“New Biologically Active Transition Metal Complexes of Ferrocene Assimilated Thiourea”

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In the name of Allah, the beneficent, the merciful

Declaration

This is to certify that this dissertation entitled *"New Biologically Active Transition metal Complexes of Ferrocene Assimilated Thiourea"* submitted by ***Mr. Zahid Nawaz*** is accepted in its present form by the Department of Chemistry, Quaid-I-Azam, Islamabad, Pakistan, as satisfying the dissertation requirements for the degree of ***Master of Philosophy*** *in* ***Inorganic/Analytical Chemistry.***

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Zahid Nawaz

I

Dedicated this work

To the Holy Prophet, Hazrat Muhammad

(PUBH),

Who is the well-wisher of us and showed us the right way.

To my father, M, Nawaz,

To myBrother, Adil Nawaz,

Who led me in every field of my life and education,

To my mother,

Who has always prayed for my success,

To my Cousin Rashid Maqsood.

Abstract

Series of ferrocene substituted organometallic thioureas complexes of general formula $[(C5H5FeC5H4)_2(C6H4)_2NC(S)NH]M$ where, M =(Au, Mn, Ni, Cu, Co, Pt., and Ru) have been synthesized and characterized by using elemental analysis, FT-IR, multinuclear (^{1}H and ^{13}C) NMR spectroscopy and UV-Visible spectroscopy. The compounds interaction with the DNA has been studied by using UV -Visible spectroscopy and cyclic voltammetry. DNA binding data showed that all the synthesized compounds are showing good interaction with the DNA. The negative and positive shift in peak potential and decrease in peak current were used to study mode of interactions and different DNA binding parameters.All the screened compounds had the electrostatic and intercalation mode of interaction with DNA. All screened compounds have good antibacterial activity.

Some of the screening compound shows a good anticancer activity. All the synthesized compounds show effective antioxidant activities.

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Abbreviations

Thiourea	(TU)
Cyclic voltammetry	(CV)
UV-visible spectrometry	(UV)

Chapter 1 Introduction

Science have made much progress to develop and rationalized the society by creating so many facilities in every field of the life. However, by the industrial revolution along with the facility and development to mankind it created many problems, like, pollution of air, water, and soil. Due to these unhealthy practices of industrial development, issue of health becomes prominent along with other problem. According to the WHO survey of 2014, 70% people are expired by health problems and all other death due to natural or others accidental/suicidal etc. Table 1 shows the list of world top ten reasons of death [1].

Table 1: Top ten causes of death world wild [1]

S.No.	Cause	S. No.	Cause
1	Ischemic heart disease	**6**	HIV / AIDS
2	Stroke and other cerebrovascular disease	**7**	Diarrheal diseases
3	COPD Chronic obstructive	**8**	Tuberculosis
4	Lower respiratory infections	**9**	Diabetes mellitus
5	Cancers	**10**	Road Traffic accidents

1. Cancer

Fifth most precarious disease of the world is cancer that causes 1.6 million deaths per year. Cancer is the combination of many diseasing group which can affect any part of the body these includes neoplasms malignant and tumors. Cancer is the unlimited and uncontrolled division of cell and metastases which affect the one part of body and extend to other organs of the body so can affect the whole body during time passing. From cancer the primary cause of death is metastases [2-4]. Cancer can be diagnosed by the normal cell cycle the human life started from signal cell divide into millions of numbers cell is the basic structural and functional units of the body. The figure below shows the explanation of normal cell cycle by Rubin *et. al.* [5].

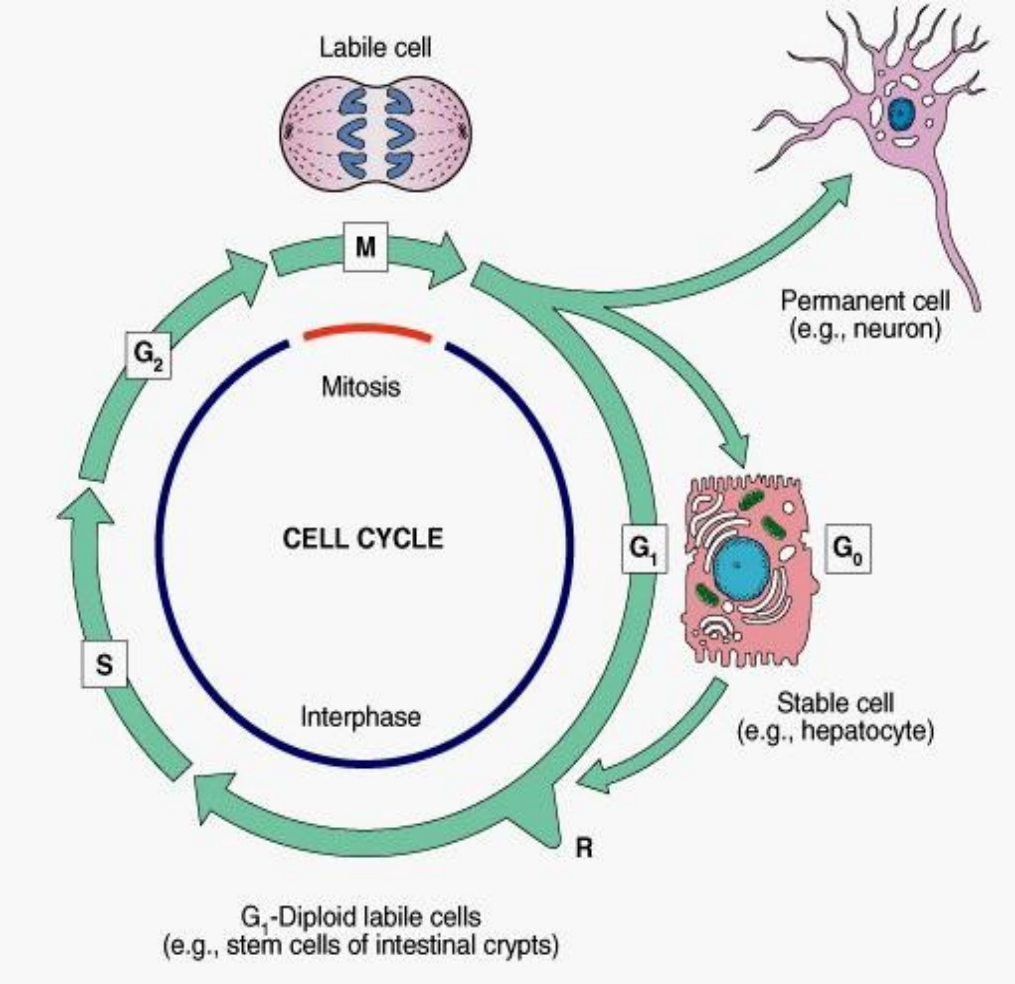


Figure 1: The cell cycle, in different types of cells is; (I) Labile cells (e.g., Intestinal crypt cells) endure continuous reproduction and the gap between two successive mitoses is called the cycle. After partition, the cells go into a gap (G_1) during which deoxyribonucleic acid (DNA) synthesis stops, while ribonucleic acid (RNA) and protein synthesis occurs as the cell build up its own particular function. Cells that carry on in the cell cycle go by the restriction point (R), which assigns them to a new round of cell replication and continuance to the synthesis (S) phase during which each and every chromosome is replicated. Followed by The S phase cell division process enter into a small gap (G2) during which DNA synthesis ceases and protein synthesis carry on. The M phase is the time of mitosis. After each cycle, one daughter cell will committed to differentiation and the other will continue cycling. (II) Some cell types, such as hepatocytes, are stable. After cell mitosis, the cells take up their specialized function (G_0) and do not reenter the cell cycle unless stimulated by the loss of other cells. (III) Permanent cells (neuron) become terminally differentiated after mitosis and cannot reenter the cell cycle.

Cancer is caused by any type of disturbance in the normal cell cycle the enhancement of cell cycle by any procedure is the result of cancer causing [6]. The mutation in single cell (monoclonal population) lead to the maximum cause of cancer, while some are due to mutation in multi cells which is the example of polyclonal population [4]. Though, by the cancer the normal cell division is disturbed and a large numbers

of the cancerous cell are formed these large number of uncontrolled cells are called tumors [7]. Classification of the tumor cell depends upon cell nature e.g. carcinomas, lymphomas, leukemia's and sarcomas. [2]. According to alike cells there are so many types of cancer in the human body [8]. Cancer can be noticeable when tumor is about 1cm in thickness or by cells divide more than 1 million times [4]. It start (noncancerous) and malignant (cancerous) are the two main type of tumors. In the start of tumor is not dangerous to life it cannot effect the other organ but the malignant one effect the other body parts and destroy them so it is the life warning cancer [9].

2. Causes of Cancer

Cancer is due to genetic makeup disorderness which controls the cell division and growth of cells. For the development of cancer several aspects of genetic and environmental factor are responsible [10]. Table 2 present the list for the reason which are creating cancer along with these some other reason are also present presenting by many authors.

Table 2: A concise list of cancer causes [10].

S.No.	Reason	Cancer caused
	Genetic causes	
1	Acquired mutation	Can cause any kind of cancer
2	Inherited mutation (Germline mutation)	Sometime causes cancer but not always
	Environmental causes	
3	Tobacco use	Larynx, lung, pancreas, bladder
4	Inorganic arsenic	Skin, bladder, lung
5	Water chlorination	Numerous
6	Aromatic hydrocarbons	Numerous
7	Heavy metals	Numerous
8	Viruses	Liver, cervix and others
9	Alcohol abuse	Breast, liver, larynx, esophagus, oropharynx
10	Recreational and prescribed drugs	Increase the risk of cancer cell development
11	Radiation	Skin, ovary, leukemia, breast sarcoma, prostate

3. Treatment of Cancer

Though disease of cancer is one of awful disease but successfully many cancer cases can be prevented, or by different treatments the patient's life can be prolonged meaningfully in early stages if the cancer is detected. The followings are the existing medications for the cancer treatment; organ transplantation, surgery, biotherapy, radiation therapy, palliative care an There are three main method for the cancer treatment which are radiotherapy chemotherapy and surgery. While in some cases biotherapy organ transplantation and palliative care method is also use for the cancer treatment [11]. Chemotherapy has advantages over surgery and radiation treatment. All these treatments are being in use combinely but that depend on the stage and nature of disease. All the above treatment have the disadvantages and side effect along with pay backs because these are so much expensive [12].

4. Chemotherapy treatment

Chemotherapy treatment is the most communal cure used against different type of cancers. In this methodology of cancer treatment the various drugs or chemical are used to eliminate the origin of cancer or cancer cells. For the minor part of effected region in body is treated by using radiotherapy and surgery, but there are two possible way internally and externally for the radiation treatment. By internal radiation therapy the successive uncontrolled division of cells is killed in metastases. The success distortion rate is 40% (with minor tumor) with surgery and radiotherapy. The remaining 60% is still vanishing due to metastasis of cancer cells [13]. Both these are not much effective and also very costly as compared to chemotherapy. Chemotherapy can be successfully operated for the metastasized tumor treatment so it has advantages over surgery and radiation therapy [14]. A perfect drug for the cancer treatment is that which destroy the cancerous cell without any changes in the normal cells. There are so many side effect of the entire drug's use for cancer treatment as they disturb the normal cells the side effect are kidney damages hair loss nervous system damages etc. Several agent of chemotherapy are present in market for the cancer treatment some other chemotherapeutic drugs are present which reduce the cells division. Figure 1.01 is for the normal cell division cycle the drug use for cancer treatment hair to goal to

stop up to some extent the 1 step of cell division cycle Chemotherapy drugs are classified into several groups based on these target (Table 3).

Table 3: A brief listing of representative commercial chemotherapeutic agents [15-23].

S.No	Chemotherapeutics	Cancer against which practiced
Alkylating agents (Interact with DNA to inhibit the cell replication process)		
1	Nitrogen Mustards (Mechlorethamine)	Hodgkin's disease, non-Hodgkin's lymphoma breast and lung
2	Nitrosoureas (Carmustine)	Brain tumors, Hodgkin's disease, non-Hodgkin's lymphoma, melanoma, lung cancer, colon cancer
3	Alkyl Sulfonates (Busulfan)	Chronic myelogenous leukemia
4	Ethylenimines (Thiotepa)	Breast cancer, ovarian cancer, Hodgkin's disease, and nonHodgkin's lymphoma
Anti-metabolites (Induce cell death during the S phase of cell growth, incorporated into RNA, DNA or inhibit enzymes)		
5	Pyrimidines (Flurouracil)	Breast, head, neck, adrenal, pancreatic, gastric, colon, rectal, esophageal, liver
6	Purines (6-Mercaptopurine)	Acute lymphocytic leukemia
7	Folate antagonists (Pemetrexed)	Mesothelioma, non-small cell lung cancer
8	Hydroxyurea	Melanoma, chronic myelogenous leukemia, squamous cell carcinomas
Topoisomerase inhibitors (Makes the enzyme nonfunctional by blocking the ability of the topoisomerase to bind the DNA)		
9	Doxorubicin	Hodgkin's lymphoma, bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma
10	Mitoxantrone	Breast cancer, acute myeloid leukemia, non-Hodgkin's lymphoma.
Mitotic inhibitors (Arrest the division of cells and cause cell death, By binding to the building blocks of a tubulin protein)		
11	Vincristine	Acute leukemia, rhabdomyosarcoma, neuroblastoma, Wilm's tumor, Hodgkin's disease
12	Vindesine	Melanoma, lung cancers, uterine cancers
Kinase Inhibitors (Blocks a kinase gene from binding to ATP, preventing the phosphorylation that would benefit the cancerous cell and promote cell division)		
13	Sorafenib	Renal cancer, Liver cancer

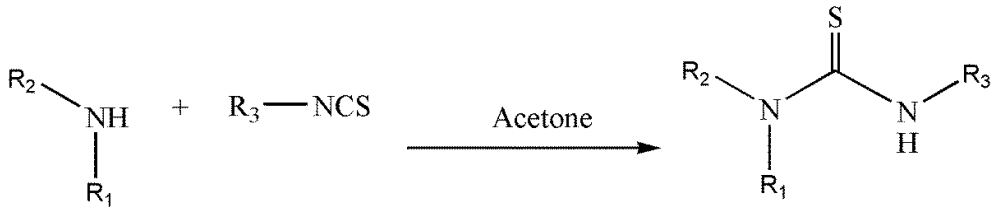
5. Thiourea

Thiourea is structurally like urea, and are the organosulfur compounds different from urea is that the oxygen of urea is replace by sulfur in thiourea. These thiourea have a general formula of (R_3R_4N)C=S(NR_1R_2), the thiourea and urea have differ in physical and chemical properties due to different functional groups.

6. Thiourea synthesis

(a) By using Isothiocynates

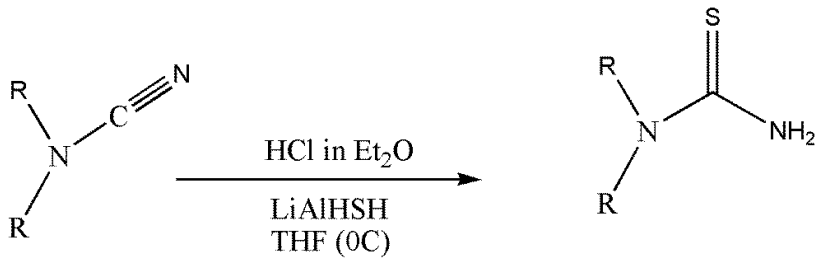
Compounds with general formula R_1NCS are known as isothiocynates, N, N`-substituted thioureas can be obtained by condensation with primary ammin or secondary (R_2R_3NH) amine produces resultant. Altered alkyl, acyl, aroyl and aryl substituted thioureas can be formed by same procedure [24, 25]. This condensations process is stated under various conditions like: solvent free microwave synthesis [26-28], phase transfer catalyst (PTC) of two different immiscible solvent. [26, 29], while some of the substituted thiourea like, N,N,N`,N`-tetra substituted cannot be prepared by same method.



Scheme 1 shows the synthesis of thioureas from isothiocyanates. R_1, R_2 and R_3 may be same or different alkyl and aryl groups, R_3 may also be alkoyl or aroyl group.

(b) By using Cyanamid's

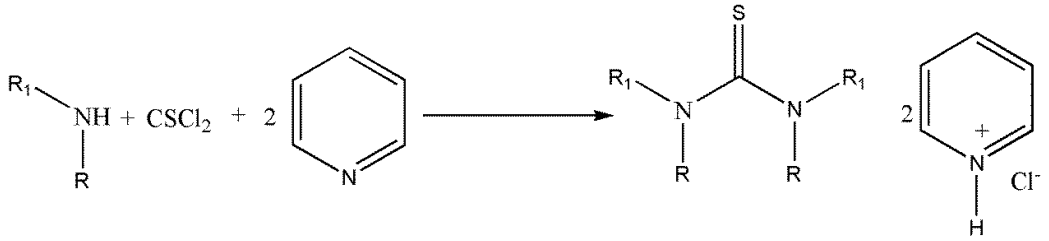
Compounds having general formula R_1R_2NCN are known as Cyanamid, reacts "LiAlHSH" in the company of 1N HCl in anhydrous diethyl ether to produce resultant N, N-substituted thioureas. In cyanamid reaction substitutions take place only on single nitrogen. The "LiAlHSH" is produced by mixing lithium aluminum hydride with elemental sulfur [25]. N,N' disubstituted thiourease cannot be synthesized by reaction.



Scheme 2 shows the synthesis of thioureas from Cyanamid's. R_1 and R_2 may be same or different alkyl and aryl groups.

(c) By using Thiophosgene

By reacting two amine (primary or secondary) molecules with thiophosgene ($CSCl_2$) coupled by pyridine desired thiourea can be synthesized [30]. Different amines mixture of thioureas synthesized can be separated by various analytical techniques like chromatography.



Scheme 3 shows the synthesis of thioureas from thiophosgene, R and R_1 may be same or different alkyl and aryl groups.

7. Importance of thioureas

The early mention derivatives thioureas have also great command over different biological activities, such as fungicidal [31, 32], antiviral [33, 34], herbicidal, [28, 35] plant growth regulating [36], antibacterial [37], tyrosine kinase [38], potent and selective inhibitors of the platelet derived growth factor (PDGF) receptor autophosphorylation [39]. It has an analgesic, local anesthetic activities and antihyperlipidemic potentials [40]. Newly formed thiourea are much important and drug subtle human oral carcinoma cells and nasopharyngeal carcinoma (CNE2) cells [41]. Coordination compounds of thiourea exhibit extra activity against numerous cancer cells as compare to that of illustrious anticancer agent cis-platin [42]. Thioureas also have fruitful applications in metal complexes and molecular electronics. Thioureas have also been successfully used for the purification and extraction of Nickel, Platinum and Palladium metals [43-47]. These thioureas

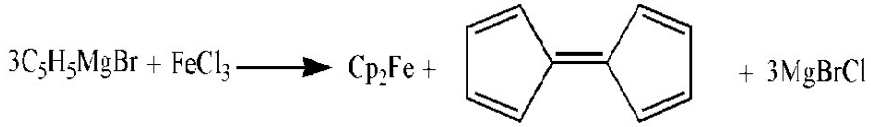
compounds are used as first source of materials for the growth of agrochemicals, pharmaceuticals products.

8. Ferrocene

Ferrocene is illustrious metallocene, ferrocene is a organometallic compound that contains a central metal atom with two cyclopentadienyl rings attached on opposite sides. Sa the metal is embedded inside the two rings that,s why ferrocene compounds are also known as sandwiched compounds [23,48]. In contrast with other metallocenes, ferrocene and its derivatives display attractive chemistry which provoked the attitude of synthetic scientist in organometallic chemistry [49].

8.1. Discovery of ferrocene

A lucky accident in 1951 leads to the revolutionary discovery of ferrocene by Pauson and Kealy [24] in an attempt to produce fulvalene by reaction known as oxidative coupling reaction of ferric chloride with C_5H_5MgBr. Against their expected product, they grown an extremely stable powder having light orange color. After investigation it was suggested that covalent bond is present between two cp rings and metal center in their proposed structure. The Equation below signifies the said reaction [25]. In February 1952, production of ferrocene was stated by Miller et al. [26, 27, 29].



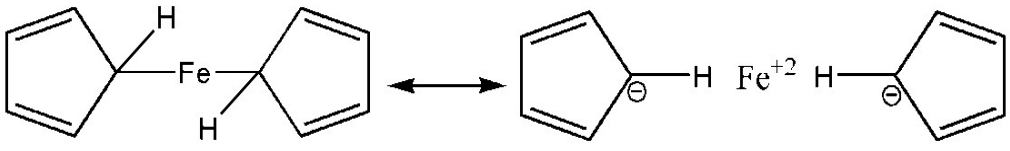


Figure 2: shows the Kealy and Pauson assumptions of resonance contributing structures of ferrocene.

8.2. Structure of ferrocene

Several structures were planned for ferrocene to clarify its strange stability. Wilkinson et al. planned a sandwiched structure that fulfilled bonding of both Cp rings to Fe [50].

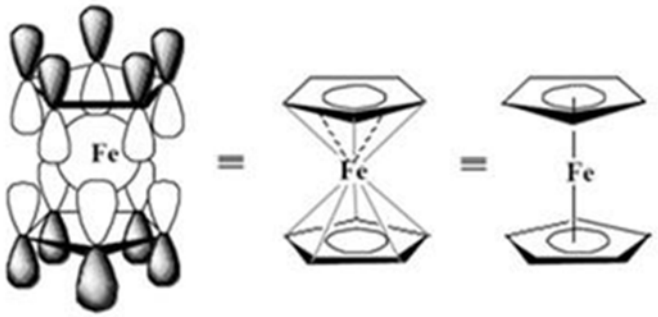


Figure 3: Structures proposed for ferrocene.

Carbon-carbon bond length of ferrocene is measured as 1.40 A° within the five membered rings, and the bond lengths of Fe-C was measured to be 2.04 A°. X-ray crystallographic data conform the staggered conformation whereas in in gas phase eclipsed form as revealed by gas phase electron diffraction[30] and computational studies [33].

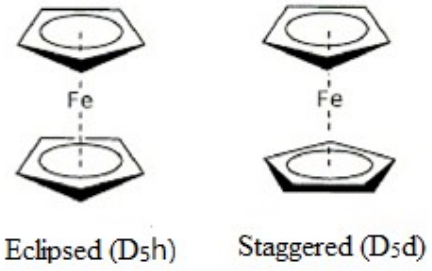


Figure 4: Conformations of ferrocene.

8.3. Electronic Structure of ferrocene

The Fe is sandwich between two Cp (that give aromatic nature due to six πbond) ring having oxidation state +2. Covalent bonding with Fe atom is formed due to these twelve electrons. Therefore the complex achieves 18 (eighteen) electron configurations [34] due to the great stability of ferrocene. Owing to aromaticity of

Cp rings the chemistry of ferrocene is comparable to that of benzene because several electrophilic substitution reactions takes place at the Cp ring like benzene.The ferrocenyl group having strong electron donar abilitiy.

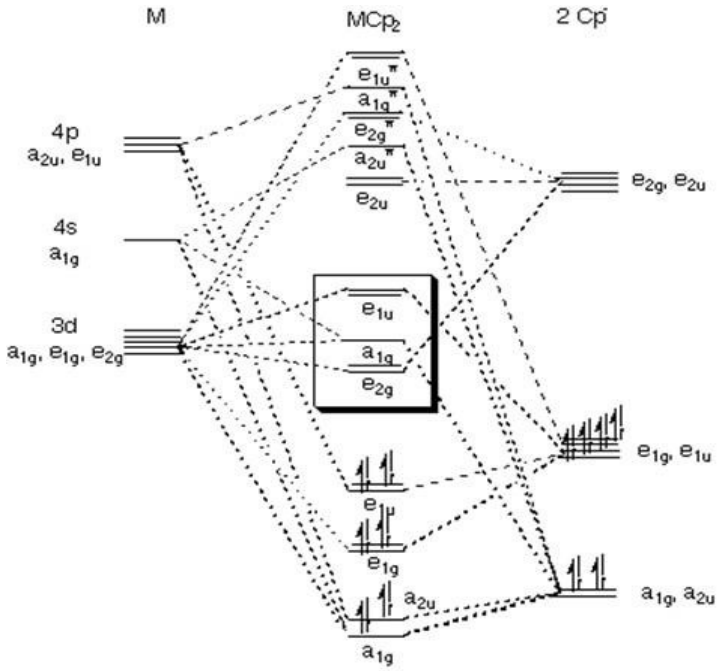


Figure 5: Molecular orbital diagram of ferrocene

MO diagram in ferrocene explain its bonding ability. Figure 5 displays that empty orbitals of the ligand and metal show interaction with each other to form molecular orbitals. The strength of the ferrocene molecule is credited to the formation of two π-bonds as a result of overlapping of the d_{xz} and d_{yz} orbitals of the Fe atom with e_{1g} orbitals of ligand [31, 37].

8.4. Physical properties of ferrocene

Ferrocene is diamagnetic in nature with orange color in solid physical state having melting point in the range of 173-174 °C with. It contains high oxidative and thermal stability. It is resistant a best solubility profile against attack of water, oxygen, non-oxidizing acids and aqueous bases under usual condition up to 450 °C [32].

8.5. Applications of ferrocene

The extraordinary steadiness of ferrocene [28], an extensive variety of ferrocene derivatives, and it's fascinating chemistry, excellent properties in optics [35]

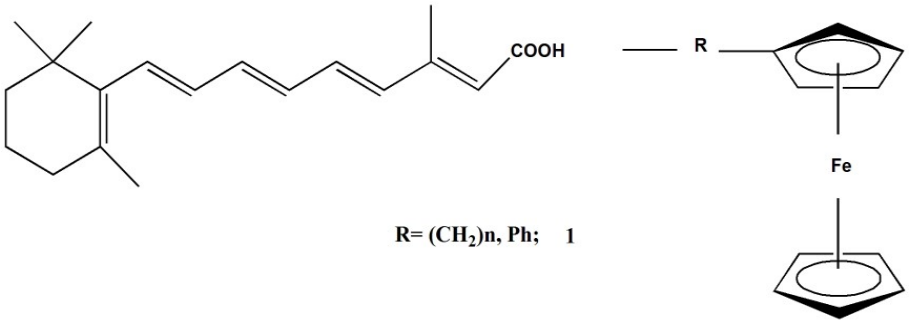
informal procedure capability of materials [36] consequence in fame of ferrocenyl compounds in diverse fields of life for their versatile applications.

9. Biological applications of ferrocene and its derivatives

Ferrocene and its derivatives have great application in medical chemistry [39-42, 51]. It has been stated that few ferrocene derivatives are effective against several diseases like, both *in-vivo* and *in-vitro* [43, 44] against malaria [45, 46], HIV aids [47] and cancer [39, 40, 42].

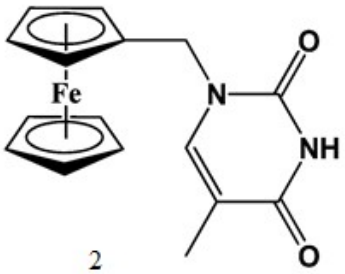
9.1. As an anticancer agent

The influence of ferrocene and their derivatives as anti-cancer proxies was initially evaluated at the end of 1970s by Brynes and his coworkers [52]. Several drugs are supported by Ferrocene due to its worthy redox properties, as Fe(III) have less redox ability than Fe(II)[53-55]. This anticancer activity may be because of strength to catalyze the fabrication of Reactive Oxygenated Species (ROS), consequential in the oxidative cleavage of DNA [56].



R= (CH_2)n, Ph; 1

Ferrocenyl derivatives of nucleosides were synthesized by Simenel et al. and one of produced compound 1-N-ferrocenylmethyl thymine **2** was selected *in vivo* against lung cancer cell line carcinoma, ensuing in 70% reticence of tumor growth [58].



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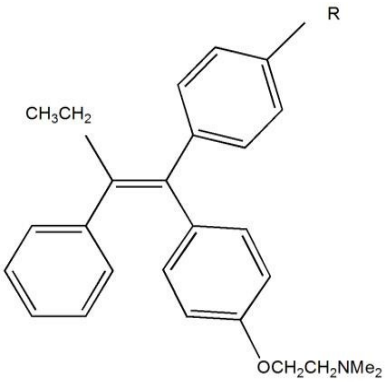
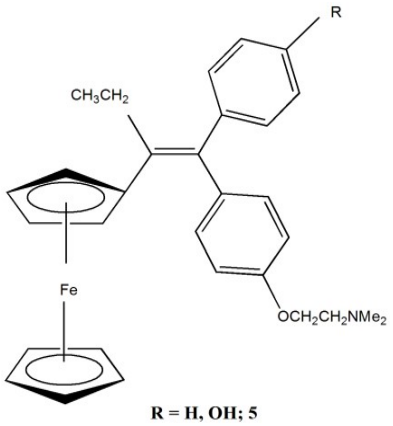
The universal drugs treated for the medication of breast cancer are hydroxy tamoxifen **4** and tamoxifen **3**, and have many side effects [59].



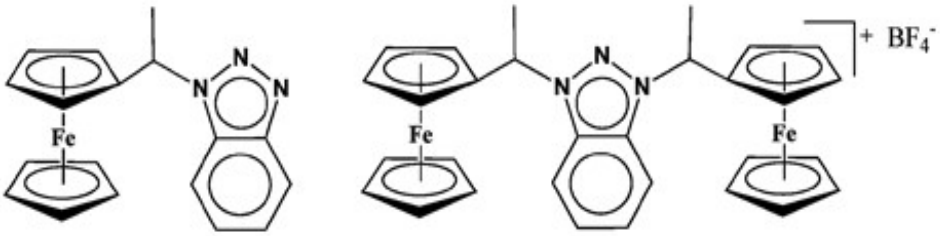
R = H, 3; R = OH, 4

The ferrocenyl derivative of tamoxifen known as ferrocifen **5** used for the treatment of hormone cells [60].



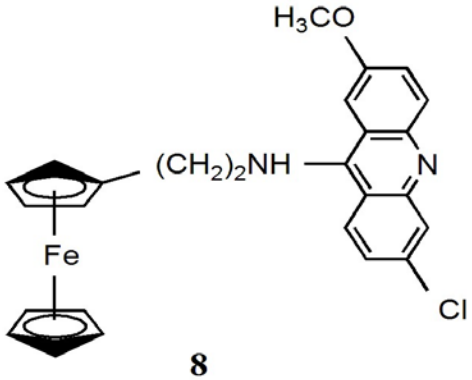
R = H, OH; 5

Snegur et al. chemically improved those azoles by ferrocene that are main component in many drugs including pyrazole, imidazole and adenine [61-64].
Ferrocenyl derivative **6** and **7** display higher action for solid tumors.



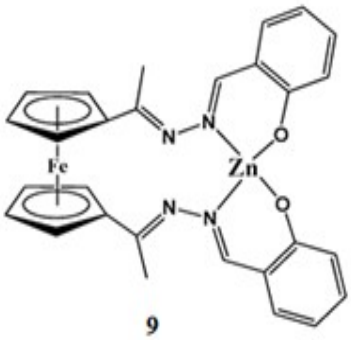
6 7

Ong and its co-worker produced ferrocenyl acridine **8**. As acridine ring is active DNA intercalator, overview of ferrocene improved its intercalation capability. *In vitro* broadcast of **8** maintained its great toxicity against cancer cell lines [65].



8

Abd –Elzaher et al. produced the complexes of Ni(II), Co(II), Cu(II) and Zn(II) along ferrocenyl ligand and studied their toxicity against human cancer cell line[66]. These metal complexes presented remarkable anticancer activity nearly similar to that of cisplatin.



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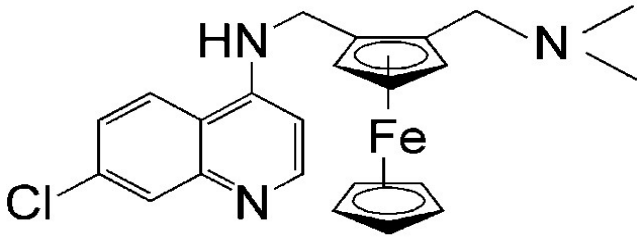
9.2 Anti-malarial agent

Malaria is a lethal illness produced by one of the organism that is transferred to fit one. It is stated by WHO, nearly 207 million malarial cases were informed in 2012 with 627000 expiries. The familiar drugs used against malarial cure are chloroquine, quinine, and qefloquine [67]. Potent to antimalarial cures is a common issue, for this purpose many researchers tried for the better amendment of medicines. These struggles leads to the production of ferrocenyl derivatives of such kinds of drugs called ferroquine [68-70]. These ferroquines presented in vitro activity beside Plasmodium falciparum [71].



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9.3. Non biological applications of ferrocene and its derivatives

Ferrocenyl derivatives display various non-biological applications because of great air, photochemical and thermal steadiness [72].

10. DNA binding studies

In recent research work drug -DNA study is one of the mainly focused areas [73]. Electrochemical and spectroscopic approaches are extensively used reliable approaches to explore the extent of interface of with DNA [74-76].

10.1. Structure of DNA

DNA is a double helical structure having two strands moving in contrary directions. It is a polymeric in nature made up of a monomer known as nucleotides. Each nucleotide comprises of three component of nitrogenous base, deoxyribose sugar and phosphate group. Due to different of nitrogenous bases there are four kinds of nucleotides.

- Adenine (A)
- Cytosine (C)
- Guanine (G)
- Thymine (T)

DNA is genetic material for all living creatures, as it contains complete genetic data.

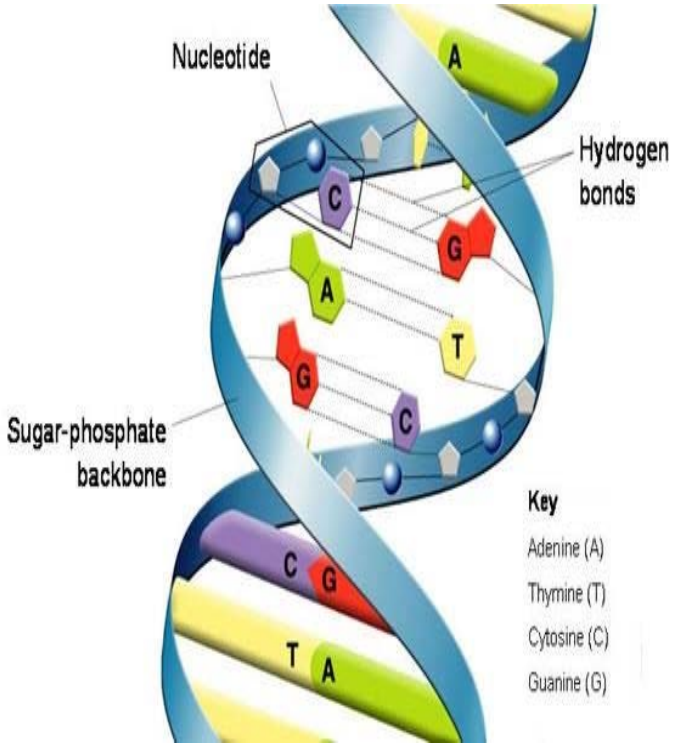


Figure 6: Structure of DNA.

10.2. Drug DNA interaction

Compounds displaying anticancer activity interact with DNA via covalent or non-covalent mode of interaction. That is why compound having non-covalent interaction is favored drug candidate [77, 78]. Drug DNA interaction is divided into three types as given.

10.3. (a) Intercalation

All those Molecules exhibit reversible inclusion between the base pairs DNA known as intercalation. Such type of interactions mostly adopted by those molecules which

are planner in nature as well as small in size. Intercalation delays the activity of DNA by bringing some conformational changes in its double helical structure [79].

10.4. (b) Electrostatic interaction

When positively charged compounds show interaction with negatively charged phosphate group of DNA is known as electrostatic interaction. Electrostatic interactions defend the steady DNA activity, causing in cell death.

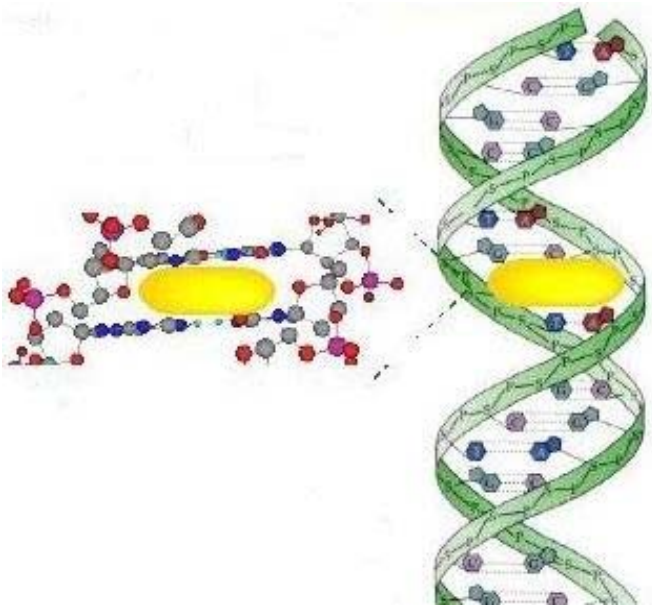


Figure 7: Intercalation between DNA base pairs.

Figure 8: Drug-DNA electrostatic interaction.

10.5. (c) Groove binding

Another type of Binding of compounds with DNA can take place through major or minor grooves called groove binding. The credit of Groove binding mainly goes to the particular structure of drug. Because such type of interaction depends upon the structure and shape of drug. All those drugs show compatibility with grooves can bind through van der Waals interactions. Large size molecules like proteins and small size molecules select bind major grooves and minor grooves of DNA respectively.

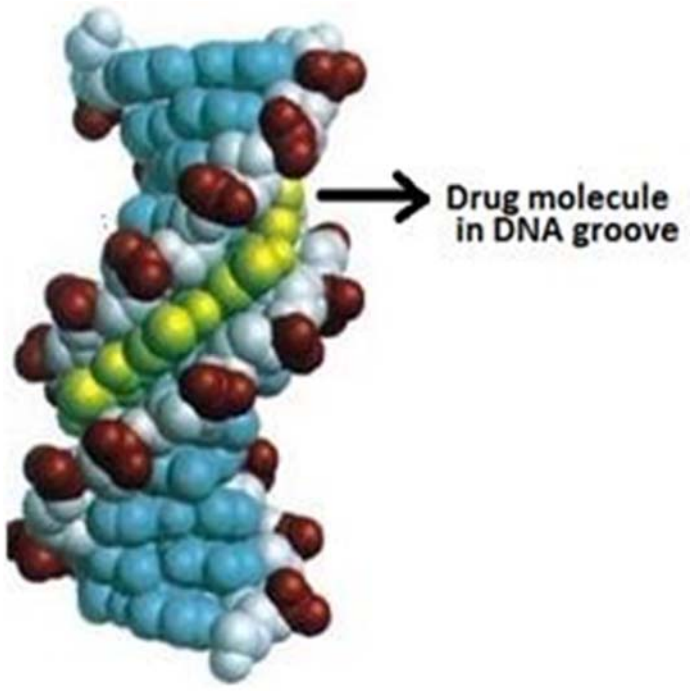


Figure 9: Drug molecule in DNA groove.

References:

1. Lee, I.-M., et al., Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. The lancet, 2012. 380(9838): p. 219-229.
2. Cooper, G.M., Elements of human cancer. 1992: Jones & Bartlett Learning.
3. Evans, C.W., The metastatic cell. 1991: Chapman a. Hall London etc.
4. Miller, B., Cancer: We Can Win The War Against Cancer By Aggresively pursuing Prevention. 2005: Oak Publication Sdn Bhd.
5. Bignold, L., Initiation of genetic instability and tumour formation: a review and hypothesis of a nongenotoxic mechanism. Cellular and Molecular Life Sciences CMLS, 2003. 60(6): p. 1107-1117.
6. Hartwell, L.H. and M.B. Kastan, Cell cycle control and cancer. Science, 1994. 266(5192): p. 1821-1828.
7. Trevor, A.J., B.G. Katzung, and S.B. Masters, Basic & clinical pharmacology. 2009: McGraw-Hill Medical.
8. Li, C., et al., Identification of pancreatic cancer stem cells. Cancer research, 2007. 67(3): p. 1030-1037.
9. Goyns, M.H., Cancer and you: how to stack the odds in your favour. 1999: CRC Press.
10. Li, Z., et al., Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. Journal of Biological Chemistry, 2009. 284(23): p. 15676-15684.
11. Trotti, A., et al. CTCAE v3. 0: development of a comprehensive grading system for the adverse effects of cancer treatment. in Seminars in radiation oncology. 2003. Elsevier.
12. Vanneman, M. and G. Dranoff, Combining immunotherapy and targeted therapies in cancer treatment. Nature Reviews Cancer, 2012. 12(4): p. 237-251.
13. Yarbro, C.H., D. Wujcik, and B.H. Gobel, Cancer nursing: Principles and practice. 2010: Jones & Bartlett Publishers.
14. Sangi, M., et al., Applying a Novel Combination of Techniques to Develop a Predictive Model for Diabetes Complications. 2015.

15. Esteller, M., et al., Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. New England Journal of Medicine, 2000. 343(19): p. 1350-1354.
16. Saffhill, R., G.P. Margison, and P.J. O'Connor, Mechanisms of carcinogenesis induced by alkylating agents. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1985. 823(2): p. 111-145.
17. Lindahl, T., et al., Regulation and expression of the adaptive response to alkylating agents. Annual review of biochemistry, 1988. 57(1): p. 133-157.
18. Arcangelo, V.P. and A.M. Peterson, Pharmacotherapeutics for advanced practice: a practical approach. Vol. 536. 2006: Lippincott Williams & Wilkins.
19. Pasut, G. and F.M. Veronese, PEG conjugates in clinical development or use as anticancer agents: an overview. Advanced drug delivery reviews, 2009. 61(13): p. 1177-1188.
20. Sharma, S.V., D.A. Haber, and J. Settleman, Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nature Reviews Cancer, 2010. 10(4): p. 241-253.
21. Drwal, M.N., et al., Exploring DNA topoisomerase I ligand space in search of novel anticancer agents. PloS one, 2011. 6(9): p. 1-12.
22. Cragg, G.M., D.G. Kingston, and D.J. Newman, Anticancer agents from natural products. 2011: CRC press.
23. Eastman, A., Activation of programmed cell death by anticancer agents: cisplatin as a model system. Cancer cells (Cold Spring Harbor, NY: 1989), 1989. 2(8-9): p. 275-280.
24. Miyabe, H. and Y. Takemoto, Discovery and application of asymmetric reaction by multi-functional thioureas. Bulletin of the Chemical Society of Japan, 2008. 81(7): p. 785-795.
25. Aly, A.A., et al., Update survey on aroyl substituted thioureas and their applications. Journal of Sulfur Chemistry, 2007. 28(1): p. 73-93.
26. Yang, G., Z. Chen, and H. Zhang, Clean synthesis of an array of N-benzoyl–N′-aryl ureas using polymer-supported reagents. Green Chemistry, 2003. 5(4): p. 441-442.
27. Wei, T.B., et al., Microwave promoted efficient synthesis of N-aryl-N′-aroyl thioureas under solvent-free and phase transfer catalysis conditions. Synthetic communications, 2004. 34(1): p. 181-186.

28. Xue, S.J., et al., Ultrasonic Irradiated Synthesis of N-(5-aryl-2-furoyl) thiourea Derivatives Containing Substituted Pyrimidine Ring under Phase Transfer Catalysis. Journal of the Chinese chemical society, 2004. 51(5A): p. 1013-1018.
29. Zhang, Y.-M., et al., An efficient synthesis of polymethylene-bis-aroyl thiourea derivatives under the condition of phase-transfer catalysis. Phosphorus, Sulfur, and Silicon, 2004. 179(10): p. 2007-2013.
30. Huang, Y.-B., W.-B. Yi, and C. Cai, Thiourea based fluorous organocatalyst, in Fluorous Chemistry. 2012, Springer. p. 191-212.
31. Ke, S.-Y. and S.-J. Xue, Synthesis and herbicidal activity of N-(o-fluorophenoxyacetyl) thioureas derivatives and related fused heterocyclic compounds. Arkivoc, 2006. 10: p. 63-68.
32. Wang, F., Z. Qin, and Q. Huang, Synthesis and fungicidal activity of 1, 3, 4-oxadiazole substituted acylthioureas. Frontiers of Chemistry in China, 2006. 1(1): p. 112-114.
33. Sun, C., et al., Synthesis and evaluation of a new series of substituted acyl (thio) urea and thiadiazolo [2, 3-a] pyrimidine derivatives as potent inhibitors of influenza virus neuraminidase. Bioorganic & medicinal chemistry, 2006. 14(24): p. 8574-8581.
34. Sun, J., et al., Molecular Docking and QSAR Studies on Substituted Acyl (thio) urea and Thiadiazolo [2, 3-α] Pyrimidine Derivatives as Potent Inhibitors of Influenza Virus Neuraminidase. Chemical biology & drug design, 2010. 76(3): p. 245-254.
35. Xiao, L., C.-J. Liu, and Y.-P. Li, Ultrasound promoted synthesis of bis (substituted pyrazol-4-ylcarbonyl)-substituted thioureas. Molecules, 2009. 14(4): p. 1423-1428.
36. Hu, J.-H., et al., Biological activities studies and phase transfer catalysts promoting the one-pot synthesis of N-Aryl-N′-(4-ethyloxy benzoyl)-thiourea derivatives. Phosphorus, Sulfur, and Silicon, 2006. 181(12): p. 2691-2698.
37. Zhong, Z., et al., Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities in vitro. Carbohydrate Research, 2008. 343(3): p. 566-570.

38. Claridge, S., et al., Discovery of a novel and potent series of thieno [3, 2-b] pyridine-based inhibitors of c-Met and VEGFR2 tyrosine kinases. Bioorganic & medicinal chemistry letters, 2008. 18(9): p. 2793-2798.
39. Furuta, T., et al., Identification of potent and selective inhibitors of PDGF receptor autophosphorylation. Journal of medicinal chemistry, 2006. 49(7): p. 2186-2192.
40. Ranise, A., et al., Synthesis of N-substituted-N-acylthioureas of 4-substituted piperazines endowed with local anaesthetic, antihyperlipidemic, antiproliferative activities and antiarrythmic, analgesic, antiaggregating actions. Il Farmaco, 2003. 58(9): p. 765-780.
41. Peng, H., et al., Efficient synthesis and biological evaluation of 1, 3-benzenedicarbonyl dithioureas. Bioorganic & medicinal chemistry letters, 2011. 21(4): p. 1102-1104.
42. Garoufis, A., S. Hadjikakou, and N. Hadjiliadis, Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents. Coordination Chemistry Reviews, 2009. 253(9): p. 1384-1397.
43. Bott, R.C., et al., Crystal Structure of [Cu4 (tu) 7](SO4) 2]⊙ H2O and Vibrational Spectroscopic Studies of Some Copper (I) Thiourea Complexes. Inorganic chemistry, 1998. 37(4): p. 651-657.
44. Golovnev, N., et al., Formation of bismuth (III) N-methylthiourea complexes in aqueous solutions. Russian Journal of Inorganic Chemistry, 2012. 57(4): p. 596-600.
45. Mlcouskova, J., et al., DNA conformation and repair of polymeric natural DNA damaged by antitumor azolato-bridged dinuclear Pt II complex. Journal of inorganic biochemistry, 2012. 114: p. 15-23.
46. Konig, K.-H., et al., Process for separation and purification of platinum group metals (II). 1986, Google Patents.
47. Schuster, M., et al., Substituted thioureas for the separation of complexly bound heavy-metal ions. 1990, Google Patents.
48. Lowe, S.W., et al., p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell, 1993. 74(6): p. 957-967.
49. Koketsu, M., C. Kobayashi, and H. Ishihara, Synthesis of N-aryl S-alkylthiocarbamates. Heteroatom Chemistry, 2003. 14(4): p. 374-378.

50. Zeng, R.S., et al., Synthesis of l-Benzoyl-3-aryl-4-hydroxy-4-phenylimidazolidin-2-thiones. Chinese Journal of Chemistry, 2003. 21(12): p. 1652-1654.
51. Saavedra, O., et al., N 3-Arylmalonamides: A new series of thieno [3, 2-b] pyridine based inhibitors of c-Met and VEGFR2 tyrosine kinases. Bioorganic & medicinal chemistry letters, 2009. 19(24): p. 6836-6839.
52. Khachatryan, V., et al., Prompt and non-prompt J/ψ production in pp collisions at\ sqrt {s}= 7 TeV. The European Physical Journal C, 2011. 71(3): p. 1-26.
53. Yuan, C., H. Zhai, and H. Liu, Angular multiplexing in pulsed digital holography for aperture synthesis. Optics letters, 2008. 33(20): p. 2356-2358.
54. Vasantha, B., H.P. Hemantha, and V.V. Sureshbabu, 1-Propanephosphonic Acid CyclicAnhydride (T3P) as an Efficient Promoter for the Lossen Rearrangement: Application to the Synthesis of Urea and Carbamate Derivatives. Synthesis, 2010. 2010(17): p. 2990-2996.
55. Hemantha, H., et al., One-Pot Synthesis of UreidoPeptides and Urea-Tethered Glycosylated Amino Acids EmployingDeoxo-Fluor and TMSN3. Synlett, 2009. 2009(03): p. 407-410.
56. Han, C. and J.A. Porco Jr, Synthesis of carbamates and ureas using Zr (IV)-catalyzed exchange processes. Organic letters, 2007. 9(8): p. 1517-1520.
57. Lee, H.-G., et al., Phenyl 4, 5-DiehIoro-6-Oxopyridazine-1 (6H)-Carboxylate as Carbonyl Source: Facile and Selective Synthesis of Carbamates and Ureas under Mild Conditions. Synlett, 2009(17): p. 2809-2814.
58. Wang, S., et al., Kinetics and mechanism of the reaction between thiourea and iodate in unbuffered medium. Science in China Series B: Chemistry, 2004. 47(6): p. 480-487.
59. Simoyi, R.H. and I.R. Epstein, Systematic design of chemical oscillators. 40. Oxidation of thiourea by aqueous bromine: autocatalysis by bromide. Journal of Physical Chemistry, 1987. 91(19): p. 5124-5128.
60. James, J.P., G.B. Quistad, and J.E. Casida, Ethylenethiourea S-oxidation products: preparation, degradation, and reaction with proteins. Journal of Agricultural and Food Chemistry, 1995. 43(9): p. 2530-2535.
61. Sahu, S., et al., Oxidation of thiourea and substituted thioureas: a review. Journal of Sulfur Chemistry, 2011. 32(2): p. 171-197.

62. Chirizzi, D. and C. Malitesta, Potentiometric urea biosensor based on urease immobilized by an electrosynthesized poly (o-phenylenediamine) film with buffering capability. Sensors and Actuators B: Chemical, 2011. 157(1): p. 211-215.
63. Hill, J. and J. Leaver, Energy and protein supplementation of lactating dairy cows offered urea treated whole-crop wheat as the sole forage. Animal feed science and technology, 1999. 82(3): p. 177-193.
64. Rochette, P., et al., Ammonia volatilization following surface application of urea to tilled and no-till soils: A laboratory comparison. Soil and Tillage Research, 2009. 103(2): p. 310-315.
65. Azizullah, A., et al., Evaluation of the adverse effects of two commonly used fertilizers, DAP and urea, on motility and orientation of the green flagellate Euglena gracilis. Environmental and Experimental Botany, 2011. 74: p. 140-150.
66. Crowe, G.A. and C.C. Lynch, Polarographic Urea--Formaldehyde Kinetic Studies. Journal of the American Chemical Society, 1949. 71(11): p. 3731-3733.
67. Wenzel, A.G. and E.N. Jacobsen, Asymmetric catalytic Mannich reactions catalyzed by urea derivatives: enantioselective synthesis of β-aryl-β-amino acids. Journal of the American Chemical Society, 2002. 124(44): p. 12964-12965.
68. Castellano, R.K. and J. Rebek, Formation of discrete, functional assemblies and informational polymers through the hydrogen-bonding preferences of calixarene aryl and sulfonyl tetraureas. Journal of the American Chemical Society, 1998. 120(15): p. 3657-3663.
69. Nguyen, T.D., et al., Application of urea-based SNCR to a municipal incinerator: On-site test and CFD simulation. Chemical Engineering Journal, 2009. 152(1): p. 36-43.
70. de Oliveira Rangel-Yagui, C., et al., Chlorophyll production from Spirulina platensis: cultivation with urea addition by fed-batch process. Bioresource technology, 2004. 92(2): p. 133-141.
71. Martin, T. and W. Jiang, Anti-Cancer agents in medicinal chemistry (Formerly current medicinal chemistry-Anti-cancer agents). Anti-cancer agents in medicinal chemistry, 2010. 10(1): p. 1.

72. Palani, A., et al., Biaryl ureas as potent and orally efficacious melanin concentrating hormone receptor 1 antagonists for the treatment of obesity. Journal of medicinal chemistry, 2005. 48(15): p. 4746-4749.
73. Singh, M., et al., Urea biosensors. Sensors and Actuators B: Chemical, 2008. 134(1): p. 345-351.
74. Johansen, M. and H. Bundgaard, Pro-drugs as drug delivery systems xiii. Kinetics of decomposition of n-mannich bases of salicylamide and assessment of their suitability as possible pro-drugs for amines. International Journal of Pharmaceutics, 1980. 7(2): p. 119-127.
75. Leuner, C. and J. Dressman, Improving drug solubility for oral delivery using solid dispersions. European journal of Pharmaceutics and Biopharmaceutics, 2000. 50(1): p. 47-60.
76. Gnewuch, C.T. and G. Sosnovsky, A critical appraisal of the evolution of N-nitrosoureas as anticancer drugs. Chemical reviews, 1997. 97(3): p. 829-1014.
77. Okada, H., T. Koyanagi, and N. Yamada, Synthesis and antitumor activities of prodrugs of benzoylphenylureas. Chemical & pharmaceutical bulletin, 1994. 42(1): p. 57-61.
78. Toth, J.E., et al., Sulfonimidamide Analogs of Oncolytic Sulfonylureas, 1. Journal of medicinal chemistry, 1997. 40(6): p. 1018-1025.
79. Liu, L.F., DNA topoisomerase poisons as antitumor drugs. Annual review of biochemistry, 1989. 58(1): p. 351-375.

CHAPTER 2 EXPERIMENTAL

2.1. Materials:

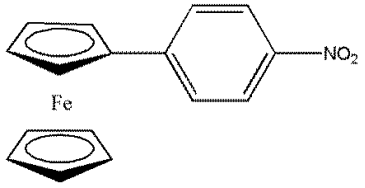
Ferrocene, sodium nitrite ($NaNO_2$), hydrochloric acid (HCl), diethyl ether, p-nitroanaline, distilled water, PTC (trimethylhexadecyl ammonium chloride), ether, Pd-charcoal (10%), hydrazine, carbon disulfide (CS_2), DMSO, methanol, metal salts (Au ,Ni , Co, Cu, Ru, Pt., Mo), were obtain from Sigma-Aldrich. Before using all the solvent are purified and dries according to the reported method [1].

2.2. Instrumentation:

1. **NMR**
2. **FT-IR spectrometer**
3. **Ultraviolet-visible spectrophotometer**
4. **Cyclovoltameter**
5. **Melting point apparatus**

2.3. Synthesis:

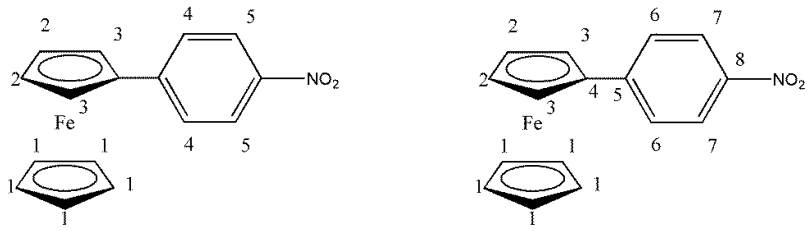
2.3.1. Synthesis of p-Nitrophenylferrocene



20ml water and 20 ml HCl are mixed together, 4-Nitroanaline (14.5g, 100mmol) was added slowly with regular stirring along with 30 ml extra HCl was added and cooled to 0-5^{0}C after complete addition. A solution of sodium nitrite (8g, 100mmol) was added drop wise in previous solution. After 30 mins of complete addition along with stirring at <5˚C, diazonium salt solution is formed [2]. Ferrocene (9.5g, 100mmol) and 0.1g hexadecyltrimethylammonium chloride (PTC) are added in 300ml of diethyl ether and cooled to 0-5^{0}C. The above prepared diazonium salt solution is added drop wise with stirring. After the complete addition, the solution is stirred for additional 24hours at room temperature. The mixture is concentrated by rotary evaporation and the product is extracted by pet-ether. The product is re-extracted

twice with pet ether and after the dryness of the filtrate, black yellow needle like crystal of 4-nitrophenylferrocene is formed with yield of approx. 73% and having melting point of 162˚C.

FT-IR (υ cm^{-1}): Fe-Cp (487), NO-asym (1555) and NO-sym (1528), sp^2 CH (3082), C=C Ar (1427-15760), Para-disub benzene (820)



^{1}H NMR **^{13}C NMR**

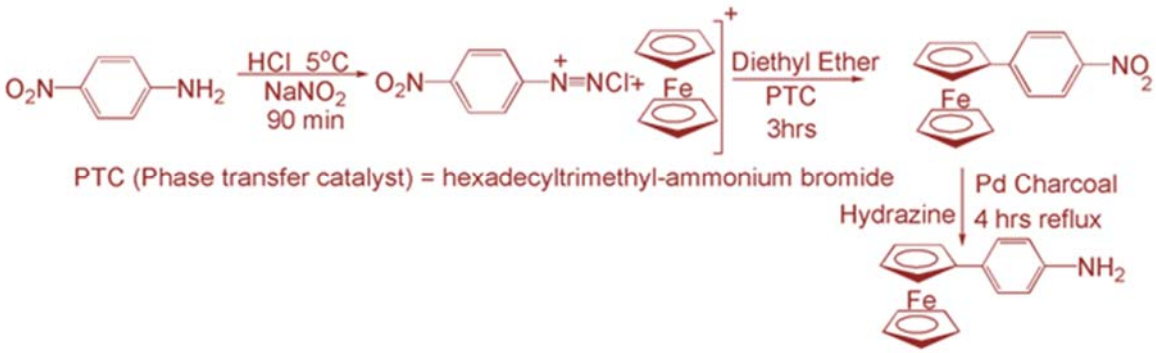
^{1}H NMR (300 MHz, DMSO ppm): δ 4.3 (s, 5H C_5H_4), 4.61 (d, 2H, C_5H_4), 4.25 (t, 2H, C_5H_4), 7.6 (d, 2H, *J* =6.3Hz, C_6H_4), 7.4 (d, 2H, *J* =7.5, C_6H_4),

^{13}C NMR (75 MHz, DMSO ppm): δ 66.8 (^{1}C), 69.4 (^{2}C), 63.52 (^{3}C), 80.6 (^{4}C), 125.4 (^{5}C), 114.5(^{6}C), 116.34 (^{7}C), 145.5 (^{8}C) ppm.

Elemental analysis Cal. (%): C, 62.54; H, 5.21; N, 4.56. Found (%) C, 62.59; H,5.34; N, 4.38.

Then reduction of 4-nitrophenylferrocen started for the formation of 4-ferrocenylaniline

2.3.2. Synthesis of 4-ferrocenylaniline:



Scheme 1: Synthesis of 4-ferrocenylaniline.

The reduction of 4-nitrophenylferrocen with 10% hydrazine and 10% Pd-charcoal form 4- ferrocenylaniline. The product of 4-nitrophenylferrocen dissolved in 200ml of methanol solvent is stirred and 0.3g palladium charcoal is added. Solution

was heated under reflux and 30ml hydrazine is added slowly drop wise when the solution become yellowish within 4-6hrs. Solution was filtered in hot condition and the solvent is removed by rotary evaporation and residue was washed with n-hexane and crude product is recrystallize by acetone **Yield** 78%, **MP,** (145˚C)

FT-IR (υ cm^{-1}): Fe-Cp (494), C=C (3025), sp^2 C-H (1528), NH (3439 cm^{-1}) P-disub benzene (883 cm^{-1})

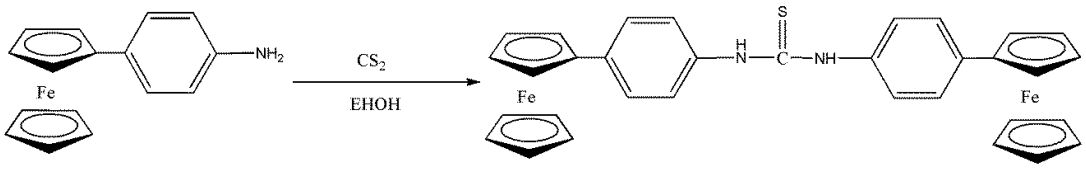
^{1}H NMR (300 MHz, DMSO ppm): δ 4.02 (s, 5H C_5H_4), 4.21 (d, 2H, C_5H_4), 4.51(t, 2H, C_5H_4), 6.32 (d, 2H, *J* =6.3Hz, C_6H_4), 7.27 (d, 2H, *J* =7.8, C_6H_4), NH (4.033).

^{13}C NMR (75 MHz, DMSO ppm): δ 69.3 (^{1}C), 67.8(^{2}C), 65.3(^{3}C), 87.3(^{4}C), 114.0(^{6}C), 126.79(^{7}C), 146.79(^{8}C),

Elemental analysis Calculated for C16H13FeNH2:

C, 69.31; H, 5.41; N, 5.05. **Found (%):** C, 68.68; H, 5.31; N, 4.90.

2.3.3. Synthesis of 1,1`(4,4`-diferrocenyl)diphenyl thiourea:



Scheme 2: Synthesis of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea.

Acetonic solution of 4- ferrocenylaniline was added drop wise to calculated amount of CS_2 (which is taken in 1:2 to aniline here aniline is taken 0.8g so 0.17ml CS_2 is added) in ice bath (0-5^{0}C) and the reaction mixture is stirred overnight in room temperature and the extent of reaction is monitor by TLC. After completion of reaction the solvent was evaporated by rotary and recrystallize the product by acetone. A yellow product of thiourea will be formed **Yield 75%,** MP**;** 179.5 ˚C.

FT-IR (υ cm^{-1}): Fe-Cp (485) 2NH (sharp) (3436) (3355) C=S (1238) C=C Ar (1408-1528) sp^2 CH=C (3098) P-benzene (816)

^{1}H NMR (300 MHz, DMSO ppm): δ 4.12 (s, 5H C_5H_4), 4.31 (d, 2H, C_5H_4), 4.61(t, 2H, C_5H_4), 6.42 (d, 2H, J=6.3Hz, C_6H_4), 7.17 (d, 2H, J=7.8, C_6H_4), NH (9.784).

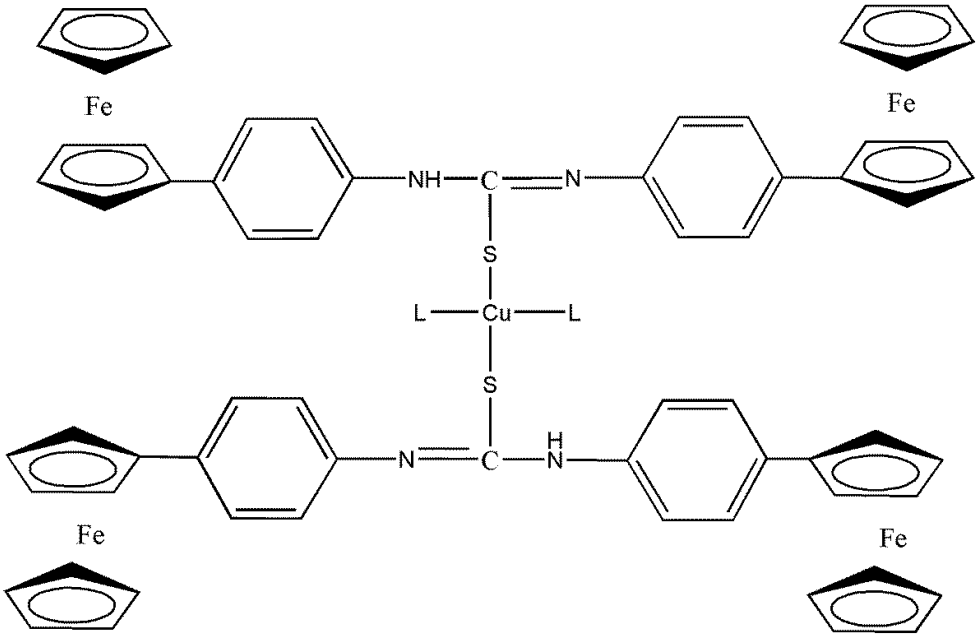
^{13}C NMR (75 MHz, DMSO ppm): δ 67.8 (^{1}C), 68.4 (^{2}C), 67.52 (^{3}C), 81.6 (^{4}C), 124.4(^{5}C), 116.5(^{6}C), 135.34 (^{7}C), 147.5 (^{8}C) C=S 179.39.

Elemental analysis Calcd (%):

C, 66.39; N, 4.68; H, 4.74. S, 5.34 **Found (%):** C, 66.81; N, 4.93; N, 4.98, S, 5.68

2.3.4. General synthesis of metal complexation:

Acetonic solution of synthesized thiourea is added to the acetonic solution of metal salts and the reaction mixture was stirred for 24 hour at room temperature and the extent of reaction was monitored on TLC. After completion of reaction solvent is removed by rotary evaporation. The crude product was recrystallized and purified by acetone to get ferrocene based bi metallic thiourea



M^{+1}, M^{+2}, M^{+3}

Scheme 3: General synthesis of metal complexation.

2.3.4.1.Copper complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: (Cu)

Copper acetate solution (0.016g, 0.19mmol) of 2:2 is added to the solution of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (0.1g, 0.43mmol) in acetone and methanol mixture use as a solvent at room temperature for 24h stirring. The improvement of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of copper. **Yield** 67% MP (230 ˚C) **FT-IR: (**υ cm^{-1}): Fe-Cp (485), NH (3372), sp^2 CH (2956), C=C Ar (1412-1527), para-disub.benzene (885), C=S (1103)

^{1}H NMR (300 MHz, DMSO ppm): δ 4.14 (s, 10H, C_5H_4), 4.39 (s, 4H, C_5H_4), 4.76 (s, 4H, C_5H_4), 7.74 (d, 2H, C_6H_4), 7.29 (d, 2H, *J* =7.5Hz, C_6H_4), 7.35 (2H *J* =7.5Hz, C_6H_4), 7.39 (d, 2H J=7.5 C_6H_4).

^{13}C NMR (75 MHz, DMSO ppm): δ 69.87 (^{1}C), 69.38(^{2}C), 66.53(^{3}C), 86.3(^{4}C), 134.0(^{6}C), 146.79(^{7}C), 176.79(^{8}C).

Elemental analysis Calcd (%) for Cu:

C, 59.71; H, 4.21; N, 4.19. S, 4.34 **Found (%):** C, 60.25; H, 4.63; N, 4.64. S, 4.56

2.3.4.2.Nickel complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: (Ni)

Nickel bromide solution (0.0184g, 0.19mmol) of 1:2 is added to the solution of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (0.1g, 0.43mmol) in acetone and methanol mixture use as a solvent at room temperature for 24h stirring. The improvement of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of nickel. **Yield** 65% MP (226 ˚C)

FT-IR: (υ cm^{-1}): Fe-Cp (482), NH (3304), sp^2 CH (2966), C=C Ar (1422-1537), para-disub.benzene (884), C=S (1108)

^{1}H NMR (300 MHz, DMSO ppm): δ 4.04 (s, 10H, C_5H_4), 4.38 (s, 4H, C_5H_4), 4.68 (s, 4H, C_5H_4), 7.7 (d, 2H, C_6H_4), 7.27 (d, 2H, *J* =7.5Hz, C_6H_4), 7.45 (2H *J* =7.5Hz, C_6H_4), 7.69 (d, 2H *J* =7.5 C_6H_4.

^{13}C NMR (75 MHz, DMSO ppm): δ 69.84 (^{1}C), 69.38(^{2}C), 66.63(^{3}C), 85.3(^{4}C), 131.0(^{6}C), 145.79(^{7}C), 175.49(^{8}C).

Elemental analysis Calcd (%) for Cu:

C, 66.39; H, 4.74; N, 4.68. S, 5.34 **Found (%):** C, 66.87; H, 5.34; N, 5.12. S, 5.75

2.3.4.3. Ruthenium complex of 1,1`(4,4`-diferrocenyl)diphenyl thiourea: (Ru)

Ruthenium(III) chloride solution (0.0156g, 0.19mmol) of 1:3 is added to the solution of 1,1`(4,4`-diferrocenyl)diphenyl thiourea (0.1g, 0.43mmol) in acetone and methanol mixture use as a solvent at room temperature for 24h stirring. The improvement of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of Ruthenium complex. **Yield** 63% MP (255 ˚C)

FT-IR: (υ cm^{-1}): Fe-Cp (490), NH (3092), sp^2 CH (2936), C=C Ar (1422- 1537), para-disub.benzene (881), C=S (1105)

Elemental analysis Calcd (%) for Ru:

C, 56.71; H, 3.21; N, 5.19. S, 4.34 **Found (%):** C, 57.25; H, 3.63; N, 5.64. S, 4.5

2.3.4.4.Manganese complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: (Mn)

Manganese(II) chloride solution (0.0166g, 0.19mmol) of 1:2 is added to the solution of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (0.1g, 0.43mmol) in acetone use as a solvent at room temperature for 24h stirring. The improvement of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of Manganese. **Yield** 70 % MP (237 ˚C)

FT-IR: (υ cm^{-1}): Fe-Cp (489), NH (3192), sp^2 CH (2946), C=C Ar (1412-1557), para-disub.benzene (885), C=S (1107)

Elemental analysis Calcd (%) for Cu:

C, 67.71; H, 4.41; N, 5.49. S, 4.14 **Found (%):** C, 67.25; H, 4.63; N, 5.64. S, 4.8

2.3.4.5.Cobalt complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: (Co)

Cobalt(II) chloride solution (0.01486g, 0.19mmol) of 1:2 is added to the solution of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (0.1g, 0.43mmol) in methanol use as a solvent at room temperature for 24h stirring. The improvement of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of Cobalt. **Yield** 71 % MP (227 ˚C)

FT-IR: (υ cm^{-1}): Fe-Cp (489), NH (3192cm^{-1}), sp^2 CH (2946cm^{-1}), C=C Ar (1412-1557 cm^{-1}), para-disub.benzene (885cm^{-1}), C=S (1107cm^{-1})

Elemental analysis Calcd (%) for Cu:

C, 65.71; H, 4.71; N, 5.59. S, 4.24 **Found (%):** C, 65.25; H, 4.63; N, 5.64. S, 4.8

2.3.4.6.Platinum complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: (Pt.)

Platinum(II) chloride solution (0.02005g, 0.19mmol) of 1:2 is added to the solution of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (0.1g, 0.43mmol) in methanol use as a solvent at room temperature for 24h stirring. The improvement of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent

are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of platinum. **Yield** 65 % MP (267 ˚C)

FT-IR: (υ cm^{-1}): Fe-Cp (491), NH (3182), sp^2 CH (2956), C=C Ar (1424-1547), para-disub.benzene (883), C=S (1103)

^{1}H NMR (300 MHz, DMSO ppm): δ 4.55 (s, 10H, C_5H_4), 4.57 (s, 4H, C_5H_4), 4.88 (s, 4H, C_5H_4), 7.65 (d, 2H, C_6H_4), 7.41 (d, 2H, *J* =7.4Hz, C_6H_4), 7.86 (2H *J* =7.87Hz, C_6H_4), 7.57 (d, 2H *J* =7.65 C_6H_4).

^{13}C NMR (75 MHz, DMSO ppm): δ 67.67 (^{1}C), 65.48(^{2}C), 65.93(^{3}C), 86.3(^{4}C), 135.0(^{6}C), 147.69(^{7}C), 175.65(^{8}C).

Elemental analysis Calcd (%) for Cu:

C, 64.71; H, 4.81; N, 5.89. S, 4.34 **Found (%):** C, 65.25; H, 4.63; N, 5.64. S, 4.86

2.3.4.7.Gold complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: (Au)

Chloroauric acid (HAuCl4) solution (0.0221g, 0.19mmol) of 1:3 is added to the solution of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (0.1g, 0.43mmol) in (methanol and DMSO mixture) use as a solvent at room temperature for 24h stirring. The extent of reaction was monitored by TLC. After a completion of reaction precipitate are formed the solvent are evaporated by rotary evaporation and the residue is washed 1st by water and then by n-hexane and will get a pure complex of Gold complex. **Yield** 74 % MP (269 ˚C)

FT-IR: (υ cm^{-1}): Fe-Cp (487), NH (3272), sp^2 CH (2986), C=C Ar (1416-1534), para-disub.benzene (878), C=S (1106)

^{1}H NMR (300 MHz, DMSO ppm): δ 4.55 (s, 10H, C_5H_4), 4.57 (s, 4H, C_5H_4), 4.88 (s, 4H, C_5H_4), 7.65 (d, 2H, C_6H_4), 7.41 (d, 2H, *J* =7.4Hz, C_6H_4), 7.86 (2H *J* =7.87Hz, C_6H_4), 7.57 (d, 2H *J* =7.65 C_6H_4).

^{13}C NMR (75 MHz, DMSO ppm): δ 65.67 (^{1}C), 63.48(^{2}C), 63.93(^{3}C), 87.3(^{4}C), 138.0(^{6}C), 144.69(^{7}C), 173.65(^{8}C).

Elemental analysis Calcd (%) for Cu: 67.71; H, 4.81; N, 5.79. S, 4.34

Found (%): C, 65.75; H, 4.83; N, 5.74. S, 4.66

2.4. Instrumentation:

CHNS analysis:

By Lecco CHNS-932 Lecco corporation USA CHNS analysis was carried out

Melting point:

By using capillary tube melting point was recorded on BIO COTE Model SMP10 melting point apparatus and reported without correction.

FT-IR spectroscopy:

For FT-IR spectra using KBr discs (4000-400) cm^{-1} on a Thermo Scientific Nicolet-6700 FT-IR USA

^{1}H & ^{13}C NMR spectroscopy:

All the synthesized compound ^{1}H & ^{13}C NMR spectra were obtained on BRUKER AVANCE 300 MHz NMR spectrometer using deuterated tetra methyl silane (TMSO) as an internal reference in deuterated acetone and DMSO-d_6 solvent working at frequencies of 300MHz and 75.47MHz respectively at 297 k. Chemical shift values are expressed in ppm. The signals for 1H splitting are given as; s = singlet, d = doublet, t = triplet, and m = multiplet

UV-visible spectrometry:

To record the UV-visible spectrometric measurements Pharmaspac UV-1800 UV-Visible Spectrophotometer Schimadzu was used at constant temperature of 298K. Samples were run with 1cm path length in quartz cell. The absorption spectra of known concentration of sample are taken then the same amount of sample with an addition of small amount of DNA aliquots is monitored. All the entire samples are taken for 5min earlier to every spectroscopic reading.

Cyclic voltammetry (CV)

Cyclic voltammetry of synthesized complexes was carried out on auto lab running with GPES 4.9 software, Eco-Chemie, Utrecht the Netherlands with three electrode cells at constant temperature of 25˚C with pH 7.

References

1 Armarego, W.L. and C.L. Chai, Purification of laboratory chemicals. Butterworth-Heinemann, Oxford (UK), 2003: p. 103-554.

2. Nadeem, S., et al., Synthesis, characterization and antibacterial activity of palladium (II) bromide complexes of thioamides; X-ray structure of [Pd (tetramethylthiourea) 4] Br2. Transition Metal Chemistry, 2010. **35**(5): p. 555-561.

3.0. Result and Discussion

3.1. P-nitrophenylferrocene

In FT-IR vibrational stretching frequency peak in p-nitrophenylferrocene for Fe-Cp has been observed at 487cm^{-1}. Another important peak for phenyl group that is (C=C) it 1427-1576 and (sp^2 C-H) appear it 3082cm^{-1}.Nitro group is also the structural component of compound having a stretching vibration peak at 1528cm^{-1},hence all the spectral peak satisfy the compound.

The unsubsitituted Cp ring gives a singlet peak at 4.3ppm in ^1H NMR spectrum. Where is substituted Cp ring the ortho an Mata proton are at δ 4.61 and δ 4.25 respectively that split into three peak one singlet which is due to 5 proton of unsubsitituted Cp ring and 2 peak of equal intensity for 2, 2 equal proton of substituted Cp ring on the formation of compound 1 of them give a pseudo triplet with *J*-value of 6.1Hz at δ 4.43 and 1 is doublet with δ 4.75ppm this splitting of one peak into 2 peak give the evidence of substitution of Cp ring of ferrocene. The phenyl ring proton attached to Cp ring of ferrocene give 2 doublet peak of equal intensity at δ 7.6 with *J*-value of 6.3Hz and δ 7.4ppm with J-value of 7.5Hz, This splitting pattern give evidence that two group are in Para position.

The ^{13}C NMR spectra signal in the region of δ 66.8-84.1 suggestive of monosubstituted ferrocene subunit. In the compound (1z) three other peak are detected along with peak of ferrocene region due to attachment of strong electron withdrawing group (NO_2). Carbon C-8 appear at most downfield. C-6, 7 appear at 114.5 and 116.34 ppm respectively due to effect of NO_2-group attached to adjacent carbon of NO_2 which is almost comparable due to NO_2 and Cp group. Due to direct attachment with Cp ring of ferrocene C-5 appear at downfield at 125.4.This spectral behavior is due to NO_2 explained on the basis of mesmeric effect due to attachment of electron withdrawing group to phenyl ring.

3.2. 4-Ferrocenylaniline:

FT-IR for 4-Ferrocenylaniline the vibrational stretching frequency peak of Fe-Cp has been detected at 494 cm^{-1}. For phenyl group the important peak that is (C=C, sp^2 C-H) appear at1528 cm^{-1} and 3025 cm^{-1} respectively. In the structural component of this compound contain amino group having stretching vibrational frequency at 3439cm^{-1}, hence satisfying the entire groups in compound.

The unsubsitituted C5H5 ring of ferrocene give a singlet in the ^{1}H NMR spectrum at δ 4.02ppm. Whereas the ortho and Meta proton on the substituted Cp ring give 1 doublet and 1 triplet of almost equal intensity of 2, 2 equal proton at δ 4.21 and δ 4.51 respectively. This splitting of signal in Cp ring gives the indication of compound formation. The phenyl ring attached Cp ring give two peak of equal intensity at δ 6.32 and δ 7.27ppm. These two peak further split in two doublets with a *J*-value of 7.8Hz for both. The NH_2 peak gives a singlet at δ 4.033ppm. This splitting pattern of phenyl ring gives evidence that the two groups are at Para position.

The ^{13}C NMR spectra signal in the region of δ 65.3-87.2 suggestive of monosubstituted ferrocene subunit. In the above compound four other peaks are detected along with peak of ferrocene region. Due to attachment of nitrogen (electronegativity effect) of (NH_2) Carbon C-8 appear at most downfield. C-6, 7 appear at 114.36 and 126.72 ppm respectively due to effect of NH_2-group attached to adjacent carbon of NH_2 (increasing the electron density). Due to direct attachment with Cp ring of ferrocene C-5 appear at downfield and C-6, 7 appear at downfield which is almost comparable due to NH_2 and Cp group. This spectral behavior is due to NH_2 explained on the basis of mesmeric effect due to attachment of electron withdrawing group to phenyl ring.

3.3. 1,1′-(4,4′-diferrocenyl)diphenyl thiourea(Tu):

The reaction of 4-Ferrocenylaniline react with Carbon disulfide (CS_2) in a fix molar ratio of 2:1 in presence of acetone use as a solvent it give a product Ferrocene incorporated N, N'-disubstituted thiourea.

In IR spectra of the ligand thiourea, the characteristic band were observed; υ(C=S) at 1238cm^{-1}, υ (N-H) for the secondary amine in this case give two sharp peak at 3436 and 3555cm^{-1}, υ(Para disubst.benzene) at 816cm^{-1}, υ(C-H) aromatic at 3098 cm^{-1}, υ(C=C) aromatic at 1408-1528 cm^{-1} and υ(Fe-Cp) at 485cm^{-1}. These indicate the formation of ferrocene assimilated thiourea. The shift in peak from initial intermediate is the conformation of required compound. In the ^{1}H NMR spectra, the indication peak is N-H that shifted from 4.033 to 9.784 is the conformation of ferrocene assimilated thiourea. A downfield shift in N-H resonance was observed by C-N bond.

In ^{13}C NMR spectra all other peak for ferrocene and benzene are present in there characteristic position while C=S peak was appeared at 179.39 ppm.

3.4. Copper complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea:

The Copper *(*II*)* acetate monohydrate (Cu $(CO_2CH_3)_2 \cdot H_2O$) reaction with ferrocene based thiourea (Fe-Tu) in a 2:2 molar ratio resulted in the product of copper complex having empirical formula *(*Cu_2 (L) $_2$). The IR spectra of free ligand (Tu) and *Copper* (*II*) complex are given

In the IR spectra all the peak are present in there characteristic regions with a slightly change. This slightly change in their region is the conformation of the complex formation υ (C=S) appear it 1103 cm^{-1} which show that a single bond character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one N-H is detected near 3372 cm^{-1} having broad peak and C=N peak appear at 1618 cm^{-1} which also conform the complex formation, Fe-Cp is observed close to 485 cm^{-1}. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand.

The ^{1}H and ^{13}C NMR chemical shift are clarified in table of copper complex in DMSO. In the ^{1}H NMR spectra the 1 NH peak in complex is disappeared it is due to the formation of complex Here the NH proton is removed C=N bond is formed and C=S convert to C-S and the ligand attached to metal through Sulfur. The ligand is symmetric, here on both side of C=S same moiety is present so there are equal chances of both side of NH and by formation of double bond in C=N there is become a conjugation in between C=N and NH so due to this the second NH proton become exchangeable which is not detected by NMR.

In ^{13}C NMR spectra the δ C=S resonance of the complex is shifted up field by about 2ppm as compared to free ligand. Due to π-character in C-N bond there is a small deshielding effect observed in other carbon atoms.

3.5. Nickel complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea: (Ni)

The Nickel bromide reaction with ferrocene based thiourea (Fe-Tu) in a 1:2 molar ratio resulted in the product of nickel complex having empirical formula *(*Ni (L) $_2$ Br_2). In the IR spectra all the peak are present in there representative regions with a slightly change. This slightly change in their region is the conformation of the complex formation υ (C=S) appear it 1103 cm^{-1} which show that a single bond

character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one

N-H is detected near 3371 cm^{-1} having broad peak and C=N peak appear at 1600 cm^{-1} which also conform the complex formation, Fe-Cp is observed close to 485 cm^{-1}. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand.

The ^{1}H and ^{13}C NMR chemical shift are clarified in table of Nickel complex in DMSO. In the ^{1}H NMR spectra the 1 NH peak in complex is disappeared it is due to the formation of complex. In ^{13}C NMR spectra the δ C=S resonance of the complex is shifted up field by about 2ppm as compared to free ligand. Due to double bond character in C-N bond there is a small deshielding effect observed in other carbon atoms.

3.6. Ru complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea: (Ru)

The Ruthenium (III) chloride reaction with ferrocene based thiourea (Fe-Tu) in a 1:3 molar ratio resulted in the product of Ruthenium complex having empirical formula *(*Ru (L) $_3$ Cl). In the IR spectra all the peak are present in there representative regions with a slightly change. This slightly change in their region is the conformation of the complex formation υ (C=S) appear it 1103 cm^{-1} which show that a single bond character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one

N-H is detected near 3092 cm^{-1} having broad peak and C=N peak appear at 1604 cm^{-1} which also conform the complex formation, Fe-Cp is observed close to 490 cm^{-1}. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand.

This is only conforming by IR and physical changes this complex is Para magnetic so not detected by NMR.

3.7. Co complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea: (Co)

The *Cobalt* (II) *chloride* reaction with ferrocene based thiourea (Fe-Tu) in a 1:2 molar ratio resulted in the product of *Cobalt* complex having empirical formula *(*Co (L) $_2$ Cl_2). In the IR spectra slightly shift in their υ (C=S) appear it 1107 cm^{-1} which show that a single bond character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one N-H is detected near 3192 cm^{-1} having broad peak and C=N peak appear at 1602 cm^{-1} which

also conform the complex formation. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand. This is only conforming by IR and physical changes this complex is Para magnetic so not detected by NMR.

3.8. Mn complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea: (Mn)

The Manganese *(II)* chloride reaction with ferrocene based thiourea (Fe-Tu) in a 1:2 molar ratio resulted in the product of manganese *complex* having empirical formula *(*Mn (L) $_2$ Cl_2). In the IR spectra slightly shift in their υ (C=S) appear it 1107 cm^{-1} which show that a single bond character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one N-H is detected near 3192 cm^{-1} having broad peak and C=N peak appear at 1605 cm^{-1} which also conform the complex formation. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand. This is only conforming by IR and physical changes this complex is Para magnetic so not detected by NMR.

3.9. Gold complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea: (Au)

The Chloroauric acid (HAuCl4) reaction with ferrocene based thiourea (Fe-Tu) in a 1:3 molar ratio resulted in the product of Gold complex having empirical formula *(*Au (L) $_3$ Cl). In the IR spectra all the peak are present in there representative regions with a slightly change. This slightly change in their region is the conformation of the complex formation υ (C=S) appear it 1106 cm^{-1} which show that a single bond character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one

N-H is detected near 3272 cm^{-1} having broad peak and C=N peak appear at 1602 cm^{-1} which also conform the complex formation, Fe-Cp is observed close to 487 cm^{-1}. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand.

The ^{1}H and ^{13}C NMR chemical shift are clarified in table of gold complex in DMSO. In the ^{1}H NMR spectra the 1 NH peak in complex is disappeared it is due to the formation of complex. In ^{13}C NMR spectra the δ C=S resonance of the complex is shifted up field by about 2ppm as compared to free ligand. Due to double bond character in C-N bond there is a small deshielding effect observed in other carbon atoms.

3.10. Platinum complex of 1, 1′-(4, 4′-diferrocenyl) diphenyl thiourea: (Pt)

The Platinum *(II)* chloride reaction with ferrocene based thiourea (Fe-Tu) in a 1:2 molar ratio resulted in the product of platinum complex having empirical formula *(*$Pt(L)_2Cl_2$). In the IR spectra all the peak are present in there representative regions with a slightly change. This slightly change in their region is the conformation of the complex formation υ (C=S) give peak it lower frequency which show that a single bond character is appear in CS bond compare to ligand which having completely double bond due to the formation of complex, and only one

N-H is detected near 3272 cm^{-1} having broad peak and C=N peak appear at 1602 cm^{-1} which also conform the complex formation, Fe-Cp is observed close to 487 cm^{-1}. A little frequency shift in υ (C=S) band and υ (NH) compared to free ligand.

The ^{1}H and ^{13}C NMR chemical shift are clarified in table of *Platinum* complex in DMSO. In the ^{1}H NMR spectra the 1 NH peak in complex is disappeared it is due to the formation of complex. In ^{13}C NMR spectra the δ C=S resonance of the complex is shifted up field by about 2ppm as compared to free ligand. Due to double bond character in C-N bond there is a small deshielding effect observed in other carbon atoms.

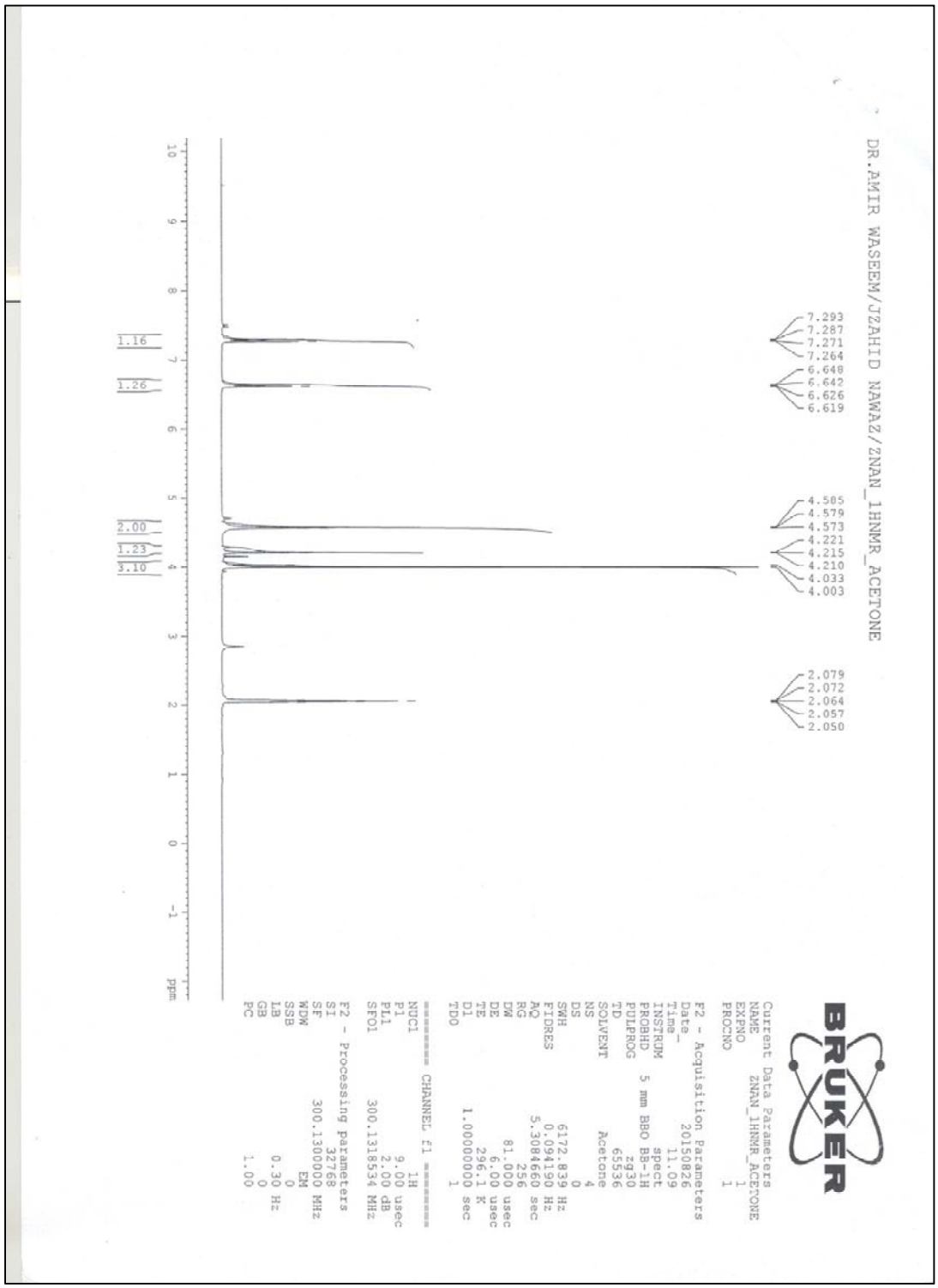


Figure 1: 1H NMR spectra of of 4-ferrocenylaniline.

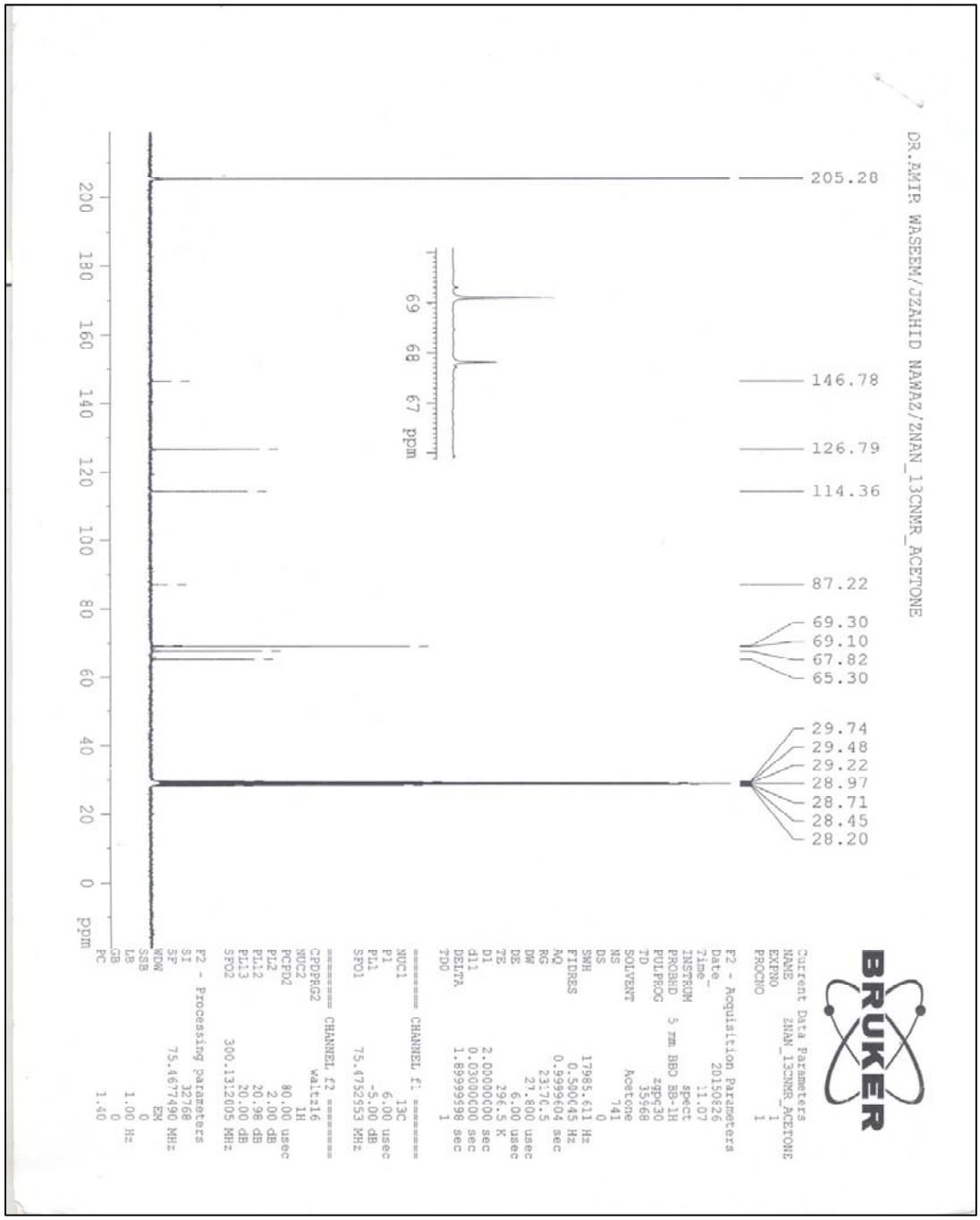


Figure 2: ^{13}C NMR spectra of of 4-ferrocenylaniline.

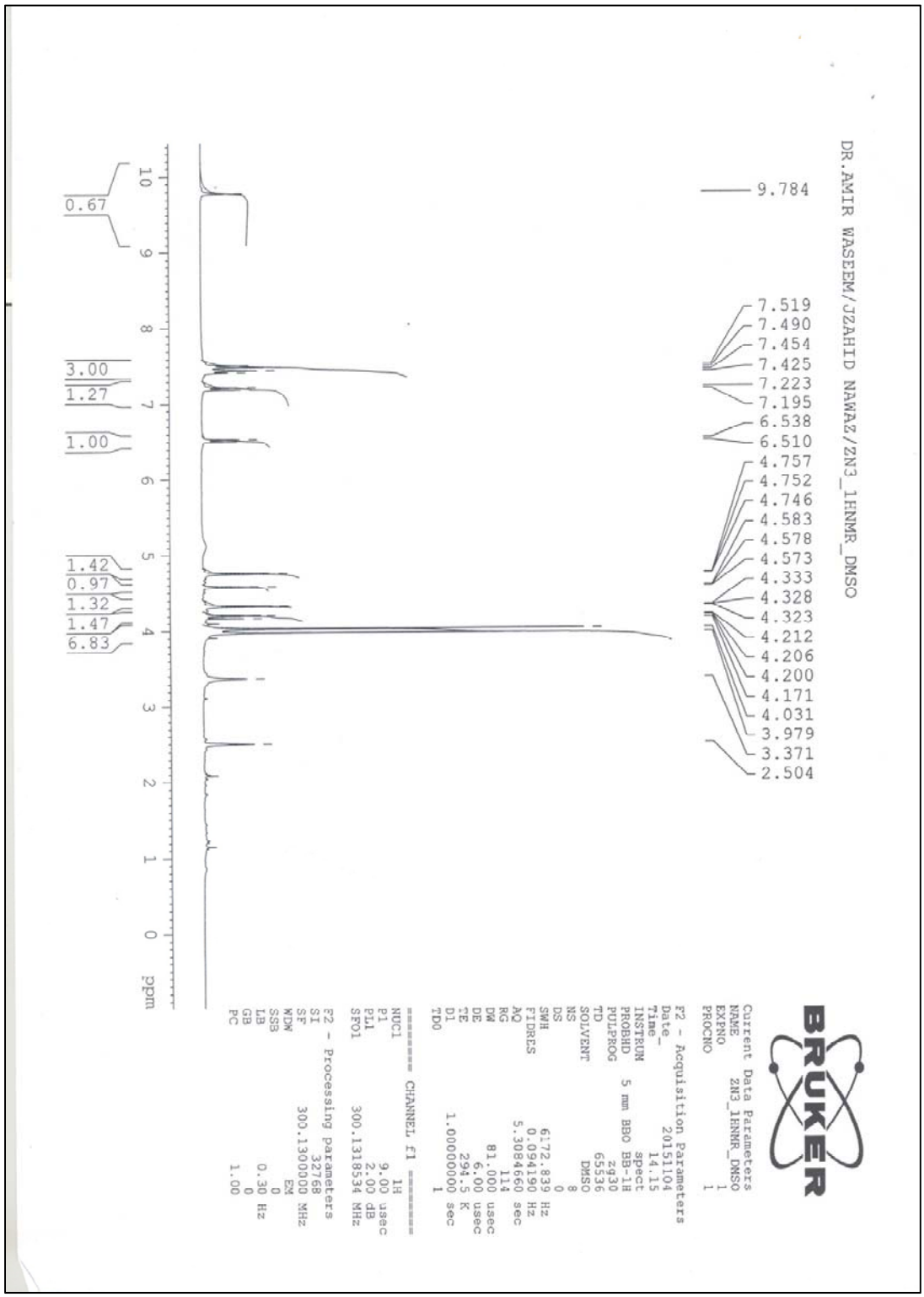


Figure 3: ^{1}H NMR spectra of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea:

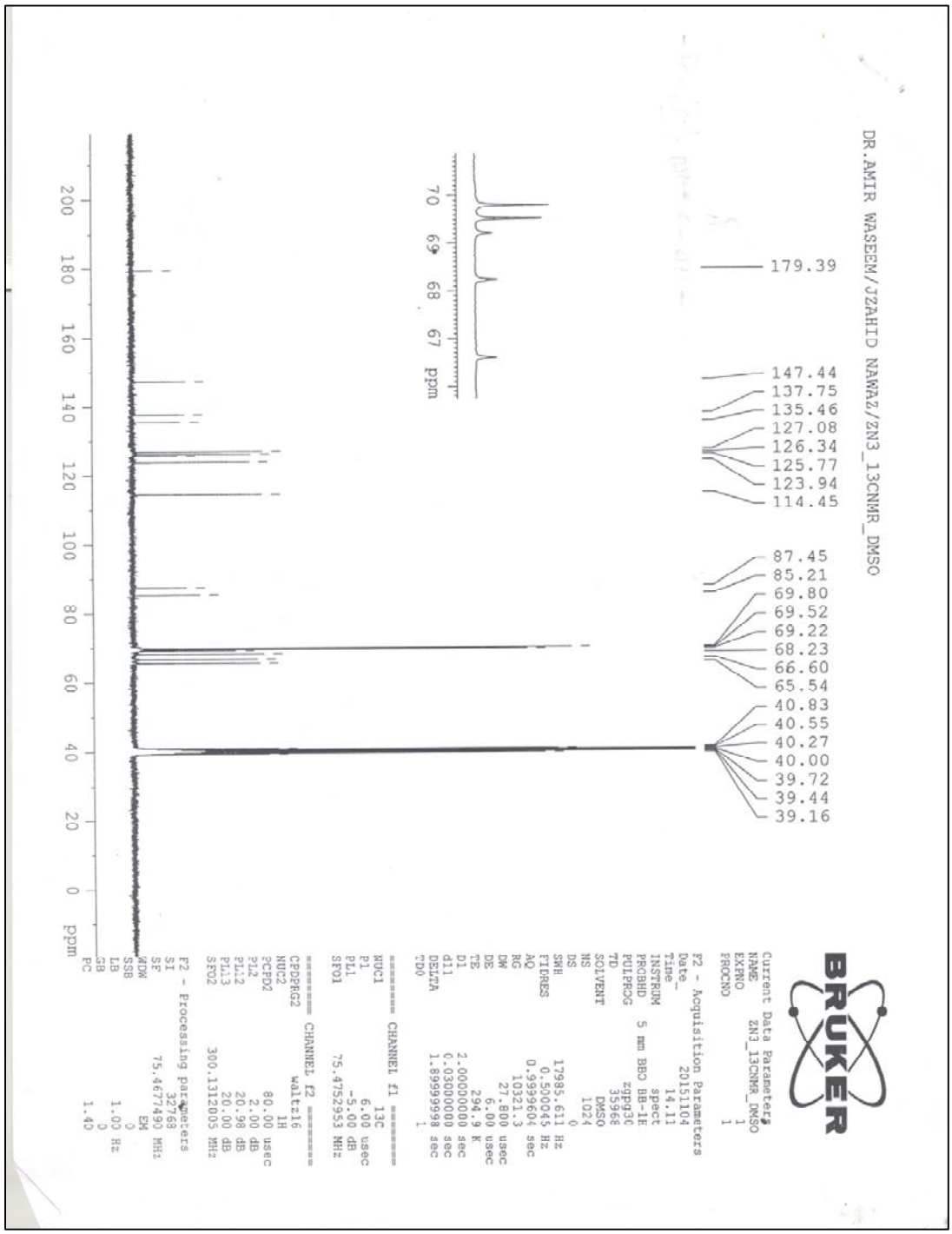


Figure 4: ^{13}C NMR spectra of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea (Tu).

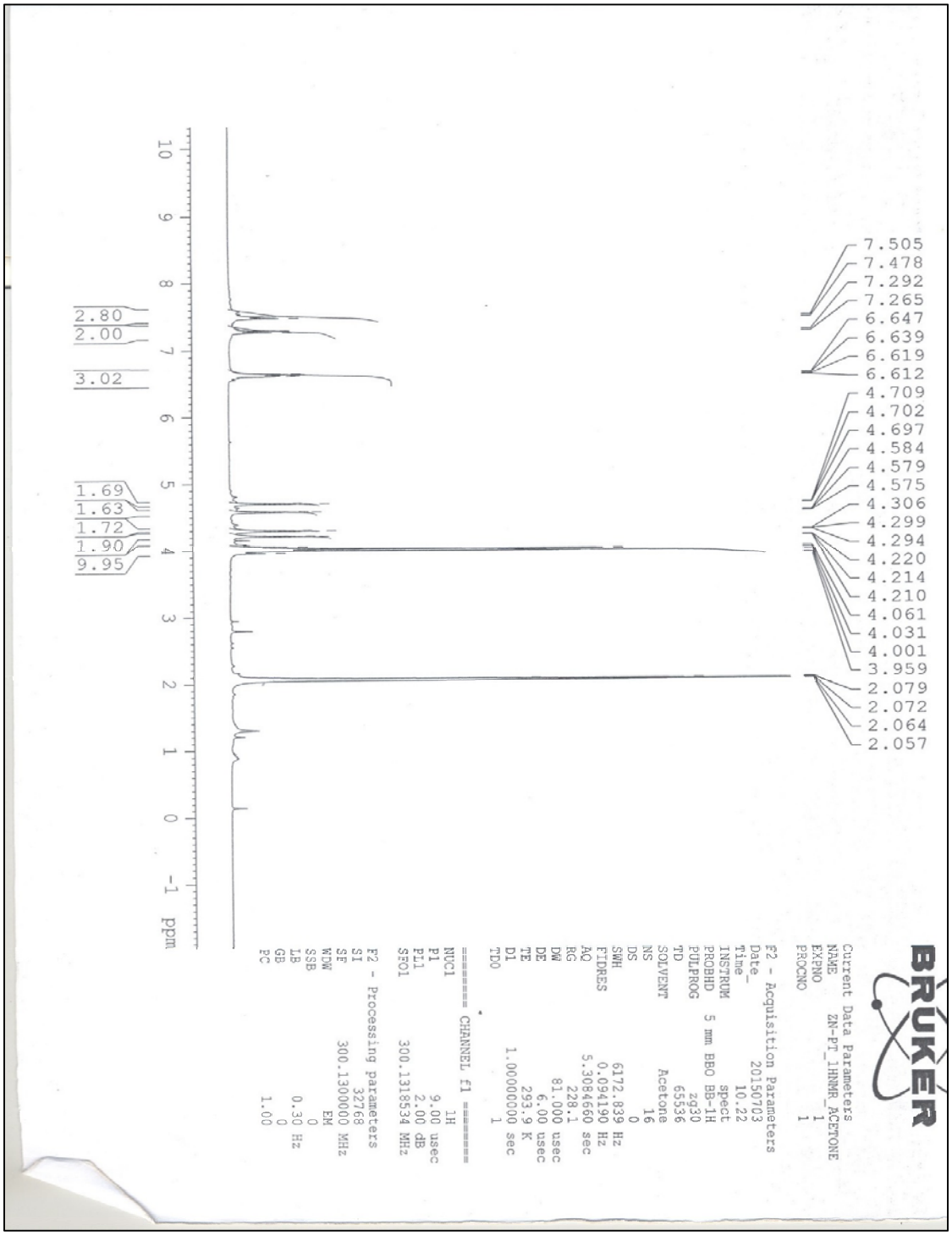


Figure 5: ^{1}H NMR spectra of Cu complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea

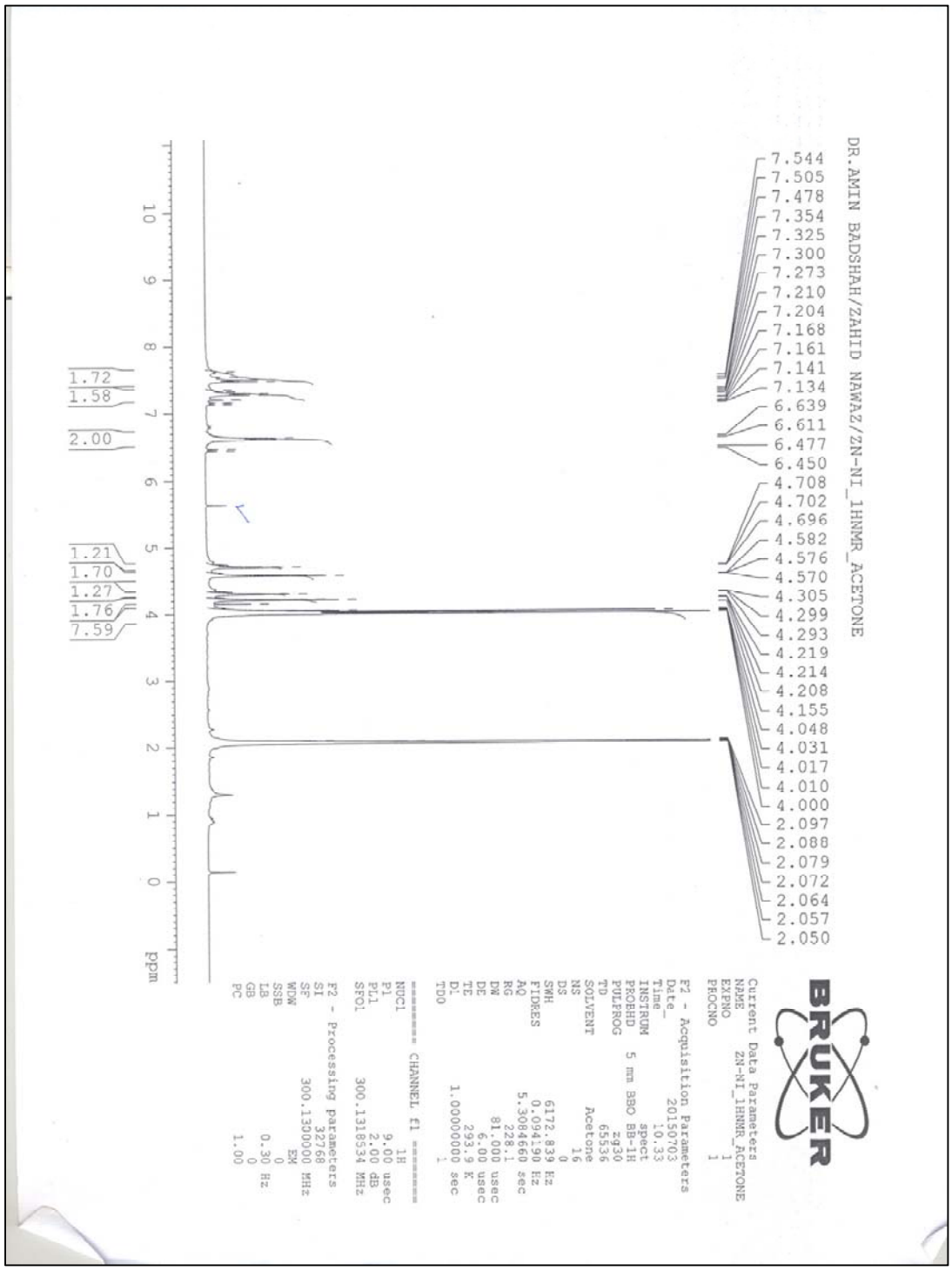


Figure 6: ^{1}H NMR spectra of Ni complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea

3.11. Procedure for cyclic voltammetry:

For the interaction of DNA with the synthesized compounds Eco Chemie Auto lab PGSTAT 12 potentiostat/galvanostat (Utrecht, The Netherlands) has been used the

solution was taken in cell of 6 mL of 1 mM of compound in DMSO. In cell of cyclic voltammetry three type of electrode are used which are counter, reference and working electrodes. Counter electrode is of platinum wire, working electrode is of 0.07 cm^2 area is made of platinum disc, the saturated calomel is used as a reference electrode. 1mL of 5M KCl solution is used as a supporting electrolyte. In the various amount of samples concentrations (10-60 μM) calf thymus DNA (CT-DNA) is adding drop wise by syringe and cyclic voltammograms was recorded at fix scan rate of 100mV/sec.

3.12. In vitro antibacterial activity:

By using 3 different strains of bacteria for their antibacterial activities all synthesized compounds are screened in vitro, two Gram-positive bacteria namely Listeria monocytogen and Klebsiella pneumoneae and one Gram-negative strains Acinetobacter baumanni and ager well diffusion method is used. Human pathogens are present in these selected 3 strains that are why they are nominated. The solutions of tested compound are prepared in DMSO by dissolving 0.25mg of sample in per mL of DMSO. Reference of antibacterial drug (Imipenem) is attendant as negative and positive control respectively with a medium of DMSO. With sterile cork tool 3well of 5 mm per plate is prepared in petri dish ager nutrient after vaccination. A solution of 100 μL of synthesized complexes is slanted in particular wells and sheltered at for 37 ˚C then measured the clear zone of inhibiting (which is due to activities of the compounds) after 24 hour. The every calculations of antibacterial are taken by mean of 3 replicates.

3.13. Properties of synthesized compounds

The impact of synthesized samples in practical field is determined by studying DNA interactions, electrochemical properties and against bacteria. As all the compounds contains ferrocene moieties in their structure due to which they show a good electrochemical and DNA binding activities. All synthesized compounds are made-up of ferrocene assimilated thiourea of N-donor ligands are detail studying for their DNA binding antioxidant antibacterial and electrochemical activities.

3.14. Electrochemical properties

Electrochemically all the synthesized compounds are active with specific voltammograms of Fc/Fc+ redox pair. All the synthesized samples with clear

oxidative/reductive peak showed a characteristic CV activities of ferrocene derivatives [1]. 0.0-0.8 V of window potential was found proper to studying the redox manners of thiourea compounds. The electrochemical activities were analyzed in relation to factors like Ep-Ep/2 (half value of difference b/w peak potential), Ipa/Ipc (the ratio of peak current in redox pair) and scan rate variant from 0.1 Vs-1 - 0.7 Vs-1. In 80% DMSO the 1 mmol solution of all the samples were prepared. By using supporting electrolyte of 1M KCl solution cyclic voltammograms was run in potential window of 0.0-0.8 V. On working electrode surface that reducing species may deposit so it was cleaned by washed with distilled water and rubbing it with alumina powder to avoid interference in the process. A couple of redox peaks is obtained in CV of all compounds with oxidation maxima at ~ 0.59 V and reduction maxima at ~ 0.52 V.

3.15. Electrochemical behavior of Synthesis ligand 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: Tu

The cyclic voltammograms of Tu indicates single electron transfer and reversible electrochemical reaction. And by the following criteria the reversible processes is determined:

- With the change in scan rate the peak potential is slightly change constant.
- $\Delta Ep = Epc - Epa$ is ~ 57mV for one electron system.
- The peak intensities of oxidation and reduction current are same having their ratio (Ipa/ Ipc) is equal to 1/one.

So for the confirmation of reversible electrochemical behavior all the conditions are fulfill [2, 3]

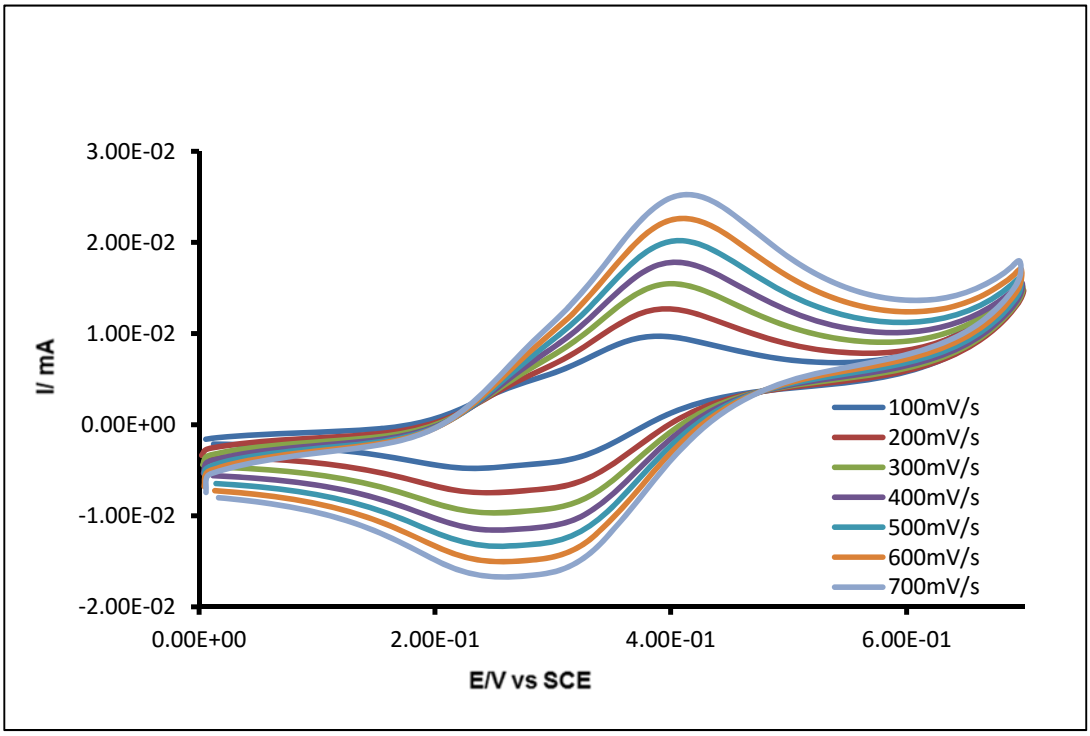


Figure 7: CV of 1mM DNA free Tu 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea at different scan rate.

A cathodic current (mA) plotted versus square root of scan rate gives straight line with the zero intercept value. This confirms that the process is diffusion controlled [4]. Show in Figure 8**.**

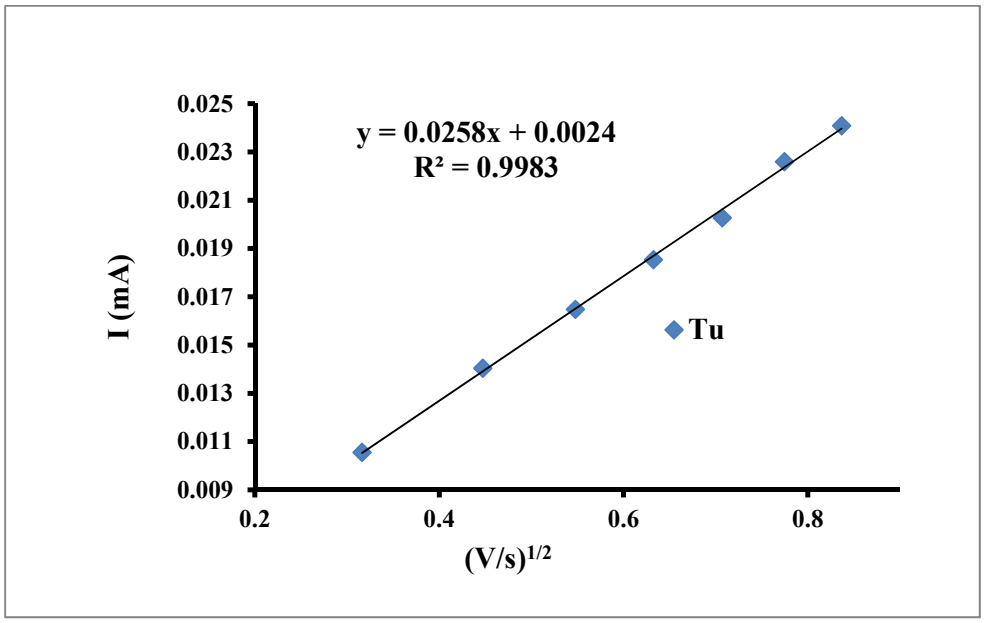


Figure 9: Diffusion coefficient of DNA free compound Tu

3.16. Electrochemical behavior Cu complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: Cu

Cyclic voltammograms of Cu in the absent of DNA in a potential window of 0.0 to 0.8 V give pair of redox peaks. Anodic peak appears at 0.43 V versus SCE while it same condition simple ferrocene becomes oxidized at 0.34 V. Due to inductive electron withdrawing effect of phenyl group attached to the cyclopentadienyl ring of ferrocene the difference of 0.09 V in the oxidation potential of compound and free ferrocene comes, which reduces oxidation process

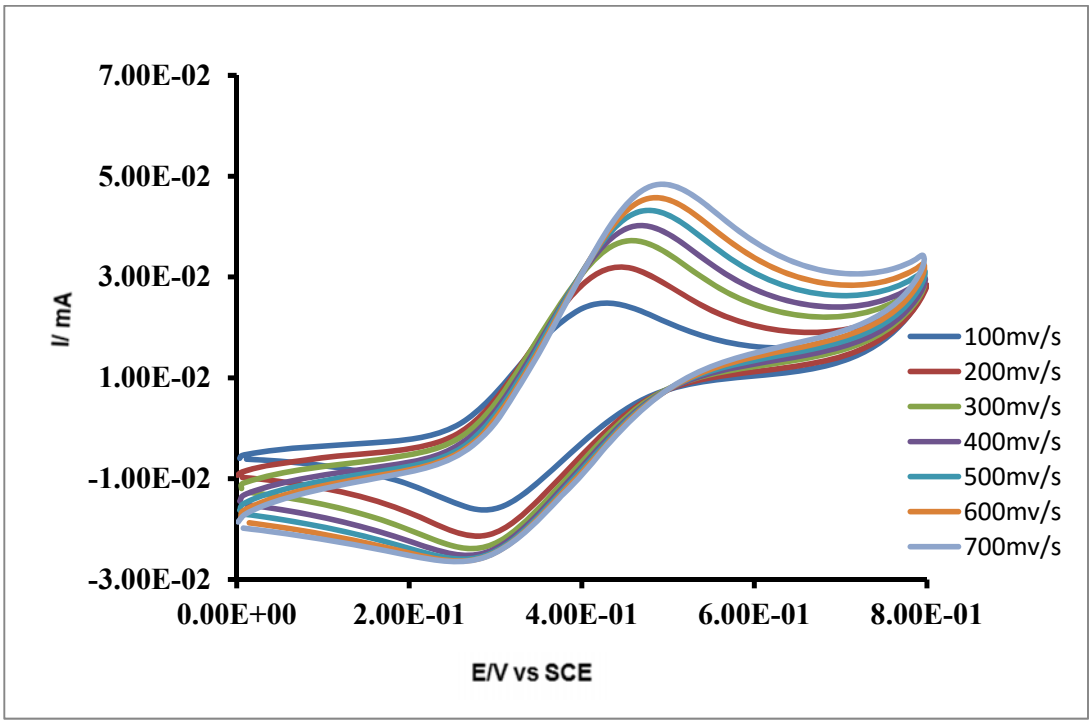


Figure 10: CV of 1mM DNA free Cu at different scan rate

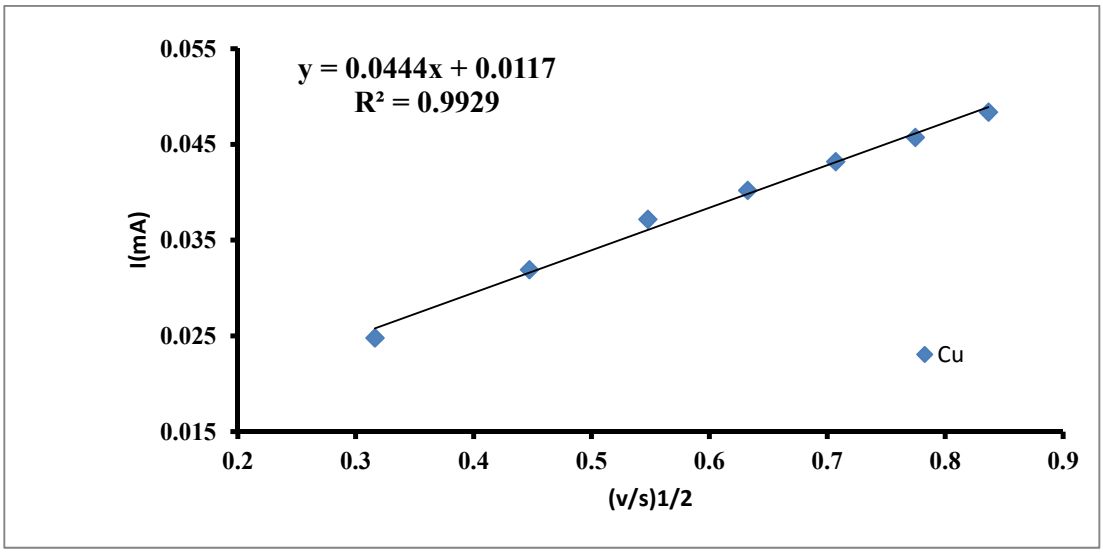


Figure 11 : Diffusion coefficient of DNA free compound Cu

3.17. Electrochemical behavior of (Ni complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea): Ni

Cyclic voltammograms of Ni complex represents quasi reversible redox behavior. By increase in scan rate there is no change in peak position while successive increase in current peak. Show in bellow Figure 12.

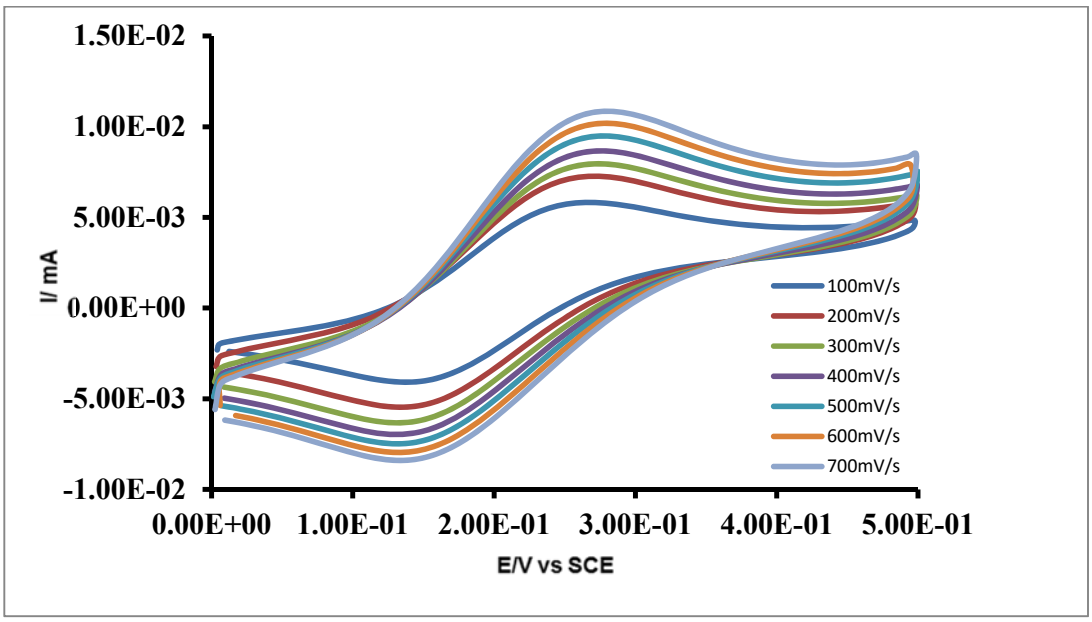


Figure 13: CV of 1mM DNA free Ni at different scan rate

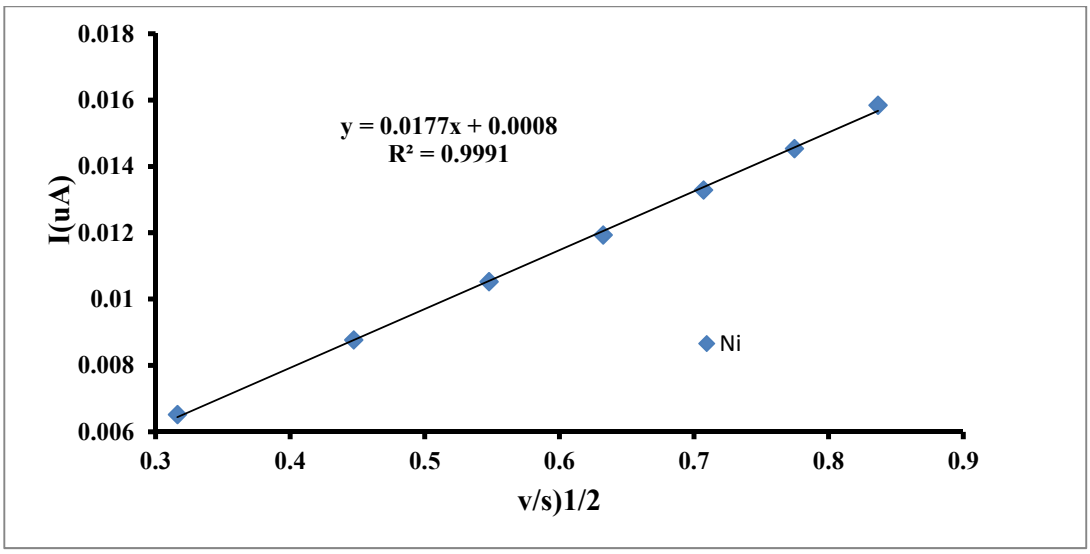


Figure 14: Plot Ip (mA) vs. $(V/s)^{1/2}$ for Ni

All others compounds show same activities towards electrochemical behavior by increasing scan rate peak current is increasing and peak position remain same for all

of them so all the compound show quasi reversible behavior due to this behavior all the compounds can be interacted electrostatically by DNA.

3.18. DNA binding study

By variations in UV visible and cyclic voltammetry spectra all the synthesized compounds were checked for their interaction with DNA double helix structure.

3.19. DNA binding study using cyclic voltammetry

For the DNA binding study of electro active species cyclic voltammetry is a good apparatus. Electrochemistry was fast of all started by Bard and co-workers they investigate the interaction of complex with DNA [6]. DNA interacts with metal complexes non-covalently through intercalation groove binding and electrostatic interaction. By the addition of DNA to compounds there will be decrease in a current which will show the interaction of DNA with a complex, this is due to the interaction there will be the formation of drug DNA adduct with a large molecular weight so due to increase in size it will diffuse slowly towards electrodes so current is decrease. And by the addition of DNA there is 1st positive and then negative shift are present in peak potential of synthesized compounds so by the rule as reported by Bard intercalation of the drug into the double helical structure of DNA is due to positive shift (anodic shift) in potential, while negative shift is produced for the electrostatic interaction between the cationic part of drug and the anionic phosphate of DNA backbone [5].

In order to studying drug-DNA interaction, 5mL volume of 1mM solution of compound in DMSO was taken in cell. Kept the compound concentration constant, various concentrations (10-60 μM) of calf thymus DNA (CT-DNA) was added drop wise. On the addition of increasing concentration of DNA there is decrease in the current of all the synthesized compounds along with cathodic shift in peak potential for all the particular compounds. The drug concentration which is free in solution is responsible for flow of current. On addition of DNA there is formation of drug-DNA adduct, which slowly diffused results in decrease of current. Which type of interaction is present b/w drug and DNA is conformed from cathodic peak shift it will show in the bellow Figure 15, 16, 18 that both electrostatic and intercalation is present between drug and DNA. There may be two way for interaction of drug DNA proposed mechanism, it may involve the binding of Fe(II) ion with DNA followed by

its oxidation or Fe(II) is first oxidize to Fe(III) followed by interaction of this oxidized form with DNA.

The mechanism is shown

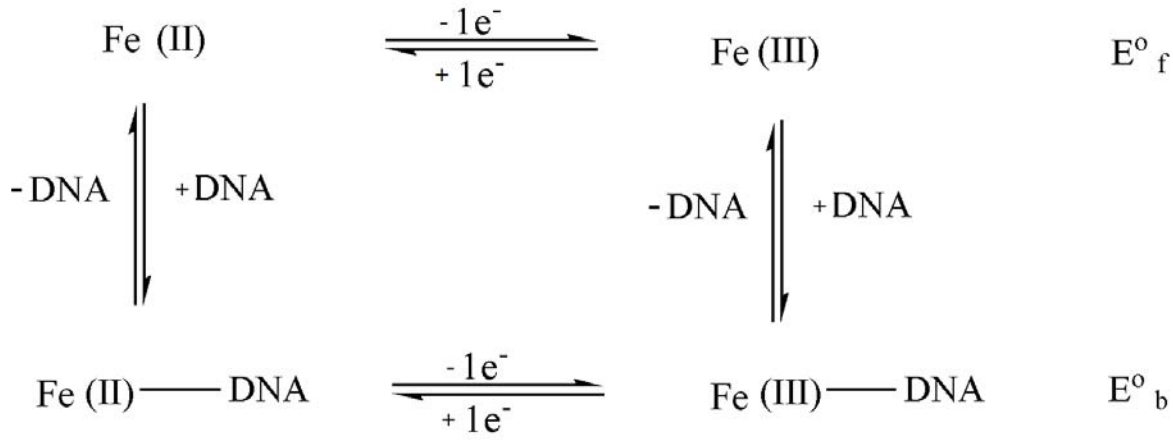


Figure 16: The General redox process of the free and DNA bound compounds

The binding constant of Drug-DNA was calculated from the following equation.

$$\text{Log } (1/[DNA]) = \log K + \log (I/I_o - I)$$

Where current peak for free drug is Io, current peak for Drug-DNA adduct is I and K is binding constant.

While the diffusion coefficient (Do) is determination of the rate of diffusion of electro active species which has been calculated from Randles-Sevcik equation at 100 m V/s [6]

$$Ipa = 2.69x10^5\ n\ 3/\ 2\ ACo\times\ Do^{1/2}\ \upsilon^{1/2}$$

In above equation Ipa is current in ampere of anodic peak , υ (volts^{-1}) is scan rate , C_o^* (mol cm^{-3}) is the concentration, A (cm^2) is the electrode surface area, n is electrons number present in the redox reaction, D_o (cm^{2}s^{-1}) is diffusion coefficient.

3.20. 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea: Tu cyclic voltammetry measurements

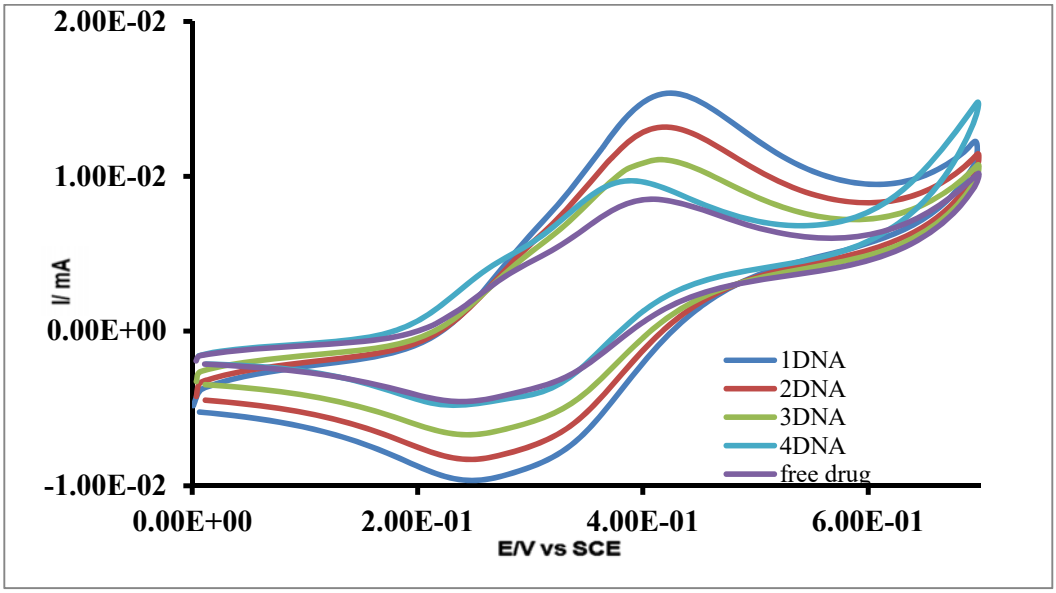


Figure 17: CV of Tu recorded at 100 m V/s scan rate in the presence of different concentrations of DNA

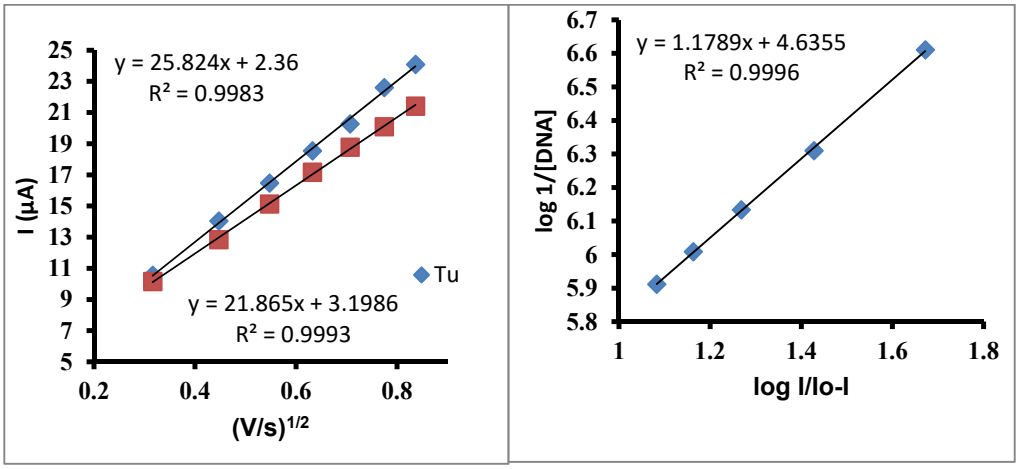


Figure 18: Plot Ip (µA) vs. (V/s) 1/2 for Tu (a), Plot of log 1/[DNA] vs. log(I/I_o-I)(b).

3.21. Cu complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea cyclic voltammetry measurements (Cu)

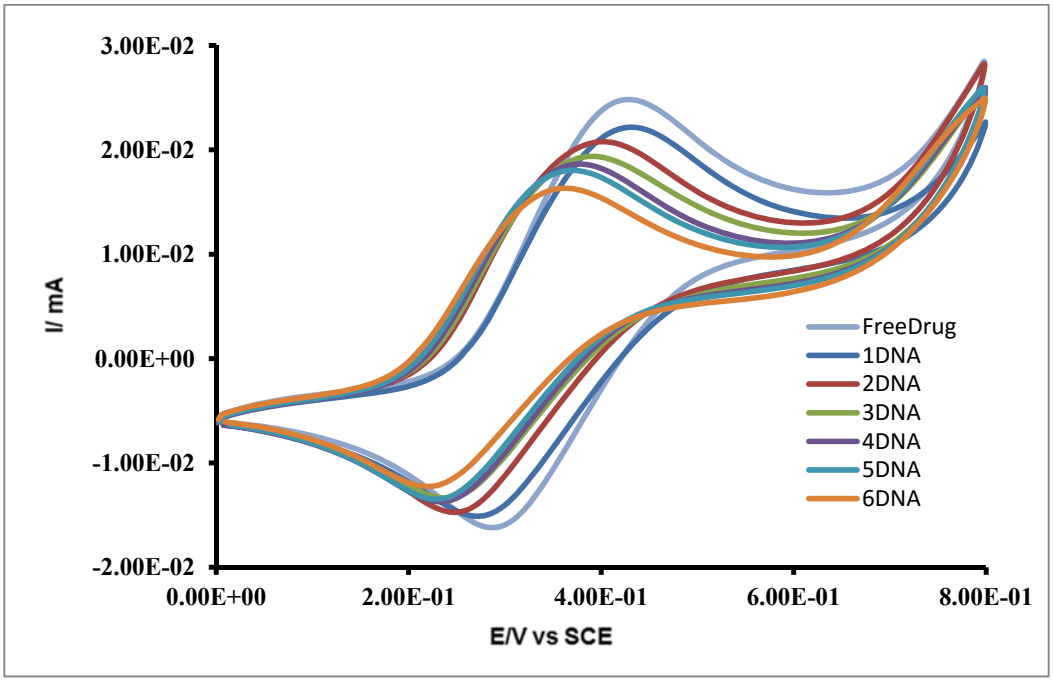


Figure 19: CV of Cu recorded at scan rate 100 m V/s in the presence of different conc of DNA

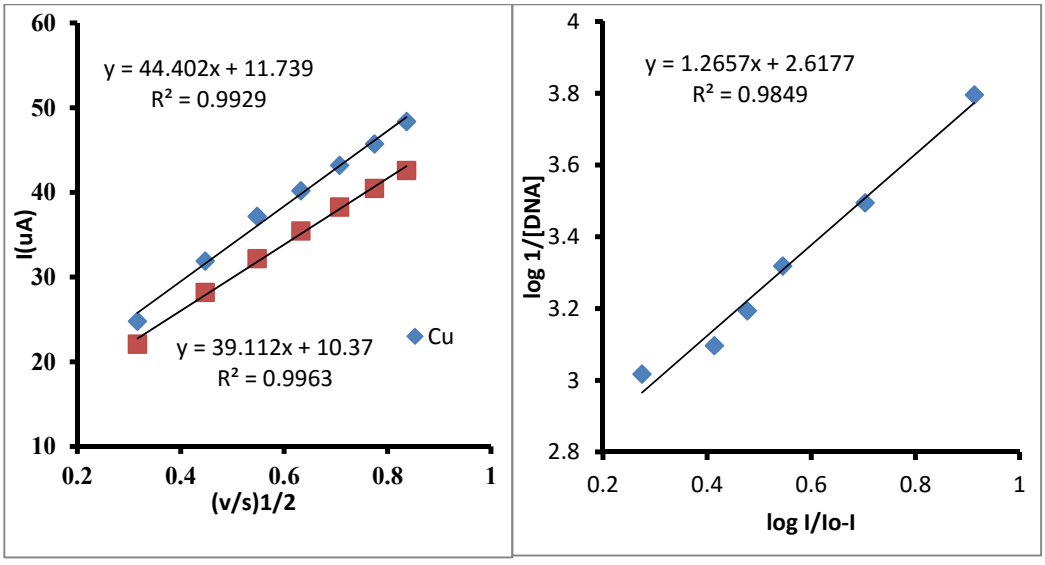


Figure 20: plot Ip (µA) vs.(V/s) 1/2 for Cu(a), Plot of log (1/[DNA] vs.log(I/l_o-l) for Cu (b).

3.22. [Ni complex of 1, 1` (4, 4`-diferrocenyl) diphenyl thiourea] cyclic voltammetry measurements (Ni)

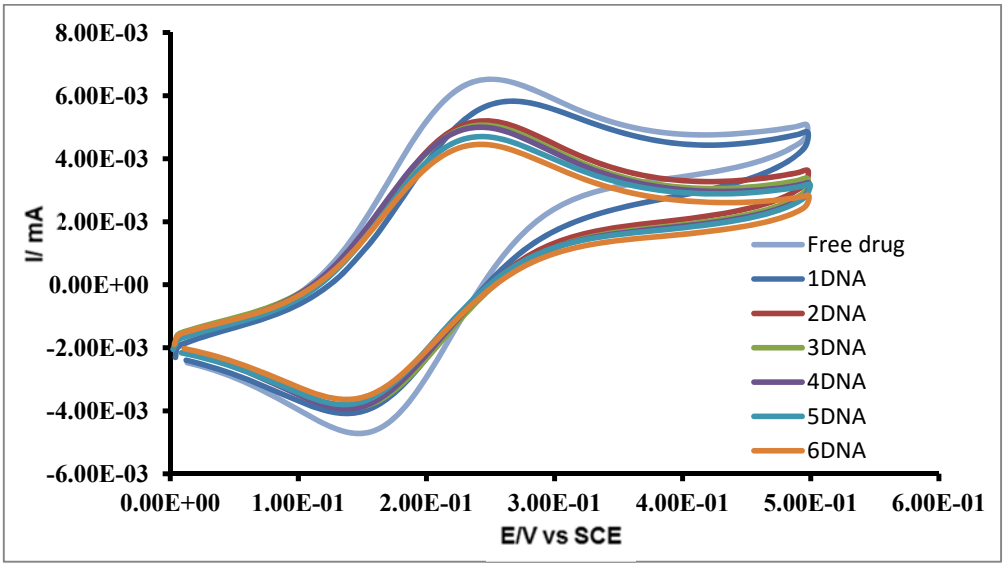


Figure 21: CV of Ni recorded at 100 mV/s scan rate in the presence of different conc. of DNA

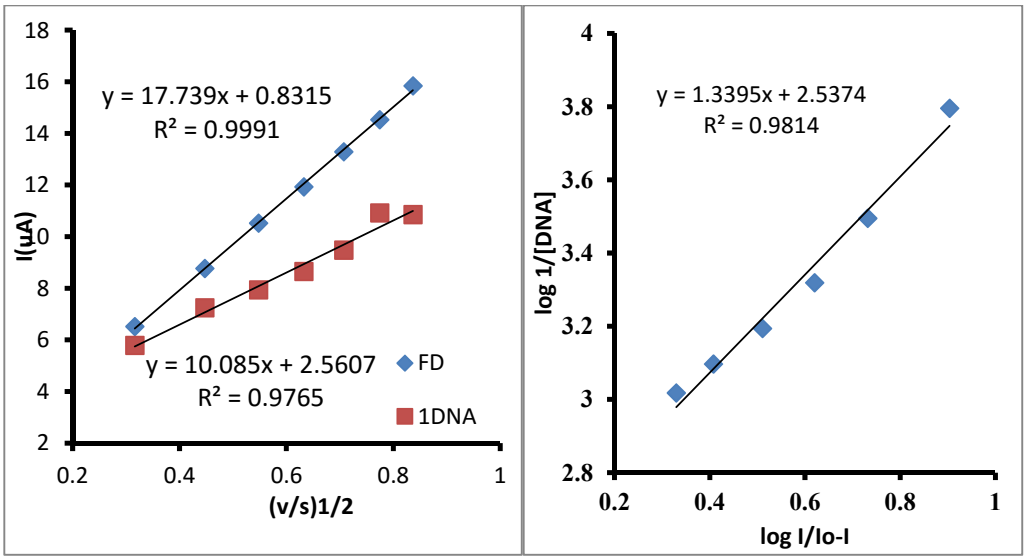


Figure 22: Plot Ip(µA) vs (V/s) 1/2 for Ni(a), plot log 1/[DNA] vs log I/Io-I (b).

Table 1: Diffusion coefficient of free drug and drug-DNA adduct.

Compound	Diffusion coefficient free drug (cm^2/s)	Diffusion coefficient drug-DNA adduct (cm^2/s)
Tu	1.34×10^{-6}	1.31×10^{-6}
Cu	5.2×10^{-7}	1.7×10^{-7}
Ni	5.3×10^{-6}	3.34×10^{-6}
Co	2.46×10^{-6}	1.4×10^{-6}
Ru	3.37×10^{-6}	1.37×10^{-6}
Pt	1.67×10^{-6}	1.1×10^{-6}
Mn	4.33×10^{-6}	2.25×10^{-6}
Au	3.29×10^{-7}	1.35×10^{-7}

It is clear from the above table 1 that the diffusion coefficient of free drug is more than the drug DNA adduct. This is because of the slow diffusion of large size drug-DNA adduct towards electrode**.**

Table 2 : Binding constant obtained from CV.

Compound	Binding constant $K(M^{-1})$	Binding Energy (kj/M)
Tu	5.9×10^4	-26.6
Cu	3.5×10^4	-20.2
Ni	4.14×10^4	-26.3
Co	4.56×10^4	-26.57
Ru	3.8×10^4	-26.1
Pt	2.8×10^5	-25.37
Mn	3.6×10^5	-25.99
Au	1.9×10^5	-24.4

From the above data in Table 3 it is clear that all the synthesis compound show high bonding constant so they can show a good activity against cancer cell.

3.23. DNA binding study using UV- visible spectroscopy

The interaction of synthesized compounds with a DNA is also confirmed by other technique of UV-visible spectroscopy. It provides a significance indication about drug DNA interaction along with the mode of interaction. Generally, bathochromic shift along with hypochromism show intercalative mode of interaction [7]. Hypochromism with no change in wavelength is symbolic of groove binding. Bensi-Heilderband equation is used to calculate the binding constant [8, 9]

$$Ao/ (A-Ao) = \varepsilon F / (\varepsilon B - \varepsilon F) + \varepsilon F / (\varepsilon B - \varepsilon F) \times 1/K [DNA]$$

Where K is the binding constant, Ao is absorbance of the free drug and A is the absorbance of compound after addition of DNA, εF is the absorption coefficient for free compound and εB is the absorption coefficients of addition of DNA. From the plot between Ao/ (A−Ao) versus 1/[DNA] slope to intercept ratio of the plot produced the binding constant K. Gibbs free energy gives an idea about the spontaneity of binding of compound with DNA. Binding constant Kb is used to calculate the Gibbs free energy by using the following relation.

$$\Delta G = -RT \ln Kb (KJ / mol)$$

A 20 μM solution of all the synthesized complexes was prepared in ethanol and interaction of DNA is study by adding different concentrations of DNA which is from 10 to 50 μM. There is a decrease in absorbance peak with consecutive addition of increased DNA concentration i.e. hypochromism. This change in absorbance shows the DNA interaction with a compound. Along with hypochromism there is bathochromic shift in the order of 3-4 nm, which approves the dominant mode of interaction being intercalation. UV- Vis spectrum of Tu has 2 peaks at 290 nm, and 250nm. The very intense peak at 290 nm is associated with $\pi \rightarrow \pi^*$ transition of phenyl ring. At 250 nm a band of less intensity corresponds to $\pi \rightarrow \pi^*$ transition of C=C group of ferrocene.

3.24. DNA binding study using UV- visible spectroscopy of Tu:

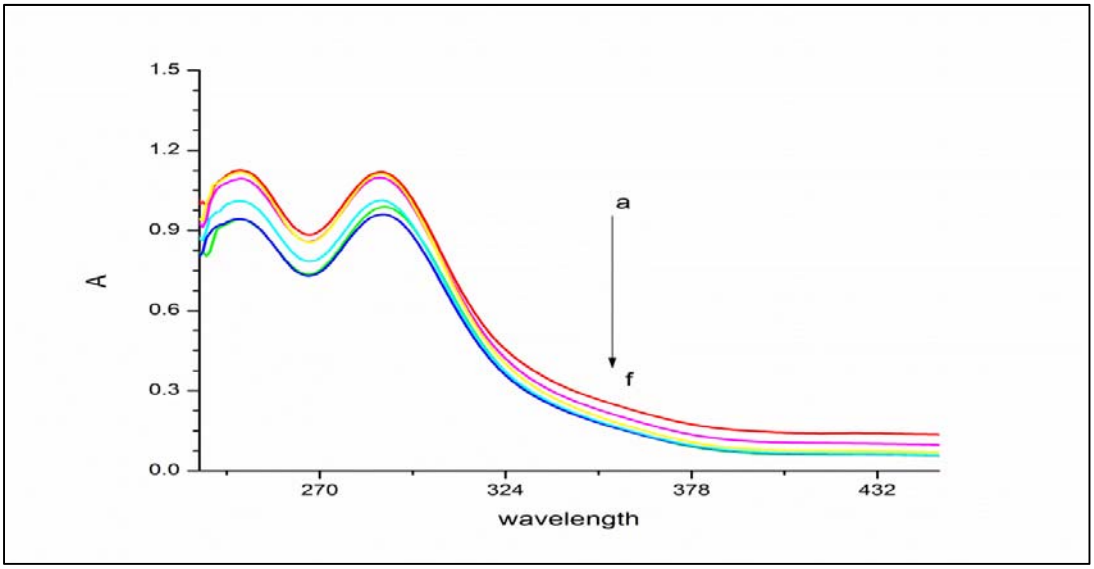


Figure 23 : UV-Vis spectra of 25 µM Tu in the absence (a) and presence of 10-50 µM DNA (b-f).

From the plot bellow of Ao/A-Ao vs. 1/[DNA] the value of binding constant was calculated which is in nearly equal with the values obtained from cyclic voltammetry. A negative value for Gibbs free energy confirms the spontaneous interaction of compound with DNA.

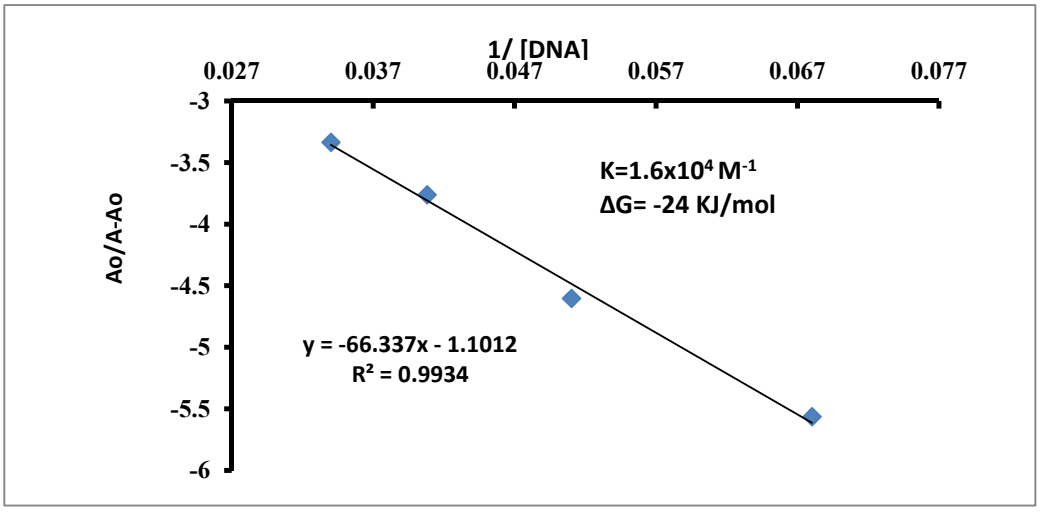


Figure 24: Plot of Ao/A-Ao vs 1/ [DNA] for Tu.

3.25. DNA binding study using UV- visible spectroscopy of Cu:

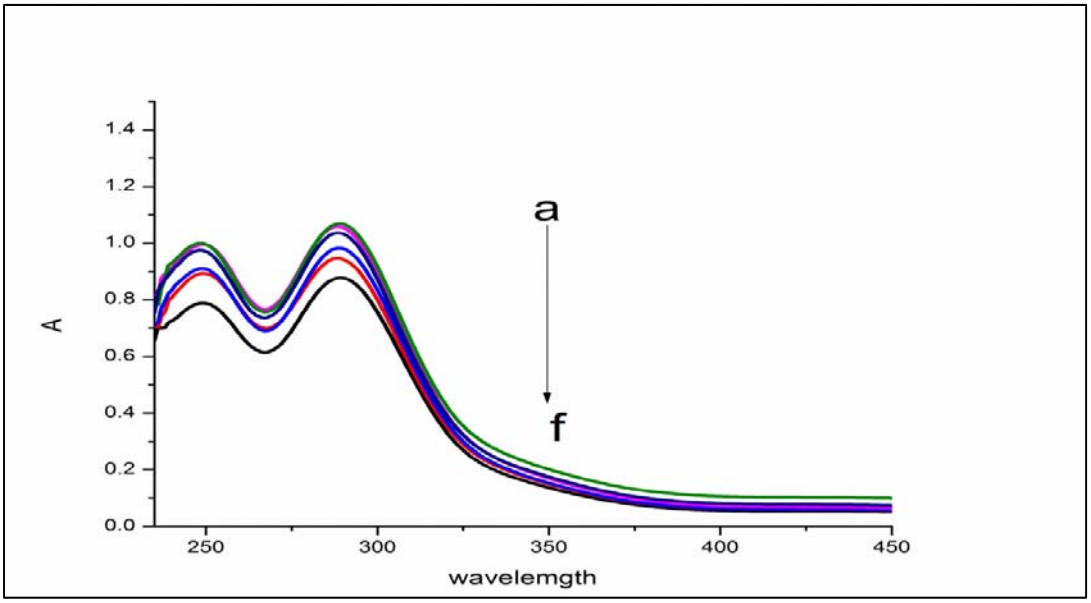


Figure 25: UV-vis spectra of 25 µM Cu in the absence (a) and presence of 10 - 50 µM DNA (b-f).

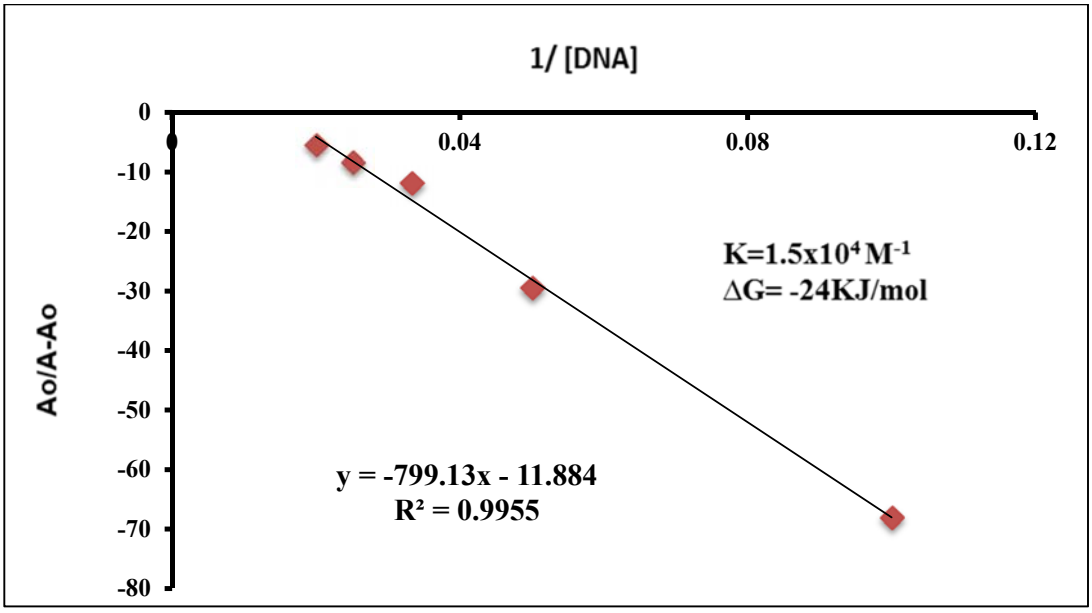


Figure 26: Plot of Ao/A-Ao vs. 1/ [DNA] for Cu.

3.26. DNA binding study using UV- visible spectroscopy of Ni:

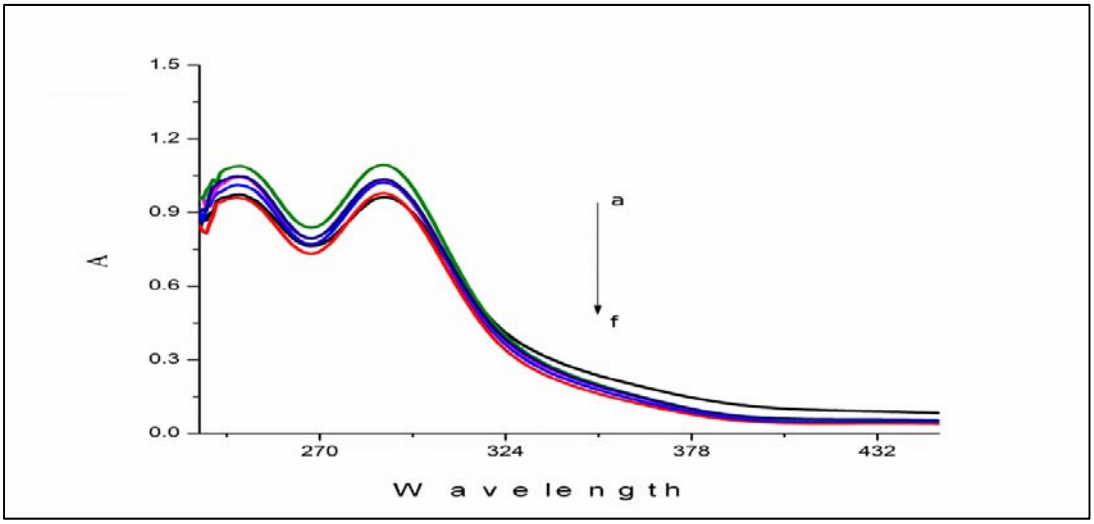


Figure 27: UV-Vis spectra of 25 µM Ni in the absence (a) and presence of 10-50 µM DNA (b-f).

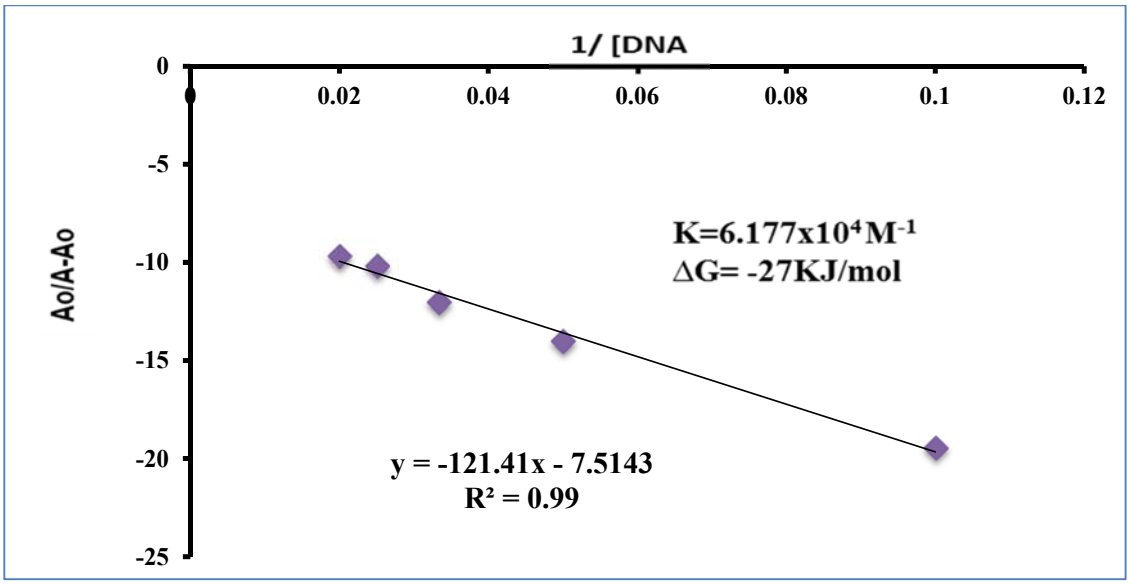


Figure 28: Plot of Ao/A-Ao vs 1/ [DNA] for Ni.

Rest of compounds Co, Mn, Ru, Au, Pt., also showed hypochromism with Red shift. Binding constant and binding energy was calculated for all compounds and mentioned in Table below.

Table 4: Binding constant and free energy values obtained from UV-Vis spectroscopy.

Compound	Binding constant K(M^{-1})	Binding Energy (kj/M)
Tu	1.6×10^4	-24
Cu	1.5×10^4	-24
Ni	6.117×10^4	-27
Co	3.55×10^4	-25.4
Ru	2.6×10^4	-25.1
Pt	2.3×10^4	-24.6
Mn	2.6×10^4	-24.1
Au	1.05×10^4	-23.5

Binding constant values calculated from UV-vis data are nearly equivalent with those gotten from CV studies. Their comparison is given in Table 4

Table 5: Comparison of Kb by CV and UV.

Compound	Kb (M^{-1}), UV	Kb (M^{-1}), CV
Tu	1.6×10^4	5.9×10^4
Cu	1.5×10^4	3.5×10^4
Ni	6.117×10^4	4.14×10^4
Co	3.55×10^4	4.56×10^4
Ru	2.6×10^4	3.8×10^4
Pt	2.3×10^4	2.8×10^4
Mn	2.6×10^4	3.6×10^4
Au	1.05×10^4	1.9×10^4

3.27. Antibacterial activities

These all products were tested against anti-bacterial activity using agar well diffusion method in vitro against Listeria monocytogenes, Klebsiella pnemoniae, Acinetobacter baumannii Imipenem as standard drug for comparison (Table 5 below)

Table 6: Antibacterial activities.

Chemical Codes	Listeria Monocytogenes (G+ve)		Klebsiella pneumoniae (G-ve)		Acinetobacter baumanni (G-ve)	
	Radius (mm)	**% Value**	**Radius (mm)**	**% Value**	**Radius (mm)**	**% Value**
Imipenem	18	100	20	100	20	100
Tu	00	00	05	28	02	11
Au	12	66	14	78	13	72
Co	11	58	07	39	14	78
Cu	02	11	03	17	02	11
Mn	09	50	03	17	05	28
Pt	02	11	00	00	00	00
Ru	14	78	00	00	02	11
Ni	05	28	05	28	03	17

Ferrocenyl assimilated thiourea is biologically active due to sulfur group [10]. It was found that produced compounds were more toxic as compared to parent ligands versus same bacterial strains. This increase in toxicity behavior was clarified by Tweedy's chelation theory [11]. Central atom become more lipophilic as chelation is reduced [12].

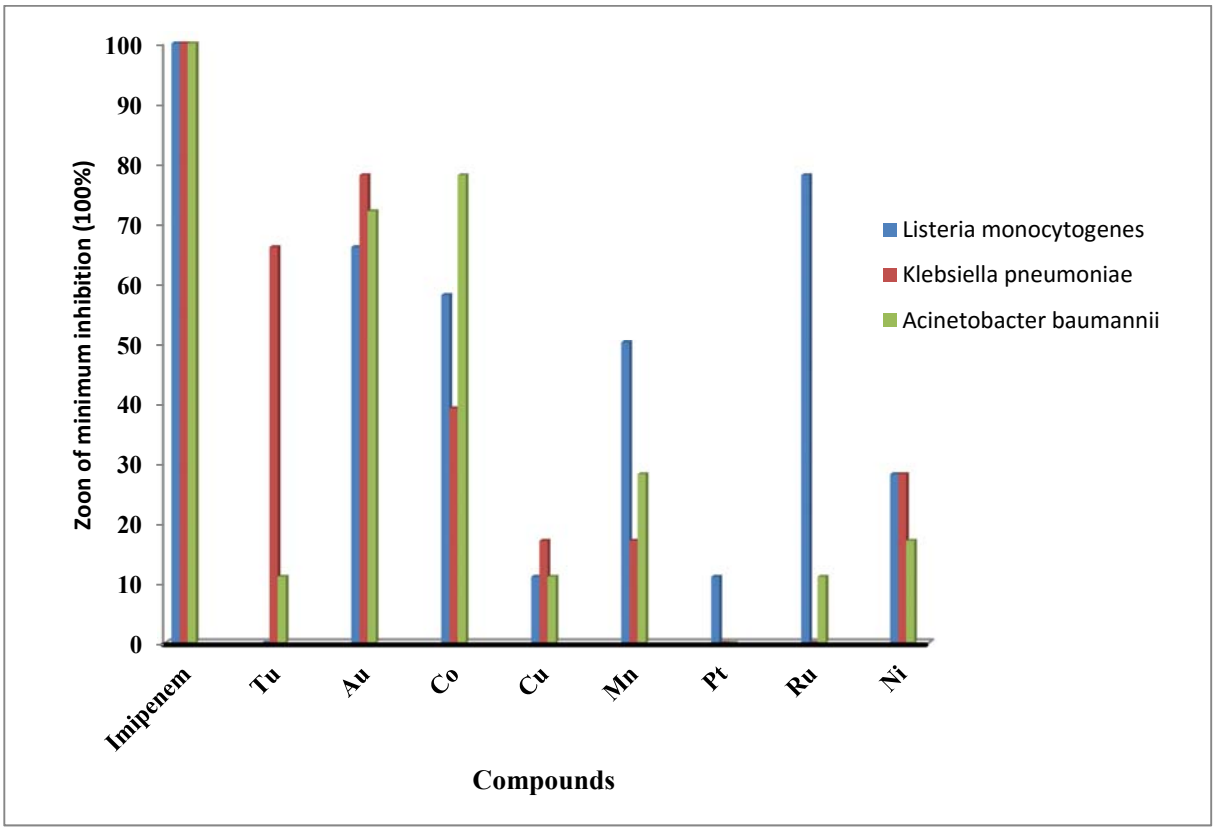


Figure 29: Comparison of antibacterial activity of all the synthesized compounds.

3.28. Antioxidant activities:

The antioxidants are the compounds which have ability to capture reactive oxidant species/free radicals.

Free radicals: Free radicals are highly reactive species such as hydrogen peroxides, peroxides and lipids peroxyl. These are found in variety of sources. These free radicals react with enzymes, hormones DNA and oxidize it. It causes degenerative diseases in the body. Thus it is significant to capture ROS after failure of natural biological antioxidant defense system the synthetic antioxidants are used to protect cell injury and ultimately to hinder the passage of degenerative diseases[13]. DPPH (2, 2-Diphenyl-1-pecrylhydazayl) is use to find all the antioxidant activities of the drugs because this analyze is simple, perform easily, low-cost and provides high degree of accuracy[14, 15]. DPPH in solution have odd number of electron having purple color which give a maximum absorption at 517nm. The intensity of color of DPPH depends upon number of odd electron. The intensity of color of DPPH decreases by the addition of antioxidant into DPPH solution[14]. Antioxidant activity

of novel synthesized ligand and complexes showed that they have good free radical scavenging activity. Antioxidant activity of some of the synthesized compounds is shown below in Figure 30.

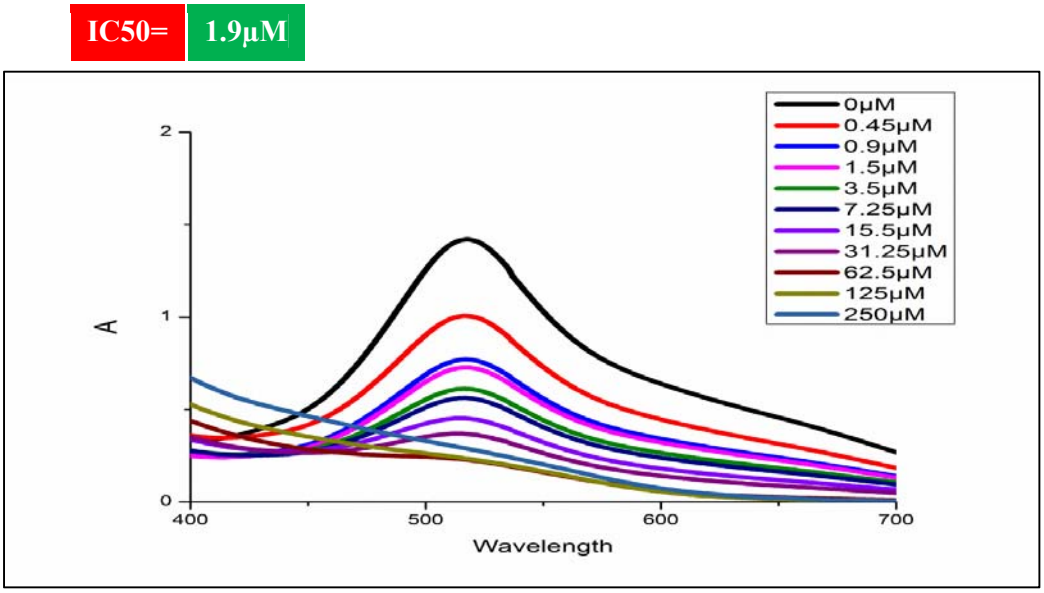


Figure 31: Antioxidant activates for 100 uM solution of copper complex (Cu**).**

In the above plot the 1st black line is for free DPPH which give a maximum absorption at 517nm by the addition of copper complex (act is antioxidant) there is decreasing in DPPH peak. At very high concentration of 250 uM there is almost complete scavenging of DPPH free radical. The IC50 vale for Cu is 1.9 uM which show that half of the DPPH free radical is scavenged at that much concentration.

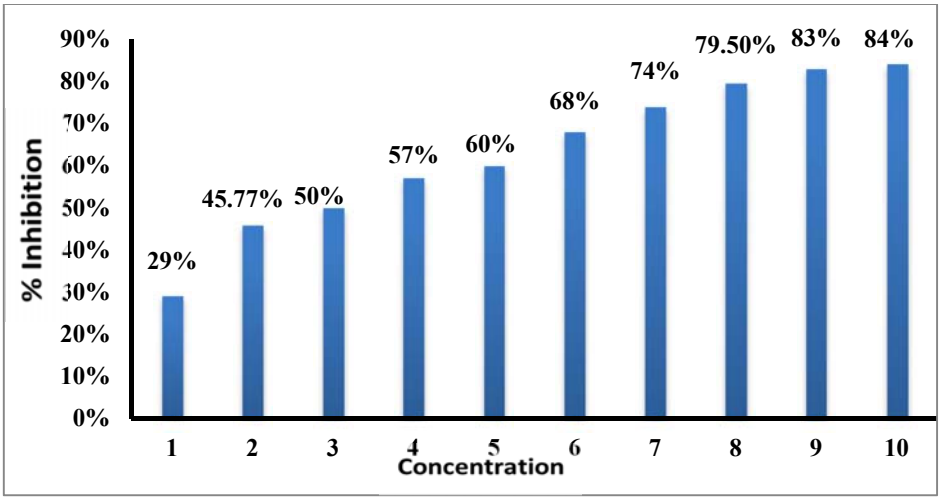


Figure 32: Percent scavenging activates of Cu-complex.

It has been observed that at lower concentration of complex, the % scavenging activity is lower which is 29%. But it increases by increasing the concentration up to 250 uM (84%). All the other synthesized compound show similar behavior towards DPPH solution.

3.29. Comparison of IC50 value of synthesis complex:

The removal of free radical of DPPH is due to capturing of N-H labile proton present in the ligand and by increasing a size of metal the ligand attached to metal will show minimum attraction to each other so proton will be easily removed by DPPH so give good antioxidant activity and vice versa to small size metal which is conform from the bellow plot

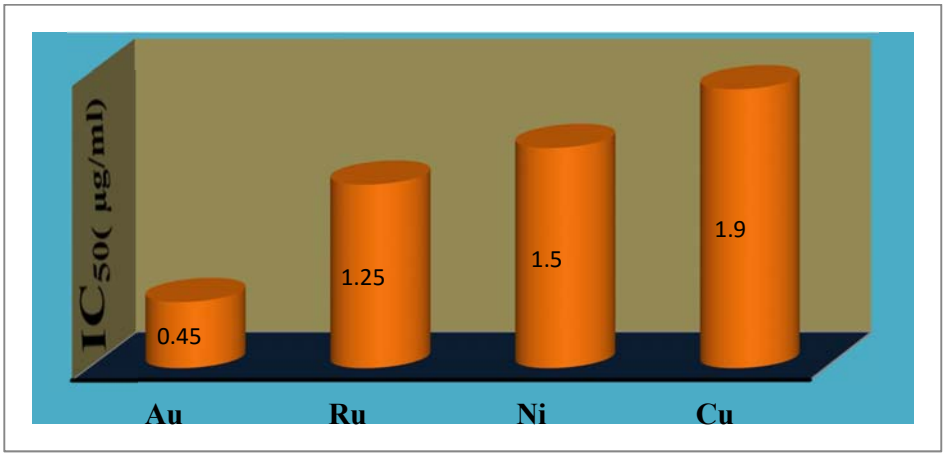


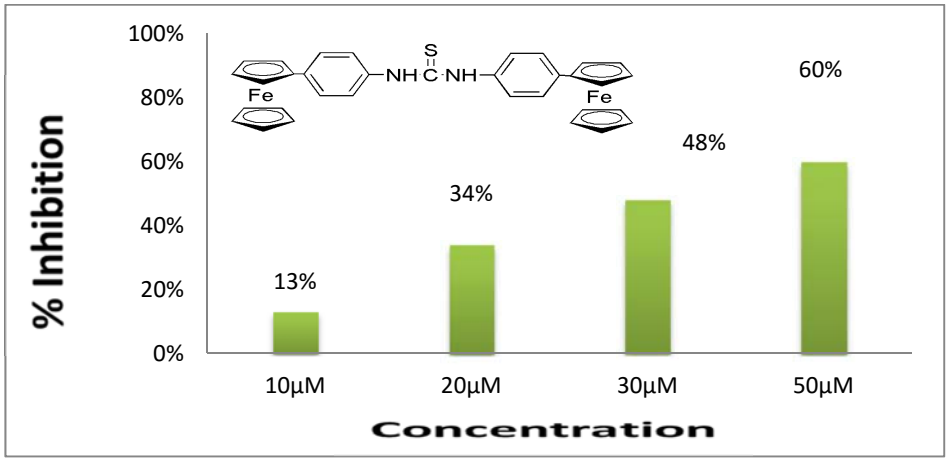
Figure 33: Comparison of IC50 value of some of the synthesis complex.

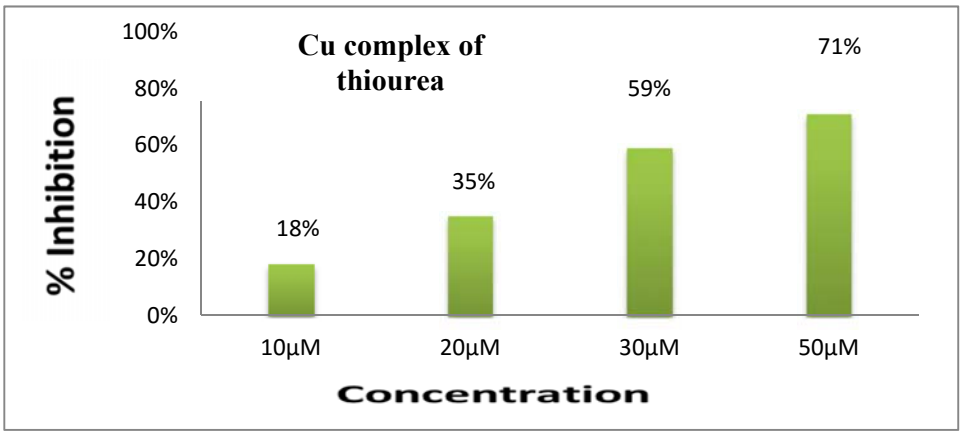
Anti-cancer activities

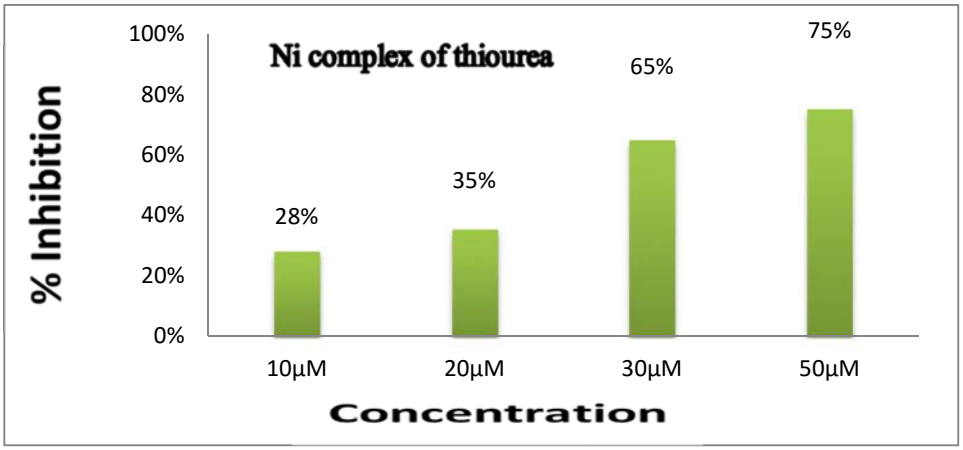
Activities against the cancer cell lines: Cytotoxic activities have been carried out against Three cancerous cell lines namely Ovarian cancer cell lines (CaOV3) liver cancer cell line (SMCC-7721) and breast cancer cell line (MCF70) The activity against this cell lines is comparatively too much inferior than MYCN2. 4.1.5.2. Cytotoxicity against Ovarian cancer and liver cancer. Liver cancer is the 5th most diagnosed cancer of the world and is mostly caused by hepatitis B and hepatitis C. The synthesized FIS have shown 27– 37% cell killings against this cancer cell line Cytotoxicity against MCF-70 and breast cancer. Breast cancer is 22.7% of all the

cancers in the world and in 2008 13.7% of cancer deaths were caused because of this cancer. It is 100 time more common in females than in males[16]. Graph presents the data for the synthesized FIS against breast cancerous cell line MCF-70. Ovarian cancer is the female cancer which ratio is increasing by increasing age. Ovarian cancer occurred in 239,000 women and resulted in 152,000 deaths worldwide in 2012. This varieties it, among women, it is the seventh-most common cancer and the eighth-most common cause of death from cancer. Death from ovarian cancer is more in North America and Europe than in Africa and Asia.

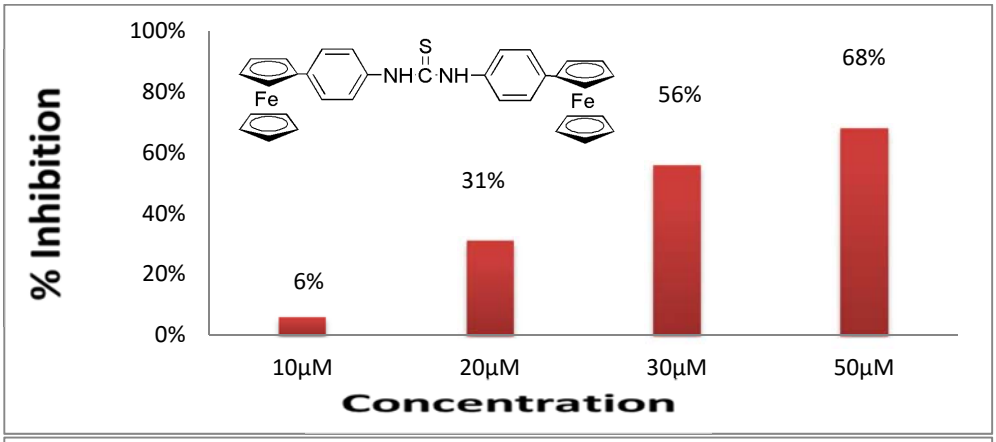
3.30. Percent inhibition it different concentration of ligand, and Cu/Ni complex against liver cancer

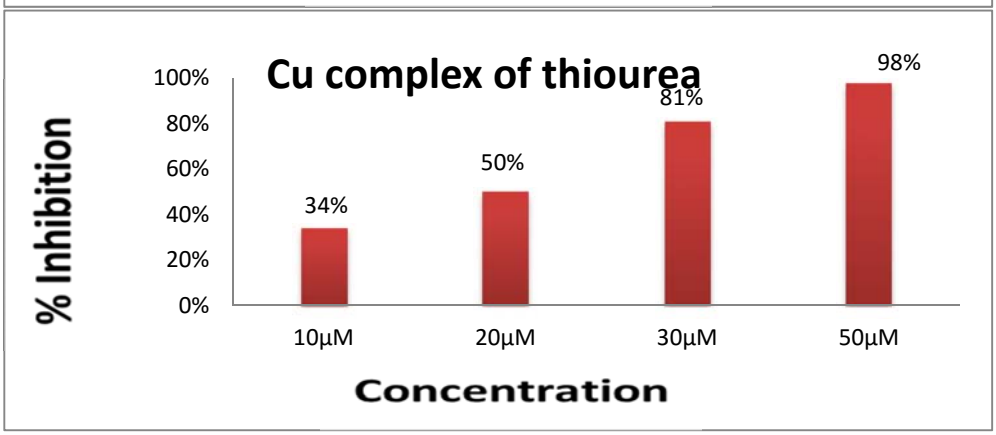


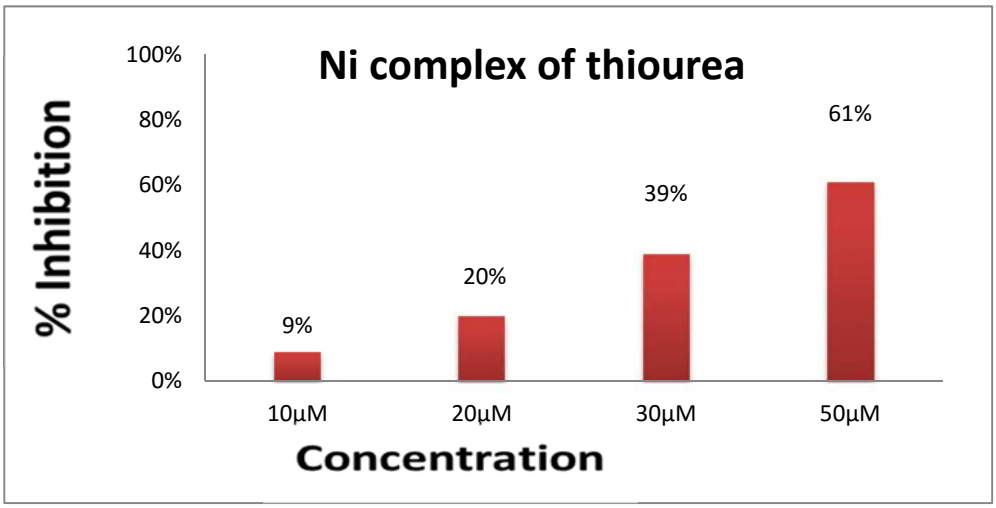




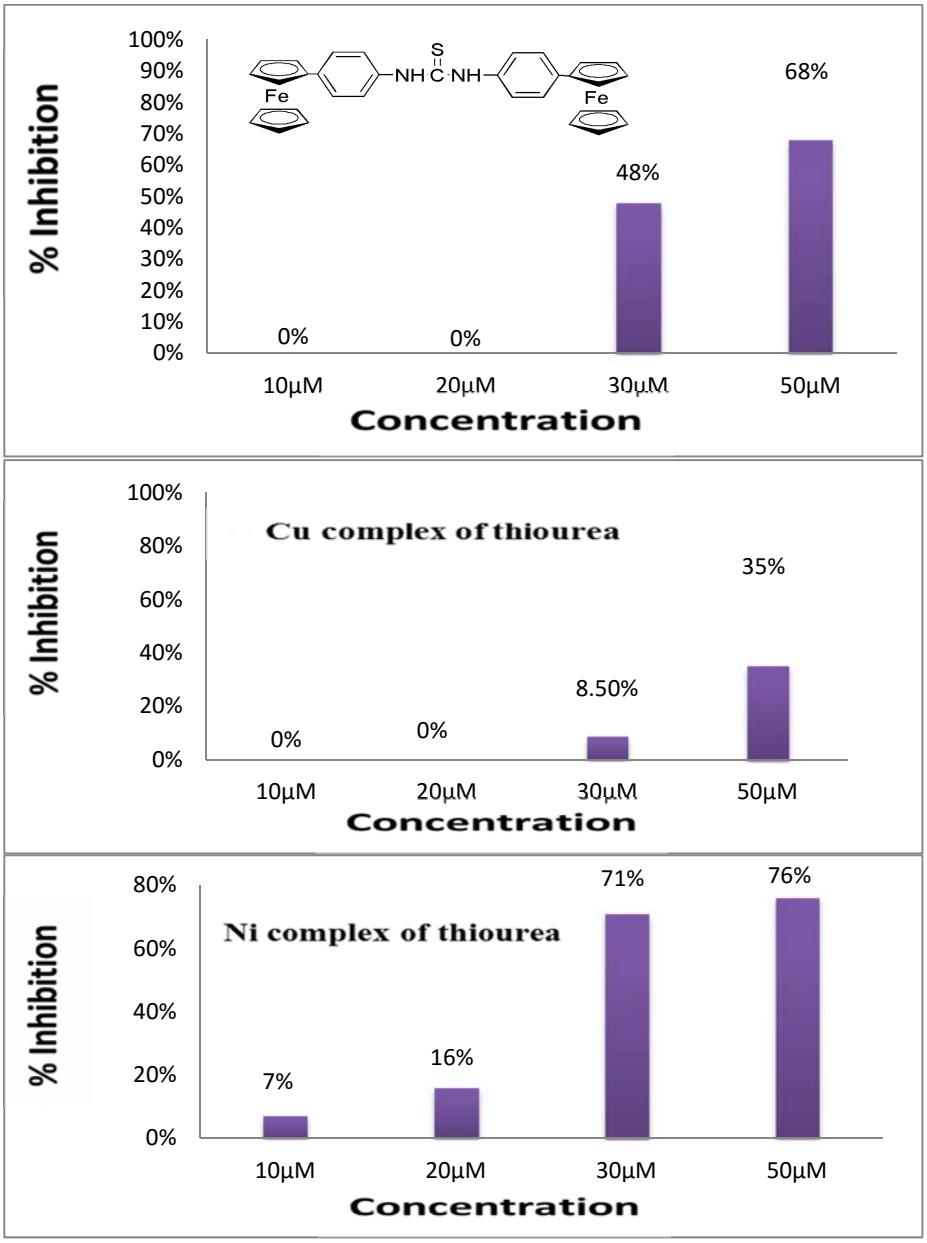
3.31. Percent inhibition it different concentration of sample against Ovarian cancer







3.32. Percent inhibition it different concentration of sample against Breast cancer.



It is clear from the above graphs that anticancer activities of Ni/Cu complexes, the percent inhibition increase by increasing concentration of complex. The IC_{50} value of the entire synthesis compound show nearly equal activity as with standard cisplatin which conform the good anticancer activities of synthesis compounds.

3.33. Summary and Conclusions

- Seven new ferrocenyl assimilated thiourea compounds of (Au, Mn, Ni, Cu, Co, Pt., and Ru) have been synthesized.
- The characterization has been done by melting point, FT-IR, ^{1}H and $^{13}CNMR$
- The compounds interaction with the DNA has been studied by using UV - Visible spectroscopy and cyclic voltammetry.
- DNA binding data showed that all the synthesized compounds are showing good interaction with the DNA.
- The negative shift in peak potential and decrease in peak current were used to study mode of interactions and different DNA binding parameters.
- All the screened compounds had the electrostatic and intercalation mode of interaction with DNA.
- All screened compounds have good antibacterial activity.
- Some of the screening compound shows a good anticancer activity.
- All the synthesized compound show effective antioxidant activities.

Referenes

1. Daniel, M.C., et al., Synthesis of Five Generations of Redox-Stable Pentamethylamidoferrocenyl Dendrimers and Comparison of Amidoferrocenyl-and Pentamethylamidoferrocenyl Dendrimers as Electrochemical Exoreceptors for the Selective Recognition of H2PO4−, HSO4−, and Adenosine 5′-Triphosphate (ATP) Anions: Stereoelectronic and Hydrophobic Roles of Cyclopentadienyl Permethylation. Chemistry-A European Journal, 2003. 9(18): p. 4371-4379.
2. Hussain, R.A., et al., Synthesis, chemical characterization, DNA interaction and antioxidant studies of ortho, meta and para fluoro substituted ferrocene incorporated selenoureas. Inorganica Chimica Acta, 2013. 402: p. 133-139.
3. Mahmoud, K.A. and H.-B. Kraatz, Synthesis and electrochemical investigation of oligomeric and polymeric ferrocenyl-amides having cyclohexyl, phenylene, and lysyl spacers. Journal of Inorganic and Organometallic Polymers and Materials, 2008. 18(1): p. 69-80.
4. Zaheer, M., et al., Synthesis, characterization, electrochemistry and evaluation of biological activities of some ferrocenyl Schiff bases. Applied Organometallic Chemistry, 2011. 25(1): p. 61-69.
5. Carter, M.T., M. Rodriguez, and A.J. Bard, Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt (III) and iron (II) with 1, 10-phenanthroline and 2, 2'-bipyridine. Journal of the American Chemical Society, 1989. 111(24): p. 8901-8911.
6. Janjua, N.K., et al., Flavonoid–DNA binding studies and thermodynamic parameters. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2011. 79(5): p. 1600-1604.
7. Rajesh, J., et al., Analytical methods to determine the comparative DNA binding studies of curcumin–Cu (II) complexes. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2012. 97: p. 223-230.
8. Randles, J., A cathode ray polarograph. Transactions of the Faraday Society, 1948. 44: p. 322-327.

9. Shah, A., et al., Electrochemical and spectroscopic investigations of protonated ferrocene-DNA intercalation. Analytical Sciences, 2008. 24(11): p. 1437-1441.
10. Zidan, A.S., Mixed Ligand Complexes of Nickel (II) Dialkyldithiophosphates with 2-Acetylpyridine Semicarbazone and 2-Acetylpyridine Benzoylhydrazone. Phosphorus, Sulfur, and Silicon and the Related Elements, 2003. 178(3): p. 567-582.
11. Tweedy, B. Possible mechanism for reduction of elemental sulfur by monilinia fructicola. in Phytopathology. 1964. AMER PHYTOPATHOLOGICAL SOC 3340 PILOT KNOB ROAD, ST PAUL, MN 55121.
12. El-Sherif, A.A. and T.M. Eldebss, Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu (II), Ni (II), Mn (II), Co (II) and Zn (II) complexes with Schiff base derived from 5-bromosalicylaldehyde and 2-aminomethylthiophene. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2011. 79(5): p. 1803-1814.
13. Beckman, J.S. and W.H. Koppenol, Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. American Journal of Physiology-Cell Physiology, 1996. 271(5): p. C1424-C1437.
14. Bondet, V., W. Brand-Williams, and C. Berset, Kinetics and mechanisms of antioxidant activity using the DPPH. free radical method. LWT-Food Science and Technology, 1997. 30(6): p. 609-615.
15. Molyneux, P., The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol, 2004. 26(2): p. 211-219.
16. Giordano, S.H., A review of the diagnosis and management of male breast cancer. The oncologist, 2005. 10(7): p. 471-479.