

# **Part IV**

## **Antitumour studies**

### **Chapter 1**

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## CHAPTER 1

### INTRODUCTION AND REVIEW

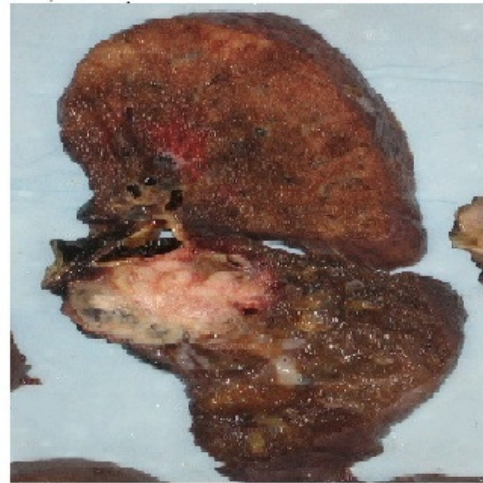
Tumour has a reputation as a deadly disease. A tumour, also known as a neoplasm, is an abnormal mass of tissue which may be solid or fluid-filled. It is a subject of widespread fear since millions of new cases of tumour were reported globally for the last few years. It is reported that about 8.2 million deaths or 14.6% of all human deaths are due to tumour<sup>1,2</sup>. Depending on the shape and the kind of tissue that tumours affect, there are over 100 different types of tumours and a variety of names for them. A tumour is a kind of lump or swelling, it does not necessarily pose a health threat. Nearly fifty percentage of cancer patients die due to the disease or because of the treatment strategies<sup>3</sup>. It is a great matter of concern that the most common types of tumour at present are not easy to treat except which arise from the modern lifestyle. The prognosis of the cancer is quite difficult once it is malignant in the body.

A tumour does not mean cancer ; tumours can be benign (not cancerous), pre-malignant (pre-cancerous) or malignant (cancerous). Tumour sizes may vary enormously. In January 2012, Nguyen Duy Hai, a 32-year-old Vietnamese man underwent a 12-hour operation to remove a 200-pound tumour from his leg. The success of the operation was rated only 50% by lead surgeon Dr. McKay McKinnon. Cancers of lung, prostate, stomach and colorectal are the common types in males whereas in females, the common types of cancers are the cervical, breast and lung<sup>4</sup>. Skin cancer is not included in these statistics and if it were it would account for at least 40% of cases<sup>5,6</sup>. Generally seen cancers in children are brain tumour and leukaemia<sup>7</sup>. Statistics indicate that the risk of cancer is increasing significantly. The increase in life span and changes in lifestyle have

major role in increasing the number of people affected with tumour. The fact that the probability of a secondary cancer is high in people who recovered from cancer primarily<sup>8,9</sup>.

### History of cancer

Cancer is known since the early stages of human history. The report of a breast cancer from circa in 1600 BC is considered as the first record of cancer<sup>10-14</sup>. Different types of cancers were described by Hippocrates who gave the Greek name *karkinos* (crab)<sup>15,16</sup>. It was Celsus who changed the *karkinos* into *cancer* (Latin word) meaning crab. He also proposed the treatment style of surgery for cancer<sup>17</sup>. In the second century Galen suggested purgatives as an alternative to surgery<sup>18</sup>.



Lung cancer

By 17<sup>th</sup> century, the doctors were able to discover the cause of death by analyzing the body<sup>19</sup>. Wilhelm Fabry reported that milk clot in the mammary duct caused breast cancer. Francois de la Boe Sylvius postulated that acidic lymph fluid was responsible for cancer development. In 1761, the physician John Hill found that the cause of nose cancer is tobacco snuff. Later it is published that cancer of the scrotum, commonly seen in chimney cleaners are due to the smoke dust in the chimney. In 1874, the English surgeon Campbell De Morgan formulated that cancer spreads through the lymph nodes to other sites by metastasis<sup>20</sup>.

## **Classifications of cancer**

Cancer is broadly classified into five classes:

1. Sarcomas: affects bone, cartilage, fat, connective tissues, muscles and other supportive tissues.
2. Lymphomas: affects lymph nodes and immune system tissues.
3. Carcinomas: affects internal and external parts of the body such as lung, breast and colon.
4. Leukemias: begin in the bone marrow and accumulate in the blood stream.
5. Adenomas: affects thyroid, the pituitary gland, the adrenal gland and other glandular tissues.

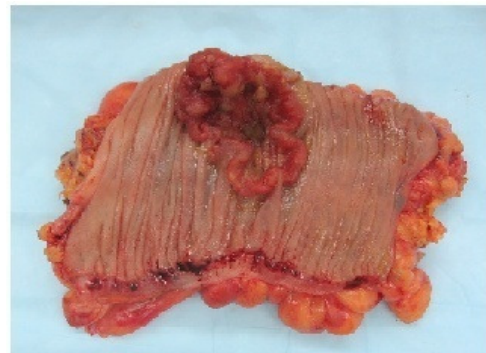
## **Carcinogens**

Carcinogens are substances, exposure to which causes specific types of cancer.

Some of the main carcinogens are described below:

### **Chemicals**

Tobacco smoke: One third of the cancer death is due to tobacco smoking. Cancers of lung, larynx, stomach and pancreas are caused due to tobacco smoking. Literature shows that more than fifty types of carcinogens are present in tobacco smoke.



Colon cancer

Alcohol: In Europe both males and females suffer from cancer because of alcohol consumption. Tumours of the liver and digestive tract are reported in people with alcohol addiction.

Chemicals at work site: It is reported that around two lakh people are affected with cancer from their worksite. Exposure to asbestos fibers and tobacco smoke causes mesothelioma and lung cancer. Frequent contact with benzene can result in leukemia<sup>21</sup>.

### **Radiations**

Radiations can cause tumour in any part of the body irrespective of the age. Ionizing radiations and non-ionizing ultraviolet radiations cause almost 10% of invasive cancers. Medical imaging and radon gas is one of the main sources of ionizing radiations. UV radiations from the sun are responsible for non-melanoma cancer. Long time exposure to radon can result in increased risk of cancer. Ionizing radiations are not a strong mutagen, but together with tobacco or radon they become a more potent source of cancer. Radiation exposure before birth has ten times the effect. Leukemia may be developed in children and adolescents as in adults on radiation exposure.

Prolonged exposure to radiations from the sun, especially the non-ionizing medium wave UVB, can lead to melanoma skin cancer which is one of the most common type of cancer. Radiations from mobile phones and similar sources are also regarded as cancer causing agents by World Health Organization.

### **Diet and exercise**

Lack of proper diet and exercise are considered as the major cause of more than thirty percentage of cancer deaths. Obesity and excess body weight can cause different types of tumours, resulting about 20% deaths due to cancer. Physical inactivity may have negative effects on immune system and endocrine system and can contribute to cancer development.

Some specific foods are linked to specific cancers. Aflatoxin B1, a frequent food contaminate, can result in liver cancer and Betel nut chewing causes oral cancer. A high-salt diet is linked to gastric cancer.

### **Heredity**

Genetic defects are responsible for the hereditary tumours. Majority of ovarian cancer and breast cancer are due to genetic disorder. More than 75% risk of breast cancer and ovarian cancer can be resulted from them. It is found that in about 3% of people suffering from colorectal cancer, hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome) is also associated.

### **Infection**

Approximately 18% of cancer deaths are related to infectious diseases. Cancer causing viruses are known as *oncovirus*. Examples are Epstein–Barr virus, human papilloma virus, hepatitis B and C viruses, Human T-cell leukemia virus-1 and Kaposi's sarcoma herpes virus. Cancers like gastric carcinoma are caused by bacteria. Infections by parasites are also responsible for cancer.

### **Physical agents**

These are substances that can cause cancer on prolonged exposure. For example, asbestos can cause mesothelioma in serous-membrane. Particulate matter which are non fibrous in nature, like crystalline silica, cobalt and nickel powders are also carcinogenic. Attapulgit, rock wool, wollastonite and glass wool are also responsible for cancer.

### **Hormones**

Some hormones can promote proliferation of cell. Differentiation, proliferation and apoptosis of cancer cells can be due to Insulin like hormones and the attached

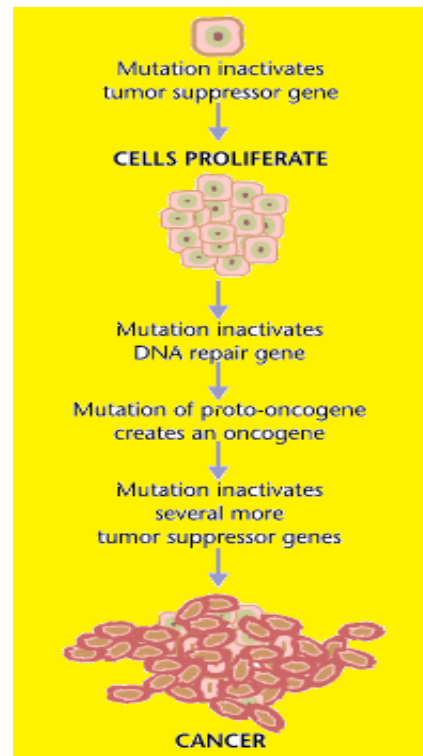
protein. Some hormones can produce tumour in endometrium, breast and prostate. Obese people have higher levels of some hormones and are associated with cancer. Growth hormones can also promote osteosarcoma. Up to a certain extent the excessive hormone levels can be reduced by regular and systematic exercises by which the risk of cancer can be minimized. These hormone levels can be reduced artificially by some specific treatments.

### Pathophysiology

Repeated mutations in DNA can result in development of tumour. Each mutation can alter the nature of the cell. Cancer is due to the uncontrolled cell growth which is the result of changes in genes. Mutations will result in two types of genes, oncogenes and tumour suppressor genes. The first one promotes tissue growth and the second one suppresses cell division. Tumours are the result of inappropriate performance of oncogenes or tumour suppressor genes. Errors during mitosis can result in complete destruction of chromosomes. Genetic deformations are possible by a number of mechanisms.

Moreover the presence of certain environments like ionising radiations, carcinogens or hypoxia can cause genetic deformations. The defects are self multiplying, resulting in excess reproduction of the cells compared to normal cells.

The conversion of normal cell into a cancer cell is similar to an uncontrolled chain reaction. These



chain processes may result in more invasive stages and thereby develop the cancer in a faster rate<sup>22</sup>.

### **Epigenetics**

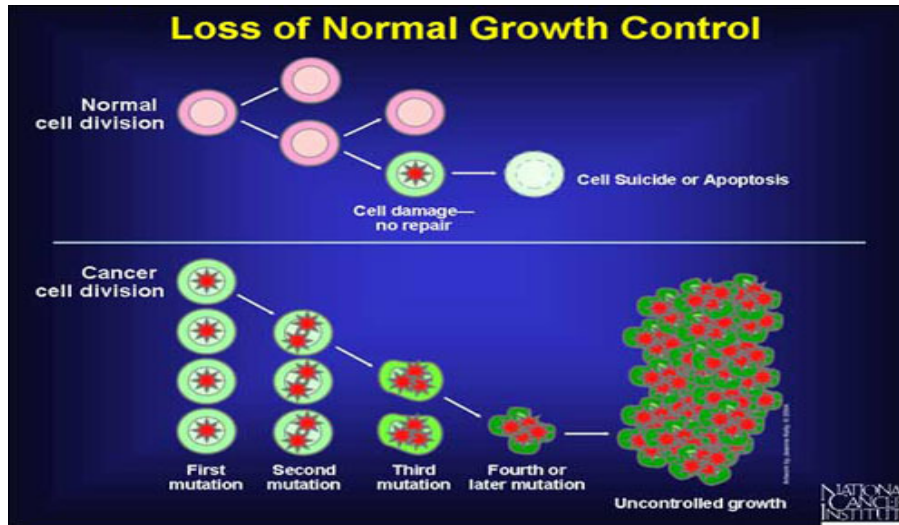
Epigenetic changes also can cause cancer<sup>23</sup>. Modifications in the genome without alteration in nucleotides are termed epigenetic alterations. Even though there are many types of epigenetic changes related to cancer, those changes in DNA repair genes are mainly responsible for cancer.

Epigenetic alterations occur frequently in cancers and will result in alteration in hyper or hypomethylation of DNA or chromosomal changes<sup>24,25</sup>. The poor performance of DNA repairing genes will allow permanent changes in DNA. When DNA repair is deficient DNA damages remain in cells at a higher level than usual level and these excess damages cause increased mutation or epimutation<sup>26-29</sup>. It is found that many heavy metals also are carcinogenic and they can reduce the activity of DNA repair genes.

### **Metastasis**

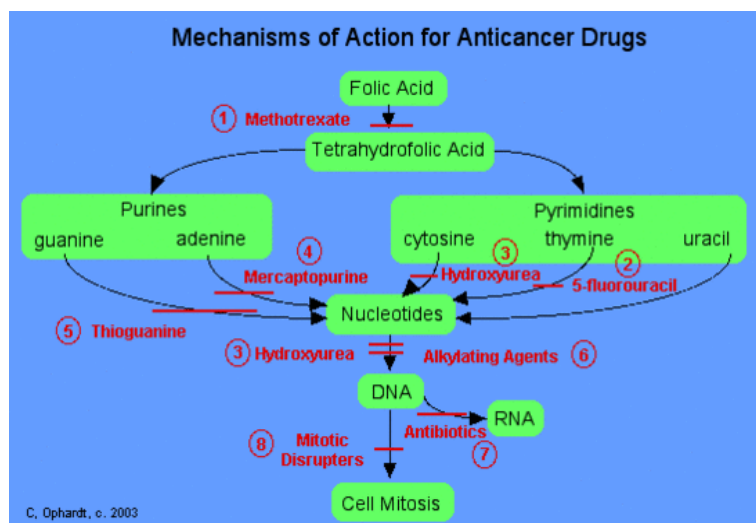
Metastasis is the spread of cancer to other parts of the body. It was found that all types of cancers has the ability to metastasize. It is the most devastating aspect of cancer and it is often resistant to conventional treatment methods. The newly formed tumour cells are known as metastatic tumours, which will ultimately result in death of the organism<sup>30</sup>. In the late stages of cancer metastasis becomes prominent either through blood or lymphatic system<sup>31-33</sup>. Metastasis is very common in liver, bones and brain. The various steps in metastasis include invasion, intravasation, extravasation, proliferation and angiogenesis<sup>34</sup>.





## Anticancer drugs

The anticancer drugs either kill cancer cells or modify their growth. They are used along with treatments like radiotherapy, immunotherapy and surgery, against solid metastatic tumours. The mechanism of action of each anticancer drug is different on normal and tumour cells. The efficiency of anticancer drugs is based on their toxicity on normal cells, since there is biochemical similarity between cancer cells and normal cells. The fact that cancer cells can develop resistance against a specific drug, suggested the possibility of using combinations of various drugs during the treatment.



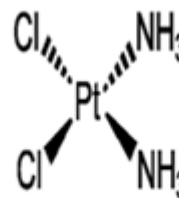
## Classification of anticancer drugs

1. Alkylating agents
  - Platinum coordination complexes: Cisplatin, Carboplatin, Oxaliplatin
  - Nitrogen mustards: Melphalan, Ifosfamide, Cyclophosphamide,
  - Nitrosoureas
  - Triazene
  - Alkylsulfonates
  - Ethyleneimines
  - Methyl hydrazines
2. Antimetabolites
  - Folate antagonists: Methotrexate
  - Pyrimidine antagonists: 5-Fluorouracil, Cytarabine
  - Purine antagonists
3. Natural products
  - a. Plant products
    - Taxanes: Paclitaxel, Docetaxel
    - Vinca alkaloids: Vincristine, Vinblastine
    - Epipodophyllotoxins: Etoposide
    - Camptothecins: Irinotecan
  - b. Microorganism products
    - Enzymes: L-Asparaginase
    - Antibiotics: Bleomycin, Doxorubicin,
4. Miscellaneous
  - Imatinib mesylate
  - Epirubicin
  - Hydroxy urea
  - Bortezomib
  - Gemcitabine
  - Leucovorin
  - Zoledronic acid
  - Gefitinib

- Rituximab
  - Pamidronate
- 5. Hormones and Antagonists**
- Estrogens: Ethinyloestradiol
  - Androgen: Testosterone propionate
  - Corticosteroids: Dexamethasone, Prednisone
  - Aromatase inhibitor: Anastrozole, Letrozole
  - Antiestrogens: Tamoxifen
  - Progesteron derivative: Megestrol Acetate
  - Antiandrogen: Bicalutamide, Flutamide

### **Coordination complexes as antitumour drugs – a review**

The therapeutic use of transition metal complexes in cancer and leukemia treatment date from the sixteenth century. In 1960 the anti-tumour activity of an inorganic complex cis-diamminedichloroplatinum(II) (cisplatin) was discovered. It has developed into one of the most frequently used and most effective cytostatic drug for treatment of solid carcinomas. Other metals like gallium, germanium, tin, bismuth, titanium, ruthenium, rhodium, iridium, molybdenum, copper and gold were shown effective against tumors in man and animals<sup>35</sup>. Research on other metals mainly transition metals as anticancer drugs have proved the dominance of these metals by low cost, increased coordination sites, changes in oxidation states, changes in ligand affinity and the substitute kinetics, as well as the excited light-emiting properties that can be used for treatment and so on. Those related coordination compounds vary in composition and



**Fig. 4.1** Structure of cisplatin

structure, including amino acid complexes, heterocyclic complexes, Schiff base complexes, dicyclopentadienyl coordination compounds, binuclear complexes and so on.

Cisplatin or *cis*-diamminedichloroplatinum(II) (Figure 4.1) is the first member of a class of platinum containing anti-cancer drugs, which also includes carboplatin and oxaliplatin. These platinum complexes react *in vivo*, binding to and causing cross-linking of DNA, which ultimately triggers apoptosis<sup>36</sup>. Cisplatin moves into the cell through diffusion and active transport. The compound *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>(Cl)<sub>2</sub>] was first described by Michele Peyrone in 1845 and known for a long time as Peyrone's salt. Inside the cell it causes platination of DNA, which involves cross-linking as well as formation of adducts, usually through guanine. Formation of cisplatin DNA adducts causes distortion and results in inhibition of DNA replication.

Gold complexes also show anti-cancer activity. These complexes act through a different mechanism as compared to cisplatin<sup>37</sup>. The target site of Au complexes is mitochondria not DNA. Certain gold complexes with aromatic bipyridyl ligands have shown cytotoxicity against cancer cells. The 2-[(dimethylamino)methyl]phenyl gold(III) complex has also proven to be antitumor agent against human cancers. Gold nano particles when used in combination with radiotherapy or chemotherapy enhance DNA damage and make the treatment target more specific<sup>38</sup>.

Ansari et. al.<sup>39</sup> in 2009 studied some complexes of Mn(III) induce tumor selective apoptosis of human cells. Some oxindole-Schiff base copper(II) complexes were analysed by Ramadoss Gomathi et. al.<sup>40</sup> to explore the potential antitumor activity towards different cells. Cytotoxicity experiments carried out towards human liver HepG2

cells confirmed its proapoptosis property and the copper complexes were found to have excellent anticancer activity against HepG2 liver cells.

Several Schiff bases and their complexes were reported to possess potential anticancer properties. Shahriar Ghammamy et. al.<sup>41</sup> synthesized complexes such as  $[\text{Fe}(\text{pythsal Br})\text{Cl}_2]$  with the NSNO-donor tetradentate Schiff base ligands. The ligand pythsal HX [(5-X-N-(2-Pyridylethyl sulfanylethyl)salicylidenemine] (X=I, Br) was obtained from the inserted condensation of (1,2-pyridyl)-3-thia-5-aminopentane with the respective derivative of salicylaldehyde in a 1:1 molar ratio and their iron (III) complexes were studied for their antitumor properties.

Kuzmin et. al.<sup>42</sup> reported that a series of macrocyclic Schiff bases of 2,6-bis (formyl aryloxymethyl)pyridines were synthesized and studied their anticancer activity relationship by the topological approach. The anticancer activity and structural parameters of the molecules were correlated and on the basis of *in vitro* screening data their structures were characterized. The influence of structure of the studied compounds, as reflected by the parameters studied on the anticancer activity, was established.

Gupta et. al.<sup>43</sup> in 2011 synthesized new ligands, N-benzyl-2-(diethylamino)acetamide, (HL<sub>1</sub>) and 2-(diethylamino)-N-phenylethylacetamide(HL<sub>2</sub>) and these ligands have been used to synthesize copper(II) complexes  $[\text{Cu}(\text{HL}_1)_2(\text{ClO}_4)_2]$  and  $[\text{Cu}(\text{HL}_2)_2(\text{ClO}_4)_2]$  respectively. Antitumour screening of these complexes against the U87 and HeLa cancerous cells revealed excellent activity. The complexes were effective for growth inhibition and cell death in a concentration and time dependent manner for both U87 and HeLa cell lines. The anticancer activity might be due to the formation of DNA adducts with cancer cell and thereby inhibiting the DNA replication.

1,2-pyrazole derivatives were found to possess various biological activities by Mathew V. et. al.<sup>44</sup>. It was also observed that incorporation of aryl substituent and halogen atoms into the heterocyclic ring systems enhanced the biological activities considerably. Non-steroidal aromatase inhibitors obtained from triazole derivatives are used in the treatment of breast cancer<sup>45</sup>.

Studies have shown that amino acid Schiff bases and their metal complexes have anticancer as well as antiviral activities and could also inhibit superoxide anion radicals<sup>46</sup>. In 1971, Hodnet et. al.<sup>47</sup> synthesized a series of Schiff base metal complexes and carried out antitumor experiments, which indicated that aldehyde substituent was superior to amine substituent in anticancer effect and salicylaldehyde Schiff bases were superior to other aldehyde Schiff bases. To overcome the toxicity and poor water-solubility of amino acid Schiff bases, either Schiff bases can be sulphonated or hydrophilic groups might be introduced in the parent compound, or a second ligand might be introduced hoping to improve its activity.

A number of Schiff base copper(II) complexes are regarded as the most promising alternatives to cisplatin as anticancer drugs. In particular, the involvement of copper in human diseases has been described from medicinal, chemical and biochemical view, focusing on the molecular physiology of Cu transport.

Hernández W. et. al.<sup>48</sup> studied the antitumor activity of the copper(II) complexes with acylthiourea ligands. The *in vitro* activity studies against the mouse mammary adenocarcinoma TA3 cell line showed that these complexes have much higher cytotoxic activity (IC(50) values in the range of 3.9-6.9 $\mu$ M) than their corresponding ligands (40-240 $\mu$ M). This clearly indicates that the antitumor activity is increased on coordination

of the chelate ligands around the Cu(II) metal. Furthermore, this result confirmed that the involvement of the nitro and chloro substituent groups in the complex activities is slightly relevant. The high accumulation of the copper complexes in TA3 tumour cells and the much faster binding to cellular DNA were consistent with the *in vitro* cytotoxic activities found for these copper complexes.

Three new copper complexes were synthesized with pyridoxal semicarbazone as ligand by Violeta Jevtovic<sup>49</sup> and were subjected to anticancer tests. Specifically, an activity was demonstrated in breast cancer cells. Birjesh Singh<sup>50</sup> synthesized a Cu(II) complex with 6-thioguanine and C-57BL/6 mice has been used for anticancer screening of metal complex for *in vitro* and *in vivo* study. The results revealed that the complex is more potent as compared to the pure drug as regards to its anticancer activity.

Investigations of D. Palanimuthu et. al.<sup>51</sup> says that copper complexes of thiosemicarbazides especially those derived from glyoxal-bis(4-methyl-4-phenyl-3-thiosemicarbazone) have great cytotoxic activity against various human cancer cells, almost same as that adriamycin drug. The corresponding zinc complex showed much less activity than the copper complex. It was found that the Cu complexes were able to inhibit the DNA synthesis and can produce apoptosis. DNA cleavage was found to occur because of these complexes. It is found that *in vivo* administration of copper complex significantly inhibited the tumour growth in HCT116 xenografts in nude mice.

Unfortunately, the majority of drugs currently on the market are not specific, which leads to many common side effects associated with cancer chemotherapy. Because the common approach of all chemotherapy is to decrease the growth rate of the cancer

cells, the side effects are seen in bodily systems that naturally have a rapid turnover of cells including skin, hair, gastrointestinal and bone marrow.

### Scope of present investigation

Copper(II) complexes are regarded as the most promising alternatives to cisplatin and other anticancer drugs. A number of Cu(II) chelate complexes that exhibit cytotoxic activity through cell apoptosis or enzyme inhibition have been reviewed. Such complexes can significantly increase the survival of the hosts. Current interest in copper complexes is stemming from their potential use as antitumor agents. The special structure and ligand diversity determine the chemical and biological significance of these Schiff base complexes.

In the present work, some new copper(II) complexes were prepared with different Schiff base ligands like 3-(1H-indol-3-yl)-2-[(thiophen-2-ylmethylidene)amino]propanoic acid (I3YT2YMAPA), 3-[thiophen-2-ylmethyleneamino]benzoic acid (T2YMABA), 4-(5-[(2-carbamothioylhydrazono)methyl]thiophen-2-yl)benzoic acid (CTHMT2YBA), 4-(5-[(2-phenylhydrazono)methyl]thiophen-2-yl)benzoic acid (PHMT2YBA), 4-(5-[(2-carbamothioylhydrazono)methyl]furan-2-yl)benzoic acid (CTHMF2YBA), 2-(1-[pyridin-3-yl]ethylidene)hydrazinecarbothioamide (P3YEHCTA), 3-(1-(2-phenylhydrazono)ethyl)pyridine (PHEP), 3-[anthracen-9(10H)-ylideneamino]propanoic acid (A9Y3APA), 2-[anthracen-9(10H)-ylideneamino]-3-(1H-imidazole-4-yl)propanoic acid (A9Y3IMPA) and 2-[anthracen-9(10H)-ylideneamino]-3-phenylpropanoic acid (A9Y3PPA).

These complexes were screened for their *in vitro* cytotoxic activity against Dalton's lymphoma ascites cells. The toxicity of the copper complexes were checked by *in vivo* studies. Then the highly active copper(II) complexes were selected for conducting



*in vivo* ascites tumour studies on Swiss albino mice, in which the mortality rate of the tumour bearing mice were noted. The percentage increase in life span was determined for different concentrations of each drug and compared with standard drug cyclophosphamide.