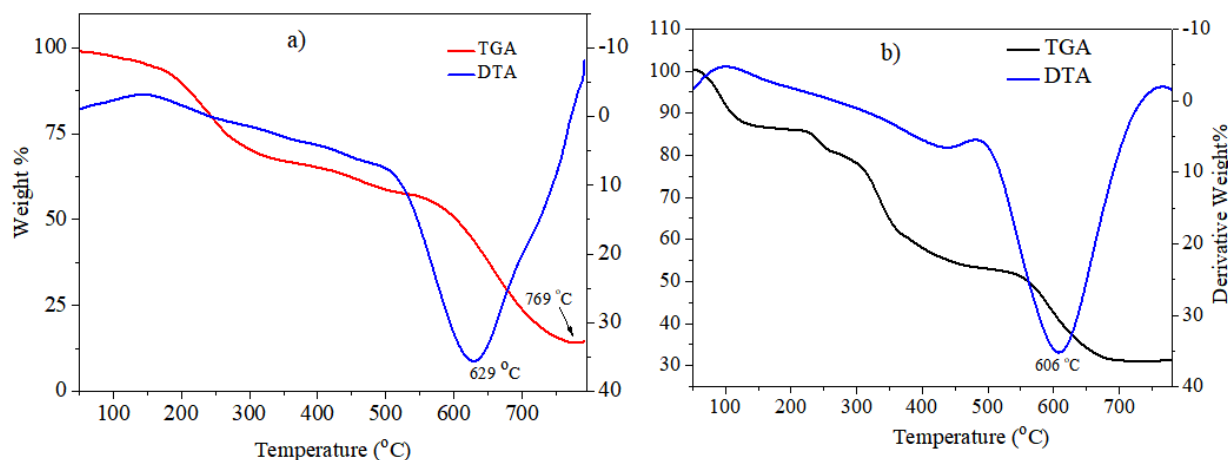


Figure 49. TGA (blue) and DTA (red) curves for complexes **60** (a) and **61**(b)

4.7.5 Powder X-ray Diffraction analysis of Cu(II) complexes (**60** - **61**)

The X-ray powder diffraction (XRD) was used for determining the structure of the synthesized mixed drug ligand complexes. The obtained XRD peaks are presented in Figure 63. The XRD patterns shows the synthesized complexes (**60** and **61**) found to be polycrystalline as confirmed by the observed intense peaks (Figure 50). Complex **60** was found to follow a monoclinic crystal system (COD = 00-721-1791) and C 1 2/c 1 space group with lattice parameters 24.189 Å, 12.933 Å, 25.434 Å, 90°, 117.000°, and 90°, respectively for a, b, c, α , β and γ . Whereas, complex **61** was found to be an orthorhombic crystal system (COD = 00-153-6726) with P b c n space group and lattice parameters 11.082 Å, 15.335 Å, 10.601 Å, 90°, 90° and 90°, respectively for a, b, c, α , β and γ . The average crystalline size of the complexes was found to be in the range of nanoparticle with calculated values of 35.760 and 33.320 nm, respectively for complexes **60** and **61**. The percent crystalline index was found to be 54.271 and 40.438 % crystallinity for **60** and **61**, respectively. Then the dislocation density (δ^*) of the complexes were calculated as 7.820×10^{-4} and $9.007 \times 10^{-4} \text{ nm}^{-2}$, respectively for **60** and **61**, confirming a crystalline material that have less dislocation density and less irregularity within the structure compared to chrysin based complexes (vide-supra).

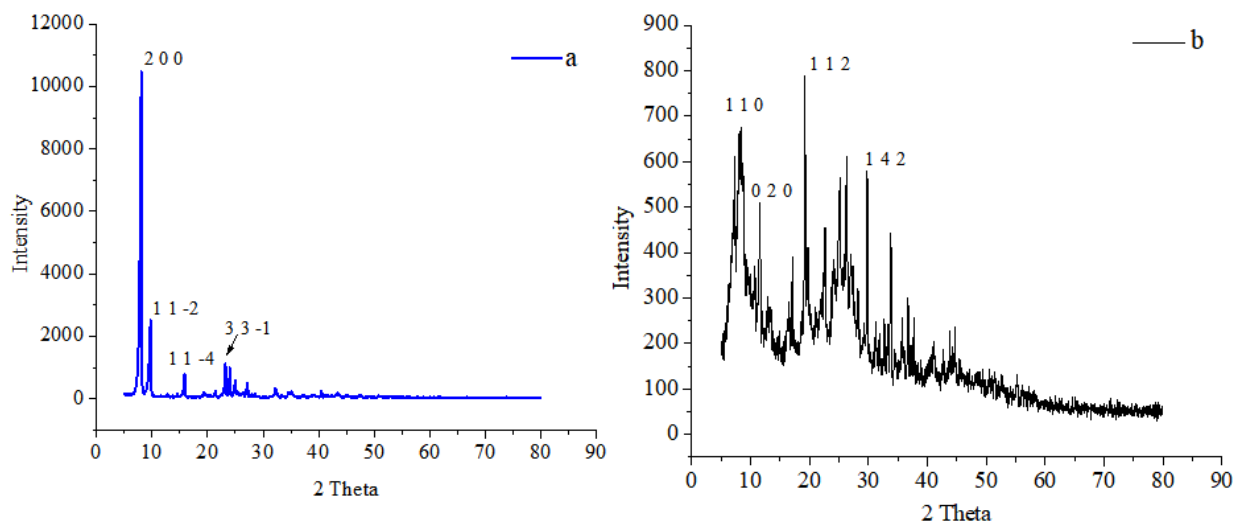


Figure 50. X-ray diffraction pattern of complex **60** (a) and **61** (b) in the powder form

4.8 Biological activity of the synthesized complexes

4.8.1 Biological activity of chrysin and 1,10-phenanthroline based Cr(III), Cu(II) and Zn(II) complexes (54 – 56)

4.8.1.1 Cytotoxicity activity of complexes (54 - 56)

The cytotoxic activity of chrysin based complexes that employ 1,10-phenanthroline co-ligand (**54** - **56**) against the MCF-7 cells, as given by their percent cell viability, is presented in Figure 51. It was notable that, upon 24 h of incubation, relevant cytotoxicity activity has been observed for all complexes. It was clearly observed that the cytotoxicity of the synthesized complexes and the positive control strongly depend on concentration (Figure 51). At 3.125 μ M concentration of complex **55**, the cell viability reduced to 67.36 % compared to complex **54** (93.32 %), **56** (95.41 %) and cisplatin (89.17 %) demonstrating high cytotoxicity of the complex to the MCF-7 cells at lowest concentration. Complex **56** showed comparable cytotoxicity to the standard cis-platin (Figure 51). At highest concentration (100 μ M) complex **56** and cis-platin reduced the cell viability to 34.30 and 32.17 %, respectively. It has been reported that the lower percentage cell viability has associated to higher cytotoxic potential of the test compound.

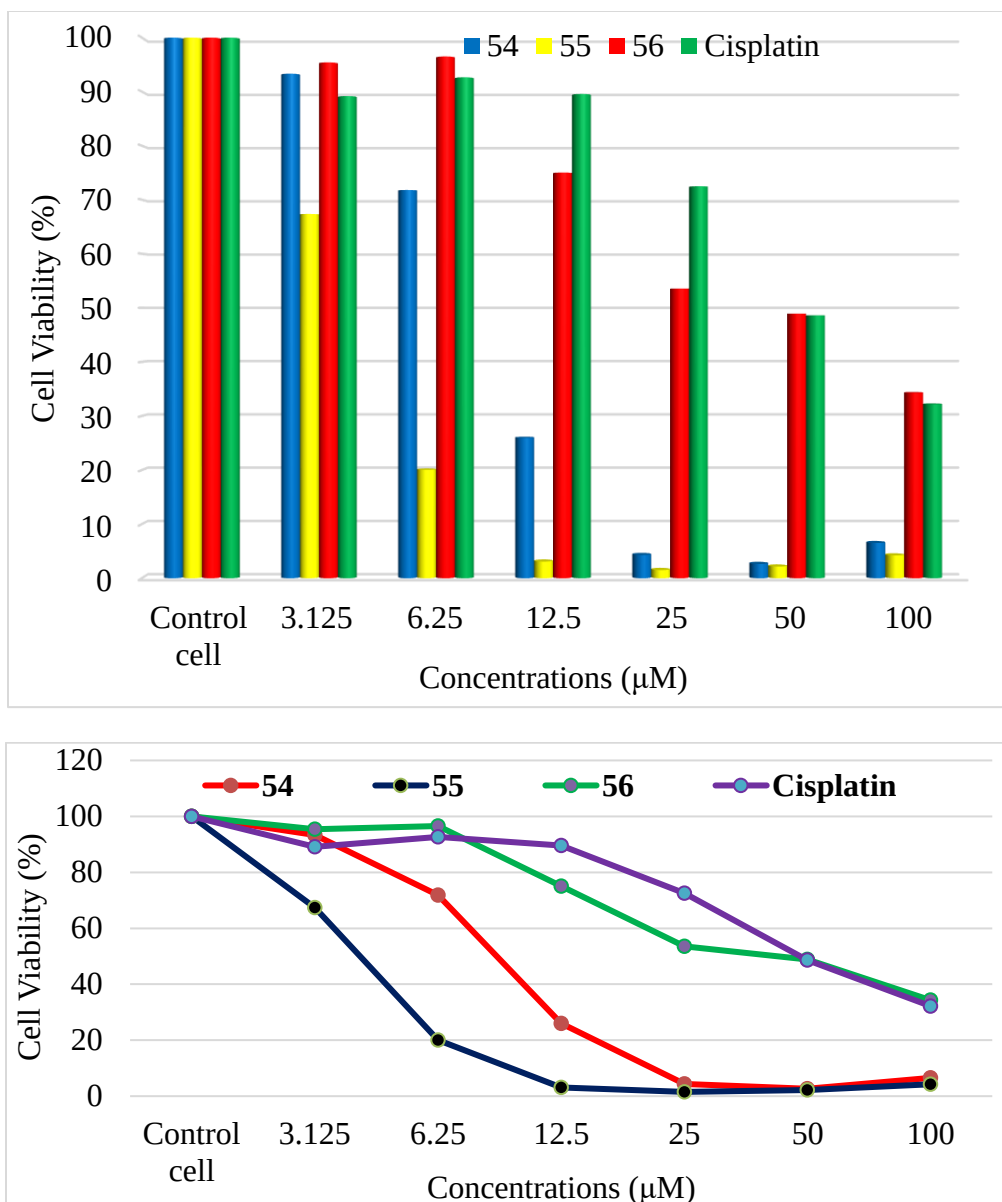


Figure 51. Cytotoxicity of chrysin based 1,10-phenanthroline complexes (**54 - 56**) and the positive control (Cisplatin) against MCF-7 cell lines

Hence, a sample is considered cytotoxic if the percentage of viability is less than 70% (Cannella et al., 2019; Ramasamy et al., 2022; Roth, Coemert, Burkhardt, Rodewald, & Lueth, 2020). Hence, all the reported compounds (**54 - 56**) were found to be cytotoxic at 25 μM at which the percent cell viability of MCF-7 cell lines were decreased to 4.31, 1.52 and 53.55 %, respectively for Cr(III) **54**, Cu(II) (**55**) and Zn(II) (**56**) compared to cisplatin (%cell viability at 25 μM = 72.49 %). Whereas, Cr(III) (**54**) and Cu(II) (**55**) complexes were found to be cytotoxic, respectively at 12.5 (%cell viability = 25.96%) and 3.125 (% cell viability = 67.36%) μM, inferring diverse

cytotoxicity of the synthesized metal complexes. In this work, we reported cytotoxic Cr(III)(**54**), Cu(II)(**55**) and Zn(II)(**56**) complexes comprising 1,10-phenanthroline and chrysin which demonstrated IC₅₀ values 8.08, 3.72 and 44.88 μ M, respectively, compared to the positive control cisplatin (IC₅₀ = 18.62 μ M). Moreover, a similar cytotoxic Cu(II) complex (**60**) consisting of 1,10-phenanthroline and metformin that shows cytotoxicity at 12.5 μ M (% cell viability = 15.19 %) with IC₅₀ of 4.29 μ M has been reported (*vide-infra*). The obtained results confirmed that Cu(II) complexes of 1,10-phenanthroline are promising potentials against MCF-7 cell lines which is in line with the previously reported works (Bravo-Gómez et al., 2009; Nunes et al., 2020). This infers the potency of Cu(II) complexes of 1,10-phenanthroline as feature cytotoxic agents. Regarding the cytotoxicity of Cu(II) complexes of 1,10-phenanthroline, different mechanisms of actions have been proposed: oxidative damage to DNA, depletion of reduced glutathione, and/or cell death by apoptotic and non-apoptotic dose-dependent DNA binding mechanism (Andiappan et al., 2018). Hence, the observed cytotoxicity would be due one or more of the above proposed mechanisms.

Cellular morphology of the microscopic images of the MCF-7 cell lines (untreated) and treated with 50 μ M of Cr(III) (**54**), Cu(II) (**55**) and Zn(II) (**56**), and cisplatin for 24 h are provided in Figure 52. The MCF-7 cells are characterized by their epithelial-like cells with irregular and polygonal structures (Andiappan et al., 2018; Razak et al., 2019). The characteristic epithelial cells with irregular and polygonal shapes are obviously observed in the untreated MCF-7 cells (Figure 52., a). However, after treatment with 50 μ M of the synthesized complexes and cisplatin for 24 h, the morphology and density of the cells was changed and decreased. This phenomenon was highly observed for complex **55** followed by **54** as observed in the IC₅₀ value (*vide-supra*). Generally, the obtained microscopic and percent cell viability results demonstrated that the reported metal complexes are cytotoxic which is in agreement with literature report for other metal complexes (Alem et al., 2022; Andiappan et al., 2018; Nunes et al., 2020; Razak et al., 2019).

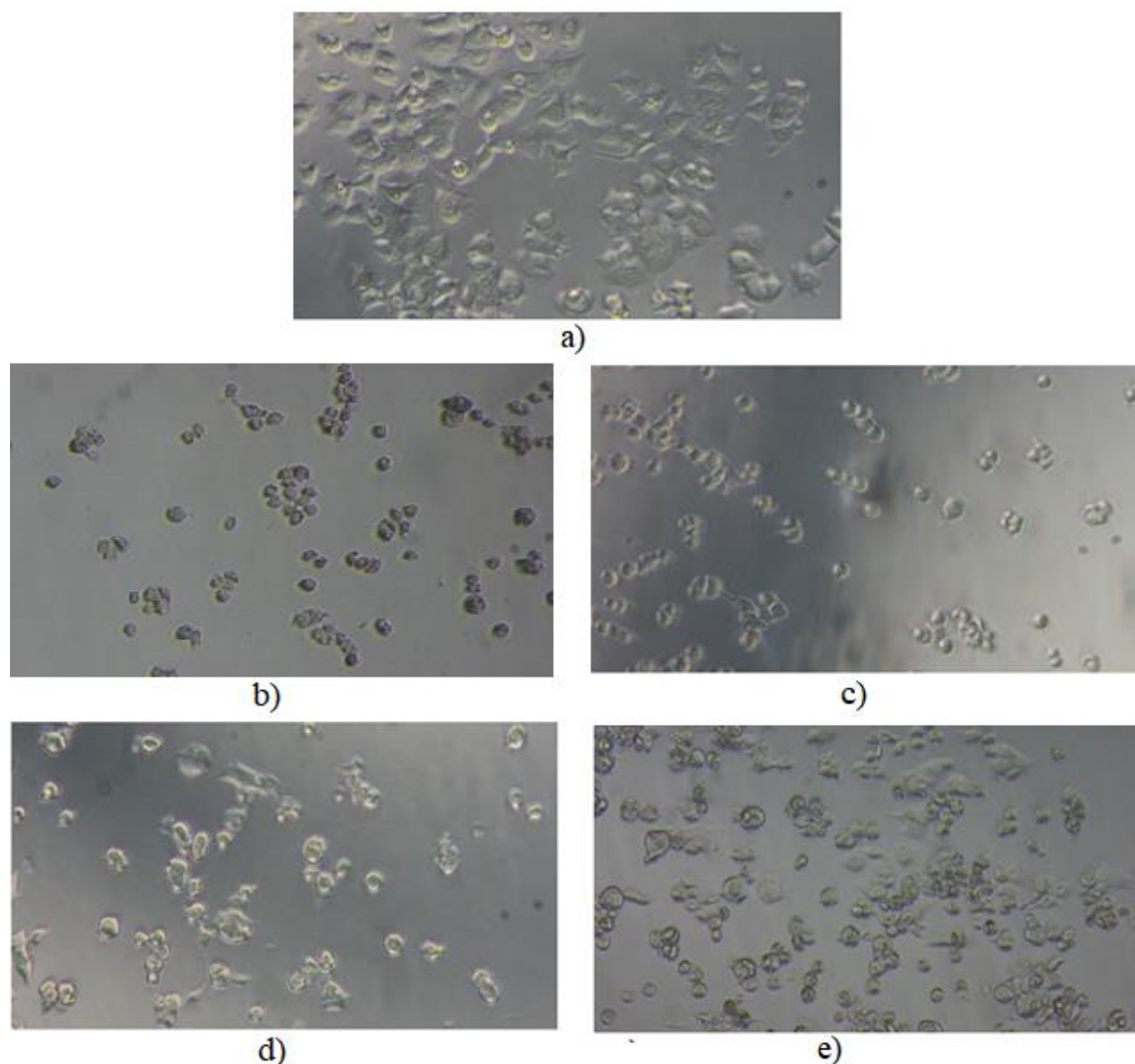


Figure 52. Photomicrographs of the cellular morphology of untreated MCF-7 (a) and the changes induced by complex **54** (b), **55** (c) **56** (d) and cisplatin (e) at 50 μ M

4.8.1.2 Antibacterial activity of chrysin and 1,10-phenanthroline complexes

The antibacterial activity of as-synthesized mixed-ligand metal complexes (**54** – **56**) were tested against four bacterial strains (Gram-negative: *E. coli* and *P. aeruginosa*, and Gram-positive: *S. aureus* and *S. pyogenes*). The complexes were found to be active against both types of bacterial strains. The obtained results are presented in Figure 53, Figure 54 and Appendix 25. All the synthesized complexes showed preference to a gram-positive bacterial strain, *S. aureus* (Figure 37). Among the mixed-ligand complexes, a Cu(II) complex, **55** showed strong antibacterial activity

at both 500 and 1000 μM concentrations against all bacterial strains, followed by Zn(II) (**56**) and Cr(III) (**54**). Detailed antibacterial activity results demonstrated that complex **54** and **55** showed better activity pattern to Gram-positive bacterial strains, *S. aureus* and *S. pyogenes* at both concentrations.

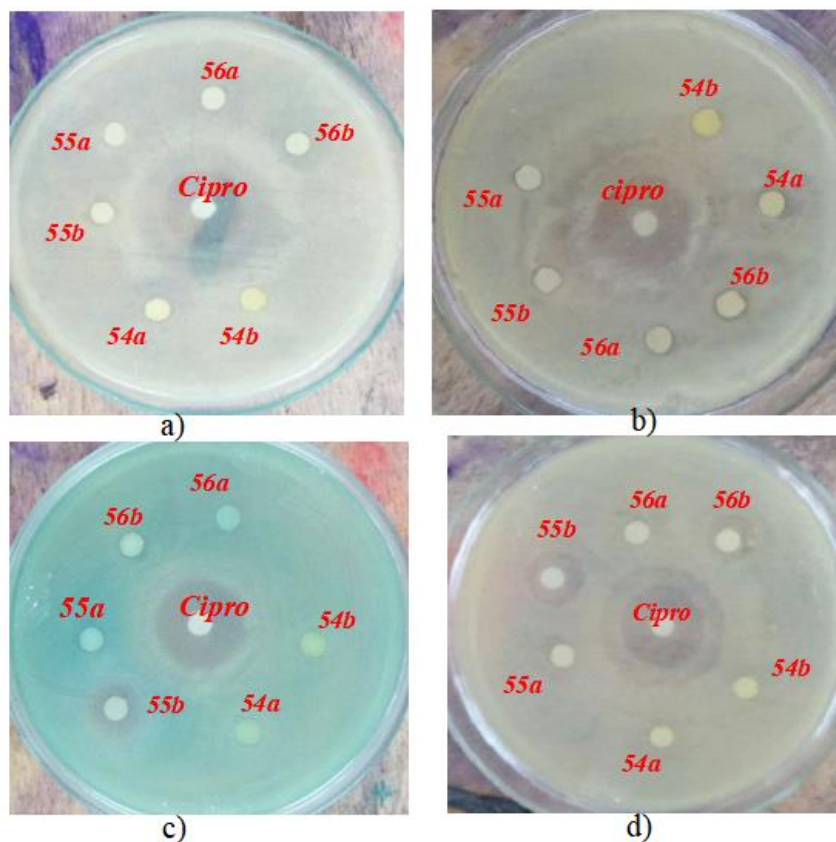


Figure 53. Antibacterial activity of 1,10-phenanthroline and chrysin mixed ligand complexes against *E. coli*(a), *S. aureus* (b), *P. aeruginosa* (c) and *S. pyogenes* (d)

The minimum inhibition zones (MIZ) values were 8.500 ± 0.408 mm and 9.167 ± 0.289 mm for Cr(III) complex (**54**), 11.000 ± 0.236 mm and 11.333 ± 0.577 mm for Cu(II) (**55**) respectively against *S. pyogenes* and *S. aureus* bacterial strains at 500 μM . The Cu(II) centered complex (**55**) showed promising activity against *S. pyogenes* and *P. aeruginosa* bacterial strains. On the other hand, complex **56** found to show preferences against *E. coli* and *S. pyogenes*, and *E. coli* and *S. aureus*, respectively at 500 and 1000 μM concentrations. A relatively small inhibition zone difference between a Cr(III) complex (**54**) was observed at both concentrations. The results showed that the MIZ values of **54** at 500 and 1000 μM are found to be 8.333 and 10.167 mm, 8.333 and 9.500 mm, 8.500 and 10.333 mm, and 9.167 mm and 10.333 mm respectively against

E. coli, *P. aeruginosa*, *S. pyogenes* and *S. aureus*. The observed minimum MIZ values for Cr(III) complex might be due to its structural difference from Cu(II) and Zn(II) analogs in which chrysin is covalently bonded.

In addition to the MIZ values, percent activity index of the synthesized metal complexes were calculated at 500 μM by comparing with the standard ciprofloxacin (Damena, Alem, et al., 2022a, 2022b). It has been found that the percent activity index (%AI) of the synthesized complexes against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa* found to be 33, 39, 33 and 36%; 45, 49, 42 and 46%; 38, 40, 40 and 38% respectively for Cr(III)(**54**), Cu(II)(**55**) and Zn(II)(**56**). The %AI data showed a structural activity relationship between the synthesized complexes as a result of change in the metal center of the complexes. In this case, the relatively small %AI shown by Cr(III) complexes (**54**) might be due to the different chemical environment in the coordination sphere.

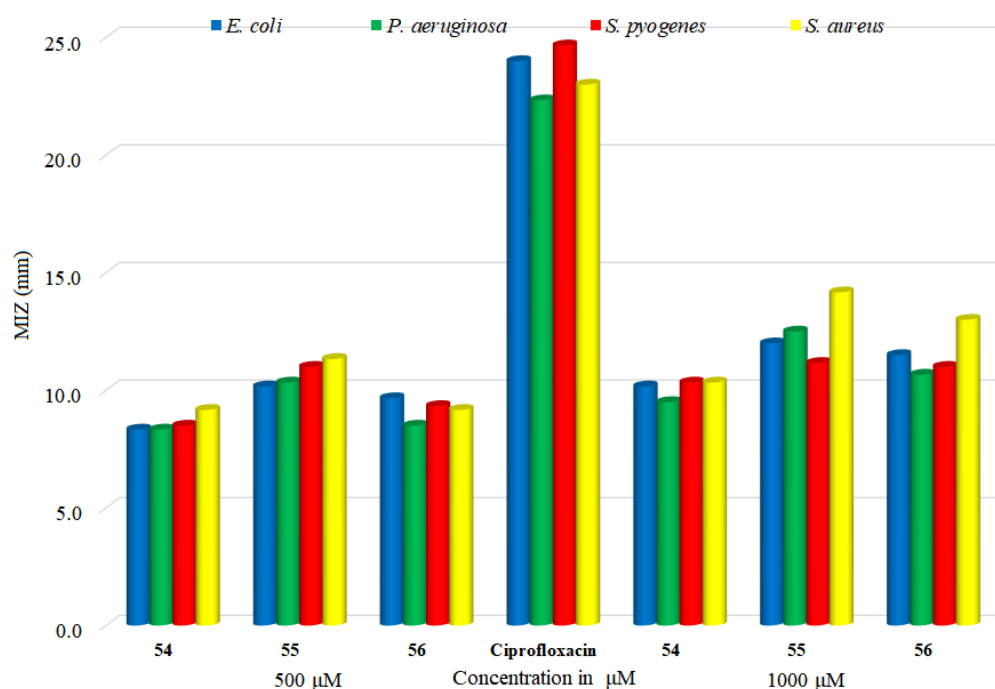


Figure 54. Minimum inhibition zone for the antibacterial activity of 1,10-phenanthroline and chrysin mixed ligand complexes of Cr(III), Cu(II) and Zn(II) (**54 – 56**)

Statistically, the univariate analysis of variance and Post Hoc Tests of the synthesized compounds are presented in Appendix 44 – 45. The univariate analysis of variance for a significance deference test ($p < 0.05$) showed that parameters concentration, type of compound and metal centers on the bacterial type are found to affect the zone of inhibition significantly. Except the Zn(II) (**56**) and Cr(III) (**57**) centered

compounds, significant difference in the minimum zone of inhibition of **54** (Cr(III)) with all compounds were observed (Appendix 45). Compound **55** (Cu(II)) showed insignificant difference to that **59** (Zn(II)) ($p = 1.00$).

4.8.1.3 Antioxidant activity

The antioxidant activities of chrysin and its heteroleptic complexes (**54**, **55** and **56**) were compared with the standard antioxidant, ascorbic acid and the obtained results are presented in Figure 55 and Appendix 26. Both the absorbance vs concentration and percent free radical scavenging results demonstrated that the designed heteroleptic complexes showed higher antioxidant activities than the free ligand chrysin, assuring our design and proposed complexation to enhance biological activity of the selected ligands agrees to the obtained results. The free radical scavenging activity can be ordered as: AA > Zn(II) (**56**) > Cu(II) (**55**) > Cr(III) (**54**) > chrysin. The obtained results indicated enhanced antioxidant activity of the metal complexes than the free ligand is in line with the previously reported works (Gençkal, 2020; Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021; Panhwar & Memon, 2014a).

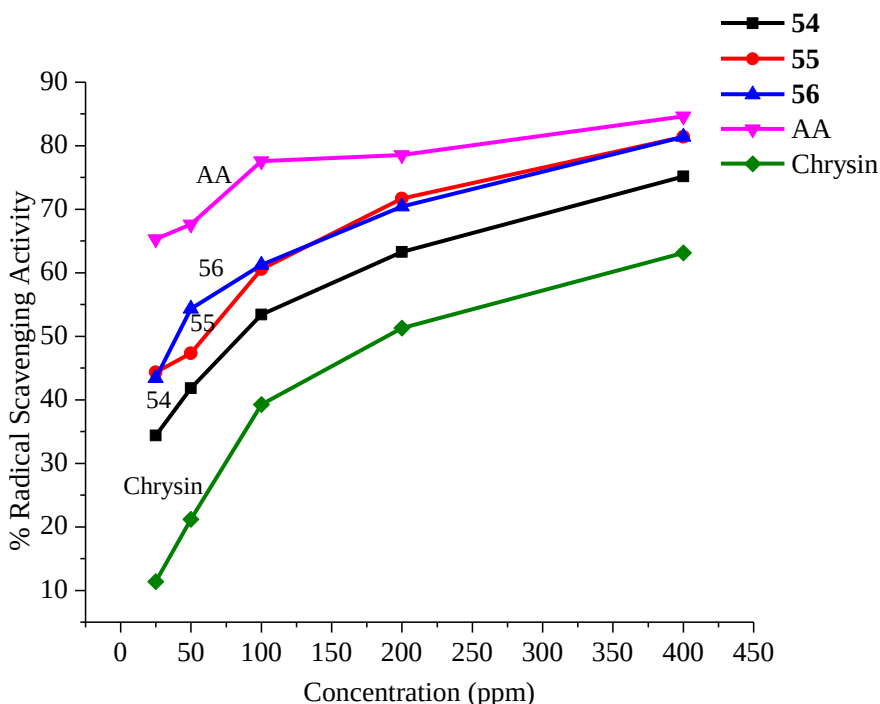


Figure 55. Antioxidant activity of the synthesized metal complexes (**54** -**56**) against DPPH radical

4.8.1.4 Quantum chemical descriptors analysis of the complexes

The quantum chemical descriptors analysis of the free ligands and the corresponding metal complexes are presented in Figure 56 and Appendix 27. It is a well-established fact that the band gap energy (E_g) has a strong correlation with various biological activities: antibacterial, antioxidant and DNA binding activities (Alem et al., 2022; Bitew et al., 2021; Damena, Alem, et al., 2022a; Lemilemu et al., 2021). Moreover, band gap energy has been used as an important stability descriptor. A compound with large band gap energy is suggested to be stable and hard, whereas that of small band gap energy becomes less stable, giving the compound softness and enhanced biological importance (Ismael et al., 2020). In this work, the band gap energy of the ligands and the synthesized metal complexes were found to be 4.755, 4.403, 2.609, 2.784 and 3.333 eV for 1,10-phenanthroline, chrysin, **54**, **55** and **56**, respectively. The band gap energies of the metal complexes were found to be less than that of the ligands, inferring that the synthesized metal complexes are softer than the ligands (Figure 56). The observed decrease in the band gap energy of the metal complexes than the ligand could be due to the presence of ligand to metal charge transfer (LMCT).

According to the HSAB concept, soft acids prefer binding to soft bases to give covalent complexes, whereas hard acids prefer binding to the hard bases to give ionic complexes (Ismael et al., 2020; LoPachin, Gavin, DeCaprio, & Barber, 2012). The formation of a covalently bonded adduct through soft-soft molecular interaction and their preference to soft biological systems can weaken the functions of soft biological molecules: enzymes, DNA, cytoskeletal proteins, and other macromolecules, leading to malfunctioning cell processes and cytotoxicity (LoPachin et al., 2012). Hence, the biological activity of a compound increases with increasing softness and decreasing hardness [39, 71]. In this work, the order of chemical hardness (η) was found to be 1,10-phen > chrysin > **56** > **55** > **54**, suggesting that complex **56** is more stable than complex **55** and **54** counter parts. Moreover, a decrease in hardness is found following the metal-ligand coordination, inferring biological significance of the metal complexes than the free ligands.

Chemical potential (μ) measures the tendency of an electron to escape from equilibrium, directly proportional with the Gibbs free energy (LoPachin et al., 2012). Also, it represents the ability of an electrophilic or nucleophilic species to undergo chemical change (LoPachin & Gavin, 2014).

The chemical reactivity of a species increases with decrease in chemical potential, thus the chemical reactivity ranking of the synthesized complexes in this study could be well-ordered as follow: **55** > **56** > **54**, with difference 0.525 eV (between **55** and **56**), 1.211 eV (between **55** and **54**) and 0.686 eV (between **56** and **54**).

The other important parameters computed for the ligands and the synthesized metal complexes are electrophilicity and nucleophilicity indexes. The electrophilicity index is associated to the ability of electron accepting, whereas nucleophilicity index associates to electron donating ability of a compound (Ismael et al., 2020). A quantitative information about the transition state energies involved in toxicant-protein adduct formation can be obtained from electrophilicity calculation. Thus, values for electrophilicity index (ω) agree to the rate constant of the adduct formation and are directly related to toxicant strength (LoPachin & Gavin, 2014).

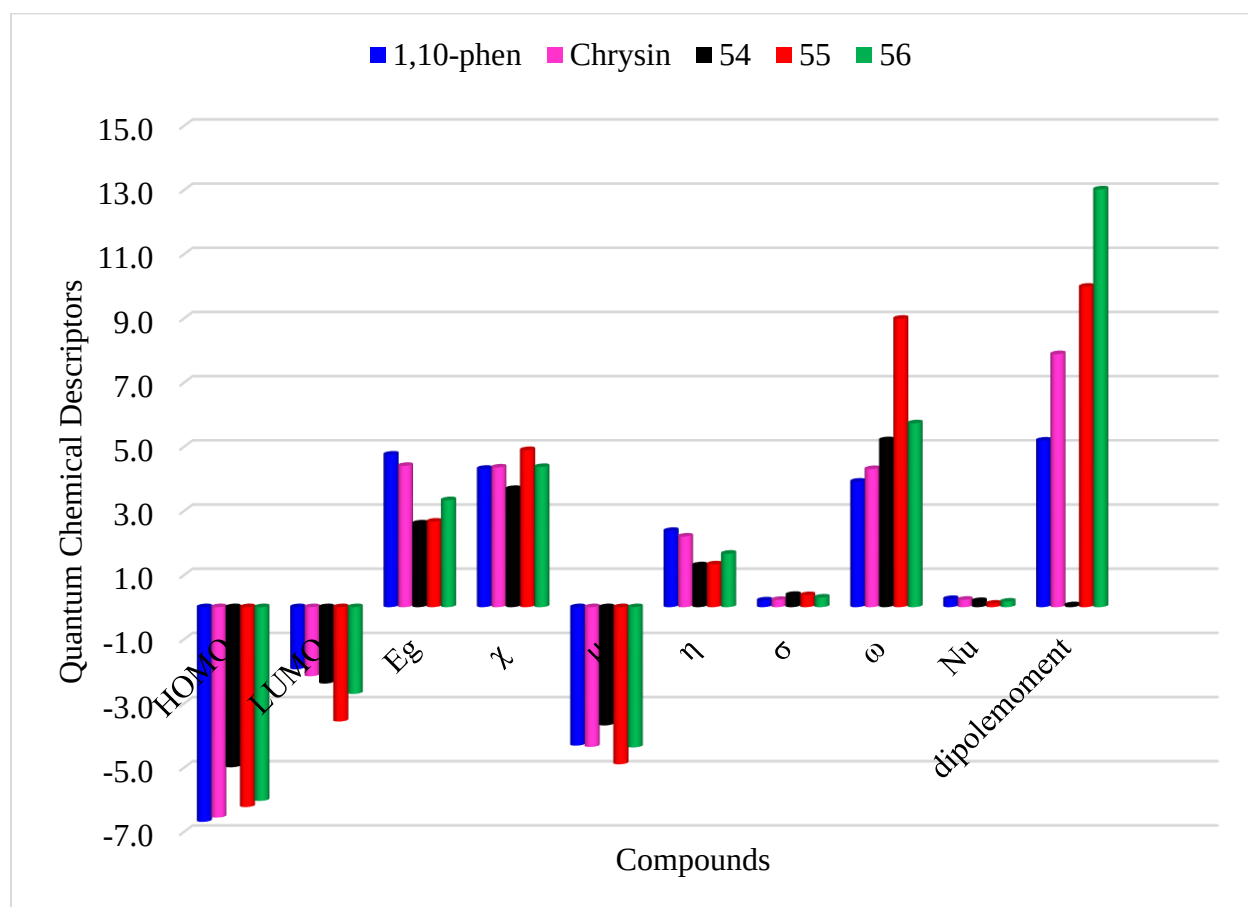


Figure 56. Graphical presentations of quantum chemical descriptors

The electrophilicity and nucleophilicity of the synthesized metal complexes are found to be 5.203 and 0.192 eV, 7.517 and 0.133 eV, and 5.729 and 0.175 eV, respectively for **54**, **55** and **56**, giving chemical reactivity rank as electrophile: **55** > **56** > **54** and vice-versa as nucleophile as reported in previous work (Ismael et al., 2020). From computed electrophile index results it can be inferred that **55** and **56** complexes could have better adduct formation. The dipole moment (in Debye) is a parameter related to the mobility of electrons. The higher the dipole moment of the compound, the higher the active electron for the compound and the higher the biological activity (Rahmouni, el Houda Bensiradj, Megatli, Djebbar, & Baitich, 2019). Except complex **54** which has different coordination chemistry, the two complexes (**55** and **56**) showed large enhancement in dipole moment relative to the ligands (Appendix 27).

4.8.1.5 Frontier molecular orbitals (FMOs) analysis (54 – 56)

The electronic features of molecular structures can be calculated from frontier molecular orbitals (FMOs): the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) (Ferchichi et al., 2022). The B3LYP calculated HOMO-LUMO distribution of the ligands and the corresponding metal complexes are presented in Figure 57 and Appendix 16. It was found that the spreading of isodensity shows different trend in the HOMO and LUMO of the three complexes. In complex **54**, the isodensity was concentrated mainly on the 1,10-phenanthroline rings in both FMOs (HOMO and LUMO) this could be due to the existence the two rigid rings in the sample plane and taking part intra-ligand charge transfer. In complex **55**, the isodensity was concentrated mainly on the 1,10-phenanthroline ring and the Cu-metal center, and the metal center and chrysin for HOMO and LUMO, respectively. This inferred that the presence of ligand to metal charge transfer and $d \rightarrow d$ electronic transition. Whereas, in complex **56** the isodensity shows concentration on the Zn-metal center and chrysin for HOMO and 1-10-phenanthroline for LUMO, inferring the possible intera-ligand and metal to ligand charge transfer.

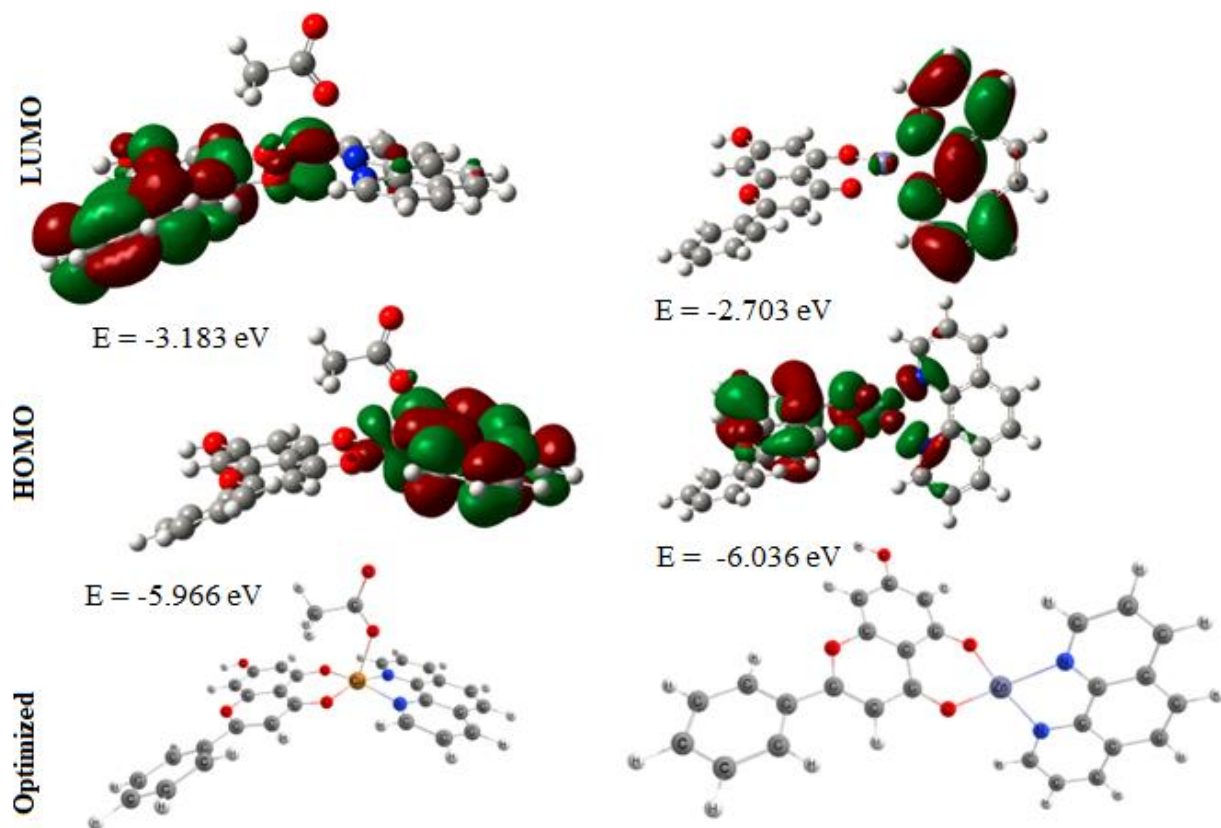


Figure 57. Optimized geometry, HOMO – LUMO and eigen values of **55** (left) and **56** (right) metal complexes

4.8.1.6 Molecular Docking study of the complexes (**54** -**56**) against estrogen receptor alpha

Estrogen receptor α (ER α) is one of the major clinical biomarkers used to subtype breast cancers. It plays an important role in the development and progression of dependent hormonal type breast cancer (Bhatt, Stender, Joshi, Wu, & Katzenellenbogen, 2016). The interaction of the synthesized complexes and the control cisplatin with estrogen receptor alpha (ER α ; PDB:5GS4) residual amino acids are well presented (Figure 58, Table 16 and appendix). The binding affinity and inhibition constant of the studied compounds were found to be -8.35 and 0.76, -7.52 and 3.07, -6.83 and 9.93 μ M, and -6.32 kcal/mol and 23.42 μ M, respectively, for **56**, **55**, **54** and Cis-platin, demonstrating the better binding affinity and the smaller amount of medication needed in order to inhibit the activity of that enzyme for the synthesized complexes compared to the standard cis-platin. Arg394, Glu 353, Leu 387 and Leu 391 are the most important active site amino acids of ER α that take part in hydrogen bonding and pi-alkyl interaction (Pang et al., 2018).

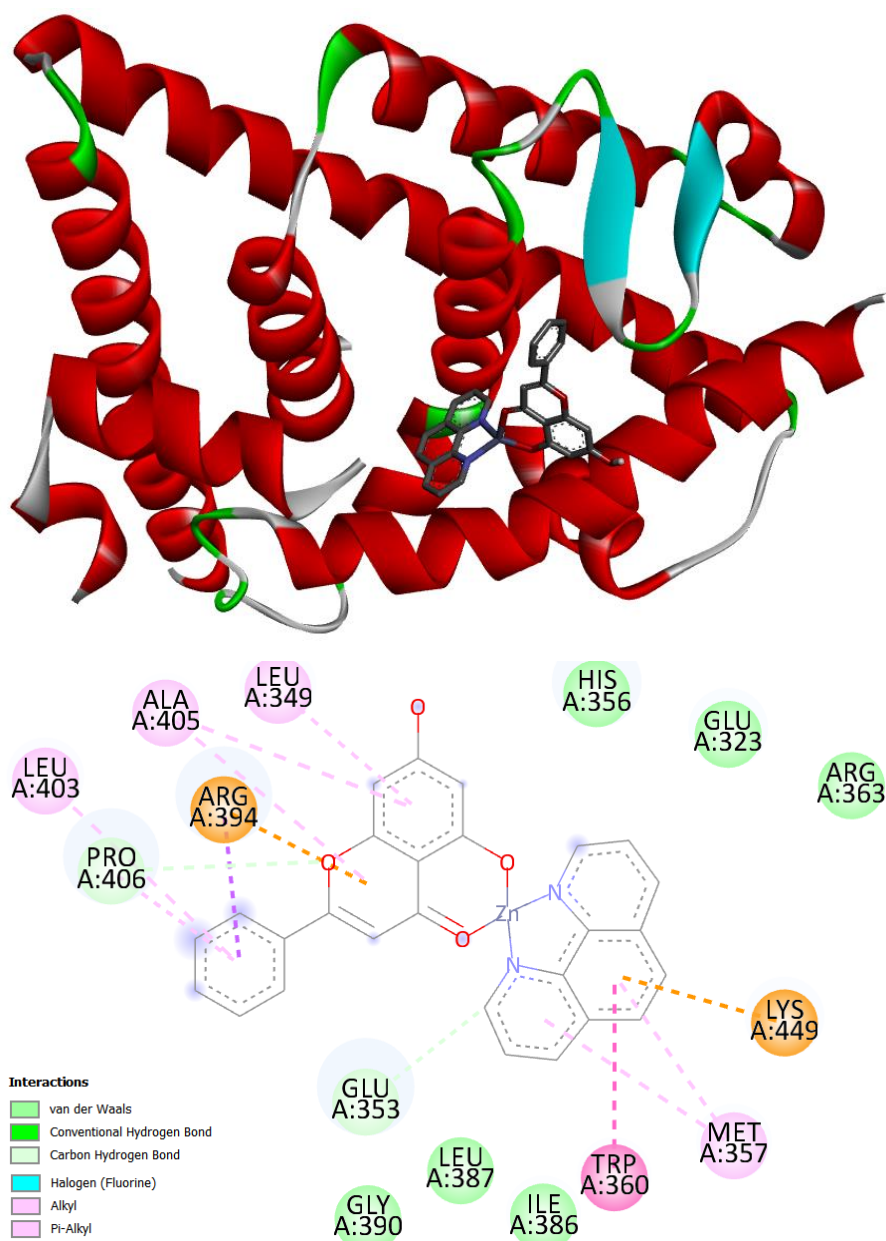


Figure 58. The binding interactions of **56** against estrogen receptor alpha (ER α ; PDB: 5GS4)

The molecular docking analysis results showed the involvement of twelve van der Waals and π -alkyl/ π -ion type of interactions, respectively for **54**: Phe 445, Gly 390, Ile 386, Leu 387, Trp 360, Asn 359 and Met 357, His 356, Arg 363, Lys 449, Arg 394, Glu 323; Thirteen for **55**: Met 357, His 356, Ile-386, Leu 387, Gly 390, Phe 445 and Met 357, His 356, Arg 363, Lys 449, Arg 394, Glu 323 and one hydrogen bonding with Trp 393; and fifteen interactions for complex **56**: Glu 387, Gly 390, Ile 386, His 356, Glu 323, Arg 363 and Arg 394, Lys 449, Pro 406, Glu 353, Leu 403, Ala 405, Leu 349, Trp 360, Met 357. Whereas, cis-platin showed a total of five interactions:

two hydrogen bonding (Ser468, Asp374), one van der Waals (Lys467) and two π -alkyl/ π -ion (Thr371, Glu471) (Figure 58 and Appendix 29 – 31). The obtained residual amino acids interactions showed that the number of interactions affects the binding affinity and K_i .

Table 16. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against estrogen receptor alpha (ER α ; PDB: 5GS4)

Cpds	RM SD	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding	van der Waals	π -alkyl/ π -ion
54 (Cr(III))	1.14	-6.83	9.93 μ M	-	Phe 445, Gly 390, Ile 386, Leu 387, Trp 360, Asn 359	Met 357, His 356, Arg 363, Lys 449, Arg 394, Glu 323
55 (Cu(II))	0.34	-7.52	3.07 μ M	Trp 393	Met 357, His 356, Ile-386, Leu 387, Gly 390, Phe 445	Met 357, His 356, Arg 363, Lys 449, Arg 394, Glu 323
56 (Zn(II))	0.31	-8.35	0.76 μ M	-	Glu 387, Gly 390, Ile 386, His 356, Glu 323, Arg 363	Arg 394, Lys 449, Pro 406, Glu 353, Leu 403, Ala 405, Leu 349, Trp 360, Met 357
Cisplatin	0.39	-6.32	23.42 μ M	Ser468, Asp374	Lys467f	Thr371, Glu471

4.8.1.7 Molecular docking against *S. aureus*, dihydrofolate reductase (DHFR)

The molecular docking analysis of the complexes were performed according to the results obtained from in-vitro antibacterial activity in which the complexes showed promising results against *S. aureus*. Complex 55 established a total of 17 interactions (Figure 59 and Table 17) two hydrogen bonding, Ile 14, Leu 20; eleven van der Walls, Asn 18, Thr 121, Gly 15, Phe 92, Leu 5, Phe 98, Ala 7, Val 6, Val 31, His 23, Trp 22; and four π -alkyl/ π -ion, Ile 50, Phe 92, Leu 20, Ser 49 with a binding affinity -7.51 kcal/mol and inhibition constant of 3.12 μ M. Complex 54 (Appendix 32)

showed small interaction: five van der Waals, His 23, Gln 19, Leu 54, Phe 92, Thr 46 and four π -alkyl/ π -ion, Ser 49, Leu 20, Ile 50, Leu 28 with -7.51 kcal/mol and 3.12 μ M, binding affinity and inhibition constant, respectively. In complex **56**, two hydrogen bonding, Leu 20, Gln 19; eight van der Waals, His 23, Asn 18, Thr 46, Gly 94, Ile 14, Gly 93, Lys 52, Ser 49; and five π -alkyl/ π -ion, Leu 20, Lys 45, Ser 49, Gln 19, Ile 50 interactions with a binding affinity of -10.75 kcal/mol and inhibition constant of 0.01 μ M (Table 17 and Appendix 33).

Table 17. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against *S. aureus* (PDB: 2w9h)

Cpds	RMS D	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding	van der Waals	π -alkyl/ π -ion
54 (Cr(III))	0.81	-7.51	3.12 μ M	-	His 23, Gln 19, Leu 54, Phe 92, Thr 46	Ser 49, Leu 20, Ile 50, Leu 28
55 (Cu(II))	0.35	-11.49	0.04 μ M	Ile 14, Leu 20	Asn 18, Thr 121, Gly 15, Phe 92, Leu 5, Phe 98, Ala 7, Val 6, Val 31, His 23, Trp 22	Ile 50, Phe 92, Leu 20, Ser 49
56 (56(II))	0.81	-10.75	0.01 μ M	Leu 20, Gln 19	His 23, Asn 18, Thr 46, Gly 94, Ile 14, Gly 93, Lys 52, Ser 49	Leu 20, Lys 45, Ser 49, Gln 19, Ile 50
Cipro.	0.92	-8.66	0.45 μ M	Asn 18, Asp 27	Lys 45, Ser 49, Thr 46, Leu 28, val 31, Thr 111, Val 6, Leu 5, Phe 98, Gly 94, Ile 14, Gly 13	Ala 17, Leu 20, Thr 46, Ser 49

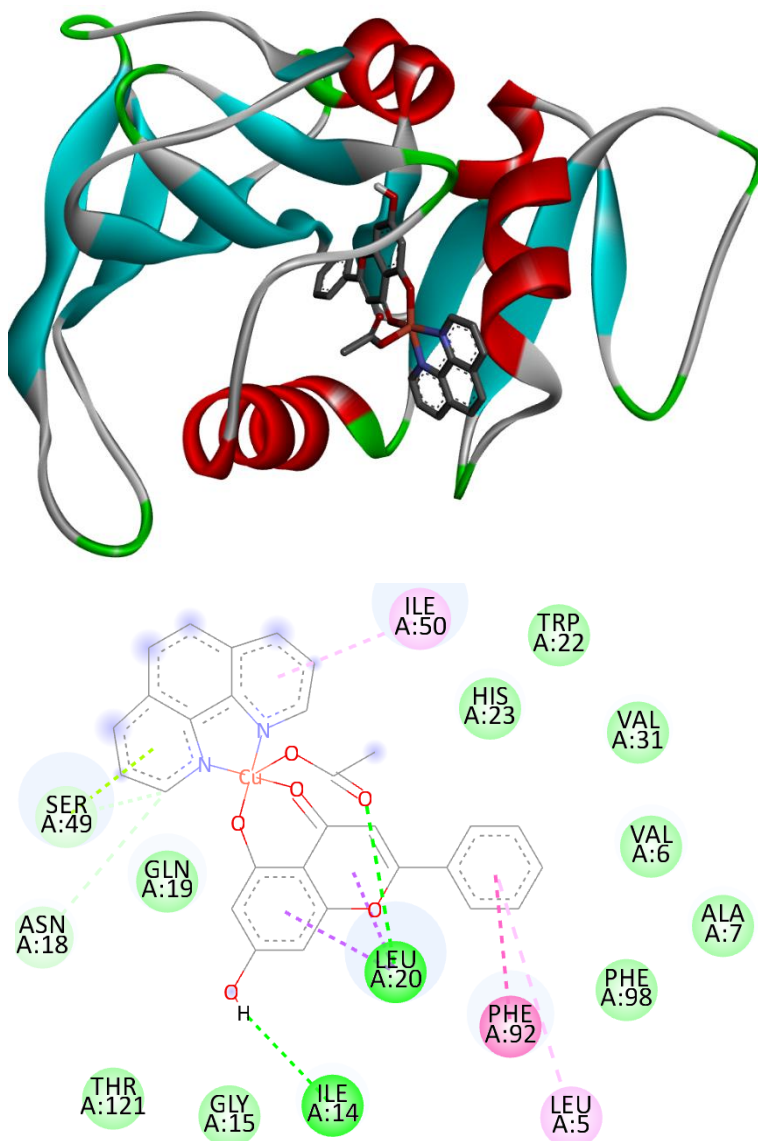


Figure 59. The binding interactions of 55 against *S. aureus* (Dihydrofolate reductase) (PDB: 2w9h)

4.8.2 Biological activity of chrysin and metformin Cr(III), Cu(II) and Zn(II) complexes (57 - 59)

4.8.2.1 Cytotoxicity study of the complexes (57 - 59)

The mixed ligand complexes of Cr(III), Cu(II) and Zn(II) that contain the natural flavonoid, chrysin and the antidiabetic drug, metformin has been synthesized and evaluated for their cytotoxicity against the MCF-7 cell line using MTT-assay. The obtained results for percent cell viability at different concentrations (0 - 100 μ M) and the cellular morphological changes before and after treatment are presented in Figure 60 and Figure 61. The cytotoxicity potential of the

synthesized complexes in a concentration dependent manner has been observed in a 24 h incubation time.

The first complex to show cytotoxicity was complex **58** at 12.5 μM concentration where the percent cell viability reduced to 64.62% relative to **57** (91.06 %), **59** (100 %) and cisplatin (89.56 %), demonstrating outperformance against MCF-7 cells at lowest concentration. Moreover, outperforming cytotoxicity results are observed by complex **58** such as 25 μM (52.99 %), 50 μM (12.98 %) and 100 μM (5.38 %).

In this study, complexes **57** and **59** were found to be cytotoxic at higher concentration (100 μM) in which the percent cell viability decreased to 62.76 and 59.57 % for complex **57** and **59**, respectively. According to cytotoxicity research reports, a sample is considered cytotoxic if the percentage of viability is less than 70% (Cannella et al., 2019; Ramasamy et al., 2022; Roth et al., 2020). Hence, all the reported complex compounds (**57** - **59**) were found to be cytotoxic at 100 μM . In this work, we reported the successful design and synthesis of cytotoxic Cr(III)(**57**), Cu(II)(**58**) and Zn(II)(**59**) mixed ligand complexes comprising antidiabetic drug, metformin and naturally available flavonoid, chrysin as ligands. In this particular study, the role of heteroleptic ligand composition on the biological activities have been uncovered. Similarly, the synergic effect of enhancing biological activity of organic ligands or drugs has been better confirmed. The IC_{50} values of the synthesized complexes were calculated from the regression equations generated using the percent cell viability versus concentration graph (Figure 60 (bottom)). And hence, the IC_{50} values were found to be 31.02, 32.74 and 44.00 μM respectively for **57**, **58** and **59**, compared to the positive control cisplatin ($\text{IC}_{50} = 18.62 \mu\text{M}$). The obtained results confirmed that Cu(II) complex are promising candidates towards MCF-7 cell lines. The finding is in agreement with previously reported works (Bravo-Gómez et al., 2009; Nunes et al., 2020).

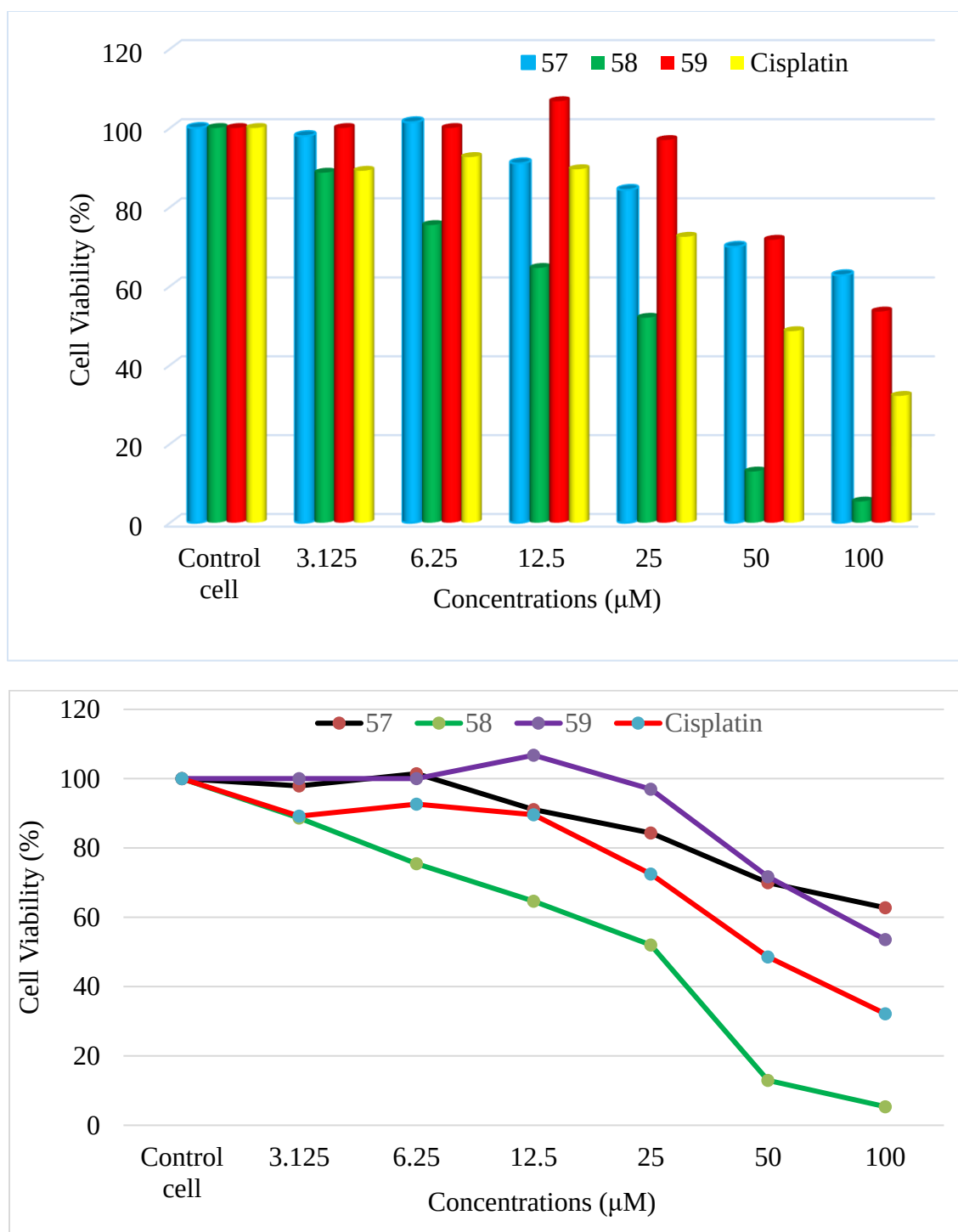


Figure 60. Cytotoxicity of chrysin based metformin complexes (57 - 59) and the positive control (Cisplatin) against MCF-7 cell lines

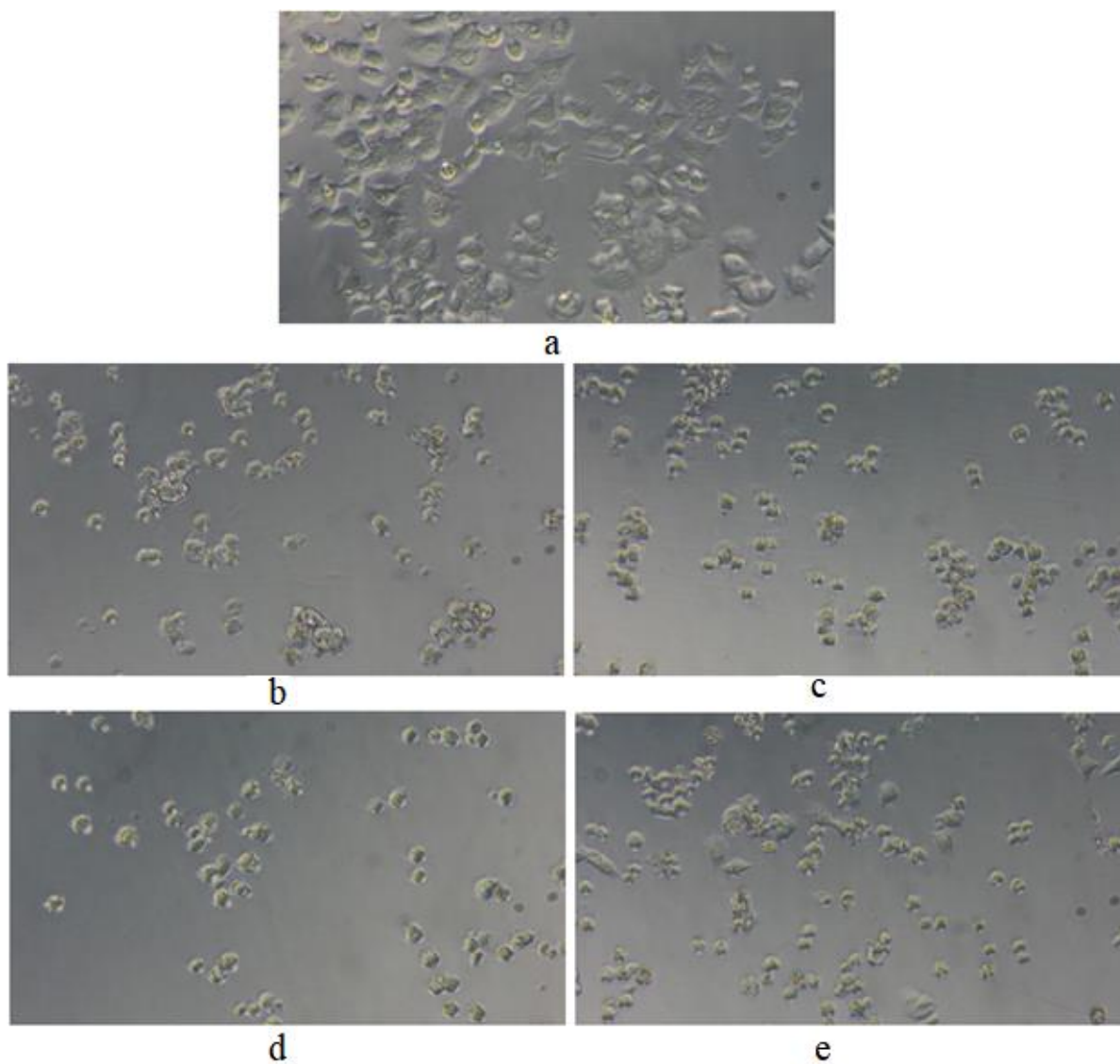


Figure 61. Photomicrographs of the cellular morphology of untreated MCF-7 (a) and the changes induced by complex **57** (b), **58** (c), **59** (d) and cisplatin (e) at 100 μ M

The epithelial like skeletons of the MCF-7 cells with polygon morphology has been clearly observed in untreated MCF-7 cells (Figure 61). However, after treatment with 1000 μ M of the synthesized complexes and cisplatin for 24 h, the cells morphology and density was changed and decreased. This phenomenon was highly observed for Cu(II) centered complex **58** followed by complex **57**. Generally, the obtained microscopic and percent cell viability results demonstrated that the metal complexes in this study are cytotoxic as to MCF-7 cell lines comparable to other metal complexes reported elsewhere (Alem et al., 2022; Andiappan et al., 2018; Nunes et al., 2020; Razak et al., 2019).

4.8.2.2 Antibacterial activity of metformin and chrysin heteroleptic complexes (57 - 59)

Metformin coordinated chrysin-based Cr(III) (57), Cu(II) (58) and Zn(II)(59) metal complexes were proved to show potent antimicrobial activity (Figure 62). In this work, the *in vitro* antibacterial activities analysis of the as-synthesized drug coordinated metal complexes were conducted against two gram negative: *E. coli* and *P. aeruginosa* and gram-positive: *S. aureus* and *S. pyogenes* bacterial strains.

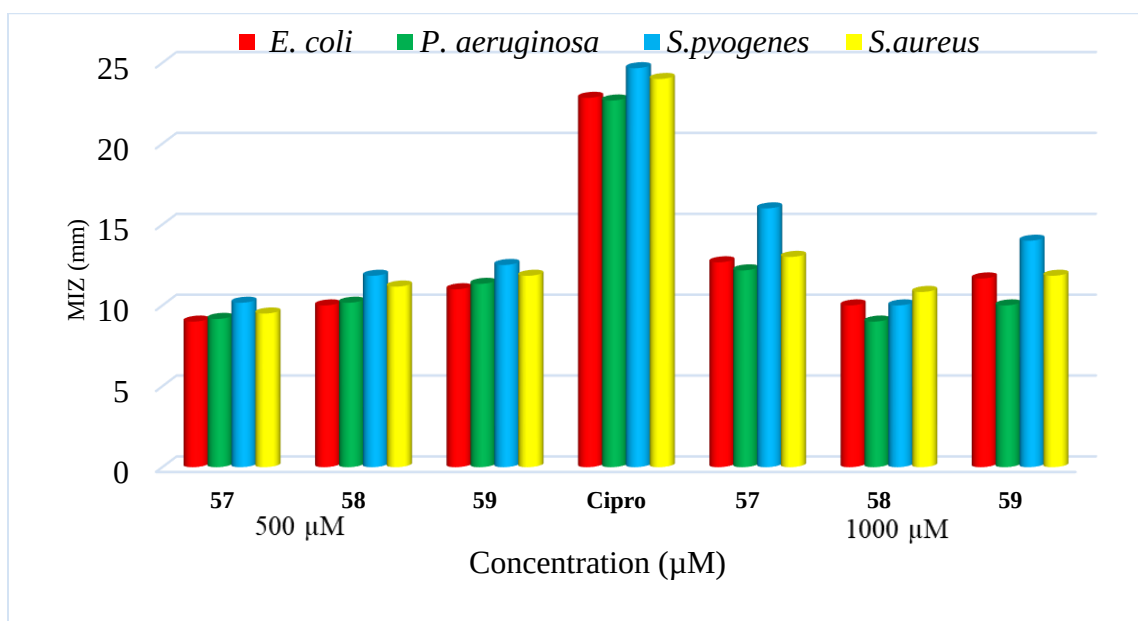


Figure 62. Antibacterial activity of metformin-based complexes (57 – 59)

The results obtained from the disc diffusion method showed that the metal complexes showed higher efficiency towards the gram-positive (*S. aureus* and *S. pyogenes*) over gram-negative (*E. coli* and *P. aeruginosa*) bacterial isolates. Among the synthesized complexes, 58 and 59 displayed better activities against *S. pyogen* with MIZ of 11.533 ± 0.236 and 12.500 ± 0.408 mm, respectively (Figure 62 and Appendix 34) at 500 μ M. Complex 57 exhibited higher activity against all the bacterial strains at higher (1000 μ M) concentration (Figure 62). Moreover, the %AI of the complexes were calculated and found to be 40, 42, 39 and 41%; 47, 48, 44 and 45%; 49, 51, 48 and 50% against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa*, respectively for Cr(III) (57), Cu(II)(58) and Zn(II)(59) complexes. In this work, the antibacterial activity of chrysin and metformin complexes showed higher efficacy than their 1,10-phenanthroline and chrysin structural analogs, confirming the importance of ligands in bioactivity testing. The change in percent activity

index (%AI) between structural analogs of same metal centers Cr(III) complexes (**54** and **57**) showed that complex **57** has better antibacterial activity than **54** with 7, 3, 6, and 5 mm inhibition zone enhancements respectively against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa*. The Cu(II) complex, **58** brought a small changes to the inhibition of *S. aureus* (by 2mm) and *E. coli* (by 2mm) compared to its analog **55**. Whereas, complex **55** was found to be better than that of **58** against *S. pyogenes* (1 mm) and *P. aeruginosa* (1 mm). The structural activity relationship was highly noticeable for Zn(II) complexes at which 7, 9, 11 and 12 mm inhibition zone enhancements against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa* when the 1,10-phenanthroline ligand is substituted by a metformin ligand.

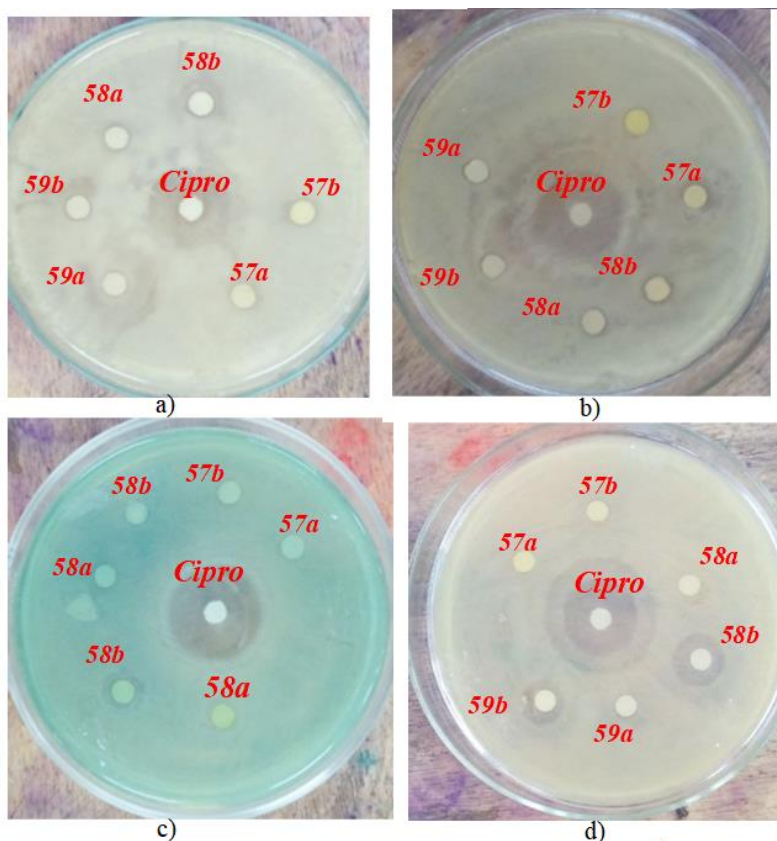


Figure 63. Zone of inhibitions of complexes against *E. coli* (a), *S. aureus* (b), *P. aeruginosa* (c) and *S. pyogenes* (d)

The statistical analysis (ANOVA) showed that concentration and the metal center in a compound are significant factors to affect the zone of inhibition (Appendix 44 – 45). Hence, compound **57** showed highly significant difference ($p = 0.000$) in zone of inhibition except compounds **54** ($p = 0.092$) and **56** (1.000). Compound **58** (Cu(II)) showed significance difference to compounds except compound **62**

(Cu(II)) ($p = 0.189$). Similarly, compound **59** (Zn(II)) showed insignificant difference to that of a Cu(II) centered complex **55** ($p = 1.00$) and significantly different to all others.

4.8.2.3 Antioxidant activity of the complexes (57 – 59)

The antioxidant activities of chrysin and its metformin-coordinated heteroleptic Cr(III), Cu(II) and Zn(II) complexes (**57 - 59**) were compared with the standard antioxidant, ascorbic acid and the obtained results are presented in Figure 64. The results demonstrated that the heteroleptic complexes had higher antioxidant activities than the free ligand chrysin, inferring that the design of the complexes to enhance biological significance has been successful. The synthesized metformin coordinated chrysin-based metal complexes (**57 - 59**) found to follow a DPPH radical scavenging activity as: AA >> **59** > **58** > **57** > chrysin. The obtained results clearly demonstrated that the enhancement of the required antioxidant activity following the coordination of the metal complexes compared to the free ligand which are in agreement with the previously reported works for the antioxidant activity of metal complexes (Gençkal, 2020; Eleftherios Halevas, Barbara Mavroidi, Maria Pelecanou, et al., 2021; Panhwar & Memon, 2014a).

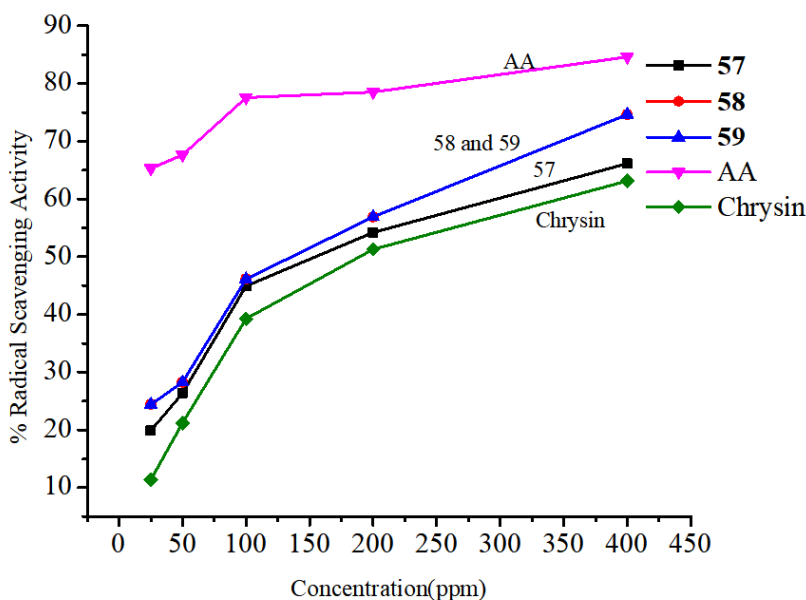


Figure 64. The antioxidant activity of the synthesized metal complexes (**57 - 59**) against DPPH radical

4.8.2.4 Quantum Chemical descriptor analysis of the complexes (57 - 59)

The results of quantum chemical descriptors analysis conducted in this study are presented in Table 16. The energy gap between the E_{HOMO} and E_{LUMO} eigen values, E_g is a significant stability index

and associated with structural and kinetic stability. A molecule with large band gap is described as a hard molecule, high kinetic stability, low chemical reactivity and high molecular stability (Choudhary, Bhatt, Dash, & Sharma, 2019; Issaoui, Ghalla, Muthu, Flakus, & Oujia, 2015; Ruiz-Morales, 2002).

The E_g values for the synthesized complexes was found to be 3.451, 2.964 and 3.648 eV, respectively for **57**, **58** and **59**, indicating that complex **59** was more stable than other complexes. On the other hand, compounds with small band gap energy are soft and easily interacts with soft molecules (DNA, proteins, receptors, etc) (Damena, Alem, et al., 2022a; Ismael et al., 2020). Accordingly, complex **58** was found to be biologically significant complex its small band gap energy. This quantum chemical descriptor was observed in the strong activity of complex **58** in the *in-vitro* antibacterial and cytotoxic study (see above).

Table 18. Quantum chemical descriptors of metal complexes (**57** – **59**)

Cpd	E_{HOMO}	E_{LUMO}	E_g	X	μ	H	σ	Ω	Nu	D
57 (Cr)	-5.959	-2.508	3.451	4.234	-4.234	1.725	0.290	5.194	0.193	10.838
58 (Cu)	-5.883	-2.919	2.964	4.401	-4.401	1.482	0.337	6.535	0.153	9.341
59 (Zn)	-5.949	-2.302	3.648	4.125	-4.125	1.824	0.274	4.666	0.214	18.794

4.8.2.5 Frontier molecular orbitals analysis of the complexes (**57** – **59**)

Frontier molecular orbitals (FMOs) play a very important role in the reactivity and stability of chemical compounds (Ashfaq et al., 2022). To understand the distribution of FMOs, we represent the HOMO and LUMO of the synthesized compounds together with their band gap energies as shown in Figure 65. In complex **57**, the electron density distribution of the HOMO found to rely on the phenyl part of the ligand chrysin. In the LUMO of **58**, this distribution was observed to be uniform on the structure of the chrysin including the metal center with small localization to the NH of metformin. This infers that the presence of π - π^* and LMCT electronic transitions agrees with the data obtained from the UV-Vis study. The HOMO and LUMO distribution of complex **58** was found to rely on the phenyl part of chrysin and the metal center, and on the metal center and metformin, respectively. This clearly demonstrates the presence of d - d and intraligand charge transfer electronic transitions in a Cu(II) complex. Whereas, in a Zn(II) centered complex (**59**), the

HOMO was found to distribute on the entire complex and the LUMO was concentrated on the chrysin part of the complex, confirming the presence of intraligand and MLCT electronic transitions.

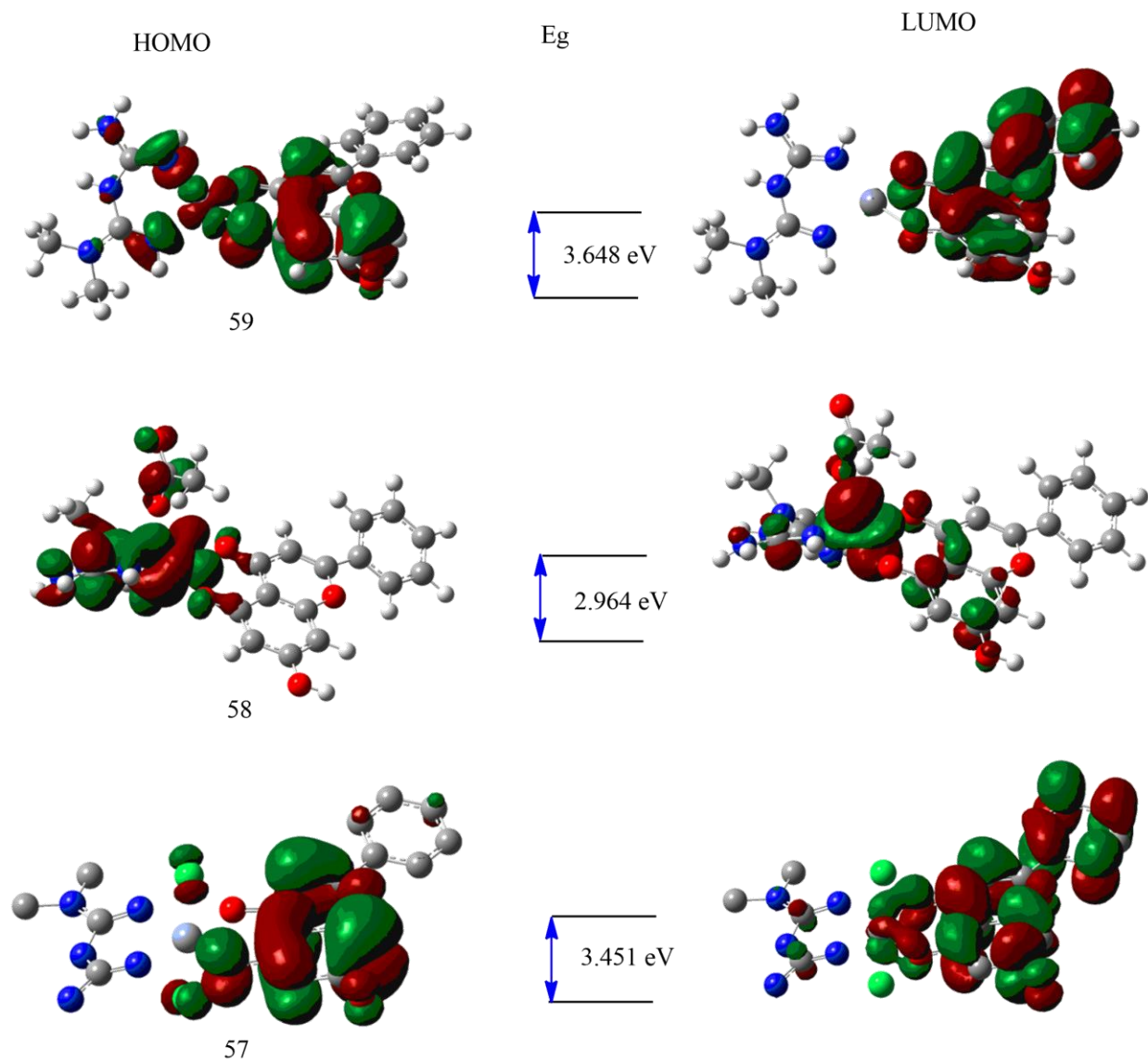


Figure 65. The frontier molecular orbitals and associated band gap energies of complexes (57 - 59)

4.8.2.6 Molecular docking study of the complexes (57 – 59) against estrogen receptor alpha (ER α ; PDB: 5GS4)

Drug discovery is a multi-step platform in which a candidate compound and biological targets are evaluated using various approaches. New drug discovery with high therapeutic potential can be fostered using new technologies. In this regard, molecular docking is one of the cutting-edge

computational drug discovery technologies in which the most stable form of drug-protein/receptor/DNA complex can be predicted (Arjmand et al., 2022). The molecular docking study of the synthesized mixed ligand complexes were conducted against estrogen receptor alpha (ER α ; PDB:5GS4) in order to associated the in-vitro cytotoxicity activity against MCF-7 cell lines in which ER α is the major clinical biomarkers used to subtype breast cancers (Bhatt et al., 2016). The interaction of the synthesized mixed-ligand metal complexes (**57** -**58**) with the prominent residual amino acid interactions of the estrogen receptor alpha (ER α ; PDB:5GS4) are well presented in Figure 66 and Appendix 35 – 37.

Table 19. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against estrogen receptor alpha (ER α ; PDB: 5GS4)

Cpds	RMS D	Binding energy (kcal/mol)	Inhibition constant (K _i)	H-bonding	van der Waals	π -alkyl/ π -ion
57	1.35	-6.26	22.99 μ M	Glu 323, His 356	Arg 394, Phe 445, Lys 449, Glu 323, Gly 390, Ala 322, Trp 360, Met 357, Glu 357	Trp 393, Glu 353, Leu 349, Arg 352
58	0.74	-5.69	51.37 μ M	Glu 323	Arg 394, Trp 393, Glu 323, Phe 445, Met 357, Ile 386, Leu 387, Leu 349	Lys 449, Glu 353, His 356, Arg 352
59	0.95	-6.12	30.12 μ M	Glu 323	Trp 393, Phe 445, Lys 449, Trp 360, Ile 386, Met 357, Leu 387, His 356, Leu 346	Glu 323, Glu 353, Arg 352
Cisplatin	0.39	-6.32	23.42 μ M	Ser468, Asp374	Lys467f	Thr371, Glu471

It is shown that complex **57** was found to bind to ER α with Glu 323 and His 356 in a conventional hydrogen bond; Arg 394, Phe 445, Lys 449, Glu 323, Gly 390, Ala 322, Trp 360, Met 357 and Glu 357 via van der Waals force; and Trp 393, Glu 353, Leu 349 and Arg 352 via pi-alkyl/pi-sigma interaction with binding affinity and inhibition constant -6.26 kcalmol⁻¹ and 22.99 μ M, respectively.

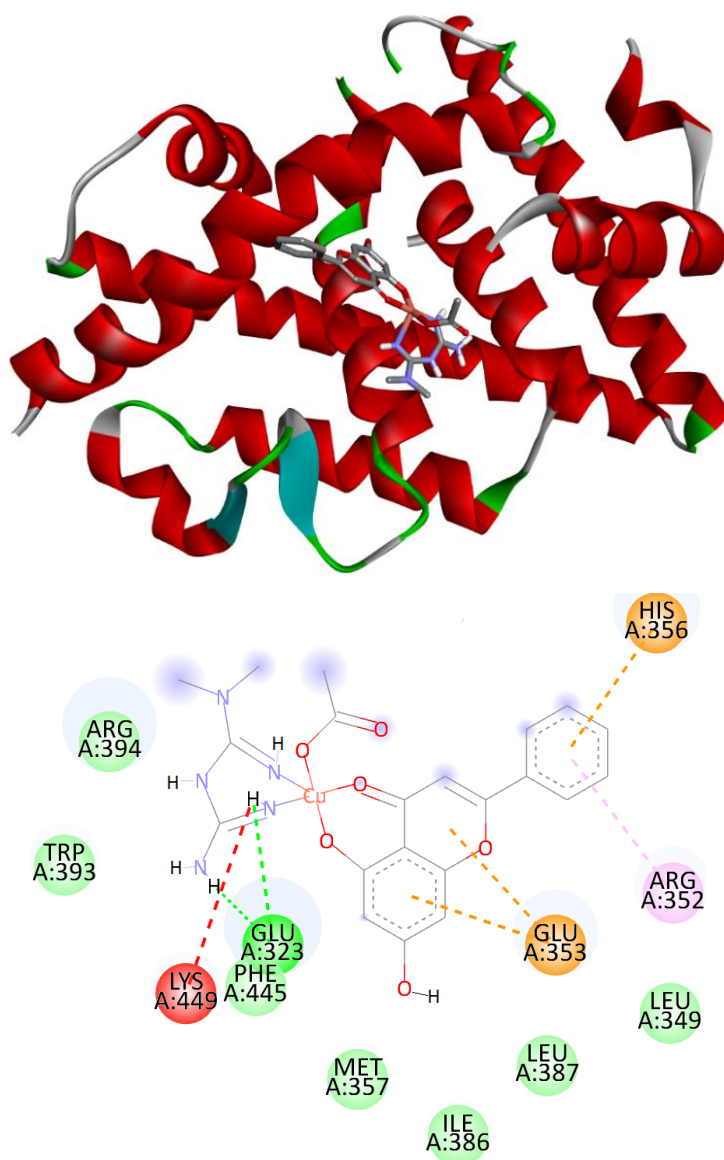


Figure 66. The binding interactions of **58** against estrogen receptor alpha (Erg; PDB: 5GS4)

4.8.2.7 Molecular docking against *S. aureus* (PDB: 2w9h)

The molecular docking analysis of the mixed-ligand metal complexes (**57** - **59**) was performed against the *S. aureus* protein (dihydrofolate reductase). The results obtained for the residual amino acid interaction, binding energy, and inhibitions constant together with the 2D and 3D representations are presented in Table 18, Figure 67, Figure 68 and Appendix 38– 39. It has been clearly shown that complex **57** established a total of sixteen interactions: two hydrogen bonding interactions with Leu 5 and Phe 92; nine van der Waals with Leu 54, Asp 27, Val 31, Thr 46, Val

6, Phe 98, Gln 19, Ser 49 and Trp 22; and five π -alkyl/ π -ion interactions with Ile 14, Ala 7, Leu 28, Leu 20 and Ile 50 with a binding affinity -8.82 kcal/mol and inhibition constant of 0.30 μ M.

Table 20. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against *S. aureus* (PDB: 2w9h)

Cpds	RMSD	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding	van der Waals	π -alkyl/ π -cation/ π -anion
57	0.86	-8.82	0.30 μ M	Leu 5, Phe 92	Leu 54, Asp 27, Val 31, Thr 46, Val 6, Phe 98, Gln 19, Ser 49, Trp 22	Ile 14, Ala 7, Leu 28, Leu 20, Ile 50
58	0.93	-9.40	0.09 μ M	Ile 94, Asn 46	Leu 98, Val 97, Ser 121, Gly 119, Val 120, Ala 47, Asp 73, Thr 165, Glu 50	Ile 78, Pro 79, Ile 94, Asn 46,
59	0.79	-9.78	0.059 μ M	Ser 49, Phe 92	Ile 50, Asn 18, Gln 19, His 23, Trp 22, Phe 98, Gly 93, Ile 14, Gly 94, Thr 121	Gln 95, Lys 45, Thr 46, Leu 20, Ser 49, Gln 19
Cip	0.92	-8.66	0.45 μ M	Asn 18, Asp 27	Lys 45, Ser 49, Thr 46, Leu 28, Val 31, Thr 111, Val 6, Leu 5, Phe 98, Ile 14, Gly 93	Leu 20, Ala 7, Phe 92, Ser 46, Ser 49

It has been also confirmed from the analysis that complexes **58** and **59** showed the highest binding energy and lower inhibition constants. A binding energy and inhibition constant values of -9.40 kcal/mol and 0.09 μ M were recorded with a total of fifteen interactions: two hydrogen bonding with Ile 94 and Asn 46; nine van der Waals interactions Leu 98, Val 97, Ser 121, Gly 119, Val 120, Ala 47, Asp 73, Thr 165 and Glu 50; and four π -alkyl/ π -ion interactions Ile 78, Pro 79, Ile 94 and Asn 46. Whereas, complex **59** was found to create a total of eighteen interactions: two hydrogen bonding with Ser 49 and Phe 92; ten van der Waals interactions with Ile 50, Asn 18, Gln 19, His 23, Trp 22, Phe 98, Gly 93, Ile 14, Gly 94, Thr 121; and six π -alkyl/ π -ion interactions with Gln 95, Lys 45, Thr 46, Leu 20, Ser 49 and Gln 19. The higher binding energy and small

inhibition constants obtained for complexes **58** and **59** would be due to the greater number of interactions with the amino acids in the binding pocket of the protein.

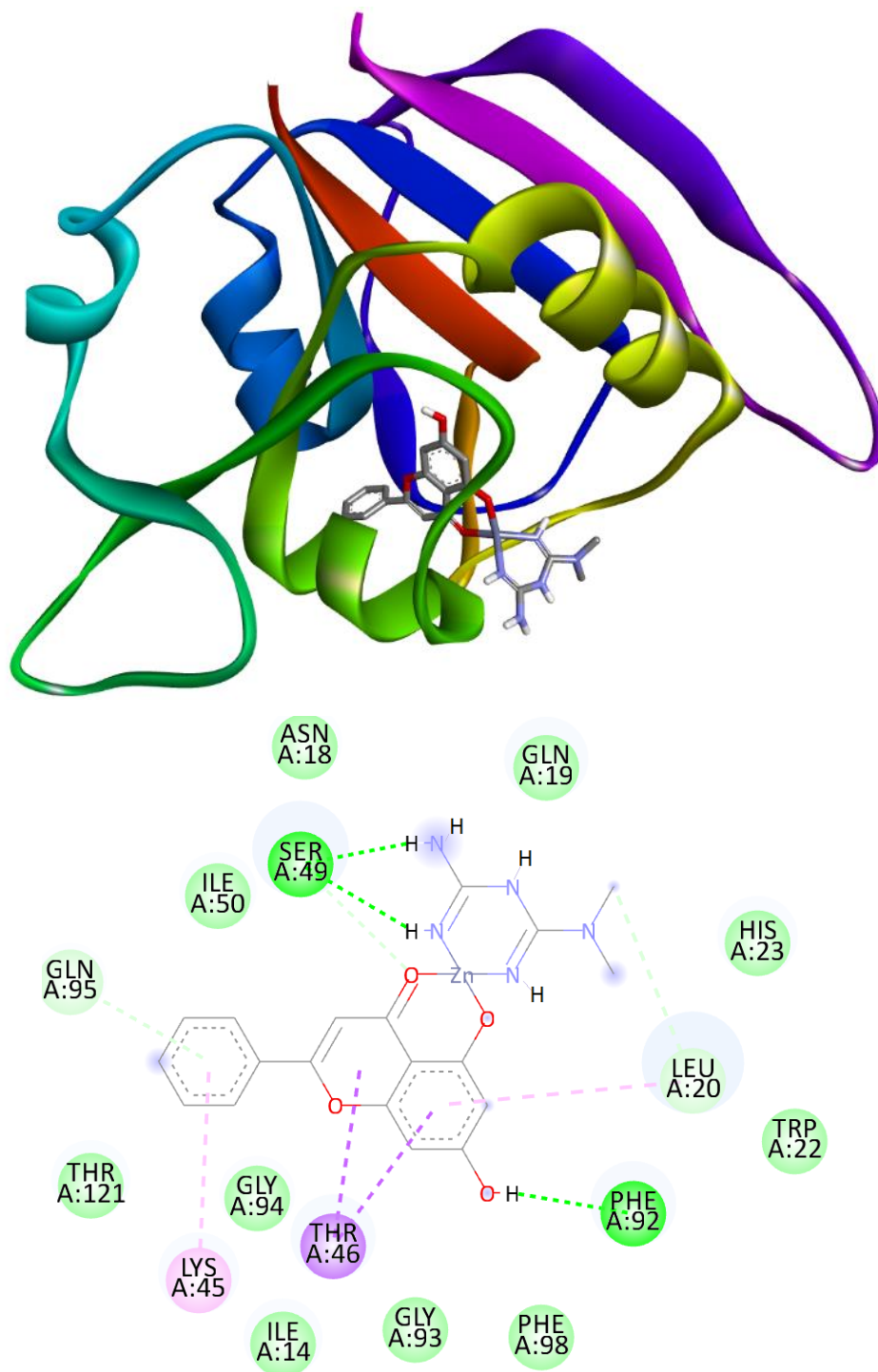


Figure 67. The binding interactions of **59** against *S. aureus* (PDB: 2w9h)

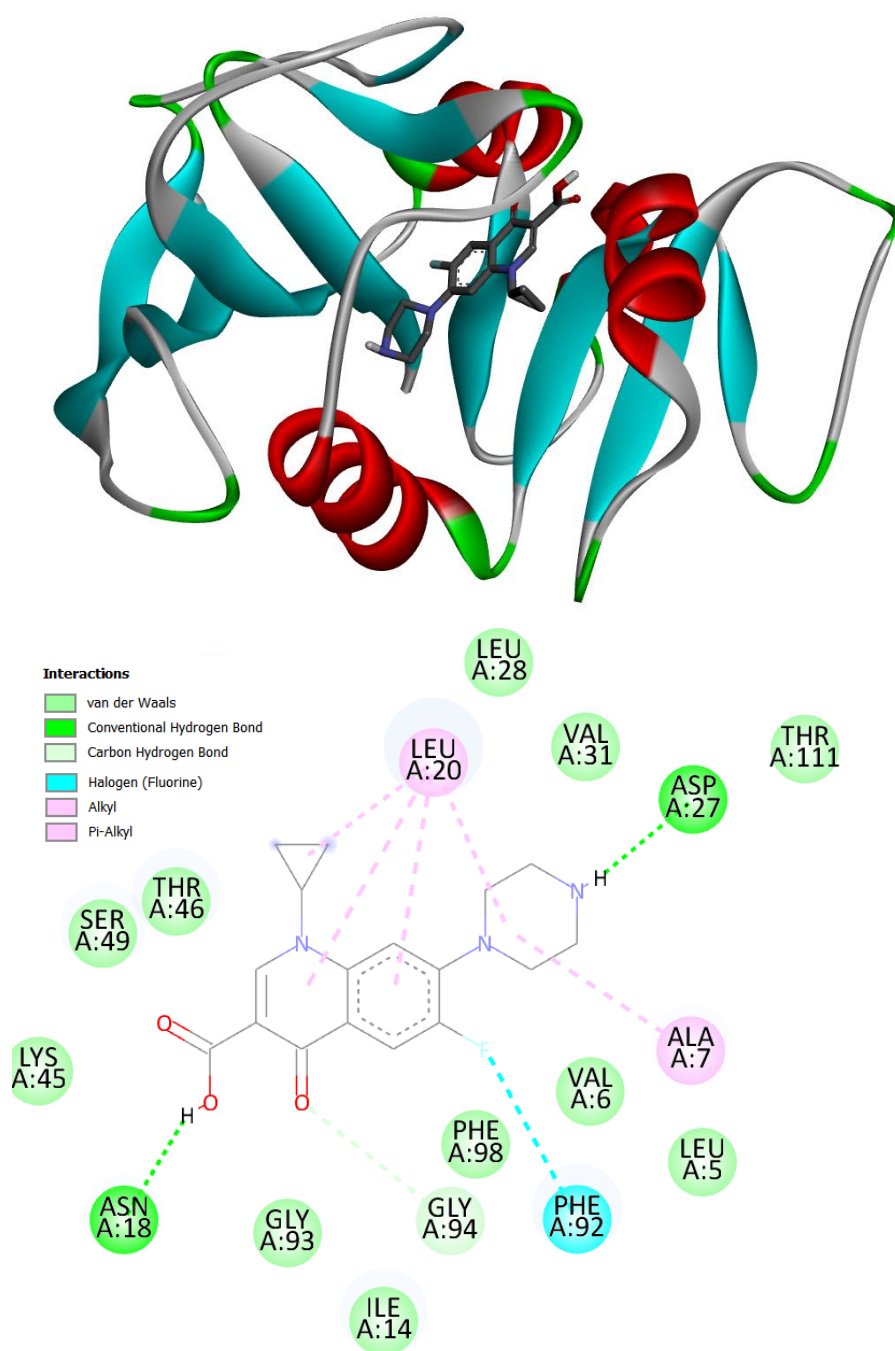


Figure 68. The binding interactions of ciprofloxacin against *S. aureus* (PDB: 2w9h)

4.8.3 Biological activity of mixed drug Cu(II) and Zn(II) metal complexes (**60** - **61**)

4.8.3.1 *In vitro* cytotoxicity studies (**60** - **61**)

The *in vitro* cytotoxicity of the synthesized complexes (**60** and **61**) at various concentrations (1.56 - 25 μM) was evaluated against human breast cancer cell line (MCF-7). Complex **62** was subjected for a similar cytotoxicity protocol, but found to be no effect in the preliminary test. The results obtained for viability of cells are presented in Figure 69. The Cu(II) complexes showed *in vitro* cytotoxicity against MCF-7 cell line. Increasing concentrations of the synthesized complexes strongly reduced the proliferation of the cancer cells, demonstrating a dose-dependent growth inhibitory effect. The IC_{50} values of the complexes were found to be $\text{IC}_{50} = 4.29 \pm 0.12 \mu\text{M}$ and ($\text{IC}_{50} = 7.58 \pm 0.10 \mu\text{M}$), respectively for **60** and **61** compared to cisplatin ($\text{IC}_{50} = 18.62 \pm 3.56 \mu\text{M}$). Our results are in the range of the IC_{50} values of previously reported mixed ligand copper(II) complexes named Casiopeinas® ($\text{IC}_{50} = 2.2$ to $103.7 \mu\text{M}$) against MCF-7 cell line (Bravo-Gómez et al., 2009; Nunes et al., 2020). These could be due to the geometrical and donor atom type resemblance between the metal complexes. The microscopic analysis of the cancer cells (Appendix 40) treated with complexes **60** and **61** shows a decrease in the number of the cancer cells, confirming the complexes' ability to induce apoptosis.

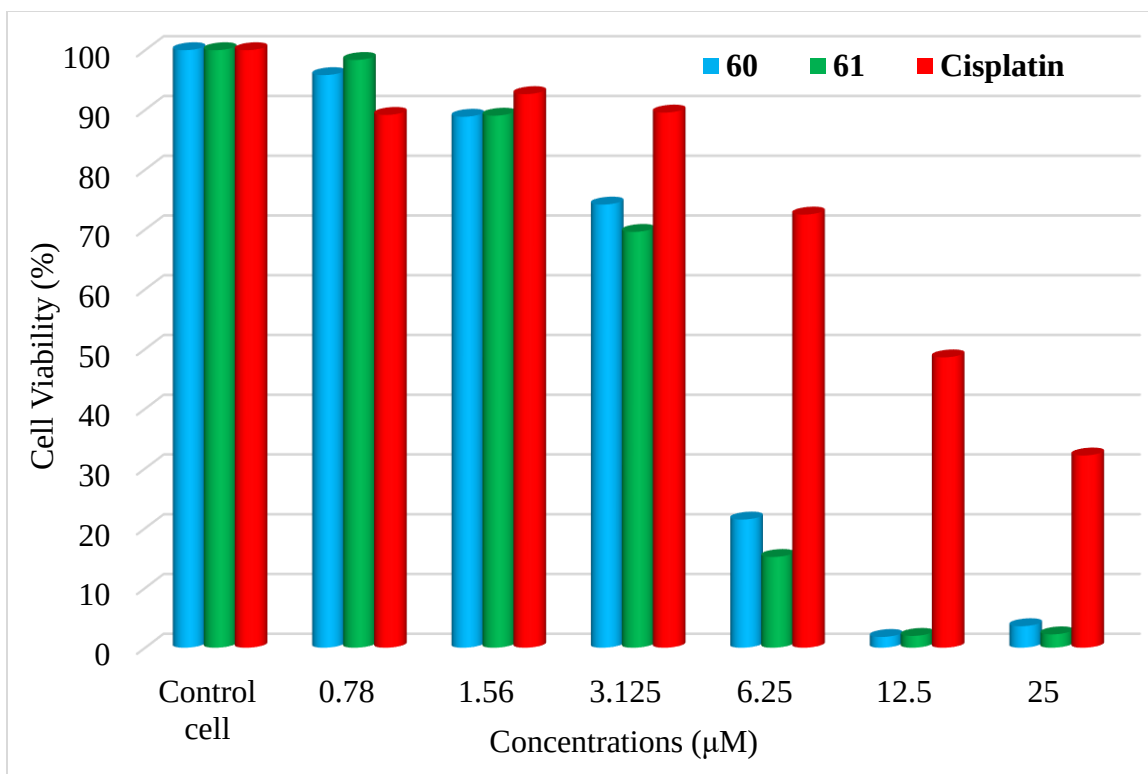


Figure 69. Cell viability of MCF-7 cancer cell line against complexes **60** and **61** and cisplatin

4.8.3.2 Antibacterial activity of the complexes

The synthesized mixed-ligand metal complexes (**60** - **62**) were tested against four bacterial strains (Gram-negative: *E. coli* and *P. aeruginosa*, and Gram-positive: *S. aureus* and *S. pyogenes*). The complexes were found to have potent antibacterial activity against both types of bacterial strains. The obtained results are presented in Figure 70 and Appendix 42. Among the mixed-ligand Cu(II) complexes, **61** showed strong antibacterial activity at both 25 and 50 μg/mL concentrations against all bacterial strains. This may be due its ligand constituents (1,10-phenanthroline and ciprofloxacin) unlike that of **60** which was derived from 1,10-phenanthroline and metformin. On the other hand, complexes **60** and **62** are found to be potent against *E. coli* and *S. pyogenes*, and *P. aeruginosa* and *S. pyogenes* bacterial strains, respectively. The activity of **62** was not promising at small concentration relative to **60** and **61**. This lowest activity is also observed in the cytotoxicity study of the metal complexes against MCF-7 cell lines.

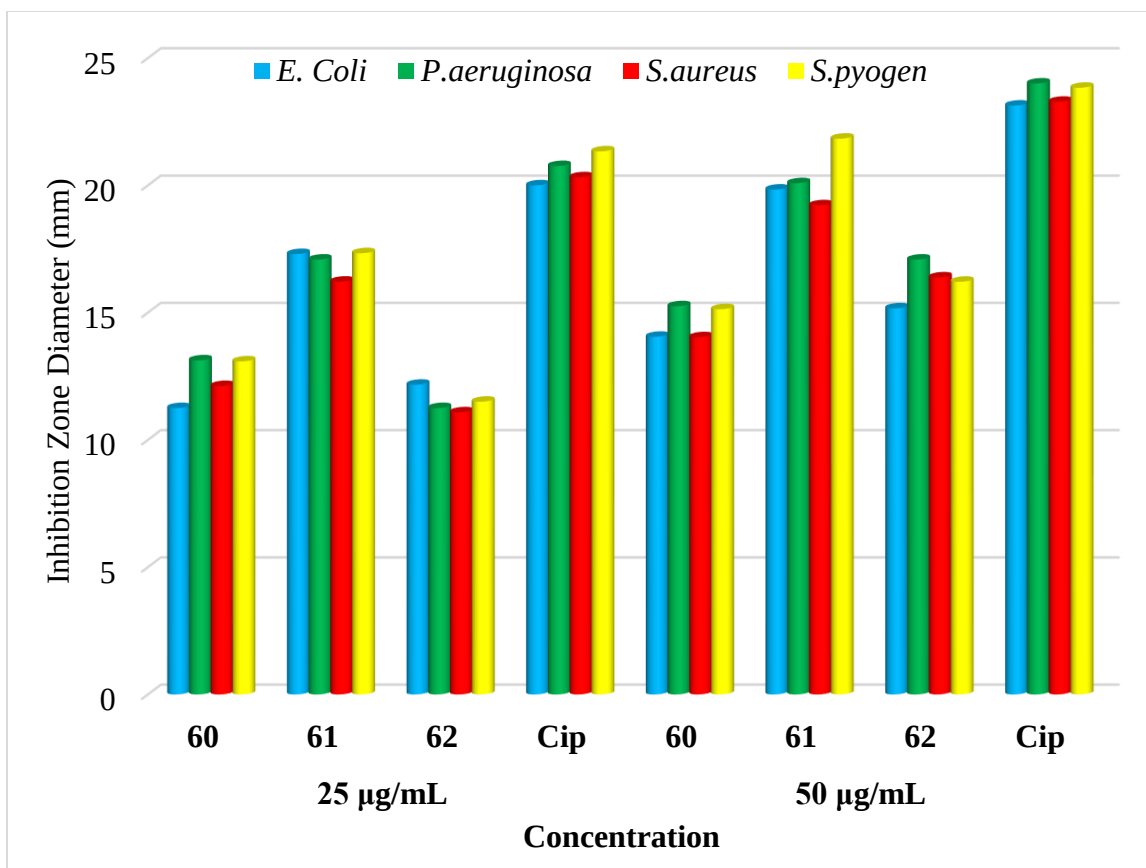


Figure 70. Mean inhibition zone diameter of mixed-drug metal complexes **60** and **61** and ciprofloxacin in mm at 25 and 50 µg/mL

The percent activity index (%AI) of the synthesized metal complexes was calculated at 25 µg/mL by comparing with the standard ciprofloxacin at the same concentration. It has been found that the %AI of the synthesized complexes against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa* found to be 60, 61, 56 and 63%; 80, 81, 86 and 82%; 55, 57, 61 and 54% respectively for Cu(II)(**60**), Cu(II)(**61**) and Zn(II)(**62**). A Cu(II) complex (**61**) showed extra ordinary antibacterial activity index compared to complexes **60** and **62** which might be due to the presence of ciprofloxacin as a ligand. The %AI data showed a structural activity relationship as result of metal center and ligand changes.

The statistical analysis (ANOVA) showed that concentration and the metal center in a compound are significant factors to affect the zone of inhibition (Appendix 44 – 45). Hence, compound **60** showed highly significant difference ($p = 0.000$) to all compounds tested except compounds ($p = 0.000$). It was found to show insignificant in zone of inhibition to the positive control, ciprofloxacin ($p = 1.000$).

Compound **61** showed significance difference to all compounds except compound **62** ($p = 0.443$). Similarly, compound **62**(Zn(II)) showed insignificant difference to compounds **58** ($p = 0.189$) and **61** (0.443) and highly significantly different to all other compounds.

4.8.3.3 Antioxidant activity evaluation

The antioxidant activities of the 1,10-phenanthroline based complexes (**60** – **62**) were evaluated using DPPH assay. The ability of the complexes as DPPH free radical scavenger at different concentrations (25 – 400 ppm) are presented in Figure 71. The free radical scavenging activity of the complexes at 25 ppm were found to be in the range of 21.857 – 33.286% compared to the standard ascorbic acid (66.150%). The overall antioxidant activity of the synthesized complexes was found to follow a concentration dependent radical scavenging activity. Accordingly, the antioxidant activity of the complexes (**60** – **62**) found to follow order **62** (Zn(II)) > **61** (Cu(II)) > **60** (Cu(II)) in their antioxidant activity with free radical scavenging activity of 69, 73 and 74 %, respectively for **60**, **61** and **62**, compared to the standard 88%, inferring the synthesis of metal-based antioxidant agents.

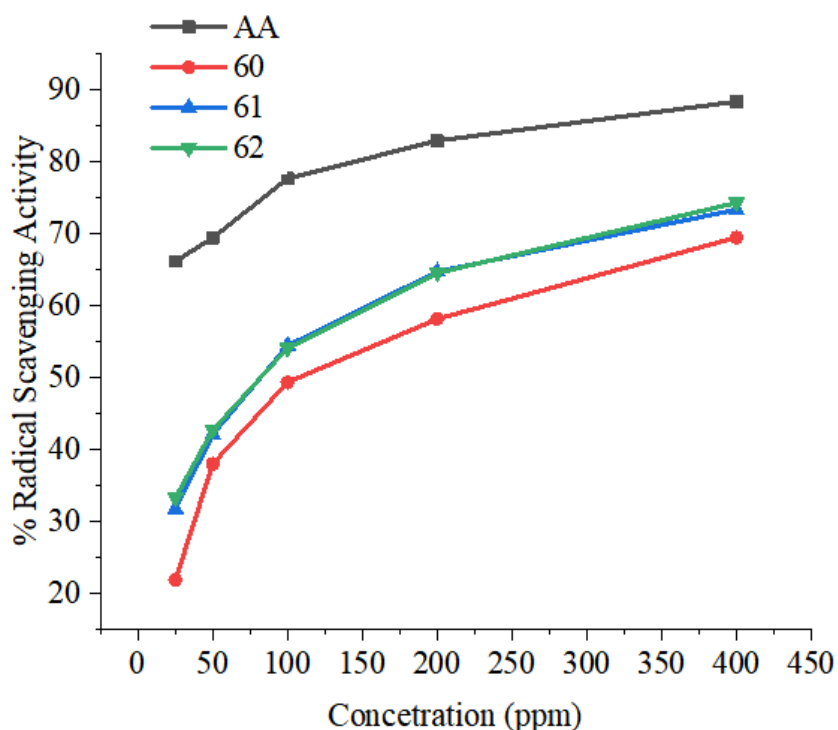


Figure 71. Graphical presentation of the antioxidant activity of complexes (**60** - **62**)

4.8.3.4 Quantum Chemical Analysis of complexes (60 – 62)

Analysis of quantum chemical descriptors would be used to relate quantum chemical descriptors of the synthesized metal complexes and their chemical and biological properties. Results obtained from DFT calculations and associated quantum chemical descriptors of the ligands and their metal complexes are presented in Table 21. The HOMO and LUMO Eigen values and band gap energy (E_g , $E_{LUMO} - E_{HOMO}$) can be related to the antibacterial, and antioxidant activities. The biological activity of the synthesized complexes towards appropriate molecules can be discussed with the hard–soft–acid–base (HSAB) theory, which states that hard acids prefer to coordinate with hard bases and soft acids with soft bases. Biological molecules such as DNA, enzyme and proteins are soft. Related to this, molecules with small band gap energy are considered soft and those with large band gap energy are hard. There is a direct relationship between biological activity and softness, and inverse relationship with hardness (Damena, Alem, et al., 2022a; Ismael et al., 2020). Consequently, soft complexes can interact easily with biological molecules than the hard ones. Hence, biological activity increases with increasing softness and decreasing hardness. Accordingly, the E_g was found to be 5.988, 4.755 and 4.096 eV for metformin, 1,10-phenanthroline and ciprofloxacin ligands, respectively. These band gap energy values are relatively decreased upon metal coordination and found to be 2.295 and 2.886 eV, respectively for **60** and **61** (Table 21), inferring the biological importance of the metal complexes than the free ligands.

Table 21. Quantum chemical descriptor analysis of the ligands and the synthesized metal complexes

CPD	HOMO	LUMO	E_g	X	M	η	σ	Ω	Nu	Dipole Moment
Met	-6.440	-0.452	5.988	3.446	-3.446	2.994	0.167	1.983	0.504	5.752
1,10-Phen	-6.690	-1.935	4.755	4.313	-4.313	2.377	0.210	3.912	0.256	5.192
Cipro	-6.155	-2.059	4.096	4.107	-4.107	2.048	0.244	4.118	0.243	15.128
60	-4.630	-2.335	2.295	3.482	-3.482	1.148	0.436	5.283	0.189	9.215
61	-6.268	-2.616	2.886	4.442	-4.442	1.826	0.274	5.402	0.185	13.759
62	-6.695	-2.343	4.352	4.519	-4.519	2.176	0.230	4.692	0.213	6.425

The band gap energy of complex **62** was found to be 4.353 eV which is even higher than that of the ligand ciprofloxacin. Moreover, results from the band gap energy and dipole moments show that the synthesized metal complexes (**60** and **61**) were found to enhance biological activities. In addition, the calculated electrophilicity (ω) and nucleophilicity index (Nu) parameters showed that **60** (5.283 eV), **61** (5.402 eV) and **62** (4.692 eV) have higher electron acceptor affinity than the constituent ligands. This implies that the synthesized Cu(II) complexes have higher Lewis acidity than that of the Zn(II) analog and the ligands (Tandon, Chakraborty, & Suhag, 2019).

The wave function distribution of the ligands (metformin, 1,10-phenanthroline and ciprofloxacin) and the synthesized metal complexes (**60** and **61**) are presented in Figure 72 and Appendix 39. The HOMO of the ligands found to reside on dimethyl and quinolone part of metformin and ciprofloxacin, respectively. Equal HOMO-LUMO distributions are observed in 1,10-phenanthroline and ciprofloxacin (Appendix 28 and 40). The HOMO and LUMO distribution of the metal complexes clearly showed the possible electronic transition. In **60** the HOMO resides on the metal center and 1,10-phenanthroline, confirming the presence of $\pi \rightarrow \pi^*$ transition. This distribution found to reside only on the metal center for the LUMO of **60** which confirms the presence of ligand to metal charge transfer (LMCT) and $d \rightarrow d$ electronic transitions in line with the experimental UV-Vis results.

In complex **62**, the HOMO was found to concentrate on the Zn(II) metal center and the nitrogen of the coordinated ligands donor atoms. Whereas, the LUMO undergoes a shift from the metal center to the one part of the ligand (1,10-phenanthroline), which confirms the possible $\pi \rightarrow \pi^*$ and metal to ligand charge transfer (MLCT). The broad and shoulder peak that appeared in the experimental UV-Vis result and TD-DFT calculation (Figure 72) would be related to either of these electronic transitions.

On the other hand, the HOMO distribution highly resides on the 1,10-phenanthroline and metal center, and the LUMO was found to reside on the metal center and ciprofloxacin parts of the molecule in complex **61**, which would be due to $\pi \rightarrow \pi^*$ and $d \rightarrow d$ electronic transitions. In all synthesized metal complexes, 1,10-phenanthroline showed to play a significant role in electron transition which would be due to its structural planarity.

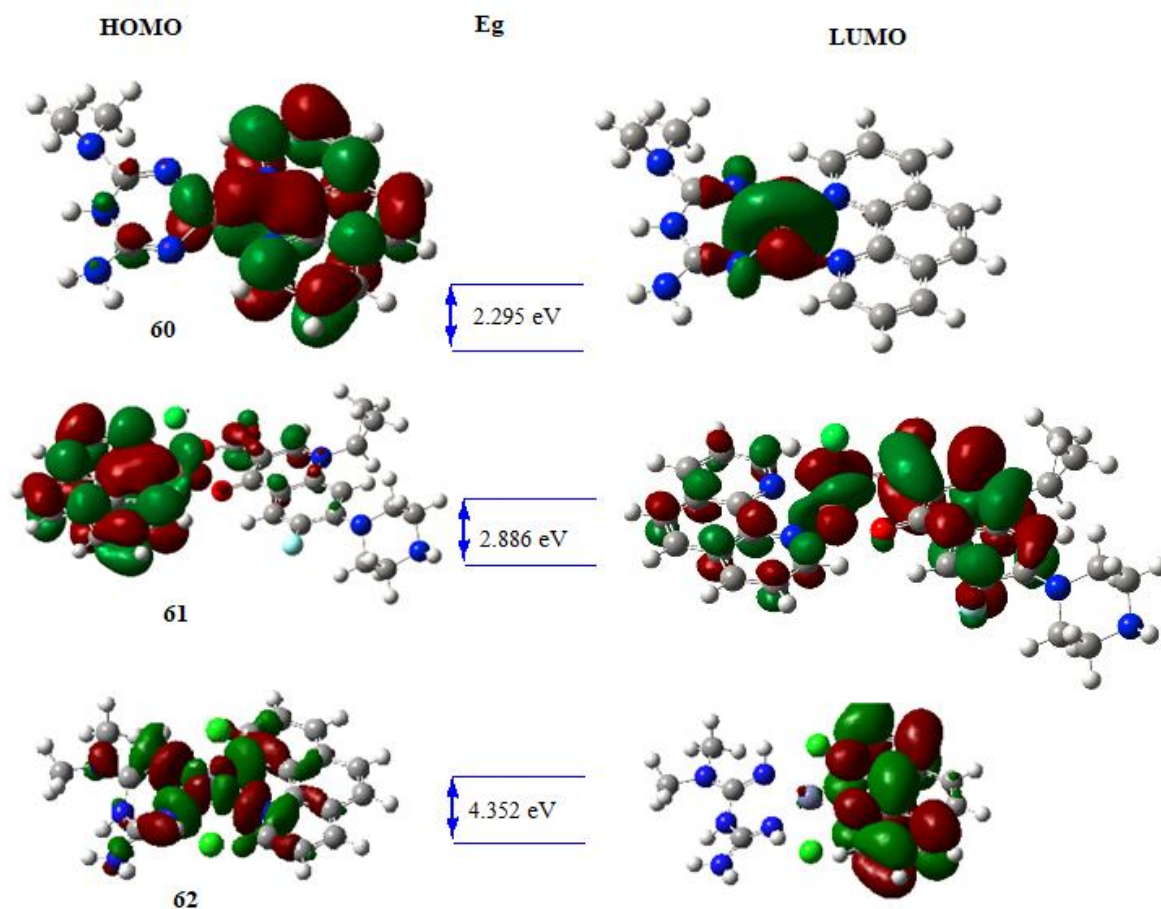


Figure 72. HOMO – LUMO distribution and band gap energy of the synthesized metal complexes

4.8.3.5 ADMET profile and BOILED Egg map evaluation

The physicochemical, drug-likeness and pharmacokinetic prediction profiles of the ligands and their metal complexes are presented in Appendix 43. A concomitant profiling of the lipophilicity and blood brain barrier permeation are presented in a BOILED egg model (Figure 73). Results obtained from pharmacokinetic profile shows modulation of pharmacokinetic profile of the synthesized metal complexes compared to the constituent ligands (Appendix 43). This is due to the fact that the metal complexes showed geometrical diversity, high electronegativity difference between the central metal and ligands, and relatively weak bonds between the atoms forming the metal-organic complexes allow the interaction of the desired complexes with biological targets unlike the free ligands (Noffke et al., 2012). The interaction of the ligands and their metal complexes as inhibitors of cytochrome family enzymes: CYP1A2, CYP2C19, CYP2C9, CYP2D6,

and CYP3A4 shows that inhibition of CYP1A2 and CYP3A4, and CYP2C19 and CYP3A4 by 1,10-phenanthroline and **61**, respectively. Cisplatin was predicted to share similar pharmacokinetic profile with that of complex **61**. It has been reported that the inhibition of CYPs is suggested to induce clinically significant drug-drug interactions (Hakkola, Hukkanen, Turpeinen, & Pelkonen, 2020), thereby drug induced toxicity. The previously reported toxicity (as neurotoxic, nephrotoxic) (Buchčík et al., 2012; C. X. Zhang & Lippard, 2003) of cisplatin would be due to its inhibitory interaction of the CYPs.

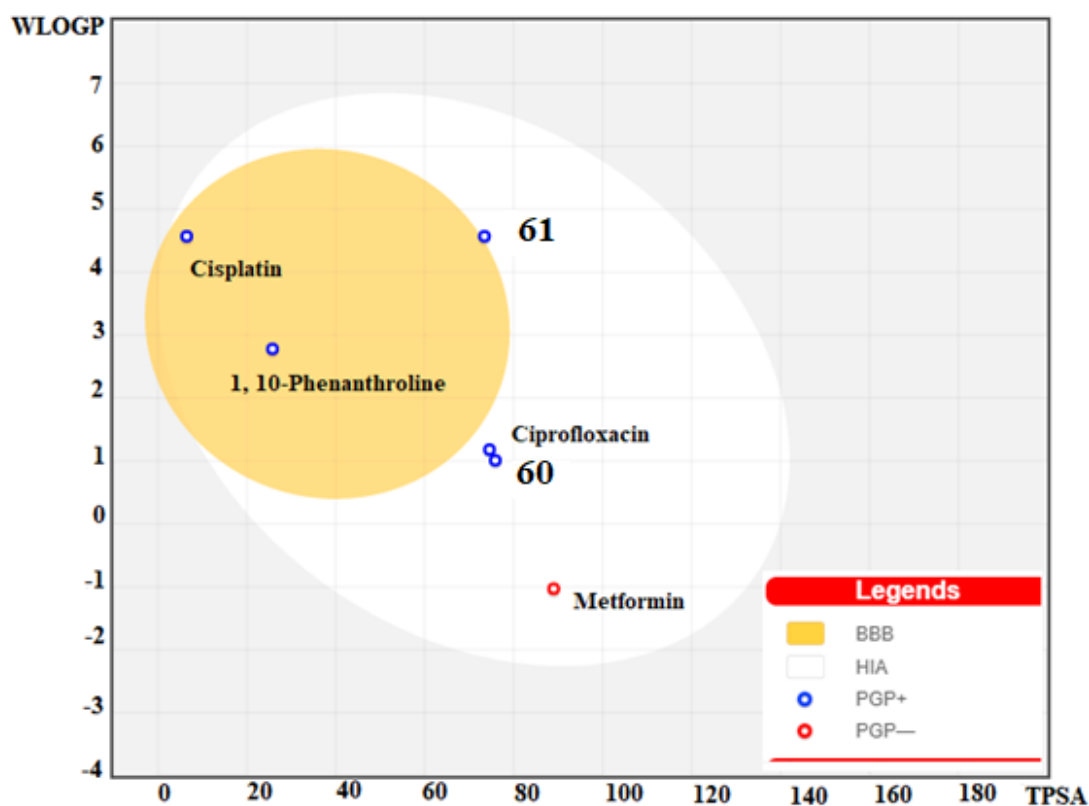


Figure 73. BOILEDegg model of the ligands (1,10-phenanthroline, Ciprofloxacin, and metformin), **60**, **61** and Cisplatin

Gastrointestinal (GI) absorption and blood-brain barrier (BBB) are vital properties to estimate at various stages of the drug discovery process (Daina & Zoete, 2016). The tendency of the synthesized metal complexes for their GI absorption and BBB permeability was predicted using boiled egg model. The obtained map for the boiled egg model of the complexes is presented in Figure 73. It is found that the lipophilicity and associated neurotoxicity of 1,10-phenanthroline has improved upon coordination to a metal center. Our strategy to use the combination of a lipophilic

ligand 1,10-phenanthroline, and a ligand that has only intestinal absorption resulted in improved metal complexes for their GI absorption, and lipophilicity. Moreover, the synthesized metal complexes showed better GI absorption than that of cisplatin, which is suspected to cause neurotoxicity.

4.8.3.6 Molecular docking analysis

Estrogen receptors (estrogen receptor α and estrogen receptor β) are the major clinical biomarkers used to subtype breast cancers. The estrogen receptor α (ER α) plays an important role in the development and progression of dependent hormonal type breast cancer (Bhatt *et al.*, 2016).

Table 22. Molecular docking scores and the corresponding prominent residual amino acid interactions of the complexes against estrogen receptor alpha (ER α ; PDB:5GS4)

Cpds	Rmsd	Binding Energy (Kcal/Mol)	Inhibition Constant (K _i)	H-Bonding With	Π -Sigma/ Π -Alkyl	Van Der Waals
60	0.10	-7.35	4.12 μ M	Leu387	Leu525, Ala350, Ile424	Phe404, Leu349, Leu428
61	0.05	-8.76	0.38 μ M	Glu542, Val376	Leu372, Ile358, Leu379	Lys362, Phe367, Glu380
62	0.18	-6.37	21.30 μ M	Glu380, Glu542	Val376	His373, Met543
Cisplatin	0.39	-6.32	23.42 μ M	Ser468, Asp374	Lys467f	Thr371, Glu471

The interaction of the synthesized Cu(II) complexes with the prominent residual amino acid interactions of estrogen receptor alpha (ER α ; PDB:5GS4) are well presented (Figure 74 and Table 22). Complex **60** was found to bind to ER α with Leu387 via conventional hydrogen bond; Leu525, Ala350 and Ile424 via pi-alkyl/pi-sigma interaction; and Phe404, Leu349 and Leu428 via van der Waals force with binding affinity of -7.35 kcalmol⁻¹. The importance of Arg394 and Glu353 amino acid residues in hydrogen bonding, and Leu387 and Leu391 in pi-alkyl interaction have been reported in a previous study (Pang *et al.*, 2018). The promising anticancer activity of **60** observed in the *in vitro* cytotoxicity study may be associated with a hydrogen bonding interaction with Leu387 amino acid of the active site (Figure 74). A minimum binding energy (-6.32 kcal/mol) and a relatively very high inhibition constant (K_i = 21.30 μ M) was found for complex **62** which

are in line with the preliminary cytotoxicity evaluation. Ile358, Val376, Leu379, Glu380, Leu539, Glu542, and Met543, Phe367, Leu372, Gln375, Val355, Lys362, and Asn359 are amino acids of ER α engaged in hydrophobic interactions. Whereas, Lys362 and Glu542 are proved to be involved in hydrogen bonding (M. Xie et al., 2017). The higher binding affinity ($-8.76 \text{ kcalmol}^{-1}$) of **61** would be due to its greater number of interactions with binding site amino acids: two hydrogen bond interaction to Glu542 and Val376, three π -Sigma/ π -Alkyl interaction to Leu372, Ile358 and Leu379; and three van der Waals interactions to Lys362, Phe367 and Glu380 (Figure 69).

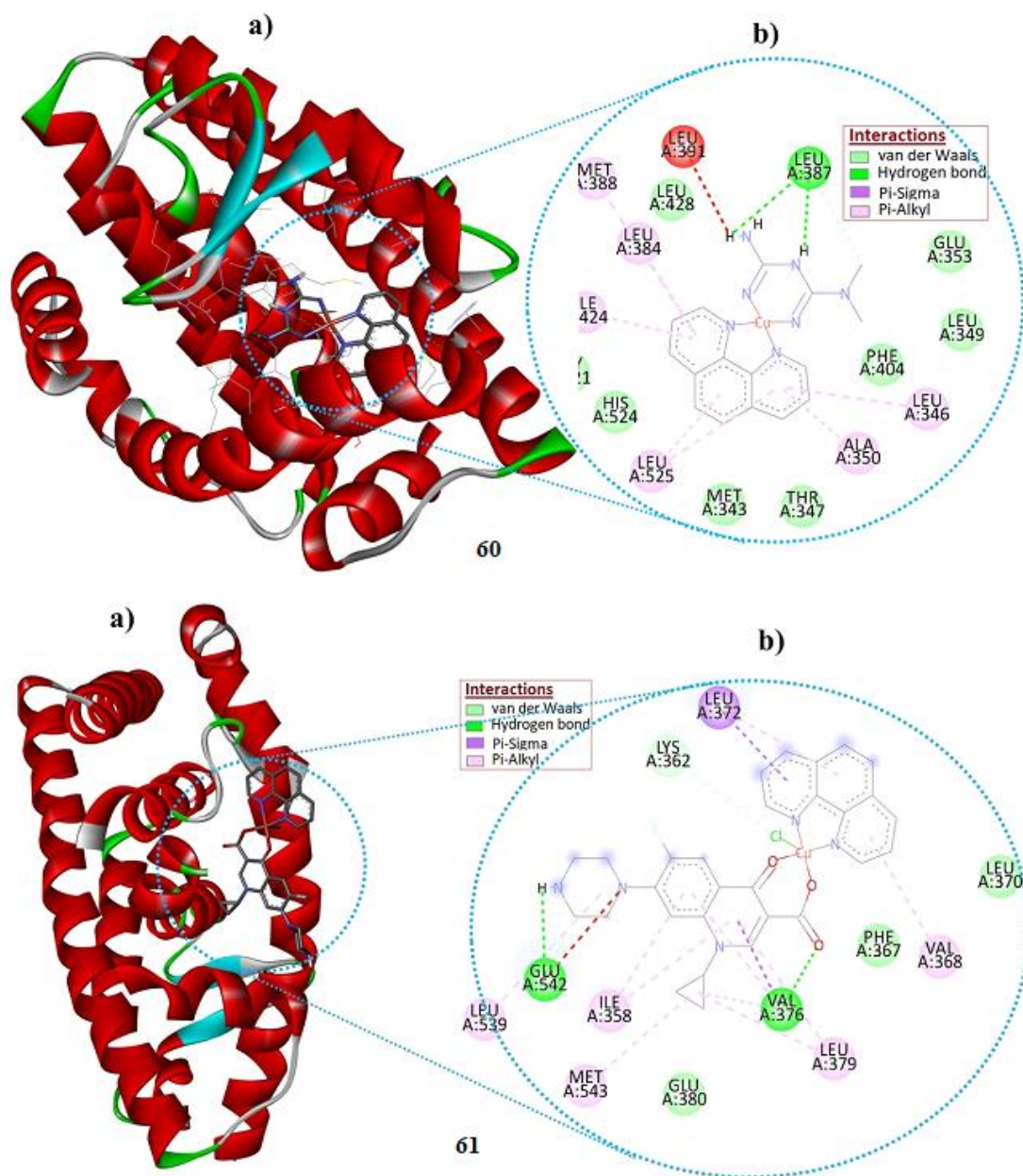


Figure 74. The (a) 3D and (b) 2D presentations of the binding interactions of complexes **60** and **61** against estrogen receptor alpha (ER α ; PDB: 5GS4)

The synthesized compounds are found to show any of the three interactions (Table 22) with the binding site amino acids residues of the receptor ER α . However, it is important to note that 1,10-phenanthroline based Cu(II) complexes follow multiple mechanism of actions: oxidative damage to DNA, depletion of reduced glutathione, and/or cell death by apoptotic and non-apoptotic dose-dependent DNA binding mechanism (Andiappan et al., 2018).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

In this work, the synthesis, characterization and biological activity of three classes of mixed ligand complexes were reported. *In-silico* aided ligand screening protocol was employed to choose a biocompatible ligand for the desired biological activities. The ADMET, DFT and molecular docking simulations clearly showed that a naturally available and biologically active chrysin is the best ligand choice for further study in metal complexation. Hence, chrysin inspired Cr(III), Cu(II) and Zn(II) complexes employing 1,10-phenanthroline and metformin co-ligands were synthesized and characterized. Moreover, metal complexes employing antidiabetic and antibacterial drugs (metformin and ciprofloxacin) as a ligand and 1,10-phenanthroline as a co-ligand were synthesized and characterized. DFT, TD-DFT, and molecular docking computational tools were employed to shed more-light on the electronic structures of the synthesized metal complexes. Spectroscopic and spectrometric techniques: UV-Vis, FTIR, and MS together with TGA/DTA, XRD and SEM-EDX were used to deduce the structure of the reported complexes. Polycrystalline natures of the complexes were confirmed using powder XRD with percent Crystalline index of 66, 27, 40, 31.4, 23, 94, 54 and 40%, respectively for Cr(III)(**54**), Cu(II) (**55**), Zn(II) (**56**), Cr(III)(**57**), Cu(II) (**58**), Zn(II) **59**, Cu(II) (**60**) and Cu(II) (**61**). Biologically, promising cytotoxicity, antibacterial and antioxidant activities were found. Cytotoxicity results demonstrated that 1,10-phenanthroline coordinated Cr(III) and Cu(II) complexes (**54**, **55**, **60** and **61**) showed promising cytotoxicity results against MCF-7 cell lines with the reported IC₅₀ values less than 10 μ M. Antibacterial and antioxidant evaluations showed that the synthesized metal complexes (**54** – **62**) are active against both gram-negative and gram-positive bacterial strains, and have the potentials to scavenge free radicals. Statistically, significant variations due to metal center and concentration are obtained ($p < 0.05$). Computationally, DFT/B3LYP method was used to infer and identify the type of vibrational frequencies and absorption energies associated with the functional groups or bonds. Moreover, quantum chemical descriptors found to show strong association with experimentally obtained anti-bacterial and cytotoxicity profiles of the complexes. In this work, it is found that the type of the ligand coordinated to the metal center has effect on the biological properties of the metal complex. 1,10-phenanthroline based complexes are promising class of metal complexes for further studies in *in-vivo* considerations. Moreover, currently available drugs for a certain disease can be used as promising metal therapeutic agents for a disease of research interest.

5.2 RECOMMENDATIONS

In this work, a novel strategy to use ligands for metal complexation, the synthesis of metal complexes, characterization, cytotoxicity, antibacterial and antioxidant activity of the metal complexes have been reported. The results demonstrated that, except a Zn(II) complex (**62**) that showed no effect for MCF-7 cell lines, all the complexes showed cytotoxicity against MCF-7 cell lines. Four of the reported complexes were more cytotoxic than the standard commercial drug, cisplatin. Moreover, all the synthesized complexes were antioxidant and active towards gram-negative: *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853), and Gram-positive strains: *Staphylococcus aureus* (ATCC25923) and *Streptococcus pyogenes* (ATCC19615). Accordingly, we would like to recommend further studies to be conducted on the synthesis of better crystalline materials of the same metal complexes, *in-vivo* antibacterial and anticancer activities on the same bacterial species and cancer cell line, and *in-vivo* antibacterial activity of the complexes against other types of gram-positive and gram-negative bacterial strains. Moreover, the *in-vitro* and *in vivo* cytotoxicity studies of the synthesized metal complexes against other types of cancer cell lines are recommend in further studies.

6.0 REFERENCES

- Abbas, B. F., Kamel, B. A., & Khamais, W. M. (2019). Preparation, diagnosis, biological activity, and theoretical studies of some mixed drug complexes. *The Scientific World Journal*, 2019.
- Abd El-Halim, H. F., Mohamed, G. G., & Khalil, E. A. (2017). Synthesis, spectral, thermal and biological studies of mixed ligand complexes with newly prepared Schiff base and 1, 10-phenanthroline ligands. *Journal of Molecular Structure*, 1146, 153-163.
- Abebe, A., Bayeh, Y., Belay, M., Gebretsadik, T., Thomas, M., & Linert, W. (2020). Mono and binuclear cobalt (II) mixed ligand complexes containing 1, 10-phenanthroline and adenine using 1, 3-diaminopropane as a spacer: synthesis, characterization, and antibacterial activity investigations. *Future Journal of Pharmaceutical Sciences*, 6, 1-9.
- Abebe, A., & Hailemariam, T. (2016). Synthesis and assessment of antibacterial activities of ruthenium (III) mixed ligand complexes containing 1, 10-phenanthroline and guanide. *Bioinorganic Chemistry and Applications*, 2016.
- Adhikary, C., Banerjee, S., Chakraborty, J., & Ianelli, S. J. P. (2013). Copper (II) azide complexes with NNO donor ligands: Syntheses, structure, catalysis and biological studies. 65, 48-53.
- Ahmad, S., Hamid, F., Hamid, M., Wattoo, S., Sarwar, S., & Nawaz, A. (2012). Spectrophotometric study of stability constants of cimetidine – Ni (II) complex at different temperatures. *Arabian Journal of Chemistry*, 5(3), 309-314.
- Ahmad, S., Isab, A. A., Ali, S., & Al-Arfaj, A. R. (2006). Perspectives in bioinorganic chemistry of some metal based therapeutic agents. *Polyhedron*, 25(7), 1633-1645.
- Ahmed, A. H., & Alkali, Y. I. (2019). In silico Pharmacokinetics and Molecular Docking Studies of Lead Compounds Derived from Diospyros Mespiliformis. *PharmaTutor*, 7(3), 31-37.
- Al-Fartusie, F. S., & Mohssan, S. N. (2017). Essential trace elements and their vital roles in human body. *Indian J Adv Chem Sci*, 5(3), 127-136.
- AL-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, 9(9), 430.
- Al-Nour, M. Y., Ibrahim, M. M., & Elsaman, T. (2019). Ellagic acid, Kaempferol, and Quercetin from Acacia nilotica: Promising combined drug with multiple mechanisms of action. *Current Pharmacology Reports*, 5(4), 255-280.
- Al-Qadisy, I., Saeed, W. S., Al-Odayni, A.-B., Ahmed Saleh Al-Faqeeh, L., Alghamdi, A. A., & Farooqui, M. (2020). Novel metformin-based schiff bases: synthesis, characterization, and antibacterial evaluation. *Materials*, 13(3), 514.
- Alem, M., Damena, T., Desalegn, T., Koobotse, M., Eswaramoorthy, R., Ngwira, K., Demissie, T. (2022). Cytotoxic mixed-ligand complexes of Cu (II): A combined experimental and computational study. *Frontiers in Chemistry*, 10.
- Alexiou, A. D., Decandio, C. C., Almeida, S. d. N., Ferreira, M. J., Romoff, P., & Rocha, R. C. (2017). Metal-ligand coordination and antiradical activity of a trichromium (III) complex with the flavonoid naringenin. *Journal of Coordination Chemistry*, 70(12), 2148-2160.
- Alexiou, A. D., Silva, D. D., Romoff, P., & Ferreira, M. J. (2016). Tris (3, 7-dihydroxyflavonolate- κ O3, O4) chromium (III) Complex. *Molbank*, 2016(1), M886.
- Alfadda, A. A., & Sallam, R. M. (2012). Reactive oxygen species in health and disease. *Journal of Biomedicine and Biotechnology*, 2012.
- Ali, I., Wani, W. A., & Saleem, K. (2013). Empirical formulae to molecular structures of metal complexes by molar conductance. *Synthesis and reactivity in inorganic, metal-organic, and nano-metal chemistry*, 43(9), 1162-1170.

- Aljazzar, S. O. (2021). Synthesis and spectral characterizations of vanadyl (II) and chromium (III) mixed ligand complexes containing metformin drug and glycine amino acid. *Open Chemistry*, 19(1), 735-744.
- Allouche, A. R. (2011). Gabedit—A graphical user interface for computational chemistry softwares. *Journal of computational chemistry*, 32(1), 174-182.
- Alper, P., Erkisa, M., Genckal, H. M., Sahin, S., Ulukaya, E., & Ari, F. (2019). Synthesis, characterization, anticancer and antioxidant activity of new nickel (II) and copper (II) flavonoid complexes. *Journal of Molecular Structure*, 1196, 783-792.
- Altomare, A., Corriero, N., Cuocci, C., Falcicchio, A., Moliterni, A., & Rizzi, R. (2015). QUALX2. 0: a qualitative phase analysis software using the freely available database POW_COD. *Journal of Applied Crystallography*, 48(2), 598-603.
- Amarsy, I., Papot, S., & Gasser, G. (2022). Stimuli-Responsive Metal Complexes for Biomedical Applications. *Angewandte Chemie*, 134(40), e202205900.
- Anderson, R. A., Cheng, N., Bryden, N. A., Polansky, M. M., Cheng, N., Chi, J., & Feng, J. (1997). Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes*, 46(11), 1786-1791.
- Andiappan, K., Sanmugam, A., Deivanayagam, E., Karuppasamy, K., Kim, H.-S., & Vikraman, D. (2018). In vitro cytotoxicity activity of novel Schiff base ligand–lanthanide complexes. *Scientific reports*, 8(1), 1-12.
- Anjaneyulu, Y., & Rao, R. P. (1986). Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu (II) with acetylacetone and various salicylic acids. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, 16(2), 257-272.
- Anthony, E. J., Bolitho, E. M., Bridgewater, H. E., Carter, O. W., Donnelly, J. M., Imberti, C., Palau, M. (2020). Metallodrugs are unique: Opportunities and challenges of discovery and development. *Chemical Science*, 11(48), 12888-12917.
- Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. J. A. (2002). Methods for testing antioxidant activity. 127(1), 183-198.
- Archibald, S. J. (2009). Coordination chemistry of macrocyclic ligands. *Annual Reports Section "A"(Inorganic Chemistry)*, 105, 297-322.
- Arjmand, B., Hamidpour, S. K., Alavi-Moghadam, S., Yavari, H., Shahbazbadr, A., Tavirani, M. R., Larijani, B. (2022). Molecular docking as a therapeutic approach for targeting cancer stem cell metabolic processes. *Frontiers in pharmacology*, 13.
- Arnott, J. A., & Planey, S. L. (2012). The influence of lipophilicity in drug discovery and design. *Expert opinion on drug discovery*, 7(10), 863-875.
- Asakura, H., & Kitahara, T. (2018). Antioxidants and polyphenols in inflammatory bowel disease: ulcerative colitis and Crohn disease *Polyphenols: Prevention and treatment of human disease* (pp. 279-292): Elsevier.
- Ashfaq, M., Tahir, M. N., Muhammad, S., Munawar, K. S., Ali, S., Ahmed, G., Ibraheem Khan, M. E. (2022). Shedding light on the synthesis, crystal structure, characterization, and computational study of optoelectronic properties and bioactivity of imine derivatives. *ACS Omega*, 7(6), 5217-5230.
- Atabey-Ozdemir, B., Demirkiran, O., Yildiz, U., Tekin, I., & Coban, B. (2017). Cytotoxicity and DNA binding of copper (II) and zinc (II) complexes of flavonoids: quercitrin, myricitrin, rutin. *BULGARIAN CHEMICAL COMMUNICATIONS*, 49(4), 901-907.

- Atakilt, A., Bayissa, G., Sendek, A., & Kibret, M. (2018). Cobalt (II) complexes with 1, 10-phenanthroline alone and mixed with cytoside: Synthesis and antibacterial activities. *Ethiopian Journal of Science and Technology*, 11(2), 79-96.
- Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Ovais, M., Ullah, I., Shahid, M. (2019). Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. *Frontiers in Aging Neuroscience*, 11.
- Ayukekbong, J. A., Ntemgwa, M., & Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance & Infection Control*, 6(1), 47.
- Azam, A., Raza, M. A., & Sumrra, S. H. (2018). Therapeutic Application of Zinc and Vanadium Complexes against Diabetes Mellitus a Coronary Disease: A review. *Open Chemistry*, 16(1), 1153-1165.
- Azminah, A., Erlina, L., Radji, M., Mun'im, A., Syahdi, R. R., & Yanuar, A. (2019). In silico and in vitro identification of candidate SIRT1 activators from Indonesian medicinal plants compounds database. *Computational biology and chemistry*, 83, 107096.
- Bailey, C. J. (2017). Metformin: historical overview. *Diabetologia*, 60(9), 1566-1576.
- Bakchi, B., Krishna, A. D., Sreecharan, E., Ganesh, V. B. J., Niharika, M., Maharshi, S., Shaik, A. B. J. J. o. M. S. (2022). An Overview on Applications of SwissADME Web Tool in the Design and Development of Anticancer, Antitubercular and Antimicrobial agents: A Medicinal Chemist's Perspective. 132712.
- Balouiri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
- Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life sciences*, 78(5), 431-441.
- Ban, K. A., & Godellas, C. V. (2014). Epidemiology of breast cancer. *Surgical Oncology Clinics*, 23(3), 409-422.
- Banerjee, P., Dehnbostel, F. O., & Preissner, R. (2018). Prediction Is a balancing act: importance of sampling methods to balance sensitivity and specificity of predictive models based on imbalanced chemical data sets. *Frontiers in Chemistry*, 6, 362.
- Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic acids research*, 46(W1), W257-W263.
- Barakat, A., Soliman, S. M., Al-Majid, A. M., Lotfy, G., Ghabbour, H. A., Fun, H.-K., Wadood, A. (2015). Synthesis and structure investigation of novel pyrimidine-2, 4, 6-trione derivatives of highly potential biological activity as anti-diabetic agent. *Journal of Molecular Structure*, 1098, 365-376.
- Bayeh, Y., Abebe, A., Thomas, M., & Linert, W. (2019). Synthesis, characterization and antimicrobial activities of new mixed ligand complexes of copper (II) with 1, 10-phenanthroline and thymine. *Journal of Transition Metal Complexes*, 2, 1-6.
- Beck, A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys*, 98(7), 5648-5646.
- Bhatt, S., Stender, J., Joshi, S., Wu, G., & Katzenellenbogen, B. (2016). OCT-4: a novel estrogen receptor- α collaborator that promotes tamoxifen resistance in breast cancer cells. *Oncogene*, 35(44), 5722-5734.
- Bitew, M., Desalegn, T., Demissie, T. B., Belayneh, A., Endale, M., & Eswaramoorthy, R. (2021). Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. *PLoS One*, 16(12), e0260853.

- Bitu, M. N. A., Hossain, M. S., Zahid, A., Zakaria, C., & Kudrat-E-Zahan, M. J. A. J. o. H. C. (2019). Anti-pathogenic activity of cu (II) complexes incorporating Schiff bases: a short review. *5*(1), 11-23.
- Boros, E., Dyson, P. J., & Gasser, G. (2020). Classification of metal-based drugs according to their mechanisms of action. *Chem*, *6*(1), 41-60.
- Bratu, M. M., Birghila, S., Miresan, H., Negreanu-Pirjol, T., Prajitura, C., & Calinescu, M. (2014). Biological activities of Zn (II) and Cu (II) complexes with quercetin and rutin: Antioxidant properties and UV-protection capacity. *Rev. Chim.(Bucharest)*, *65*, 544-549.
- Bravo-Gómez, M. E., García-Ramos, J. C., Gracia-Mora, I., & Ruiz-Azuara, L. (2009). Antiproliferative activity and QSAR study of copper (II) mixed chelate [Cu (N–N)(acetylacetonato)] NO₃ and [Cu (N–N)(glycinato)] NO₃ complexes,(Casiopeínas®). *Journal of inorganic biochemistry*, *103*(2), 299-309.
- Buchtík, R., Trávníček, Z., & Vančo, J. (2012). In vitro cytotoxicity, DNA cleavage and SOD-mimic activity of copper (II) mixed-ligand quinolinonato complexes. *Journal of inorganic biochemistry*, *116*, 163-171.
- Buchy, P., Ascioğlu, S., Buisson, Y., Datta, S., Nissen, M., Tambyah, P. A., & Vong, S. (2020). Impact of vaccines on antimicrobial resistance. *International Journal of Infectious Diseases*, *90*, 188-196.
- Cannella, V., Altomare, R., Chiaramonte, G., Di Bella, S., Mira, F., Russotto, L., Guercio, A. (2019). Cytotoxicity evaluation of endodontic pins on L929 cell line. *BioMed Research International*, 2019.
- Cassini, A., Högberg, L. D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G. S., Cecchini, M. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *The Lancet infectious diseases*, *19*(1), 56-66.
- Chen, W., Sun, S., Liang, Y., & Song, J. (2009). Antioxidant property of quercetin–Cr (III) complex: The role of Cr (III) ion. *Journal of Molecular Structure*, *918*(1-3), 194-197.
- Chiniforoshan, H., Radani, Z. S., Tabrizi, L., Tavakol, H., Sabzalain, M. R., Mohammadnezhad, G., Plass, W. (2015). Pyrazinamide drug interacting with Co (III) and Zn (II) metal ions based on 2, 2'-bipyridine and 1, 10-phenanthroline ligands: Synthesis, studies and crystal structure, DFT calculations and antibacterial assays. *Journal of Molecular Structure*, *1081*, 237-243.
- Chordia, N., & Kumar, A. (2018). Bioinformatics in Drug Discovery. *SciFed Journal of Protein Science*, *1*(1).
- Choudhary, V., Bhatt, A., Dash, D., & Sharma, N. (2019). DFT calculations on molecular structures, HOMO–LUMO study, reactivity descriptors and spectral analyses of newly synthesized diorganotin (IV) 2-chloridophenylacetohydroxamate complexes. *Journal of computational chemistry*, *40*(27), 2354-2363.
- Christaki, E., Marcou, M., & Tofarides, A. (2020). Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. *Journal of molecular evolution*, *88*(1), 26-40.
- Ciarimboli, G. (2014). Membrane transporters as mediators of cisplatin side-effects. *Anticancer research*, *34*(1), 547-550.
- Clark, D. E. (1999). Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. *Journal of pharmaceutical sciences*, *88*(8), 807-814.

- Claudel, M., Schwarte, J. V., & Fromm, K. M. (2020). New antimicrobial strategies based on metal complexes. *Chemistry*, 2(4), 849-899.
- Collins-Burow, B. M., Antoon, J. W., Frigo, D. E., Elliott, S., Weldon, C. B., Boue, S. M., McLachlan, J. A. (2012). Antiestrogenic activity of flavonoid phytochemicals mediated via the c-Jun N-terminal protein kinase pathway. Cell-type specific regulation of estrogen receptor alpha. *The Journal of steroid biochemistry and molecular biology*, 132(1-2), 186-193.
- Collins-Burow, B. M., Burow, M. E., Duong, B. N., & McLachlan, J. A. (2000). Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and independent mechanisms. *Nutrition and cancer*, 38(2), 229-244.
- Correia, I., Roy, S., Matos, C. P., Borovic, S., Butenko, N., Cavaco, I., Moreno, V. (2015). Vanadium (IV) and copper (II) complexes of salicylaldehydes and aromatic heterocycles: Cytotoxicity, DNA binding and DNA cleavage properties. *Journal of inorganic biochemistry*, 147, 134-146.
- Cuprys, A., Pulicharla, R., Lecka, J., Brar, S. K., Drogui, P., & Surampalli, R. Y. (2018). Ciprofloxacin-metal complexes—stability and toxicity tests in the presence of humic substances. *Chemosphere*, 202, 549-559.
- d'Angelo, J., Morgant, G., Ghermani, N. E., Desmaële, D., Fraisse, B., Bonhomme, F., Journaux, Y. (2008). Crystal structures and physico-chemical properties of Zn (II) and Co (II) tetraaqua (3-nitro-4-hydroxybenzoato) complexes: their anticonvulsant activities as well as related (5-nitrosalicylato)—metal complexes. *Polyhedron*, 27(2), 537-546.
- Da Silva, A. D., De Almeida, M. V., De Souza, M. V., & Couri, M. R. (2003). Biological activity and synthetic methodologies for the preparation of fluoroquinolones, a class of potent antibacterial agents. *Current Medicinal Chemistry*, 10(1), 21-39.
- Dai, W., Bi, J., Li, F., Wang, S., Huang, X., Meng, X., Jiang, C. (2019). Antiviral efficacy of flavonoids against enterovirus 71 infection in vitro and in newborn mice. *Viruses*, 11(7), 625.
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, 7, 42717.
- Daina, A., & Zoete, V. (2016). A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*, 11(11), 1117-1121.
- Damena, T., Alem, M. B., Zeleke, D., Desalegn, T., Eswaramoorthy, R., & Demissie, T. B. (2022a). Novel Zinc (II) and Copper (II) Complexes of 2-((2-Hydroxyethyl) amino) quinoline-3-carbaldehyde for Antibacterial and Antioxidant Activities: A Combined Experimental, DFT, and Docking Studies. *ACS Omega*.
- Damena, T., Alem, M. B., Zeleke, D., Desalegn, T., Eswaramoorthy, R., & Demissie, T. B. (2022b). Synthesis, characterization, and biological activities of zinc (II), copper (II) and nickel (II) complexes of an aminoquinoline derivative. *Frontiers in Chemistry*, 10, 1385.
- Damena, T., Zeleke, D., Desalegn, T., B. Demissie, T., & Eswaramoorthy, R. (2022). Synthesis, Characterization, and Biological Activities of Novel Vanadium(IV) and Cobalt(II) Complexes. *ACS Omega*, 7(5), 4389-4404. doi: 10.1021/acsomega.1c06205
- Das, S., Jana, A., Ramanathan, V., Chakraborty, T., Ghosh, S., Das, P. K., & Bharadwaj, P. K. (2006). Design and synthesis of 1, 10-phenanthroline based Zn (II) complexes bearing 1D push-pull NLO-phores for tunable quadratic nonlinear optical properties. *Journal of organometallic chemistry*, 691(11), 2512-2516.

- Deb, J., Lakshman, T. R., Ghosh, I., Jana, S. S., & Paine, T. K. (2020). Mechanistic studies of in vitro anti-proliferative and anti-inflammatory activities of the Zn (ii)–NSAID complexes of 1, 10-phenanthroline-5, 6-dione in MDA-MB-231 cells. *Dalton Transactions*, 49(32), 11375-11384.
- Dehghan, G., & Khoshkam, Z. (2012). Tin (II)–quercetin complex: Synthesis, spectral characterisation and antioxidant activity. *Food Chemistry*, 131(2), 422-426.
- Demissie, T. B., & Hansen, J. H. (2016). Mechanism and site selectivity in visible-light photocatalyzed C–H functionalization: insights from DFT calculations. *The Journal of Organic Chemistry*, 81(16), 7110-7120.
- Demissie, T. B., Ruud, K., & Hansen, J. H. (2015). DFT as a Powerful Predictive Tool in Photoredox Catalysis: Redox Potentials and Mechanistic Analysis. *Organometallics*, 34(17), 4218-4228.
- Dennington, R., Keith, T., & Millam, J. (2009). GaussView, version 5.
- Devadasu, V. R., Deb, P. K., Maheshwari, R., Sharma, P., & Tekade, R. K. (2018). Physicochemical, Pharmaceutical, and Biological Considerations in GIT Absorption of Drugs *Dosage Form Design Considerations* (pp. 149-178): Academic Press.
- Dharmaraj, N., Viswanathamurthi, P., & Natarajan, K. J. T. M. C. (2001). Ruthenium (II) complexes containing bidentate Schiff bases and their antifungal activity. 26(1), 105-109.
- Dong, J., Liu, B., Liang, G., & Yang, B. (2018). Synthesis, biological activity and toxicity of chromium (III) metformin complex as potential insulin-mimetic agent in C57BL/6 mice. *Journal of Coordination Chemistry*, 71(10), 1526-1541.
- Dong, J., Wang, N.-N., Yao, Z.-J., Zhang, L., Cheng, Y., Ouyang, D., . . . Cao, D.-S. (2018). ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. *Journal of Cheminformatics*, 10(1), 29.
- Dong, J., Wang, N. N., Yao, Z. J., Zhang, L., Cheng, Y., Ouyang, D., . . . Cao, D. S. (2018). Admetlab: A platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. *Journal of Cheminformatics*, 10(1), 1-11.
- dos Santos, P. R., Ely, M. R., Dumas, F., & Moura, S. (2015). Synthesis, structural characterization and previous cytotoxicity assay of Zn (II) complex containing 1, 10-phenanthroline and 2, 2'-bipyridine with valproic acid. *Polyhedron*, 90, 239-244.
- Drwal, M. N., Banerjee, P., Dunkel, M., Wettig, M. R., & Preissner, R. (2014). ProTox: a web server for the in silico prediction of rodent oral toxicity. *Nucleic acids research*, 42(W1), W53-W58.
- Dunn, C. J., & Peters, D. H. (1995). Metformin. *Drugs*, 49(5), 721-749.
- Dutta, T. K., & Mukta, V. (2012). Trace elements. *Medicine*, 22, 352-357.
- Efthimiadou, E. K., Psomas, G., Sanakis, Y., Katsaros, N., & Karaliota, A. (2007). Metal complexes with the quinolone antibacterial agent N-propyl-norfloxacin: synthesis, structure and bioactivity. *Journal of Inorganic Biochemistry*, 101(3), 525-535.
- Ekins, S., Mestres, J., & Testa, B. (2007). In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. *British journal of pharmacology*, 152(1), 9-20.
- El-Megharbel, S. (2015). Synthesis, characterization and antidiabetic activity of chromium (III) metformin complex. *J. Microb. Biochem. Technol*, 7, 65-75.
- El-Sonbati, A., Omar, N., Abou-Dobara, M., Diab, M., El-Mogazy, M., Morgan, S. M., . . . El-Ghettany, A. (2021). Structural, molecular docking computational studies and in-vitro evidence for antibacterial activity of mixed ligand complexes. *Journal of Molecular Structure*, 1239, 130481.

- El-Sonbati, A. Z., Diab, M. A., Eldesoky, A. M., Morgan, S. M., & Salem, O. L. (2019). Polymer complexes. LXXVI. Synthesis, characterization, CT-DNA binding, molecular docking and thermal studies of sulfoxine polymer complexes. *Applied Organometallic Chemistry*, 33(5), e4839.
- El Omari, N., Sayah, K., Fettach, S., El Blidi, O., Bouyahya, A., Faouzi, M. E. A., Barkiyoun, M. (2019). Evaluation of in vitro antioxidant and antidiabetic activities of Aristolochia longa extracts. *Evidence-Based Complementary and Alternative Medicine*, 2019.
- Eleraky, N. E., Allam, A., Hassan, S. B., & Omar, M. M. (2020). Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. *Pharmaceutics*, 12(2), 142.
- Farrell, N. (2003). Metal complexes as drugs and chemotherapeutic agents.
- Ferchichi, A., Makhoulouf, J., El Bakri, Y., Saravanan, K., Valkonen, A., Hashem, H. E., Smirani, W. (2022). Self-assembly of new cobalt complexes based on [Co (SCN) 4], synthesis, empirical, antioxidant activity, and quantum theory investigations. *Scientific reports*, 12(1), 1-18.
- Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D. M., Piñeros, M., Znaor, A., & Bray, F. (2021). Cancer statistics for the year 2020: An overview. *International Journal of Cancer*, 149(4), 778-789.
- Fjellaksel, R., Dugalic, D., Demissie, T. B., Riss, P. J., Hjelstuen, O. K., Sundset, R., & Hansen, J. H. (2018). An acylation-Finkelstein approach to radioiodination of bioactives: The role of amide group anchimeric assistance. *Journal of Physical Organic Chemistry*, 31(7), e3835.
- Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., Chun, O., & analysis. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. 24(7), 1043-1048.
- Frei, A., Zuegg, J., Elliott, A. G., Baker, M., Braese, S., Brown, C., Jung, N. (2020). Metal complexes as a promising source for new antibiotics. *Chemical Science*, 11(10), 2627-2639.
- Frisch, M. J. (2016). Gaussian, Inc. Wallingford, CT, 32, 5648-5652.
- Fujimoto, S., Yasui, H., & Yoshikawa, Y. (2013). Development of a novel antidiabetic zinc complex with an organoselenium ligand at the lowest dosage in KK-Ay mice. *Journal of inorganic biochemistry*, 121, 10-15.
- Gao, J. (2007). A weak hydrolytical copper (II) complex derived from condensation of N, N-Dimethylbiguanide with 2-Pyridinecarbaldehyde synthesis, crystal structure and biological activity. *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry*, 37(8), 621-625.
- Gao, X. (2011). cis-Dichloridobis (1, 10-phenanthroline) chromium (III) chloride. *Acta Crystallographica Section E: Structure Reports Online*, 67(2), m139-m139.
- Gençkal, H. M. (2020). New heteroleptic Cu (II) complexes of chrysin with 2, 2'-bipyridine and substituted 1, 10-phenanthrolines: Synthesis, characterization, thermal stability and antioxidant activity. *Journal of Molecular Structure*, 1209, 127917.
- Gezhagn, T. M., Temam, A. G., & Lelisho, T. A. (2021). Theoretical study on chemical fixation of carbon dioxide with aziridine into cyclic carbamate catalysed by purine/HI system. *Molecular Physics*, 119(5), e1831637.

- Ghose, A. K., Herbertz, T., Salvino, J. M., & Mallamo, J. P. (2006). Knowledge-based chemoinformatic approaches to drug discovery. *Drug discovery today*, 11(23-24), 1107-1114.
- Görlach, A., Dimova, E. Y., Petry, A., Martínez-Ruiz, A., Hernansanz-Agustín, P., Rolo, A. P., . . . Kietzmann, T. (2015). Reactive oxygen species, nutrition, hypoxia and diseases: problems solved? *Redox biology*, 6, 372-385.
- Grimme, S., Furche, F., & Ahlrichs, R. (2002). An improved method for density functional calculations of the frequency-dependent optical rotation. *Chemical physics letters*, 361(3-4), 321-328.
- Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. 94(3), 651-715.
- Guo, C., Zhang, H., Guan, X., & Zhou, Z. (2019). The Anti-Aging Potential of Neohesperidin and Its Synergistic Effects with Other Citrus Flavonoids in Extending Chronological Lifespan of *Saccharomyces Cerevisiae* BY4742. *Molecules*, 24(22), 4093.
- Hakkola, J., Hukkanen, J., Turpeinen, M., & Pelkonen, O. (2020). Inhibition and induction of CYP enzymes in humans: An update. *Archives of toxicology*, 94(11), 3671-3722.
- Halevas, E., Mavroidi, B., Antonoglou, O., Hatzidimitriou, A., Sagnou, M., Pantazaki, A. A., Pelecanou, M. (2020). Structurally characterized gallium–chrysin complexes with anticancer potential. *Dalton Transactions*, 49(8), 2734-2746.
- Halevas, E., Mavroidi, B., & Pelecanou, M. (2021). Structurally characterized zinc complexes of flavonoids chrysin and quercetin with antioxidant potential. 523, 120407.
- Halevas, E., Mavroidi, B., Pelecanou, M., & Hatzidimitriou, A. G. (2021). Structurally characterized zinc complexes of flavonoids chrysin and quercetin with antioxidant potential. *Inorganica Chimica Acta*, 523, 120407.
- Halevas, E., Mitrakas, A., Mavroidi, B., Athanasiou, D., Gkika, P., Antoniou, K., Pantazaki, A. (2021). Structurally characterized copper-chrysin complexes display genotoxic and cytotoxic activity in human cells. *Inorganica Chimica Acta*, 515, 120062.
- Hao, C., Hao, J., Wang, W., Han, Z., Li, G., Zhang, L., Yu, G. (2011). Insulin sensitizing effects of oligomannuronate-chromium (III) complexes in C2C12 skeletal muscle cells. *PLoS One*, 6(9), e24598.
- Hay, P. J., & Wadt, W. R. (1985). Ab initio effective core potentials for molecular calculations. Potentials for the transition metal atoms Sc to Hg. *The Journal of chemical physics*, 82(1), 270-283.
- Hazra, M., Dolai, T., Pandey, A., Dey, S. K., & Patra, A. (2014). Synthesis and characterisation of copper (II) complexes with tridentate NNO functionalized ligand: Density function theory study, DNA binding mechanism, optical properties, and biological application. *Bioinorganic chemistry and applications*, 2014.
- He, J.-W., Yang, L., Mu, Z.-q., Zhu, Y.-Y., Zhong, G.-Y., Liu, Z.-Y., Cheng, F. (2018). Anti-inflammatory and antioxidant activities of flavonoids from the flowers of *Hosta plantaginea*. *RSC advances*, 8(32), 18175-18179.
- Hemmateenejad, B., Akhond, M., Mohammadpour, Z., & Mobaraki, N. (2012). Quantitative monitoring of the progress of organic reactions using multivariate image analysis-thin layer chromatography (MIA-TLC) method. *Analytical Methods*, 4(4), 933-939.
- Heslop-Harrison, J. (1990). Energy dispersive X-ray analysis. *Physical Methods in Plant Sciences*, 244-277.
- Hollenberg, P. F. (2002). Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug metabolism reviews*, 34(1-2), 17-35.

- Holzwarth, U., & Gibson, N. (2011). The Scherrer equation versus the 'Debye-Scherrer equation'. *Nature nanotechnology*, 6(9), 534-534.
- Hsiao, S.-H., Liao, L.-H., Cheng, P.-N., & Wu, T.-J. (2006). Hepatotoxicity associated with acarbose therapy. *Annals of Pharmacotherapy*, 40(1), 151-154.
- Huang, D., Ou, B., Prior, R. L. J. J. o. a., & chemistry, f. (2005). The chemistry behind antioxidant capacity assays. 53(6), 1841-1856.
- Hussain, A., AlAjmi, M. F., Rehman, M., Amir, S., Husain, F. M., Alsalmeh, A., Khan, R. A. (2019). Copper (II) complexes as potential anticancer and Nonsteroidal anti-inflammatory agents: In vitro and in vivo studies. *Scientific reports*, 9(1), 1-17.
- Hyun, T. K., Eom, S. H., & Kim, J.-S. (2014). Molecular docking studies for discovery of plant-derived α -glucosidase inhibitors. *Plant Omics*, 7(3), 166-170.
- Ikeda, N. E. A., Novak, E. M., Maria, D. A., Velosa, A. S., & Pereira, R. M. S. (2015). Synthesis, characterization and biological evaluation of Rutin–zinc (II) flavonoid-metal complex. *Chemico-biological interactions*, 239, 184-191.
- Imran, M., Iqbal, J., Iqbal, S., & Ijaz, N. (2007). In vitro antibacterial studies of ciprofloxacin-imines and their complexes with Cu (II), Ni (II), Co (II), and Zn (II). 31(2), 67-72.
- Ismael, M., Abdel-Mawgoud, A.-M. M., Rabia, M. K., & Abdou, A. (2020). Design and synthesis of three Fe (III) mixed-ligand complexes: Exploration of their biological and phenoxazinone synthase-like activities. *Inorganica Chimica Acta*, 505, 119443.
- Issaoui, N., Ghalla, H., Muthu, S., Flakus, H., & Oujia, B. (2015). Molecular structure, vibrational spectra, AIM, HOMO–LUMO, NBO, UV, first order hyperpolarizability, analysis of 3-thiophenecarboxylic acid monomer and dimer by Hartree–Fock and density functional theory. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 136, 1227-1242.
- Jabeen, E., Janjua, N. K., Ahmed, S., Murtaza, I., Ali, T., Masood, N., Murtaza, G. (2017). DFT predictions, synthesis, stoichiometric structures and anti-diabetic activity of Cu (II) and Fe (III) complexes of quercetin, morin, and primuletin. *Journal of Molecular Structure*, 1150, 459-468.
- Jadeja, R., Chhatrola, M., & Gupta, V. K. (2013). Zn (II) coordination compounds derived from 4-acyl pyrazolones and 1, 10 phenanthroline: Syntheses, crystal structures, spectral analysis and DNA binding studies. *Polyhedron*, 63, 117-126.
- John, L., Dasan, A., Joseyphus, R. S., & Joe, I. H. (2019). Molecular docking, structural characterization, DFT and cytotoxicity studies of metal (II) Schiff base complexes derived from thiophene-2-carboxaldehyde and L-histidine. *Journal of Molecular Structure*, 1198, 126934.
- Johnstone, Park, G. Y., & Lippard, S. J. (2014). Understanding and improving platinum anticancer drugs—phenanthriplatin. *Anticancer research*, 34(1), 471-476.
- Johnstone, Suntharalingam, K., & Lippard, S. (2016). Chem. Rev.
- Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. *Manual of clinical microbiology*, 1253-1273.
- Kacsir, I., Sipos, A., Kiss, T., Major, E., Bajusz, N., Tóth, E., . . . Bokor, É. Half Sandwich-Type Osmium, Ruthenium, Iridium and Rhodium Complexes with Bidentate Glycosyl Heterocyclic Ligands Induce Cytostasis in Platinum-Resistant Ovarian Cancer Cells and Bacteriostasis in Gram-Positive Multiresistant Bacteria. *Frontiers in Chemistry*, 11, 11.
- Karabacak, M., Cinar, Z., Kurt, M., Sudha, S., & Sundaraganesan, N. (2012). FT-IR, FT-Raman, NMR and UV–vis spectra, vibrational assignments and DFT calculations of 4-butyl

- benzoic acid. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 85(1), 179-189.
- Karges, J., Stokes, R. W., & Cohen, S. M. (2021). Metal complexes for therapeutic applications. *Trends in chemistry*, 3(7), 523-534.
- Kebede, B., Yihunie, W., Abebe, D., Addis Tegegne, B., & Belayneh, A. (2022). Gram-negative bacteria isolates and their antibiotic-resistance patterns among pediatrics patients in Ethiopia: A systematic review. *SAGE open medicine*, 10, 20503121221094191.
- Khater, M., Ravishankar, D., Greco, F., & Osborn, H. M. (2019). Metal complexes of flavonoids: their synthesis, characterization and enhanced antioxidant and anticancer activities. *Future Medicinal Chemistry*, 11(21), 2845-2867.
- Kilpin, K. J., & Dyson, P. J. (2013). Enzyme inhibition by metal complexes: concepts, strategies and applications. *Chemical Science*, 4(4), 1410-1419.
- Kim, H. W. (2021). Metabolomic approaches to investigate the effect of metformin: an overview. *International Journal of Molecular Sciences*, 22(19), 10275.
- Kobayashi, A., Tanaka, H., Kumasaki, M., Torii, H., Narymbetov, B., & Adachi, T. (1999). Origin of the High Electrical Conductivity of Neutral [Ni (ptdt) ₂](ptdt₂=propylenedithiotetrathiafulvalenedithiolate): A Route to Neutral Molecular Metal. *Journal of the American Chemical Society*, 121(46), 10763-10771.
- Koleša-Dobravc, T., Maejima, K., Yoshikawa, Y., Meden, A., Yasui, H., & Perdih, F. (2018). Bis (picolinato) complexes of vanadium and zinc as potential antidiabetic agents: synthesis, structural elucidation and in vitro insulin-mimetic activity study. *New Journal of Chemistry*, 42(5), 3619-3632.
- Koobotse, M. O., Schmidt, D., Holly, J. M., & Perks, C. M. (2020). Glucose concentration in cell culture medium influences the BRCA1-mediated regulation of the lipogenic action of IGF-I in breast cancer cells. *International Journal of Molecular Sciences*, 21(22), 8674.
- Kralovec, J. A., Potvin, M. A., Wright, J. H., Watson, L. V., Ewart, H. S., Curtis, J. M., & Barrow, C. J. (2009). Chromium (III)–docosahexaenoic acid complex: Synthesis and characterization. *Journal of Functional Foods*, 1(3), 291-297.
- Krasnovskaya, O., Naumov, A., Guk, D., Gorelkin, P., Erofeev, A., Beloglazkina, E., & Majouga, A. (2020). Copper Coordination Compounds as Biologically Active Agents. *International Journal of Molecular Sciences*, 21(11), 3965.
- Kreger, B. E., Craven, D. E., & McCabe, W. R. (1980). Gram-negative bacteremia: IV. Re-evaluation of clinical features and treatment in 612 patients. *The American journal of medicine*, 68(3), 344-355.
- Krishnan, R., Binkley, J. S., Seeger, R., & Pople, J. A. (1980). Self-consistent molecular orbital methods. XX. A basis set for correlated wave functions. *The Journal of chemical physics*, 72(1), 650-654.
- Krüger, A., Maltarollo, V. G., Wrenger, C., & Kronenberger, T. (2019). ADME Profiling in Drug Discovery and a New Path Paved on Silica *Drug Discovery and Development-New Advances*: IntechOpen.
- Krych, J., & Gebicka, L. (2013). Catalase is inhibited by flavonoids. *International journal of biological macromolecules*, 58, 148-153.
- Kubinyi, H., Mannhold, R., Krosgaard, L., & Timmerman, H. (1993). Methods and principles in medicinal chemistry. *Mannhold, R. et al., eds.*

- KWON, Y. I., Apostolidis, E., & Shetty, K. (2008). Inhibitory potential of wine and tea against α -Amylase and α -Glucosidase for management of hyperglycemia linked to type 2 diabetes. *Journal of Food Biochemistry*, 32(1), 15-31.
- Laguta, I. (2012). Antioxidant properties of Zn (II) and Fe (III) complexes with flavonols. *Хімія, фізика та технологія поверхні*(3, № 4), 448-454.
- Lawal, M., Obaleye, J., Jadeja, R., Bamigboye, M., Gupta, V., Roy, H., & Shaikh, I. (2020). Copper (II) mixed-ligand complexes with fluoroquinolones and an N-donor co-ligand: Structures and biological application. *Polyhedron*, 190, 114753.
- Lee, C., Yang, W., & Parr, R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical review B*, 37(2), 785.
- Lee, C. S., Gorton, E., Neumann, H., & Hunt Jr, H. R. (1966). Optically Active Tris (1, 10-phenanthroline) Complexes of Chromium (III) and Cobalt (III) by Resolution and Synthesis. *Inorganic Chemistry*, 5(8), 1397-1399.
- Lee, S. H., Lee, Y.-s., Song, J. G., & Han, H.-K. (2019). Improved In vivo Effect of Chrysin as an Absorption Enhancer Via the Preparation of Ternary Solid Dispersion with Brij® L4 and Aminoclay. *Current Drug Delivery*, 16(1), 86-92.
- Lemilemu, F., Bitew, M., Demissie, T. B., Eswaramoorthy, R., & Endale, M. (2021). Synthesis, antibacterial and antioxidant activities of Thiazole-based Schiff base derivatives: a combined experimental and computational study. *BMC Chem*, 15(1), 1-18.
- Li, F., Wu, X., Zou, Y., Zhao, T., Zhang, M., Feng, W., & Yang, L. (2012). Comparing anti-hyperglycemic activity and acute oral toxicity of three different trivalent chromium complexes in mice. *Food and chemical toxicology*, 50(5), 1623-1631.
- Li, W., Zhou, J., & Xu, Y. (2015). Study of the in vitro cytotoxicity testing of medical devices. *Biomedical reports*, 3(5), 617-620.
- Liang, J., Sun, D., Yang, Y., Li, M., Li, H., & Chen, L. (2021). Discovery of metal-based complexes as promising antimicrobial agents. *European Journal of Medicinal Chemistry*, 224, 113696.
- Liberta, A. E., & West, D. X. (1992). Antifungal and antitumor activity of heterocyclic thiosemicarbazones and their metal complexes: current status. *Biometals*, 5(2), 121-126.
- Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., . . . Bonaduce, D. (2018). Oxidative stress, aging, and diseases. *Clinical interventions in aging*, 13, 757.
- Lin, S., Zeng, L., Zhang, G., Liao, Y., & Gong, D. (2017). Synthesis, characterization and xanthine oxidase inhibition of Cu (II)–chrysin complex. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 178, 71-78.
- Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews*, 23(1-3), 3-25.
- Loginova, N. V., Harbatsevich, H. I., Osipovich, N. P., Ksendzova, G. A., Koval'chuk, T. V., & Polozov, G. I. (2020). Metal complexes as promising agents for biomedical applications. *Current Medicinal Chemistry*, 27(31), 5213-5249.
- LoPachin, R. M., & Gavin, T. (2014). Molecular mechanisms of aldehyde toxicity: a chemical perspective. *Chemical research in toxicology*, 27(7), 1081-1091.
- LoPachin, R. M., Gavin, T., DeCaprio, A., & Barber, D. S. (2012). Application of the hard and soft, acids and bases (HSAB) theory to toxicant–target interactions. *Chemical research in toxicology*, 25(2), 239-251.

- Lu, P., Wu, Y., Kang, H., Wei, H., Liu, H., & Fang, M. (2014). What can p K a and NBO charges of the ligands tell us about the water and thermal stability of metal organic frameworks? *Journal of Materials Chemistry A*, 2(38), 16250-16267.
- Lusti-Narasimhan, M., Pessoa-Silva, C. L., & Temmerman, M. (2013). Moving forward in tackling antimicrobial resistance: WHO actions. *Sexually transmitted infections*, 89(Suppl 4), iv57-iv59.
- M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, . . . Fox, D. J. (2009). Gaussian 09, Revision A.02. Wallingford CT: Gaussian Inc.
- Mahmoud, M. A., Ammar, A. A., & Sallam, S. A. (2017). Synthesis, characterization and toxicity of Cu (II) complexes with metformin Schiff-bases. *Journal of the Chinese Advanced Materials Society*, 5(2), 79-102.
- Martinez-Gonzalez, A. I., Díaz-Sánchez, Á., de La Rosa, L., Bustos-Jaimes, I., & Alvarez-Parrilla, E. (2019). Inhibition of α -amylase by flavonoids: Structure activity relationship (SAR). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 206, 437-447.
- May, M., & Schindler, C. (2016). Clinically and pharmacologically relevant interactions of antidiabetic drugs. *Therapeutic advances in endocrinology and metabolism*, 7(2), 69-83.
- Merkys, A., Vaitkus, A., Grybauskas, A., Konovalovas, A., Quirós, M., & Gražulis, S. (2023). Graph isomorphism-based algorithm for cross-checking chemical and crystallographic descriptions. *Journal of Cheminformatics*, 15(1), 25.
- Minkel, D. T., Chan-Stier, C., & Petering, D. H. (1976). Reactions of 3-Ethoxy-2-oxobutyraldehyde Bis (N4-dimethylthiosemicarbazono)-Zinc (II) with tumor cells and mitochondria. *Molecular pharmacology*, 12(6), 1036-1044.
- Mitra, K. (2016). Platinum complexes as light promoted anticancer agents: a redefined strategy for controlled activation. *Dalton Transactions*, 45(48), 19157-19171.
- Moges, F., Endris, M., Mulu, A., Tessema, B., Belyhun, Y., Shiferaw, Y., . . . Kassu, A. (2014). The growing challenges of antibacterial drug resistance in Ethiopia. *Journal of global antimicrobial resistance*, 2(3), 148-154.
- Moroy, G., Martiny, V. Y., Vayer, P., Villoutreix, B. O., & Miteva, M. A. (2012). Toward in silico structure-based ADMET prediction in drug discovery. *Drug discovery today*, 17(1-2), 44-55.
- Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., & Olson, A. J. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*, 19(14), 1639-1662.
- Mukherjee, A., & Sadler, P. J. (2007). Metals in medicine: therapeutic agents. *Wiley Encyclopedia of Chemical Biology*, 1-47.
- Mule, N. K., & Singh, J. N. (2018). Diabetes mellitus to neurodegenerative disorders: is oxidative stress fueling the flame? *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, 17(9), 644-653.
- Munteanu, I. G., & Apetrei, C. J. I. J. o. M. S. (2021). Analytical methods used in determining antioxidant activity: A review. 22(7), 3380.
- Murakami, H., Yasui, H., & Yoshikawa, Y. (2012). Pharmacological and pharmacokinetic studies of anti-diabetic tropolonato–Zn (II) complexes with Zn (S2O2) coordination mode. *Chemical and Pharmaceutical Bulletin*, 60(9), 1096-1104.
- Naglah, A. M., Al-Omar, M. A., Almehizia, A. A., AlKahtani, H. M., Bhat, M. A., Al-Shakliah, N. S., Adam, A. M. A. (2021). Synthesis, thermogravimetric, and spectroscopic characterizations of three palladium metal (II) ofloxacin drug and amino acids mixed

- ligand complexes as advanced antimicrobial materials. *Journal of Molecular Structure*, 1225, 129102.
- Nakamoto, K. (2009). *Infrared and Raman spectra of inorganic and coordination compounds, part B: applications in coordination, organometallic, and bioinorganic chemistry*: John Wiley & Sons.
- Nandanwar, S. K., & Kim, H. J. (2019). Anticancer and antibacterial activity of transition metal complexes. *ChemistrySelect*, 4(5), 1706-1721.
- Nathan, C. (2020). Resisting antimicrobial resistance. *Nature Reviews Microbiology*, 18(5), 259-260.
- Nazarian-Samani, Z., Sewell, R. D., Lorigooini, Z., & Rafieian-Kopaei, M. (2018). Medicinal plants with multiple effects on diabetes mellitus and its complications: a systematic review. *Current diabetes reports*, 18(10), 1-13.
- Ndong, P. R., Signé, M., Kenfack, P. T., Mbiangué, Y. A., Bebga, G., & Wenger, E. (2020). Synthesis, Characterization and Thermal Analysis of an Organic-Inorganic Hybrid Salt Involving Trans-Diaquabis (oxalato- κ 2O1, O2) chromate (III) Complex Anion with Piperidinium as Counter Cation. *Crystal Structure Theory and Applications*, 9(2), 36-47.
- Neha, K., Haider, M. R., Pathak, A., & Yar, M. S. (2019). Medicinal prospects of antioxidants: A review. *European journal of medicinal chemistry*, 178, 687-704.
- Nijs, A., Cartuyvels, R., Mewis, A., Peeters, V., Rummens, J., & Magerman, K. (2003). Comparison and evaluation of Osiris and Sirscan 2000 antimicrobial susceptibility systems in the clinical microbiology laboratory. *Journal of clinical microbiology*, 41(8), 3627-3630.
- Noffke, A. L., Habtemariam, A., Pizarro, A. M., & Sadler, P. J. (2012). Designing organometallic compounds for catalysis and therapy. *Chemical Communications*, 48(43), 5219-5246.
- Nunes, P., Correia, I., Marques, F., Matos, A. P., Dos Santos, M. M., Azevedo, C. G., Pinheiro, T. (2020). Copper complexes with 1, 10-phenanthroline derivatives: underlying factors affecting their cytotoxicity. *Inorganic Chemistry*, 59(13), 9116-9134.
- Ojha, H., Gahlot, P., Tiwari, A. K., Pathak, M., & Kakkar, R. (2011). Quantitative Structure Activity Relationship Study of 2, 4, 6-Trisubstituted-s-triazine Derivatives as Antimalarial Inhibitors of Plasmodium Falciparum Dihydrofolate Reductase. *Chemical biology & drug design*, 77(1), 57-62.
- Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., . . . Klugman, K. P. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet infectious diseases*, 5(8), 481-493.
- Olelewe, C., & Awuah, S. G. (2023). Mitochondria as a target of third row transition metal-based anticancer complexes. *Current Opinion in Chemical Biology*, 72, 102235.
- Ong, Y. C., Roy, S., Andrews, P. C., & Gasser, G. (2018). Metal compounds against neglected tropical diseases. *Chemical reviews*, 119(2), 730-796.
- Ördög, R., & Grolmusz, V. (2008). *Evaluating genetic algorithms in protein-ligand docking*. Paper presented at the International Symposium on Bioinformatics Research and Applications.
- Organization, W. H. (2014). WHO Global Epidemiological Surveillance Standards for Influenza (January 2014): Geneva.
- Pal, T. K., Alam, M. A., Paul, S., & Sheikh, M. C. (2019). Spectral, magnetic, thermal, antioxidant and biological studies on new mixed ligand complexes. *Journal of King Saud University-Science*, 31(4), 445-451.

- Pandey, G., Jain, G. C., & Mathur, N. (2012). Therapeutic potential of metals in managing diabetes mellitus: a review. *J. Mol. Pathophysiol*, 1(1), 63-76.
- Pang, X., Fu, W., Wang, J., Kang, D., Xu, L., Zhao, Y., Du, G.-H. (2018). Identification of estrogen receptor α antagonists from natural products via in vitro and in silico approaches. *Oxidative medicine and cellular longevity*, 2018.
- Panhwar, Q. K., & Memon, S. (2014a). Synthesis of Cr (III)-morin complex: Characterization and antioxidant study. *The Scientific World Journal*, 2014.
- Panhwar, Q. K., & Memon, S. (2014b). Synthesis, characterisation, and antioxidant study of Cr (III)-rutin complex. *Chemical Papers*, 68(5), 614-623.
- Patel, Chhasatia, M., & Gandhi, D. (2009). DNA-interaction and in vitro antimicrobial studies of some mixed-ligand complexes of cobalt (II) with fluoroquinolone antibacterial agent ciprofloxacin and some neutral bidentate ligands. *Bioorganic & medicinal chemistry letters*, 19(10), 2870-2873.
- Patel, M. N., Patel, C. R., & Joshi, H. N. (2013). Synthesis, characterization and biological studies of mononuclear copper (II) complexes with ciprofloxacin and N, O donor ligands. *Inorganic Chemistry Communications*, 27, 51-55.
- Pathak, M., Ojha, H., Tiwari, A. K., Sharma, D., Saini, M., & Kakkar, R. (2017). Design, synthesis and biological evaluation of antimalarial activity of new derivatives of 2, 4, 6-s-triazine. *Chemistry Central Journal*, 11(1), 1-11.
- Pechova, A., & Pavlata, L. (2007). Chromium as an essential nutrient: a review. *Veterinarni Medicina-Praha-*, 52(1), 1.
- Pellei, M., Del Bello, F., Porchia, M., & Santini, C. (2021). Zinc coordination complexes as anticancer agents. *Coordination Chemistry Reviews*, 445, 214088.
- Peng, M., & Yang, X. (2015). Controlling diabetes by chromium complexes: The role of the ligands. *Journal of inorganic biochemistry*, 146, 97-103.
- Pereira, A. S., den Haan, H., Peña-García, J., Moreno, M. M., Pérez-Sánchez, H., & Apostolides, Z. (2019). Exploring african medicinal plants for potential anti-diabetic compounds with the DIA-DB inverse virtual screening web server. *Molecules*, 24(10), 2002.
- Pereira, R., Andrades, N. E., Paulino, N., Sawaya, A. C., Eberlin, M. N., Marcucci, M. C., . . . Bydlowski, S. P. (2007). Synthesis and characterization of a metal complex containing naringin and Cu, and its antioxidant, antimicrobial, antiinflammatory and tumor cell cytotoxicity. *Molecules*, 12(7), 1352-1366.
- Pernicova, I., & Korbonits, M. (2014). Metformin—mode of action and clinical implications for diabetes and cancer. *Nature Reviews Endocrinology*, 10(3), 143.
- Pietta, P.-G. (2000). Flavonoids as antioxidants. *Journal of natural products*, 63(7), 1035-1042.
- Piñeros, M., Mery, L., Soerjomataram, I., Bray, F., & Steliarova-Foucher, E. (2021). Scaling up the surveillance of childhood cancer: a global roadmap. *JNCI: Journal of the National Cancer Institute*, 113(1), 9-15.
- Piotto, S., Sessa, L., Diana, R., Torrens, G., Juan, C., Caruso, U., & Iannelli, P. (2017). Synthesis and antimicrobial studies of new antibacterial azo-compounds active against staphylococcus aureus and listeria monocytogenes. *Molecules*, 22(8), 1372.
- Pisoschi, A. M., & Negulescu, G. P. J. B. A. B. (2011). Methods for total antioxidant activity determination: a review. 1(1), 106.
- Plewczynski, D., Łażniewski, M., Grotthuss, M. V., Rychlewski, L., & Ginalska, K. (2011). VoteDock: consensus docking method for prediction of protein–ligand interactions. *Journal of computational chemistry*, 32(4), 568-581.

- Popolin, C. P., Reis, J. P., Becceneri, A. B., Graminha, A. E., Almeida, M. A., Correa, R. S., . . . Cominetti, M. R. (2017). Cytotoxicity and anti-tumor effects of new ruthenium complexes on triple negative breast cancer cells. *PLoS One*, 12(9), e0183275.
- Porchia, M., Pellei, M., Del Bello, F., & Santini, C. (2020). Zinc complexes with nitrogen donor ligands as anticancer agents. *Molecules*, 25(24), 5814.
- Porkodi, J., & Raman, N. (2018). Synthesis, characterization and biological screening studies of mixed ligand complexes using flavonoids as precursors. *Applied Organometallic Chemistry*, 32(2), e4030.
- Prati, F., Bergamini, C., Fato, R., Soukup, O., Korabecny, J., Andrisano, V., . . . Bolognesi, M. L. (2016). Novel 8-hydroxyquinoline derivatives as multitarget compounds for the treatment of Alzheimer' s disease. *ChemMedChem*, 11(12), 1284-1295.
- Pratiwi, R., Ibrahim, S., & Tjahjono, D. H. (2020). Reactivity and Stability of Metalloporphyrin Complex Formation: DFT and Experimental Study. *Molecules*, 25(18), 4221.
- Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and global health*, 109(7), 309-318.
- Prouse, J. (2010). The impact of methods of information on chemotherapy-related side effects. *Clinical journal of oncology nursing*, 14(2).
- Ragab, F., Yahya, T., El-Naa, M., & Arafa, R. (2014). Design, synthesis and structure–activity relationship of novel semi-synthetic flavonoids as antiproliferative agents. *European journal of medicinal chemistry*, 82, 506-520.
- Rahmouni, N. T., el Houda Bensiradj, N., Megatli, S. A., Djebbar, S., & Baitich, O. B. (2019). New mixed amino acids complexes of iron (III) and zinc (II) with isonitrosoacetophenone: Synthesis, spectral characterization, DFT study and anticancer activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 213, 235-248.
- Rajendiran, V., Karthik, R., Palaniandavar, M., Stoeckli-Evans, H., Periasamy, V. S., Akbarsha, M. A., Krishnamurthy, H. (2007). Mixed-ligand copper (II)-phenolate complexes: effect of coligand on enhanced DNA and protein binding, DNA cleavage, and anticancer activity. *Inorganic chemistry*, 46(20), 8208-8221.
- Ramadan, A. E.-M. M., Shaban, S. Y., Ibrahim, M. M., Sallam, S. A., El-Shami, F. I., & Al-Juaid, S. (2020). Metformin-based copper (II) complexes: synthesis, structural characterization and mimicking activity of catechol oxidase and phenoxazinone synthase. *Journal of Materials Science*, 55(15), 6457-6481.
- Raman, N., Muthuraj, V., Ravichandran, S., & Kulandaisamy, A. J. J. o. C. s. (2003). Synthesis, characterisation and electrochemical behaviour of Cu (II), Co (II), Ni (II) and Zn (II) complexes derived from acetylacetone and p-anisidine and their antimicrobial activity. *115*(3), 161-167.
- Ramasamy, S., Muthusamy, S., Nagarajan, S., Nath, A. V., Savarimuthu, J. S., Jayaprakash, J., & Gurunadhan, R. M. (2022). Fabrication of collagen with polyhexamethylene biguanide: A potential scaffold for infected wounds. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 110(3), 535-546.
- Ranasinghe, P., Pigera, S., Galappaththy, P., Katulanda, P., & Constantine, G. R. (2015). Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications. *DARU Journal of Pharmaceutical Sciences*, 23(1), 44.
- Razak, N. A., Abu, N., Ho, W. Y., Zamberi, N. R., Tan, S. W., Alitheen, N. B., . . . Yeap, S. K. (2019). Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells

- via cell cycle arrest, anti-angiogenesis and induction of apoptosis. *Scientific reports*, 9(1), 1-12.
- Refaat, H. M., & El-Din, D. A. N. (2018). Chemical and biological evaluation of moxifloxacin-benzimidazole mixed ligands complexes: Anti-cancer and anti-oxidant activities. *Journal of Molecular Structure*, 1163, 103-113.
- Refat, M. S. (2007). Synthesis and characterization of norfloxacin-transition metal complexes (group 11, IB): spectroscopic, thermal, kinetic measurements and biological activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 68(5), 1393-1405.
- Reina, M., Contreras, L. G. T., De Paz, Y. F., Ruiz-Azuara, L., & Ayala, L. H. (2022). Casiopeinas® as SARS-CoV-2 main protease (M pro) inhibitors. A combined DFT, molecular docking and ONIOM approach. *New Journal of Chemistry*.
- Reina, M., Hernández-Ayala, L. F., Bravo-Gómez, M. E., Gómez, V., & Ruiz-Azuara, L. (2021). Second generation of Casiopeinas®: A joint experimental and theoretical study. *Inorganica Chimica Acta*, 517, 120201.
- Remko, M. (2009). Theoretical study of molecular structure, pKa, lipophilicity, solubility, absorption, and polar surface area of some hypoglycemic agents. *Journal of Molecular Structure: THEOCHEM*, 897(1-3), 73-82.
- Renfrew, A. K. (2014). Transition metal complexes with bioactive ligands: mechanisms for selective ligand release and applications for drug delivery. *Metallomics*, 6(8), 1324-1335.
- Rivera, E., & Gomez, H. (2010). Chemotherapy resistance in metastatic breast cancer: the evolving role of ixabepilone. *Breast Cancer Research*, 12(2), 1-12.
- Rizzotto, M. J. A. s. f. a. a. (2012). Metal complexes as antimicrobial agents. 73-88.
- Roguin, L. P., Chiarante, N., Vior, M. C. G., & Marino, J. (2019). Zinc (II) phthalocyanines as photosensitizers for antitumor photodynamic therapy. *The international journal of biochemistry & cell biology*, 114, 105575.
- Rojas, K., & Stuckey, A. (2016). Breast cancer epidemiology and risk factors. *Clinical obstetrics and gynecology*, 59(4), 651-672.
- Ronconi, L., & Sadler, P. J. (2007). Using coordination chemistry to design new medicines. *Coordination Chemistry Reviews*, 251(13-14), 1633-1648.
- Roope, L. S., Smith, R. D., Pouwels, K. B., Buchanan, J., Abel, L., Eibich, P., . . . Robotham, J. V. (2019). The challenge of antimicrobial resistance: what economics can contribute. *Science*, 364(6435).
- Rosu, T., Pahontu, E., Maxim, C., Georgescu, R., Stanica, N., Almajan, G. L., & Gulea, A. (2010). Synthesis, characterization and antibacterial activity of some new complexes of Cu (II), Ni (II), VO (II), Mn (II) with Schiff base derived from 4-amino-2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one. *Polyhedron*, 29(2), 757-766.
- Roth, R., Coemert, S., Burkhardt, S., Rodewald, K. S., & Lueth, T. C. (2020). A process towards eliminating cytotoxicity by removal of surface contamination from electrical discharge machined nitinol. *Procedia CIRP*, 89, 45-51.
- Ruiz-Azuara, L., & E Bravo-Gomez, M. (2010). Copper compounds in cancer chemotherapy. *Current Medicinal Chemistry*, 17(31), 3606-3615.
- Ruiz-Morales, Y. (2002). HOMO– LUMO gap as an index of molecular size and structure for polycyclic aromatic hydrocarbons (PAHs) and asphaltenes: A theoretical study. I. *The Journal of Physical Chemistry A*, 106(46), 11283-11308.

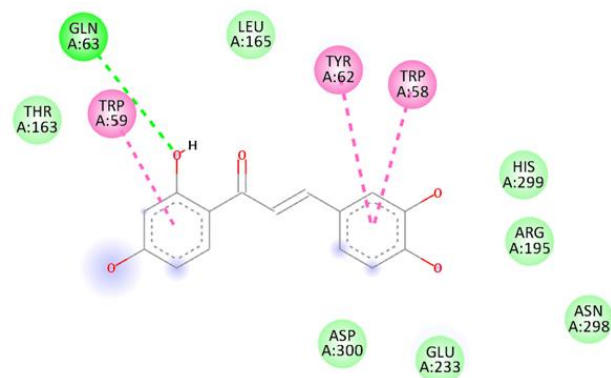
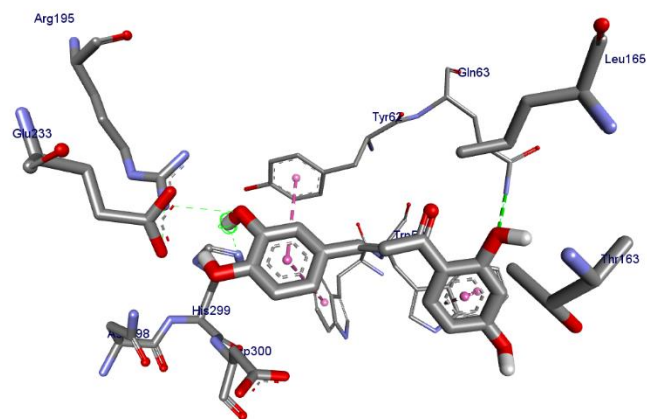
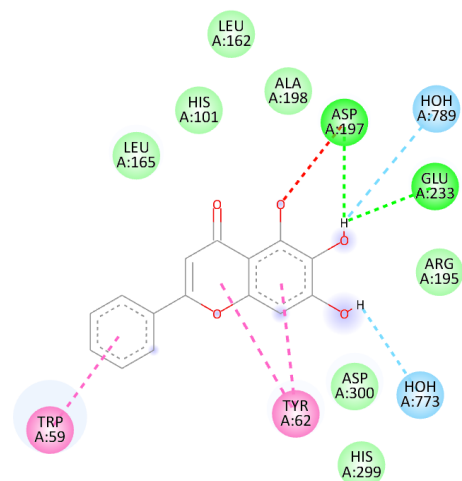
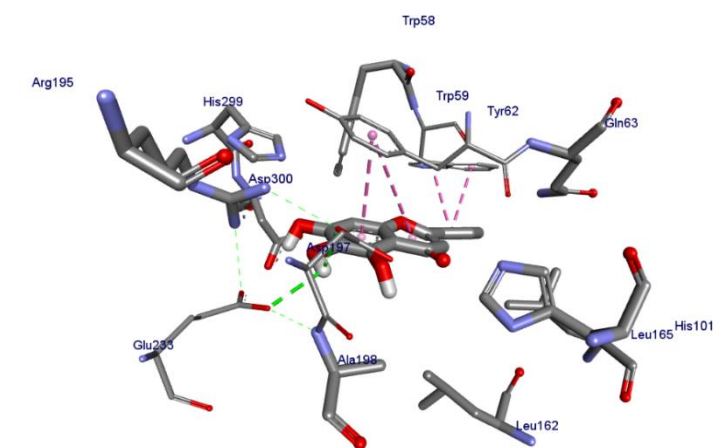
- Sadeghi, M., Moradi, M., Madanchi, H., & Johari, B. (2021). In silico study of garlic (*Allium sativum* L.)-derived compounds molecular interactions with α -glucosidase. *In Silico Pharmacology*, 9(1), 1-8.
- Saha, D. K., Sandbhor, U., Shirisha, K., Padhye, S., Deobagkar, D., Anson, C. E., & Powell, A. K. (2004). A novel mixed-ligand antimycobacterial dimeric copper complex of ciprofloxacin and phenanthroline. *Bioorganic & medicinal chemistry letters*, 14(12), 3027-3032.
- Sahoo, S., Chakraborti, C. K., & Mishra, S. C. (2011). Qualitative analysis of controlled release ciprofloxacin/carbopol 934 mucoadhesive suspension. *Journal of advanced pharmaceutical technology & research*, 2(3), 195.
- Salehi, B., Ata, A., V Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., Amiruddin Zakaria, Z. (2019). Antidiabetic potential of medicinal plants and their active components. *Biomolecules*, 9(10), 551.
- Samsonowicz, M., & Regulska, E. (2017). Spectroscopic study of molecular structure, antioxidant activity and biological effects of metal hydroxyflavonol complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 173, 757-771.
- Scalese, G., Kostenkova, K., Crans, D. C., & Gambino, D. (2022). Metallomics and other omics approaches in antiparasitic metal-based drug research. *Current Opinion in Chemical Biology*, 67, 102127.
- Schattschneider, C., Kettenmann, S. D., Hinojosa, S., Heinrich, J., & Kulak, N. (2019). Biological activity of amphiphilic metal complexes. *Coordination Chemistry Reviews*, 385, 191-207.
- Schyman, P., Liu, R., Desai, V., & Wallqvist, A. (2017). vNN web server for ADMET predictions. *Frontiers in pharmacology*, 8, 889.
- Shin, H. K., Kang, Y.-M., & No, K. T. (2017). Predicting ADME properties of chemicals. *Handbook of Computational Chemistry*, Springer, Cham, 2265-2301.
- Sies, H. (1997). Oxidative stress: oxidants and antioxidants. 82(2), 291-295.
- Singh, R., Arif, T., Khan, I., & Sharma, P. (2014). Phytochemicals in antidiabetic drug discovery. *J Biomed Ther Sci*, 1, 1-33.
- Spoerlein, C., Mahal, K., Schmidt, H., & Schobert, R. (2013). Effects of chrysin, apigenin, genistein and their homoleptic copper (II) complexes on the growth and metastatic potential of cancer cells. *Journal of inorganic biochemistry*, 127, 107-115.
- Srivastava, G. K., Alonso-Alonso, M. L., Fernandez-Bueno, I., Garcia-Gutierrez, M. T., Rull, F., Medina, J., Pastor, J. C. (2018). Comparison between direct contact and extract exposure methods for PFO cytotoxicity evaluation. *Scientific reports*, 8(1), 1-9.
- Stefanidou, M., Maravelias, C., Dona, A., & Spiliopoulou, C. (2006). Zinc: a multipurpose trace element. *Archives of toxicology*, 80, 1-9.
- Stephens, P. J., Devlin, F. J., Chabalowski, C. F., & Frisch, M. J. (1994). Ab initio calculation of vibrational absorption and circular dichroism spectra using density functional force fields. *The Journal of physical chemistry*, 98(45), 11623-11627.
- Sulpizio, C., Müller, S. T., Zhang, Q., Brecker, L., & Rompel, A. (2016). Synthesis, characterization, and antioxidant activity of Zn ²⁺ and Cu ²⁺ coordinated polyhydroxychalcone complexes. *Monatshefte für Chemie-Chemical Monthly*, 147(11), 1871-1881.
- Sun, Y., Ma, C., Sun, H., Wang, H., Peng, W., Zhou, Z., He, X. (2020). Metabolism: a novel shared link between diabetes mellitus and Alzheimer's disease. *Journal of diabetes research*, 2020.

- Sundaraganesan, N., Mariappan, G., & Manoharan, S. (2012). Molecular structure and vibrational spectroscopic studies of chrysin using HF and density functional theory. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 87, 67-76.
- Tamiru, G., Abebe, A., Abebe, M., & Liyew, M. (2019). Synthesis, structural investigation and biological application of new mono-and binuclear cobalt (II) mixed-ligand complexes containing 1, 10-phenanthroline, acetamide and ethylenediamine. *Ethiopian Journal of Science and Technology*, 12(1), 69-91.
- Tandon, H., Chakraborty, T., & Suhag, V. (2019). A new scale of the electrophilicity index invoking the force concept and its application in computing the internuclear bond distance. *Journal of Structural Chemistry*, 60(11), 1725-1734.
- Tapiero, H., & Tew, K. D. (2003). Trace elements in human physiology and pathology: zinc and metallothioneins. *Biomedicine & Pharmacotherapy*, 57(9), 399-411.
- Testai, L., Martelli, A., Cristofaro, M., Breschi, M. C., & Calderone, V. (2013). Cardioprotective effects of different flavonoids against myocardial ischaemia/reperfusion injury in Langendorff-perfused rat hearts. *Journal of Pharmacy and Pharmacology*, 65(5), 750-756.
- Tiwari, A. K., Ojha, H., Kaul, A., Dutta, A., Srivastava, P., Shukla, G., Mishra, A. K. (2009). Quantitative Structure–Property Relationship (Correlation Analysis) of Phosphonic Acid-Based Chelates in Design of MRI Contrast Agent. *Chemical biology & drug design*, 74(1), 87-91.
- Tweedy, B. J. P. (1964). Plant extracts with metal ions as potential antimicrobial agents. 55, 910-914.
- Ulas, M., Orhan, C., Tuzcu, M., Ozercan, I. H., Sahin, N., Gencoglu, H., Sahin, K. (2015). Anti-diabetic potential of chromium histidinate in diabetic retinopathy rats. *BMC complementary and alternative medicine*, 15(1), 16.
- Vaitkus, A., Merkys, A., & Gražulis, S. (2021). Validation of the crystallography open database using the crystallographic information framework. *Journal of Applied Crystallography*, 54(2), 661-672.
- van Waterschoot, R. A., & Schinkel, A. H. (2011). A critical analysis of the interplay between cytochrome P450 3A and P-glycoprotein: recent insights from knockout and transgenic mice. *Pharmacological reviews*, 63(2), 390-410.
- Vasanth, P., Shekhar, B., & PV, A. L. (2018). Copper-metformin ternary complexes: Thermal, photochemosensitivity and molecular docking studies. *Materials Science and Engineering: C*, 90, 621-633.
- Viganor, L., Howe, O., McCarron, P., McCann, M., & Devereux, M. (2017). The antibacterial activity of metal complexes containing 1, 10-phenanthroline: potential as alternative therapeutics in the era of antibiotic resistance. *Current topics in medicinal chemistry*, 17(11), 1280-1302.
- Vijayakumar, B. G., Ramesh, D., Joji, A., & Kannan, T. (2020). In silico pharmacokinetic and molecular docking studies of natural flavonoids and synthetic indole chalcones against essential proteins of SARS-CoV-2. *European Journal of Pharmacology*, 173448.
- Vijayaraghavan, K., Pillai, S. I., & Subramanian, S. P. (2012). Design, synthesis and characterization of zinc-3 hydroxy flavone, a novel zinc metallo complex for the treatment of experimental diabetes in rats. *European journal of pharmacology*, 680(1-3), 122-129.
- Villamizar-Delgado, S., Porras-Osorio, L. M., Pineros, O., Ellena, J., Balcazar, N., Varela-Miranda, R. E., & D'Vries, R. F. (2020). Biguanide–transition metals complexes as potential drug for hyperglycemia treatment. *RSC Advances*, 10(38), 22856-22863.

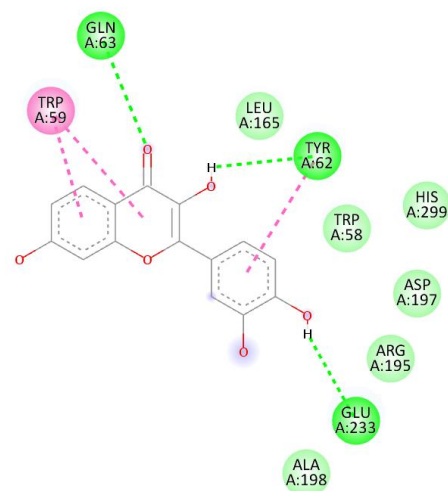
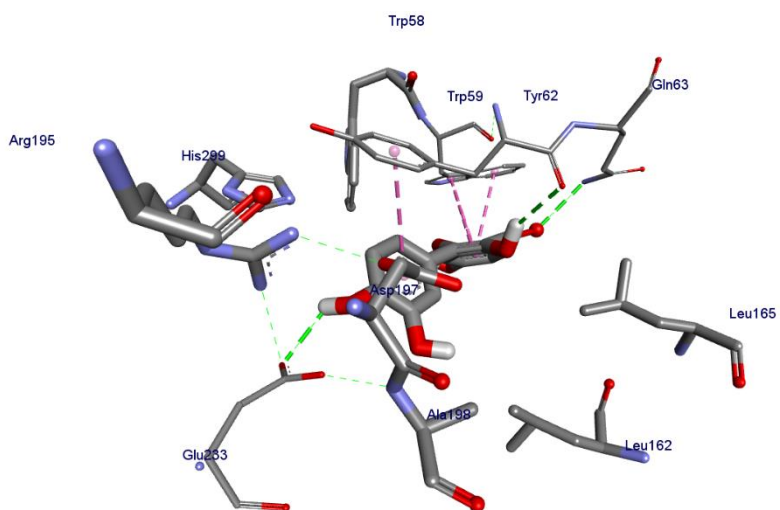
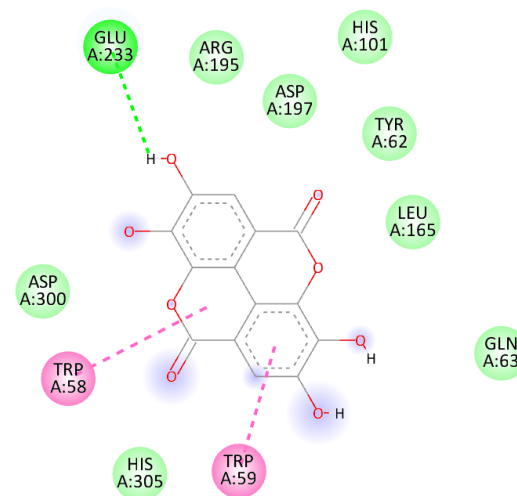
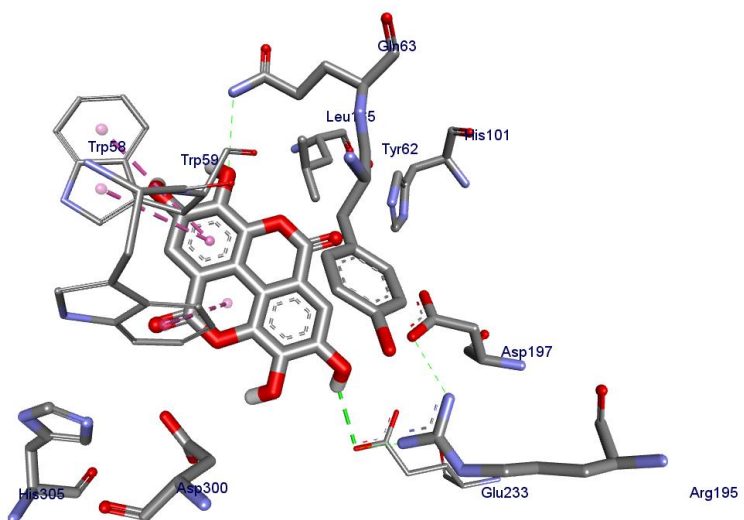
- Villamizar-Delgado, S., Porras-Osorio, L. M., Piñeros, O., Ellena, J., Balcazar, N., Varela-Miranda, R. E., & D'Vries, R. F. (2020). Biguanide–transition metals complexes as potential drug for hyperglycemia treatment. *RSC Advances*, 10(38), 22856-22863.
- Vinayagam, R., & Xu, B. (2015). Antidiabetic properties of dietary flavonoids: a cellular mechanism review. *Nutr Metab (Lond)*, 12, 60. doi: 10.1186/s12986-015-0057-7
- Vincent, J. B. (2004). Recent advances in the nutritional biochemistry of trivalent chromium. *Proceedings of the Nutrition Society*, 63(1), 41-47.
- Wang, C., Chen, Z., Pan, Y., Gao, X., & Chen, H. (2017). Anti-diabetic effects of Inonotus obliquus polysaccharides-chromium (III) complex in type 2 diabetic mice and its sub-acute toxicity evaluation in normal mice. *Food and Chemical Toxicology*, 108, 498-509.
- Wang, C., Gao, X., Santhanam, R. K., Chen, Z., Chen, Y., Xu, L., Chen, H. (2018a). Effects of polysaccharides from Inonotus obliquus and its chromium (III) complex on advanced glycation end-products formation, α -amylase, α -glucosidase activity and H₂O₂-induced oxidative damage in hepatic L02 cells. *Food and Chemical Toxicology*, 116, 335-345.
- Wang, C., Gao, X., Santhanam, R. K., Chen, Z., Chen, Y., Xu, L., Chen, H. (2018b). Effects of polysaccharides from Inonotus obliquus and its chromium (III) complex on advanced glycation end-products formation, α -amylase, α -glucosidase activity and H₂O₂-induced oxidative damage in hepatic L02 cells. *Food Chem. Toxicol.*, 116, 335-345.
- Wang, Y., Lin, G.-W., Hong, J., Li, L., Yang, Y.-M., & Lu, T. (2010). Synthesis, structure, DNA binding and cleavage ability of a new copper ciprofloxacin complex. *Journal of Coordination Chemistry*, 63(20), 3662-3675.
- Waring, M. J., Arrowsmith, J., Leach, A. R., Leeson, P. D., Mandrell, S., Owen, R. M., Wang, J. (2015). An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nature reviews Drug discovery*, 14(7), 475-486.
- Wei, W., Liu, Q., Tan, Y., Liu, L., Li, X., & Cai, L. (2009). Oxidative stress, diabetes, and diabetic complications. *Hemoglobin*, 33(5), 370-377.
- Williams, L. K., Zhang, X., Caner, S., Tysoe, C., Nguyen, N. T., Wicki, J., Yuen, V. (2015). The amylase inhibitor montbretin A reveals a new glycosidase inhibition motif. *Nature chemical biology*, 11(9), 691-696.
- Willyard, C. (2017). The drug-resistant bacteria that pose the greatest health threats. *Nature News*, 543(7643), 15.
- Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., Sayeeda, Z. (2018). DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic acids research*, 46(D1), D1074-D1082.
- Xie, M., Zhao, H., Liu, Q., Zhu, Y., Yin, F., Liang, Y., Li, Z. (2017). Structural Basis of Inhibition of ER α -Coactivator Interaction by High-Affinity N-Terminus Isoaspartic Acid Tethered Helical Peptides. *Journal of Medicinal Chemistry*, 60(21), 8731-8740. doi: 10.1021/acs.jmedchem.7b00732
- Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current Medicinal Chemistry*, 22(1), 132-149.
- Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Lu, A. J. N. A. R. (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. 49(W1), W5-W14.
- Xue, H., Li, J., Xie, H., & Wang, Y. (2018). Review of drug repositioning approaches and resources. *International journal of biological sciences*, 14(10), 1232.

- Yu, L.-C., Chen, Z.-F., Liang, H., Zhou, C.-S., & Fun, H.-K. (2006). Hydrothermal synthesis, crystal structure and antibacterial studies of nickel (II) and manganese (II) complexes with ciprofloxacin. *Journal of Coordination Chemistry*, 59(9), 953-959.
- Yu, L.-C., Tang, Z.-L., Yi, P.-G., & Liu, S.-L. (2009). Hydrothermal syntheses, crystal structures and antibacterial activities of two transition metal complexes with ciprofloxacin. *Journal of Coordination Chemistry*, 62(6), 894-902.
- Yu, L.-C., Tang, Z.-L., Yi, P.-G., Liu, S.-L., & Li, X. (2008). Hydrothermal syntheses, crystal structures and antibacterial activities of two Cu (II) complexes with quinolones. *Journal of Coordination Chemistry*, 61(18), 2961-2967.
- Zafar, R., Naureen, H., Zubair, M., Shahid, K., Saeed Jan, M., Akhtar, S., Ali, S. (2021). Prospective Application of Two New Pyridine-Based Zinc (II) Amide Carboxylate in Management of Alzheimer's Disease: Synthesis, Characterization, Computational and in vitro Approaches. *Drug Design, Development and Therapy*, 2679-2694.
- Zhang, C. X., & Lippard, S. J. (2003). New metal complexes as potential therapeutics. *Current Opinion in Chemical Biology*, 7(4), 481-489.
- Zhang, J., Yue, L., Hayat, K., Xia, S., Zhang, X., Ding, B., Chen, Z. (2010). Purification of flavonoid from Gingko biloba extract by zinc complexation method and its effect on antioxidant activity. *Separation and purification technology*, 71(3), 273-278.
- Zhang, L., Chen, Q., Li, L., Kwong, J. S., Jia, P., Zhao, P., . . . Sun, X. (2016). Alpha-glucosidase inhibitors and hepatotoxicity in type 2 diabetes: a systematic review and meta-analysis. *Scientific reports*, 6, 32649.
- Zhang, X., Huang, H., Zhao, X., Lv, Q., Sun, C., Li, X., & Chen, K. (2015). Effects of flavonoids-rich Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) pulp extracts on glucose consumption in human HepG2 cells. *Journal of Functional Foods*, 14, 144-153.
- Zhou, Y., Wu, F., Li, L., Shen, X., Chen, G., Wang, X., Huang, Z. (2020). Computational approaches in preclinical studies on drug discovery and development. *Frontiers in Chemistry*, 8, 726.
- Zhu, Y., Xiong, X., Suo, Z., Tang, P., Sun, Q., Ding, X., & Li, H. (2019). Synthesis, structure, and DNA-binding study of a novel Zn (II) complex with fleroxacin and 1, 10-phenanthroline monohydrate. *Inorganic Chemistry Communications*, 103, 6-11.

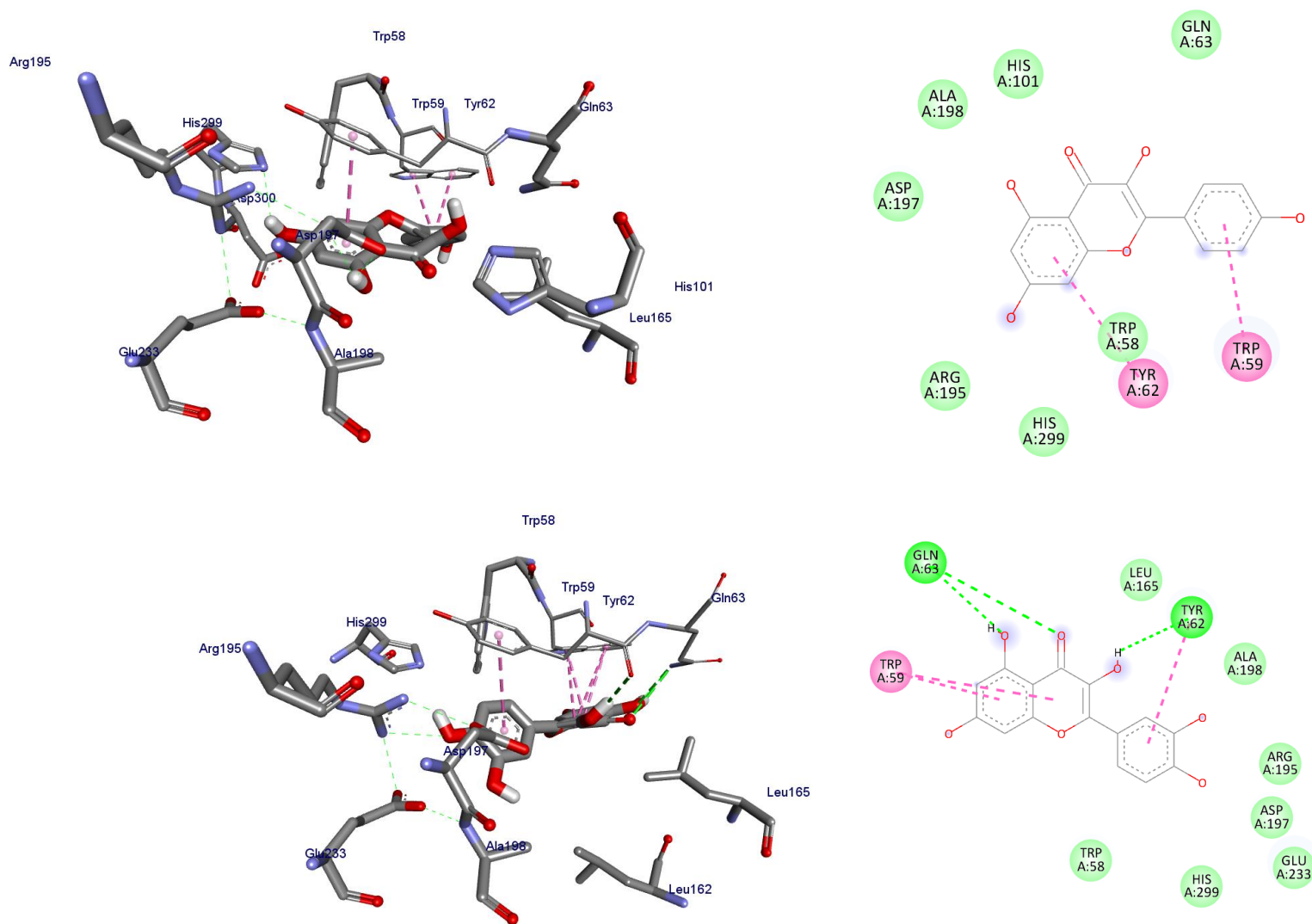
APPENDIX



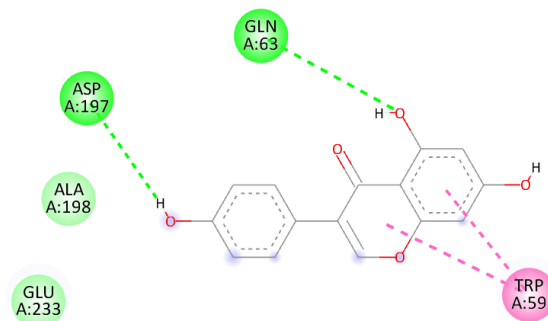
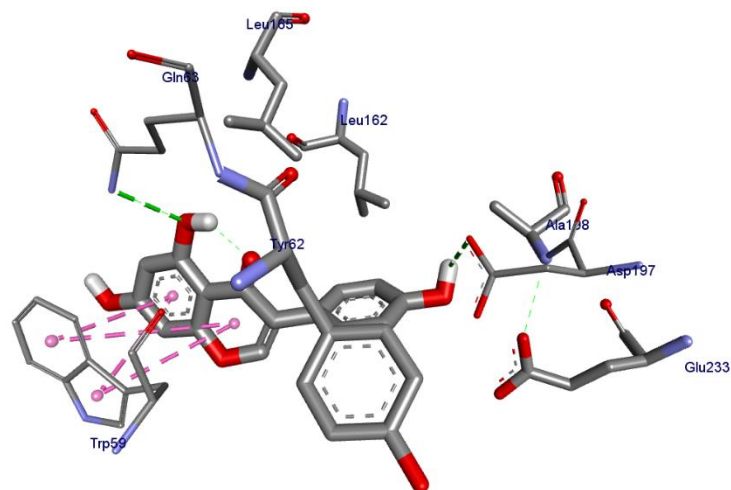
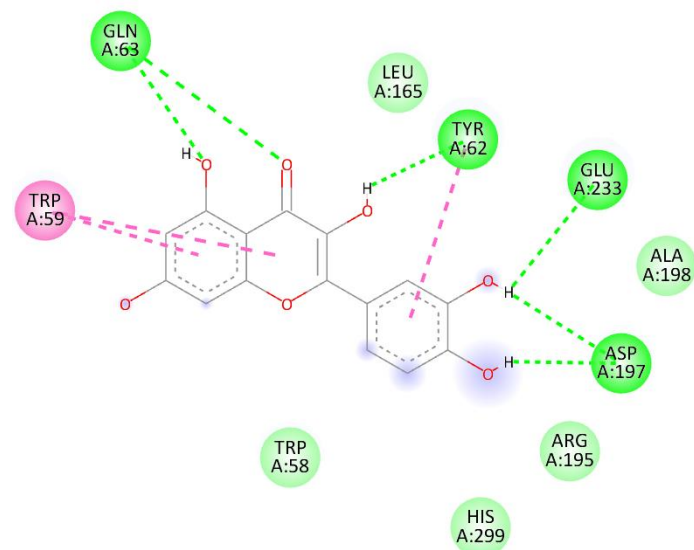
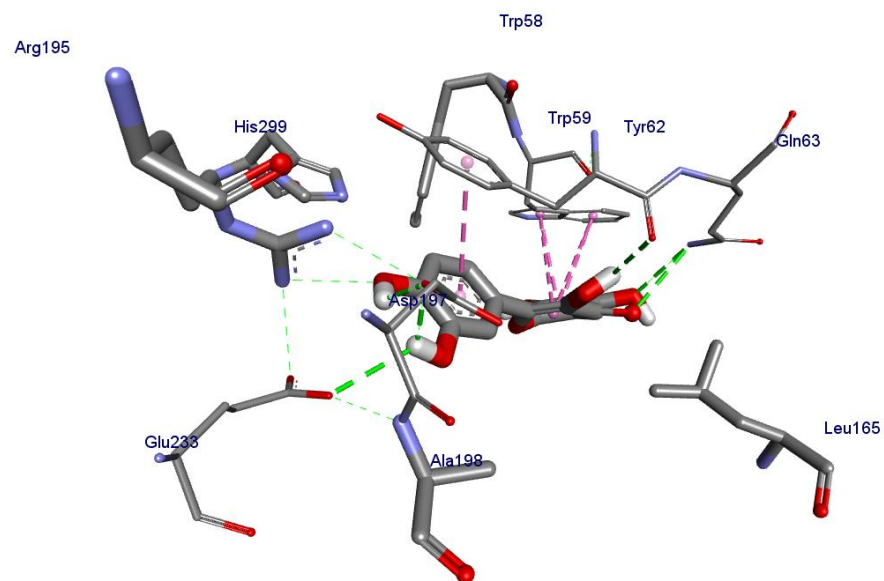
Appendix 1. 2D and 3D representations of the binding modes of compounds Bicalien (37) and Butien (38) to Human α -amylase.



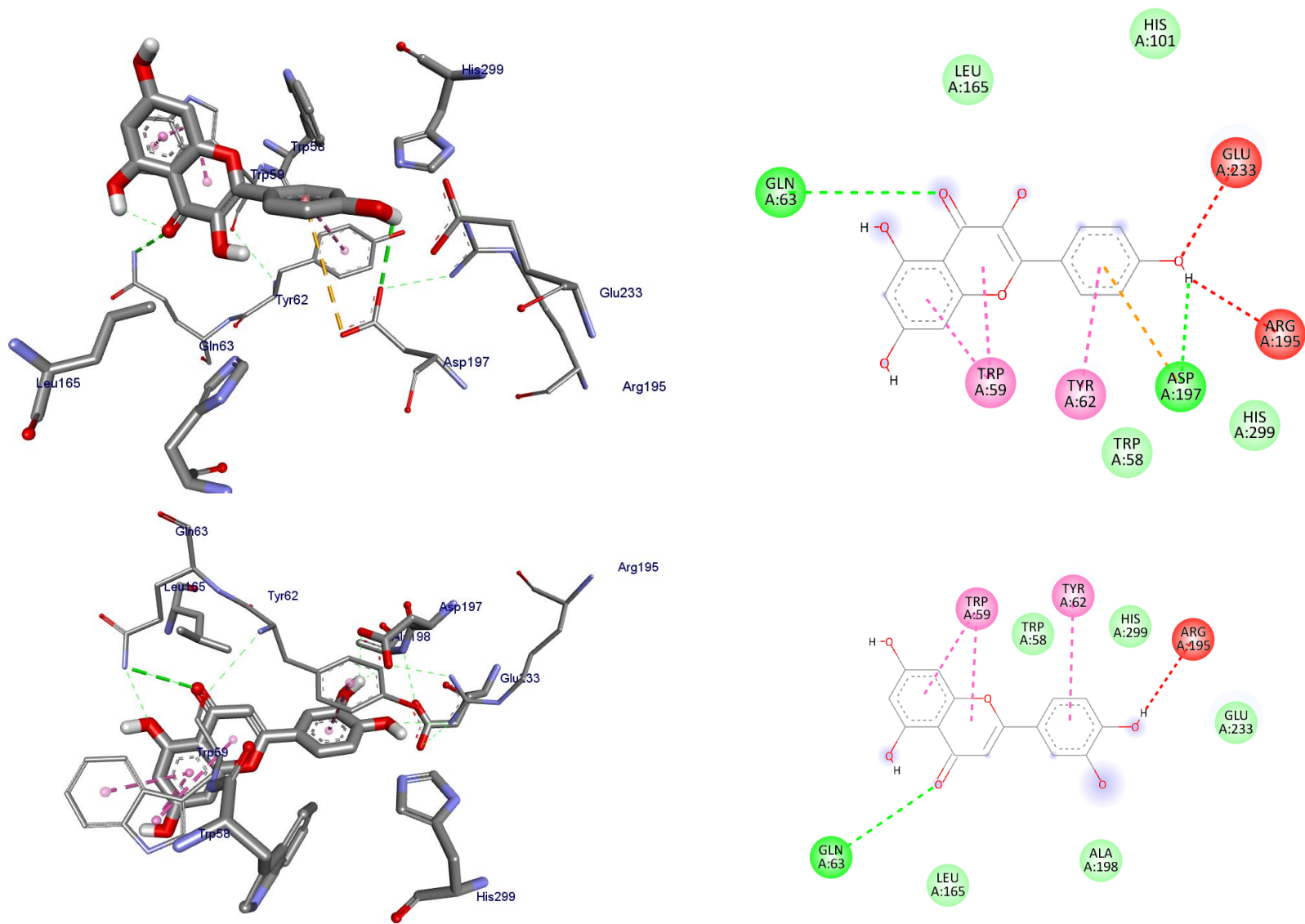
Appendix 2.2D and 3D representations of the binding modes of Ellagic acid (40) and Eriodyctiol (41).



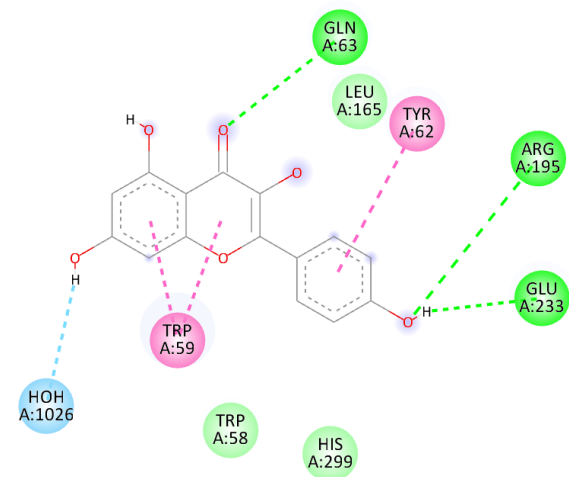
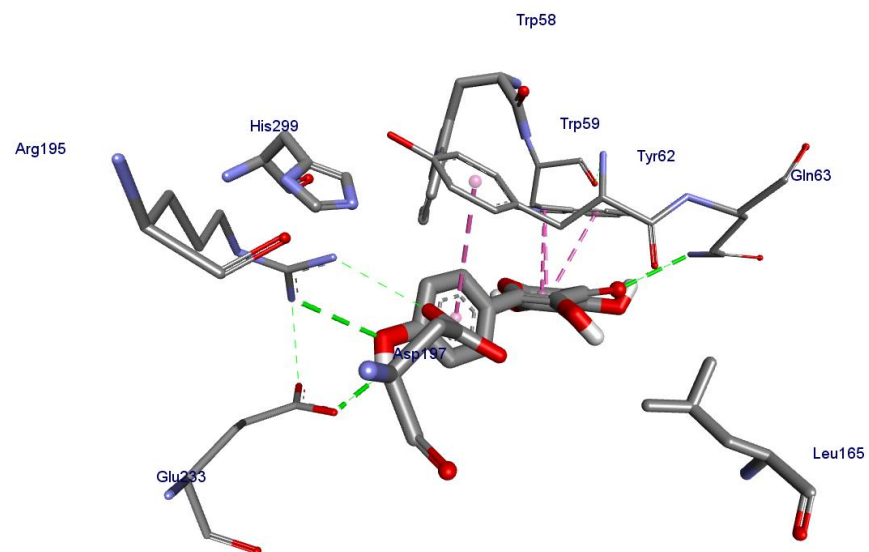
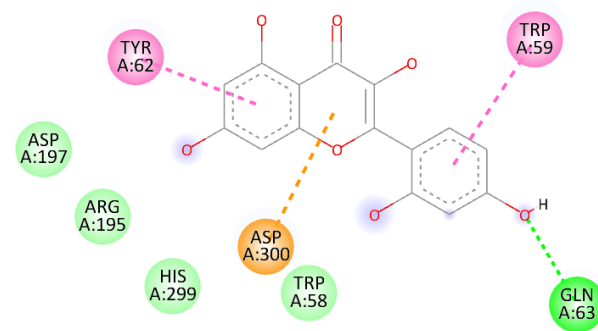
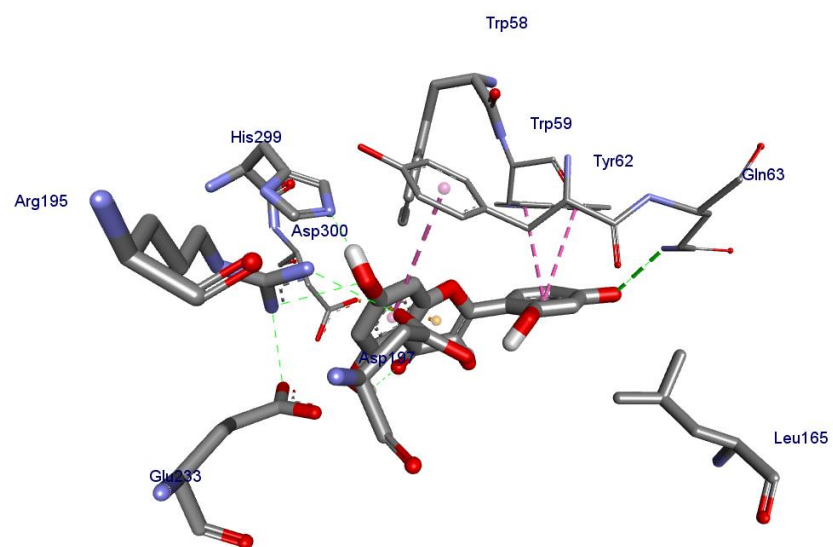
Appendix 3. 2D and 3D representations of the binding modes of Fisetin (42) and Kaempferol (43).



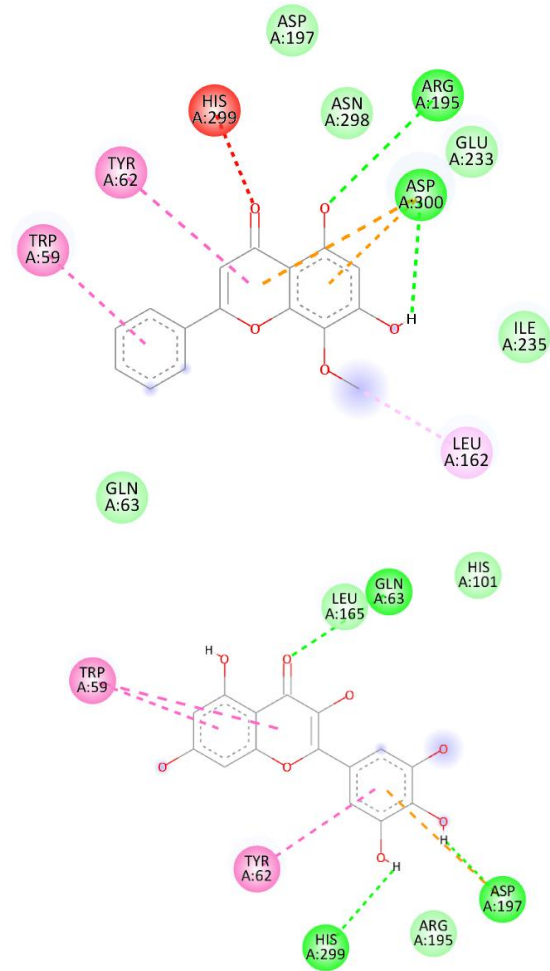
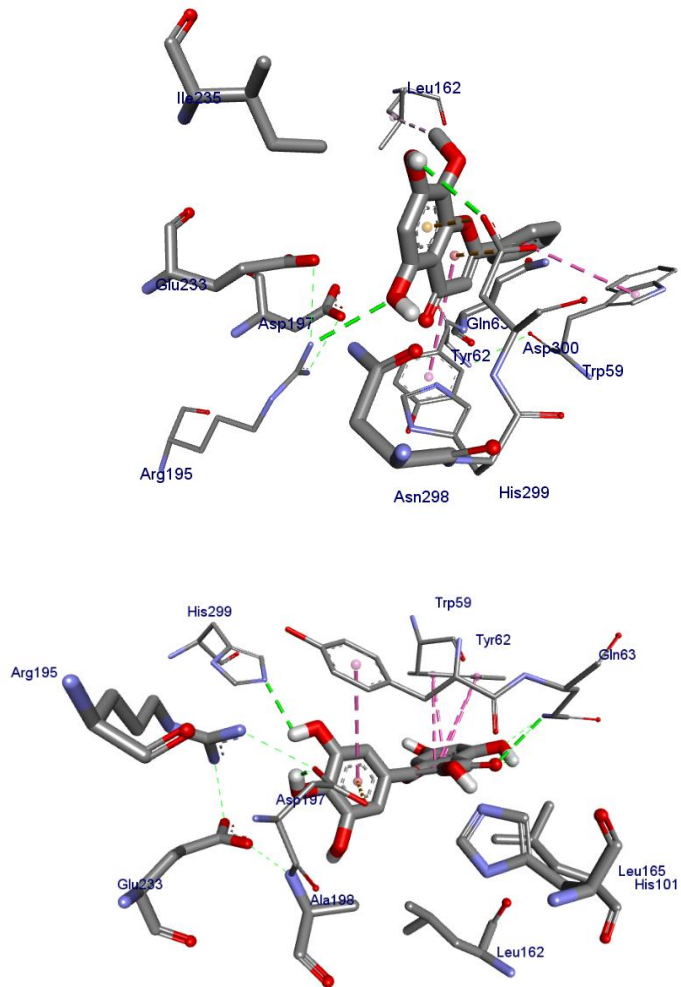
Appendix 4. 2D and 3D representations of the binding modes of Quercetin (44) and Genistein (45).



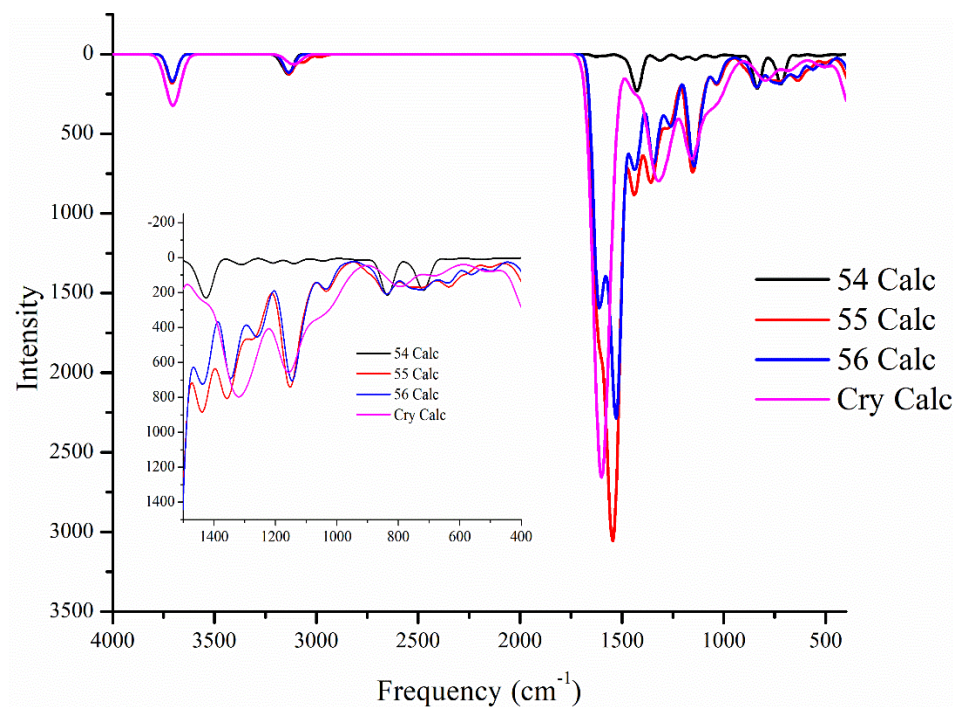
Appendix 5. 2D and 3D representations of the binding modes of Isorhamnetin (46) and Luteolin (47).



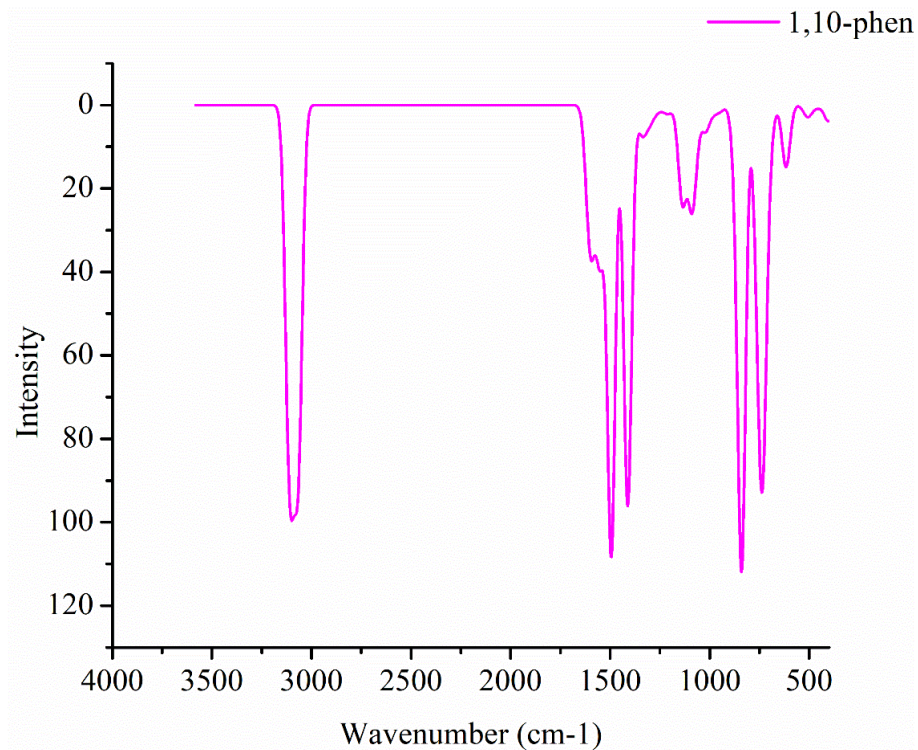
Appendix 6. 2D and 3D representations of the binding modes of Morin (48) and Naringenin (49)



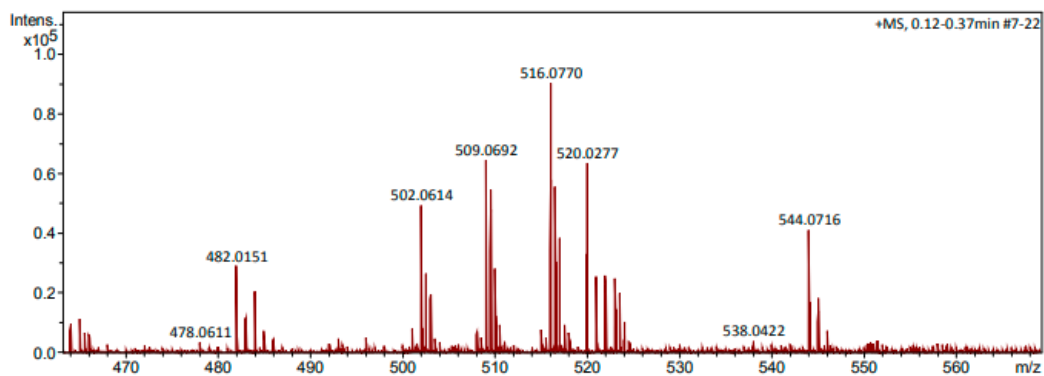
Appendix 7. 2D and 3D representations of the binding modes of Wogonin (50) and Myricetin (51)



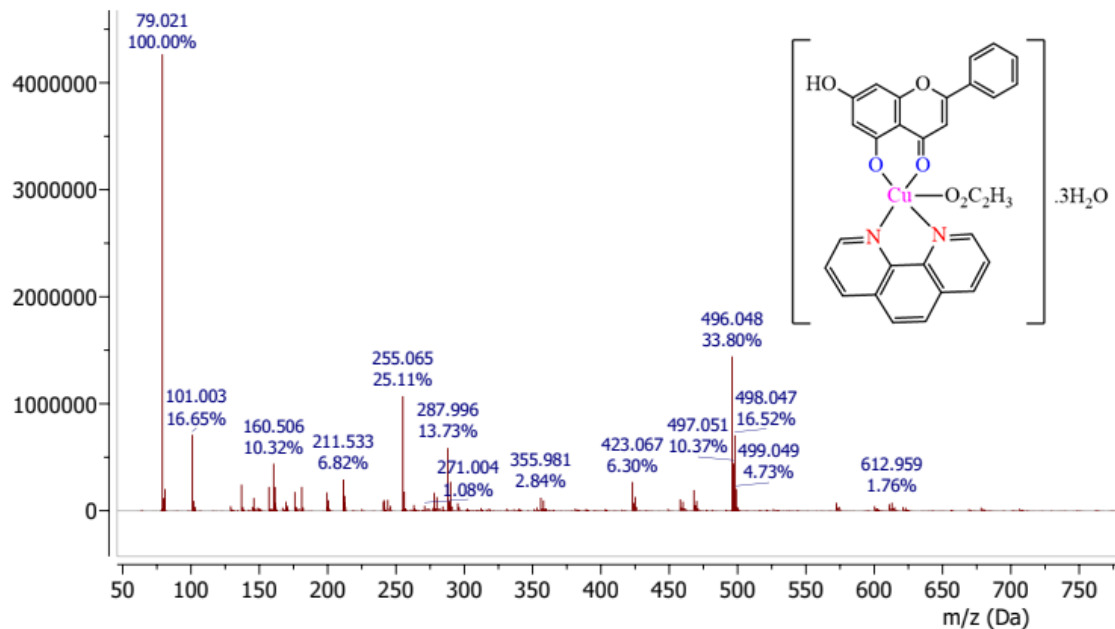
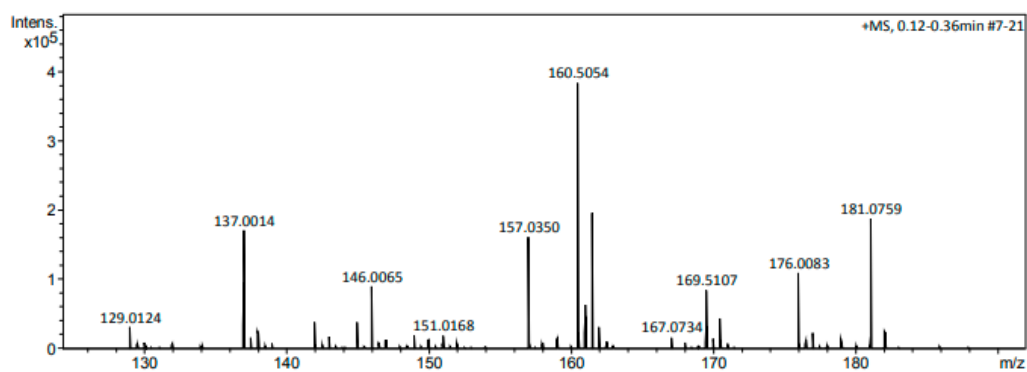
Appendix 8. Calculated FTIR spectra of chrysin and its complexes (54 – 56)



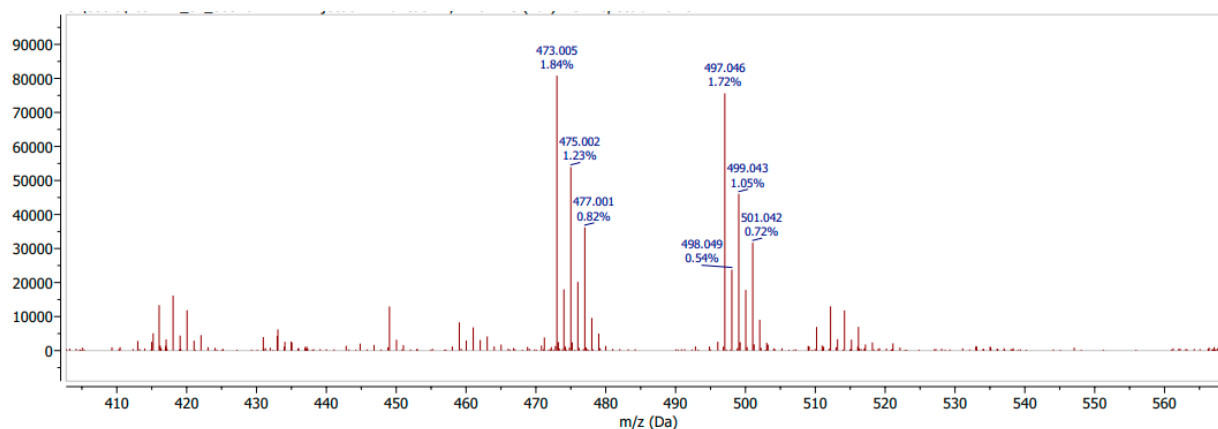
Appendix 9. Calculated FTIR spectra of 1,10-phenanthroline



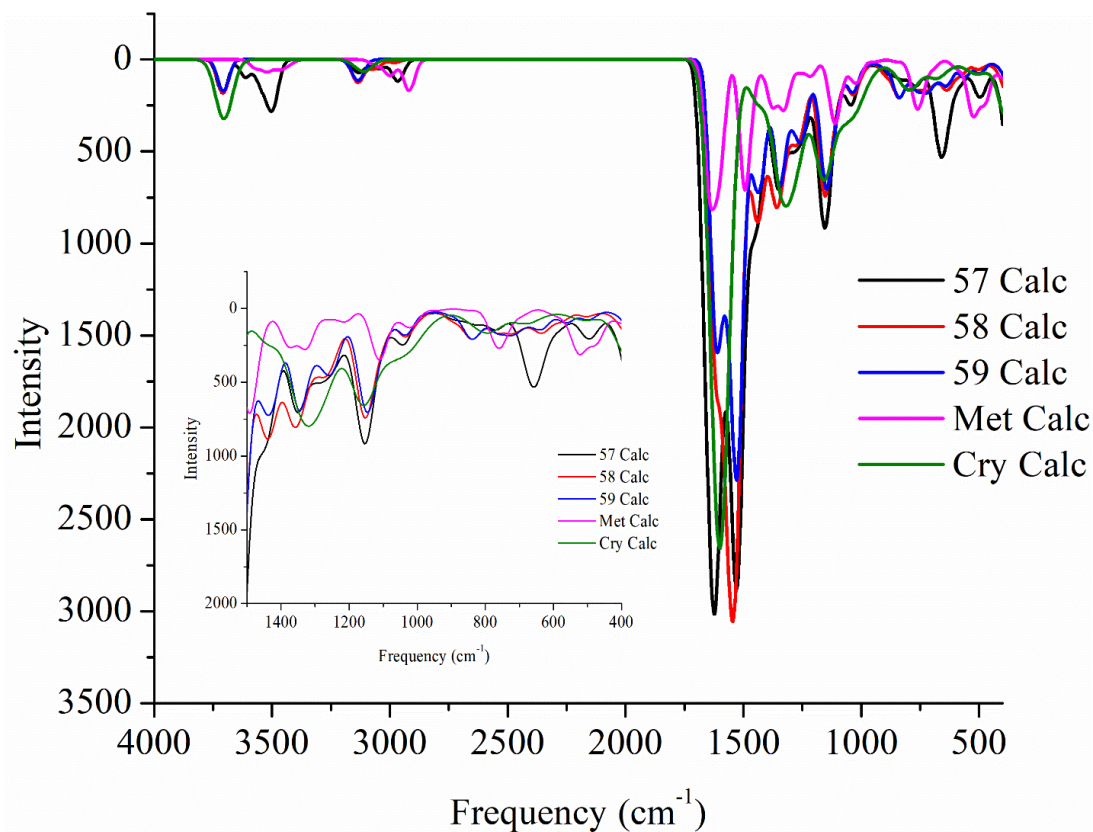
Appendix 10. Mass spectrometric analysis of complex **54**



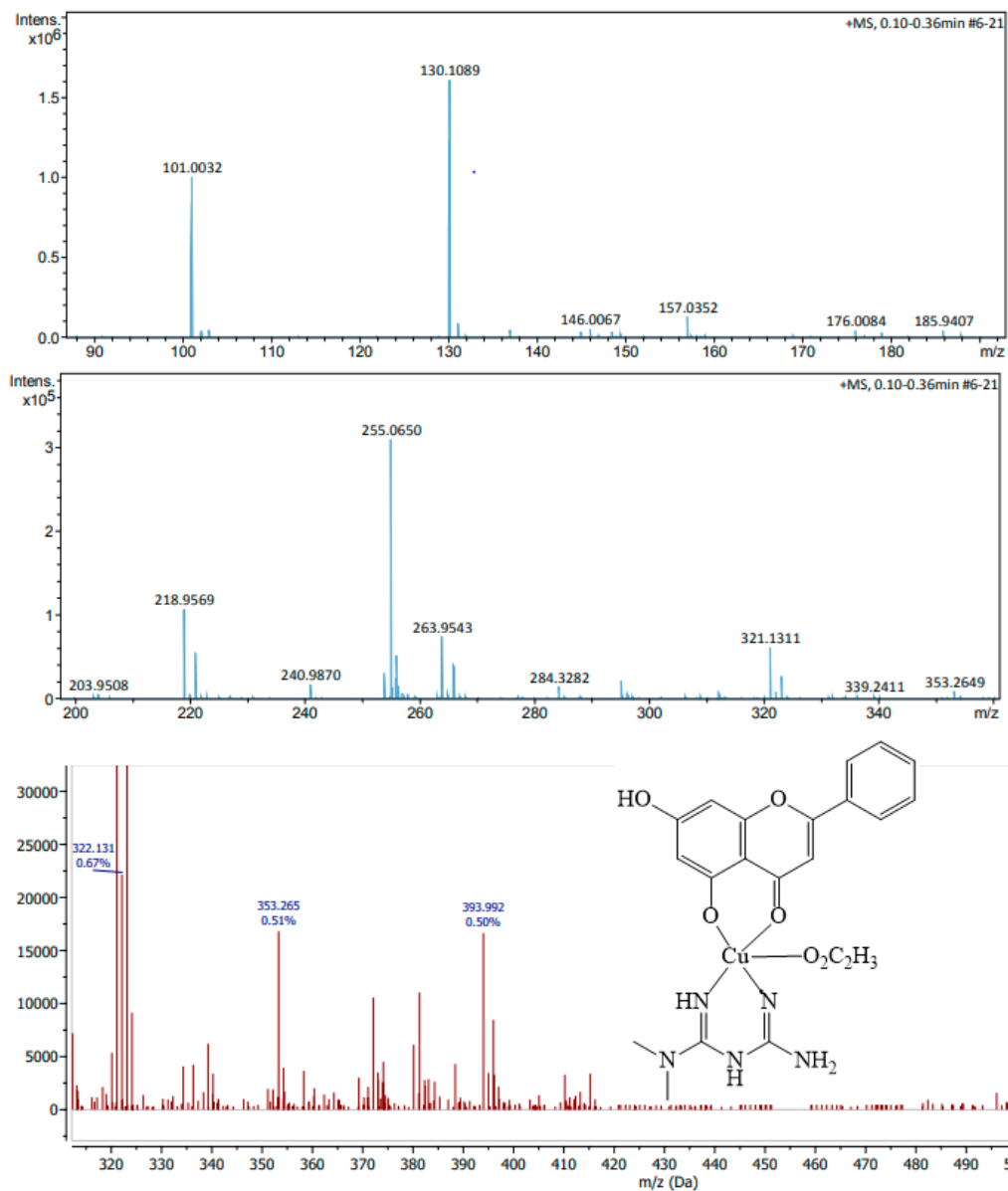
Appendix 11. Mass-spectrometric analysis of complex **55**



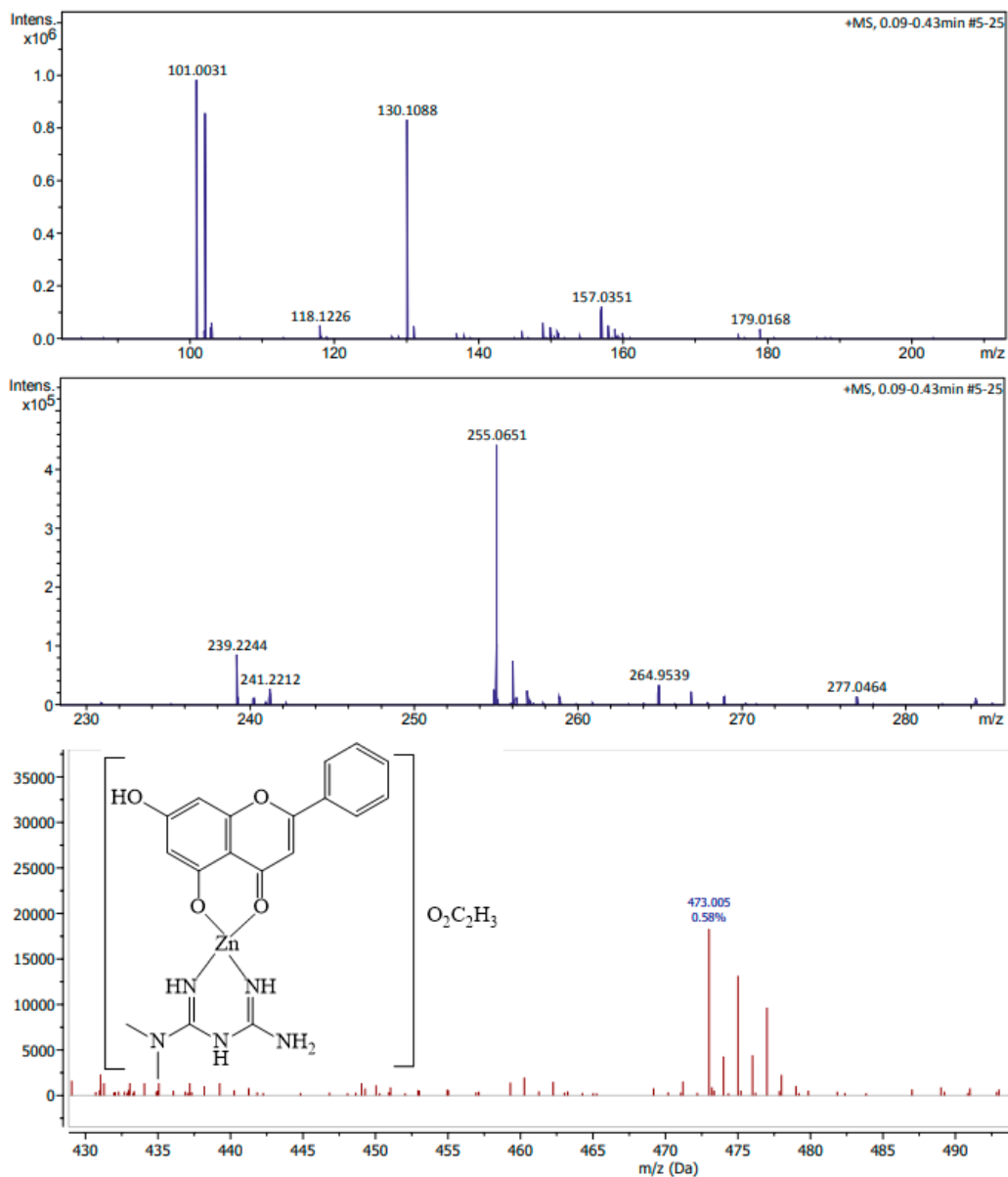
Appendix 12. Mass spectrometric analysis of complex **56**



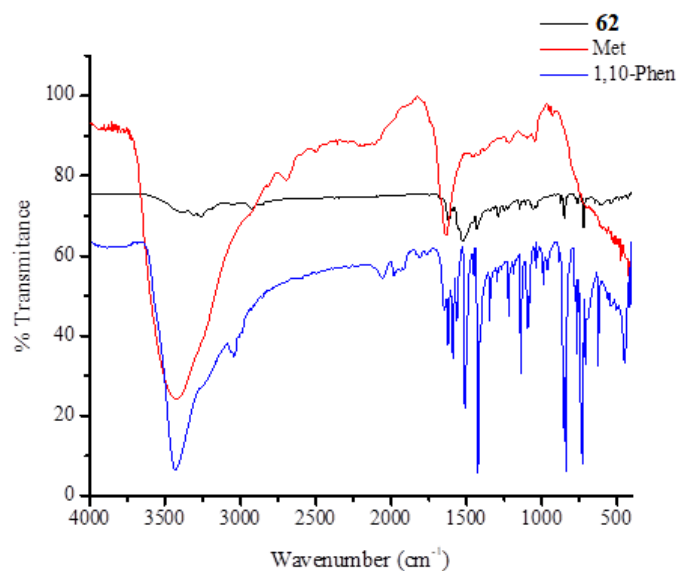
Appendix 13. Calculated FTIR spectra of the ligands (metformin and chrysin) and their complexes (**57 – 59**)



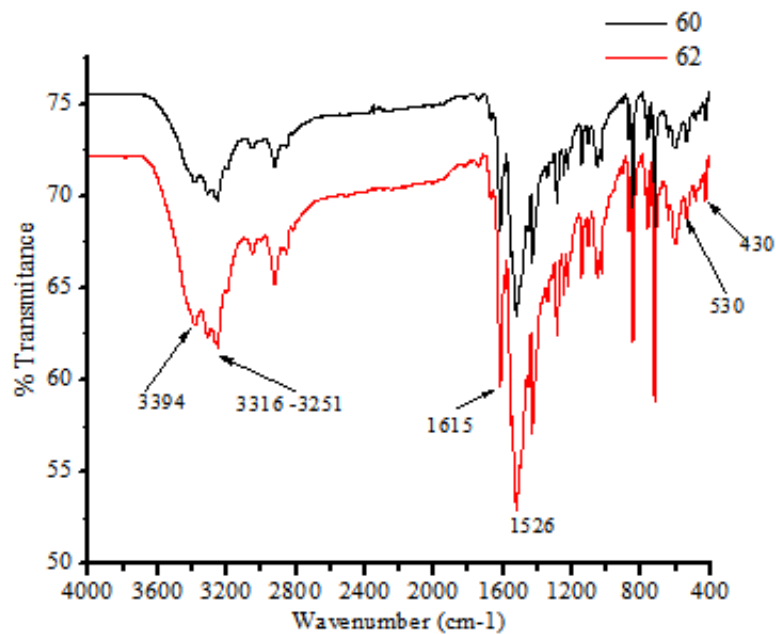
Appendix 14. Mass spectrometric analysis of complex **58**



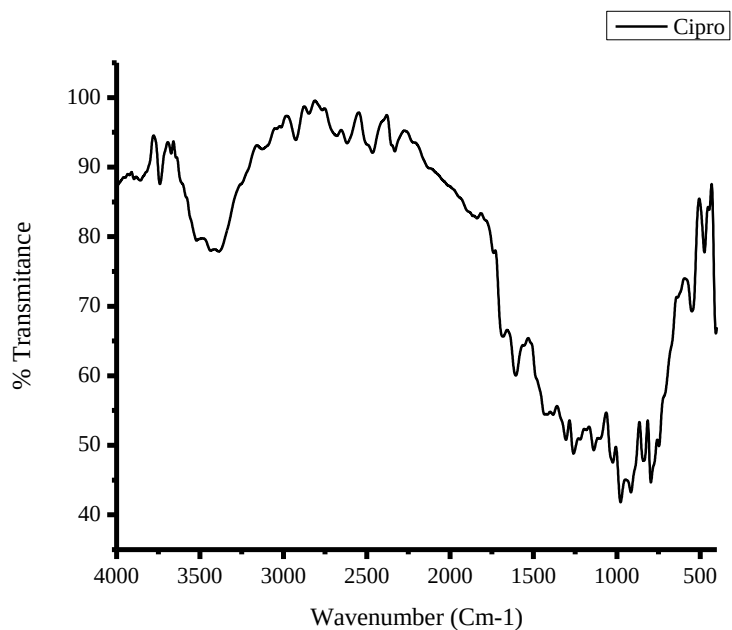
Appendix 15. Mass spectrometric analysis of complex **59**



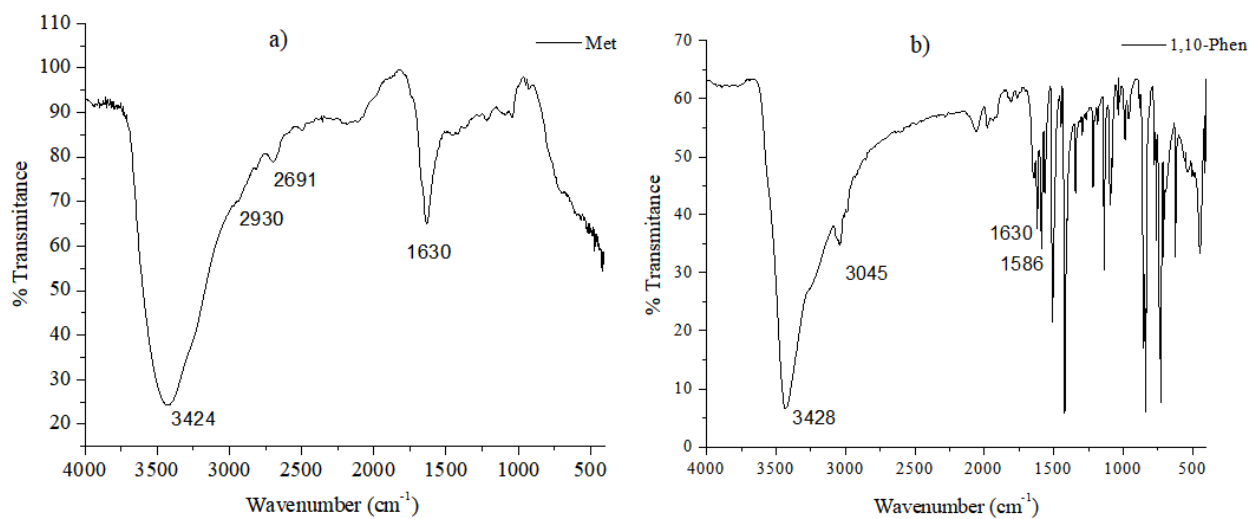
Appendix 16. FTIR spectra of the ligands and complex **62**



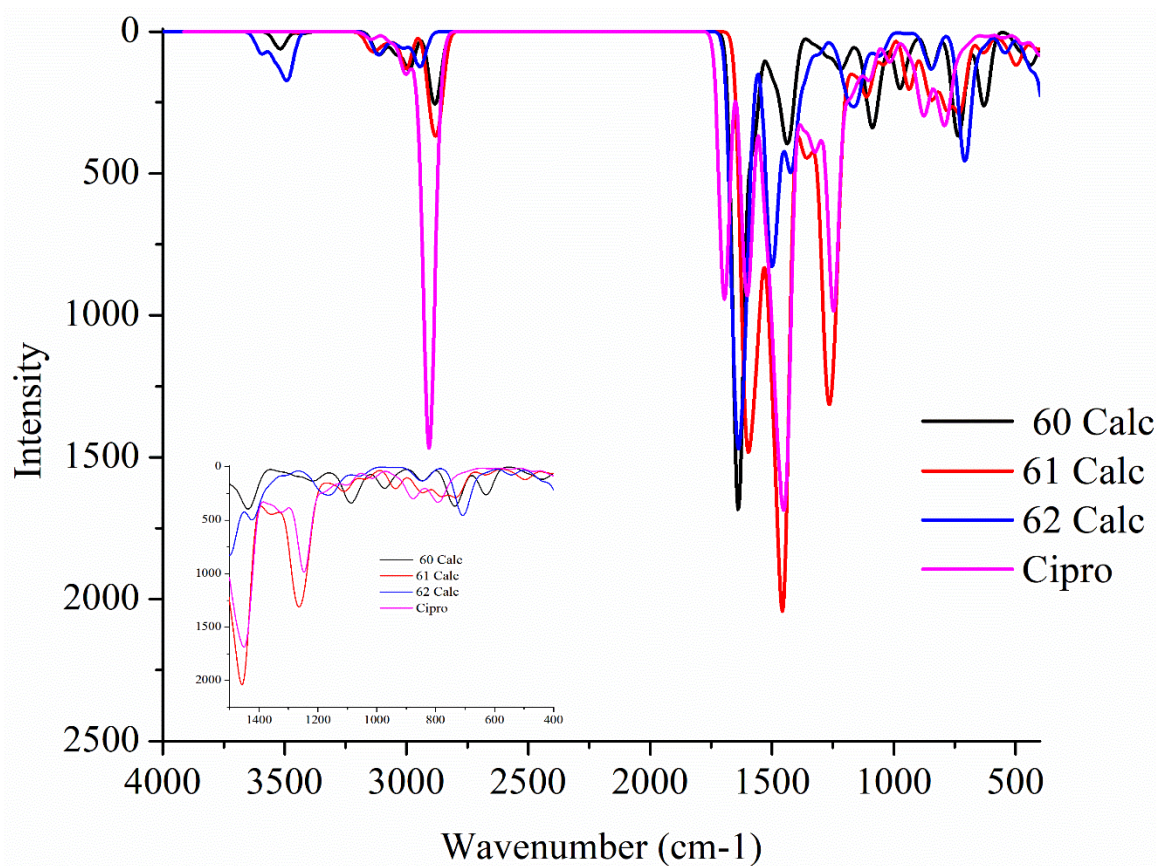
Appendix 17. Overlaid FTIR spectra of Cu(II) (**60**) and Zn(II) (**62**) complexes of metformin and 1,1-phenanthroline.



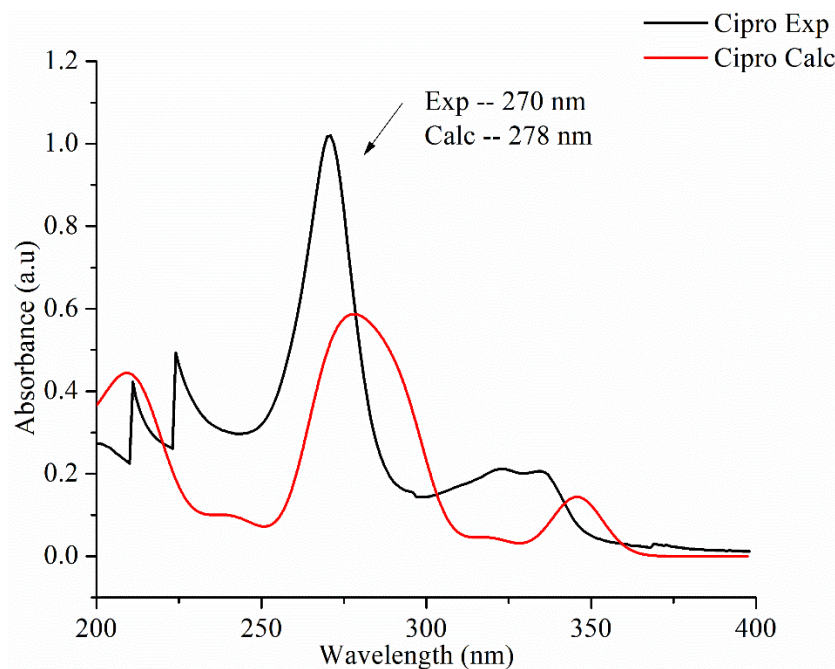
Appendix 18. FTIR Spectra of ciprofloxacin hydrogen chloride (Cip)



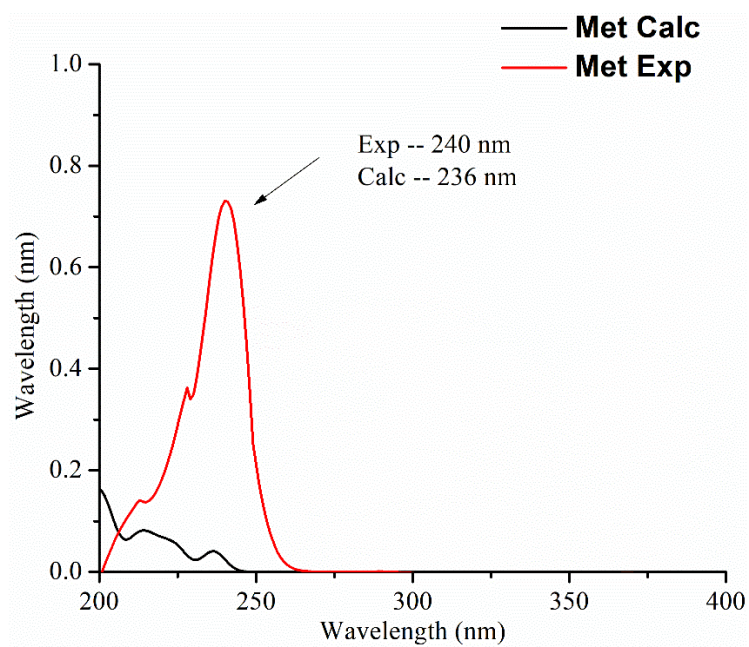
Appendix 19. FT-IR spectra of metformin hydrogen chloride (a) and 1,10-phenanthroline monohydrate (b)



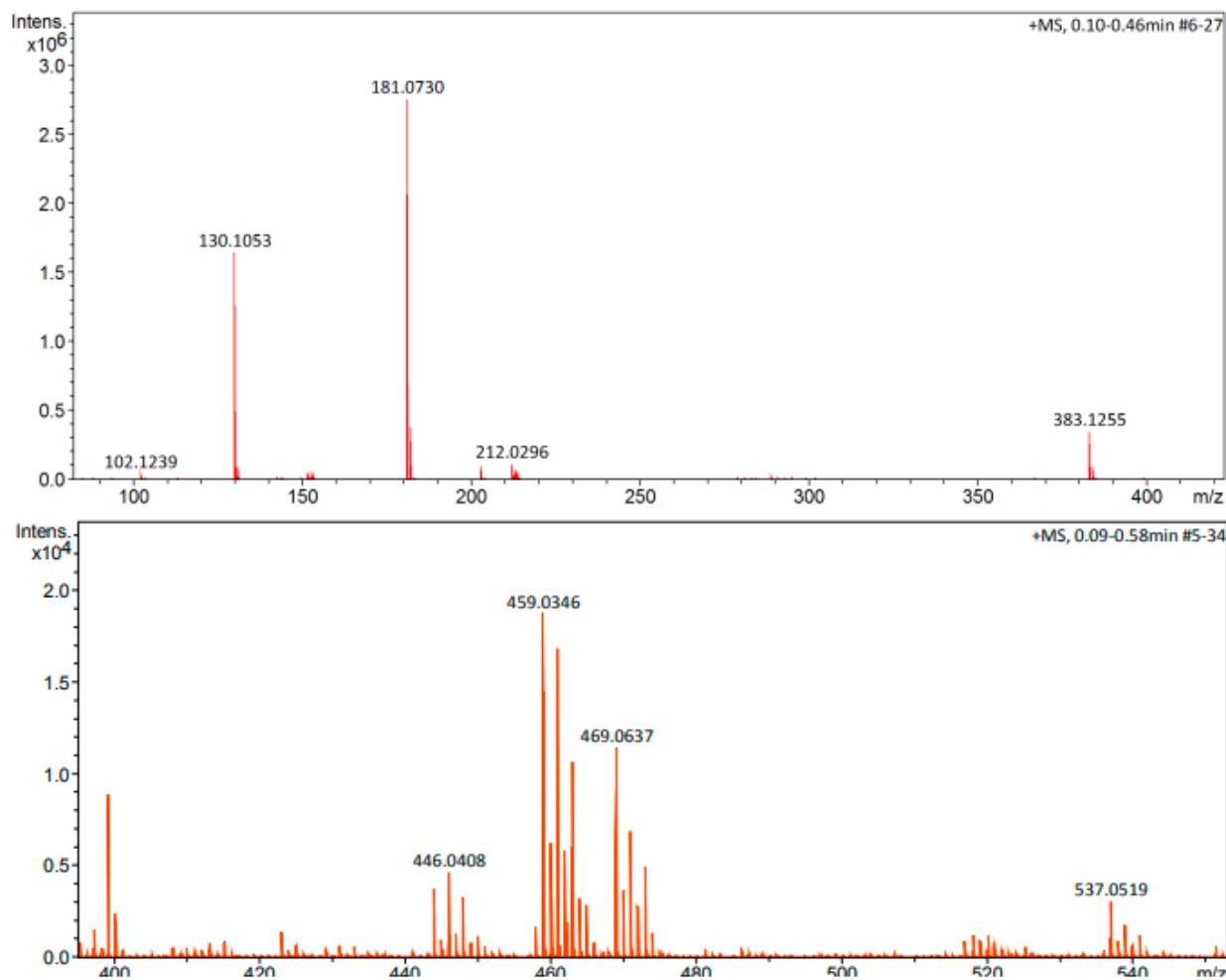
Appendix 20. Calculated FTIR spectra of ciprofloxacin (cipro) and complexes (60 – 62)



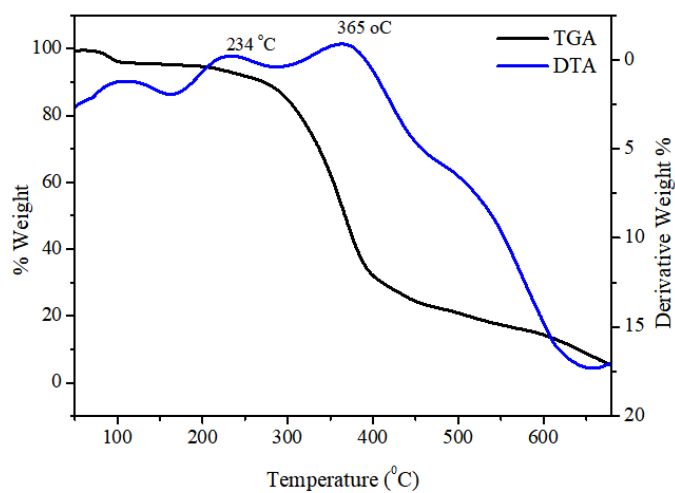
Appendix 21. TD-DFT calculated spectra of ciprofloxacin hydrogen chloride



Appendix 22. TD-DFT calculated spectra of ciprofloxacin hydrogen chloride



Appendix 23. Mass spectrometric analysis of complex 62

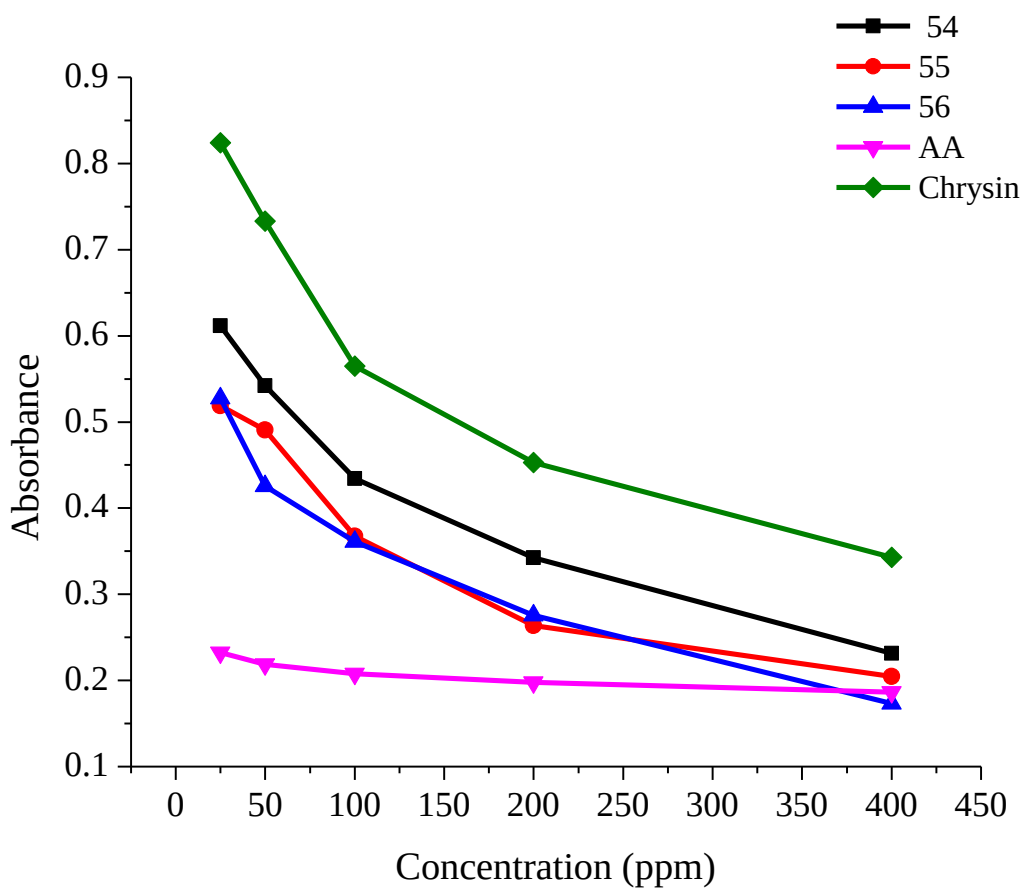


Appendix 24. TG and DTG curves for complex 62

Appendix 25. Antibacterial activity of 1,10-phenanthroline and chrysin mixed ligand complexes

(MIZ and SD values are given)

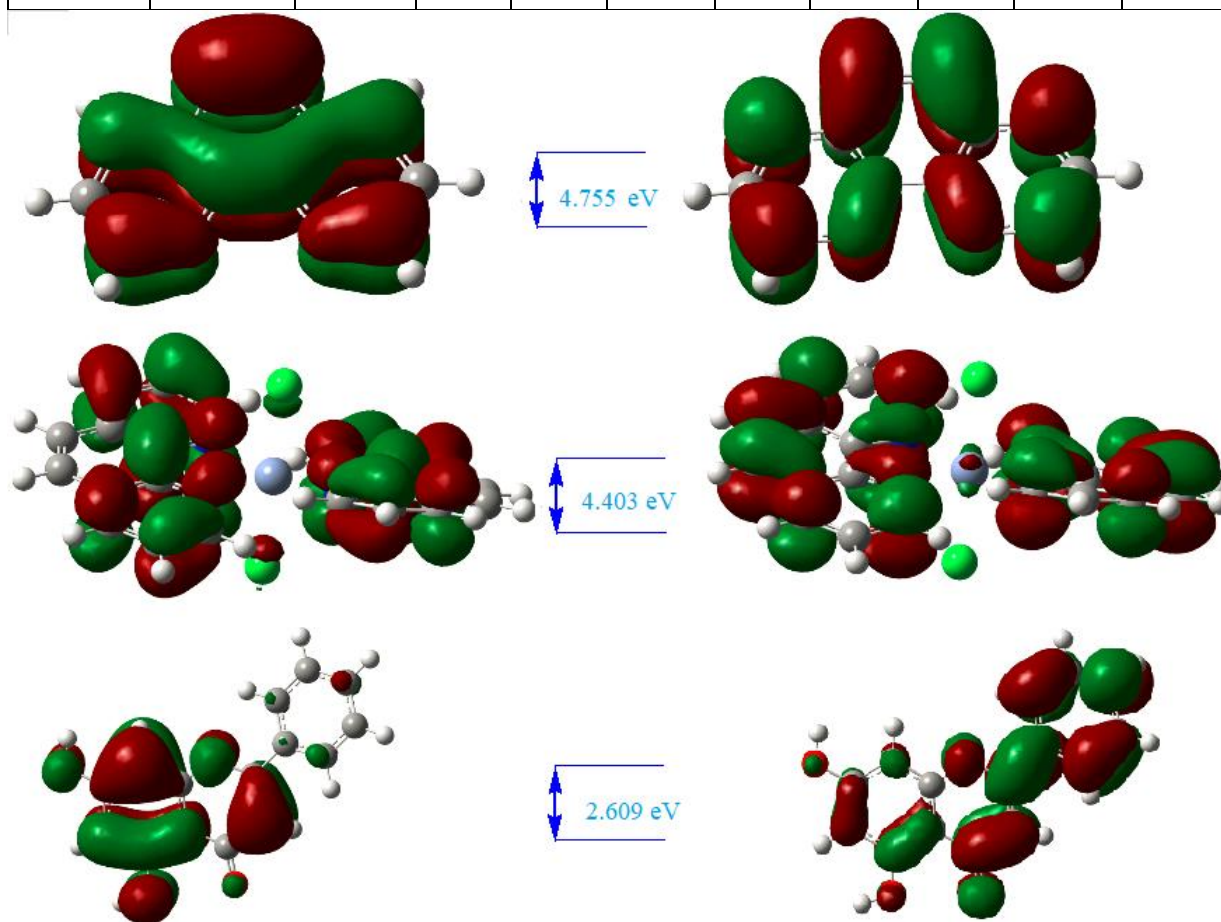
Concentration	Cpd	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. pyogen</i>	<i>S. aureus</i>
500 μ M	54	8.333 $\pm$ 0.581	8.333 $\pm$ 0.236	8.500 $\pm$ 0.408	9.167 $\pm$ 0.289
	55	10.167 $\pm$ 0.624	10.333 $\pm$ 0.408	11.000 $\pm$ 0.236	11.333 $\pm$ 0.577
	56	9.667 $\pm$ 0.624	8.500 $\pm$ 0.850	9.333 $\pm$ 0.408	9.167 $\pm$ 0.289
500 μ M	Cipro	24.000 $\pm$ 0.408	22.333 $\pm$ 0.408	24.667 $\pm$ 0.624	23.000 $\pm$ 0.289
1000 μ M	54	10.167 $\pm$ 0.471	9.500 $\pm$ 0.408	10.333 $\pm$ 0.236	10.333 $\pm$ 0.764
	55	12.000 $\pm$ 0.408	12.500 $\pm$ 0.236	11.167 $\pm$ 0.408	14.167 $\pm$ 0.500
	56	11.500 $\pm$ 0.816	10.667 $\pm$ 0.471	11.000 $\pm$ 0.408	13.000 $\pm$ 0.236



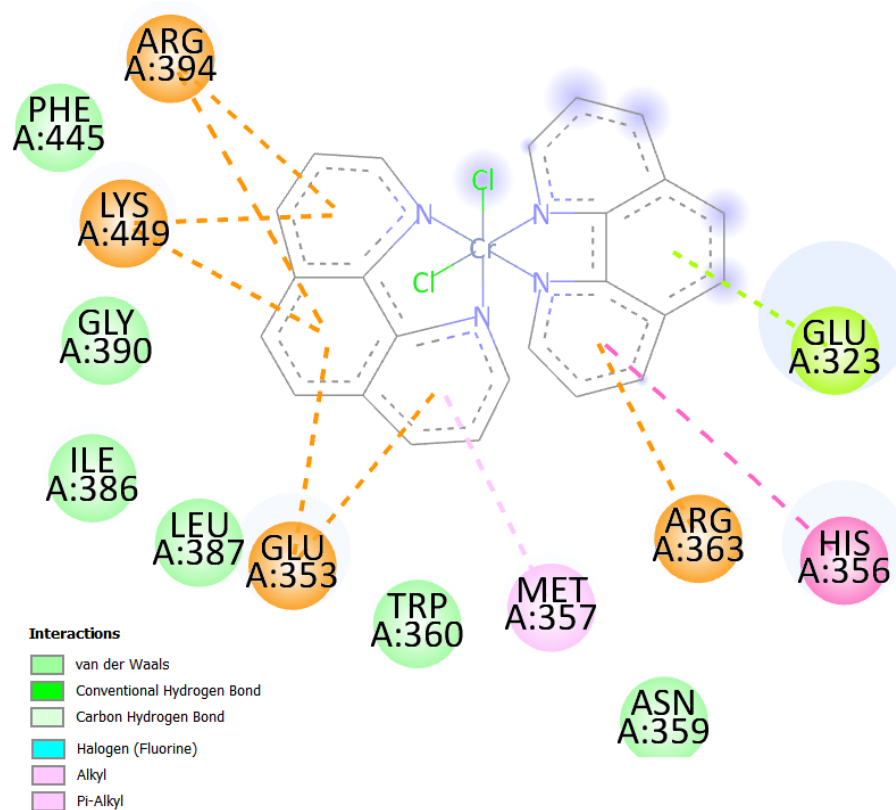
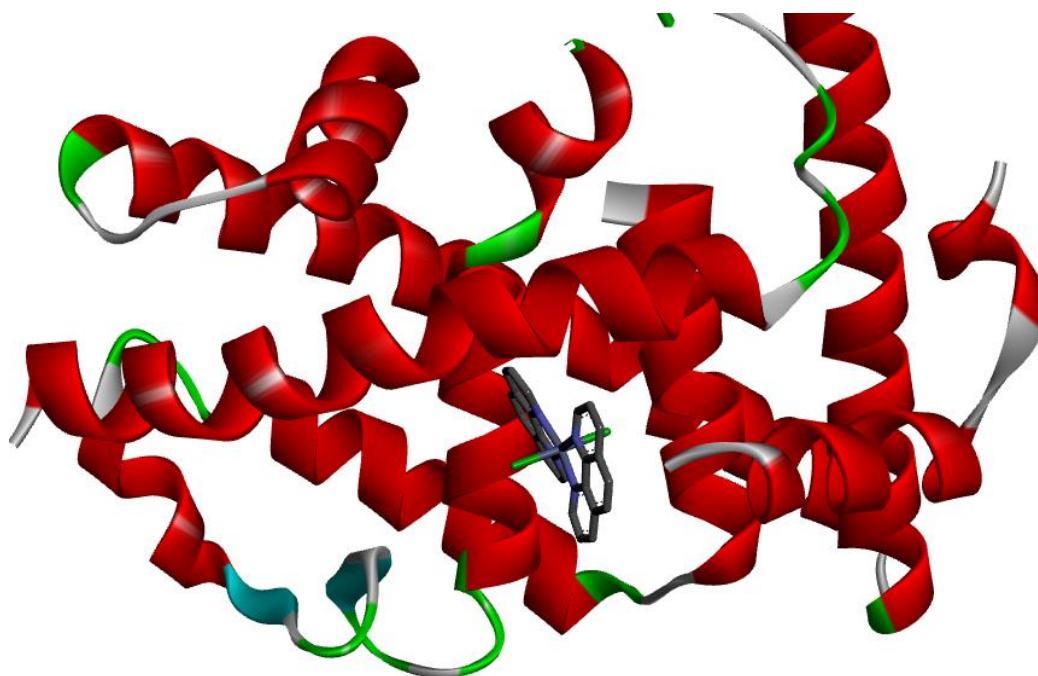
Appendix 26. Concentration Vs absorbance plot for the antioxidant activity of chrysin-based metal complexes (**54 - 56**)

Appendix 27. Quantum chemical descriptors of the ligands and the complexes (54 – 56)

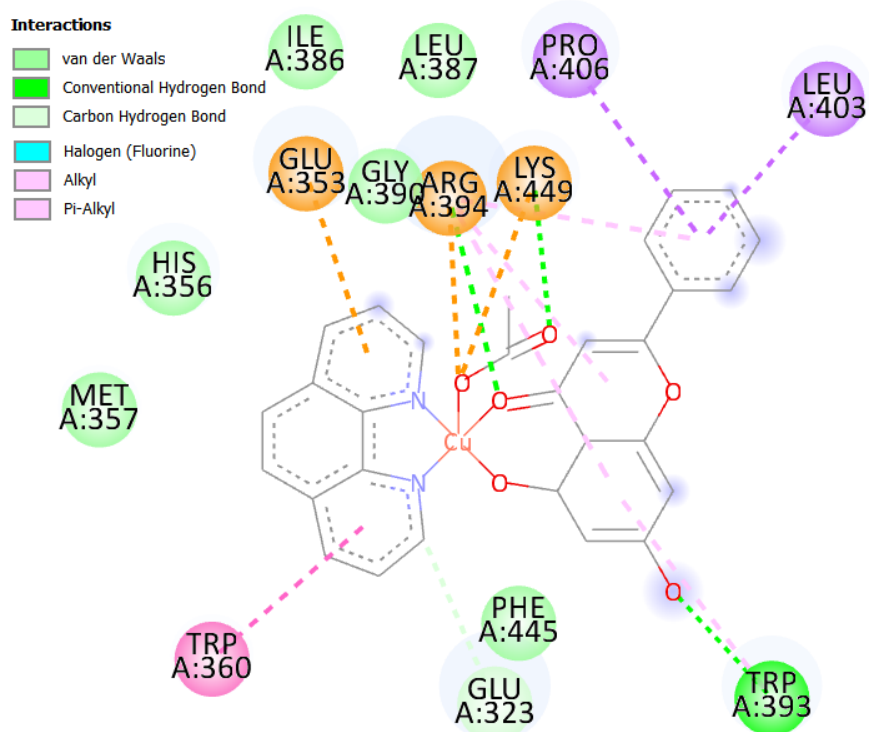
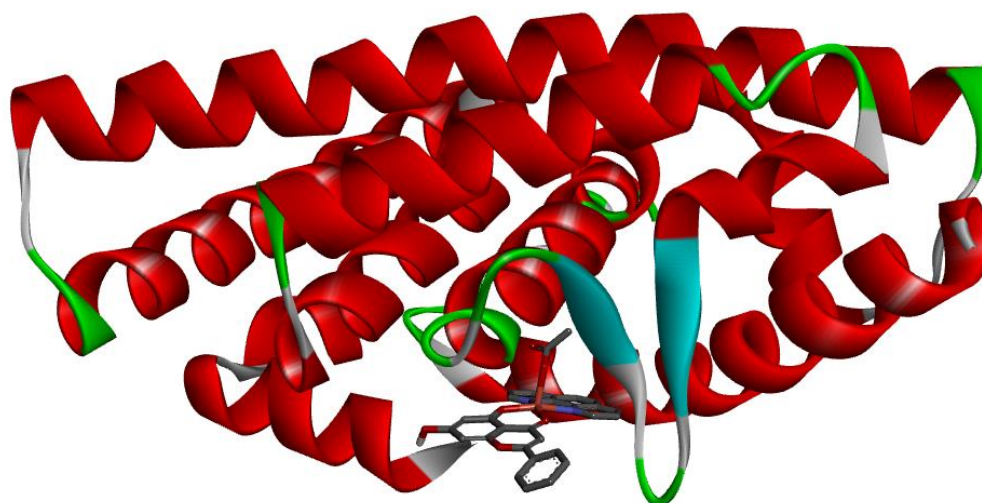
Cpd	<i>HOMO</i>	<i>LUMO</i>	<i>E_g</i>	χ	μ	<i>H</i>	σ	ω	<i>Nu</i>	dipole moment
1,10-phen	-6.690	-1.935	4.755	4.313	-4.313	2.377	0.210	3.912	0.256	5.192
Chrysin	-6.555	-2.152	4.403	4.353	-4.353	2.202	0.227	4.304	0.232	7.884
54	-4.989	-2.380	2.609	3.684	-3.684	1.304	0.383	5.203	0.192	0.062
55	-5.966	-3.183	2.784	4.574	-4.574	1.392	0.359	7.517	0.133	17.370
56	-6.036	-2.703	3.333	4.370	-4.370	1.667	0.300	5.729	0.175	13.018



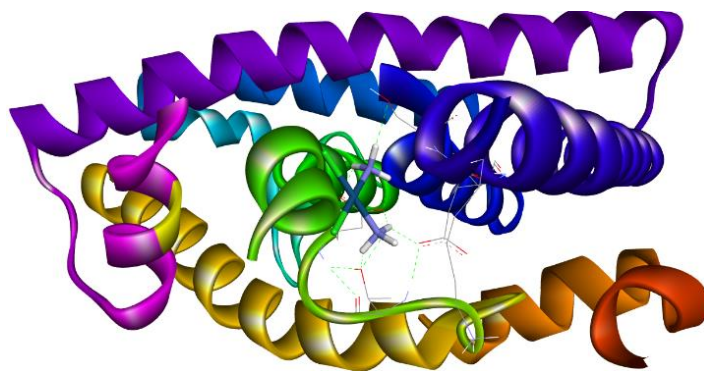
Appendix 28. HOMO - LUMO distribution of chrysin (bottom), complex 54 (center) and 1,10-phenanthroline (top).



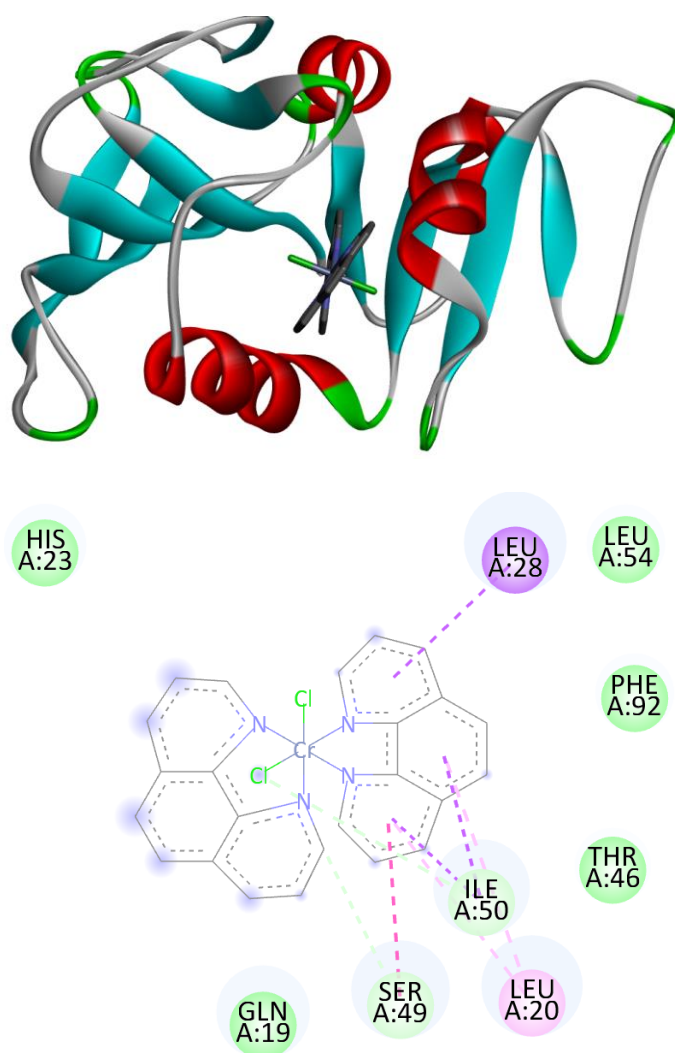
Appendix 29. The binding interactions of 54 against estrogen receptor alpha (ERα; PDB: 5GS4)



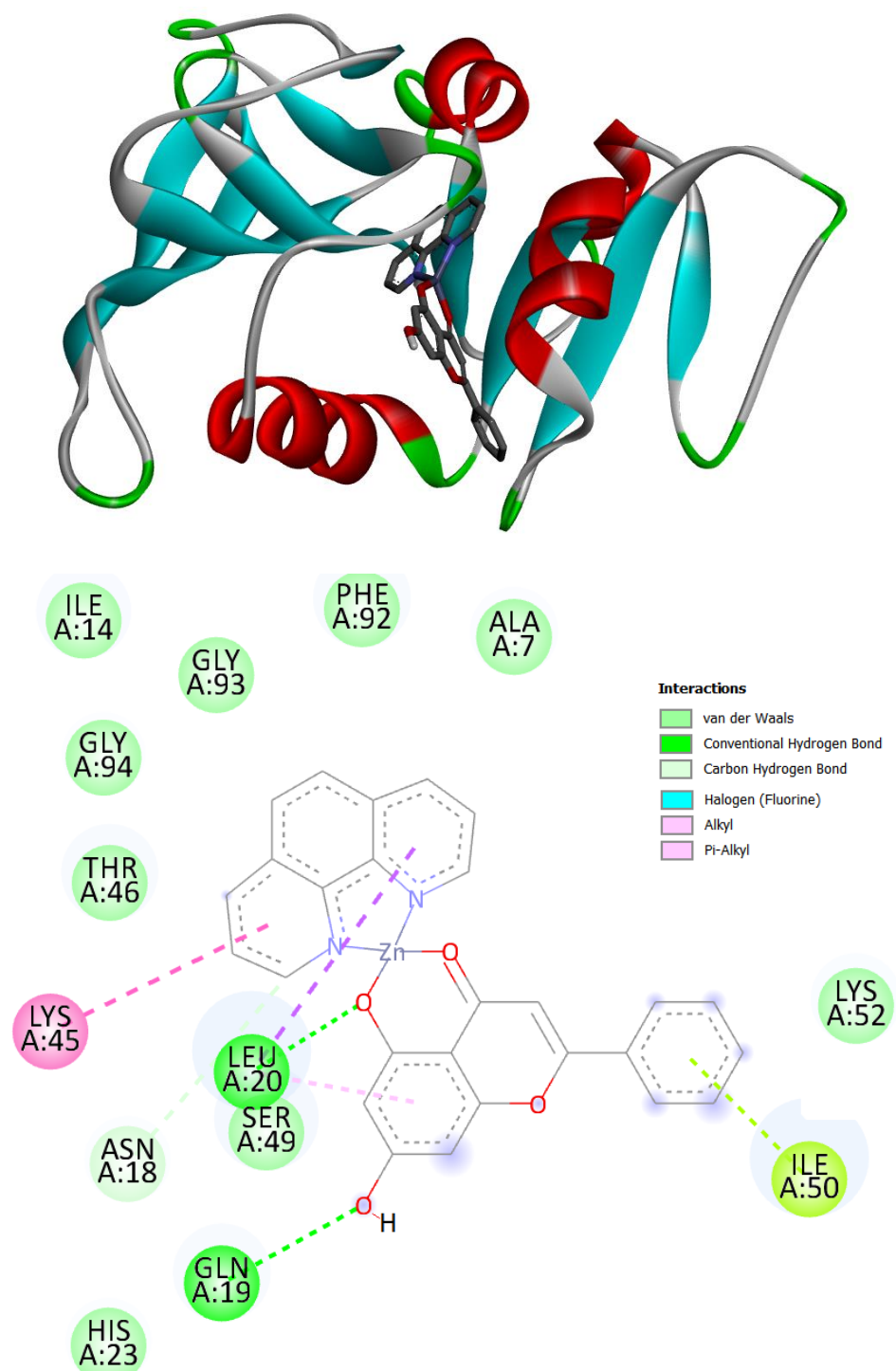
Appendix 30. The binding interactions of 55 against estrogen receptor alpha (ERα; PDB: 5GS4)



Appendix 31. The binding interactions of cisplatin against estrogen receptor alpha (Er α ; PDB: 5GS4)



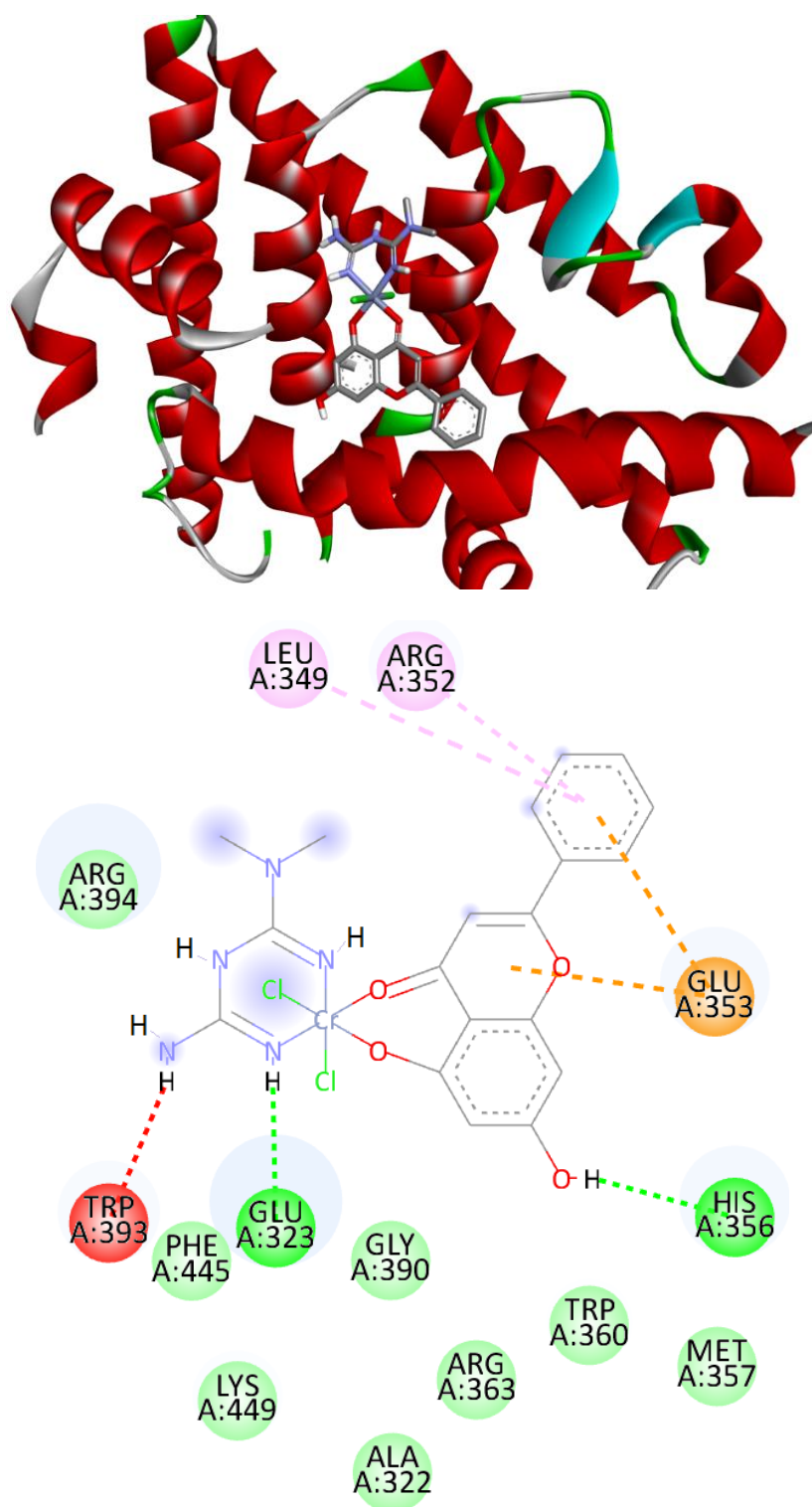
Appendix 32. The binding interactions of **54** against *S. aureus* (PDB: 2w9h)



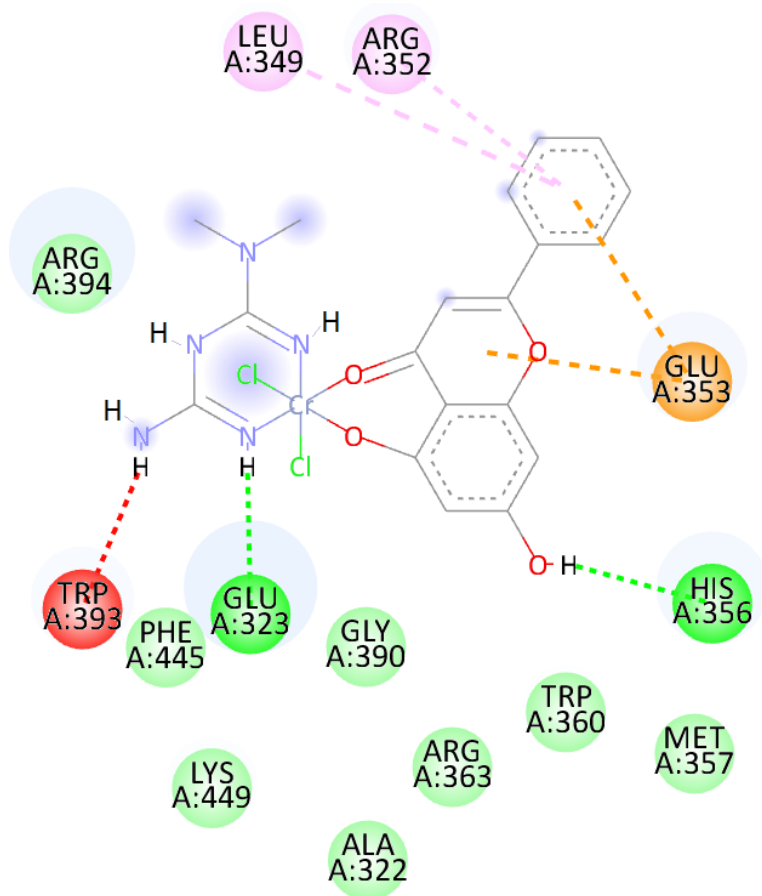
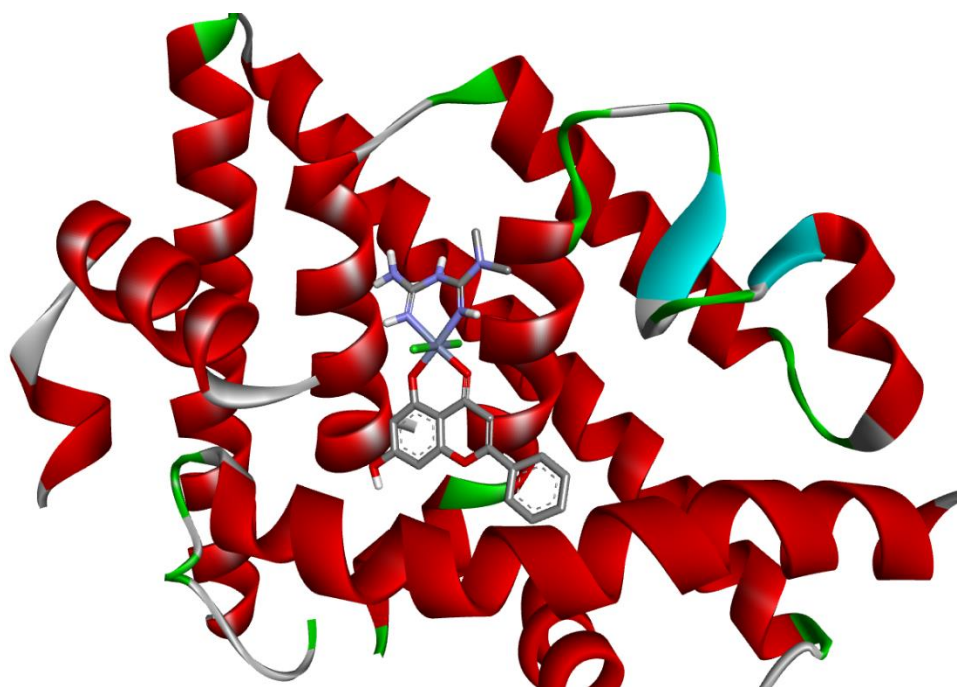
Appendix 33. The binding interactions of **56** against *S. aureus* (PDB: 2w9h)

Appendix 34. Antibacterial activity of metformin and chrysin mixed ligand complexes (57 – 59)
(SD values are given)

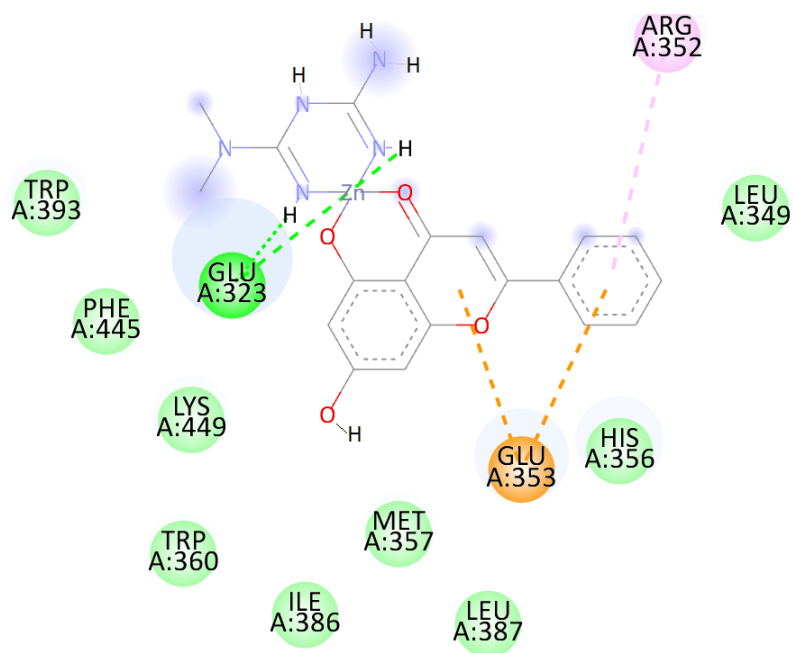
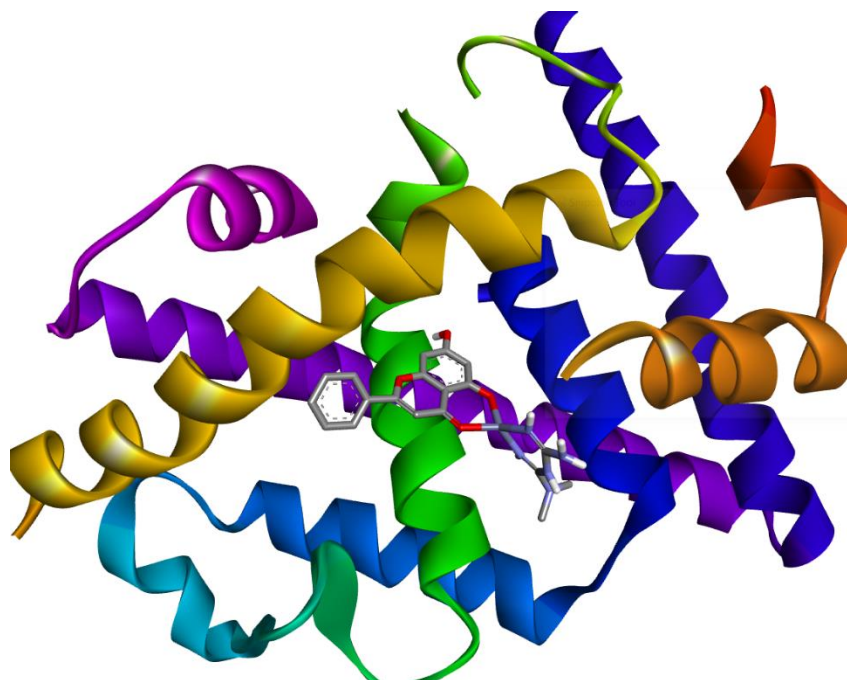
Concentration	Cpd	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. pyogenes</i>	<i>S. aureus</i>
500 μ M	57	9.000 $\pm$ 0.408	9.167 $\pm$ 0.236	10.167 $\pm$ 0.236	9.500 $\pm$ 0.408
	58	10.000 $\pm$ 0.408	10.167 $\pm$ 0.624	11.833 $\pm$ 0.236	11.167 $\pm$ 0.236
	59	11.000 $\pm$ 0.408	11.333 $\pm$ 0.236	12.500 $\pm$ 0.408	11.833 $\pm$ 0.236
	Cipro	22.833 $\pm$ 0.236	12.167 $\pm$ 0.471	24.667 $\pm$ 0.408	24.000 $\pm$ 0.408
1000 μ M	57	12.667 $\pm$ 0.408	9.000 $\pm$ 0.408	16.000 $\pm$ 0.000	13.000 $\pm$ 0.236
	58	10.000 $\pm$ 0.236	10.000 $\pm$ 0.408	10.000 $\pm$ 0.000	10.833 $\pm$ 0.236
	59	11.667 $\pm$ 0.236	22.667 $\pm$ 0.236	14.000 $\pm$ 0.236	11.833 $\pm$ 0.408



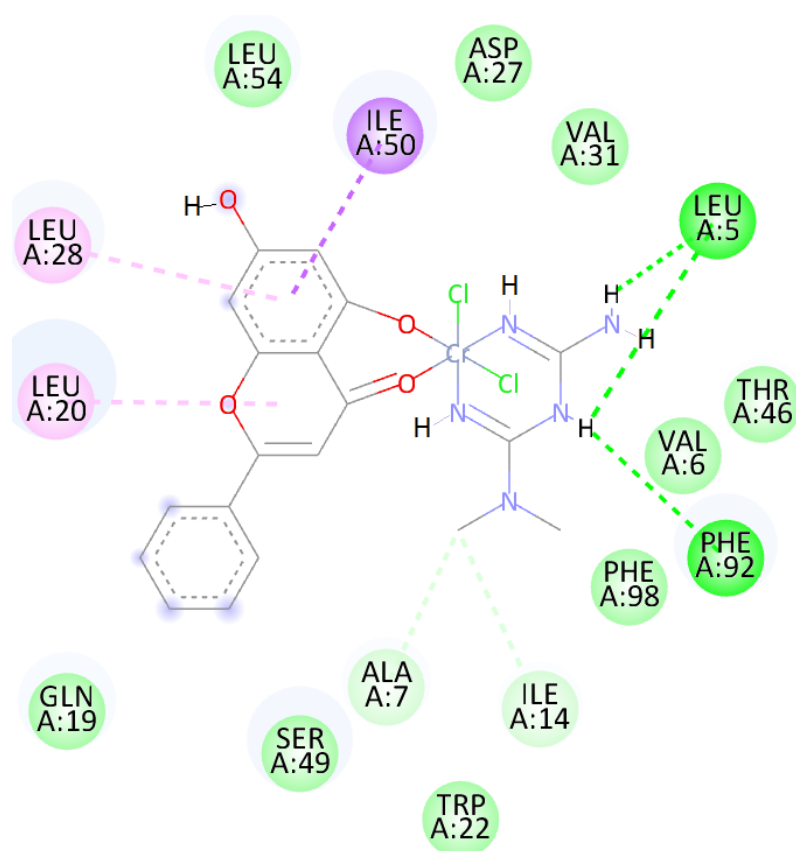
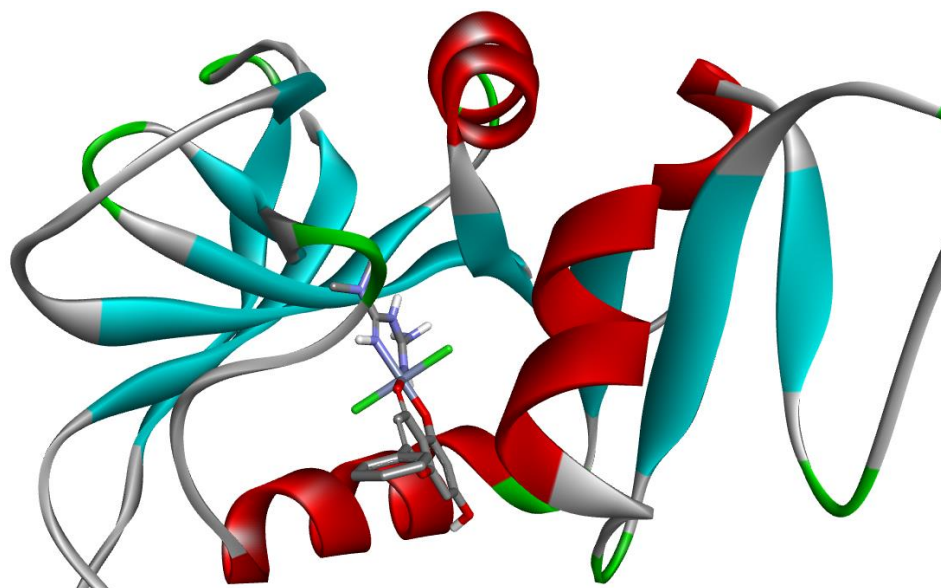
Appendix 35. The binding interactions of 57 against estrogen receptor alpha (ERα; PDB: 5GS4)



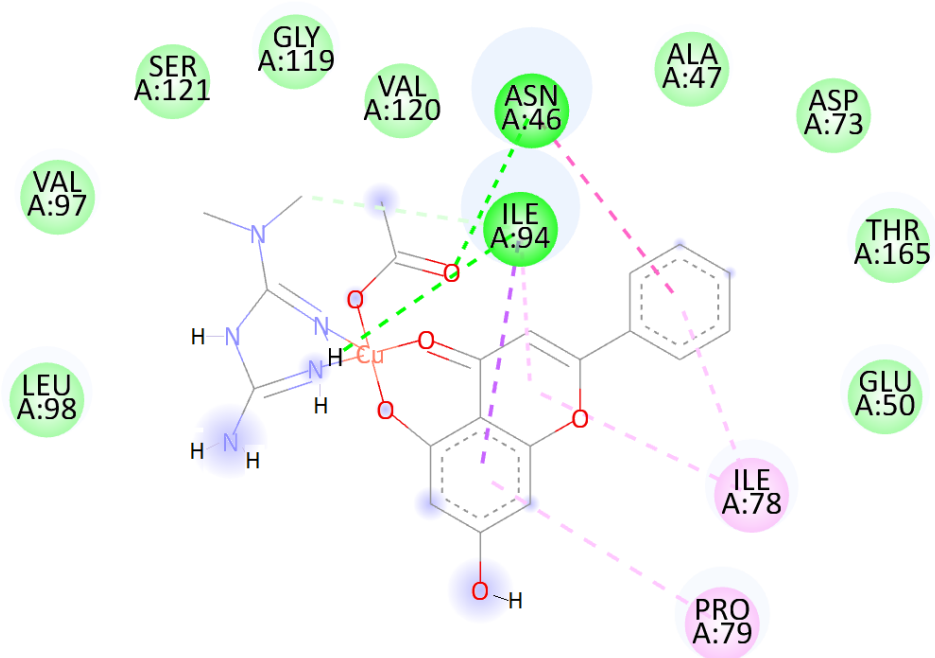
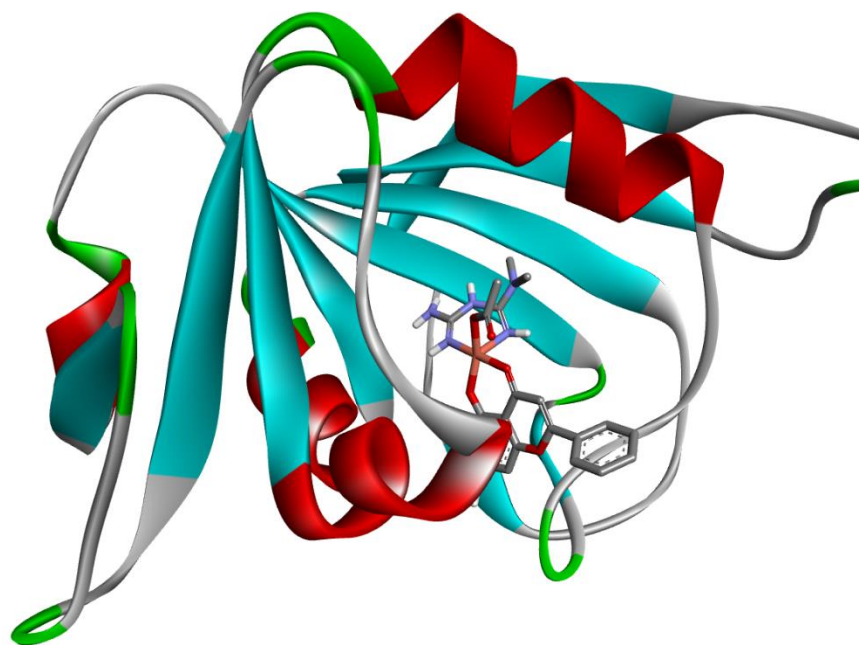
Appendix 36. The binding interactions of 57 against estrogen receptor alpha (ER α ; PDB: 5GS4)



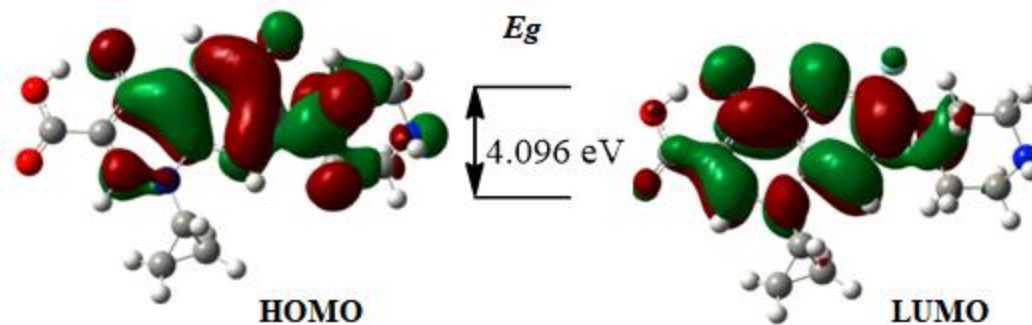
Appendix 37. The binding interactions of 59 against estrogen receptor alpha (Er α ; PDB: 5GS4)



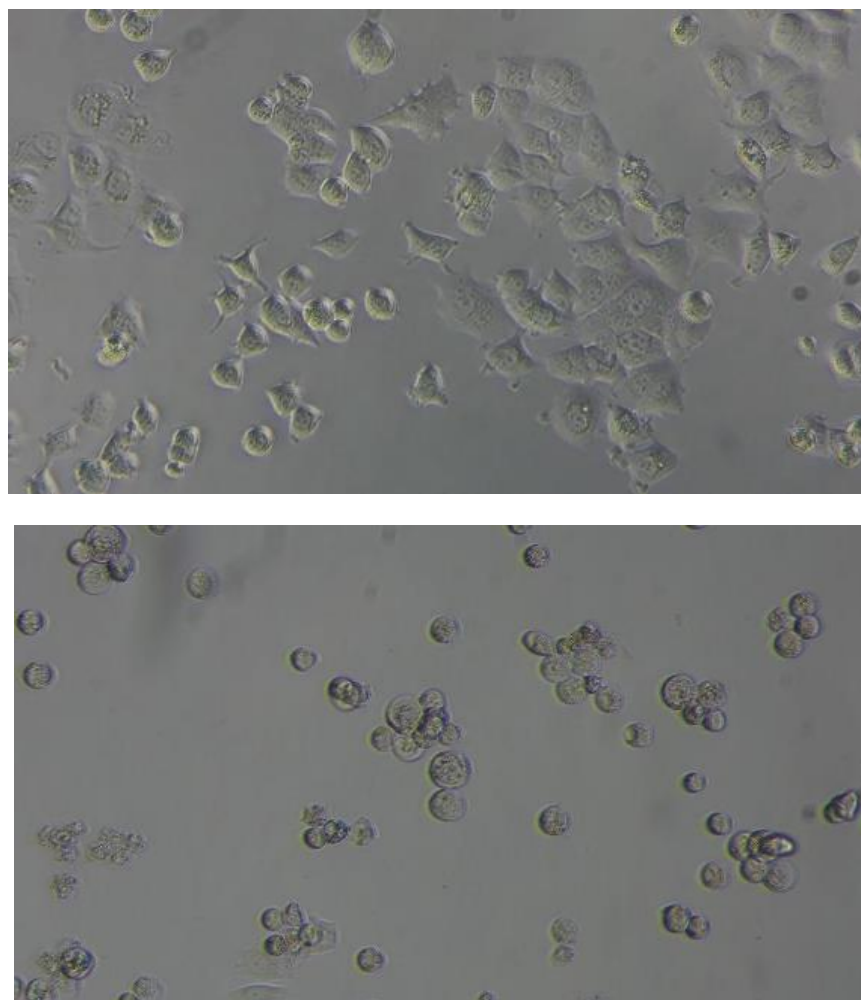
Appendix 38. The binding interactions of 57 against *S. aureus* (PDB: 2w9h)



Appendix 39. The binding interactions of **58** against *S. aureus* (PDB: 2w9h)



Appendix 40. HOMO-LUMO distribution of ciprofloxacin



Appendix 41. Morphological changes of MCF-7 cell lines induced by: (top) with PBS, and (bottom) with $12.5 \mu\text{M}$ complex **61**

Appendix 42. Mean inhibition zone of the synthesized mixed ligand metal complexes in mm (mean $\pm$ SD)

Cpd & Conc.	Bacteria Species and Minimum Zone of Inhibition in mm			
	<i>E. coli</i> ATCC25922	<i>P. aeruginosa</i> ATCC27853	<i>S. aureus</i> ATCC25923	<i>S. pyogen</i> ATCC19615
60 (25 μ g/mL)	11.250 $\pm$ 0.204	13.125 $\pm$ 0.102	12.117 $\pm$ 0.103	13.083 $\pm$ 0.312
61	17.300 $\pm$ 0.216	17.083 $\pm$ 0.118	16.217 $\pm$ 0.165	17.333 $\pm$ 0.471
62	12.167 $\pm$ 0.236	11.250 $\pm$ 0.204	11.083 $\pm$ 0.118	12.083 $\pm$ 0.118
Ciprofloxacin	20.000 $\pm$ 0.354	20.758 $\pm$ 0.722	20.317 $\pm$ 0.131	21.333 $\pm$ 0.471
60 (50 μ g/mL)	14.050 $\pm$ 0.268	15.250 $\pm$ 0.540	14.033 $\pm$ 0.205	15.133 $\pm$ 0.189
61	19.830 $\pm$ 0.118	20.083 $\pm$ 0.118	19.217 $\pm$ 0.165	21.833 $\pm$ 0.624
62	18.167 $\pm$ 0.236	17.083 $\pm$ 0.118	16.383 $\pm$ 0.103	18.167 $\pm$ 0.850
Ciprofloxacin	23.133 $\pm$ 0.189	24.000 $\pm$ 0.204	23.283 $\pm$ 0.085	23.833 $\pm$ 0.312

Appendix 43. Physicochemical and drug-likeness screening of the ligands, cisplatin and complexes (**60** – **61**)

Formula	MW	#HBAs	#HBDs	MR	TPSA	iLOGP	Lipinski #violations
C ₁₂ H ₈ N ₂	180.21	2	0	57.04	25.78	1.65	0
C ₄ H ₁₁ N ₅	129.16	2	4	36.93	88.99	0.77	0
C ₁₇ H ₁₈ FN ₃ O ₃	331.34	5	2	95.25	74.57	2.24	0
C ₁₇ H ₂₁ CuN ₇ (60)	386.94	2	2	109.54	75.87	0	0
C ₂₉ H ₂₅ ClCuFN ₅ O ₃ (61)	609.54	5	1	156.6	73.43	0	1
Cl ₂ H ₆ N ₂ Pt (cisplatin)	300.05	5	1	21.16	6.48	0	1

Appendix 44. Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: minimum inhibition zone

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	3906.821 ^a	71	55.026	51.038	.000
Intercept	37692.046	1	37692.046	34960.659	.000
Concentration	217.014	1	217.014	201.288	.000
Compound	1111.942	6	185.324	171.894	.000
Bacteria	.000	0	.	.	.
bacteria1	5.917	2	2.958	2.744	.068
compund1	.000	0	.	.	.
Concentration * Compound	26.600	5	5.320	4.934	.000
Concentration * Bacteria	.000	0	.	.	.
Concentration * bacteria1	.949	2	.474	.440	.645
Concentration * compund1	.000	0	.	.	.
Compound * Bacteria	.000	0	.	.	.
Compound * bacteria1	32.899	12	2.742	2.543	.004
Compound * compund1	.000	0	.	.	.
Bacteria * bacteria1	.000	0	.	.	.
Bacteria * compund1	.000	0	.	.	.
bacteria1 * compund1	.000	0	.	.	.
Concentration * Compound * Bacteria	.000	0	.	.	.
Concentration * Compound * bacteria1	19.581	10	1.958	1.816	.063
Error	155.250	144	1.078		
Total	39824.843	216			
Corrected Total	4062.072	215			

R Squared = .962 (Adjusted R Squared = .943)_a

Appendix 45. Post Hoc Tests of the synthesized compounds

Multiple Comparisons							
Dependent Variable: minimum inhibition zone							
(I) (II) (III)	Compounds	synthesized (J) synthesized compounds (IV)	Std. Error	Mean Differen ce (I-J)	Sig.	95% Confidence Interval	
						Lowe Bound	Upper Bound
Tuke y HSD	54	55	-2.25292*	.299740	.000	-3.21626	-1.28957
		56	-.75000	.299740	.276	-1.71335	.21335
		Control/Cipro	-14.16667*	.367105	.000	-15.34652	-12.98681
		57	-.87821	.293919	.092	-1.82285	.06644
		58	-3.34848*	.306476	.000	-4.33348	-2.36349
		59	-2.04667*	.296727	.000	-3.00033	-1.09300
		60	-14.30303*	.378066	.000	-15.51811	-13.08795
		61	-4.83958*	.299740	.000	-5.80293	-3.87624
		62	-4.17188*	.299740	.000	-5.13522	-3.20853
	55	54	2.25292*	.299740	.000	1.28957	3.21626
		56	1.50292*	.299740	.000	.53957	2.46626
		Control/Cipro	-11.91375*	.367105	.000	-13.09361	-10.73389
		57	1.37471*	.293919	.000	.43007	2.31935
		58	-1.09557*	.306476	.017	-2.08057	-.11057
		59	.20625	.296727	1.000	-.74742	1.15992
		60	-12.05011*	.378066	.000	-13.26520	-10.83503
		61	-2.58667*	.299740	.000	-3.55001	-1.62332
		62	-1.91896*	.299740	.000	-2.88231	-.95561
	56	54	.75000	.299740	.276	-.21335	1.71335
		55	-1.50292*	.299740	.000	-2.46626	-.53957
		Control/Cipro	-13.41667*	.367105	.000	-14.59652	-12.23681
		57	-.12821	.293919	1.000	-1.07285	.81644
		58	-2.59848*	.306476	.000	-3.58348	-1.61349
		59	-1.29667*	.296727	.001	-2.25033	-.34300
		60	-13.55303*	.378066	.000	-14.76811	-12.33795
		61	-4.08958*	.299740	.000	-5.05293	-3.12624

	Control/Cipro	62	-3.42188*	.299740	.000	-4.38522	-2.45853
		54	14.16667*	.367105	.000	12.98681	15.34652
		55	11.91375*	.367105	.000	10.73389	13.09361
		56	13.41667*	.367105	.000	12.23681	14.59652
		57	13.28846*	.362368	.000	12.12383	14.45309
		58	10.81818*	.372625	.000	9.62058	12.01578
		59	12.12000*	.364649	.000	10.94804	13.29196
		60	-.13636	.433423	1.000	-1.52936	1.25664
		61	9.32708*	.367105	.000	8.14723	10.50694
		62	9.99479*	.367105	.000	8.81494	11.17465
	57	54	.87821	.293919	.092	-.06644	1.82285
		55	-1.37471*	.293919	.000	-2.31935	-.43007
		56	.12821	.293919	1.000	-.81644	1.07285
		Control/Cipro	-13.28846*	.362368	.000	-14.45309	-12.12383
		58	-2.47028*	.300786	.000	-3.43699	-1.50357
		59	-1.16846*	.290846	.004	-2.10323	-.23370
		60	-13.42483*	.373468	.000	-14.62513	-12.22452
		61	-3.96138*	.293919	.000	-4.90602	-3.01674
		62	-3.29367*	.293919	.000	-4.23831	-2.34903
	58	54	3.34848*	.306476	.000	2.36349	4.33348
		55	1.09557*	.306476	.017	.11057	2.08057
		56	2.59848*	.306476	.000	1.61349	3.58348
		Control/Cipro	-10.81818*	.372625	.000	-12.01578	-9.62058
		57	2.47028*	.300786	.000	1.50357	3.43699
		59	1.30182*	.303531	.001	.32629	2.27735
		60	-10.95455*	.383428	.000	-12.18686	-9.72223
		61	-1.49110*	.306476	.000	-2.47610	-.50610
		62	-.82339	.306476	.189	-1.80839	.16161
	59	54	2.04667*	.296727	.000	1.09300	3.00033
		55	-.20625	.296727	1.000	-1.15992	.74742
		56	1.29667*	.296727	.001	.34300	2.25033
		Control/Cipro	-12.12000*	.364649	.000	-13.29196	-10.94804
		57	1.16846*	.290846	.004	.23370	2.10323
		58	-1.30182*	.303531	.001	-2.27735	-.32629
		60	-12.25636*	.375682	.000	-13.46378	-11.04894
		61	-2.79292*	.296727	.000	-3.74658	-1.83925

	60	62	-2.12521*	.296727	.000	-3.07887	-1.17154
		54	14.30303*	.378066	.000	13.08795	15.51811
		55	12.05011*	.378066	.000	10.83503	13.26520
		56	13.55303*	.378066	.000	12.33795	14.76811
		Control/Cipro	.13636	.433423	1.000	-1.25664	1.52936
		57	13.42483*	.373468	.000	12.22452	14.62513
		58	10.95455*	.383428	.000	9.72223	12.18686
		59	12.25636*	.375682	.000	11.04894	13.46378
		61	9.46345*	.378066	.000	8.24836	10.67853
		62	10.13116*	.378066	.000	8.91607	11.34624
	61	54	4.83958*	.299740	.000	3.87624	5.80293
		55	2.58667*	.299740	.000	1.62332	3.55001
		56	4.08958*	.299740	.000	3.12624	5.05293
		Control/Cipro	-9.32708*	.367105	.000	-10.50694	-8.14723
		57	3.96138*	.293919	.000	3.01674	4.90602
		58	1.49110*	.306476	.000	.50610	2.47610
		59	2.79292*	.296727	.000	1.83925	3.74658
		60	-9.46345*	.378066	.000	-10.67853	-8.24836
		62	.66771	.299740	.443	-.29564	1.63106
	62	54	4.17188*	.299740	.000	3.20853	5.13522
		55	1.91896*	.299740	.000	.95561	2.88231
		56	3.42188*	.299740	.000	2.45853	4.38522
		Control/Cipro	-9.99479*	.367105	.000	-11.17465	-8.81494
		57	3.29367*	.293919	.000	2.34903	4.23831
		58	.82339	.306476	.189	-.16161	1.80839
		59	2.12521*	.296727	.000	1.17154	3.07887
		60	-10.13116*	.378066	.000	-11.34624	-8.91607
		61	-.66771	.299740	.443	-1.63106	.29564
		62					
LSD	54	55	-2.25292*	.299740	.000	-2.84537	-1.66046
		56	-.75000*	.299740	.013	-1.34246	-.15754
		Control/Cipro	-14.16667*	.367105	.000	-14.89228	-13.44106
		57	-.87821*	.293919	.003	-1.45916	-.29725
		58	-3.34848*	.306476	.000	-3.95426	-2.74271
		59	-2.04667*	.296727	.000	-2.63317	-1.46016
		60	-14.30303*	.378066	.000	-15.05031	-13.55576

		61	-4.83958*	.299740	.000	-5.43204	-4.24713
		62	-4.17188*	.299740	.000	-4.76433	-3.57942
	55	54	2.25292*	.299740	.000	1.66046	2.84537
		56	1.50292*	.299740	.000	.91046	2.09537
		Control/Cipro	-11.91375*	.367105	.000	-12.63936	-11.18814
		57	1.37471*	.293919	.000	.79376	1.95566
		58	-1.09557*	.306476	.000	-1.70134	-.48979
		59	.20625	.296727	.488	-.38025	.79275
		60	-12.05011*	.378066	.000	-12.79739	-11.30284
		61	-2.58667*	.299740	.000	-3.17912	-1.99421
		62	-1.91896*	.299740	.000	-2.51142	-1.32650
	56	54	.75000*	.299740	.013	.15754	1.34246
		55	-1.50292*	.299740	.000	-2.09537	-.91046
		Control/Cipro	-13.41667*	.367105	.000	-14.14228	-12.69106
		57	-.12821	.293919	.663	-.70916	.45275
		58	-2.59848*	.306476	.000	-3.20426	-1.99271
		59	-1.29667*	.296727	.000	-1.88317	-.71016
		60	-13.55303*	.378066	.000	-14.30031	-12.80576
		61	-4.08958*	.299740	.000	-4.68204	-3.49713
		62	-3.42188*	.299740	.000	-4.01433	-2.82942
	Control/Cipro	54	14.16667*	.367105	.000	13.44106	14.89228
		55	11.91375*	.367105	.000	11.18814	12.63936
		56	13.41667*	.367105	.000	12.69106	14.14228
		57	13.28846*	.362368	.000	12.57221	14.00471
		58	10.81818*	.372625	.000	10.08166	11.55470
		59	12.12000*	.364649	.000	11.39924	12.84076
		60	-.13636	.433423	.754	-.99306	.72033
		61	9.32708*	.367105	.000	8.60147	10.05269
		62	9.99479*	.367105	.000	9.26918	10.72040
	57	54	.87821*	.293919	.003	.29725	1.45916
		55	-1.37471*	.293919	.000	-1.95566	-.79376
		56	.12821	.293919	.663	-.45275	.70916
		Control/Cipro	-13.28846*	.362368	.000	-14.00471	-12.57221
		58	-2.47028*	.300786	.000	-3.06481	-1.87575
		59	-1.16846*	.290846	.000	-1.74334	-.59358
		60	-13.42483*	.373468	.000	-14.16301	-12.68664

		61	-3.96138*	.293919	.000	-4.54233	-3.38043
		62	-3.29367*	.293919	.000	-3.87462	-2.71272
	58	54	3.34848*	.306476	.000	2.74271	3.95426
		55	1.09557*	.306476	.000	.48979	1.70134
		56	2.59848*	.306476	.000	1.99271	3.20426
		Control/Cipro	-10.81818*	.372625	.000	-11.55470	-10.08166
		57	2.47028*	.300786	.000	1.87575	3.06481
		59	1.30182*	.303531	.000	.70187	1.90177
		60	-10.95455*	.383428	.000	-11.71242	-10.19667
		61	-1.49110*	.306476	.000	-2.09687	-.88532
		62	-.82339*	.306476	.008	-1.42916	-.21762
	59	54	2.04667*	.296727	.000	1.46016	2.63317
		55	-.20625	.296727	.488	-.79275	.38025
		56	1.29667*	.296727	.000	.71016	1.88317
		Control/Cipro	-12.12000*	.364649	.000	-12.84076	-11.39924
		57	1.16846*	.290846	.000	.59358	1.74334
		58	-1.30182*	.303531	.000	-1.90177	-.70187
		60	-12.25636*	.375682	.000	-12.99893	-11.51380
		61	-2.79292*	.296727	.000	-3.37942	-2.20641
		62	-2.12521*	.296727	.000	-2.71171	-1.53870
	60	54	14.30303*	.378066	.000	13.55576	15.05031
		55	12.05011*	.378066	.000	11.30284	12.79739
		56	13.55303*	.378066	.000	12.80576	14.30031
		Control/Cipro	.13636	.433423	.754	-.72033	.99306
		57	13.42483*	.373468	.000	12.68664	14.16301
		58	10.95455*	.383428	.000	10.19667	11.71242
		59	12.25636*	.375682	.000	11.51380	12.99893
		61	9.46345*	.378066	.000	8.71617	10.21072
		62	10.13116*	.378066	.000	9.38388	10.87843
	61	54	4.83958*	.299740	.000	4.24713	5.43204
		55	2.58667*	.299740	.000	1.99421	3.17912
		56	4.08958*	.299740	.000	3.49713	4.68204
		Control/Cipro	-9.32708*	.367105	.000	-10.05269	-8.60147
		57	3.96138*	.293919	.000	3.38043	4.54233
		58	1.49110*	.306476	.000	.88532	2.09687

		59	2.79292*	.296727	.000	2.20641	3.37942
		60	-9.46345*	.378066	.000	-10.21072	-8.71617
		62	.66771*	.299740	.027	.07525	1.26017
	62	54	4.17188*	.299740	.000	3.57942	4.76433
		55	1.91896*	.299740	.000	1.32650	2.51142
		56	3.42188*	.299740	.000	2.82942	4.01433
		Control/Cipro	-9.99479*	.367105	.000	-10.72040	-9.26918
		57	3.29367*	.293919	.000	2.71272	3.87462
		58	.82339*	.306476	.008	.21762	1.42916
		59	2.12521*	.296727	.000	1.53870	2.71171
		60	-10.13116*	.378066	.000	-10.87843	-9.38388
		61	-.66771*	.299740	.027	-1.26017	-.07525
		Based on observed means.					
		The error term is Mean Square(Error) = 1.078.					
		*. The mean difference is significant at the 0.05 level.					

List of publications

1. **Bitew M**; Desalegn, T., Belayneh A, Endale M, Eswaramoorthy R., Demissie T.B., (2021) Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. PLoS ONE 16(12). <https://doi.org/10.1371/journal.pone.0260853>

IF = 3.24

2. **Bitew M**; Desalegn, T., Damena, T., Koobotse, M., Eswaramoorthy, R., J Ngwira, K., ... & Demissie T.B. (2022). Cytotoxic mixed-ligand complexes of Cu (II): A combined experimental and computational study. *Frontiers in Chemistry*. <https://doi.org/10.3389/fchem.2022.1028957>

IF = 5.545

3. **Bitew M**; Desalegn, T., Damena, T., Alemayehu Bayle, E., Koobotse, M. O., Ngwira, K. J., ... & Demissie, T. B. (2023). Cytotoxicity and Antibacterial Potentials of Mixed Ligand Cu (II) and Zn (II) Complexes: A Combined Experimental and Computational Study. *ACS Omega*. <https://doi.org/10.1021/acsomega.3c00916>

IF = 4.132

4. **Bitew M**; Desalegn, T., Damena, T., Alemayehu Bayle, E., Koobotse, M. O., Ngwira, K. J., ... & Demissie, T. B. Organic-Inorganic Hybrid Salt and Mixed Ligand Cr(III) Complexes Containing the Natural Flavonoid Chrysin: Synthesis, Characterization, Computational and Biological Studies. *Frontiers in Chemistry*. <https://doi.org/10.3389/fchem.2023.1173604>.

IF = 5.545