A multi-cellular organism can grow and develop well only when all cells of the body, functions in accordance with the rules and regulations that govern cell growth and reproduction (Aktipis et al. 2015). Quite different from normal cells, cancer cells defiance to the normal control, starts dividing, devastates their hierarchy, invading distant and surrounding tissues, usurp resources and ultimately can leads to no longer survival of the body in which it lives. Cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis. The term cancer used to describe more than 100 forms of disease which possess the common characteristic features like self-sufficiency in growth signals, insensitivity to growth inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011).
Figure 1: Acquired six major capabilities of cancer. (Source: Hanahan D and Weinberg RA, The hallmarks of cancer. Cell 100: 57-70, 2000).

Acquired growth signals (GS) independency is the first of the six capabilities of cancer cells. Normal cells require mitogenic signals before entering into an active proliferative state from a quiescent state. Cancer cells strongly contrast to such behavior to that of normal cells, which invariably show a very less extent dependency on exogenous growth stimulation and generate many of their own growth signals. Insensitivity of antiproliferative signals is the second of six capabilities of cancer cells. Most of the signaling pathways that enable normal cells to respond to antigrowth signals are associated with the cell cycle regulation, particularly for G1 phase of cell cycle. Cells scrutinize their internal as well as external environment and on the basis of perceived signals, decide whether to proliferate or to be into G0 (quiescent) state (Weinberg 1995). Cancer cells are deaf
and have no regulation in response to antiproliferative signals which operate to maintain cellular quiescence and tissue homeostasis. To maintain the tissue homeostasis, the rate of cell proliferation and destruction should be maintained. Growth of tumor cells not only determined by cell proliferation rate but also picked up the pace by decreased destruction rate. Apoptosis i.e. programmed cell death represents a major source of careful destruction of cells. It was evidenced from extensive studies of biopsied human carcinogenic samples, in vitro as well as in vivo mouse models that cancer cells acquired ability to evade apoptosis which is a hallmark of most and perhaps all types of cancer. A certain number of doublings due to limited replicative potential (Hayflicks limit) and followed by senescence is a basic features of normal cells however most types of tumor cells that are propagated in culture appear to be immortalized i.e. limitless replicative potential (Hayflick 1997). Telomere maintenance is evident in almost all types of malignant cells (Shay and Bacchetti 1997) and maintenance of telomeres above a critical threshold length provides/permits unlimited multiplication of descendant cells (Bryan et al. 1995; Bryan and Cech 1999). Oxygen and nutrient supplementation are crucial for cell survival and tightly regulated through angiogenesis, which restrict all cells in a tissue to live within 100nm of a capillary blood vessel. But in case of tumor progression the balance between angiogenic inducer and countervailing inhibitor were disturbed and angiogenic switch were activated (Hanahan and Folkman 1996). The exact mechanisms for imbalance between angiogenic regulators remain incompletely understood but tumor angiogenesis may be a uniquely attractive therapeutic target.
About 90% of human cancer deaths occur due to metastasis i.e. distant invasions of tumor cells (Sporn 1996). The capability for metastasis and invasion facilitate cancer cells to inhabitate at new terrain in the body where at least initially, nutrients and space are not limiting. Moreover, genome instability was also an enabling characteristic of cancer cells may be obtained due to frequent mutations, defected repair mechanism and altered check points proteins (Jeggo et al. 2016). Initiation and progression of cancer is no longer a mystery. All through in last two decades, researchers accomplished amazing progress in the etiology of cancer even at molecular level. The molecular events that lead to the cancer-initiating cell involve critical mutations in genes regulating normal cell growth and differentiation. Cancer initiation and progression is regarded as a multi-step process, which is reflected by progressive genetic alterations that drive the transformation of normal human cells into highly malignant derivates (Zhou et al. 2009). At least three important classes of genes play key roles in tumor initiation: proto-oncogenes, tumor suppressor genes, and genes involved in DNA repair mechanisms. Mutations, amplifications or deletions in these genes may lead to a de-coupling of biological mechanisms involved in the regulation of normal cell growth and differentiation (Lee and Muller 2010).

Cancer causing factors can be divided in three broad categories:

**Environmental factors:**

The term "environment" as used by biologist, refers to everything outside the body that interacts with humans and it accounts for 50–60% of cancer deaths word wide.
In this sense, the environment is not limited to the biophysical environment (e.g. exposure to factors such as air pollution or sunlight, encountered outdoors or indoors, at home or in the workplace), but also includes lifestyle, economic and behavioral factors (Anand et al. 2008). Further it can be divided into three subgroups:

A. Chemical carcinogens (25 - 30% of cancer death)

B. Physical carcinogens (Up to 10% of cancers)

C. Infections (18% of cancer)

**Dietary factors and physical inactivity:**

Diet, physical inactivity and obesity are related to approximately 30–35% of cancer deaths (Kushi et al. 2012). Diets that are low in vegetables, fruits and whole grains, and high in processed or red meats are linked with a number of cancers (Park et al. 2008). A high-salt diet is linked to gastric cancer, aflatoxin B1, a frequent food contaminate, with liver cancer, and Betel nut chewing with oral cancer (Brenner et al. 2009). Physical inactivity is believed to contribute to cancer risk not only through its effect on body weight but also through negative effects on immune system and endocrine system.

**Other factors:**

Up to 3-10% of cancer death occurs due to other factors like Heredity, hormones, age and life style. Among all, most cancers are non-hereditary ("sporadic cancers") and less than 0.3% of the populations are carriers of a genetic mutation which has a
large effect on cancer risk and these causes less than 3–10% of all cancer (Roukos 2009).

Generally (not in all cases) cancer starts from tumor and can be classified in two types, primary tumor and secondary/advanced/malignant tumor. In healthy human body, approximately $3 \times 10^{18}$ cells are there and each cell can be transformed into malignant one. Almost every tissue in the body can spawn malignancies some even yield several types. The 30 trillion cells of the normal, healthy human body live in a complex, interdependent condominium, regulating one another proliferation.

According to primary site of origin from which organ cancer cells originated, cancers may be of specific types like breast cancer, lung cancer, prostate cancer, liver cancer, renal cell carcinoma (kidney cancer), oral cancer, brain cancer etc (Shalini et al. 2011). Based on cell types, cancer can be classified into four major categories:

**Carcinoma (epithelial origin):**

This type of cancer originates from the epithelial layer of cells that form the lining of external parts of the body or the internal linings of organs within the body. Carcinomas, malignancies of epithelial tissue, account for 80 to 90% of all cancer cases since epithelial tissues are most abundantly found in the body. Carcinomas can be further divided into two groups, Adenocarcinoma and Squamous cell carcinoma.

**Sarcoma (connective and supportive tissue origin):**

These cancers originate in connective and supportive tissues including muscles, bones, cartilage and fat. Different types of sarcomas are Osteosarcoma (bone), Chondrosarcoma (cartilage) Mesothelial sarcoma or mesothelioma (membranous
lining of body cavities), Fibrosarcoma (fibrous tissue), Angiosarcoma or hemangioendothelioma (blood vessels), Liposarcoma (adipose or fatty tissue) and Glioma or astrocytoma (neurogenic connective tissue found in the brain).

**Leukemia (blood cancer) and Lymphoma:**

Leukemia is a group of cancers that affects blood cells. These cancers affect the bone marrow which is the site for blood cell production. In this condition, bone marrow starts to produce excessive immature Red blood cells (RBCs) that fail to perform their usual actions. Unlike the leukemia it affects lymph nodes of lymphatic system at specific sites like stomach, brain, intestines etc (Cancer Research UK 2014). Lymphomas may be of two types, Hodgkin’s lymphoma and Non-Hodgkin’s lymphomas. In Hodgkin lymphoma there is characteristic presence of Reed-Sternberg cells in the tissue samples which are not present in Non-Hodgkin lymphoma.

**International and national cancer statistics:**

Latest online database of WHO, International Agency for Research on Cancer (IARC), GLOBOCAN 2012, provides a comprehensive overview of global cancer status of 28 types of cancer in 184 countries. According to GLOBOCAN 2012, it accounts for 14.1 million new cancer cases and 8.2 million cancer related deaths occurred in 2012 and if compared with year 2008 statistical data it increased up to 1.4 million and 0.6 million respectively.

Among all types of cancer diagnosed, Lung cancer is the most common cancer worldwide (1.8 million, 13% of the total), breast cancer (1.7 million, 11.9%), and
colorectal cancer (1.4 million, 9.7%). Similar kind of data were estimated in respect
to cancer mortality, the most common causes of cancer death were cancers of the
lung (1.6 million, 19.4%), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%).
Breast cancer incidence in 2012 has increased by more than 20%, while mortality
has increased by 14%. Breast cancer is also the most frequently diagnosed cancer
and most common cause of cancer death among women (approximately 5.2 lacks
deaths) worldwide (Ferlay 2013).

On the basis of previous data, GLOBOCAN 2012 predicted a substantive increase to
19.3 million new cancer cases per year by 2025. More than half of all cancers
(56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions of the
world, and these proportions will increase further by 2025. General worldwide trends
show that in developing countries like India, going through rapid changes in social
and economical status. People’s lifestyle in developing countries shifted towards
typical industrialized countries leads to increase burden of cancers associated with
reproductive, dietary, and hormonal risk factors (Weiderpass 2010).

![Figure 2: Estimated incidence of cancer and mortality rates in men and women worldwide (Source: IARC, GLOBOCAN 2012 Section of Cancer Surveillance, WHO).](image-url)
In India it accounts for 1014.9 thousands new cancer cases and 682.8 thousands cancer related deaths occurred in 2012. In all types of cancer diagnosed, breast cancer is the most common cancer in India (14.3% of the total cancer incidence), cervix cancer (12.1%), lip and oral cancer (7.6%) and lung cancer (6.9%). Whereas top four cancer mortality occurs in India due to breast cancer (10.3%), cervix-uteri cancer (9.9%), lung cancer (9.3%) and stomach cancer (8.6%). In India, lip, oral cavity cancers (11.3%) and lung cancer (11.3%) are more common in men where as breast cancer (21.5%) and cervix-uteri cancer (20.7%) are highly incident in women.

Figure 3: Estimated incidence of cancer and mortality rates in men and women in India (Source: IARC, GLOBOCAN 2012 Section of Cancer Surveillance, WHO).

The origins of therapy for cancer are evidenced in ancient documents. Malignancy and their treatments are described in the Ramayana, the Edwin Smith papyrus and the Ebers papyrus. Since ancient times, topical preparations were used for the most cancer treatment although removal of neoplasm had been practiced in ancient times (Papac 2001). In the eleventh century, Systemic arsenical therapy was used by an Arabic physician, Ibn Sina however Arsenical preparations known as Unguentum Aegypticum were used topically until the sixteenth century (Shen et al. 1997). Many consider the use of potassium arsenite and arsenicals (Fowler's solution) to treat
chronic myelogenous leukemia in 1865 by Lissauer as the first instance of effective chemotherapy for malignant disease and continued until the 1930s (Forkner and Scott 1931). The origins of effective cancer chemotherapy considered from World War I when mustard gas (sulfur mustard) was used and the advent of World War II stimulated further research on chemical warfare. A series of analogues of sulfur mustards were produced as potential offensive agents (Krumbhaar and Krumbhaar 1919). Further in 1969, Barnett Rosenberg serendipitously discovered the anticancer activity of platinum complexes which was one of the milestone developments in the field of metal complexes in medicine which remains the world’s highest selling anticancer drug (Rosenberg et. al., 1969; van Rijt et al. 2009). Cis-diamminedichloroplatinum (ii) or cisplatin is the first member of inorganic complexes discovered and now has comparable to organic drugs, such as Adriamycin and Fluorouracil (Frezza et al. 2010). However, drugs resistance to tumor cells, severe systemic toxicity and several other side effects frequently limit the clinical usefulness of cisplatin. This has led to the development of 2nd generation platinum drugs, such as carboplatin and oxaliplatin having lower toxicity and are being used for the treatment of ovarian and colon cancer, respectively. Third generation compounds are also being explored, including the first orally active platinum drug, Satraplatin and is currently undergoing clinical trials. The discovery of anticancer activity of cisplatin and its modification to develop its analogs that stimulated research in the area of metal-based antitumor drugs (inorganic medicinal chemistry) worldwide (Ali et al. 2013).
Figure 4: The chemical structures of the 2\textsuperscript{nd} generation platinum anticancer agents Carboplatin and Oxaliplatin and the 3\textsuperscript{rd} generation orally active drug Satraplatin.

Chemotherapy drugs interfere with properties of fast proliferating cells in various possible ways e.g. DNA replication phenomenon or the separation of newly formed chromosomes. Most chemotherapeutic agents target all rapidly dividing cells and are not specific to cancer cells. Although some degree of specificity may come due to specific characteristic features of cancer cells like inability to repair DNA damage or high expression of receptors. Therefore chemotherapy shows side effects on normal and healthy tissue, especially those tissues that have a high replacement rate (e.g. intestinal lining, skin cells and hair cells) and these normal cells usually repair themselves after chemotherapy (Liang et al. 2010). There are several different approaches to treat the cancer depending on the form of cancer and how advance it is but complete cure for advanced stages of cancer is still needed. However, continuous efforts are going on to develop new drugs with novel mechanisms of action to overcome the side effects and limitations of existing drugs.

Now, in recent years, several other metal (non-platinum) complexes have been extensively investigated for their antitumor activity and it has been observed that the derivatives of ruthenium, rhodium, iridium, and palladium as well as other
complexes containing copper, gallium, titanium, tin and germanium are having ability to developed as anticancer drugs (Rafique et al. 2010). In order to search an alternative anticancer drug, several metals including ruthenium, rhodium and iridium have been investigated as possible cancer chemotherapeutics. Ruthenium compounds having potential to develop new anticancer drugs as alternative to current chemotherapeutic drugs, due to the decreased toxicity and variable oxidation states accessible under physiological conditions (Jungwirth et al. 2011). Two ruthenium drugs, NAMI-A (New Anti-tumor Metastasis Inhibitor) and KP1019, or trans-[tetrachlorobis(1 H-indazole)ruthenate(III)] are currently in clinical trials (Antonarakis and Emadi 2010).

Figure 5: Chemical structure of NAMI-A and KP1019 drugs currently in clinical trials.

NAMI-A is an arene Ru(III) drug and antitumor metastatic inhibitor in nature that is used for treatment of non-small cell lung carcinoma (NSCLC) through reducing metastases rate without affecting the primary tumor (Antonarakis and Emadi 2010). KP-1019 is activated by reduction of RuIII to RuII in tumor tissues with hypoxic
environment by reducing sulfur rich biomolecules such as glutathione (Gransbury et al. 2016). KP-1019 has been used in clinical trials for colorectal cancer and both drugs described above are well tolerated by patients (Graf and Lippard 2012). Studies on NAMI-A and KP-1019 offer novel chemotherapeutics outside of platinum related drugs and display how transition metals can provide several interesting complexes may be capable to induce apoptosis. Alternatively, the DNA binding ruthenium compounds provides an alternative to platinum drugs with a non-covalent mechanism of DNA interactions (Adhireksan et al. 2014). But the problems are remain persist with all these approaches like lack of specificity, as these drugs do not discriminate between cancer cells and healthy cells.

Based on these facts it is worthy to undertake this investigation on three different series of newly synthesized Ru, Rh and Ir complexes. The advantages of these new complexes are that these have DNA and protein binding abilities and well characterized by physical and electrochemical studies like Elemental analysis, ESI-MS and NMR. Structure has been authenticated by X-ray single crystal analysis. The following three series of novel Ru Rh and Ir complexes are given below:
**Series 1:** Twelve (12) newly synthesized complexes of a series of organometallic Ru, Rh and Ir arene complexes.

![Comp. 1](image1)
![Comp. 2](image2)
![Comp. 3](image3)
![Comp. 4](image4)

![Comp. 5](image5)
![Comp. 6](image6)
![Comp. 7](image7)
![Comp. 8](image8)

![Comp. 9](image9)
![Comp. 10](image10)
![Comp. 11](image11)
![Comp. 12](image12)

**Series 2:** Four (4) novel heteroleptic dipyrrinato arene complexes of transition metal Ru, Rh and Ir containing a new chelating ligand [4-(2-methoxypyridyl)-phenylidipyromethene (2-pcdpm)].

![D 1](image13)
![D 2](image14)
![D 3](image15)
![D 4](image16)
Series 3: Four (4) novel arene complexes of transition metal Ru, Rh and Ir containing chelating ligand [4-mtdpm = 5-(4-methylthiophenyl)-dipyrrromethene].

Since complexes described in this thesis are evaluated primarily in perspective for their potential to act as anticancer drugs whose findings may led to the modern era of cancer treatment. The step by step strategy and work plan will be adapted to screen most appropriate complexes for specific types of cancer. In this regard, the complexes will be passed though short term toxicity test in vitro and most active complexes will be selected for further studies like cytotoxicity and antiproliferation on different cancer and normal cell lines, antitumor study in vivo, apoptosis inducing properties and molecular basis of probable mechanism of apoptosis induction.
Therefore to screen and study for the anticancer activity of above novel complexes, following objectives was set:

**Objectives:**

1. Evaluation of cytotoxicity and antiproliferation activity of arene complexes *in vitro* and *in vivo*.

2. Detection of apoptotic induction and effect on cell cycle phase distribution.

3. To determine the possible molecular mechanism of anticancer activity of arene complexes.