CHAPTER - 1

GENERAL INTRODUCTION
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1.1. Cancer:

Cancer is a devastating disease - but largely preventable. Its impact can be reduced through basic research and improvements in treatment and care. It is estimated that there would be over 12 million people diagnosed with cancer in 2008. The global cancer burden doubled in the last thirty years of the twentieth century, and it is estimated that this would double again between 2000 and 2020 and nearly triple by 2030 according to the World health organization (WHO) world cancer report (Boyle et al., 2008). According to the report, in the year 2000, malignant tumors were responsible for 12 per cent of the nearly 56 million deaths worldwide from all causes. In many countries, more than a quarter of deaths are attributable to cancer. In 2000, 10 million people developed a malignant tumor and altogether 6.2 million died from the disease. Cancer has emerged as a major public health problem in developing countries, matching its effect in industrialized nations. The most common cancers worldwide, excluding non-melanoma skin cancers, are cancers of the lung, breast and colorectal tissue. As per figures for the year 2000, lung cancer is the most common cancer worldwide, accounting for 1.2 million new cases annually; followed by cancer of the breast, just over 1 million cases; colorectal, 940,000; stomach, 870,000; liver, 560,000; cervical, 470,000; esophageal, 410,000; head and neck, 390,000; bladder, 330,000; malignant non-Hodgkin lymphomas, 290,000; leukemia, 250,000; prostate and testicular, 250,000; pancreatic, 216,000; ovarian, 190,000; kidney, 190,000; endometrial, 188,000; nervous system, 175,000; melanoma, 133,000; thyroid, 123,000; pharynx, 65,000; and Hodgkin disease, 62,000 cases. The cancers which caused the greatest proportion of deaths were those of the lung, stomach and liver, because of the relative success of early intervention in breast and colorectal cancers (Stewart et al., 2003).

Investigations into cancer causation had revealed that the most important human carcinogens include tobacco, asbestos, aflatoxins and ultraviolet light. In addition, nearly 20 percent of cancers are associated with chronic infections, the most significant ones being hepatitis B and C viruses (liver cancer), human papilloma viruses (cervical and anogenital cancers) and Helicobacter pylori (stomach cancer). In developed countries chronic infection causation amounted to only 8 percent of all malignancies, whereas in developing
countries up to 25 percent of tumors were associated with chronic infections. “Once considered a "Western" disease, more than 50 per cent of the world’s cancer burden, in terms of both numbers of cases and deaths, already occurs in developing countries. In addition to substantial opportunities for primary prevention, the emphasis is on the potential of early detection, treatment and palliative care (Stewart et al., 2003).

1.2. Need for the development of anti-cancer drugs:

Great strides have been made in the effective treatment of some forms of cancer by means of chemotherapy used alone or in combination with other modalities (Siemann et al., 2005). Unfortunately, however, the number of available clinically active antitumor agents remains quite small and the spectrum of clinical antitumor activity is generally rather limited. The ultimate potential of chemotherapy in cancer treatment still remains unrealized (Andrew T et al., 1997).

A quantum leap in effective cancer chemotherapy requires the discovery and development of new anticancer drugs with unprecedented antitumor activities, specificities, and mechanism of action. Anticancer drugs have well known therapeutic limitations, which have continued to stimulate the search for new agents with enhanced therapeutic efficacy. Earlier studies recognized that the growth of both normal and neoplastic cells is affected by intracellular levels of chemotherapeutic agents. Modification of drug activity, however, involved the use of modulating agents that may offer selective protection against toxicity of normal tissue without compromising anti-tumor activity (Indap MA et al., 1991). Increasing understanding of cellular and molecular biology of normal cell growth and proliferation appears to offer potentially important new targets for drug design and synthesis.

1.3. Natural products and their significance:

There are three main reasons why natural compounds are worth studying. First, natural compounds that show anticancer potential inhibit cancer by interfering with one or more of the mechanisms that researches now feel are central to cancer progression and fit into the mechanism-based approach as perfectly as a hand fits into a glove. Second,
although the future does look bright for eventual success in the fight against cancer, we are not there yet. Much more work remains to be done. As a science, the field of natural compound research can contribute to a greater understanding of cancer and a faster development of successful therapies. Third, we must study natural compounds because they are already being used in cancer treatment. For better or worse, hundreds of thousands if not millions of patients around the world are experimenting with natural compounds in their efforts to heal themselves of cancer. Because the popularity of using natural compounds in cancer treatment appears to be growing rather than declining, we are compelled to study natural compounds so that we can properly guide the public (Boik, 2001(a)).

1.4. Plant derived anti-cancer drugs

Historically, plants were a folkloric source of medicinal agents, and as modern medicine developed, numerous useful drugs were developed from lead compounds discovered from medicinal plants. Today, this strategy remains an essential route to new pharmaceuticals. Since 1961, nine plant-derived compounds have been approved for use as anticancer drugs in the US: vinblastine, vincristine, navelbine, etoposide, teniposide, taxol, taxotere, topotecan and irinotecan (Lee, 1999; Cragg et al., 2005) (Figure – 1.1).

![Figure 1.1](image-url)
Accordingly, the preclinical development of bioactive natural products and their analogs as chemotherapeutic agents is a major objective of anti-cancer research programs. Three main research approaches in the drug discovery and development process are:

(1) Bioactivity or mechanism-of-action-directed isolation and characterization of active compounds.

(2) Rational drug design-based modification and analog synthesis.

(3) Mechanism of action studies.

Structural derivatization of natural compounds is aimed at increasing activity, decreasing toxicity, or improving other pharmacological profiles. Preclinical screening using \textit{in vitro} human cell line panels and selected \textit{in vivo} xenograft testing is a major tool in identifying the most promising anticancer drug development targets.

Structure refinement is also aided by four types of studies:

(1) Structure-activity relationship (SAR) studies including qualitative and quantitative methods.

(2) Mechanism of action studies including drug receptor interactions and specific enzyme inhibitions.

(3) Drug metabolism studies including identification of bioactive metabolites and blocking of metabolic inactivation.

(4) Molecular modeling studies including determination of three-dimensional pharmacophores.

Toxicological, production, and formulation concerns are addressed before clinical trials can begin (Lee, 1999).

\textbf{1.5. Background of Combretastatin}

Combretastatin is a small organic molecule found in the bark of the African bush willow tree \textit{Combretum caffrum}, identified 27 years ago by Professor George R. Pettit, the Director of the Cancer Research Institute based at Arizona State University in the USA (Pettit \textit{et al.}, 1982). Scientists at the CRI have not only completed a total synthesis for
combretastatin, but have isolated it on scales large enough to allow clinical testing, and they have also produced water-soluble phosphate derivatives, which are helping the drug's delivery to patients. Professor Pettit, like many great scientists, is driven towards a goal, that of finding treatments for cancers. He has many lead compounds and is a well-known scientist in the world of Natural Products Chemistry (Pettit et al., 1982).

1.6. Broad objectives of the research study

The first objective of this thesis work was to study the potential of combretastatin A4 and its 56 (Fifty six) derivatives with structural modifications in ring B belonging to class of 2,3-Diaryl-4/5-hydroxycyclopent-2-en-1-one for anti-cancer activity using a panel of human tumor and endothelial cell lines. The derivatives would be screened using rapid cytotoxicity screening assays that will enable the selection of derivatives, which are more potent or equivalent to combretastatin A4. Efforts would be directed towards identifying the structure activity relationships. Further, the short-listed derivatives would be subjected to tubulin polymerization assay and in vivo efficacy studies.

The second objective of this thesis work was to study the anti-angiogenic potential of derivatives short-listed based on their anti-cancer and ability to inhibit tubulin polymerization. The anti-angiogenic studies would be carried out to determine the effect of these derivatives on stimulated primary human endothelial cells. The derivatives will be assessed on their ability to modify three key processes involved in angiogenesis i.e. growth, migration and tube formation by endothelial cells. The anti-angiogenic effect of selected derivative(s) would then be assessed in an in vivo efficacy model.

The third objective of this thesis work was attempt to carry out apoptosis of short listed derivatives using endothelial cells by performing cell cycle analysis, DNA fragmentation assay, DAPI staining for visualization of apoptotic bodies, annexin V assay and active caspase-3 immunoassay.

The broad objectives described above and the scheme of the research work to be carried out is summarized in the flow chart given in Figure – 1.2.
Design/synthesize derivatives based on structure activity relationships

*In vitro* cytotoxicity screening in human cancer & endothelial cell lines

Selection of potent derivatives based on cytotoxicity and selective to endothelial cells

*In vitro* tubulin polymerization  *In vivo* activity in animal models  Anti-angiogenic activity

Mechanism of action

Figure – 1.2: Summary of objectives and scheme of research work
1.7. Scope of the research study

Since more than 60% of anti-cancer drugs are of natural origin, the present research work on combretastatin A4 and derivatives is an effort directed towards identifying potential anti-cancer drugs from the natural source for the treatment of certain forms of cancer where combretastatin A4 derivatives are found to be effective. In general, treatment options have been found inadequate with several drugs providing either partial remissions of tumor or marginally extending the survival time. For example, in adult acute leukemia, which affects approximately five persons per 100,000 long term disease free survival is currently achieved in less than 50% of patients. In all solid tumor, once the cancer advances and metastasizes (spreads) to other parts of the body, it is hard to treat and can be deadly. During the past 10 years the number of cases of common cancer (cancers that are diagnosed with the greatest frequency in the United States) has increased more. The latest figures from U.S. state that there were about 49,960 deaths from cancers of the colon and rectum (National Cancer Institute, 2008). The studies that are proposed to be done in this thesis would help in identifying potent molecules of 2,3-diaryl-4/5-hydroxy-cyclopent-2-en-1-one (combretastatin A4 derivatives) specific for such cancers, inhibit metastasis by inhibiting process of angiogenesis, which have desirable drug-like properties that will enable better success rate in treating human cancers. The findings of this research study would fulfill the requirements of the investigational new drug application (IND) to be filed to the drug approval authorities.

1.8. Limitations of the research study

The research work involves the use of in vitro systems (cell lines) and animal models for drug discovery. The correlation of these results to clinical situation would emerge only once clinical trials are performed. It is a well known fact that several drugs with good pre-clinical activity fail in clinical trials due to several factors, but are not limited to the differences between in vitro and in vivo systems and the large differences that exist between animal and human species due to differences in drug metabolism and other complex and yet unexplained factors. These factors have been taken care of to some extent in this research study by incorporating human tumor cell lines, human tumor xenografts
and human endothelial cells in the research models in which the compounds were tested. In general, it has been suggested that the *in vitro* human tumor cell line, human endothelial cells and the human xenograft models might have good clinical predictive value in some solid tumors (such as ovary and NSCLC) under both the disease and compound oriented strategies, as long as an appropriate panel of tumors is used in preclinical testing (Nomikos *et al.*, 2003).

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