Anticancer Applications of New Nickel(II) Complexes with Detoxicant Ligands

ISLAMABAD

A Thesis Submitted to the Department of Chemistry, Quaid-i-Azam University, Islamabad, in partial fulfillment of the requirement for the degree of

Master of Philosophy

In

Inorganic/Analytical Chemistry

By

Nazish Dalil

Department of Chemistry

Quaid-i-Azam University

Islamabad, Pakistan

(2017)

DECLARATION

This is to certify that this dissertation entitled "***Anticancer Applications of New Nickel(II) Complexes with Detoxicant Ligands***" submitted by ***Miss Nazish Dalil*** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, as satisfying the partial requirement for the degree of ***Master of Philosophy*** in ***Inorganic/Analytical Chemistry***.

External (1): ______________________

Supervisor: ______________________

Dr. Zia-Ur-Rehman
Department of Chemistry
Quaid-i-Azam University
Islamabad.

Head of Section ______________________

Prof. Dr. Amin Badshah
Department of Chemistry
Quaid-i-Azam University
Islamabad.

Chairman: ______________________

Prof. Dr. Siddique
Department of Chemistry
Quaid-i-Azam University
Islamabad.

ACKNOWLEDGEMENTS

All praise is due to God, who has created the heavens and the earth, and brought into being deep darkness as well as light. Allah! There is no god but He - the Living, The Self-subsisting, Eternal. All praises be to the **Holy Prophet** (Peace Be Upon Him), the city of knowledge, whose teaching enlighten my heart and flourished my thoughts.

I feel much pleasure in expressing my heartiest gratitude and sincere appreciation to my ever affectionate supervisor **Dr. Zia-ur-Rehman** for the continuous support of my MPhil study and research, for his patience, motivation, enthusiasm, and immense knowledge

I want to express my deep thanks to **Prof. Dr. Muhammad Siddique**.

My sincere thanks also goes to **Prof. Dr. Amin Badshah** head of Inorganic/Analytical chemistry for his full support motivation encouragement and valuable advises.

I express my heartiest and sincerest gratitude to my lab colleagues **Israr, Abdul Ghafoor, Noor Islam, Salma, Hira**, **Imran, Wagma, Faisal Bhai, Maria, Nafeesa, Jamal Abdul Nasir and Ibrar Ahmed** and my friends **Laiba, Nadia, Sehrish, Laraib, Asma and Ain-ul-Fatima** for the stimulating discussions and opinions during this work. A very special thanks goes out to **Muhammad Kashif**. I am indebted to him for his support and help.

My heart-felt thanks goes out to **Sadia** for the love and laughter we shared together, for always being there and bearing with me the good and bad times. Thanks to one and all.

My humble obligation for my affectionate brother **Muhammad Zeeshan** & sisters **Uzma, Amina & Asma for** their sacred prayers and gracious behavior. Thank you for being there for me when I call you and need someone to just listen.

Last but certainly not least, deepest appreciations are extended to my beloved Parents who mean world to me. I extend my respect to my **Taya jee** and **Tai jee**, my paternal and maternal

grandparents. I don't imagine a life without their love and blessings. Thank you **Ammi** & **Abu** for showing faith in me and giving me liberty to choose what I desired. May Almighty Allah bless them with good health and prosperous long lives and be a source of prayer for me.

Nazish Dalil

This dissertation is dedicated to my grandparents who paved the way before me upon whose shoulders I stand & to My Parents (M. DALIL KHAN & ZAHIDA BIBI) who instilled in me the virtues of perseverance and commitment and relentlessly encouraged me to strive for excellence.

Table of Contents

Abstract…………………………………………………………………...………. 1

Chapter 1 Introduction ……………………………………………………………… 2

1.1 Cancer………………………………………………………………………… 2

1.2 Causes of Cancers………………………………………………….…………. 4

1.2.1 Proto-oncogens and Tumor Suppressors Genes……………….………… 4

1.3 Treatment of Cancer…………………………………………..………..… 5

1.4 Metal Complexes as Potential Anticancer Agents……………….………… 6

1.4.1 Exceptional Properties of Metal Ions and Metal-based Complexes…...… 7

1.5 Cisplatin, A Chemotherapy Drug………………………………………...… 8

1.5.1 Pathway to Discovery…………………………………….…………… 8

1.5.2 Cisplatin was used to Control Tumor Cell division and Proliferation…… 8

1.5.3 The Action Mechanism of Cisplatin……………………………...…… 8

1.5.4 Hydrolysis of Cisplatin…………………………………………………... 9

1.5.5 Cisplatin cytotoxic action………………………………………………… 10

1.5.6 The Complexities of Cisplatin Sensitivity and Resistance………………. 10

1.5.7 Pleiotropic Phenotype Associated with Cisplatin Resistancec……… ….. 11

1.5.8 Reduced Accumulation is a Prominent Feature of Cisplatin Resistance… 11

1.5.9 Role of Copper Transporter CTR1 in Cisplatin Uptake…………………. 12

1.5.10 Increased Inactivation by Thiol-containing Molecules…………………. 12

1.5.11 Increase in DNA Damage Repair………………………………………. 12

1.6 Toxicological Effects and Common Side Effects of Cisplatin……………… 13

1.7 The Thread of Life “DNA”…………………………………………………… 13

1.7.1 Possible Conformations of DNA………………………………………… 15

1.8 Interaction of Metal Ions and Metal Complexes with Nucleic Acids………… 18

1.8.1 Covalent Mode of Binding………………………………………………. 20

1.8.2 Non-covalent Mode of Binding…………………………………………. 21

1.8.2.1 Intercalation………………………………………………………... 21
1.8.2.2 Groove Binding……………………………………………………….. 22
1.8.2.3 External Binding……………………………………………………… 23
1.9 Dithiocarbamates………………………... 23
1.9.1 Synthesis of Dithiocarbamates……………………………………...... 24
1.9.2 Biological role of Dithiocarbamates…………………………………….. 25
1.9.3 Medicinal Diversity of Dithiocarbamates……………………………… 26
1.10 Nickel in biological system ………………………………………………… 27
1.11 Aims and Objectives of Present Work……………………………………… 27
Chapter 2 Experimental……………………………………………………... 34
2.1 Chemicals……………………………………………………………….. 35
2.2 Instrumentation ………………………………………………………………. 35
2.3 Synthesis of Piperazine-1-carbodithioate Derivatives………………………. 36
2.4 Synthesis of Dithiocarbamate Ni(II) Complexes……………………………... 36
2.5 Biological studies……………………………………………………………….. 39
2.5.1Evaluation of anti-proliferative activity of synthesized complexes (Sulforhodamine b assay)………………………………………………………… 39
2.5.2 DNA Binding Experiments of Complexes by Uv-Vis Spectroscopy 40
2.5.3 DNA Binding Study by Viscosity Measurement……………….............. 41
2.6 Computational Studies……………………………………………………….. 41
Chapter 3 Results and Discussion……………………………………………...… 44
3.1 Physical data………………………………………………………….………. 44
3.2 Spectroscopic Characterization of the Complexes…………………………..... 46
3.2.1 FTIR Spectra Studies of the Metal Complexes………………………… 46
3.2.2 Multi-nuclear (^{1}H, ^{13}C) NMR Spectroscopy…………………………… 47
3.3 Single Crystal X-ray Crystallographic Description…………………………... 49

3.4 Biological Activity………………………………………………………… 52
3.4.1 Cytotoxic Assay by Sulforhodamine B Dye (SRB Assay) against MCF-7…… 52
3.4.2 DNA Interaction Studies by UV-Vis and Evaluation of Binding Parameters… 53
3.4.3 Viscosity measurement of Nickel (II) complexes……………………… 62
3.5 DFT calculations…………………………………………………………… 63
Conclusions…………………………………………………………………… 67
References…………………………………………………………………… 68

List of Abbreviations

DNA	Deoxyribonucleic acid
MSU	Michigan State University
FDA	Food and Drug Administration
EMT	Epithelial-Mesenchymal Transition
GSH	Glutathione
NER	Nucleotide Excision Repair
RNA	Ribo Nucleic Acid
HIV	Human Immunodeficiency Virus
SAR	Structure-activity Relationship
$\mathbf{IC_{50}}$	Inhibitory Concentration of 50%
DFT	Density Functional Theory

List of Figures

Figure 1.1 A malignant tumor……………………………………………....... 4

Figure 1.2 Different chemotherapeutic drugs for anticancer………………….. 5

Figure 1.3 Activation of the cisplatin compound by hydrolysis or reaction with S-donors before interacting with DNA of cells……………………… 9

Figure 1.4 Interaction of cisplatin with DNA base pairs…………………… 10

Figure 1.5 Different reasons asssociated with cispaltin resistance………… 11

Figure 1.6 Inactivation of cisplatin by GSH. X-SH=glutathione or cysteinylglycine………………………………………………………… 12

Figure 1.7 Factors modulating repair of cisplatin-induced DNA adducts and regulating replicative bypass………………………………………………. 13

Figure 1.8 Cytotoxicities associated with cisplatin………………………….. 14

Figure 1.9 Double stranded structure of DNA helix (major and minor grooves)……………………………………………………………………. 15

Figure 1.10 Different conformations adopted by DNA………………………. 16

Figure 1.11 Red dots symbolize the position of the helical axis of A, B, and Z-DNA with respect to a Guanine-Cytosine base pair……………………….. 17

Figure 1.12 Cisplatin forms adducts with the N7 nitrogen from guanine bases 20

Figure 1.13 Intercalation binding of a ruthenium complex with B-DNA form 22

Figure 1.14 Major groove binding…………………………………………. 23

Figure 1.15 Soft dithiocarbamate and hard thioureide Resonance forms…… 24

Figure 3.1 ^{1}H NMR spectroscopy of C1………………………………….. 48

Figure 3.2 ^{13}C NMR spectroscopy of C1………………………………… 49

Figure 3.3 Molecular structure of C5 ($C_{24}H_{30}N_4NiO_2S_4$)………………… 50

Figure 3.4 (a) 2D (b) 3D structures for C5……………………………… 51

Figure 3.5 IC_{50} values of complexes against MCF-7 (human breast cancer cell line)……………………………………………………………………. 52

Figure 3.6 IC_{50} values of complexes against MCF-7 (human breast cancer cell line)……………………………………………………………………. 53

Figure 3.7 Absorption spectrum of C1……………………………………… 56

Figure 3.8 Absorption spectrum of C2……………………………………… 57

Figure 3.9 Absorption spectrum of C3………………………………………. 58

Figure 3.10 Absorption spectrum of C4……………………………………… 59

Figure 3.11 Absorption spectrum of C5……………………………………… 60
Figure 3.12 Absorption spectrum of C6……………………………………… 61
Figure 3.13 Plot of [η/ηo] $^{1/3}$ versus [Complex]/[DNA] for complexes C1-C6 62
Figure 3.14 Optimized structures of C2 at B3LY P/LANL2DZ level………... 64
Figure 3.15 Optimized structures of C4 at B3LY P/LANL2DZ level……….. 64
Figure 2.16 The atomic orbital of the frontier molecular orbital for
$C_{18}H_{36}N_2NiS_4$ at B3LYP/3-21G level of theory……………………………… 65
Figure 3.17 The atomic orbital of the frontier molecular orbital for
$C_{14}H_{26}N_4NiO_2S_4$ at B3LYP/3-21G level of theory…………………………… 65
Figure 3.18 Optimized structures of C1 at B3LY P/LANL2DZ level……… 66
Figure 3.19 The atomic orbital of the frontier molecular orbital for
$C_{26}H_{32}N_2NiS_4$ at B3LYP/3-21G level of theory……………………………… 66

List of Tables

Table 3.1 Physical data for Ni(II) complexes……………………………………… 43

Table 3.2 FTIR data of complexes C1-C6 ……………………………………… 44

Table 3.3 Crystal data and structure and refinement parameters for C5 ……… 48

Table 3.4 Selected bond lengths (Å) and bound angles (°) …………………… 49

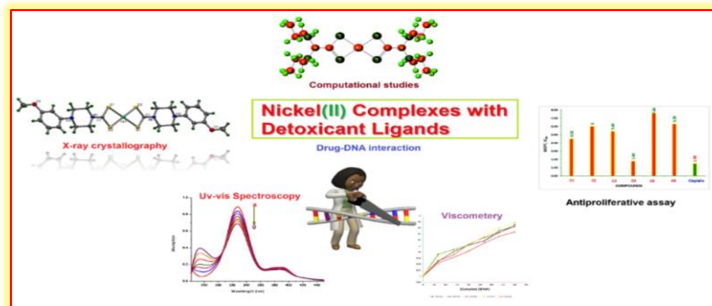
Table 3.5 IC_{50} values of the Ni(II) complexes …………………………………. 50

Table 3.6 Binding parameters of complex (1-6) based upon UV-Vis spectroscopic data…. 51

Table 3.7 Energies (eV) of HOMO and LUMO, chemical hardness (η), electronic chemical potential (μ), and electrophilicity (ω) ……………………… 62

Abstract

Herein a new series of nickel(II) dithiocarbamates has been synthesized by reacting nickel(II) chloride with sodium salts of dithiocarbamates in methanol solvent. The ligands used were sodium 4-benzylpiperidine-1-carbodithioate **(L-1),** sodium di-sec-butylcarbamodithioiate **(L-2),** sodium 4-(pyridin-2-yl)piperazine-1-carbodithioate **(L-3),** sodium 4-(2-hydroxyethyl)piperazine-1-carbodithioate **(L-4),** sodium 4-(3-methoxyphenyl)piperazine-1-carbodithioate **(L-5),** sodium 4-(pyrimidin-2-yl)piperazine-1-carbodithioate **(L-6).** The coordination mode of ligands towards Ni(II), structural confirmation and geometry assignment of the complexes was made using different analytical techniques such as elemental analysis, FT-IR, multinuclear (^{1}H and ^{13}C) NMR and single crystal X-ray diffraction technique. Single crystal X-ray analysis has confirmed the anisobidentate nature of dithiocarbamate ligand resulting in ideal square planer geometry for **C5**. UV–Vis and viscometric titration techniques have been employed to probe the details of DNA binding. The hypochromic effect for all the complexes was observed, indicating intercalative mode of interaction and intrinsic binding constants (K_b) have been estimated under a similar set of experimental conditions. Antiproliferative activity of all synthesized compounds was assessed against MCF-7 (human breast cancer cell line). Results showed that all the complexes were significantly active against MCF-7 (human breast cancer cell line) but the **C4** exhibit pronounced activity. A brief account of computational technique is described using B3LYP/LANL2DZ level of Density Functional Theory in *Gaussian 09* that can be used as a dynamic tool with a specific end goal to achieve profound information regarding the parameters requisite for the stabilization of coordination compound-DNA adduct.



Chapter 1

Introduction

1.1 Cancer

Living cells comprise the basic structure of all living organisms that frequently require to separate to produce more cells for life processes, furthermore, to take place of injured cells or those have been kicked the bucket. Genes inherited to offspring regulate cell proliferation and confer innumerable other distinct attributes. These processes are well-controlled but upon loss or damage of genomic control, cells are not any more absolutely sensitive to signals that are responsible for regulation of cell survival, differentiation, proliferation and ultimately death [1]. Subsequently these cells amass inside the tissues cause damage and continue to divide, thus increase in number when and where they aren't needed. This is known to be cancer and it is major cause of death and affecting more people present at time than ever before. Above 200 different kinds of cancer are identified [2].

So cancers are the large family of ailments in which cluster of abnormal cells show incontrollable growth by ignoring the standard rules and regulations of process of cell division and can be best described as a systematic disease resulting from an accretion of genetic variations. This accrual of transmutations results in the collapse of cooperation among cells in a tissue in such a way that single cells relapse to self-seeking behavior evocative of single celled organisms [3]. Normal cells are continually controlled by signals that command the processes like whether cell ought to divide, distinguish into another cell or fate to death. Cancer cells start to build up a degree of self-government from these signals and bringing about unrestrained growth and multiplication and this proliferation can proved to be lethal if permitted to sustain and grow for an extended period of time. Nearly 90% deaths associated with cancer are owing to tumor proliferation a process named as metastasis [1]. The foundation of present-day cancer study is based on a simple theory; almost all the mammalian cell lines share comparable molecular systems that govern processes of multiplying and differentiating cells and of course their death. The existing theory which forms the foundation of research for origin and therapy of cancer is that, as an outcome of

variations in these systems at molecular, biochemical as well as cellular level normal cells are altered into cancerous cells. In addition to that for each cell there is a fixed number of methods this disturbance can happen [4].

From every part of recorded history it is evident that all living organisms (human beings as well as other animals) have had cancer, so it’s no wonder that people have recorded about cancer since the inception of history. Fossilized bone tumors and human mummies of the bygone age are the primitive evidences of cancer found in prehistoric Egypt and manuscript [5]. The most seasoned explanation of cancer (despite the fact that word cancer wasn’t in use) was originated in Egypt around 3000 BC. It's known as the Edwin Smith Papyrus and is similar to an old Egyptian course reading on trauma surgery which narrates about eight examples of cancers relating to breast that were detached by burning through fire drill, an instrument known at that time. The written work says about the illness, "There is no treatment” [6].

Hippocrates a Greek physician, deemed as “Father of Medicine” had the recognition to coin the terms *carcinos* and *carcinoma* to give a detailed account of ulcer-forming tumors in addition to those that don’t cause ulcer. These terms allude to a crab in Greek since the protrusion resembling a finger from a cancer is similar in shape to a crab. A Roman physician Celsus, translated the Greek expression into *cancer*, a word used for crab in Latin. Another Greek physician, Galen used the term *oncos* (bulge in Greek) to give a description of tumors.

Since the most primitive eras physicians have confused over the causes behind the cancer. In the prehistoric ages the Egyptians accused the divine beings for cancers. More than a few theories were presented regarding the cancer, Humoral, Lymph, Blastema, Chronic irritation, Trauma and Infectious disease theory [7].

1.2 Causes of Cancers

Both external and internal factors account for the commencement and development of tumor. Both these factors can proceed either jointly or in succession with subsequent unnecessary multiplication or abnormality. These results in growth of cell masses which expand to nearby normal tissues or can also proliferate to other sites resulting metastasis. Though it is important that majority of tumors take long duration even years to accumulate these DNA alterations for some observable cancer. Environmental

factors are major cause of the larger part of tumors, nearly 90–95% of problems. The term environmental implies any cause that is not acquired hereditarily. Some statistics of environmental factors are (25–30%, tobacco), (30–35% diet and obesity), (15–20% infections) and (up to 10% radiation). Other factors include apoptosis, carcinogens, nutritional deficiencies and food practices, betel nut, alcohol, heredity, sunshine, industrial irritants, viruses, hormones, some pre-existing abnormalities and physical agents etc. [8].

1.2.1 Proto-oncogenes and Tumor Suppressors Genes

There is a braking mechanism in every normal genomic make-up which ceases cell growth upon fulfilment of need. The terms proto-oncogenes and tumor suppressors are sets of genes which either act as promoters or suppressors of cell division and development. Proto-oncogenes are recognized as positive regulators that control growth and differentiation of cell [9]. Contrary, tumor suppressor genes act as negative regulators and stop unrestricted growth [10]. Proto-oncogene is thus a normal gene in every aspect often controlling vital processes of cell multiplication, diversity and existence. A proto-oncogene turns into cancer causing oncogene on receiving activating mutation. Activation may be brought out by mutation, gene duplication and chromosomal translocation. Normal cells will go through a planned cell death known as apoptosis [11]. Oncogenes cause cells destined to death to survive and proliferate further and some tumor suppressor genes become inactivated. Unwanted growth of cells takes place along with proliferating to other parts of body as well resulting in cancer.

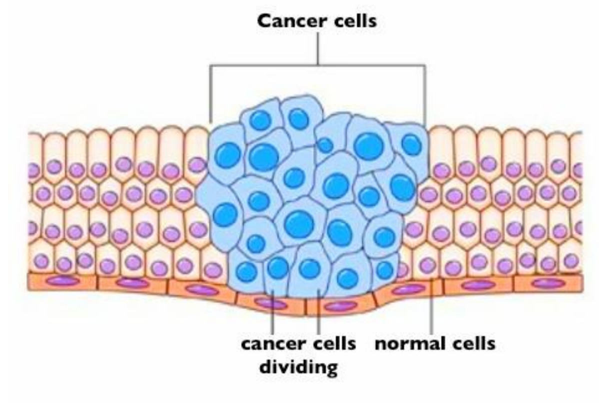


Figure 1.1 A malignant tumor

The loss of tumor suppressor genes is comparatively more essential than oncogene activation for cancer formation in humans. Lately, tumor suppressor genes are categorized into three groups, first being “gatekeepers”. Generally they are negative regulators and act as “brakes” to regulate cell division. The second group “caretakers” genes and their function is to stabilize genome and in DNA repair. The third group is named as “landscapers” [12, 13].

1.3 Treatment of Cancer

The cure of cancer has gone through developmental changes as understanding of the basic biological processes has become better. Several remedial choices exist for cancer. The prime ones are treatment by surgery, using drugs (chemotherapy), radiation therapy, hormonal therapy, targeted therapy as well as palliative care but chemotherapy is primarily focused. Which method is used relies upon on the sort, position, patient's health and main concern as well as grade of the cancer. The proposed action may or may not be remedial. Chemotherapy refers to the use of diverse cytotoxic anti-neoplastic drugs to treat cancer as a part of regular routine. It includes a variability of drugs, which are divided into wide groups such as cytotoxic antibiotics, some alkylating agents, antimetabolites, anti-microtubule agents and topoisomerase inhibitors [14]. Effectiveness of chemotherapy is determined by the type of cancer and the stage and is frequently restricted by its harmfulness to various other tissues in the body.

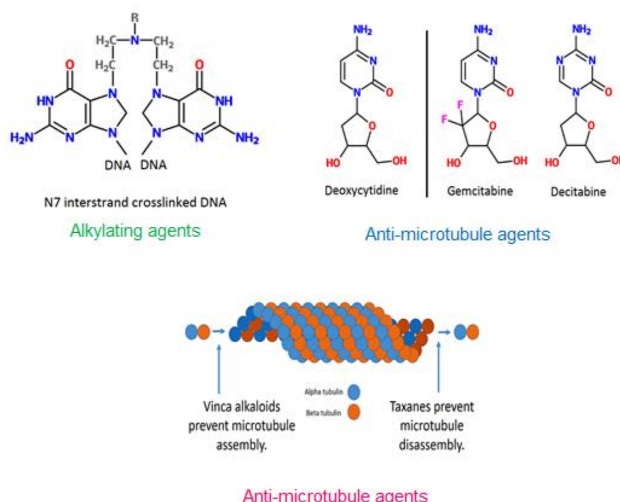


Figure 1.2 Different chemotherapeutic drugs for anticancer

1.4 Metal Complexes as Potential Anticancer Agents

Everything is poisonous, and nothing is harmless. The dose (amount) alone defines whether something isn't poison" Paracelsus, 1493-1541.

The implementation of inorganic chemistry in therapeutics is a promptly growing domain and innovative medicinal complexes have a strong influence in therapeutic applications. Developments in biocoordination chemistry are pivotal to lessen the toxic effects and mechanisms of activities of compounds. The medicinal use of metals found nearly 5000 years back. Biomolecules being negatively charged offer outstanding ligands to bind with positively charged metal centers, thus metal complexes has phenomenal pharmaceutical potential [15].

Metals are requisite cell parts selected by nature to work in a few essential biochemical procedures for living beings. Metals are furnished with distinctive features that comprise inconstant coordination modes, redox activity in addition to reactivity to biological substrates. Metals are firmly managed in typical conditions owing to their reactivity and different metal particles are related with different obsessive issue, including abnormal growth. Therefore, coordination compounds moreover as medications or prodrugs, turn out to be exceptionally alluring tests as potential agents against cancer. Since iatrochemistry to advanced, the utilization of metals besides their salts has been available all through mankind's history for therapeutic purposes [16]. The present-day tool case of dynamic antitumor agents is wide-ranging and aims at various cellular and organic properties over a few tumor kinds. The antitumor medications progressed far from common cytotoxicity towards the perceptive plan of particular agents in the course of the most recent fifty years, that follow up on particular cell targets [17, 18]. Be that as it may, critical difficulties remain and the admix between chemistry and biology may give the most profitable intends aimed at finding and enhancing innovative anticancer agents [19].

Naturally numerous organic frameworks make broad utilization of metal particles for example, copper and zinc which assume basic parts in the typical working of living beings [15]. Numerous complexes containing metals have been used throughout history in order to treat a wide assortment of ailments [20]. In therapeutic science

generally ruled by organic chemistry, complex systems containing metals have acquired approval as symptomatic tools and performers of anticancer activity [21].

1.4.1 Exceptional Properties of Metal Ions and Metal-based Complexes

Therapeutic inorganic chemistry includes, yet is not constrained to delivering or expulsion of a metal particle into living system for either remedial or indicative causes [22]. Formation of positively charged ions in aqueous media is a significant attribute of metals owing to that they can attach to contrarily charged organic molecules [15]. In advancement of new medicinal field metal-containing compounds present many preferences over routine carbon-based complexes. These favorable circumstances are because of their capacity to arrange ligands in a three dimensional design, in this way permitting functionalization of sets that can be customized to characterized atomic targets. Diverse distinguishing structures that present a wide range of geometries, coordination numbers moreover kinetic properties are figured by a rich domain proffered by metal-based complexes that can't be achieved by means of traditional complexes based on carbon [21, 23, 24]. Riveting electronic properties are conferred to transition metals by their partly filled *d* orbitals and might go about like appropriate tests with the aim to design antitumor agents [25]. Likewise oxidation state of a metal is a vital idea to design coordination mixtures, thus permitting involvement within organic redox science and assumes a powerful part in optimum dosage in addition to bioavailability of the drug directed [15]. Besides, a multitude of chances for metals to interrelate in addition to coordinate to living molecules are proposed by ligand interchange reactions as exhibited by the extensively utilized medicine cisplatin [26]. The inadvertent disclosure of cisplatin triggered an investigation about anticancer agents. The revelation of cisplatin, cis-$[PtII(NH_3)_2Cl_2]$ was a vital turning point which set off the initiative for structures of platinum(II) and others metal as prospective innovative anticancer medications. It was a successful anti-tumor drug being widely used for the treatment of ovarian and testicular cancer and progressively used for cervical, small-cell lung, head and neck cancer and is often used in combination. Nevertheless its extensive clinical utilize had been hindered by toxicity and resistance whichever inherent or acquired. These shortcomings were addressed by platinum

analogs, most notable are carboplatin and oxaliplatin and are clinically accepted that sustain a more controllable toxicity profile [24, 27].

1.5 Cisplatin, a Chemotherapy Drug

1.5.1 Pathway to Discovery

Cisplatin, first and dynamic member of platinum complexes was an accidental discovery by a biophysics researcher Dr. Rosenberg at MSU, when an experiment was devised by him to find either electrical field could have an influence on cell division or not. It was believed that platinum has no biological application, so platinum electrodes were dipped in to solution of bacteria *E. coli* and power was turned on. With the passage of current bacterial cells ceased to divide, though growth was up to 300 times more than usual length which led Dr. Rosenberg to assume that effect was because of electric current. Two years were spent in an attempt to find why the electrical field had such a significant impact. At long last, they understood that current had no effect; in fact cell division was being stopped because of platinum discharge from electrodes. A dramatic effect was noticed after an additional two years by Dr. Rosenberg's group. It was named as cisplatin later on [28].

1.5.2 Cisplatin was used to Control Tumor Cell division and Proliferation

In order to find whether cisplatin would likewise stop cell division in tumors, an experiment was devised by Rosenberg’s team to test it on sarcoma mouse and it showed promising results. Kidney damage was a problem associated with cisplatin as it was extremely toxic however, mice survived at low doses of drug. After six months, the mice stayed all right with no tumor return [29]. Clinical trials on small-scale were began in 1972 [30] with human cancer patients and cisplatin was accepted in 1978 by FDA and proved a massive discovery [31].

1.5.3 The Action Mechanism of Cisplatin

A noteworthy part is supposed to be played by significant perceptions; kinetic constancy and *trans* orientation of ligands in the effectiveness of antitumor activity of platinum(II) compounds. A square planar complex cisplatin is kinetically inactive, so it does not simply undertakes ligand replacement reactions and so increase its coordination number. It is absorbed by cells either passively or actively. In blood

plasma cisplatin remains stable to hydrolysis because of high chloride ions concentration (100 mM). Nonetheless the notably lesser (4-23 mM) intracellular concentrations ease fast hydrolysis. Primarily it was supposed that passive transport played imperative role in cellular uptake of cisplatin, but recent studies strongly suggest that active transport through copper transporters CTR1(Copper TRansport protein 1) and CTR2 (Copper TRansport protein 2) regulates the routes of cisplatin into the cell [32].

1.5.4 Hydrolysis of Cisplatin

On entering cell, cisplatin turns into activated complex. Earlier to react with DNA in the cytoplasm, owing to the much lower concentration of chloride ions (2-10mM), cisplatin undergoes solvation process and produce "activated" monoaqua cis-$[PtCl(H_2O)(NH_3)_2]^+$ as well as diaqua cis-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ molecules via easily replacing chloride ions by water molecules. The first and second rate constant for hydrolysis having the value $k_1 = 5.18 \times 10^{-5}$ ($t_{1/2}$= 3.4h) and $k_2 = 2.75\times10^{-5}$ ($t_{1/2}$= 7h) respectively. This hydrolyzed species is strongly electrophilic in nature and is much reactive towards nucleophilic sites at nucleic acids, thus resulting in precisely 1,2-intrastrand d(GpG) cross links that are the major adducts made by cisplatin with deoxyribonucleic acid [33, 34].

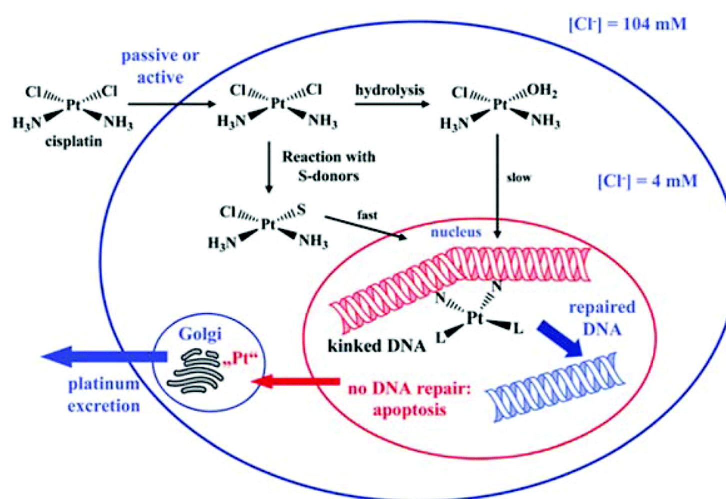


Figure 1.3 Activation of the cisplatin compound by hydrolysis or reaction with S-donors before interacting with DNA of cells

1.5.5 Cisplatin cytotoxic action

There seems to be manifold mechanisms that cause the cytotoxic and antiproliferative potential of cisplatin but mostly it is due to its interaction with DNA through linkage with nitrogen-7 position of Guanine. Its cytotoxic approach of action has been connected to its ability of crosslinking with the purine bases, mainly intrastrand crosslink adducts, impeding with DNA repair mechanisms, initiating DNA damage and consequently resulting apoptosis in cancer cells altering the progression of curative management of numerous tumors [35].

Before reaching the target DNA it passes through the cytosol where many potential targeting agents are available for this positivity charge aquated species for example, phospholipids RNA and proteins present in the cytosol and nucleus. These interactions may results in either apoptosis (programmed cell death) or necrosis (disorderly cell death) where apoptosis is important for cisplatin antitumor effect.

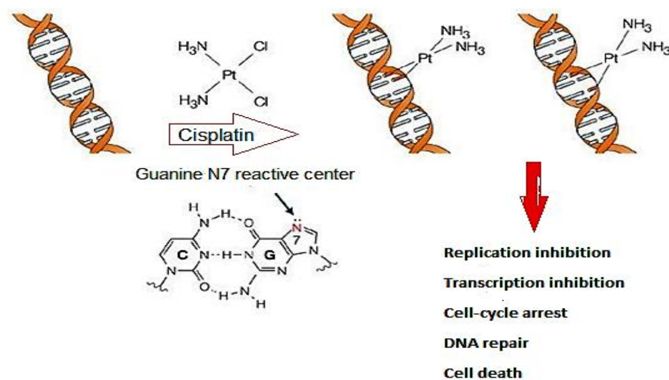


Figure 1.4 Interaction of cisplatin with DNA base pairs

1.5.6 The Complexities of Cisplatin Sensitivity and Resistance

Even though cisplatin is a very effective inducer of cell death and its clinical implementation in the late 1970s was a landmark in the progress of effective chemotherapeutic drugs. Initially a good response is observed in patients to cisplatin-based chemotherapy. Nevertheless, later decline as of resistance develops either acquired or inherent, which significantly decreases its clinical efficacy. Tissue culture investigations propose that this resistance can be consequence of epigenetic variations at molecular and also to cellular levels, which includes decreased accretion of the

platinum compounds by either active sequestration or reduced influx, better levels of DNA damage repair, inactivation by GSH and metallothioneins besides additional antioxidants, increased expression of chaperones, inactivation of the apoptosis and activation of the EMT pathways etc. Investigations of the resistance mechanisms of platinum have exposed an overabundance of complex resistance mechanisms [36].

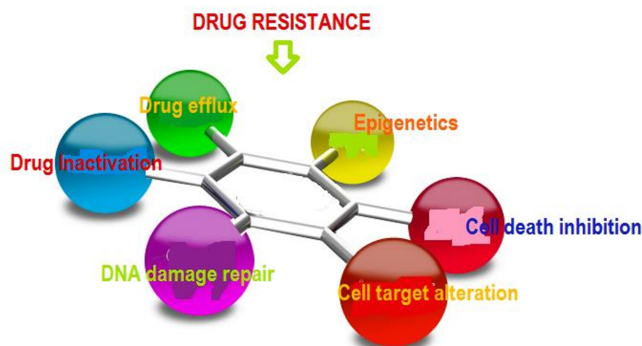


Figure 1.5 Different reasons asssociated with cispaltin resistance

1.5.7 Pleiotropic Phenotype Associated with Cisplatin Resistancec

Innumerable phenotypic variations found in human cisplatin-resistant (CP-r) cells have been well recognized. These consist of cross-resistance to various structurally linked or dissimilar drugs, decreased intake of drug in CP-r cells in connection with a reduction in platinum-DNA adduct formation, alterations in levels of gene expression concerned with practically every facet of cell existence like apoptosis, chaperones, DNA repair, the cell cycle, transporters, transcription factors, protein transferring, oncogenes, small GTPases, GSH and linked enzymes, mitochondria etc. [36].

1.5.8 Reduced Accumulation is a Prominent Feature of Cisplatin Resistance

Decreased accretion of the compound is one of the utmost leading features of cellular resistance to cisplatin. As an outcome of decreased uptake, platinum-DNA adducts formation is likewise reduced, decreasing cytotoxicity, resulting in more resistance to the platinum compound. Reduced influx might be outcome of malfunctioning transporters or channels, functional/structural alterations in cell organs or membrane potential whereas increased efflux can result from greater export, emission or exocytosis of the platinum drugs [37].

1.5.9 Role of Copper Transporter CTR1 in Cisplatin Uptake

Recently, it has been examined that the copper transporter CTR1, a main influx transporter, performs a vital part in facilitating uptake of platinum drugs. Genomic sensation of CTR1 brings cellular resistance to cisplatin in vivo. Cells with greater CTR1 expression show increased platinum accretion and in most cases, great sensitivity to cisplatin [38]. Howell- et al. studied the role of CTR1 in CP-r cells.

1.5.10 Increased Inactivation by Thiol-containing Molecules

Lower concentration of chloride ions (~4 mmol/l) inside the cell facilitates aquation of cisplatin. This activated cisplatin reacts with many cytoplasmic ingredients, including the plentiful nucleophilic GSH as well as the cysteine-rich metallothionein. These thiol-containing molecules decrease the level of the antitumor drug accessible for interaction with the objective DNA [39].

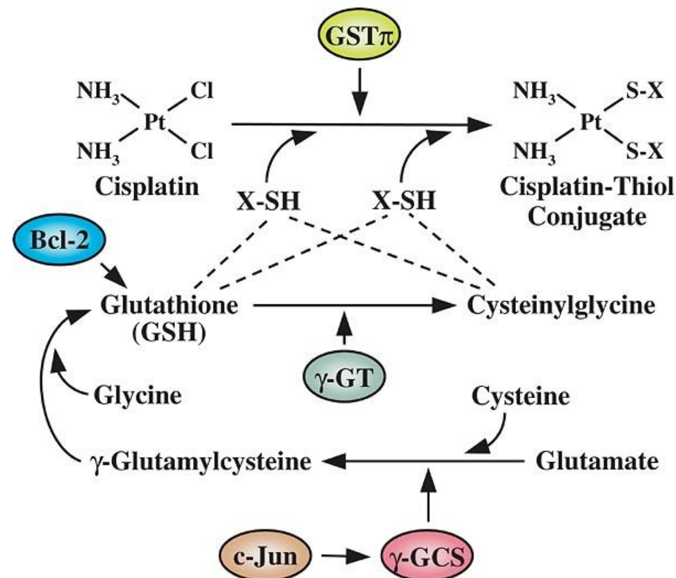


Figure 1.6 Inactivation of cisplatin by GSH. X-SH=glutathione or cysteinylglycine

Certainly, the greater conjugation among GSH and cisplatin is usually considered as an important aspect in resistance, but the role of raised GSH in either increasing DNA-damage repair or increasing the restraining influence on cell death via shielding an endogenous drug-induced oxidative stress is also interesting [40].

1.5.11 Increase in DNA Damage Repair

Formation and perseverance of DNA-drug adducts is vital in bringing apoptosis. Hence, an increased level of adduct repair will decrease the apoptotic process.

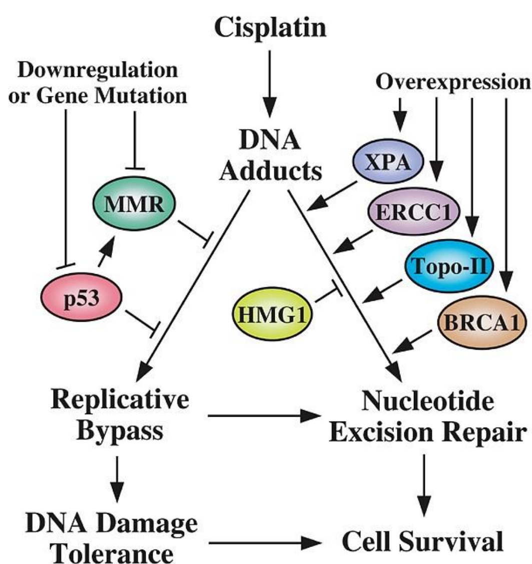


Figure 1.7 Factors modulating repair of cisplatin-induced DNA adducts and regulating replicative bypass

Increased level of NER in the resistance cell line is accountable for elimination of cisplatin lesion, which involves identification and specification of the affected area and lesion is excised followed by DNA repair. More than 90% treatment of testicular cancer by cisplatin therapy is because of reduced level of NER in cells [41].

1.6 Toxicological Effects and Common Side Effects of Cisplatin

Cisplatin has a number of aftermaths that can restrict its use including ototoxicity, cardiotoxicity, neurotoxicity, nephrotoxicity, gastrointestinal toxicity, hemolytic anemia, electrolytic disturbance and numbness etc. [42].

1.7 The Thread of Life "DNA"

The thread of life "DNA" (deoxyribonucleic acid) possesses the quality of being worthy of attention because of countless biological significance and known to be athenaeum of life having all the knowledge encoded therein turns out to be more accessible each day.

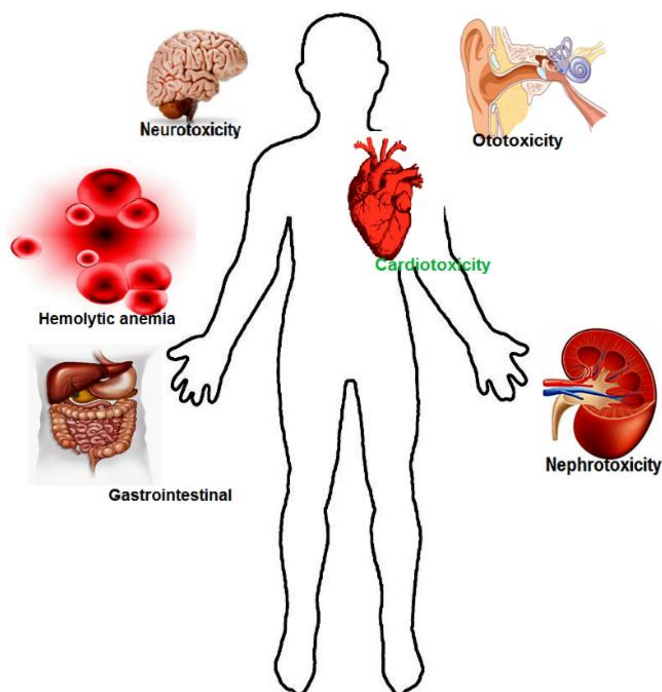


Figure 1.8 Cytotoxicities associated with cisplatin

Therefore, DNA like an instruction manual plays a significant role in processes of life for the reason that it transmits the genetic information and commands the production of proteins as well as enzymes in living cells by the procedure of replication and transcription of genomic library. This hereditary instruction stays as a part of all vital life processes of all known living life forms and numerous viruses as well. The historical framework of hereditary research started with Gregor Mendel the "Father of Genetics". His experiments with plants in 1857 resulted in keen attention towards genetic studies greater than before. A substance was found by Friedrich Miescher and in 1869 he named it "nuclein". Later from the sperm of salmon he isolated a material now recognized as DNA and in his understudy, Richard Altmann in 1889, termed it "nucleic acid”. Phoebus Levene recognized that in addition to sugar and phosphate backbone, four bases constitute DNA i.e. Purines (adenine and guanine) as well as Pyrimidines (thymine and uracil). Molecular structure of DNA was identified in 1953 by Francis Crick and James Watson [43, 44]. Various physical laws and speculations are investigated by researchers by using DNA as a molecular tool. DNA has become

an alluring particle for material researchers concerned with micro and nano assemblage because of its special material characteristics.

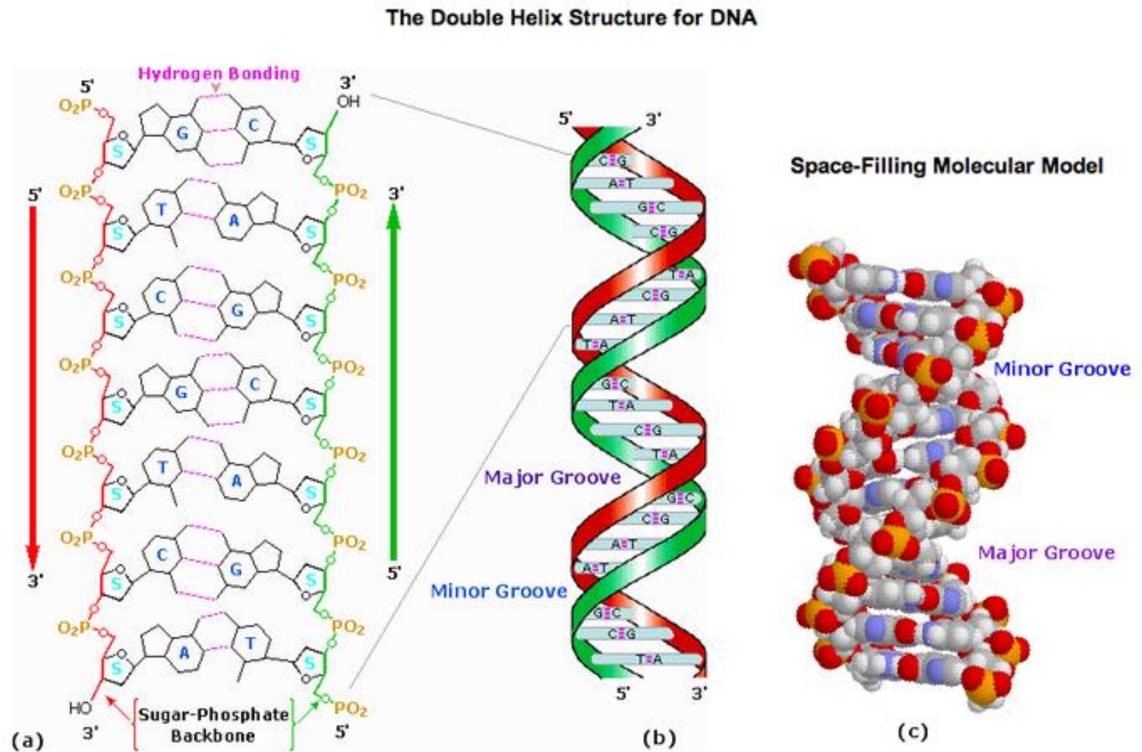


Figure 1.9 Double stranded structure of DNA helix (major and minor grooves)

DNA is a long polymer made from repeating units entitled as nucleotides. Five-membered ring carbohydrate deoxyribose constitutes a nucleotide by attaching to C1 and C5 of a heterocyclic base that possibly will be an adenine, guanine, cytosine or thymine. 5'-end of one and the 3'-end of other sugar is bridged by a phosphodiester linker. Consequently, extensive single-stranded polyanionic chains are resulted by the polymerization of nucleotides [45].

1.7.1 Possible Conformations of DNA

Diverse unusual conformations are acquired by DNA and are influenced by environmental factors including hydration level, ionic strength as well as base sequence. A, B, C, D and Z forms are broadly grouped as different conformations acquired by DNA. Still B form of DNA is pre-eminent in normal functioning of an organism. Right-handed orientation of helices is attributed to A and B-DNA, however Z-DNA is a left-handed helix [46].

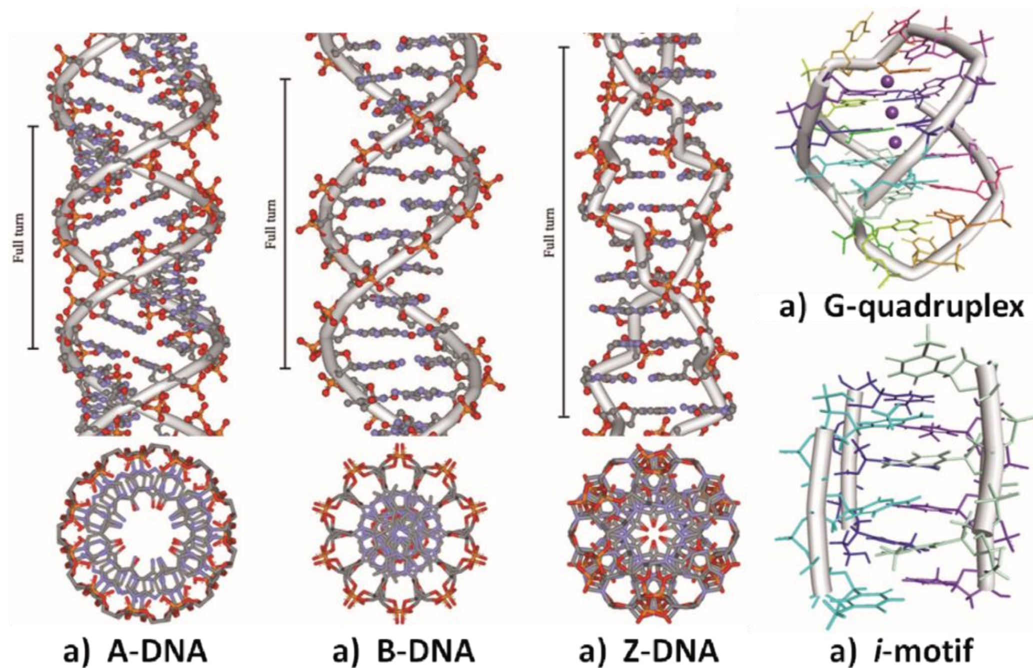


Figure 1.10 Different conformations adopted by DNA

A-DNA is one of the most likely double helical structures which DNA can have and was first recognized at low humidity conditions (75%) from fibre diffraction investigations of DNA. Usually A-DNA form is preferred in dehydrating conditions; however, even when hydration level is greater, A conformation is favored in purine stretches. Eleven base residues per helical turn of A-DNA are present with 2.25 Å distance between bases and a more compact structure. The A-DNA has a deep and narrow major groove whereas a wide and shallow minor groove [47].

The most common and adjustable structure in nature is B-DNA among three conformations of DNA and is known as Watson–Crick form of DNA, first found in fibres at 92% relative moisture. Approximately 10–10.5 base pairs are present per turn and it is right-handed. In B-DNA major groove is much wider as compared to minor groove with lengths 10.5 Å and. 4.8 Å respectively. Allomorphs C-DNA and D-DNA are alike to B-like in structure. However, rise per residue is reduced (3.31 and 3.05 A correspondingly). In low hydration surroundings these are found with lithium and sodium ions. D-DNA form is also produced by alternation in purine and pyrimidine sequences.

Z-DNA was firstly discovered by Robert Wells and colleagues. It is identified as left-handed orientation of helix and this conformation is mostly adopted at higher salt concentration.

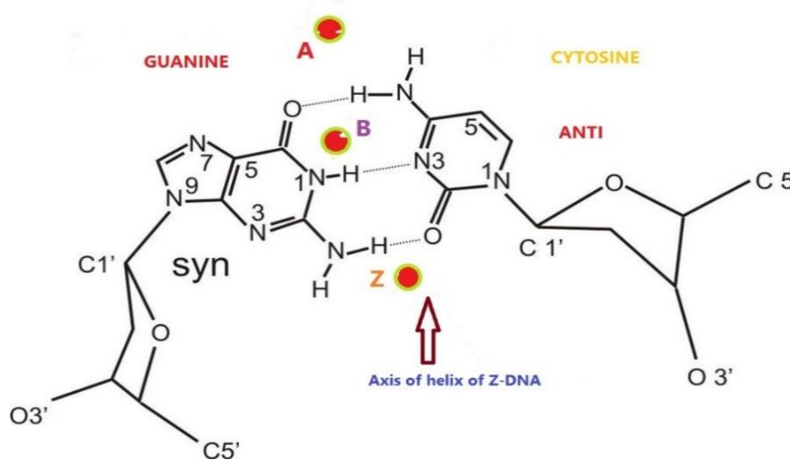


Figure 1.11 Red dots symbolize the position of the helical axis of A, B, and Z-DNA with respect to a Guanine-Cytosine base pair

Twelve base pairs and a sugar phosphate comprise each turn. Thus it assumes a bent form and is narrower than the A and B conformations. For instance triplex (H-DNA), hairpin, tetraplex (G-quadruplex and *i*-motif) are further conformations likewise assumed by DNA. An unusual change takes place in B-DNA or infrequent formation of base pairs resulting in DNA strands to bend with a specific end goal to design these structures. These are different pairs of hydrogen bonding amongst nucleic bases in comparison to Watson-Crick base pairs. Hairpin structures are regarded as possible locations for regulating gene expression have been found in genetic DNA [48].

DNA owns a non-static structure. It does not typically be present like single molecule, but as tightly held pair of molecules. Double helix shape is adopted by two extensive intertwine strands. Diverse altered conformations are assumed by DNA and are affected via environmental aspects (hydration and ionic strength). Anticancer and antibacterial drugs have their anticancer activities emerged as of their interactions by nucleic acid substrates.

1.8 Interaction of Metal Ions and Metal Complexes with Nucleic Acids

A pivotal role is played by metals in biological processes and key part in cellular and subcellular functions is universally acknowledged. The prominence of metals is inescapable owing to the fact that they are intrinsic part of enzymes associated with metabolic or biochemical activities. With the use of highly up to date and advanced machines to investigate biochemical and biological systems, the exact part of inorganic salts in all living forms can be disclosed. Inorganic chemistry is not the "Dead Chemistry" and it has recognized that metals are as much important as organic molecules. Metals are known since earliest eras. Egyptians used copper to purify water and Chinese used gold for medicinal purposes in 2500 BC. There are number of metal complexes which take part in biological processes in some way. Transition metal complexes have variable oxidation states which made them noteworthy in bioinorganic chemical and redox enzymes systems. Numerous reactive sites and site precise substructures prompt metal complexes as a hopeful candidate intended for artificial metallonucleases.

Metal complexes represent a significant group of compounds furnished with biological attention being extensively used for medicinal purposes for chemotherapy, in Radiopharmaceuticals, in MRI as contrast agents, ulcer treatment as well as for degenerative arthritis. Regardless of the fact that, comparatively limited number of coordination complexes have been put into practice as a drug, yet being marker agents as well as promoters in anchoring procedures these complexes are employed to greater extent in order to reveal the structural behavior and nucleic acid functions. The examination of the cis- $[Pt(NH_3)_2Cl_2$ in1964 instantly elicited an attention in the progress of metal-based drugs owing to its potential to inhibit cell proliferation and treatment of wide range of cancers with exceptional results by its mysterious clinical utilization. Carboplatin and other similar complexes of the cis-Platinum family that might attack nucleobases covalently has been the object of a comprehensive review for long. However cisplatin has some severe outcomes like nephrotoxicity, nausea, hair loss etc. The notable side effects of this drug mostly emerge from the manner of its

binding with DNA as it forms covalent crosslinks which impeded the success of cisplatin as a therapeutic agent.

Consequently, scientist paid plentiful interest in designing and synthesizing more biocompatible metal-based drugs which show less toxicity, high medicinal effect, target specificity and non-covalent mode of interaction with DNA. Study regarding complexes and DNA interaction has gained much significance in present-day scenario because medicinal effect of various metal-based anticancer drugs is exercised by interacting with either protein or DNA. Because of multiple proteins in human body which all are given separate biological tasks, these drug proteins interactions are of significance. Transition metal complexes present unusual benefits as DNA-binding agents. First and foremost transition metal complexes show well-defined coordination geometries that make them appealing components aimed at reversible recognition of nucleic acid study. Furthermore, electrochemical and photophysical properties are the distinguishing features of these complexes which encouraged them to be use in large number of implementations as fluorescent markers, in DNA foot-printing and in electrochemical research [49].

Despite of any specific type, interaction of drugs with nucleic acid produce alteration in their structure, therefore altering the DNA sequence and disturbing the constancy of genomic makeup as a result of conformational changes in DNA in addition to substitution, loss or addition of bases. Drug–DNA interaction can be discerned in three distinct ways. First DNA-binding proteins like polymerases as well as transcription factors might interact with drugs. The second approach infers binding of RNA to the DNA double helix forming triple helix hybrids and thus hindering the transcription. Interaction of DNA with small molecules denoted as drugs is considered as last method. Either via covalent or non-covalent interactions, nearly all anticancer drugs exercise their effect by impairing the DNA's replication mechanism. Thus DNA-binding drugs can be categorized based on the kind of attachment with DNA as covalent binders, intercalators, groove binders and newly categorized phosphodiester backbone binders. A well found sign for exactly how guest and host molecule bind, is provided by K_b (inherent binding constant). It constitutes a valuable parameter to assess drug-DNA interaction affinity [50].

Inorganic chemistry is conveniently divided in two main classes in medicinal field, firstly with targeted metal ions ligands act like medications either free or bound to protein. Furthermore, metal-based medications having central metal ion is frequently vital. Processes of transcription and replication are vital to cell existence and growth as well as for normal working of body. DNA transcribes or replicates only when a signal, often a regulatory protein is received by it which bind to a specific site on DNA [51].

Drug-DNA interactions studies initiates around 1960s. DNA shows both covalent as well as non-covalent mode of interaction. Relying on the size and form, coordination compounds might demonstrate an inclination for a specific mode of binding or nucleotide arrangement.

1.8.1 Covalent Mode of Binding

Numerous anticancer medicines exhibiting both covalent as well as non-covalent interactions are used clinically by interacting with DNA. Drug–DNA interaction by covalent mode is irreparable and always results in hindering DNA procedures and successive apoptosis. Great binding strength is foremost benefit of covalent binders. Best-selling anticancer medicine 'cisplatin' shows an intra/interstrand cross-linking with the nitrogen on guanine base by the chloro groups.

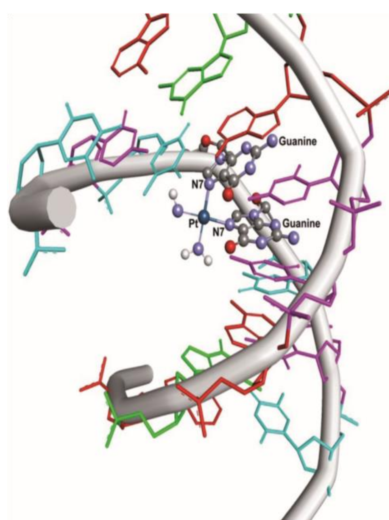


Figure 1.12 Cisplatin forms adducts with the N7 nitrogen from guanine bases

The first group of anticancer drugs is alkylating agents still frequently utilized in the treatment of cancers of numerous sorts. They are being used as anticancer agents

because of substantial DNA damage produced by them to kill cancer cells by involving reactions with guanine bases. Though some alkylating agents apply their strongest effects through alkylation at different sites and formation of different sorts of cross-links.

Methyl or different alkyl groups are added by these agents onto those molecules, they are not fit therein. Their accurate utilization thus in turn constrained by base pairing and DNA miscoding. Three types of mechanisms are exhibited by alkylating agents. In an attempt intended to substitute the alkylated bases by repair enzymes, fragmentation of DNA takes place as a result attachment of alkyl groups to DNA bases by an alkylating agent. This is first type of mechanism.

Cross-links formation among atoms of DNA is the second type, thus an alkylating agent links together two bases having two sites for DNA to bind. Because of cross-link formation, DNA is avoided for being used as a template for more DNA as well as RNA formation, thus hindering replication and transcription and subsequently apoptosis. Impairing of the nucleotides resulting in mutations is the third type of mechanism involved [52].

1.8.2 Non-covalent Mode of Binding

Keeping in mind the drug metabolism and toxic aftermaths, non-covalent interactions are usually favored over covalent and are reversible. Interacting agents such as groove binders and intercalators are normally thought to be less toxic in comparison to those making covalent adducts with DNA resulting in additional DNA damage. Moreover non-covalent interactions are categorized into three classes as intercalation, groove and external binding (outwards of the helix).

1.8.2.1 Intercalation

Mechanism of intercalation was first proposed by Leonard Lerman et al. in 1961 [50] and is well-known for introducing usually heterocyclic planar aromatic systems among adjacent stacked base pairs present in DNA causing significant *p*-electron overlap [50]**.** Short of breaking up hydrogen bonds and lacking covalent bonds amongst the DNA bases intercalators heap to the DNA in perpendicular fashion. The firmness of the DNA–intercalator complex is endured by hydrogen bonding, van der Waals, hydrophobic and/or charge transfer forces. Intercalators are utilized to prevent DNA

replication in fast developing cancer cells. Intercalation elongates, stabilizes, hardens and unwinds the DNA helix. A space is created by DNA among its base pairs via unwinding in order to fit intercalator. Two most important manners of intercalation are classical intercalation as well as threading intercalation. Intercalators bring about strong perturbations in DNA structure.

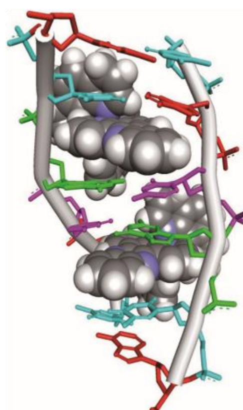


Figure 1.13 Intercalation binding of a ruthenium complex with B-DNA form

1.8.2.2 Groove Binding

Via either of the grooves, molecules generally extend to DNA and respond to either backbone or nucleobases. Few small compounds exhibit minor groove binding by Vander-Walls interaction as well as hydrogen bonding. Medicines exhibiting minor groove binding usually are narrow curved shaped which matches the groove shape and enables binding and have numerous aromatic rings. Moreover, hydrogen bonds are formed by these drugs between bases normally nitrogen (3) of adenine and oxygen (2) of thymine [53].

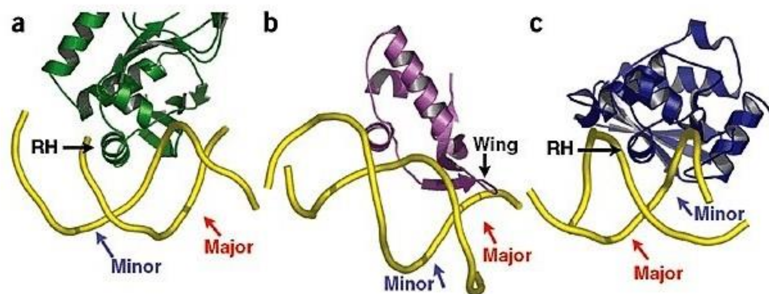


Figure 1.14 Major groove binding

1.8.2.3 External Binding

Non-specific, outside edge stacking interactions are likewise formed by some ligands with the phosphate backbone of DNA. This mode usually occurs by self-association of ligands to form aggregates of higher-order, which might heap on the anionic backbone of DNA to decrease repulsion among ligand molecules.

1.9 Dithiocarbamates

As an outcome of cancer treatment, surgical transplants and HIV disease there is an increasing number of immunocompromised patients that result in a marked rise in invasive as well as systemic pathogenic contaminations in the previous two decades. Thusly resistance to drugs is a growing concern in the therapy of various contagious diseases caused by microorganisms. There has been an exceptional advancement in understanding mechanisms of cytotoxicity at cellular and molecular level after the disclosure of anticancer activity of traditional platinum-based cisplatin (*cis*-diamminedichloroplatinum (II). Cisplatin was claimed to be absolutely innovative anti-cancer drug and it tempted inorganic chemists to invent and trial different metal-based remedies. Disappointingly carboplatin and oxaliplatin are only two more anticancer drugs which have been approved worldwide in 1993 and 2002 respectively. Henceforth, development of efficacious drugs with unique ways of mechanisms of action has become important task for research. In order to regulate the activity and to lessen the cytotoxicity of cisplatin, novel metal complexes comprising donor ligands (N and S) are designed, probably because of detoxicant properties of ligands containing sulphur as a protector from heavy metal intoxication. As of late, DNA

binding interactions of dithiocarbamate metal complexes are significantly focused [54]. A diverse chemistry has been developed around dithiocarbamates (R_2CNS_2), class of monoanionic 1,1-dithio ligands with versatile nature and can be easily prepared. By one-electron process which takes place upon adding iodide or ferric salts, they can be related to thiuram disulfides. Dithiocarbamates are lipophilic in nature [55]. In general they show symmetrical chelation to bind with metals, however, other modes of coordination are also well known, and among them the monodentate and anisobidentate modes are most prevailing. By wisely choosing the substituents they can be electronically adjusted as they are planer and sterically non-demanding ligands. Dithiocarbamate moiety stabilizes metals in wide range of oxidation states and this is ascribed to the two resonance forms present [56].

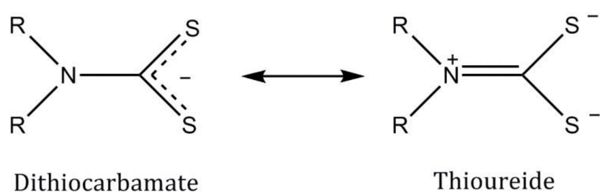


Figure 1.15 Soft dithiocarbamate and hard thioureide Resonance forms

In the first resonance form there is a single bond between nitrogen atom and carbon atom bearing sulphur and -1 charge is delocalizing among carbon and two Sulphur atoms. While in the second form delocalized lone pair is present on nitrogen atom, as a result double bond is formed between it and carbon bearing S_2 both of which have negative charges. Nitrogen has sp^2 hybridization in this resonance form. The former conformation is designated as a soft dithiocarbamate while the later is hard thioureide (stabilize hard metals at higher oxidation state and soft metal centers at lower oxidation state respectively. This resonance behavior has another outcome that dithiocarbamates can said to be both *strong* and *weak-filed* ligands determined by the substituents attached. It is a *strong-field* ligand in case if 'dithiocarbamate' conformation dominates and *weak-field* if thioureide form dominates [56].

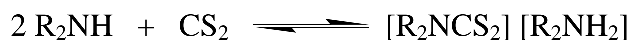
1.9.1 Synthesis of Dithiocarbamates

The first DTCs was synthesized in 1934 [57]. Normally synthesis of Dithiocarbamates can takes place in one of the two ways. The most usual way is the exothermic reaction of a secondary amine with carbon disulfide in the presence of a base. Generally

reactions are performed in methanol or ethanol, water and a base normally potassium or sodium hydroxide. Low temperature is recommended for some reactions but mostly room temperature is adequate. Reactions typically proceed rapidly, cleanly and give high yield [58].

$$R_2NH + \text{Carbondisulfide} + MOH \rightarrow R_2NCS_2M + \text{water}$$

Nearly entire secondary dialkylamines follow the same method of preparation. Although very often the salts of sodium and potassium might be separated if needed but mostly dissolve in water and used as such. NaS_2CNR_2 (R=Me, Et) are sold as $NaS_2CNR_2.3H_2O$ (tris (aqua) salts) can be hydrated. The potassium and sodium salts of dithiocarbamates exhibit good water solubility while poor solubility in organic solvents. Contrary the ammonium salts; [R_2NH_2], [S_2CNR_2] exhibit satisfactory solubility in organic solvents. The later can be prepared in the absence of base by reacting carbon disulfide and two equivalents of secondary amine. One equivalent performsas a base and other as a nucleophile.



$$2\ R_2NH + CS_2 \rightleftharpoons [R_2NCS_2]\ [R_2NH_2]$$

1.9.2 Biological role of Dithiocarbamates

Numerous biologically active molecules are synthesized by using organosulfur compounds which serve as important intermediates. Dithiocarbamates are considered as exceedingly versatile mono-anionic chelating ligands with strong complexing ability towards transition elements and likewise with the most of main group elements as well as d and f block elements. Analog of carbamate, Dithiocarbamate is well studied functional group of organic chemistry and found as a pharmacophore in a numerous biologically active molecules showing diverse chemical and pharmaceutical versatility. For over 50 years dithiocarbamates (DTCs) have largely been used in agriculture as pesticides. Tetramethylthiuram disulfide (TMTDS) a fungicide all the more generally known as thiram was first derivative of a DTC attained much eminence. Disodium ethylene bisdithiocarbamate known as Hester's compound might be thought as the first true ethylene bisdithiocarbamate (EBDC). Tetraethylthiuram disulfide (TETDS) known as disulfiram was firstly prepared in 1881 and used to speed up rubber vulcanization. Disulfiram was in medical used as a scabicide and vermicide in 1930s. Subsequently in 1948 it was suggested to use as alcohol aversion therapy for

chronic alcoholism [59, 60]. DTC is notable in many therapeutic agents and serve as an anesthetic, anti-HIV, fungicidal, microbicidal spermicides, anti-cancer, mono glyceride lipase inhibitors, antialcoholism etc. Sulfur atom have strong nucleophilic nature and unusual redox properties make it crucial residue in protein folding, enzyme catalysis and redox processes which are vital for cellular systems. Because of their potential of metal-binding they can also perform as enzyme inhibitors, for instance indoleamine 2,3-dioxygenase which can serve as potential candidate for tumor growth. Moreover, benzamide-based thiocarbamates evolved as HIV-I NCp7 inhibitors. Subsequently with the development of diverse dithiocarbamate-linked peptidomimetics, drug-like character is introduced alongside enhanced potency, longer term of action and target specifity. Besides DTCs have been utilized for the protection of amino groups in peptide chemistry [61].

1.9.3 Medicinal Diversity of Dithiocarbamates

In the course of past decade in the area of medicinal chemistry, DTCs arose as a significant pharmacophore and prosperous structure and various DTCs were produced and evaluated for numerous biological applications. A widely known DTC comprising drug, disulfiram has been recently attributed a number of vital biological properties, including anticancer, antimycobacterial, antileishmanial, monoacylglycerol lipase inhibitor and platelet aggregation inhibitor [62, 63].

Many DTCs adjoining diverse heterocycles have been investigated in past era for wide-ranging spectrum of medicinal activities which includes:

- Anti-cancer agents
- Anti-mycobacterial agents
- Antimicrobial agents
- Double-edged spermicidal agents
- Anti-HIV agents

Cao *et al.* have investigated a DTC moiety with potential anticancer activity. The piperazine-1-carbodithioate moiety was incorporated into its C-6 site by keeping in vision the 2,4-diaminoquinazoline center of anticancer medications, an innovative sequences of anticancer drugs, 4-substituted-piperazine-1-carbodithioate derivatives of 2,4-diaminoquinazoline were produced. Their anticancer activities were investigated

against five different human cancer cell lines including, MCF-7 (breast cancer cell line), A549 (lung cancer), HT29 and HCT-116 (colorectal cancer) and *HeLa* (cervical carcinoma). Among the tested compounds noticeable results are exhibited by a, b and c against the multiplication of five different cell lines with IC_{50} values ranging from 1.47-4.68 μM. SAR shows that a phenyl group attached at N-4 position of piperazine ring show extra promising results for the anticancer than a cyclohexyl methyl, benzyl, pyridin-2-yl or pyrimidin-2-yl group [64].

1.10 Nickel in biological system

Nickel (Ni) is a naturally existing metal present in numerous mineral forms and it is component of all parts of the environment and present everywhere in the environment, for instance nickel compounds and complexes. Even though not documented till the 1970s nickel is recognized to play an imperative part in some plants, archaebacteria, eubacteria and fungi. Urease, a nickel enzyme is considered as a virulence aspect in some organisms. Biochemical as well as physiological working of nickel is still to some extent uncertain. In the body highest concentrations of nickel are found in nucleic acids particularly in RNA and are thought to be somehow involved structure and function of proteins.

1.11 Aims and Objectives of Present Work

Consequently, the deficits associated with cisplatin have provided motivation for further research into other transition metal complexes with an aim to develop innovative drugs that would overcome the shortcomings related with cisplatin therapy. With this in mind research is focused primarily on synthesis of cheaper Ni(II) dithiocarbamate complexes and to explore the potency of synthesized complexes on cancer cell lines as promising therapeutic DNA-binding agents with minimized toxicity, low reactivity towards off-targeted thiol containing biomolecules, coordinatively unsaturated molecules, DNA target oriented, protection from off-targeted molecules and reasonable cost factor.

[1] B. Vogelstein, K.W. Kinzler, The genetic basis of human cancer, McGraw-Hill, 2002.

[2] J.F. Kerr, C.M. Winterford, B.V. Harmon, Apoptosis. Its significance in cancer and cancer therapy, Cancer, 73 (1994) 2013-2026.

[3] J.L. Spivak, Cancer-related anemia: its causes and characteristics, in: Seminars in oncology, 1994, pp. 3-8.

[4] M. Hejmadi, Introduction to cancer biology, Bookboon, 2009.

[5] F. Michor, Y. Iwasa, M.A. Nowak, Dynamics of cancer progression, Nature reviews cancer, 4 (2004) 197-205.

[6] R.A. Smith, V. Cokkinides, O.W. Brawley, Cancer screening in the United States, 2009: a review of current American Cancer Society guidelines and issues in cancer screening, CA: a cancer journal for clinicians, 59 (2009) 27-41.

[7] A. Sudhakar, History of cancer, ancient and modern treatment methods, Journal of cancer science & therapy, 1 (2009) 1.

[8] P. Anand, A.B. Kunnumakara, C. Sundaram, K.B. Harikumar, S.T. Tharakan, O.S. Lai, B. Sung, B.B. Aggarwal, Cancer is a preventable disease that requires major lifestyle changes, Pharmaceutical research, 25 (2008) 2097-2116.

[9] J.I. Morgan, T. Curran, Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun, Annual review of neuroscience, 14 (1991) 421-451.

[10] C.J. Sherr, J.M. Roberts, Inhibitors of mammalian G1 cyclin-dependent kinases, Genes & development, 9 (1995) 1149-1163.

[11] R.A. Weinberg, Tumor suppressor genes, Science, 254 (1991) 1138-1146.

[12] C.J. Marshall, Tumor suppressor genes, Cell, 64 (1991) 313-326.

[13] C.A. Stratakis, Genetics of adrenocortical tumors: gatekeepers, landscapers and conductors in symphony, Trends in Endocrinology & Metabolism, 14 (2003) 404-410.

[14] M. Lind, Principles of cytotoxic chemotherapy, Medicine, 36 (2008) 19-23.

[15] C. Orvig, M.J. Abrams, Medicinal inorganic chemistry: introduction, Chemical Reviews, 99 (1999) 2201-2204.

[16] M. Frezza, S. Hindo, D. Chen, A. Davenport, S. Schmitt, D. Tomco, Q. Ping Dou, Novel metals and metal complexes as platforms for cancer therapy, Current pharmaceutical design, 16 (2010) 1813-1825.

[17] V.T. DeVita, E. Chu, A history of cancer chemotherapy, Cancer research, 68 (2008) 8643-8653.

[18] T.W. Hambley, Is anticancer drug development heading in the right direction?, Cancer research, 69 (2009) 1259-1262.

[19] S. Neidle, D.E. Thurston, Chemical approaches to the discovery and development of cancer therapies, Nature Reviews Cancer, 5 (2005) 285-296.

[20] D. Chen, V. Milacic, M. Frezza, Q.P. Dou, Metal complexes, their cellular targets and potential for cancer therapy, Current pharmaceutical design, 15 (2009) 777-791.

[21] Y.K. Yan, M. Melchart, A. Habtemariam, P.J. Sadler, Organometallic chemistry, biology and medicine: ruthenium arene anticancer complexes, Chemical communications, (2005) 4764-4776.

[22] L.E. Scott, C. Orvig, Medicinal inorganic chemistry approaches to passivation and removal of aberrant metal ions in disease, Chemical Reviews, 109 (2009) 4885-4910.

[23] S.M. Cohen, New approaches for medicinal applications of bioinorganic chemistry, Current opinion in chemical biology, 11 (2007) 115-120.

[24] I. Ott, R. Gust, Non Platinum Metal Complexes as Anti-cancer Drugs, Archiv der Pharmazie, 340 (2007) 117-126.

[25] T.W. Hambley, Developing new metal-based therapeutics: challenges and opportunities, Dalton Transactions, (2007) 4929-4937.

[26] S.P. Fricker, Metal based drugs: from serendipity to design, Dalton Transactions, (2007) 4903-4917.

[27] A.d. de Gramont, A. Figer, M. Seymour, M. Homerin, A. Hmissi, J. Cassidy, C. Boni, H. Cortes-Funes, A. Cervantes, G. Freyer, Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer, Journal of Clinical Oncology, 18 (2000) 2938-2947.

[28] B. Rosenberg, L. Van Camp, T. Krigas, Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode, Nature, 205 (1965) 698-699.

[29] A.H. Rossof, R.E. Slayton, C.P. Perlia, Preliminary clinical experience with cis-diamminedichloroplatinum (II)(NSC 119875, CACP), Cancer, 30 (1972) 1451-1456.

[30] Y. Shi, J. Goodisman, J.C. Dabrowiak, Cyclodextrin capped gold nanoparticles as a delivery vehicle for a prodrug of cisplatin, Inorganic chemistry, 52 (2013) 9418-9426.

[31] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, Nature reviews Drug discovery, 4 (2005) 307-320.

[32] B. Lippert, Cisplatin: chemistry and biochemistry of a leading anticancer drug, John Wiley & Sons, 1999.

[33] B. Michalke, Platinum speciation used for elucidating activation or inhibition of Pt-containing anti-cancer drugs, Journal of Trace Elements in Medicine and Biology, 24 (2010) 69-77.

[34] S.J. Berners-Price, T.A. Frenkiel, U. Frey, J.D. Ranford, P.J. Sadler, Hydrolysis products of cisplatin: p K a determinations via [1 H, 15 N] NMR spectroscopy, Journal of the Chemical Society, Chemical Communications, (1992) 789-791.

[35] A.R. Timerbaev, C.G. Hartinger, S.S. Aleksenko, B.K. Keppler, Interactions of antitumor metallodrugs with serum proteins: advances in characterization using modern analytical methodology, Chemical reviews, 106 (2006) 2224-2248.

[36] D.-W. Shen, L.M. Pouliot, M.D. Hall, M.M. Gottesman, Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes, Pharmacological reviews, 64 (2012) 706-721.

[37] L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo, G. Kroemer, Molecular mechanisms of cisplatin resistance, Oncogene, 31 (2012) 1869-1883.

[38] S. Ishida, J. Lee, D.J. Thiele, I. Herskowitz, Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals, Proceedings of the National Academy of Sciences, 99 (2002) 14298-14302.

[39] Z.H. Siddik, Cisplatin: mode of cytotoxic action and molecular basis of resistance, Oncogene, 22 (2003) 7265-7279.

[40] A.-M. Florea, D. Büsselberg, Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects, Cancers, 3 (2011) 1351-1371.

[41] L.P. Martin, T.C. Hamilton, R.J. Schilder, Platinum resistance: the role of DNA repair pathways, Clinical Cancer Research, 14 (2008) 1291-1295.

[42] E.S. El Daly, Protective effect of cysteine and vitamin E, Crocus sativus and Nigella sativa extracts on cisplatin-induced toxicity in rats, Journal de pharmacie de Belgique, 53 (1997) 87-93; discussion 93-85.

[43] J.D. Watson, A. Berry, DNA: The secret of life, Knopf, 2009.

[44] R. Dahm, Discovering DNA: Friedrich Miescher and the early years of nucleic acid research, Human genetics, 122 (2008) 565-581.

[45] P.D. Hebert, A. Cywinska, S.L. Ball, Biological identifications through DNA barcodes, Proceedings of the Royal Society of London B: Biological Sciences, 270 (2003) 313-321.

[46] J.C. García-Ramos, R. Galindo-Murillo, F. Cortés-Guzmán, L. Ruiz-Azuara, Metal-based drug-DNA interactions, Journal of the Mexican Chemical Society, 57 (2013) 245-259.

[47] D.R. Whelan, T.J. Hiscox, J.I. Rood, K.R. Bambery, D. McNaughton, B.R. Wood, Detection of an en masse and reversible B-to A-DNA conformational transition in prokaryotes in response to desiccation, Journal of the Royal Society Interface, 11 (2014) 20140454.

[48] R.M. Wadkins, B. Vladu, C.-S. Tung, Actinomycin D binds to metastable hairpins in single-stranded DNA, Biochemistry, 37 (1998) 11915-11923.

[49] F.R. Keene, J.A. Smith, J.G. Collins, Metal complexes as structure-selective binding agents for nucleic acids, Coordination Chemistry Reviews, 253 (2009) 2021-2035.

[50] L. Lerman, Structural considerations in the interaction of DNA and acridines, Journal of molecular biology, 3 (1961) 18IN13-30IN14.

[51] N. Hadjiliadis, E. Sletten, Metal complex-DNA interactions, John Wiley & Sons, 2009.

[52] M. Sirajuddin, S. Ali, A. Badshah, Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry, Journal of Photochemistry and Photobiology B: Biology, 124 (2013) 1-19.

[53] R.V. Gessner, G.J. Quigley, A.H. Wang, G.A. Van der Marel, J.H. Van Boom, A. Rich, Structural basis for stabilization of Z-DNA by cobalt hexaammine and magnesium cations, Biochemistry, 24 (1985) 237-240.

[54] V. Bala, G. Gupta, V. L Sharma, Chemical and medicinal versatility of dithiocarbamates: an overview, Mini reviews in medicinal chemistry, 14 (2014) 1021-1032.

[55] G. Hogarth, Transition metal dithiocarbamates: 1978–2003, Progress in Inorganic Chemistry, Volume 53, (2005) 71-561.

[56] G. Hogarth, Metal-dithiocarbamate complexes: chemistry and biological activity, Mini reviews in medicinal chemistry, 12 (2012) 1202-1215.

[57] M.L. Gullino, F. Tinivella, A. Garibaldi, G.M. Kemmitt, L. Bacci, B. Sheppard, Mancozeb: past, present, and future, Plant Disease, 94 (2010) 1076-1087.

[58] S. Kanchi, P. Singh, K. Bisetty, Dithiocarbamates as hazardous remediation agent: A critical review on progress in environmental chemistry for inorganic species studies of 20 th century, Arabian Journal of Chemistry, 7 (2014) 11-25.

[59] R. Room, T. Babor, J. Rehm, Alcohol and public health, The lancet, 365 (2005) 519-530.

[60] Z.E. Sauna, S. Shukla, S.V. Ambudkar, Disulfiram, an old drug with new potential therapeutic uses for human cancers and fungal infections, Molecular bioSystems, 1 (2005) 127-134.

[61] R. Schreck, B. Meier, D.N. Männel, W. Dröge, P.A. Baeuerle, Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells, The Journal of experimental medicine: JEM, 175 (1992) 1181-1194.

[62] Y. Horita, T. Takii, T. Yagi, K. Ogawa, N. Fujiwara, E. Inagaki, L. Kremer, Y. Sato, R. Kuroishi, Y. Lee, Antitubercular activity of disulfiram, an antialcoholism drug, against multidrug-and extensively drug-resistant Mycobacterium tuberculosis isolates, Antimicrobial agents and chemotherapy, 56 (2012) 4140-4145.

[63] P. Liu, S. Brown, T. Goktug, P. Channathodiyil, V. Kannappan, J. Hugnot, P. Guichet, X. Bian, A. Armesilla, J. Darling, Cytotoxic effect of disulfiram/copper on human glioblastoma cell lines and ALDH-positive cancer-stem-like cells, British journal of cancer, 107 (2012) 1488-1497.

[64] S.-L. Cao, Y. Han, C.-Z. Yuan, Y. Wang, Z.-K. Xiahou, J. Liao, R.-T. Gao, B.-B. Mao, B.-L. Zhao, Z.-F. Li, Synthesis and antiproliferative activity of 4-substituted-piperazine-1-carbodithioate derivatives of 2, 4-diaminoquinazoline, European journal of medicinal chemistry, 64 (2013) 401-409.

Chapter 2

Experimental

2.1 Chemicals

All reagents employed in this research were of high grade and employed as they were obtained. Nickel(II) chloride and piperazine derivatives of analytical grade were bought from Sigma-Aldrich and Fluka Co., while other reagent i.e. CS_2 from Reidel-de-Haen. A number of analytical grade solvents such as methanol, chloroform, toluene, ethanol, dichloromethane as well as DMSO were purchased from Dae-Jung and Fluka chemical company. The solvents were dried and purified according to standard procedures [65]. CT-DNA (Calf thymus) was bought from Across company USA.

2.2 Instrumentation

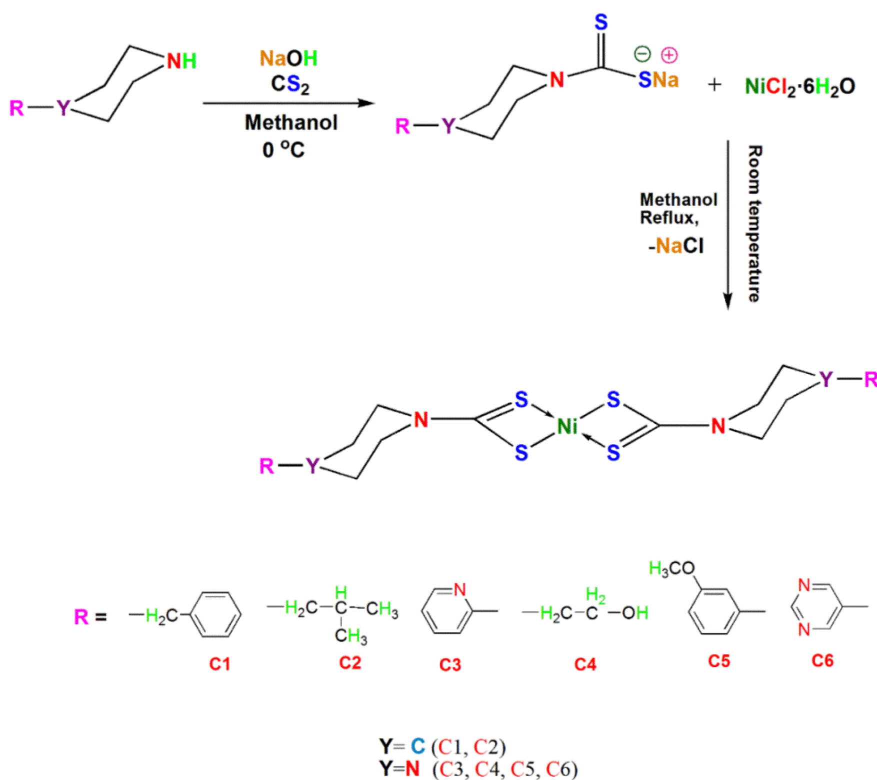
Melting points of the synthesized complexes were determined by Gallenkamp (UK) electro thermal melting point apparatus in open capillary-tube and are uncorrected. Perkin Elmer spectrophotometer was used to record infra-red absorption spectra in the frequency range of 4000-200 cm^{-1}. Elemental analysis was conducted by means of a CE-440 Elemental Analyzer (Exeter Analytical, Inc.). By using deuterated solvents as well as TMS as an internal reference (300 and 75.5 MHz) respectively, data of ^{1}H and ^{13}C NMR spectra was documented on Bruker AV 300 MHz spectrometer. Chemical shift values are given in ppm while *J* (coupling constant) values in Hz. Signals multiplicities in ^{1}H NMR spectra are presented as singlet (s), doublet (d) and multiplet (m). X-ray measurement of a selected crystal of $C_{24}H_{30}N_4NiO_2S_4$ was performed on a Bruker Venture Metaljet diffractometer by keeping the crystal at temperature of 150 K in collection of data. The structure was solved using Olex2 [66] with the XT [67] structure solution program and structure was refined with the XL [67] refinement package via least-squares minimization and later on the MERCURY package was employed intended for producing the structures. UV-Vis absorbance experiments involving DNA-drug interaction were carried out in doubly deionized water and electronic spectra were recorded in a Shimadzu UV-240 instrument. Viscosity measurements were conducted by using an Ubbelodhe viscometer.

2.3 Synthesis of Piperazine-1-carbodithioate Derivatives

Sodium hydroxide was added to piperazine derivatives solution in equimolar amount (1:1) and stirred for one hour. Equal molar solution of CS_2 in methanol was added in drop wise manner to the reaction mixture at 0 ^{o}C and allowed to stir further for 24 h. The resulting solution was obtained, filtered off and evaporated at rotary evaporator to get the solid product. The product was then dried and recrystallized in methanol.

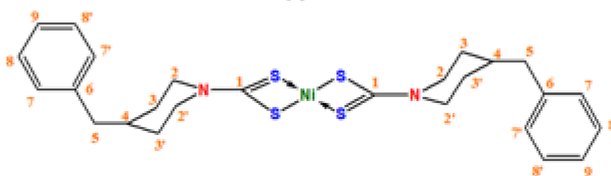
2.4 Synthesis of Dithiocarbamate Ni(II) Complexes

An aqueous solution of the sodium salt of the appropriate dithiocarbamate ligand prepared previously was added in drop wise manner to $NiCl_2.6H_2O$ for the synthesis of title complex in 1:2 molar ratio in the presence of methanol used as a solvent. The reaction mixture was allowed to stir for 4 hours at room temperature. The resultant crude green product was filtered off, washed with water and ice-cold methanol. The product was crystallized in the mixture of chloroform and DMSO and needle like crystals were obtained for C5.



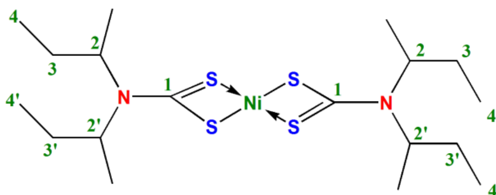
Scheme: Synthesis of Bis(dithiacarbamato) nickel(II) complexes

***Bis*-(4-benzylpiperidine-1-carbodithioate)nickel(II) (C1)**



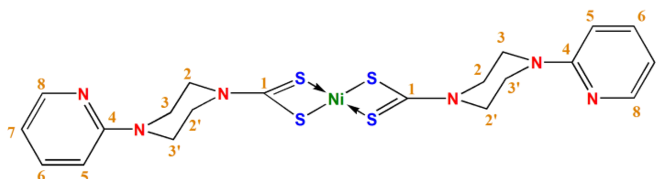
Green-solid, Yield: 82%. m.p. 237-239°C**.** Elemental analysis, % Calculated (Found) for $C_{26}H_{32}N_2NiS_4$ (Mol. Wt. 559.5 g/mol): C, 55.81(55.78); H, 5.76(5.74); N, 5.01(5.00); S, 22.92(22.90). FT-IR (4000–200 cm^{-1}): 1514 *v*(N-CSS), 1249 *v*($N\text{-}CH_2$), 2886 v($C\text{-}H_{nonaromatic}$), 1002 *v*(C-S). ^1H NMR (300MHz $CDCl_3$): δ (ppm): 7.13-7.34 (m, 10H, phenyl), 4.52-4.58 (m, 8H, $H_{2,\ 2'}$), 2.85-2.94 (m, 8H, $H_{3,\ 3'}$), 2.58 (d, Hz, 4H, H_5 ^1J = 6.9 Hz), 1.23-1.93 (m, 2H, H_4). ^{13}C NMR {75 MHz, $CDCl_3$) δ *(*ppm)}: 204.9 (C_1); 46.8 ($C_{2,\ 2'}$), 31.3 ($C_{3,\ 3'}$), 38.0 (C_4), 42.5 (C_5), 139.44 (C_6), 128.42 ($C_{7,\ 7'}$), 129.0 ($C_{8,\ 8'}$), 126.27(C_9).

***Bis*-(di-sec-butylcarbamodithioiate)nickel(II) (C2)**



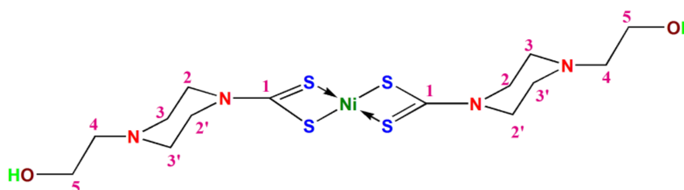
Green-solid, Yield: 92%. m.p. 173°C. Elemental analysis, % Calculated (Found) for $C_{18}H_{36}N_2NiS_4$ (Mol. Wt. 467.45 g/mol): C, 46.25(46.23); H, 7.76(7.74); N, 5.99(5.96); S, 27.44(27.42). FT-IR (4000–200 cm^{-1}): 1501 *v*(N-CSS), 1240 *v*($N\text{-}CH_2$), 2884 *v*($C\text{-}H_{nonaromatic}$), 1002 *v*(C-S). ^1H NMR (300MHz DMSO-d_6): δ (ppm)): 3.42 (s, 12H, $H_{4,\ 4'}$), 2.4-2.9 (m, 8H, $H_{3,\ 3'}$), 2.07-2.27 (m, 4H, $H_{2,\ 2'}$), 0.85 (d, 12H, methyl, ^1J = 6.6 Hz).

***Bis*-(4-(pyridin-2-yl)piperazine-1-carbodithioate)nickel(II) (C3)**



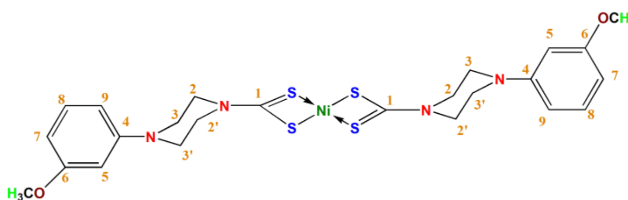
Green-solid, Yield: 86%. m.p. 343-344°C. Elemental analysis, % Calculated (Found) for $C_{20}H_{24}N_6NiS_4$ (Mol. Wt. 537.41 g/mol): C, 44.70(44.68); H, 4.88(4.85); N, 15.64(15.62); S, 23.87(23.85). FT-IR (4000–200 cm^{-1}): 1499 *v*(N-CSS), 1241 *v*(N-CH_2), 2885 *v*($C-H_{nonaromatic}$), 1001 *v*(C-S). ^1H NMR (300MHz DMSO-d_6): δ (ppm)): 6.79-8.10 (m, 8H, H_{5-8}), 3.15-3.19 (m 8H, $H_{3,3'}$), 2.72-2.78 (m, 8H, $H_{2,2'}$).

***Bis*-(4-(2-hydroxyethyl)piperazine-1-carbodithioate)nickel(II) (C4)**



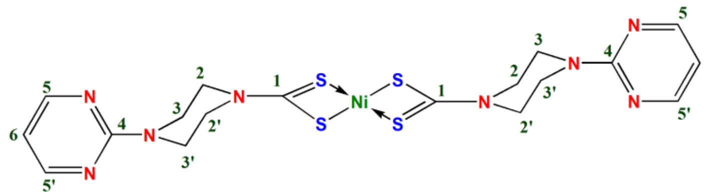
Green-solid, Yield: 83%. m.p. 276-278°C. Elemental analysis, % Calculated (Found) for $C_{14}H_{26}N_4NiO_2S_4$ (Mol. Wt. 469.34 g/mol): C, 35.83 (35.81), H, 5.58 (5.56); N, 11.94 (11.92); S, 27.33 (27.31). FT-IR (4000–200 cm^{-1}): 1501 v N-CSS), 1240 *v*(N-CH_2), 2885 *v*($C-H_{nonaromatic}$), 999 *v*(C-S). ^1H NMR (300MHz DMSO-d_6): δ (ppm)): 4.49 (t, 4H, H_4, $^1J = 5.1$ Hz), 3.68 (s (b), 8H, $H_{2,2'}$), 3.34 (s (b), 8H, $H_{3,3'}$), 3.47-3.53 (m, 4H, H_5), 2.44(s, 2H, OH).

***Bis*-(4-(3-methoxyphenyl)piperazine-1-carbodithioate)nickel(II) (C5)**



Green-solid, Yield: 85%. m.p. 310°C**.** Elemental analysis, % Calculated (Found) for $C_{24}H_{30}N_4NiO_2S_4$ (Mol. Wt. 593.47 g/mol): C, 48.57(48.55); H, 5.10(5.07); N, 9.44(9.42); S, 21.61(21.58). FT-IR (4000–200 cm^{-1}): 1508 *v*(N-CSS), 1242 *v*(N-CH_2), 2886 *v*($C-H_{nonaromatic}$), 962, *v*(C-S). ^1H NMR (300MHz DMSO-d_6): δ (ppm)): 6.42-6.79 (m, 8H, H_{5-9}), 3.89-3.95 (m 8H, $H_{3,3'}$), 3.22-3.28 (m, 8H, $H_{2,2'}$), 3.75 (s, 6H, OCH_3).

***Bis*-(4-(pyrimidin-2-yl)piperazine-1-carbodithioate)Nickel(II) (C6)**



Green-solid, Yield: 89%. m.p. 372-373°C. Elemental analysis, % Calculated (Found) for $C_{18}H_{22}N_8NiS_4$ (Mol. Wt. 537.37 g/mol): C, 40.23(40.20); H, 4.13(4.12); N, 20.85(20.84); S, 23.87(23.84). FT-IR (4000–200 cm^{-1}): 1507 *ν*(N-CSS), 1242 *ν*(N-CH_2), 2886 *ν*($C\text{-}H_{nonaromatic}$), 962 *ν*(C-S). ^{1}H NMR (300MHz DMSO-d_6): *δ* (ppm)): 6.65-8.43 (m, 6H, $H_{5,5',6}$), 3.84 (d, 8H, $H_{3,3'}$, $^1J = 6.6$ Hz), 2.08-2.28 (m, 8H, $H_{2,2'}$).

2.5 Biological studies

2.5.1 Evaluation of anti-proliferative activity of synthesized complexes (Sulforhodamine b assay)

Antiproliferative action of synthesized compounds (1-6) was screened against MCF-7 (human breast cancer cell line) employing the SRB (sulforhodamine B) cellular protein-staining procedure [68]. In short suspension of cancerous cells was grown in 96-well tissue culture plate (1×10^4 in 190 µL of the whole medium) comprising compounds put to the test, prepared in 100% dimethyl sulfoxide as well as incubated at 37°C in the presence of 90% humidified air and 5% CO_2 intended for 72 hours. Using TCA the cell growth was arrested proceeded by washing with distilled water and then air-dried as well as stained with SRB solution. All tests were done thrice and values are described as mean ± SD of three repeats.

Using a microplate reader ODs (optical densities) were measured at 515 nm. Via addition of an equivalent number of cells to several wells, a zero-day control was done for each case thus incubating at 37°C for half hour and processed further as explained earlier. The 50% inhibitory concentrations (IC_{50}) for synthesized complexes against MCF-7 (human breast cancer cell line) were evaluated and compared with cisplatin a notable anticancer drug.

2.5.2 DNA Binding Experiments of Complexes by Uv-Vis Spectroscopy

Possibly the simplest and frequently employed instrumental procedure to study drug-DNA interactions is UV-visible absorption spectroscopy. Such study can be carried

out by observing variance in absorption properties of either drug or DNA molecule [69]. The binding of Ni complexes with CT-DNA was carried out using solution of calf thymus DNA (CT-DNA) prepared by using deionized water having pH = 7.0 preceded by stirring for 24 hours and kept at 4 ^{o}C and for not more than 4 days. The experimental CT-DNA used was satisfactorily free from proteins evident from ratio of UV absorbance at 260 and 280 (A_{260}/A_{280}) of ca. 1.9 [70]. DNA concentration was found out by its extinction coefficient ε of 6600 L mol^{-1} cm^{-1} at 260 nm [71]. Working solutions of different concentrations were prepared from stock solution. Stock solutions of complexes of known concentrations were prepared by dissolving calculated amount of complexes (1-6) in 98 % DMSO because of their fair solubility and diluting aptly to get requisite concentration for experiments. Absorption spectral experiments were performed by keeping constant the concentration of each Ni(II) metal complex (1-6) with varying the CT-DNA concentration and absorbance values were recorded after addition of each DNA solution consecutively. Equivalent amount of solution of CT-DNA was added to the complex and reference solution respectively in an order to remove the absorbance of DNA itself. Compound-CTDNA solutions were permitted to incubate for 30 min at room temperature beforehand measurements were made. Single beam spectrophotometer was used to perform spectrophotometric measurements. Airtight quartz cuvettes having 1 cm optic pathway length with alike optic factors were used to record absorption measurement at room temperature (25 ± 1 °C). Micropipette was used to accomplish the titration of the solutions. Relying on variation in absorption the data were fitted into Benesi-Hildebrand equation to find out the intrinsic binding constant K_b [72].

$$\frac{A_0}{\mathrm{A} - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G}\frac{1}{K\,[\mathrm{DNA}]}$$

Where K represents the binding constant, A_0 and A are the absorbances of the free drug and adduct with DNA correspondingly. e_G and $e_{H–G}$ denote absorption coefficients of the drug and drug–DNA adduct correspondingly. Association constant (K) can be obtained from the intercept-to-slope ratios of A_0/(A-A0) vs. 1/[DNA] plots.

Using the relation $\Delta G^o = - RT \ln k$ Gibb's free energy (ΔG) was determined where R represents general gas constant (8.314 $JK^{-1}mol^{-1}$) as well as T is the temperature.

2.5.3 DNA Binding Study by Viscosity Measurement

Viscosity experimentations were conducted on Ubbelohde viscometer dipped in a thermostatic water-bath kept at 30±0.1 ºC. Measurements were performed by introducing each compound in solution of CT-DNA (150 μM) already present in viscometer. Digital stopwatch was employed to record time flow of each sample thrice and an average flow time was computed. Comparative viscosities for DNA were measured from the relation $\eta \propto (t - t_0)$ in the absence as well as presence of complexes where t is the practical flow time of DNA containing solution and t_0 is that of without DNA. Data was presented as $(\eta/\eta o)^{1/3}$ set against ratio of complex to CT-DNA, where η and η_O represent relative viscosities of CT-DNA both in the presence and absence of complex [73].

2.6 Computational Studies

DFT calculations have been carried out by means of the *Gaussian 09* software package [74]. Results were attained using *Gauss View 5.0.8* software [75]. B3LYP functional together with LANL2DZ basis set comprising an effective core potential function was employed for calculations. Gaussian checkpoint files of optimized geometries were utilized for additional calculations [75].

[1] W.L. Armarego, C.L.L. Chai, Purification of laboratory chemicals, Butterworth-Heinemann, 2013.

[2] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program, Journal of Applied Crystallography, 42 (2009) 339-341.

[3] G.M. Sheldrick, Crystal structure refinement with SHELXL, Acta Crystallographica Section C: Structural Chemistry, 71 (2015) 3-8.

[4] M. You, D.M. Wickramaratne, G.L. Silva, H. Chai, T.E. Chagwedera, N.R. Farnsworth, G.A. Cordell, A.D. Kinghorn, J.M. Pezzuto, (-)-Roemerine, an aporphine alkaloid from Annona senegalensis that reverses the multidrug-resistance phenotype with cultured cells, Journal of natural products, 58 (1995) 598-604.

[5] G. Barone, A. Terenzi, A. Lauria, A.M. Almerico, J.M. Leal, N. Busto, B. García, DNA-binding of nickel (II), copper (II) and zinc (II) complexes: Structure–affinity relationships, Coordination Chemistry Reviews, 257 (2013) 2848-2862.

[6] Y. Zhang, X. Wang, L. Ding, Synthesis and DNA binding studies of Mg (II) complex of Schiff base derived from vanillin and l-tryptophan, Nucleosides, Nucleotides and Nucleic Acids, 30 (2011) 49-62.

[7] M. Reichmann, S. Rice, C. Thomas, P. Doty, A further examination of the molecular weight and size of desoxypentose nucleic acid, Journal of the American Chemical Society, 76 (1954) 3047-3053.

[8] A. Shah, E. Nosheen, S. Munir, A. Badshah, R. Qureshi, N. Muhammad, H. Hussain, Characterization and DNA binding studies of unexplored imidazolidines by electronic absorption spectroscopy and cyclic voltammetry, Journal of Photochemistry and Photobiology B: Biology, 120 (2013) 90-97.

[9] R. Sinha, M.M. Islam, K. Bhadra, G.S. Kumar, A. Banerjee, M. Maiti, The binding of DNA intercalating and non-intercalating compounds to A-form and protonated form of poly (rC)· poly (rG): Spectroscopic and viscometric study, Bioorganic & medicinal chemistry, 14 (2006) 800-814.

[10] D. Sholl, J.A. Steckel, Density functional theory: a practical introduction, John Wiley & Sons, 2011.

[11] A. Frisch, A. Nielsen, A. Holder, Gaussview user manual, Gaussian Inc., Pittsburgh, PA, 556 (2000).

Chapter 3

Results and Discussion

3.1 Physical data

Physical statistics of synthesized complexes is presented in Table 3.1. Synthesized complexes were pure and exhibited appreciable solubility in dimethyl sulfoxide (DMSO) while moderately soluble in chloroform ($CHCl_3$) and dichloromethane (DCM). Complexes exhibit green color with sharp melting points.

Table 3.1: Physical data for Ni(II) complexes

Compound no.	Molecular Formula	Physical state (color)	Melting Point	Solubility	%Yield
C1	$C_{26}H_{32}N_2NiS_4$	Green-solid	237-239 °C	DMSO, Chloroform	88 %
C2	$C1_8H_{36}N_2NiS_4$	Green-solid	173 °C	DMSO	92 %
C3	$C_{20}H_{24}N_6NiS_4$	Green-solid	343-344 °C	DMSO, Chloroform, Dichloromethane	86 %
C4	$C1_4H_{26}N_4NiO_2S_4$	Green-solid	276-278 °C	DMSO	83 %
C5	$C_{24}H_{30}N_4NiO2S_4$	Green-solid	310 °C	DMSO, Chloroform	85 %
C6	$C_{18}H_{22}N_8NiS_4$	Green-solid	372-373 °C	DMSO, Chloroform, Dichloromethane	89 %

3.2 Spectroscopic Characterization of the Complexes

3.2.1 FTIR Spectra Studies of the Metal Complexes

FT-IR spectra studies affords valuable evidence regarding the formation of the title complexes C**1-C6** by appearance of characteristic peaks which estimate the nature of bonding and coordination mode of dithiocarbamate with nickel metal centre and data collected for complexes is presented in Table 3.2. The characteristic frequencies have been identified by comparing the complexes spectra with the literature.

Table 3.2 FTIR data of complexes C1-C6

Compounds	*ν*(C-S) cm^{-1}	*N*(N-CH_2) cm^{-1}	*N*(N-CSS) cm^{-1}	*ν*(C-H)$_{nonaromatic}$ cm^{-1}
C1	1002	1249	1514	2886
C2	1002	1240	1501	2884
C3	1001	1241	1499	2885
C4	999	1240	1501	2885
C5	962	1242	1508	2886
C6	962	1243	1507	2886

The ν(C┈N) thioureide band was noticed around 1499-1514 cm^{-1} intermediate between (C—N) (1250-1350 cm^{-1}) and (C═N) (1640-1690cm^{-1}) bond character, inferring coordination of the metal owing to the delocalization of electrons, resultant a partial double bond character [76]. Investigation of complex by X-ray crystallographic studies additionally reinforced the statement. Another structural importance is appearance of peak of ν(C═S) band in the range 1055-950cm^{-1}. This band specify the coordination of ligand with metal [77]. Furthermore it was likewise found that the ν(C—N) occurs at lower wavenumber in this case as compared to equivalent diethyl as well as dimethyl equivalents which may be attributed to the heterocyclic ring system

of piperazine molecule which has little inclination to release electrons to the C—N bond thus reducing its double bond nature.

3.2.2 Multi-nuclear (^{1}H, ^{13}C) NMR Spectroscopy

Figures 3.1 and 3.2 represent the ^{1}H NMR and ^{13}C NMR spectra of one of the representative complex 1. The ^{1}H NMR spectrum was recorded in $CDCl_3$. Proton resonances were assigned by their peak's multiplicity, intensity pattern and comparing integration values of protons with probable composition. Protons of phenyl ring appears at 7.13-7.34 ppm as multiplet and methylene protons of N-(CH2) of piperadine at position 1, 1' resonate at higher value (4.52-4.58 ppm) in the complex while other protons of piperdine ring appear at 2.85-2.94 ppm at position 2, 2' respectively.

In ^{13}C NMR the carbon signals for methylene groups adjacent to the nitrogen atom in piperadine ring appeared in the region 46.8 ppm while the other methylene carbon signals are observed in the upfield region of 31.3-42.5 ppm. The downfield shift of the methylene carbons adjacent to nitrogen atoms in the complexes is a sign of the significant result of the complexation process viz. a significant thioureide influence to the stability of the complexes as well as subsequent decrease in the electron density [78]. Diagnostic peak for C=S band appeared at 204.9 which supports the complex formation.

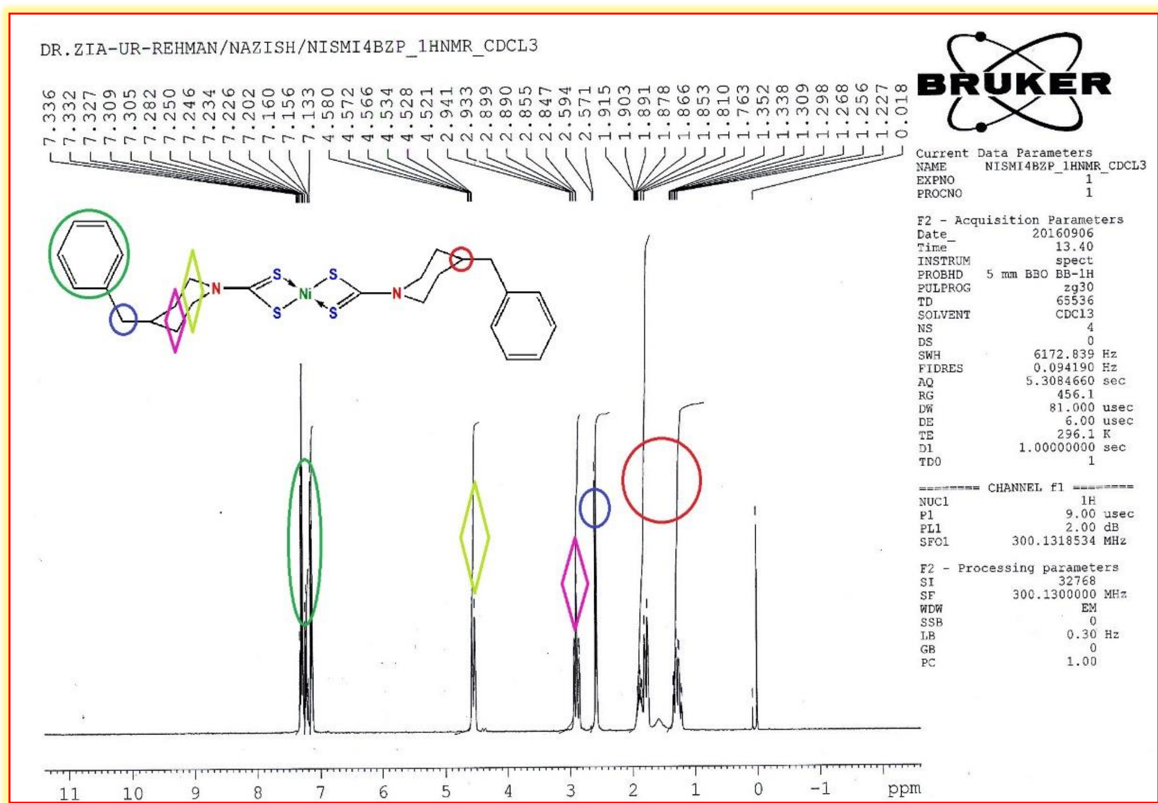


Figure 3.1 ^{1}H NMR spectroscopy of C1

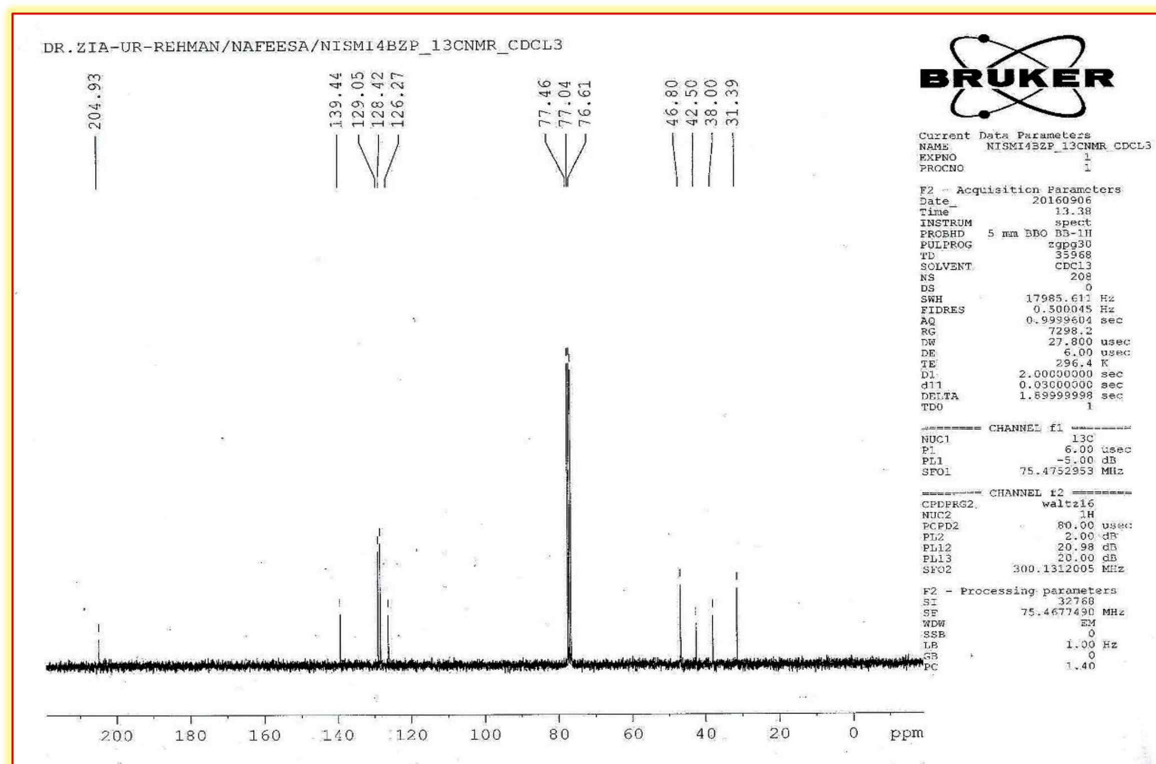


Figure 3.2 ^{13}C NMR spectroscopy of C1

3.3 Single Crystal X-ray Crystallographic Description

The molecular structure of the **C5** and with the atomic labeling is presented in the figure 3.3. Crystal data and structural refinement parameters related to **C5** are listed in Table 3.3 and particular inter-atomic bond distances as well as bond angles are presented in Table 3.4. The complex has orthorhombic crystal system, Pccn space group and a square planar geometry is acquired by nickel(II), tetracoordinated by four sulfur atoms of two bidentate carbodithioate moieties with the cis and trans angels are 79.39(3) and 180.00(3) degree which revealed perfect square planer geometry for nickel moiety. The elements present in structure are linked together in the crystal packing via a prolonged system of H-bonds. Intermediate bond lengths, between single (1.82 Å) and double (1.60 Å) bond, are shown by carbon–sulfur bond within the chelate ring with an average bond lengths C1–S1= 1.716(3), C1–S2 =1.723(3) Å. This proposes a significant charge delocalization [79]. Likewise, the C1–N1 bond length

1.314(4) Å, is considerably smaller than a normal C–N bond (1.47 Å) and longer than C=N bond (1.28 Å), evidently exhibit the resonance phenomenon occurring in the NSCS moiety [80]. Ni–S1 and Ni–S2 bond lengths are 2.2232(7) and 2.1970(8) respectively which reveal the anisobidentate mode of coordination of 1,1-dithiolate moiety to nickel center [81].

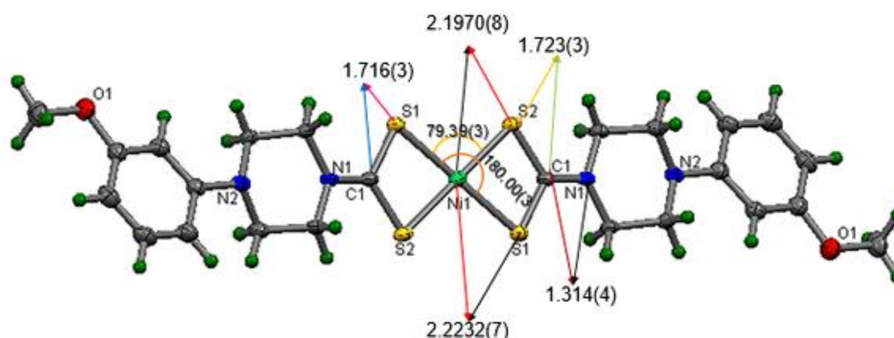


Figure 3.3 Molecular structure of C5 ($C_{24}H_{30}N_4NiO_2S_4$)

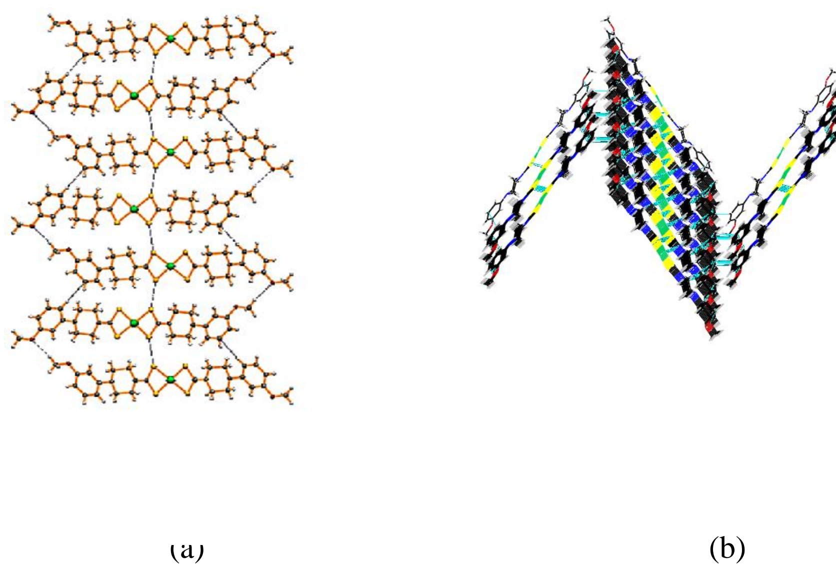
Table 3.3 Crystal data and structure and refinement parameters for C5

Crystal data	C5
Empirical formula	$C_{24}H_{30}N_4NiO_2S_4$
Formula weight	593.47
Temperature/K	150
Crystal system	orthorhombic
Space group	Pccn
a/Å	11.6577(4)
b/Å	31.8753(11)
c/Å	6.7919(2)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2523.82(14)
Z	4
ρ_{calc}mg/mm^3	1.562
m/mm^{-1}	6.471
F(000)	1240.0
Crystal size/mm^3	0.05 × 0.02 × 0.02
Radiation	GaKα (λ = 1.34139)
2Θ range for data collection	7.024 to 121.332°
Index ranges	$-15 \le h \le 15$, $-41 \le k \le 41$, $-8 \le l \le 8$
Reflections collected	40577
Independent reflections	2902 [R_{int} = 0.1121, R_{sigma} = 0.0459]
Data/restraints/parameters	2902/0/161
Goodness-of-fit on F^2	1.042
Final R indexes [I>=2σ (I)]	R_1 = 0.0455, wR_2 = 0.1006
Final R indexes [all data]	R_1 = 0.0753, wR_2 = 0.1132

Table 3.4 Selected bond lengths (Å) and bound angles (°)

Type of bond	Bond length (°A)	Type of angle	Bond Angel
Ni1–S1	2.2232(7)	S1-Ni1-S1[1]	180.00(3)
Ni1–S1[1]	2.2232(7)	S2[1]-Ni1-S1[1]	79.39(3)
Ni1–S2[1]	2.1970(8)	S2-Ni1-S1	79.39(3)
Ni1–S2	2.1970(8)	S2[1]-Ni1-S1	100.61(3)
S1–C1	1.716(3)	S2-Ni1-S1[1]	100.61(3)
S2–C1	1.723(3)	S2[1-]Ni1-S2	180.00(3)
O1–C8	1.381(4)	C1-S1-Ni1	84.52(10)
O1–C12	1.433(4)	C1-S2-Ni1	85.18(11)

Both the intermolecular (C–H---O, C–H---C and C–H---π) and intramolecular (C–H---O, C–H---π) types of van der Waals non-covalent contacts are involved in the crystal structure. Therefore, forming fascinating 2D and 3D supramolecular architectures.



(a) (b)

Figure 3.4 (a) 2D (b) 3D structures for C5. The hydrogen atoms not involved in the depicted non-covalent contacts are omitted for clarity

3.4 Biological Activity

3.4.1 Cytotoxic Assay by Sulforhodamine B Dye (SRB Assay) against MCF-7

The synthesized complexes were tested for their antiprolifertive activity against MCF-7 (human breast cancer cell line) using sulforhodamine B (SRB) method. The outcomes shown as concentration of complex requisite to hinder the growing cancerous cells by 50 %(**IC_{50}**) are presented in the table and it is witnessed that moderate to good anticancer activity is shown by Ni(II) complexes (1-6) against MCF-7 cell line.

Table 3.5 IC_{50} values of the Ni(II) complexes

Compound	C1	C2	C3	C4	C5	C6	*Cisplatin*
IC_{50}	4.50	6.00	5.40	1.80	7.60	6.30	1.50

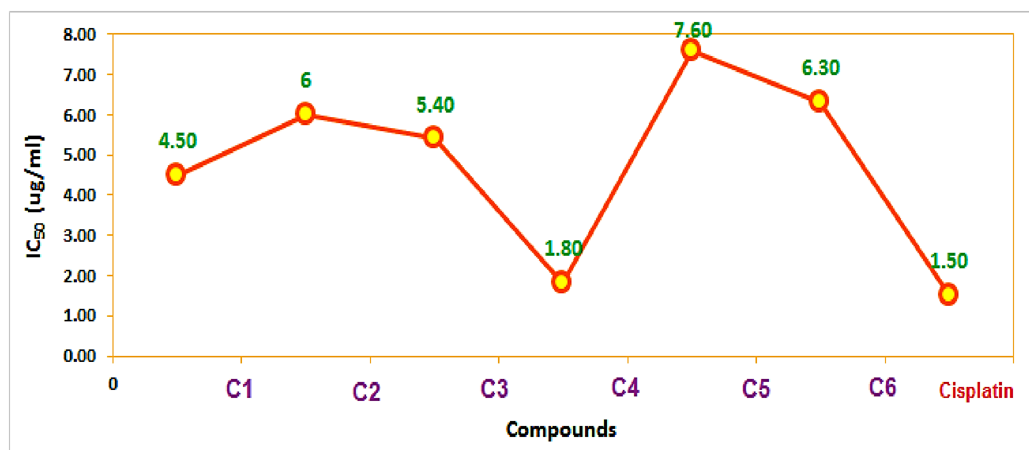


Figure 3.5 IC_{50} values of complexes against MCF-7 (human breast cancer cell line)

From the 50% inhibitory concentrations (**IC_{50}**) values for complexes listed in table, which are lying in the low range of microgram concentrations, it is revealed that all the six tested complexes are significantly active against MCF-7 with **C4** has most pronounced **IC_{50}** value of 1.80 ug/ml while the **C5** has the highest **IC_{50}** value of 7.60

ug/ml having binding constant value (K= 6.207×10^2) although complexes **C4** and **C6** both have alike dithiocarbamate ligand but differ in the attached organic moiety. The higher antiprolifertive activity of **C4** is probably because of likelihood of its strong interaction with nucleotides of DNA via hydrogen bonding by OH group to halt its replication [82]. Based on drug-DNA interaction studies, it might be anticipated that compounds interact with the base pairs on DNA, thus inhibit cell proliferation by interfering its replication and transcription. In spite of prominent anticancer activity of **C4**, binding constant (K= 7.385×10^2) is not that exceptional. This shows that drug-DNA interaction is merely not the mean of cell death. Complexes can also deactivate various enzymes governing the replication and transcription processes of nucleic acids [83]. Certainly this can also be anticipated that protons from OH group attached with **C4** can be removed by water molecules inside cell and this negatively charged body is unable to reach its target DNA efficiently, hence lowering its binding constant value [84].

C1, C2 and **C3** showed weak cytotoxicity against tested cell line regardless of their good binding constants and this can be attributed to the absence of any labile moiety in structure

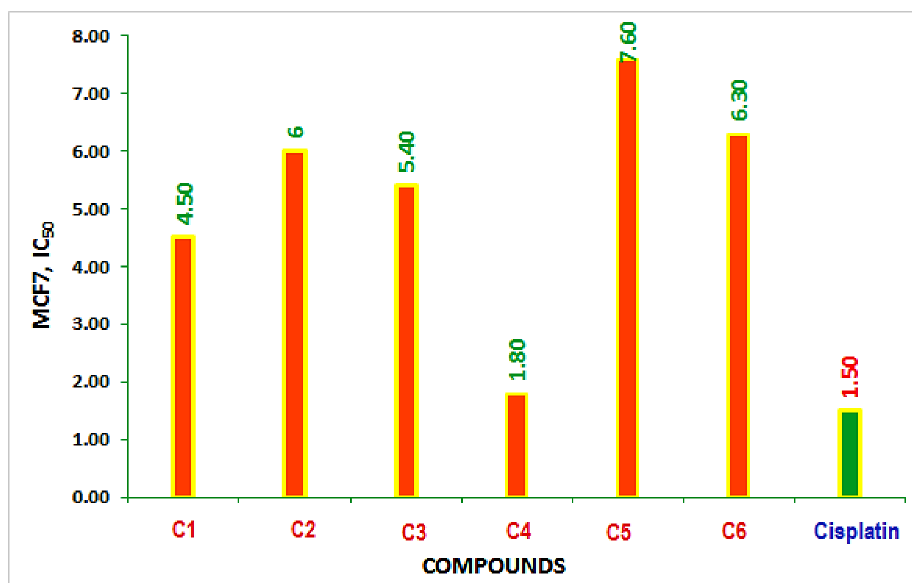


Figure 3.6 IC_{50} values of complexes against MCF-7 (human breast cancer cell line)

3.4.2 DNA Interaction Studies by UV-Vis and Evaluation of Binding Parameters

For the sake information regarding the extent of interaction of drug with DNA as well as binding strength, UV-spectroscopy was used. Hence to determine the binding affinity and binding mode of complexes **C1**-**C6** to DNA, UV-Vis spectroscopic titrations were performed. The experiential changes in the UV spectra of the complexes after mixing them with DNA is evident of their interaction with DNA, which can be either increase or decrease in the intensity or shifting in the wavelength region owing to formation of a new complex with the double-helix structure (DNA). Absorption bands of the complexes are affected with an increment in concentration of CT-DNA [85]. The strong absorption shown by complexes in region (300–330 nm) is ascribable to intra-ligand π–π^* transitions whereas broad absorption band near (350-470 nm) owing to the metal-to-ligand charge-transfer (MLCT) transitions resulting from an excitation of an electron from the metal t_{2g} orbitals to empty π^* molecular orbital of ligand [52]. The absorption spectra of complexes **C1**-**C6** in the absence as well as presence of calf thymus DNA (CT-DNA) at varying concentrations are shown in figures (2.6-2.11). The absorption band corresponding to the metal perturbed intra-ligand π-π* transition (300–330 nm) was observed to decrease at the time of adding aliquots of Calf Thymus DNA (CT-DNA), in the µM range, to a solution of complex of known concentration. Increment of DNA brought about substantial decrease in absorbance with sharp isobestic points with change in the wavelength of CT-DNA in complex. Long and Barton [86] had mentioned that binding mode of small molecules can be evaluated by exploiting the absorptions peak's shift. If binding mode is intercalative, a hypochromic effect together bathochromism will be evident. Interaction of electronic states of intercalating chromophore and those of stacked base pairs of DNA are responsible for special hypochromic effect. The π* orbital of binding ligand couple with π orbital of DNA base pairs and partially filled by electrons thus lowering the π and π* transition energy of the complexes because of their ordered stacking between base pairs after intercalation takes place, therefore decreasing the chances of transitions and result in hypochromocity. Thus the complexes (1-6) shown the intercalation with DNA. Reduction in absorbance was because of drop in the

availability of free compound as it binds more and more by increasing the DNA concentration.

In Figures (3.7-3.12) the spectra of complexes (**C1**-**C6**) with the absorbance of each complex both in the absence as well as presence of CT-DNA at varied concentrations varying from 5-30μM (**C1**), 10-60μM (**C2**), 5-30 μM, (**C3**), 10-50 μM (**C4**), 5-30 μM (**C5**, **C6**) are shown with decrease in absorption intensity and isobestic points at 310 nm (**C1**, **C3**, **C4**, **C5** and **C6**) and 315nm (**C2**) respectively. The negative values of Gibbs free energy suggest that drug-DNA interaction is spontaneous in nature.

Table 3.6 Binding parameters of complex (1-6) based upon UV-Vis spectroscopic data

Compound	Binding Constant (K)	Gibbs free energy (kJ mol^{-1})
C1	2.785×10^4	-25.354
C2	1.476×10^4	-23.783
C3	1.2895×10^4	-21.555
C4	7.385×10^2	-16.362
C5	6.207×10^2	-15.932
C6	7.914×10^4	-27.987

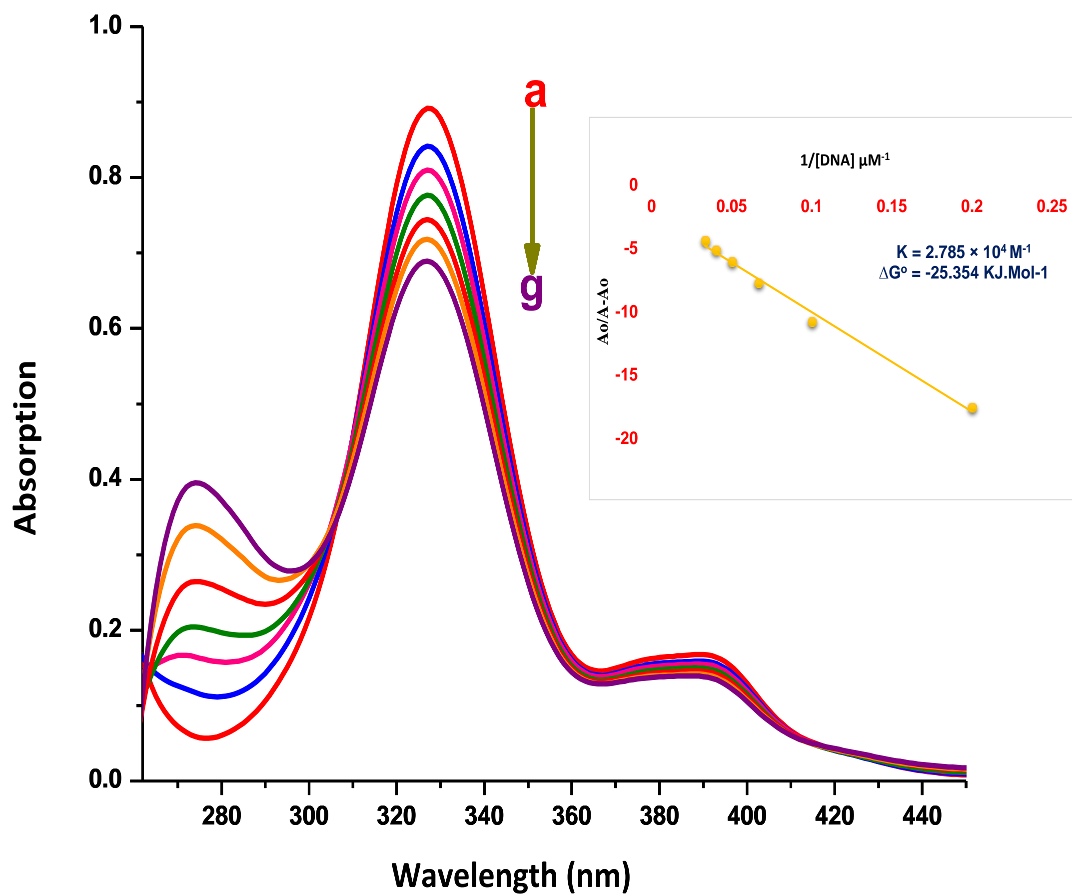


Figure 3.7 Absorption spectrum of C1 (20 µM) in the absence (a) and presence of (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30 µM DNA. The inset graph represents the plot of Ao/A-Ao vs. 1/[DNA] (μM^{-1}) for the calculation of binding parameters (K and ΔG^0).

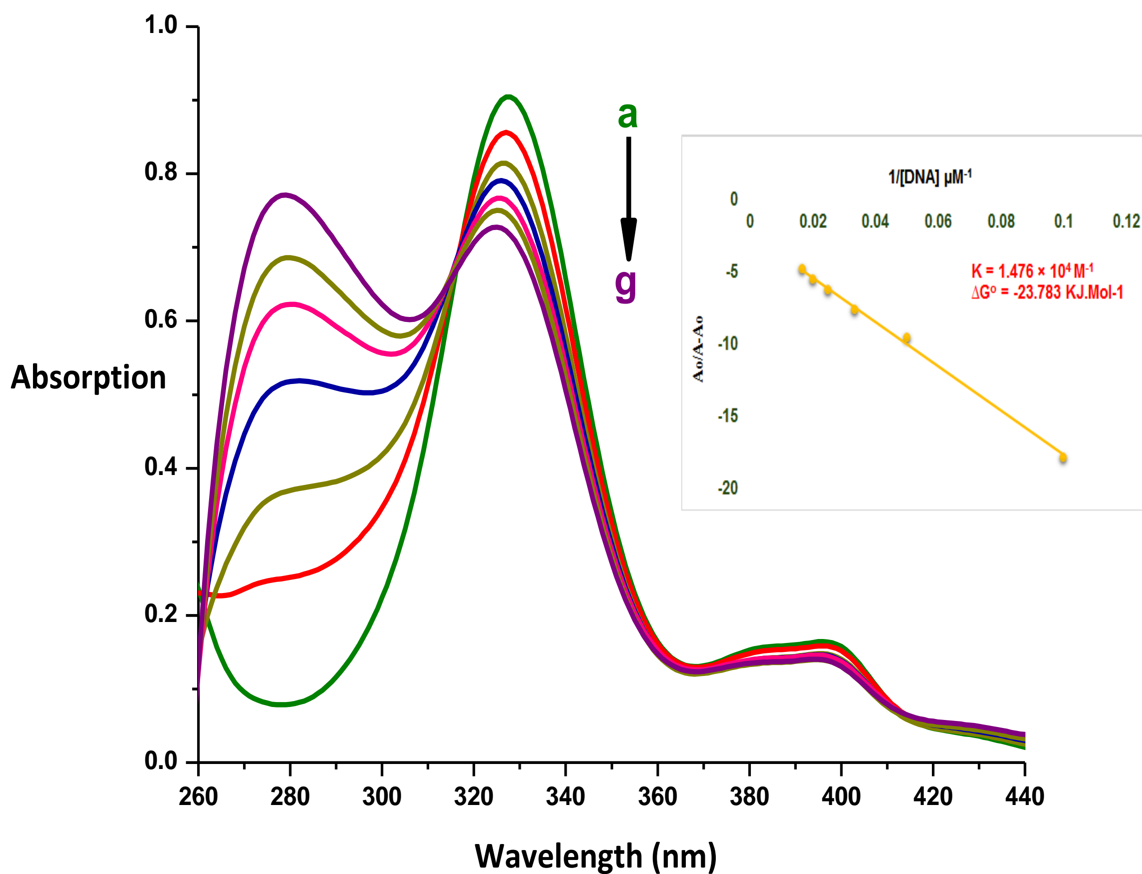


Figure 3.8 Absorption spectrum of C2 (25 μM) in the absence (a) and presence of (b) 10, (c) 20, (d) 30, (e) 40, (f) 50, (g) 60 μM DNA. The inset graph represents the plot of Ao/A-Ao vs. 1/[DNA] (μM^{-1}) for the calculation of binding parameters (K and ΔG^0).

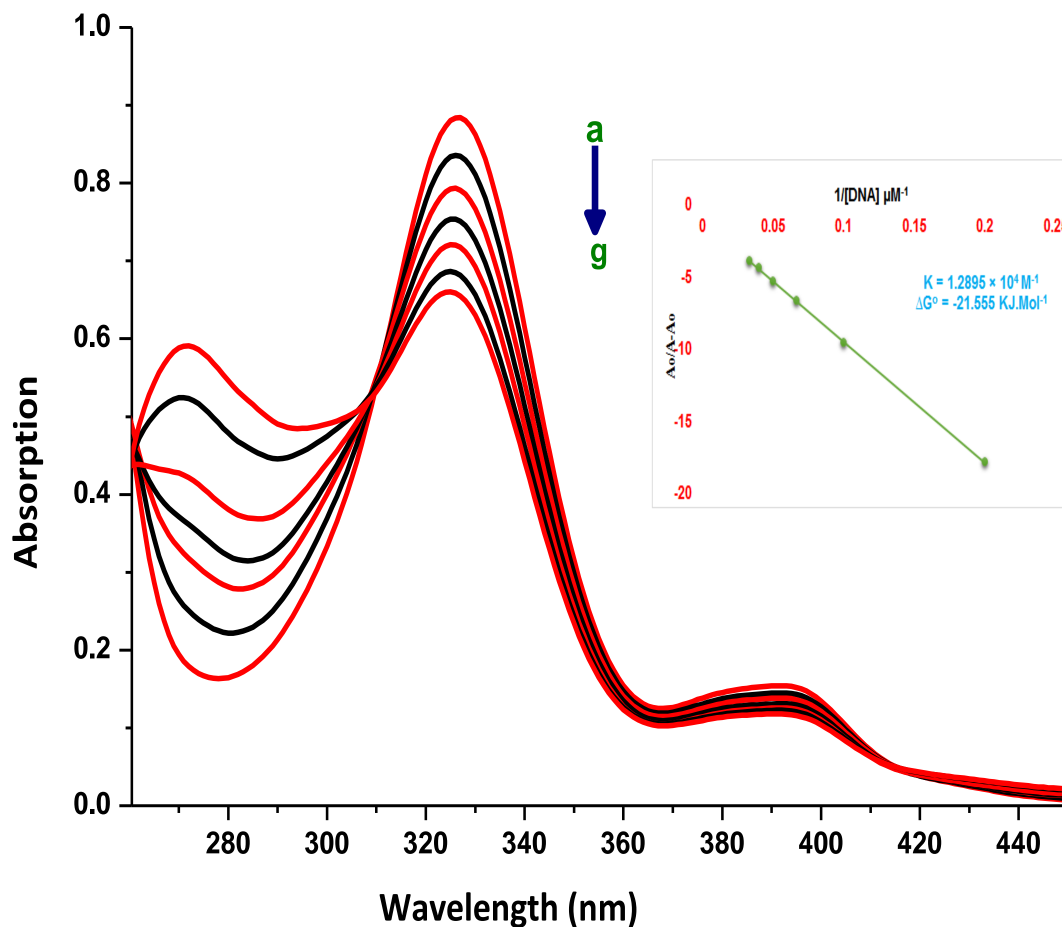


Figure 3.9 Absorption spectrum of C3 (20 µM) in the absence (a) and presence of (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30 µM DNA. The inset graph represents the plot of Ao/A-Ao vs. 1/[DNA] (μM^{-1}) for the calculation of binding parameters (K and ΔG^0).

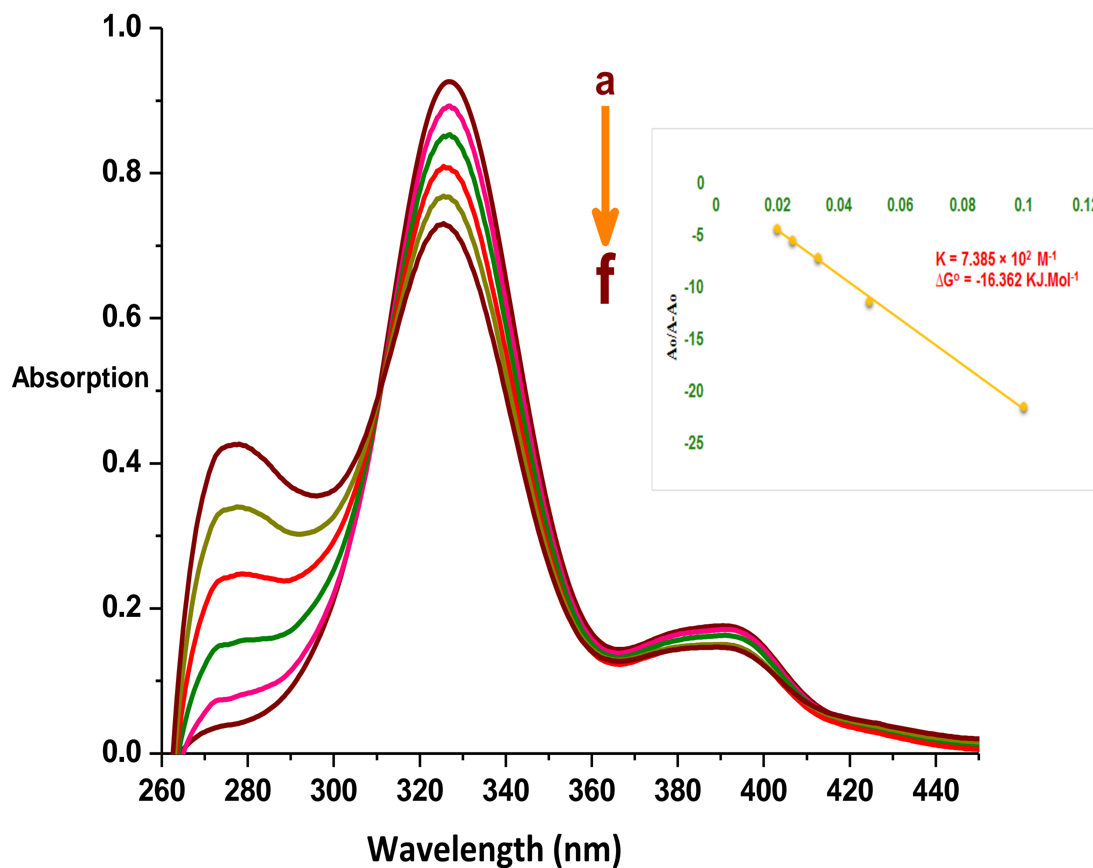


Figure 3.10 Absorption spectrum of C4 (20 µM) in the absence (a) and presence of (b) 10, (c) 20, (d) 30, (e) 40, (f) 50, (g) 30 µM DNA.The inset graph represents the plot of Ao/A-Ao vs. 1/[DNA] (μM^{-1}) for the calculation of binding parameters (K and ΔG^0).

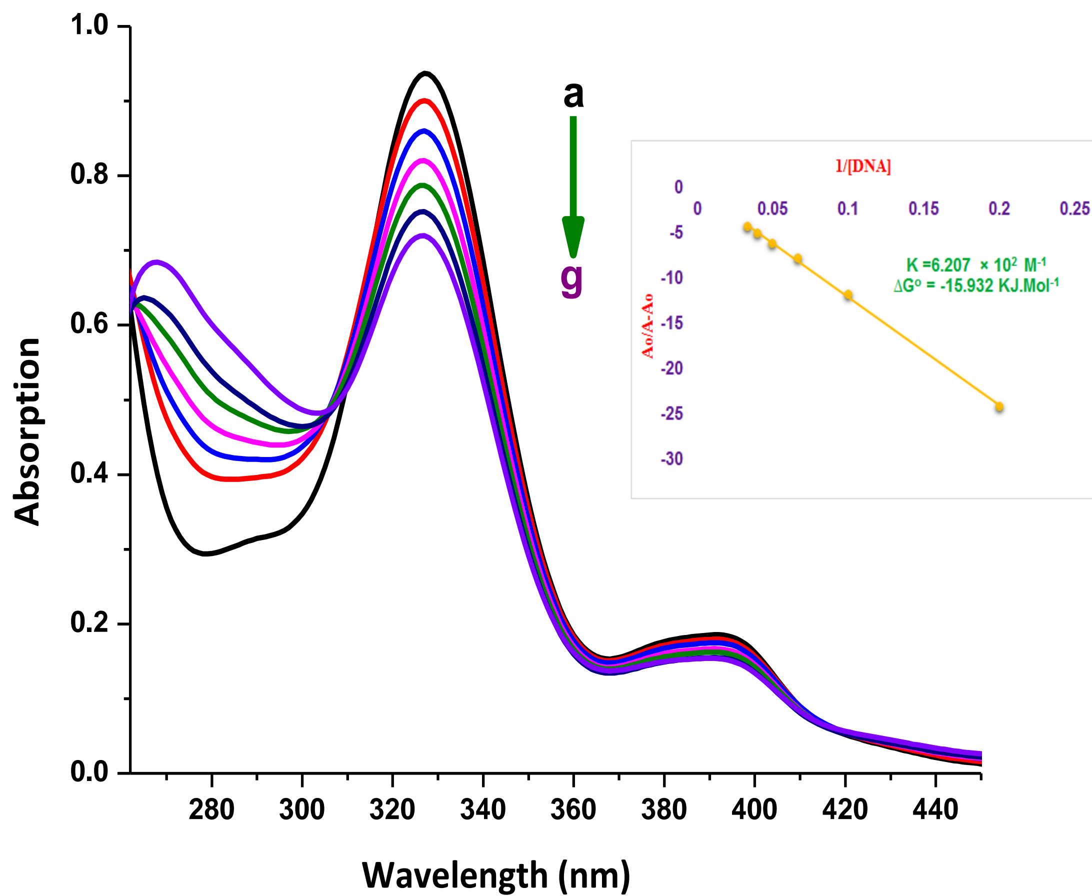


Figure 3.11 Absorption spectrum of C5 (20 µM) in the absence (a) and presence of (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30 µM DNA. The inset graph represents the plot of Ao/A-Ao vs. 1/[DNA] (μM^{-1}) for the calculation of binding parameters (K and ΔG^0).

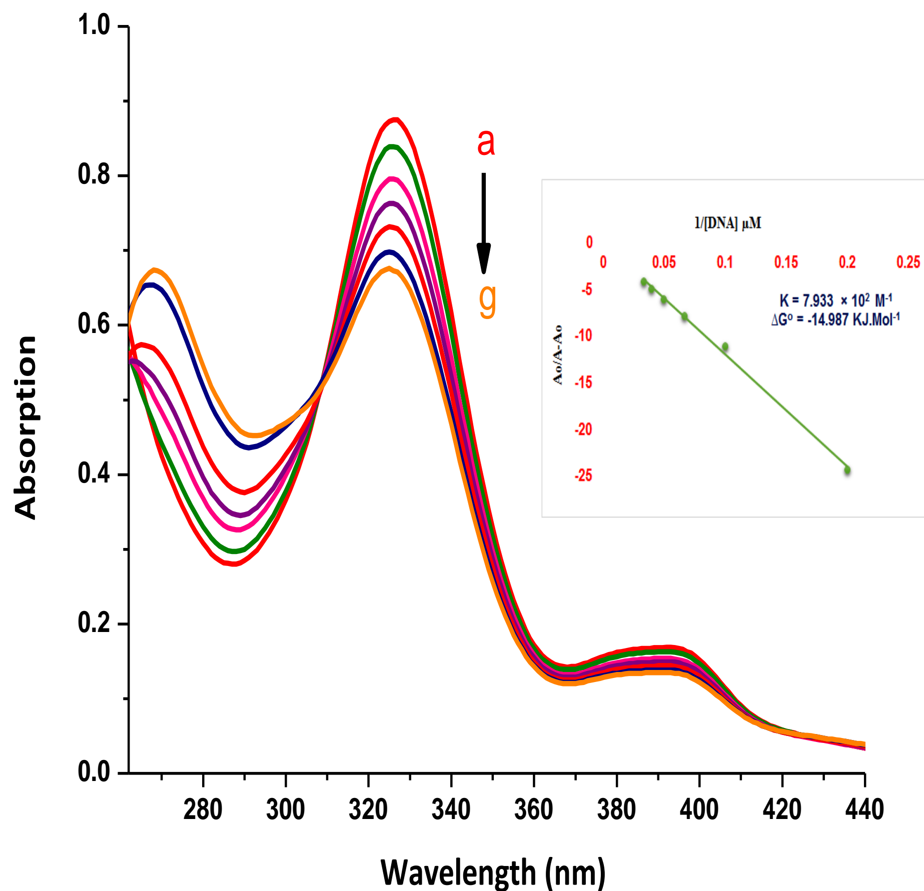


Figure 3.12 Absorption spectrum of C6 (20 µM) in the absence (a) and presence of (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30 µM DNA. The inset graph represents the plot of Ao/A-Ao vs. 1/[DNA] (μM^{-1}) for the calculation of binding parameters (K and ΔG^0).

3.4.3 Viscosity measurement of Nickel (II) complexes

Even though optical photophysical procedures are extensively used to elucidate the binding strength and mode of interaction of metal complex with DNA, however, adequate indication is not provided to confirm the binding mode. Henceforth, for further clarification of the binding model, viscosity measurement; a well-established technique is carried out. In a classical intercalative mode, at intercalation sites separation of base pairs result in an expansion of DNA helix which leads to noteworthy increase in viscosity. By contrast, partial or non-classical intercalative mode results in bending in DNA helix, hence decreasing its length and so its viscosity. Historically hydrodynamic measurements are thus deemed to be utmost stringent tests of the binding mode and present convincing reasoning for intercalative mode of binding [87]. The effects of the nickel(II) complexes on the viscosity of CT-DNA are presented in figure 3.13. As demonstrated in figure 3.13, upon increment in the quantity of the nickel(II) complexes, the relative viscosity of CT-DNA increased gradually, which is an evidence for intercalative mode of binding of nickel(II) complexes with CT-DNA. This is elucidated by the fitting of the complexes in between the base pairs causing them to separate at intercalation sites in order to adjust compounds, thus lengthening in the helix and consequently increasing the viscosity. It is obvious form the figure that all these complexes demonstrate an increase in the relative viscosity of CT-DNA however less in contrast with that of ethidium bromide.

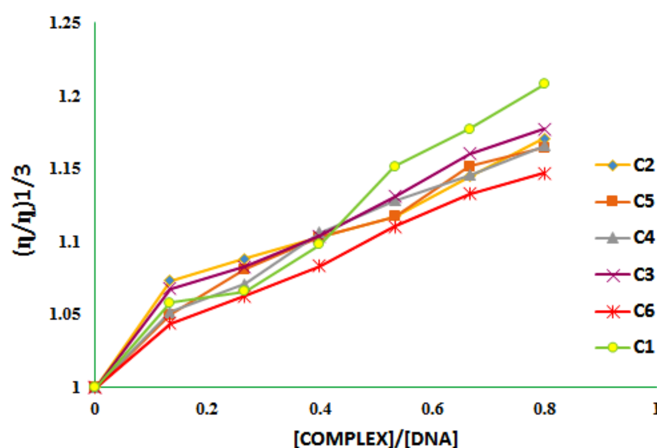


Figure 3.13 Plot of [η/ηo] $^{1/3}$ versus [Complex]/[DNA] for complexes C1-C6

The results may reflect the tendency of each ligand to intercalate into DNA base pairs. The experiential increase in viscosity proposes an intercalative mode binding [73].

3.5 DFT calculations

Gas-phase DFT optimization of complexes was accomplished using B3LYP/LANL2DZ level. The HOMO-LUMO gap and optimized structures for **C1**, **C2** and **C4** are presented in the figures 3.14-3.19. The energy difference between the LUMO and the HOMO determines the chemical reactivity and kinetic stability related with a chemical species. Little energy gap implies to a soft molecule with additional polarizability, great chemical reactivity in addition to low kinetic stability. Relatively greater HOMO and lesser LUMO energy infers well electron-donating and electron-accepting potential correspondingly. Values of HOMO–LUMO gap for **C1** (3.56), **C2** (3.592 eV) and **C4** (3.534 eV) specifies the more stable nature of the second.

Global chemical reactivity indices such as electrophilicity (ω), chemical hardness (η), and electronic chemical potential (μ) can assess the chemical reactivity and stability associated with any chemical species [88]. Chemical hardness can be explained as "half of the difference of LUMO–HOMO band gap" i.e. $\eta = (E_{LUMO} - E_{HOMO})/2$. Compounds having high η values are regarded as hard species (more stablility and less reactivity) while with smaller values are soft ones (less stability and more reactivity).

Electronic chemical potential (μ) is elucidated as "half of the HOMO–LUMO combined energies" i.e. $\mu = (E_{HOMO} + E_{LUMO})/2$ or "negative of the electronegativity" [89]. It measures the tendency of electrons to escape from the equilibrium state. Species having higher μ values seems to be more reactive than the ones having smaller values. Electrophilicity index (ω) evaluates the useful change in energy when a chemical system gets saturated by addition of electrons. Precisely $\omega = \mu^2/2\eta$. High ω values signify high electrophilic character whereas lesser relate to nucleophilicity [90].

Table 3.7 Energies (eV) of HOMO and LUMO, chemical hardness (η), electronic chemical potential (μ), and electrophilicity (ω)

Compound	E_{LUMO}	E_{HUMO}	$\Delta E(E_{LUMO}-E_{HOMO})$	η	μ	ω
C1	-1.980	-5.540	3.56	1.78	-3.76	3.97
C2	-1.850	-5.442	3.592	1.796	-3.646	3.700
C4	-2.065	-5.599	-5.599	1.767	-3.832	4.155

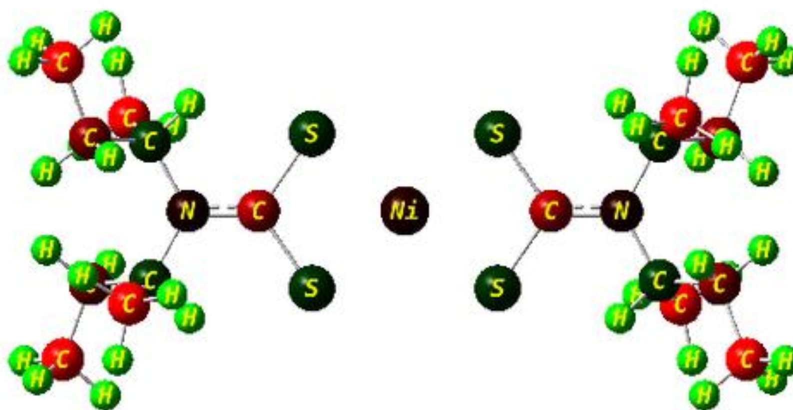


Figure 3.14 Optimized structures of C2 at B3LY P/LANL2DZ level

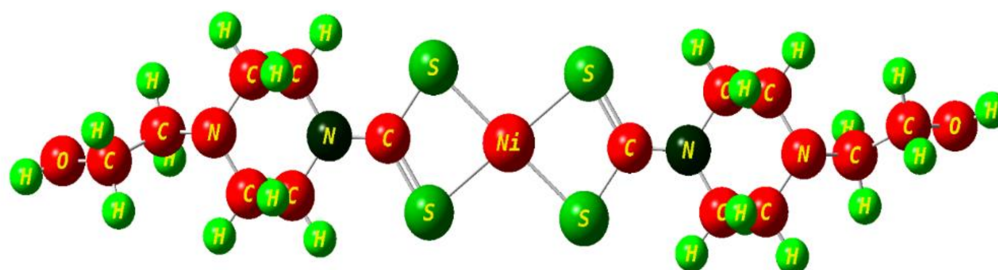


Figure 3.15 Optimized structures of C4 at B3LY P/LANL2DZ level

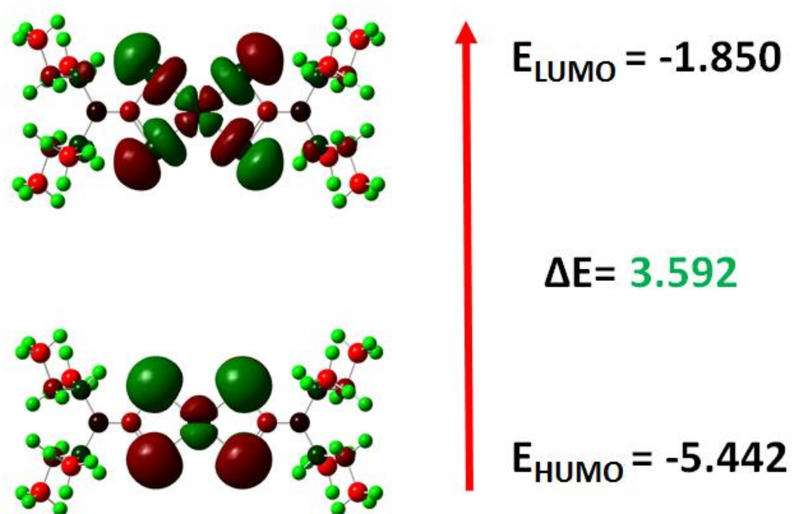


Figure 2.16 The atomic orbital of the frontier molecular orbital for $C_{18}H_{36}N_2NiS_4$ at B3LYP/3-21G level of theory

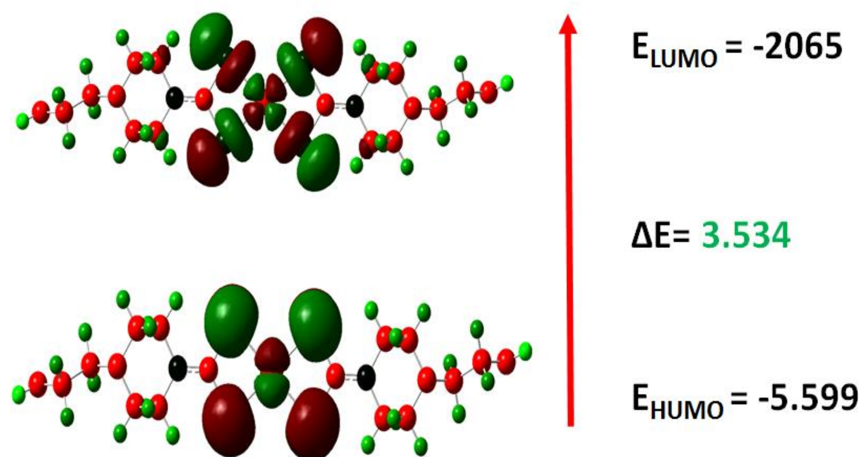


Figure 3.17 The atomic orbital of the frontier molecular orbital for $C_{14}H_{26}N_4NiO_2S_4$ at B3LYP/3-21G level of theory

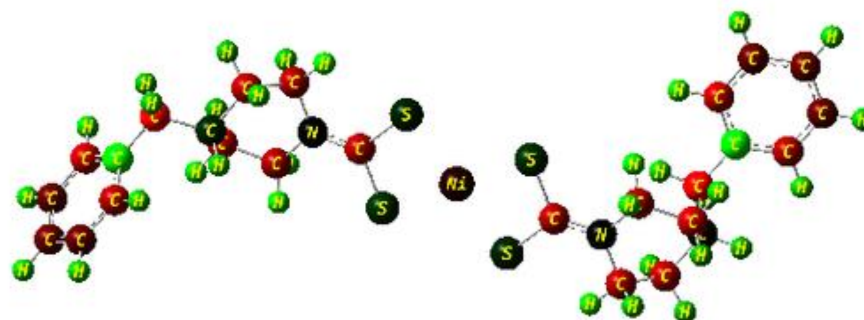


Figure 3.18 Optimized structures of C1 at B3LY P/LANL2DZ level

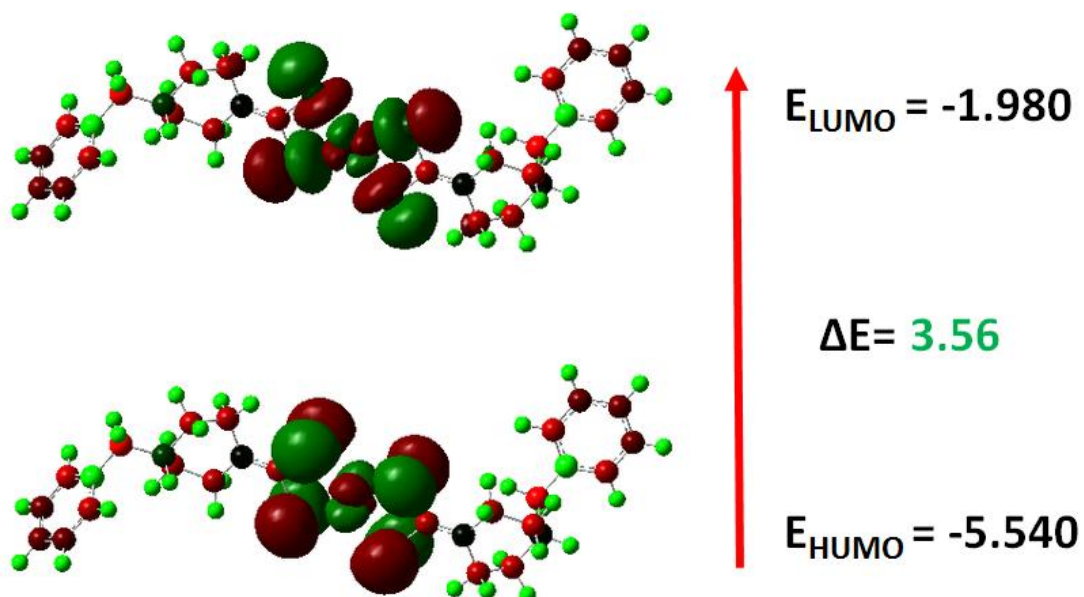


Figure 3.19 The atomic orbital of the frontier molecular orbital for $C_{26}H_{32}N_2NiS_4$ at B3LYP/3-21G level of theory

Conclusions

New bis(dithiocarbamato)Ni(II) complexes have been prepared and characterized by different analytical techniques viz. elemental analysis, FT-IR, multinuclear (^{1}H and ^{13}C) NMR and single crystal X-ray analysis. In FT-IR spectra, the ν(C⎓N) thioureide band was noticed around 1499-1514 cm^{-1} which is intermediate between (C—N) single (1250-1350 cm-1) and (C═N) double (1640-1690cm^{-1}) bond character, inferring coordination of the metal owing to the delocalization of electrons, resultant a partial double bond character. Investigation of complex by X-ray crystallographic studies additionally reinforced the statement. In ^{13}C NMR, signal for C=S band appeared at 204.9 ppm confirmed the coordination of ligand via two sulfur atoms. Single crystal X-ray analysis has confirmed the anisobidentate nature of dithiocarbamate ligand with a $Ni\text{-}S_{long}$ [2.2232 (7) Å] and $Ni\text{-}S_{short}$ [2.1970 (8) Å] resulting in ideal square planer geometry for **C5**. Studies revealed ideal square planer geometry for **C5**. Intermediate bond lengths, between single (1.82 Å) and double (1.60 Å) bond, are shown by carbon–sulfur bond within the chelate ring with an average bond lengths C1–S1= 1.716(3), C1–S2 =1.723(3) Å, which proposed a significant charge delocalization. Furthermore the S_2CN bond length is 1.314(4) Å, considerably smaller than a C–N bond (1.47 Å) and longer than C=N bond (1.28 Å), hence signify a resonance phenomenon in NSCS moiety. Synthesized complexes (**C1-C6**) were screened against MCF-7 (human breast cancer cell line) employing the sulforhodamine B (SRB) cellular protein-staining procedure. Ni(II) complexes were significantly active, with the complex **C4** has shown most pronounced activity with IC_{50} value of 1.80 µg/mL, most probably because of likelihood of its strong interaction with nucleotides of DNA via hydrogen bonding by OH group to halt its replication. Binding affinity and binding mode of complexes to DNA were determined by UV-Vis spectroscopic and viscometric titrations. The outcomes revealed the hypochromic effect for all the complexes, indicating intercalative mode of interaction of these complexes with DNA. Global chemical reactivity indices and LUMO–HOMO band gap was evaluated by performing gas-phase DFT optimizations of complexes by using B3LYP/LANL2DZ level

[1] B. Vogelstein, K.W. Kinzler, The genetic basis of human cancer, McGraw-Hill, 2002.

[2] J.F. Kerr, C.M. Winterford, B.V. Harmon, Apoptosis. Its significance in cancer and cancer therapy, Cancer, 73 (1994) 2013-2026.

[3] J.L. Spivak, Cancer-related anemia: its causes and characteristics, in: Seminars in oncology, 1994, pp. 3-8.

[4] M. Hejmadi, Introduction to cancer biology, Bookboon, 2009.

[5] F. Michor, Y. Iwasa, M.A. Nowak, Dynamics of cancer progression, Nature reviews cancer, 4 (2004) 197-205.

[6] R.A. Smith, V. Cokkinides, O.W. Brawley, Cancer screening in the United States, 2009: a review of current American Cancer Society guidelines and issues in cancer screening, CA: a cancer journal for clinicians, 59 (2009) 27-41.

[7] A. Sudhakar, History of cancer, ancient and modern treatment methods, Journal of cancer science & therapy, 1 (2009) 1.

[8] P. Anand, A.B. Kunnumakara, C. Sundaram, K.B. Harikumar, S.T. Tharakan, O.S. Lai, B. Sung, B.B. Aggarwal, Cancer is a preventable disease that requires major lifestyle changes, Pharmaceutical research, 25 (2008) 2097-2116.

[9] J.I. Morgan, T. Curran, Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun, Annual review of neuroscience, 14 (1991) 421-451.

[10] C.J. Sherr, J.M. Roberts, Inhibitors of mammalian G1 cyclin-dependent kinases, Genes & development, 9 (1995) 1149-1163.

[11] R.A. Weinberg, Tumor suppressor genes, Science, 254 (1991) 1138-1146.

[12] C.J. Marshall, Tumor suppressor genes, Cell, 64 (1991) 313-326.

[13] C.A. Stratakis, Genetics of adrenocortical tumors: gatekeepers, landscapers and conductors in symphony, Trends in Endocrinology & Metabolism, 14 (2003) 404-410.

[14] M. Lind, Principles of cytotoxic chemotherapy, Medicine, 36 (2008) 19-23.

[15] C. Orvig, M.J. Abrams, Medicinal inorganic chemistry: introduction, Chemical Reviews, 99 (1999) 2201-2204.

[16] M. Frezza, S. Hindo, D. Chen, A. Davenport, S. Schmitt, D. Tomco, Q. Ping Dou, Novel metals and metal complexes as platforms for cancer therapy, Current pharmaceutical design, 16 (2010) 1813-1825.
[17] V.T. DeVita, E. Chu, A history of cancer chemotherapy, Cancer research, 68 (2008) 8643-8653.
[18] T.W. Hambley, Is anticancer drug development heading in the right direction?, Cancer research, 69 (2009) 1259-1262.
[19] S. Neidle, D.E. Thurston, Chemical approaches to the discovery and development of cancer therapies, Nature Reviews Cancer, 5 (2005) 285-296.
[20] D. Chen, V. Milacic, M. Frezza, Q.P. Dou, Metal complexes, their cellular targets and potential for cancer therapy, Current pharmaceutical design, 15 (2009) 777-791.
[21] Y.K. Yan, M. Melchart, A. Habtemariam, P.J. Sadler, Organometallic chemistry, biology and medicine: ruthenium arene anticancer complexes, Chemical communications, (2005) 4764-4776.
[22] L.E. Scott, C. Orvig, Medicinal inorganic chemistry approaches to passivation and removal of aberrant metal ions in disease, Chemical Reviews, 109 (2009) 4885-4910.
[23] S.M. Cohen, New approaches for medicinal applications of bioinorganic chemistry, Current opinion in chemical biology, 11 (2007) 115-120.
[24] I. Ott, R. Gust, Non Platinum Metal Complexes as Anti-cancer Drugs, Archiv der Pharmazie, 340 (2007) 117-126.
[25] T.W. Hambley, Developing new metal-based therapeutics: challenges and opportunities, Dalton Transactions, (2007) 4929-4937.
[26] S.P. Fricker, Metal based drugs: from serendipity to design, Dalton Transactions, (2007) 4903-4917.
[27] A.d. de Gramont, A. Figer, M. Seymour, M. Homerin, A. Hmissi, J. Cassidy, C. Boni, H. Cortes-Funes, A. Cervantes, G. Freyer, Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer, Journal of Clinical Oncology, 18 (2000) 2938-2947.
[28] B. Rosenberg, L. Van Camp, T. Krigas, Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode, Nature, 205 (1965) 698-699.

[29] A.H. Rossof, R.E. Slayton, C.P. Perlia, Preliminary clinical experience with cis-diamminedichloroplatinum (II)(NSC 119875, CACP), Cancer, 30 (1972) 1451-1456.
[30] Y. Shi, J. Goodisman, J.C. Dabrowiak, Cyclodextrin capped gold nanoparticles as a delivery vehicle for a prodrug of cisplatin, Inorganic chemistry, 52 (2013) 9418-9426.
[31] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, Nature reviews Drug discovery, 4 (2005) 307-320.
[32] B. Lippert, Cisplatin: chemistry and biochemistry of a leading anticancer drug, John Wiley & Sons, 1999.
[33] B. Michalke, Platinum speciation used for elucidating activation or inhibition of Pt-containing anti-cancer drugs, Journal of Trace Elements in Medicine and Biology, 24 (2010) 69-77.
[34] S.J. Berners-Price, T.A. Frenkiel, U. Frey, J.D. Ranford, P.J. Sadler, Hydrolysis products of cisplatin: p K a determinations via [1 H, 15 N] NMR spectroscopy, Journal of the Chemical Society, Chemical Communications, (1992) 789-791.
[35] A.R. Timerbaev, C.G. Hartinger, S.S. Aleksenko, B.K. Keppler, Interactions of antitumor metallodrugs with serum proteins: advances in characterization using modern analytical methodology, Chemical reviews, 106 (2006) 2224-2248.
[36] D.-W. Shen, L.M. Pouliot, M.D. Hall, M.M. Gottesman, Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes, Pharmacological reviews, 64 (2012) 706-721.
[37] L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo, G. Kroemer, Molecular mechanisms of cisplatin resistance, Oncogene, 31 (2012) 1869-1883.
[38] S. Ishida, J. Lee, D.J. Thiele, I. Herskowitz, Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals, Proceedings of the National Academy of Sciences, 99 (2002) 14298-14302.
[39] Z.H. Siddik, Cisplatin: mode of cytotoxic action and molecular basis of resistance, Oncogene, 22 (2003) 7265-7279.

[40] A.-M. Florea, D. Büsselberg, Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects, Cancers, 3 (2011) 1351-1371.
[41] L.P. Martin, T.C. Hamilton, R.J. Schilder, Platinum resistance: the role of DNA repair pathways, Clinical Cancer Research, 14 (2008) 1291-1295.
[42] E.S. El Daly, Protective effect of cysteine and vitamin E, Crocus sativus and Nigella sativa extracts on cisplatin-induced toxicity in rats, Journal de pharmacie de Belgique, 53 (1997) 87-93; discussion 93-85.
[43] J.D. Watson, A. Berry, DNA: The secret of life, Knopf, 2009.
[44] R. Dahm, Discovering DNA: Friedrich Miescher and the early years of nucleic acid research, Human genetics, 122 (2008) 565-581.
[45] P.D. Hebert, A. Cywinska, S.L. Ball, Biological identifications through DNA barcodes, Proceedings of the Royal Society of London B: Biological Sciences, 270 (2003) 313-321.
[46] J.C. García-Ramos, R. Galindo-Murillo, F. Cortés-Guzmán, L. Ruiz-Azuara, Metal-based drug-DNA interactions, Journal of the Mexican Chemical Society, 57 (2013) 245-259.
[47] D.R. Whelan, T.J. Hiscox, J.I. Rood, K.R. Bambery, D. McNaughton, B.R. Wood, Detection of an en masse and reversible B-to A-DNA conformational transition in prokaryotes in response to desiccation, Journal of the Royal Society Interface, 11 (2014) 20140454.
[48] R.M. Wadkins, B. Vladu, C.-S. Tung, Actinomycin D binds to metastable hairpins in single-stranded DNA, Biochemistry, 37 (1998) 11915-11923.
[49] F.R. Keene, J.A. Smith, J.G. Collins, Metal complexes as structure-selective binding agents for nucleic acids, Coordination Chemistry Reviews, 253 (2009) 2021-2035.
[50] L. Lerman, Structural considerations in the interaction of DNA and acridines, Journal of molecular biology, 3 (1961) 18IN13-30IN14.
[51] N. Hadjiliadis, E. Sletten, Metal complex-DNA interactions, John Wiley & Sons, 2009.

[52] M. Sirajuddin, S. Ali, A. Badshah, Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry, Journal of Photochemistry and Photobiology B: Biology, 124 (2013) 1-19.

[53] R.V. Gessner, G.J. Quigley, A.H. Wang, G.A. Van der Marel, J.H. Van Boom, A. Rich, Structural basis for stabilization of Z-DNA by cobalt hexaammine and magnesium cations, Biochemistry, 24 (1985) 237-240.

[54] V. Bala, G. Gupta, V. L Sharma, Chemical and medicinal versatility of dithiocarbamates: an overview, Mini reviews in medicinal chemistry, 14 (2014) 1021-1032.

[55] G. Hogarth, Transition metal dithiocarbamates: 1978–2003, Progress in Inorganic Chemistry, Volume 53, (2005) 71-561.

[56] G. Hogarth, Metal-dithiocarbamate complexes: chemistry and biological activity, Mini reviews in medicinal chemistry, 12 (2012) 1202-1215.

[57] M.L. Gullino, F. Tinivella, A. Garibaldi, G.M. Kemmitt, L. Bacci, B. Sheppard, Mancozeb: past, present, and future, Plant Disease, 94 (2010) 1076-1087.

[58] S. Kanchi, P. Singh, K. Bisetty, Dithiocarbamates as hazardous remediation agent: A critical review on progress in environmental chemistry for inorganic species studies of 20 th century, Arabian Journal of Chemistry, 7 (2014) 11-25.

[59] R. Room, T. Babor, J. Rehm, Alcohol and public health, The lancet, 365 (2005) 519-530.

[60] Z.E. Sauna, S. Shukla, S.V. Ambudkar, Disulfiram, an old drug with new potential therapeutic uses for human cancers and fungal infections, Molecular bioSystems, 1 (2005) 127-134.

[61] R. Schreck, B. Meier, D.N. Männel, W. Dröge, P.A. Baeuerle, Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells, The Journal of experimental medicine: JEM, 175 (1992) 1181-1194.

[62] Y. Horita, T. Takii, T. Yagi, K. Ogawa, N. Fujiwara, E. Inagaki, L. Kremer, Y. Sato, R. Kuroishi, Y. Lee, Antitubercular activity of disulfiram, an antialcoholism drug, against multidrug-and extensively drug-resistant Mycobacterium tuberculosis isolates, Antimicrobial agents and chemotherapy, 56 (2012) 4140-4145.

[63] P. Liu, S. Brown, T. Goktug, P. Channathodiyil, V. Kannappan, J. Hugnot, P. Guichet, X. Bian, A. Armesilla, J. Darling, Cytotoxic effect of disulfiram/copper on human glioblastoma cell lines and ALDH-positive cancer-stem-like cells, British journal of cancer, 107 (2012) 1488-1497.

[64] S.-L. Cao, Y. Han, C.-Z. Yuan, Y. Wang, Z.-K. Xiahou, J. Liao, R.-T. Gao, B.-B. Mao, B.-L. Zhao, Z.-F. Li, Synthesis and antiproliferative activity of 4-substituted-piperazine-1-carbodithioate derivatives of 2, 4-diaminoquinazoline, European journal of medicinal chemistry, 64 (2013) 401-409.

[65] W.L. Armarego, C.L.L. Chai, Purification of laboratory chemicals, Butterworth-Heinemann, 2013.

[66] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program, Journal of Applied Crystallography, 42 (2009) 339-341.

[67] G.M. Sheldrick, Crystal structure refinement with SHELXL, Acta Crystallographica Section C: Structural Chemistry, 71 (2015) 3-8.

[68] M. You, D.M. Wickramaratne, G.L. Silva, H. Chai, T.E. Chagwedera, N.R. Farnsworth, G.A. Cordell, A.D. Kinghorn, J.M. Pezzuto, (-)-Roemerine, an aporphine alkaloid from Annona senegalensis that reverses the multidrug-resistance phenotype with cultured cells, Journal of natural products, 58 (1995) 598-604.

[69] G. Barone, A. Terenzi, A. Lauria, A.M. Almerico, J.M. Leal, N. Busto, B. García, DNA-binding of nickel (II), copper (II) and zinc (II) complexes: Structure–affinity relationships, Coordination Chemistry Reviews, 257 (2013) 2848-2862.

[70] Y. Zhang, X. Wang, L. Ding, Synthesis and DNA binding studies of Mg (II) complex of Schiff base derived from vanillin and l-tryptophan, Nucleosides, Nucleotides and Nucleic Acids, 30 (2011) 49-62.

[71] M. Reichmann, S. Rice, C. Thomas, P. Doty, A further examination of the molecular weight and size of desoxypentose nucleic acid, Journal of the American Chemical Society, 76 (1954) 3047-3053.

[72] A. Shah, E. Nosheen, S. Munir, A. Badshah, R. Qureshi, N. Muhammad, H. Hussain, Characterization and DNA binding studies of unexplored imidazolidines by

electronic absorption spectroscopy and cyclic voltammetry, Journal of Photochemistry and Photobiology B: Biology, 120 (2013) 90-97.

[73] R. Sinha, M.M. Islam, K. Bhadra, G.S. Kumar, A. Banerjee, M. Maiti, The binding of DNA intercalating and non-intercalating compounds to A-form and protonated form of poly (rC)· poly (rG): Spectroscopic and viscometric study, Bioorganic & medicinal chemistry, 14 (2006) 800-814.

[74] D. Sholl, J.A. Steckel, Density functional theory: a practical introduction, John Wiley & Sons, 2011.

[75] A. Frisch, A. Nielsen, A. Holder, Gaussview user manual, Gaussian Inc., Pittsburgh, PA, 556 (2000).

[76] N. Manav, A. Mishra, N. Kaushik, In vitro antitumour and antibacterial studies of some Pt (IV) dithiocarbamate complexes, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 65 (2006) 32-35.

[77] S. Khan, S.A. Nami, K. Siddiqi, Piperazine pivoted transition metal dithiocarbamates, Journal of Molecular Structure, 875 (2008) 478-485.

[78] E. Sathiyaraj, S. Thirumaran, Synthesis and spectral studies on Pb (II) dithiocarbamate complexes containing benzyl and furfuryl groups and their use as precursors for PbS nanoparticles, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 97 (2012) 575-581.

[79] S.Z. Khan, M.K. Amir, I. Ullah, A. Aamir, J.M. Pezzuto, T. Kondratyuk, F. Bélanger-Gariepy, A. Ali, S. Khan, Zia-ur-Rehman, New heteroleptic palladium (II) dithiocarbamates: synthesis, characterization, packing and anticancer activity against five different cancer cell lines, Applied Organometallic Chemistry, 6 (2016) 392-398.

[80] H. Khan, A. Badshah, G. Murtaza, M. Said, Zia-ur-Rehman, C. Neuhausen, M. Todorova, BJ Jean-Claude, IS Butler, Eur. J. Med. Chem, 46 (2011) 4071.

[81] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettìo, R. Graziani, G. Pilloni, D. Fregona, Gold dithiocarbamate derivatives as potential antineoplastic agents: design, spectroscopic properties, and in vitro antitumor activity, Inorganic chemistry, 44 (2005) 1867-1881.

[82] D. Kanduc, A. Ghoshal, E. Quagliariello, E. Farber, DNA hypomethylation in ethionine-induced rat preneoplastic hepatocyte nodules, Biochemical and biophysical research communications, 150 (1988) 739-744.

[83] M. Sirajuddin, S. Ali, V. McKee, H. Ullah, Synthesis, spectroscopic characterization and in vitro antimicrobial, anticancer and antileishmanial activities as well interaction with Salmon sperm DNA of newly synthesized carboxylic acid derivative, 4-(4-methoxy-2-nitrophenylamino)-4-oxobutanoic acid, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 138 (2015) 569-578.

[84] D. Kanduc, M.R. Rossiello, A. Aresta, E. Quagliariello, C. Cavazza, E. Farber, Transitory DNA hypomethylation during liver cell proliferation induced by a single dose of lead nitrate, Archives of biochemistry and biophysics, 286 (1991) 212-216.

[85] S. Rauf, J. Gooding, K. Akhtar, M. Ghauri, M. Rahman, M. Anwar, A. Khalid, Electrochemical approach of anticancer drugs–DNA interaction, Journal of pharmaceutical and biomedical analysis, 37 (2005) 205-217.

[86] E.C. Long, J.K. Barton, On demonstrating DNA intercalation, Accounts of Chemical Research, 23 (1990) 271-273.

[87] P.U. Maheswari, M. Palaniandavar, DNA binding and cleavage properties of certain tetrammine ruthenium (II) complexes of modified 1, 10-phenanthrolines–effect of hydrogen-bonding on DNA-binding affinity, Journal of Inorganic Biochemistry, 98 (2004) 219-230.

[88] C.A. Mebi, DFT study on structure, electronic properties, and reactivity of cis-isomers of [(NC5H4-S) 2Fe (CO) 2], Journal of Chemical Sciences, 123 (2011) 727-731.

[89] S. Špirtović-Halilović, M. Salihović, H. Džudžević-Čančar, S. Trifunović, S. Roca, D. Softić, D. Završnik, DFT study and microbiology of some coumarin-based compounds containing a chalcone moiety, (2014).

[90] F. Hayat, Zia-ur-Rehman, M.H. Khan, Two new heteroleptic ruthenium (II) dithiocarbamates: synthesis, characterization, DFT calculation and DNA binding, Journal of Coordination Chemistry, 70 (2017) 279-295.