INTRODUCTION

The ever increasing activities in the field of cancer biology and medicine are stimulating the chemist to compose new variations on the steroid nucleus. Selected by the evolutionary process to perform some of the most fundamental biological functions, this ring system not only has inspired the endocrinologists, but has also become the basis for some of the most phenomenal developments in organic chemistry. The medicinal chemists look at the steroid nucleus with wonder and delight, as this provides them with a vast field to test their ingenious skill at structure modification with the hope of discovering new and better drugs.

The steroid drugs occupy a conspicuous place in the modern therapeutic practice. The broad spectrum of biological activity within the group and the multiplicity of actions displayed by certain individual members make the steroids one of the most intriguing classes of biologically active compounds.

STEROIDS AND NEOPLASTIC DISEASES CHEMOTHERAPY

Clinically, hormonal compounds still seem to have place in cancer therapy. Androgens are used in the treatment
of advanced breast cancer, and estrogens in the therapy of carcinoma of the prostate. Less successfully, ACTH and some corticoids are used in the treatment of acute leukemia.

It is felt that cancer is largely a problem of faulty growth regulation which results from an abnormality in information control within the cell. The normal cells of the body are, of course, potentially malignant, but differ from neoplastic cells in their response to those homeostatic mechanisms which control the extent of their mitotic activity and inhibit the emergence of invasive tendencies. Some investigators, therefore, believe that the future of cancer chemotherapy lies not with the so-called 'killer' drugs—the alkylating agents, the antimetabolites and the antibiotics, with which we currently treat the cancer patients—but with homeostatic regulators, natural or artificial.

It is considered that more attractive possibilities may exist with hormonal substances of the steroid and ACTH types. "If their action and resistance mechanism could be fully elucidated and structures synthesized that would carry, on the basis of these elucidations more specific therapeutic features, then considerable advances could be predicted."¹

There have of recent appeared many excellent reviews which discuss various aspects of steroids in relation to cancer.²⁻¹³

The mechanism whereby the steroids produce hormonal effect is not necessarily the same that causes tumor
regression, exception being those neoplasms which retain some degree of hormonal dependence, e.g., tumors susceptible to androgens and prostatic tumors caused to regress by estrogen. Though the initial effect may be the same, subsequent events may transpire to bring about changes apparently unrelated to hormonal effect. A more detailed knowledge of tumor tissue, in particular the tissue that has apparently become resistant, could well lead to further insight into the primary mechanisms.

As such, while examining steroid analogs as anticancer agents, one need not necessarily focus attention on the concomitant hormonal activity, since some 'inactive' steroids might be capable of modifying the course of cancer. For example, \( \Delta^1 \)-testololactone (1) is remarkably free of biologic activity other than the production of objective remission of breast cancer.\(^{10,14}\)

One of the steroid groups of interest is heterosteroids, to which \( \Delta^1 \)-testololactone belongs. The heterosteroids are nuclear, having heteroatom as member of the steroid nucleus, or extranuclear where heteroatom is part of the attached side chain or fused ring system. Heterosteroids,
particularly azasteroids, are being prepared and investigated at various centers for their potential biological activities.

Certain azasteroids have shown some anticancer activity. Various medicinal and biochemical thoughts have inspired their synthesis and testing.

AZASTEROIDS AS POTENTIAL ANTICANCER AGENTS

One of the concepts which has motivated investigation of azasteroids, particularly nuclear, is that nitrogen, which could be in the form of amide or amine function, in an azasteroid would have a stronger affinity for a protein receptor than the methylene group which it replaced. The methylene group would be attracted only by van der Waals forces, the nitrogen would be attracted in addition through the electrostatic attraction. Maybe, an azasteroid would fit the enzyme site of the parent hormone in such a way that only a carcino-lytic effect would result.

It has been said "as part of a search for antiandrogens and antiestrogens, it would be logical to examine the so-called azasteroids and other steroids in which a carbon atom has been replaced by a nitrogen, sulfur, or other heteroatom. These are difficult syntheses but more chemical activity is expected in this area. In addition, it would be expected that transformation products of the triterpenes might possess unusual physiological activities." Martin-Smith and Sugrue also predict that perhaps more potent agents
exhibiting antitumor type of activity await discovery within the nitrogen-containing steroid group.

Various azasteroids have been prepared and tested against experimental neoplasms. A number of steroidal dihydro-1,3-oxazines and amines (e.g., 17β-amino-5-androstene-3β-ol) have been claimed to possess a certain degree of antitumor activity when tested in mammary carcinoma in mice.\(^{17}\)

A new anabolising hormone, 2α,17α-dimethyl-5α-androstane-17β-ol-3,3'-azine (dimethazine) (2) has been tested for its effect on the immunological response of mice to Ehrlich ascites carcinoma.\(^{18}\) Animals administered dimethazine alone were found to be moderately resistant to tumor take, and dimethazine in combination with immunization markedly inhibited tumor growth.

The observation that steroidal isoxazoles\(^{19}\) of the type (3) and (4) were tumor inhibitory when assayed by the method
of Abe et al.\textsuperscript{20}, led Caspi and Piatak\textsuperscript{21} to explore further azasteroids with an isoxazole or a pyrazole system attached to ring A, but the new compounds prepared showed no consistent enhancement of antitumor activity in preliminary tests. However, 2,3-diaza analogs of the type (5) and (6) have shown antitumor activity when tested on rat mammary fibroadenoma.\textsuperscript{22}

There is an increase of cholesterol content in tumor cells in comparison with normal cells. This increase is not due to enhanced synthesis inside the tumor cells but to their capacity to accumulate cholesterol.\textsuperscript{23,24} This has prompted studies on sterols and related analogs, with the expectation of discovering antineoplastic activity in such compounds.\textsuperscript{25} 6-Aza-3,5-cholestadiene and its salts are claimed to have a cytostatic effect of value in treatment of nonmalignant and malignant tumors.\textsuperscript{26} 2-Oximino-8,21-lanostadien-3-one\textsuperscript{27}
shows an inhibition of tumor growth of \(40\%\) in the cheekpouch test\(^{28}\).

25-Azacholesterol (7) has been found to be cytostatic in tissue cultures.\(^{25}\) Treatment of mice having experimental brain tumors with 20,25-diazacholesterol (8) induced an accumulation of desmosterol in the tumors but not in normal brain.\(^{29}\)

The activity of these basic sterol analogs leads one to consider the possibility of getting antineoplastic agents among the naturally occurring derivatives such as alkaloids. In fact, Kupchan et al.\(^ {30}\) have isolated \(\alpha\)-solamarine (9), a

\[\text{Diagram of 7, 8, and 9}\]
steroid alkaloid glycoside, from *Solanum dulcamara*, which has been shown to possess tumor-inhibitory activity against Sarcoma 180 in mice.

Taking hint from anticancer activity of certain nitrogen mustards, various steroid nitrogen mustards have been prepared with a view to utilize the steroid nucleus as a supporting moiety.\textsuperscript{31-37} Some of these have shown variable antitumor activity.\textsuperscript{34-37} Other steroidal alkylating agents that have been claimed to possess a varying degree of antitumor activity include certain C-16 aziridines\textsuperscript{38-39}.

The pteridino-steroids, prepared primarily as folic acid antagonists,\textsuperscript{40,41} showed little antitumor activity though 17β-acetoxy-5α-androstano[4,3-g]-2',4'-diaminopteridine (10)

![Chemical Structure](image.png)

is stated to have given a statistically significant inhibition in Sarcoma 180 assay. The results of antitumor activity in the same assay of a related analog, 17β-acetoxy-5α-androstano-[2,3-g]-2',4'-diaminopteridine, have been reported to be inconclusive.\textsuperscript{42}
A mention may also be made of 3-guanylhydrazone-androst-17-ol which has been shown to possess a certain degree of antimitotic activity, both \textit{in vitro}^{43} and \textit{in vivo}^{44}.

OTHER BIOLOGICAL ACTIONS OF AZASTEROIDS

It is not only the possibility of getting antineoplastic agents but also the hope of finding analogs with other biological actions, which evinces a steady and continued interest in the azasteroids, both of synthetic and natural origin. The literature on biological activity of azasteroids has been the subject of reviews by Martin-Smith \textit{et al.}\textsuperscript{16,45,46} and Singh, Padmanabhan and Parashar\textsuperscript{47}.

Out of the numerous synthetic azasteroids prepared, there are many which have shown one kind or the other of biological activity and some of these are of clinical significance. In most of the cases synthesis of azasteroids has been conducted on empirical grounds but research in certain instances in the preparation of new azasteroids has been the result of sound reasoning based on theoretical concepts of drug action. The reported antineoplastic activity of various azasteroids has been reviewed in the preceding section.

There are also known azasteroids which have anabolic, antifertility, antihormonal, antihypercholesterolaemic, antimicrobial, CNS-depressant, neuromuscular blocking, or vasodilatory activity.
It is of interest to mention that recently Witkop et al. have investigated the venom of the Colombian arrow poison frog, *Phyllobates aurotaenia*. The active fraction was separated as under:

<table>
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<th>Active Fraction</th>
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<td>Batrachotoxin (\text{LD}_{50}^{50}) subcutaneous in mice, 2 (\mu g/kg)</td>
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| Batrachotoxin is the most active cardiotoxin and has \(\text{ED}_{50}\), intravenous in anaesthetized dog, 0.5 \(\mu g/kg\). The venom constituents are steroidal in nature. Batrachotoxinin A which retains only 1/500 of the toxicity of the original venom is still as toxic as strychnine \(\text{LD}_{50}^{50} 0.5 \text{ mg/kg}\). The structure of Batrachotoxinin A (11) has been determined through X-ray diffraction analysis of its \(p\)-bromobenzoate ester. From the pharmaceutical view point, batrachotoxin and the related congeners in the venom are interesting because they or a synthetic variation might have medicinal applications. The

![Diagram of Batrachotoxinin A (11)](image-url)
structure of Batrachotoxinin A is strikingly novel. It is unusual for a steroid to be so extremely active. This observation gives added fillip to the scope of research on heterosteroids.

SYNTHESIS OF AZASTEROIDS

The number of naturally occurring extranuclear azasteroids is considerable. Several categories of steroidal alkaloids belonging to this group are well known. These include alkaloids present in Solanum, Veratrum, Holarrhena, Buxus, Sarcococca and Pachysandra spp. Martin-Smith et al. have listed literature on the steroid alkaloids of Funtumia, Paravallaris, Chonemorpha, Fritillaria and Malouetia spp.

Among the naturally occurring azasteroids, the only example where nitrogen forms an integral part of the steroid nuclear skeleton is of the entities obtained from the parotid and skin glands of salamanders. Hara and Oka have recently accomplished total synthesis of some of these constituents, samandarone (12) and samamine (13).
There are known hundreds of nuclear azasteroids which are purely of synthetic origin. These have been prepared by total and partial syntheses. The reviews by Morand and Lyall\textsuperscript{60}, Gogte\textsuperscript{61} and Huisman\textsuperscript{62} cover the relevant literature on total synthesis of azasteroids and analogs. There have resulted certain genuine isosteres of natural hormones. Such relations obtained by total syntheses include (±)-6-azaequilenin (14)\textsuperscript{63}, (±)-6-azaestradiol methyl ether (15)\textsuperscript{64}, (±)-8-azaestrone (16)\textsuperscript{65} and (±)-8-azaestradiol (17)\textsuperscript{66}.

![Chemical Structures](image)

Of the partial synthetic routes, Beckmann rearrangement and Schmidt reactions are the most widely used procedures for the preparation of azasteroids. Simple steroid ketones have been suitably reacted to introduce nitrogen at different positions. α,β-Unsaturated ketones have also been
used as starting materials. The rearrangement of α-oximino ketones, α-hydroxyketoximes and α-nitroketones has also been studied. The literature on Beckmann rearrangement and Schmidt reactions as applied to the synthesis of azasteroids has been reviewed by Singh, Parashar and Padmanabhan. Insertion of nitrogen has thus been effected into rings A, B, C and D, and position 5.

The other procedure which is widely used, starts with secketo acids. For example, many of the 3,5-seco-4-nor-5-oxo-3-oic, 5,7-seco-6-nor-5-oxo-3-oic and 9,12-seco-11-nor-9-oxo-12-oic acids have been suitably reacted to obtain 4-aza, 68-91, 6-aza, 69,81,82,92-98 and 11-aza99-101 steroids, respectively.

The steroid ring A has been modified in various ways resulting in the incorporation of more than one heteroatom. Ring size is maintained in some derivatives, for example, pyridazones,102,103 pyridazinones,102-104 pyrimidines105-107 and oxazines108,109. However, five-membered analogs are also known in the form of pyrazoles110 and isoxazolines111.

The Curtius and Hofmann rearrangements have been used to introduce nitrogen at position 3 (Ref. 112), 6 (Ref. 96, 113-118), 15 (Ref. 119,120) and 16 (Ref. 121). Secodicarboxylic acids have also been processed through the imide synthesis, and 7-aza-B-homo,122 17-aza-D-homo,123,126 and N-hydroxy-17-aza-D-homo127 steroids have been obtained.
Rakhit and Gut\textsuperscript{128} paved the way for converting 17\textsubscript{a}-aza-D-homosteroids to 17-azasteroids by developing method for C-17 elimination, and succeeded in preparing 17-azorpregn-20-one (18)\textsuperscript{129,130}. The conformation of side chain of 17-aza-azapregnan-20-one was studied\textsuperscript{131} by dipole moment method, and nmr spectra of some 17-azasteroids were examined\textsuperscript{132}.

The photochemical rearrangement of steroidal 17-nitrite esters has been found to result in ring expansion giving rise to the N-hydroxy-17\textsubscript{a}-aza-D-homo-17-one system.\textsuperscript{133-135}

Kierstead, Faraone and Boris\textsuperscript{121} prepared 16-azaestrone methyl ether (19) from monomethyl ester of marrianolic acid methyl ether. Baran\textsuperscript{136,137} obtained this analog through a different procedure.
Secodialdehydes have been submitted to reductive amination. 12-Aza-C-homo-(25R)-5α-spirostan-3α-ol has been thus prepared and used to obtain various pregnane and androstane analogs.

An interesting case of microbiological transformation producing an aza analog has been reported by Sih et al. Exposure of estrone to Nocardia spp. (E 110) resulted in the formation of (20) in addition to some rings A and B ruptured and degraded products.

![Chemical Structure](image)

(20)

Regarding azasteroids in which nitrogen forms part of the hetero ring attached to the steroid nucleus, a mention may be made of two recent reviews which cover the literature in the area.

Lastly, several synthetic azasteroids are known in which N is part of the group or the side chain attached to the steroid nucleus (see Ref. 16,45,46 for some selected cross references).
RESEARCH ENVISAGED

The above survey of literature on biological activity and synthesis of azasteroids highlights the interest in the field which remains unabated.

At the Panjab University Department of Pharmaceutical Sciences, there is in progress an active program of research on the synthesis of azasteroids. The selected compounds are sent for biological screening to some institutions in India and abroad.

The author thought of preparing aza analogs with the major objective of getting tested some of the resulting compounds for their anticancer activity. The Council of Scientific and Industrial Research, Government of India, came forth to support this costly work. In addition to the interest from the biological viewpoint, the details of synthetic methods and structures were envisaged for exploration. The 'Resume' and Discussion' part which follows, outlines the synthetic work that has been accomplished, and the 'Experimental Work' part gives the description of the synthetic procedures and the analytical data.

[Note: The chemical nomenclature followed in writing of this thesis, is mostly in accordance with the IUPAC-IUB Revised Tentative Rules for Nomenclature of Steroids, Steroids, 13, 277 (1969)]