PREPARATION OF SOME ARYLHYDRAZONES OF MANNICH BASES DESIGNED AS CYTOTOXIC AGENTS

A Thesis

Submitted to the College of Graduate Studies

and Research in Partial Fulfilment of the Requirements

For the Degree of

Master of Science

in Pharmacy

by

Kavitha Advikolanu, M.Sc.

Saskatoon, Saskatchewan

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. J. R. Dimmock for his constant guidance, encouragement and personal concern throughout the course of this project. I also acknowledge the helpful suggestions given by the members of my advisory committee. The help and encouragement of Dr. R. A. Hickie and B. Elane during the tissue culture experiments are gratefully acknowledged. I wish to thank Professors, Dr. P. K. Jadhav and Dr. A. N. Kothare for their encouragement and guidance during the course of my academic pursuit.

My thanks are also due to the following people: Mr. K. Thoms for the microanalytical data, the scientific staff at Synphar Laboratories Inc.,for providing the KB in vitro screening data, Dr. Allen, University of Alberta for the in vitro antitumor evaluation using the mouse L1210 leukemia cells, the National Cancer Institute, U.S.A., for providing the anti-HIV screening data and Z. Jia for data on X-ray crystallographic studies. The financial aid provided during the course of this project by Medical Research Council of Canada is acknowledged.

I wish to thank all who in one way or the other have contributed to the successful completion of this project.

Dedicated

To

Mummy, Daddy

and

Kiran

ABSTRACT

Alkylating agents represent a class of chemotherapeutic anticancer agents used in the treatment of cancer. A large number of alkylating agents have been synthesized and several of them are in clinical use today. In an attempt to develop drugs to combat the disease, it has become increasingly important to synthesize new agents based on rational design along with systematic strategies and advances in biology. The fundamental requirement of an anticancer agent would be the selective susceptibility of the tumor to the agent's cytotoxic action.

Mannich bases have a broad spectrum of activity and earlier work from these laboratories has demonstrated the antineoplastic activity of these compounds. Mannich bases were synthesized by varying the substituent pattern in the α and α' positions to the carbonyl group and their corresponding arylhydrazones in order to determine the cytotoxic activity of these compounds. Variation in the substituents in the aryl ring in the hydrazones of the following two series of compounds was carried out: namely 1-aryl-3-dimethylamino-1-propanone hydrochloride (II) and 1-aryl-5-dimethylamino-1-penten-3-one hydrochloride (IIIa) and examined as candidate cytotoxic agents.

The Human Tumor Colony Forming Assay, the KB screen and L1210 cytotoxic assay was undertaken on some of the compounds. In the in vitro Human Tumor Colony Forming Assay two

concentrations of compounds were used namely 100 and 10μM and it was found that the compounds were cytotoxic to the cells at 100μM (except for 1-phenyl-4,4,-dimethyl,-5-dimethylamino-1-penten-3-one phenylhydrazone hydrochloride). In the KB screen some of the Mannich bases and the corresponding phenylhydrazones were studied for their cytotoxic action. Compound 1-phenyl-4,4,-dimethyl,-5-1dimethylamino-1-penten-3-one hydrochloride was active in this assay having an ED_{50} value of 2.63μg/ml (the criteria for activity is 50% inhibition of KB cell replication by 4μg/ml or less of the compound). The in vitro results obtained in the L1210 assay indicated that all the compounds were less active than melphalan, the reference antineoplastic drug (ED_{50} = 0.12μM). The most active Mannich base IIIa had an ED_{50} value of 5.1μM and among the arylhydrazones, 1-phenyl-3-dimethylamino-1-propanone 4-nitro phenylhydrazone hydrochloride was the most active derivative with an ED_{50} value of 4.9μM. The melting temperature studies on some of the compounds, however, did not show that these compounds bound with calf-thymus DNA, poly d(A-T) and, therefore, it may be inferred that intercalation may be only a minor cause of cytotoxic action of active compounds. Under the NCI'S Developmental Therapeutic Program the compounds were studied for anti-HIV activity using the XTT tetrazolium assay. 1-phenyl-5-dimethylamino-1-penten-3-one pentafluorophenyl hydrazone hydrochloride in the series of arylhydrazones prepared was found to be the most active to the

infected cells at 8.60×10^{-8} M with an IC_{50} value of $>8.57 \times 10^{-5}$ M followed by compound 1-phenyl-5-dimethylamino-1-penten-3-one-2,4-dichloro phenylhydrazone hydrochloride with an IC_{50} value of $>9.02 \times 10^{-6}$ and an EC_{50} value of approximately 20%. The structure of compound X was studied by the X-ray crystallographic technique.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

Chapter 1

1.0.0.0 Introduction

1.1.0.0 The disease of cancer

The nature of disease is reflected in the light of its pathogenesis. In the U.S.A. and probably in other countries, cancer is the second most common fatal disease; only cardiovascular problems cause more deaths. Since the early 1970s countries including the United States have made the greatest impetus to the scientific search for knowledge and understanding of the control and elimination of cancer.

Bauer et al., 1973 reported the decreased undiagnosed or incorrectly diagnosed cases of cancer is correlated with the rise in number of hospital admissions. Thus in the near future,the situation of undiagnosed or incorrectly diagnosed tumors probably will be a rarity in society. The survival rates of people with cancer has improved due to a number of reasons including the following factors.

(1) Increase in diagnosis of cancers at a controllable stage of development resulting from improved diagnostic techniques.

(2) Improvements in surgical and supportive techniques.

(3) Improvements in radiotherapy, endocrine therapy and chemotherapy.

James Ewing (1940) described neoplasia with reference to in vivo systems and the recent advances of the study of in vitro characteristics of cancer cell growth have been described. Pitot (1986a) has slightly modified pathologist

James Ewing's definition of neoplasia as follows namely "a neoplasm is a heritably altered, relatively autonomous growth of tissue."

Neoplasia exhibits a latency phenomenon or tumor induction time between the initial contact period and the commencement of the biochemical aberrations leading to the commencement of tumor formation. In 1941, Rous and his associates coined the term "initiate" within the context of the application of tar to the ears of rabbits. The wounding of the treated area "promoted" the appearance of neoplasms growing along the edge of the wound.

Mottram (1944) demonstrated skin carcinogenesis in mouse with the irritant croton oil. However not until 1947, was it clearly demonstrated that skin carcinogenesis in the mouse could be divided into two stages viz. initiation and promotion(Berenblum et al., 1947). Foulds (1964) postulated that the early stages of initiation and promotion were really part of a "progression" leading to host neoplasms.

Initiation results from the application of a chemical carcinogen and promotion requires only the repeated application of a second agent which by itself does not have neoplastic transformation properties. The gap between these two stages may be as long as a year or more without any appearance of neoplasms (Loehrke et al., 1983). The initiation process is most effective at certain stages of the cell cycle, principally at the beginning of DNA synthesis (McCormick et

al.,1982) and also on the ability of the cell to metabolize the agent to its ultimate carcinogenic form(s). Several in vivo and in vitro studies have shown the importance of one or more cycles of cell division in the presence of the initiating agent such that the cell becomes initiated with the capacity to result in neoplasms (Columbano et al., 1981; Kakunaga, 1975).

An initiating agent can be defined as a chemical, physical or biological agent that is capable of altering irreversibly the genetic component (DNA) of the cell. Butwell (1974) proposed the concept that promoting agents exert their effect by altering the expression of genetic information within the cell. The evidence that skin tumor promotion is a two-stage process has been confirmed by Slaga et al., 1980 and Furstenberger et al., 1981.

Although the two-stage process of carcinogenesis has been based on studies of the mouse skin system, evidence within the past decade has shown that a number of other neoplasms exhibit processes during their early development that are analogous to intiation and promotion in mouse skin. For example, development of "preneoplastic" or early lesions in bladder carcinogenesis in the dog and rat occur whereby the essential aminoacid, L-tryptophan acts as the promoting agent (Cohen et al., 1979). The two-stage concept of carcinogenesis in humans has been supported by morphological evidence in the case of diseases such as leukoplakia which is an atypical

proliferation of mucous epithelium. The resultant lesion, if untreated, could result in malignancy (Pitot, 1986b).

Tumor progression has a number of characteristics. Foulds (1965) was responsible for assigning these characteristics to this stage of neoplastic development. Abnormal cellular replication is one of the characteristics of the progression of the neoplasm. It is during this stage that cellular replication becomes a major factor influencing the survival and development of the neoplasms as well as the effects on the host. Fig. 1.1 (page 7) represents the events occurring during cellular division. Mitosis is the ultimate sequence of processes in the cycle (four separate components). In addition there is a fifth phase, Go, in which cells appear to leave the normal cell cycle but tend to re-enter the cycle by specific stimuli. Neoplastic cells may undergo the normal cell cycle or else leave it and enter G_o. The cells which enter Go state may never reenter the cycle and eventually die or alternatively some cells may remain in the Go state as dormant or latent neoplastic cells (Berg et al .,1971). These phases of the cell cycle appear to be individually unique and chemotherapists today are utilizing this knowledge to obtain specific actions of drugs and chemicals to these various periods of the cell cycle .

A neoplasm can arise as a consequence of one or few relatively rare random events which have a single-cell origin (Fialkow,1976). Multicellular origins of neoplasms occur with

an infectious agent such as a virus. An alternative view of the clonality of neoplasms is that taken by Nowell (1976) and others. This concept of the clonality of a neoplastic cell population in the stage progression may be due to the selective growth advantage of a clone of cells appearing during the neoplastic development, thus resulting in overgrowth of this population.

These clone cells originate from "stem" cells which are cells of a particular tissue type and have the potential to divide and reproduce the entire tissue.

1.2.0.0 Approaches to the treatment of cancer

Surgical removal of any apparently localized cancer is one of the established ways to cure cancer. Radiation treatment of solid tumors is an alternative approach to surgery on occasions although it can be used in conjunction with or to complement the radical surgery concept. Tumor response to radiation can be assessed by tumor cell survival curves. Radiocurability of a tumor will depend on the proliferative rate of its cells and the more the number of proliferative cells the faster will the tumor regress. However radiation damage is not specific for tumors and thus the therapeutic dose of radiation which can be delivered to a tumor is limited by the possibility of normal tissue damage. Besides, although radioresponsive, the tumor may contain surviving stem cells which can eventually lead to tumor

recurrence. Thus improved approaches to uses of radiotherapy in the future are to increase the therapeutic efficacy of the radiation dose delivered to tumor in relation to normal tissues and to increase the response of the tumor to that of surrounding normal tissues.

Significant advances have been made in the understanding of the basic molecular nature of neoplastic transformations and the successful treatment of neoplasms that were not curable by either surgery or radiotherapy has been achieved. The use of chemotherapeutic agents is also an established treatment modality. Chemotherapy either alone or in conjunction with surgery and/or ionising radiation has contributed to the increase in survival rates of cancer patients (Enstrom et al., 1977). Many of the solid neoplasms in both children and adults are being effectively treated with chemotherapy as the principal therapeutic modality. A variety of newer techniques using chemotherapy in combination with hyperthermia and in a variety of drug delivery systems are in the testing stages (Markham, 1984). Progress in combination drug therapy will lead to a bright future in cancer chemotherapy.

There are several 'pet' theories of investigators to prepare compounds with selective toxicity to cancer cells rather than normal tissue. However the compounds used in cancer chemotherapy are found to inhibit cell division by affecting cells during DNA synthesis or mitosis.

The types of compounds used in cancer chemotherapy are as

follows.

1. Alkylating agents. The classic alkylating agents include the nitrosoureas, antitumor antibiotics and miscellaneous agents.
2. Antimetabolites.
3. Hormonal therapy.
4. Immunotherapy.
5. Plant alkaloids.
6. Miscellaneous agents.

A detailed classification of these agents is given in the section 1.3. A pictorial model indicating the sites of action of chemotherapeutic agents in the cell cycle is represented in Fig. 1.1.

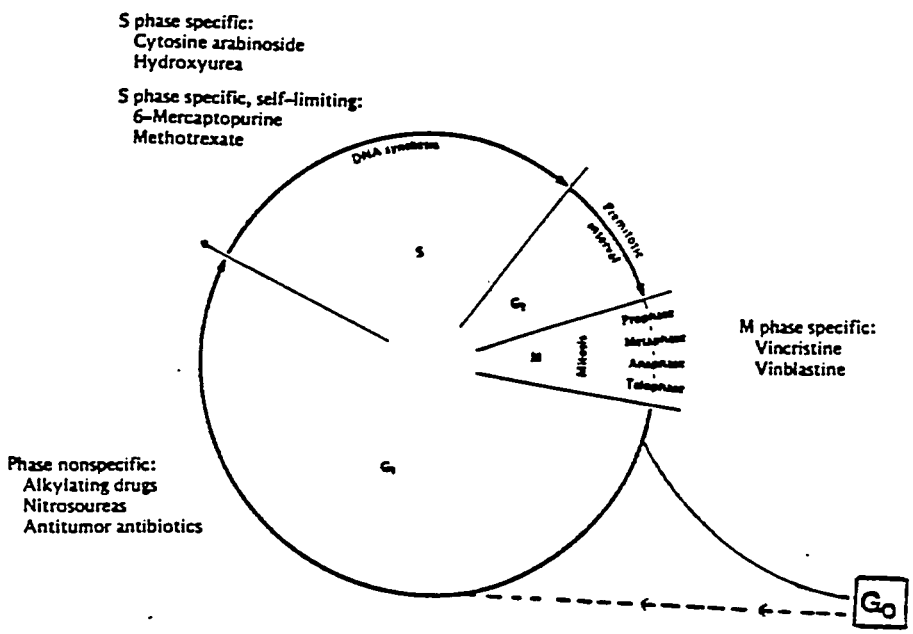

Fig. 1.1. A pictorial representation of sites of action of chemotherapeutic agents in the cell cycle, (Pratt and Ruddon, page 21).

The antitumor agents affect the tumors by interfering with the events occurring in the phases of the cell cycle. There are two basic types of agents namely either cell cycle (phase)-specific agents or cell cycle (phase)-nonspecific agents. Cell cycle (phase) specific agents are also called 'schedule'-dependent and basically are cytotoxic to the cells in a particular phase. These agents are much more effective when the cells are actively dividing which is usually the case of tumors with low cell mass. Whereas the cell cycle (phase) non-specific agents are usually responsible for activity in large tumors, their activity depends on a single dose given at a time, that is they are not schedule dependent. Alkylating agents are found in this latter group. Table 1.1. indicates this type of classification.

Table 1.1 Type of cell cycle (phase) specific or cell cycle (phase)non-specific agents used as chemotherapeutic agents.

Group	Type	Example
I	Cell cycle (phase) specific	Antimetabolites (methotrexate, 5-fluorouracil), vinca alkaloids, bleomycin
II	Cell cycle (phase) non-specific	Alkylating agents, antitumor antibiotics, nitrosoureas, miscellaneous compounds

1.3.0.0 Classification of anticancer drugs

Cancer chemotherapeutic agents have been classified into six general categories based on their mechanisms of action.

I. Alkylating agents

A. Classic alkylating agents

These alkylating agents have been further divided into three classes viz. 1. bis(chloroethyl)amines e.g.,

(i) Chlorambucil

(ii) Mechlorethamine

(iii) Cyclophosphamide

(iv) Melphalan

2. Ethyleneimines e.g.,

(i) Triethylene thiophosphoramide

3. Alkyl sulphonates e.g.,

(i) Busulfan

B. Nitrosoureas e.g.,

1. Carmustine

2. Lomustine

3. Streptozotocin

C. Antitumor antibiotics have been divided into two classes:

1. Anthracyclines e.g.,

(i) Daunorubicin

(ii) Doxorubicin

2. Other antitumor antibiotics e.g.,

(i) Dactinomycin

(ii) Mithramycin

(iii) Mitomycin

(iv) Bleomycin

D. Miscellaneous alkylator-like agents e.g. cisplatin

II. Antimetabolites

A. Folate antagonists e.g.,

1. Methotrexate

2. Dichloromethotrexate

B. Purine antagonists e.g.,

1. Mercaptopurine

2. Azathioprine

C. Pyrimidine antagonists e.g.,

1. 5-Fluorouracil

2. Cytarabine

III. Hormonal therapy

A. Estrogens

B. Progestins

C. Androgens

D. Corticosteroids

E. Antihormonal agents e.g.,

1. Antiestrogens

IV. Immunotherapy

A. Compounds acting on the immune system

B. Clinical considerations of nonspecific adjuvants

1. Bacillus Calmette-Guerin (BCG) vaccine

2. Methanol extracted residue of BCG vaccine

C. Immunorestorative agents e.g.,

1. Levamisole

V. Plant alkaloids e.g.,

1. Vinblastine

2. Vincristine

VI. Miscellaneous agents

1. Hydroxyurea

2. Asparaginase

3. Procarbazine

1.4.0.0 Alkylating agents

Alkylating agents are compounds which are able to function as monofunctional or bifunctional agents by alkylating cellular constituents. The major effect is the covalent linking of an alkyl or a substituted alkyl group to compounds in the cells and this linkage is often to the N-7 position of the guanine residue of nucleic acids (Brookes and Lawley, 1960). The mechanism of action of mechlorethamine is shown in Fig.1.2. The alkylation process takes place via an intermediate three membered ring namely an imonium ion. The process is then repeated with the other chloroethyl side chain and the result is either a cross-linking between DNA strands or linking between the same strand of DNA.

Bone marrow toxicity is a most serious complication of therapy using alkylating agents. α,ß-Unsaturated ketones are a class of alkylating agents which undergo a Michael-type addition with nucleophiles such as thiols to give ß-

ketothioethers (Friedman et al., 1965) in preference to reaction with amino functions. Hall et al., 1977 has reported the antitumor properties of compounds containing the α,ß-unsaturated ketone moiety to demonstrate thiol alkylation.

Although the bifunctional alkylating agents e.g. the nitrogen mustards interact with DNA by cross-linking, the monofunctional alkylating agents are quite different in some of their biological properties. The carcinogenic or other mutagenic properties, via their interaction with amino and hydroxyl groups of nucleic acids (Ludlum, 1975), is a disadvantage of many classic monofunctional and bifunctional alkylators. However some Mannich bases derived from conjugated styryl ketones did not show any mutagenic activity when examined in the Ames test (Dimmock et al., 1980). Thus the primary interest of making Mannich bases and related prodrugs is to obtain compounds with selective release of the ketones at the site of the tumor.

1.5.0.0 Tumor cell pH

A number of human tumors exhibit an anaerobic metabolism of substrates with the production of lactic acid. This property of tumor cells can be exploited to yield a therapeutic approach for agents which are designed to require low pH milieu to generate the cytotoxic derivative(s).

Tumor pH is generally more acidic than normal cells (Wike-Hooley et al., 1984) and attempts have been made to measure

the tissue pH in various extracts and homogenates (Pitot, 1981). This offers a diagnostic tool to the clinical oncologist and also the possibility of better determining the metabolic conditions of tumor cells during therapy. von Ardenne (1976) has reported that the low pH of malignant cells sensitizes them to alkylating agents. There are a number of techniques to measure the pH of tissues including the use of microelectrodes (Jadhe et al., 1982) and flow cytometry (Wirsching et al., 1982).

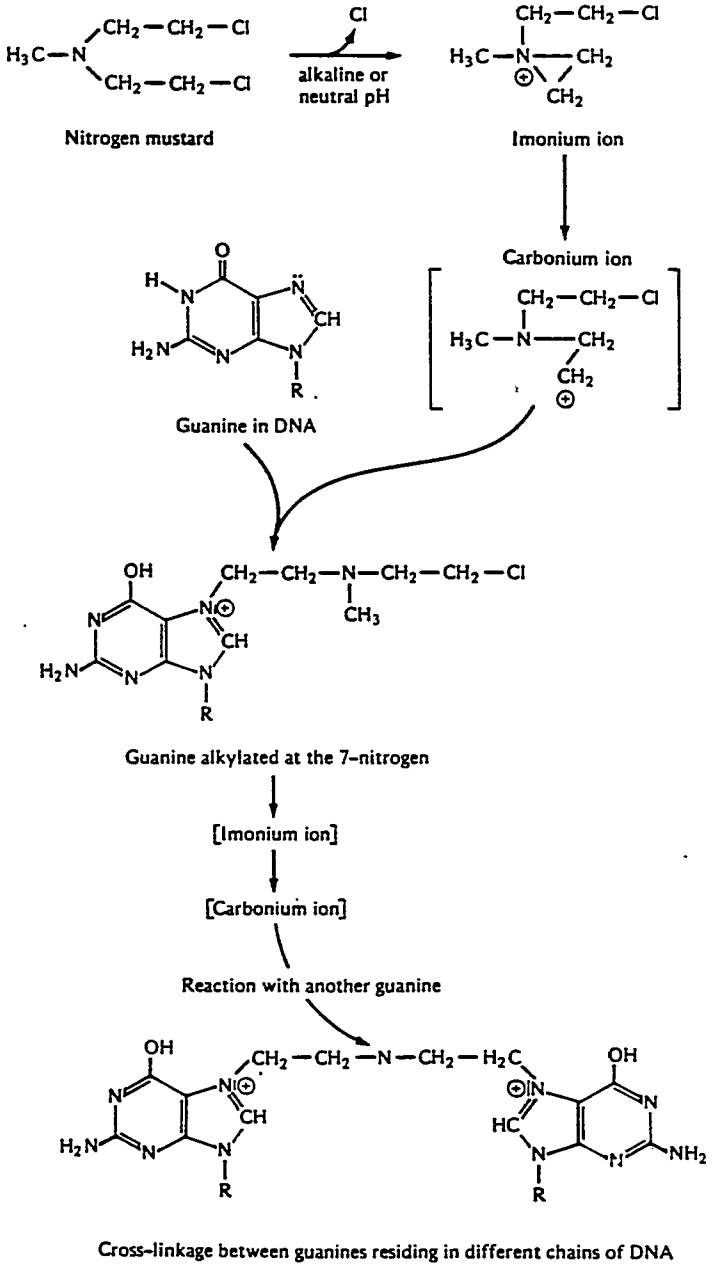

Fig.1.2. The mechanism by which nitrogen mustards are covalently bonded to the 7-nitrogen of two guanine residues (Pratt and Ruddon, 1977).

1.6.0.0 Quantitative structure-activity relationships

The concept that biological activity could be related to chemical structure (Albert, 1985) and further to physicochemical properties of a particular compound, or class of compounds, has increased the number of techniques for the design of drugs for specific purposes. Quantitative structure activity relationships can be determined when a set of physicochemical properties of a group of compounds can explain the biological activities of the compounds.

1.6.1.0 The Hansch approach

Linear multiple regression analysis (Snecdor et al., 1967) is a commonly used correlative method in drug development. This method involves finding the best fit of a dependent variable (the biological activity) to a linear combination of independent variables (parameters) by the method of least squares. Equation (1) represents the basic Hansch equation (Hansch and Fujita, 1964) which utilizes this method with physicochemical substituents and quantitative biological data. The Hansch concept relates biological responses to one or more physicochemical constants such as hydrophobic, electronic and steric factors.

$$\log 1/C = -k_1\pi^2+k_2\pi+k_3\sigma+k_4E_s+k_5 \quad \text{----------(1)}$$

In this equation, C is the concentration of drug required to produce a standard biological effect, π is the hydrophobic parameter, σ is the sum of the Hammett substituents, E_s is

the sum of the Taft's steric constants for each substituent on the ring and k_1, k_2, k_3, k_4, and k_5 are constants.
However a practical limitation to the Hansch approach is the requirement to synthesize and screen a number of compounds before a meaningful correlation can be derived and the next step taken towards optimization.

1.6.1.1 The correlation with partition coefficient

The Hansch hydrophobic substituent constant or π value correlates biological activity with the partition coefficient and is represented by equation (2):

$$\log (P_X/P_H) = \pi_X \qquad \text{----------(2)}$$

where P_H = partition coefficient of the parent molecule, P_X = partition coefficient of the parent molecule containing a substituent X, and pi (π) is the measure of the contribution of the substituent to solubility. If the pi (π) value is positive then the substituent group increases the solubility of the compound in nonpolar solvents (increases lipid solubility) and if the pi value is negative the subtituent group increases the solubility of the compound in polar solvents. The Hansch pi values enable the estimation of partition coefficients for most structures. Groups which make an equivalent contribution to partition coefficient may be referred to as isolipophilic groups. If lipophilicity is an important criterion in determining biological activity, then substitution of one isolipophilic group for another may allow