INTRODUCTION
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Nature cherishes an abundant source of chemical substances of both plant and animal origin. Steroids are one of these vital classes of chemical compounds which are extensively used for the benefits of mankind. There continues a considerable interest in steroid modification. A numerous synthetic routes have been developed by medicinal chemists in structurally modifying the molecule in order to achieve different biological activities. Synthesis of steroid nucleus and the study of reaction mechanisms and stereochemistry along with the investigation of their biological potency pose a considerable challenge to the medicinal chemists to obtain lead structures. Heterosteroids are molecules containing heteroatom(s). Nuclear heterosteroids contain nitrogen, sulphur or oxygen in the steroid nucleus. When heteroatom(s) forms part of a fused ring system, attached group or a side chain of the steroid nucleus, they are known as extranuclear heterosteroids. Rational drug design in the area of heterosteroids is a fascinating task in the optimal evaluation of therapeutic/toxic ratio. A general outline of both natural and synthetic heterosteroids is described.

NATURALLY OCCURING HETEROSTEROIDS

Extranuclear azasteroids have more prominent occurrence in nature. Several categories of steroidal alkaloids present in plant of genus Veratrum, Buxus, Sarcococca, Solanum, Malouetia, Kibatalia and Paravallaris, Chonemorpha and Fritillaria are quite popular. Steroidal alkaloidal constituents were observed in Colombian arrow poison obtained from frog Phyllobates aurotaenia and have been studied extensively. Some nucleoazasteroids
have been derived from the parotid and skin glands of salamanders\textsuperscript{7,13,14} and from the crude fermentation broth of fungus \textit{Geotrichum flavobrunneum} NRRL-3862.\textsuperscript{15} A plant growth promoter brassinolide (1) was isolated from bee-collected \textit{Brassica napus} Linn. (rape) pollen grains.\textsuperscript{16-18}

Viridin (2)\textsuperscript{19} and wortmannin (3)\textsuperscript{20} are two heterosteroidal antibiotics produced from microbial sources as \textit{Gliocladium virens} and \textit{Penicillium wortmanni}, respectively. Certain cardiac steroids obtained from plants were used as venoms and cardiovascular agents since 1500 B.C.; squill being mentioned in the Ebers papyrus of ancient Egypt. The cardioactive cardenolides and bufadienolides are well known. Digitalis contains the major cardenolides whereas the steroidal constituents of toad venom possess the latter class.
SYNTHETIC HETEROSTEROIDS

Hundreds of known nucleo-heterosteroids have been documented which are purely of synthetic origin. The total synthesis of heterosteroids and their congeners is covered in the relevant literature and reviewed by Morand and Lyall,22 Gogte23 and Huisman.24 To introduce a heteroatom (or atoms) by total synthesis, suitable mono-or bicyclic heterocyclic systems have been employed to construct the heterosteroid skeleton. Construction of heterocyclic component of the steroid nucleus through a sequence of special synthetic techniques constitutes the second approach.

The early selected references to the partial synthesis of heterosteroids were compiled by Tökés.25 Among the other available reviews are by Ninomiya26 on azasteroids and Pappo27, Ramdas and Chaudhuri28 on oxasteroids. Reviews of Zhungietu29 and Akhrem30 cover information regarding the synthesis of nucleo-heterosteroids while Martin-Smith and associates31-33 listed selected references regarding synthetic extranuclear heterosteroids.

CONCEPT OF DRUG DESIGN IN HETEROSTEROIDS

Singh, Kapoor and Paul34 have published a comprehensive review on heterosteroids and drug research. The literature on biological activity of heterosteroids,35 particularly azasteroids, has been the subject of some earlier reviews.26,36,37,38-41 The synthetic heterosteroids, from medicinal chemists' point
of view, may be said to have been prepared on empirical grounds but the research in the synthesis of new heterosteroids has been accomplished by sound reasoning based on the concepts of rational drug design. This involves:
(i) study of the physiological mechanisms, (ii) pathophysiology of the disease, (iii) chemical exploration and its biological counterpart and (iv) heteromodifications of the basic steroid nucleus leading to the development of the new compounds possessing altered physicochemical properties with a potential of providing some useful drugs. Various synthetic heterosteroids possessing antineoplastic, neuromuscular blocking, androgen antagonistic and biosynthesis inhibitory, estrogenic, antiestrogenic, adrenocorticoidal, antimineralocorticoidal, anabolic, progestational, antiprogestational, central nervous system acting, antifertility, catatotic, cardiac, antilipemic, local anaesthetic, diagnostic and antimicrobial activities are well known in the global drug market.

This thesis embodies the synthesis, study and biological activities of some heterosteroids in androstane and estrone series related to various activities. Therefore, it is in order to review the literature of the earlier work in the pertaining areas.

NEOPLASTIC DISEASES AND THEIR CHEMOTHERAPY

Medical oncology is one of the most important and impact-creating subspecialties in the changing field of medicine in the past two decades, as cure for various fatal malignancies as lymphomas, leukaemia and testicular cancer have been available. Although cancer chemotherapy has not received spectacular breakthrough due to the unknown etiology of the disease but recent
advances in rational design and study of the mechanism of action of antineoplastic agents have led to the enhanced emergence of newer and more potent compounds. Cancer is the second-seeded disease after cardiovascular ailments and is the major cause of death. Cure is defined here as an expectation of normal longevity. Presently adjuvant therapy, radiation and chemotherapy seems sure to provide better response rates; but again there are limits to reasonable expectations. Colon cancer, breast cancer and rectal cancer are best treated locally by adjuvant chemotherapy whereas, high-dose chemotherapy employed in patients with paediatric sarcomas, relapsed breast cancer, soft-tissue sarcomas and lymphoma.

Cancer cells are the target of anticancer drugs. Since it is a disease caused by uncontrolled proliferation of cells that have mutated from normal body cells, novel protocols are now exploring towards strengthening of the immune system, gene therapies, stimulation of normal hematopoietic elements, induction of differentiation in tumour tissues and inhibition of angiogenesis. Most of the lymphomas and leukaemias are sensitive to drugs. Due to continuous cell cycle, i.e. mitosis, the cells in the cycle are more sensitive to all types of anticancer agents than resting cells. On the other hand, most of the cells of the slower growing solid tumours that resist therapy are in the Go or resting state and are less sensitive to all agents. Multidrug regimens are used to reduce drastically the number of neoplastic cells.

In 1941, when Huggins demonstrated that the administration of estrogens produced regressions of metastatic prostate cancer, the new era of chemotherapy of malignant diseases emerged. In the following year, Gilman and
coworkers began clinical studies on the nitrogen mustards and found mechlorethamine effective against Hodgkin’s disease and lymphosarcoma.\textsuperscript{48}

The outstanding design and discovery of antimetabolites: methotrexate in 1949, 6-mercaptopurine in 1952, and 5-fluorouracil in 1957 marked to a pioneering work. Alkylating agents, such as melphalan and cyclophosphamide, were developed during this period and the activity of natural products such as actinomycin, mitomycin C, and the vinca alkaloids was discovered. The year 1960 witnessed the continued progress in the discovery of cytarabine, bleomycin, doxorubicin, carmustine and other plant products such as taxol. Highly potent molecules such as procarbazine, dacarbazine and cisplatin complexes were investigated.\textsuperscript{49}

**Heterosteroidal Antineoplastic Agents**

Several nucleoheterosteroids, steroidal nitrogen mustards and analogues with fused heterocyclic systems have been examined clinically for their antineoplastic profile and some were found to be potent.\textsuperscript{50-54}

The passage of the alkylating agent across cell membranes is thought to be facilitated by a more lipophilic linkage like a steroid molecule. Two cytotoxic actions achieved in case of the use of hormonally active steroids result from hormone-receptor interaction and due to a nitrogen mustard effect.

Estramustine phosphate sodium, estra-1,3,5 (10)-triene-3,17\textbeta -diol 3-[bis (2-chloroethyl)carbamate] 17-(disodium phosphate) (Estracyt) (4),\textsuperscript{55} is orally used in the palliative treatment of patients with metastatic and/or progressive carcinoma of the prostate.\textsuperscript{56-58} In humans, the drug is rapidly dephosphorylated to release
estramustine, thus readily oxidizing it to the 17-keto congener of estramustine,
i.e., estromustine.\(^{59}\) The carbamate ester linkage on hydrolysis yields estradiol
and estrone to exert their hormonal effect on the prostatic tumour.\(^{60}\)
Estramustine was observed to inhibit mitosis in prostate tumour cells in culture,\(^{61}\)
implicating an involvement of microtubules in its mode of action.

Scientists of USSR\(^{62\text{-}65}\) developed 5-cholesten-3\(\beta\)-yl p-\([N,N\text{-bis(chloroethyl)}
\text{amino}]\) phenyl acetate (phenesterin) (5) and studied its activity against a variety
of solid tumour systems by subcutaneous administration in olive oil.\(^{62\text{-}64}\) The
significance of ester function was claimed to be important in explaining the action
of phenesterin which was different from that of the parent free acid.\(^{63}\)
Phenesterin was clinically tested.\(^{66\text{-}67}\)
A series of steroid esters of \(p\)-\([N,N\text{-bis}(2\text{-chloroethyl})amino\] phenylacetic acid (BCAPAA),\(^{68,69}\) steroidal sulphides of \(p\)-\([N,N\text{-bis}(2\text{-chloroethyl})amino\] thiophenyl and a variety of steroidal ethylenimine derivatives\(^{68}\) were extensively studied. The steroid and potential oncolytic agent conjoined by a readily cleaved ester or heterocyclic ether linkage was found to possess antitumour activity. The steroidal BCAPAA esters showed excellent inhibition of a DMBA-induced and transplantable mammary adenocarcinoma and marked potency on a variety of rat leukaemias. The nature of steroid, vehicle and route of administration have definite effects on activity. Clinical studies of estradiol diester (6) were conducted.\(^{70}\)

The BCAPAA ester (ASE) (7)\(^71\) has been shown to be active against

\[
\begin{align*}
(6) & \quad \text{ClCH}_2\text{CH}_2 - N - \text{CH}_2\text{COO} \\
(7) & \quad \text{ClCH}_2\text{CH}_2 - N - \text{CH}_2\text{COO} \\
(8) & \quad \text{ClCH}_2\text{CH}_2 - O - \text{CH}_2\text{COO} 
\end{align*}
\]

L1210 and P388 leukaemias in mice.\(^{72}\) The oxa isostere (8) is less effective than
(7) in tumour T8, melanoma B16 and Th-B angiosarcoma. The synthesis of p-[N,N-bis(2-chloroethyl)amino] phenylbutyric acid (BCAPBA, chlorambucil) esters of different steroids have also been performed. The chlorambucil esters of cholesterol and estrone exhibited excellent inhibition of mammary tumour in rats – the BCAPBA ester was more active than its BCAPAA derivative (phenesterin).

The chlorambucil 21-ester of prednisolone, prednimustine (Leo 1031) (9), has been proved to be active against the L1210 murine leukaemia model and tested in patients with chronic lymphocytic leukaemia and lymphocytic lymphoma. It may also be useful in the treatment of acute leukaemia. It has been shown that prednimustine is more effective than chlorambucil against a sensitive line of the Yoshida ascites sarcoma but ineffective against Ehrlich ascites tumour resistant to alkylating agents.

Cyclophosphamide moiety has been attached to the steroid nucleus in steroidal mustards to obtain potential antineoplastic derivatives (10) and (11). The nitrogen mustard moiety is linked to the steroid nucleus in 2,4-bis(2-chloroethyl)amino-3-hydroxy-1,3,5 (10)-estratrien-17-ones and they get irreversibly
bound to the cytoplasmic estrogen receptor of the rat uterus.\textsuperscript{82} 6α-Bis(2-chloroethyl) amino-1,3,5 (10)-estratriene-3,17β-diol has been prepared.\textsuperscript{83}

Steroidal nitrosoureas were prepared to generate potential anticancer agents. Compounds (12) and (13)\textsuperscript{84} in the estrane series have free 3-OH. They inhibited the growth of the DMBA-induced transplantable rat mammary tumour and were shown to compete with estradiol for binding to cytosolic estrogen receptor in rat uterus.\textsuperscript{85} The binding possibly is irreversible and the compound (12) had higher RBA. An earlier study revealed that 13 and 14 possessed a relatively high affinity for calf and lamb uterine estradiol receptors.\textsuperscript{86} The growth of the androgen-dependent R-3327 rat prostate adenocarcinoma was not affected by 3-oxo-4-estren-17β-yl N-2-chloro-N-nitrosocarbamate (LS-1727).\textsuperscript{87}
Other heterosteroidal systems include 15-aza steroid, 1,10,11,11a-tetrahydro-11a-methyl-2H-naphtho[1,2-g]-indol-7-ol (15) which inhibited the growth of mouse L-M cells and human tumour cell line, KB, at concentration of $10^{-5}$M\textsuperscript{88,89}

The expectation of discovering antineoplastic activity on sterols and related analogues\textsuperscript{90} has evolved from the fact that cholesterol accumulates in tumour cells.\textsuperscript{91,92} 6-Aza-3,5-cholestdiene and its salts\textsuperscript{93} and 2-oximino-8,24-lanostadien-3-one\textsuperscript{94} were designed for the treatment of non-malignant and malignant tumours.

25-Azacholesterol (16) was found to be cytostatic in tissue culture.\textsuperscript{90}

Treatment of mice having experimental brain tumours with 20, 25-diazacholesterol
(17) induced an accumulation of desmosterol in the tumours but not in normal brain.95

The antiestrogen, epitiostanol (2α,3α-epithio-5α-androstan-17β-ol) (10275-S), inhibited both ductal development of the mammary gland in the mouse and an estrogen-dependent mammary fibroadenoma in rats.96 Mepitiostane (2α, 3α-epithio-5α-androst-17β-yl 1-methoxycyclopentyl ether) (10364-S) (18), is an orally active derivative of epitiostanol which has been tried with some advantage in advanced human carcinomas97: chlormadinone acetate, a potent antiandrogen, has been used for prostatic hypertrophy and prostatic carcinoma.98

Anabolic heterosteroid stanozolol (19) has been found suitable for palliative use in
the patient with active or arrested cancer.

Dimethazine (20) has been tested effectively on the immunological response of mice to Ehrlich ascites carcinoma.

The examination of several compounds has introduced two steroidal lactones 16β-hydroxy-3,11-dioxo-4,17(20)-pregnadien-21-oic acid γ-lactone (21) and

3β,16β-dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid γ-lactone as potent cytotoxic agents in a growing mammalian cell culture system. These compounds possess relatively low order of whole animal toxicity and endocrine activity and give good blood levels. They showed marginal inhibitory activity against S-180 and T-4 lymphoma implanted in mice.
Several steroidal $\alpha$-methylene-$\gamma$-lactones and their analogues, for example, 22 were active in tissue culture experiments, and preliminary in vivo tumour assay against Walker 256 carcinomas.\textsuperscript{101} 2$\beta$,16$\beta$-Dipiperidino-5$\alpha$-androstane-3$\alpha$,17$\beta$-diol dipivalate hydrochloride has been shown to be effective against Walker ascites in rats\textsuperscript{102} and in tissue culture experiments.\textsuperscript{103, 104}

2-[2-(3,17$\beta$-Dihydroxy-1,3,5(10)-estratrien-17$\alpha$-yl methyl(carbonyl)amino)ethyl] ellipticinium bromide, has a DNA intercalating property and low affinity for estrogen receptor.\textsuperscript{105} Estyramine [17$\alpha$-(3-amino-1-propyn-1-yl)estradiol] and related compounds have weak antitumour activity,\textsuperscript{106} whereas, 3,17$\beta$-dihydroxy-20,21-epoxy-19-nor-17$\alpha$-pregna-1,3,5 (10)-triens were cytotoxic to mammalian cells in culture.\textsuperscript{107}

Adenine and adenosine methylene-bridged 4-nitroestriones were more potent than 4-nitroestrone 3-O-methyl ether in inhibiting human breast cancer cell line.\textsuperscript{108} 4-Nitro-1,3,5 (10)-estratrien-17$\beta$-ol (3-deoxy compound), an effective inhibitor of porcine endometrial estrogen sulphotransferase, inhibits the growth of murine mammary tumours.\textsuperscript{109-111} 3$\alpha$-(Uracil-1-yl)-5-pregn-20-ene,\textsuperscript{112} some
androstane derivatives of 5-fluorouracil,\textsuperscript{113} and 5'-(prednisolone or prednisone-21-phosphoryl)1-(\(\beta\)-D-arabinofuranosyl) cytosines\textsuperscript{114} have been reported to be active against L-1210 lymphoid leukaemia in mice. \textit{In vitro} growth of L-1210 lymphoid leukaemia cells\textsuperscript{115} has been effectively inhibited by certain potent 9-(\(\beta\)-D-arabinosyl) adenine conjugates of corticosteroids.

Preparations of different \(\alpha\)-methylene lactones were carried out and tested \textit{in vitro} for cytotoxicity. They are 3-hydroxy-16-methylene-17a-oxa-D-homo-1,3,5-estratriene-17-one and derivatives (highly toxic towards HeLaS\textsubscript{3} cells),\textsuperscript{116} 17\(\beta\)-hydroxy-2-methylene-4-oxa-5\(\alpha\)-androstan-3-one and 3\(\beta\)-hydroxy-16-methylene-17a-oxa-D-homo-5\(\alpha\)-androstan-17-one (active against human nasopharyngeal carcinoma cells)\textsuperscript{117} and androstane-3/17-spiro-\(\alpha\)-methylene-\(\gamma\)-lactones (active against mouse Ehrlich ascites tumour cells).\textsuperscript{118,119}

In the later years, considerable amounts of progress were made in developing enzyme inhibitors capable of restricting biosynthesis of hormones in the treatment of breast and prostate-related carcinomas. Current approaches of such inhibitors are highlighted under the headings : Aromatase inhibitors and 5\(\alpha\)-reductase inhibitors mainly of steroidal origin.

\textbf{Aromatase Inhibitors}

The development and maintenance of female sex organs, the secondary sex characteristics and the mammary glands, all the requirements indirectly and directly associated with reproduction is controlled by estrogenic hormones.

In mammals the estrogens are primarily produced by the ovaries, adrenal cortex, placenta and testes. In premenopausal women, ovaries produce
The peripheral aromatisation of circulating androgens, androstenedione and testosterone in different tissues is the major source of plasma estrogens in menopausal women. Estrogens are used in a wide variety of menstrual disturbances, as amenorrhea, oligomenorrhea and dysmenorrhea. These hormones are also effective in the treatment of acne, ovarian development and senile vaginitis. Estrogens play an important role in birth control. The female sex hormones have been implicated in the treatment of breast cancer, which is the most common malignancy affecting women of the age group of 34-54 years. The inhibition of aromatase enzyme is a vital strategy in the treatment of several estrogen-dependent mammary carcinomas, endometriosis, gynecomastia, precocious puberty and for contraception. Competitive aromatase inhibitors are called as ‘substrate mimics’ and the agents causing mechanism based inhibition are called as ‘suicide inhibitors’ causing irreversible inhibition. Several authors have reviewed aromatase inhibitors.

In recent years, several new aromatase inhibitors have entered clinical trials and each succeeding generation of inhibitors have exhibited increased selectivity and potency. They exhibit a very low toxicity profile. Enhanced specificity implies that they selectively inhibit aromatase without exerting effects on the synthesis of aldosterone, thyroid hormone or cortisol.

There are several advantages of newer aromatase inhibitors (1) Well defined mechanism of action, (2) Specificity, (3) Potency, (4) Favourable toxicity profile, (5) Convenient dosage schedules, (6) Corticosteroids not required, (7) Nonestrogenic, (8) Noncarcinogenic and (9) No excessive weight gain.
Aromatase, a microsomal cytochrome P-450-dependent enzyme present in the ovary, muscle and adipose tissue, catalyzes the conversion of androgens to estrogens. Estrogen deprivation induced by aromatase inhibitors has demonstrated utility as a second-line therapy for estrogen-dependent metastatic breast cancer, and may also be useful for the management of other estrogen-
dependent processes and disease such as benign prostatic hyperplasia and endometriosis.\textsuperscript{121,138-147}

Clinically relevant aromatase inhibition has been found in nonsteroidal as well as steroidal structures. Some examples of the former include imidazoles such as fadrazole (CGS 16949A) (28) and triazoles, for example, (29) and letrozole (30).\textsuperscript{148-151} Antiestrogens such as clomiphene (31), naphoxidine (U-11,100A) (32) and tamoxifen (Nolvadex) (33) have all been tested in clinical trial but only tamoxifen (33) is available for hormone-dependent breast cancer treatment\textsuperscript{152,153} because of the low incidence of side effects.\textsuperscript{154-156} Aminoglutethimide (34), initially evaluated for its effects on the central nervous system, (anticonvulsant),
was found to be a steroid hormone biosynthesis inhibitor. Its recognition as drug decreased for it also interferes with the enzyme desmolase leading to depletion

![Chemical structure of a steroid hormone biosynthesis inhibitor](image)

(33) Z-isomer

of corticosteroid production along with other side effects.\textsuperscript{157} An analogue, roglitimid (35), has been shown to be more selective for aromatase inhibition.\textsuperscript{158,159}

Many research groups have reported variety of compounds as aromatase inhibitors.\textsuperscript{160-179}

The successful design and development of a wide variety of modified androgens have been made by the knowledge of mechanism of aromatisation.\textsuperscript{180-185}

Steroids exhibiting clinically significant aromatase inhibition are

![Chemical structure of aromatase inhibitors](image)

(36) \( R^1 = H, R^2 = \text{OH} \)

(37) \( R^1 = -CH=CH_2, R^2 = H \)

anallogues of 23 modified in the A or B ring. A prime example is 4-hydroxy
androstenedione (4-OHA, formestane) (36). In vitro studies indicated that 36 caused rapid competitive inhibition followed by inactivation of aromatase. Administration of 36 to animals with chemically induced mammary tumours, as well as to postmenopausal patients with breast cancer, resulted in tumour regression. 4-Hydroxyandrostenedione was launched in 1992 by Ciba-Geigy. Three steroidal compounds atamestane, exemestane and NKS-01 are in clinical trials.

 Mechanism-based irreversible aromatase inhibition has been achieved with propargyl-substituted steroid (37). Further studies suggested that interaction of 37 with aromatase results in oxygen incorporation into the acetylenic bond, generating an electrophilic metabolite which irreversibly alkylates aromatase. The incorporation of 19,19-difluoro-4-androstene-3,17-dione (38) and its 4-hydroxy derivative (39) involved the strategic interference with the oxidative processes in removal of the 19-methyl group of 4-androstene-3,17-dione (23). Eventually, both the fluorinated steroids were seen to be considerably less potent in 50% inhibition of enzyme activity as that of hydroxyandrostenedione.
The synthesis of diastereoisomeric 10-(epoxyethyl)estr-4-ene-3,17-diones as (19R)-(40) and (19S)-(41) isomers is of considerable interest both as active site probes of aromatase, and as inhibitors of estrogen synthesis. They unusually inhibit a cytochrome P-450 system. The (19R)-compound proved to be more effective as a competitive inhibitor of human placental microsomal aromatase (Ki=7nM) than the (19S)-isomer (Ki=75nM).\textsuperscript{196}

The fact that iodine in the 19 position of steroids can be smoothly displaced by reactive nucleophiles (CN\textsuperscript{-}, MeSO\textsubscript{2}S\textsuperscript{-}, N\textsubscript{3}\textsuperscript{-}) provided a convenient synthesis of a series of steroid derivatives, containing sulfur and nitrogen at C-19, which were tested as aromatase inhibitors. Of potential interest were the 19-
thioalkyl compounds which showed increasing competitive inhibition as the size of the alkyl substituents decreased. The most potent was the 19-thiomethyl-4-androstone-3,17-dione (42). The most potent of the 19-aza steroids was 19-azido-4-androstone-3,17-dione (43). It is proposed that both these inhibitors act by providing a sixth ligand to the haem iron of cytochrome P-450 (arom).\textsuperscript{197}

Microbial transformation of 23 produced 14\textalpha-hydroxy-4-androstone-3,6,17-trione, a very potent aromatase inhibitor \textit{in vitro}. It has almost no androgenic activity.\textsuperscript{198}

Several 7\textalpha-thio-substituted derivatives of androstenedione have been found effective as inhibitors of aromatase.\textsuperscript{199-203} Among the compounds synthesized, 7\textalpha-[(4\textprime-aminophenyl)thio]-4-androstone-3,17-dione (44) and 45\textsuperscript{204} are of interest. Reports about androstenedione derivatives with extended linear conjugation in ring A and/or B being effective inhibitors are available.\textsuperscript{205} The introduction of substituents at C-7 of 4,6-androstadiene-3,17-dione may lead to enhanced affinity of these analogues for the aromatase complex. Furthermore, replacement of the carbon-sulfur bond with a carbon-carbon bond would yield analogues with similar lipophilic character and eliminate potential metabolic oxidation of the
thioether linkage. 7-Benzyl (46-48) and 7-phenethyl-4,6-androstadiene-3,17-
diones (49-51) are found to be highly effective inhibitors of aromatase.\textsuperscript{206}

Numazawa and associates\textsuperscript{207,208} observed 3-deoxyandrostenedione to be

a potent competitive inhibitor of aromatase and analogues possessing various
substituents at C-16 or C-19 were synthesised as potential reversible or enzyme-activated inhibitors of aromatase. 19,19-Difluoro steroid (52) and 19-acetylenic alcohol has been incubated with the placental microsomes, resulting in inactivation of aromatase in a suicide manner.208

A new series of 4-(alkylthio)-substituted androstenedione analogues was designed as potential suicide inhibitors of aromatase on the basis of mechanistic considerations on the mode of action of the enzyme. Compound (53) was a reversible inhibitor of aromatase while compounds (54) and (55) displayed time-dependent kinetics of inhibition. The inhibition of aromatase by 55 was NADPH-dependent and was protected by the presence of substrate.209

Further work has been carried out in the synthesis of a group of steroids, the 6-hydroximinoandrost-4-en-3-ones, which show a high affinity for human placental aromatase, and function as competitive inhibitors of this enzyme. The oxime, (56), as both (E)-isomer in good yield and (Z)-isomer in traces has been prepared and then oxidized to the compounds (57) and (58), and have been found to bind to human placental aromatase with high efficiency.210 The 1,4-
diene-3,17-dione (59) was obtained as the sole product of biotransformation of the oxime diol, (56) by Arthobacter simplex ATCC 6946, an organism known to be capable of 1(2) desaturation of a range of 3-oxygenated steroids. The 1,4-diene (59) remains to be examined for enzyme activity.

The novel 10β-aziridinylestr-4-ene-3,17-diones (60) and (61) and the corresponding 10β-aziridinyl-17β-hydroxyestr-4-en-3-ones (62) and (63) have been synthesised and found to be powerful and stereoselective inhibitors of human placental microsomal aromatase and compound (60) was the most potent
The synthesis of 6β- and 6α-substituted androst-4-ene-3,17-diones were accomplished as aromatase inhibitors and the 6β-alkyl steroids essentially had higher affinity for the enzyme than the corresponding 6α-isomers indicating that aromatase has a hydrophobic binding pocket with a limited accessible volume in the active site in the region corresponding to the β-side rather than the α-side of the C-6 position of the substrate.

A series of androst-5-en-7-ones and androsta-3,5-dien-7-ones have been described by Numazawa and coworkers. An androst-4-ene-3,17-dione based
been devised to prepare 17-ethylenedioxy-6α-hydroxy-2-oxa-4-androsten-3-one (73) for evaluation as potential aromatase inhibitors and anabolic agents.\textsuperscript{226}

7-substituted estradiol derivatives ICI 164,384 (74)\textsuperscript{227} and ICI 182,780 (75)\textsuperscript{228} are being evaluated for pure antiestrogen activity.

**5α-Reductase Inhibitors**

Prostate cancer accounts for \~10% of all cancer cases\textsuperscript{229,230} Surgery or radiation treatment has been recommended for early, localized prostate cancer. Antihormonal therapy (normally not curative) is carried out in more advanced stages of prostate cancer which slow disease progression, relieve symptoms and prolong the lives of patients. Treatment encompassing agents like estramustine, 5-fluorouracil, cyclophosphamide and cisplatin at advanced stages suffers low response rates. Prostate cancers have been found to express steroid hormone receptors.\textsuperscript{231} Recently, it has been shown that prostate carcinomas which become refractory to androgen ablation therapies even have an increased receptor content and such tumours are therefore still a target for therapies directed towards the androgen receptor.\textsuperscript{232}

Benign prostatic hyperplasia (BPH) is the most common neoplastic condition and is virtually universal in the aging male which currently accounts for more than 0.5 million transurethral prostatectomies performed globally each year.\textsuperscript{233} Although the etiology of BPH is unclear, the permissive role of dihydrotestosterone (78) in the hyperplastic growth of the prostate is well established\textsuperscript{233,234}
5α-Reductase is an NADPH dependent membrane-bound enzyme which catalyzes the conversion of testosterone (76) to the more potent 78 which has 4-5 fold higher affinity for the androgen receptor than testosterone,\textsuperscript{235} comprises approximately 90% of the total androgen.\textsuperscript{236} Two isozymes of 5α-reductase have been identified and cloned, 5α-reductase 1 (5α-R1) and 5α-reductase 2 (5α-R2).\textsuperscript{237} The major human prostatic enzyme is 5α-R2 being found in prostate, genital skin, epididymis, seminal vesicles, and liver while the type 1 enzyme is predominant in nongenital skin and also in liver.\textsuperscript{237} 5α-R deficient male pseudohemaphrodites have mutation in 5α-R2, vestigial prostates, decreased acne, facial and body hair and do not develop male pattern baldness.\textsuperscript{238} They experience varying degree of virilization of their external genitalia at puberty, and this coincides with expression of 5α-R1 in the skin.\textsuperscript{239}

The mechanism of action of the enzyme, 5α-reductase operates via delivery of the pro S-hydrogen of the cofactor to the less hindered α-face of the

\[ \text{NADPH} \rightarrow \text{NADP}^+ \]

\[ (76) \rightarrow (77) \rightarrow (78) \]
substrate testosterone. The enolate (77) thereby generated is stabilized by the enzyme and subsequently protonated to generate dihydrotestosterone. The kinetic mechanism proceed via a preferentially ordered binding of substrates and release of products from the enzyme.\textsuperscript{240}

Potent inhibition of type-2 5α-reductase in androgen target tissue and the resultant decrease in dihydrotestosterone levels will provide selective interference with androgen action within those target tissues and no alterations of other effects produced by testosterone, other structurally related steroids, and other hormones such as corticoids and progesterone.

The most extensively studied class of 5α-reductase inhibitors are the 4-azasteroids.\textsuperscript{241} The azasteroid series of inhibitors was first disclosed by Merck in the early 1980s.\textsuperscript{242} The key 4-aza-3-oxo-5α-androstane pharmacophore and basic SAR have been studied.\textsuperscript{242} In summary, the steroidal pharmacophore provides an anchor between the key A-ring lactam and the C-17 substituent. The former acts as a transition state mimic of the intermediate enolate (77), whereas the latter significantly enhances potency via binding at a pocket largely lipophilic in character.

Merck exploited the 4-azasteroid series leading to the discovery of potent inhibitors of human 5α-reductase with \textit{in vivo} efficacy. 4-MA (79) is a potent dual inhibitor of both human 5α-reductase isozymes which was halted in clinical development due to hepatic toxicity.\textsuperscript{243} The Δ\textsuperscript{1}-unsaturated analogue MK-906 (finasteride, proscar) (80) has since been marketed for the treatment of BPH. It is a potent inhibitor of 5α-R2 with only weak \textit{in vitro} activity versus the 5α-R1
Isozyme. Clinical trials demonstrated sustained improvement in BPH disease and reduction in prostate specific androgen levels.\textsuperscript{244,245}

A number of investigators have hypothesised that a dual inhibitor of both isozymes would lead to greater reduction of both plasma and prostatic dihydrotestosterone therefore greater clinical efficacy. Variation of the C-17-amide-substituent on the optimal 4-aza-3-androstane skeleton is of particular interest in the search for potent dual azasteroid inhibitors.\textsuperscript{246} Anilides show good potency versus both 5α-R1 and 5α-R2 where the introduction of an alkyl group at the anilide nitrogen reduces the potency. This alkyl group reduces the conformational preference for the s-trans-amide conformer which is assumed to be the binding conformation (\textsuperscript{81} and \textsuperscript{82}). The indolinyl amide (\textsuperscript{83}), retains only 5α-R2 activity indicating that the 5α-R1 isozyme has stricter conformational requirements. The introduction of an o-CF\textsubscript{3} group to the anilide increases 5α-R1 potency (\textsuperscript{84}) and the naphthyl or biphenyl analogues (\textsuperscript{85}) and (\textsuperscript{86}) indicate the presence of extended lipophilic interactions in this region which may be exploited to enhance 5α-R1 potency in the optimal s-
trans-amide conformer. GG 745 (87) is among the most potent dual
inhibitors which is 60-fold more potent than finasteride versus 5α-R1.\textsuperscript{247}

One of the most potent analogues, turosteride (88) is a close analogue of
4-MA but unlike 4-MA is devoid of binding at the rat androgen receptor.\textsuperscript{248}

Glaxo has reported a series of 6-azaandrost-4-en-3-one inhibitors of 5α-
reductase where the ketoenamine functionality mimics the transition state of
NADPH-dependent hydride addition to the $\Delta^4$ alkene of testosterone.\textsuperscript{249} In
common with the SAR of the 4-azasteroids, good \textit{in vitro} potency versus 5α-R1 is
more elusive than for the 5α-R2 isozyme. The more general in vitro SAR features of the 6-azasteroidal skeleton have been reported by Frye and associates.\textsuperscript{250}

Incorporation of the optimal A-ring substitution pattern with the best C-17β-substituent led to very potent dual inhibitors (89 and 90) of both 5α-R isozymes with good oral activity.\textsuperscript{250}

It has been shown that activity resides with 3-oxo-4-aza-5α-steroids optimally substituted with methyl group at 4-position. The bridged analogue (91)

has been prepared as a potential 5α-reductase inhibitor.\textsuperscript{251}
A diazoketone, \((20\text{s})\)-4-diazo-21-hydroxy-20-methyl-5\(\alpha\)-pregnan-3-one (MDL-18, 341) (92) has been designed by Marell Dow Research Institute, as an irreversible inhibitor of 5\(\alpha\)-reductase.\(^{252-254}\)

A new class of 3-androstene-3-carboxylic acids, "steroidal acrylates", has been prepared from estrone, generally employing a differentiated bis-triflate carbonylation strategy. These exhibit potent (nanomolar), uncompetitive (vs T) inhibition of human steroidal 5\(\alpha\)-reductase via a novel association with an enzyme - NADP\(^+\) complex.\(^{255}\)

A series of estratriene-3-carboxylates have been synthesised. Despite lacking the 19-methyl group, an element shown to enhance binding in the acrylate and 4-aza series\(^{256}\) by 3-5 fold, the aryl acids \((94)\) and \((95)\) are potent inhibitors of human 5\(\alpha\)-reductase, exhibiting apparent inhibition constant equal to or lower than those of analogous acrylates \((93)\). Unlike the steroidal acrylates, however, these aryl acids exhibit greatly reduced affinity for the rat enzyme.\(^{257}\)

Holt and coworkers on the basis of their observation concluded that, 17\(\beta\)-carboxamides appear to exert the greatest positive effect on steroid binding to human 5\(\alpha\)-reductase. In the 3-carboxy series of compounds, the 17\(\beta\)-diisopropylamides displayed slightly greater affinity for the enzyme than the tert-
butylamide-containing analogues. Activity is also enhanced in analogues possessing an additional $\Delta^5$-olefin and diminished in analogues lacking the $\Delta^3$-olefin.\textsuperscript{258}

Stabilization of the proposed enolate intermediate proved to be most evident in 6-methylene-3-oxosteroid\textsuperscript{259} and epristeride (SK&F 105,657, 96) and used for the treatment of prostatic hyperplasia. Epristeride has demonstrated the ability to lower serum dihydrotestosterone levels by 50% in clinical trials.\textsuperscript{260,261}

4-Fluoroprogesterone (97) was an inhibitor of both aromatase and 5$\alpha$-

reductase at the micromolar level, probably due to the electron-withdrawing

\textsuperscript{37}
nature of the fluorine atom. In order to assess improved biological potency, other 4-substituted progesterones were prepared and 4-cyanoprogesterone (98) was found to possess marked inhibiting activity of the enzyme 5α-reductase, both in vivo and in vitro.²⁶²

Several 3-carboxymethyl steroids have been synthesised and the regioselective acrylate homologues (99) and (100) exhibited potent inhibition of human prostatic steroid 5α-reductase activity. Homologues in the estrane series also demonstrated potent inhibition, for example, compound (101). Substitution
of the benzylic carbon with gem-difluoro group (102) resulted in a maintenance of
activity.

Frye and associates\textsuperscript{264} reported on novel 6-azaandrost-4-en-3-ones (103-107) that are potent inhibitors of both human 5α-reductases with exceptional-
picomolar-potency versus the type-2 enzyme.

\begin{align*}
(103) & \quad R = O - 2\text{-adamantyl} \\
(104) & \quad R = NH - 1\text{-adamantyl} \\
(105) & \quad R = NHCH(C_6H_5)_2 \\
(106) & \quad R = NHCH(4\text{-fluorophenyl})_2 \\
(107) & \quad R = NHCH(4\text{-chlorophenyl})_2
\end{align*}
Novel 5α-reductase inhibitors 6-bromo-17α-butyryloxy-16β-methyl-4,6-pregnadiene-3,20-dione, 6-bromo-17α-propionyloxy-4,6-pregnadiene-3,20-dione and 17α-hydroxy-16β-methyl-1,4,6-pregnatriene-3,20-dione were synthesised as potential drugs for the treatment of androgen mediated prostatic cancer and benign prostatic hyperplasia.\textsuperscript{265}

Ishibashi and coworkers\textsuperscript{266} focussed attention on synthesising B-nor-4-aza-5α-androstane derivatives, since the conformation of the molecule is not much different from that of 4-aza-5α-androstene based on molecular model inspection. The compound (108) had a 63\% in vitro inhibition rate of rat 5α-

\[ \text{O} \quad \text{H} \quad \text{C}_6\text{H}_5 \]
\[ \text{CH}_3 \quad \text{N} \quad \text{C}_6\text{H}_5 \]
\[ \text{O} \quad \text{N} \quad \text{H} \]
\[ \text{H} \]

(reductase at 10\textsuperscript{-8} M concentration which was more potent than finasteride (28\% inhibition).

There have been a constant effort to design 5α-reductase inhibitors based on the concept of “inverted” or “backward-binding” steroids. The lactam steroids, e.g. (109), were less active than the parent azasteroids. Molecular modelling
studies, as well as chemical intuition strongly suggest that other molecules of the

\[ \text{(109)} \]

\[ \text{(110)} \]

generalized structure (110) will better fit the active site of the enzyme.\(^{267}\)

The preparation of a novel steroidal B-ring homologated analogue of 17β-
N,N-diethylcarboxy-6-azaandrost-4-ene-3-one (111) was accomplished and

\[ \text{(111)} \]

found to be a potent inhibitor of type-2 steroidal 5α-reductase.\(^{268}\)

Based on the concept of transition state mimickry, steroidal 3-pyridyl-
N-oxides (112 and 113) were synthesised and then assayed against the
enzyme 5α-reductase which proved to be potent inhibitors of type-2 enzymes.269

Inhibition of 5α-reductase potency was observed with 16,16-dimethyl-17β-hydroxyestr-4-en-3-one which proved to be an antiandrogen.270

Kurata and associates271 demonstrated that compounds with one aromatic moiety in the carbamoyl group of 4-aza-5α-androstane-17-carboxamide (114) showed high inhibitory activity for rat 5α-reductase, but little for human prostatic 5α-reductase. On the other hand, compounds with two aromatic moieties (115) had potent inhibitory activities for both rat and human 5α-reductase.
N-Amyl substituted 17β-formamide (116) has appeared to be one of the most potent inhibitors of human type-1 5α-reductase known so far.\(^{272}\)

The synthesis and activity of a series of 4,17-diazasteroids have been reported in a communication. The finasteride 17-aza isomer (117) proved to be quite a potent inhibitor of type-2 5α-reductase although it is less active than finasteride and its congeners. 4-Methylation (118) lowered the inhibition of the type-2 enzyme. Compound (119) was found to be an inhibitor of both type-1 and type-2 5α-reductases. 4-Methylation (120) increased the activity. Compound
with a C(5)-C(6) double bond shows only moderate inhibition of human type-2 5α-reductase activity.\textsuperscript{273}

19-Nor-10-azasteroids are a new class of inhibitors whose activity is dependent on the presence of the bridgehead N-10 atom conjugated with the 4-ene-3-one moiety in the A ring. 19-Nor-10-azasteroids (122-124), as well as testosterone having low transitional barrier energy values, are flexible molecules as compared to 6- and 4-azasteroids. The molecular flexibility of 19-nor-10-azasteroids can be considered "substrate-like" transition state analogues.\textsuperscript{274} The same group of scientists have reported a new route for the synthesis of 19-nor-
10-azasteroids (125) and (126) based on a tandem N-(acyloxy)iminium-Michael addition reaction for the construction of the A ring at a reduced number of steps and appreciable yield.  

Recently, McGuire and coworkers devised more cost effective alternate synthetic approaches to epristeride (96) a potent 5α-reductase inhibitor, utilizing a palladium or nickel catalyzed hydroxy carbonylation of a bromodiene (127).  

affinities to the androgen receptor, the CNC-alanine conjugate (129) displaying a considerably higher relative binding affinity (RBA) value to the androgen receptor, whereas 128 showed better binding to the progesterone receptor. The results demonstrated that the 19-nortestosterone conjugates of CNC-amino acids may be valuable antineoplastic agents for treatment of human prostate tumours.277

While the presence of androgens and androgen receptors and the ability to convert testosterone to dihydrotestosterone through the action of 5α-
reductase are absolute requirements for the development of BPH, a number of studies have shown that dihydrotestosterone has only minor effects on prostatic cells in culture, and the presence of other causative agents is strongly implicated.\textsuperscript{278,279} Stromal-epithelial interactions appear to be important in this respect, and a number of growth factors (such as EGF, IGF, FGF and TGF) may have a role in the pathogenesis of BPH.\textsuperscript{280} Estrogens have also been implicated in the development of BPH since changing levels associated with increasing age may affect androgen receptor density or stromal/epithelial cell death. Thus, while BPH development is age dependent and has an absolute requirement for the presence of dihydrotestosterone as a permissive factor, a definitive causative factor remains elusive.

**NEUROMUSCULAR BLOCKING AGENTS**

Neuromuscular blockade is the mechanism that results in the inhibition of nervous transmission at motor nerve endings in skeletal muscle leading to muscle relaxation. The neuromuscular blocking agents are clinically used as adjuncts to surgical anaesthesia, to prevent shock-induced dislocation and fractures, and to control muscle spasm in tetanus.\textsuperscript{281}

Neuromuscular blocking agents and drugs produce muscle relaxation and thus obviate the need for the use of high doses of volatile agents and deep planes of anaesthesia. Both anaesthetists and patients benefit from these agents for a safer and pleasant anaesthesia and quick recovery from surgery.\textsuperscript{282}

All neuromuscular blocking agents either depolarise the motor end-plate or compete with the chemical transmitter, acetylcholine (130), at the
neuromuscular junction. On the contrary, "curariform" or "curarimimetic" drugs include only those neuromuscular blocking agents which like (+)-tubocurarine, appear to act predominantly at the postjunction to induce a block of the nondepolarising type.\textsuperscript{283}

The rational design of neuromuscular blocking agents is based on the knowledge of the intimate structural features of the neuromuscular junction. A typical \textit{en plaque} neuromuscular junction consists of the axon impinging onto a specialized area of the muscle known as the muscle end-plate. The axon is covered with a myelin sheath, containing the nodes of Ranvier, but is bare at the ending. The nerve terminal is separated from the end-plate by a gap of 200 Å. The subsynaptic membrane of the end-plate contains the cholinergic receptor, the ion-conducting channels (which open under the influence of acetylcholine), and acetylcholine. There is high concentration of acetylcholinesterase, catalysing the hydrolytic destruction of acetylcholine at the neuromuscular junction.\textsuperscript{284,285}

The steric model of acetylcholine shows that the nitrogen atom is free to rotate with relation to carbon chain. The anionic site on the receptor accommodates two methyl groups which helps to stabilise the acetylcholine-receptor complex through Vanderwaal forces. The carbon atoms of the main chain lie in close approximation to a flat portion of the receptor surface,
contributing further Vanderwaal's attractions to overall binding. The carbonyl oxygen atom participates in hydrogen bond formation with an appropriate receptor group thus stabilising the interaction.²⁸⁶

Acetylcholine released into the synaptic cleft by nerve stimulation immediately combines with a specific lipoprotein receptor at the postjunctional motor end-plate. On combination, there is a change in receptor-membrane conformation.²⁸⁷ This causes a massive inward movement of sodium ions and much smaller and slower outward movement of potassium ions, across the muscle membrane through the opening of ion channels thus releasing acetylcholine molecule.²⁸⁸ The ionic imbalance lowers the normal negative charge on the inside of the muscle membrane, thereby reducing the normal resting end-plate 70-90 mV potential which is the so-called end-plate depolarisation.²⁸⁹ The movement of ions across the muscle membrane is such that the potential difference between muscle and end-plate is rapidly and completely reversed before fading to zero. The rapid passage of this self-propagating action potential over the muscle fibre membrane sets off the contraction mechanism.

The neuromuscular junction is devoid of a membrane barrier or sheath that envelops the ganglia or constitutes the blood-brain barrier. This causes the drugs accessible to the site of action, particularly so for quaternary ammonium compounds which suffers an obstructed passage through living membranes than do compounds that can exist in a nonionized species.

Bisquaternary (bisonium) salts with interonium distance of the order of 10⁻¹⁴A° selective at nicotinic sites²⁹⁰ tend to show a high specificity for
neuromuscular junction and increased potency as neuromuscular blockers. Based on this fundamental requisite rational approaches should be made in the synthesis of novel neuromuscular blockers.

Early research established two types of neuromuscular blockade—competitive, non-depolarising block (antagonist in nature) and depolarising block (agonistic). The distinction between the agonist and antagonist drugs is less clear as presynaptic desensitization or reduced acetylcholine release is readily caused by depolarising drugs that is indistinguishable from the block produced by nondepolarising agents.

**Depolarising Drugs**

Drugs in this category bring about a depolarisation of the muscle end-plate membrane which is similar to that produced by high doses of acetylcholine itself

\[
\begin{align*}
\text{H}_3\text{C} & \quad + \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{N} \quad (\text{CH}_2)_{10} \quad \text{N} \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{CH}_3
\end{align*}
\]

\[2X^-\]

\((131)\) \(X = I, Br\)

\[
\begin{align*}
\text{H}_3\text{C} & \quad + \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{N} \quad (\text{CH}_2)_2 \quad \text{OOC} \quad \text{CH}_2 \\
\text{H}_3\text{C} & \quad \text{CH}_3 \\
\text{H}_2\text{C} & \quad \text{COO} (\text{CH}_2)_2 \quad \text{N} \quad \text{CH}_3 \quad \text{CH}_3 \\
\end{align*}
\]

\[2\text{Cl}^-\]

\((132)\)

at ganglia and neuromuscular junctions in the presence of anticholinesterase agents. The muscle end-plate becomes desensitised and eventually experiences...
a block. Furthermore, a depolarisation induced by increasing the potassium ion concentration does not prevent impulse transmission. The drugs falling into this classification are decamethonium iodide/bromide (131) and succinylcholine dichloride (suxamethonium) (132). Bovet identified a class of long, thin molecules as leptocurares and a group of more bulky molecules as pachycurares. Leptocurares are depolarising agents such as decamethonium and suxamethonium. Pachycurares are nondepolarising (competitive) blocking agents represented by d-tubocurarine. The former can penetrate the muscle end-plate due to slender depolarising group and can possess maximal depolarising activity by bearing substituents of minimal steric bulk on the onium head. Progressive increase in size of the substituents on the onium centres transforms a depolarising to a competitive neuromuscular blockade, concurrently decreasing the potency.

Decamethonium has been used as a skeletal muscle relaxant, especially in combination with the anaesthetic barbiturates and is about five times as potent as (+)-tubocurarine. It is no longer in clinical use. Suxamethonium produces profound neuromuscular block and rapid recovery and its use is associated with muscle fasciculations. Its potential lethal side effects include cardiac arrest due to massive potassium ion shift from the intracellular to extracellular fluid, malignant hyperpyrexia, myalgia, myoglobinuria, bradycardia and increased intraocular pressure.
Nondepolarising Drugs

These drugs are also termed as competitive, antidepolarising, stabilising neuromuscular blocking agents or curariform drugs whose action is reversed or inhibited by anticholinesterase inhibitors. They prevent the access of acetylcholine to its receptors at the end-plate (without causing depolarisation themselves), thus preventing muscle contraction. During the progressive curarisation of muscle, the end-plate potential gradually diminishes till it falls below 70% of its normal value which is insufficient for the propagation of action potential and subsequent contraction.

The early agents, d-tubocurarine [(+)-tubocurarine]chloride (133),

\[ R^1 = H, \ R^2 = CH_3, \ R^3 = R^4 = H; \ 2 \text{Cl}^- \]

\[ R^1 = R^2 = R^3 = R^4 = CH_3; \ 2 \text{I}^- \]

The early agents, d-tubocurarine [(+)-tubocurarine]chloride (133).
metocurine iodide (134), β-erythroidine (135), gallamine triethiodide (136) and alcuronium chloride (137) have largely been superseded by the aminosteroids

![Chemical structure of metocurine iodide (134)](image1)

![Chemical structure of alcuronium chloride (137)](image2)

and the newer benzylisoquinolinium compounds. The original King formula suggested that d-tubocurarine was a bisquaternary compound. Everett and
coworkers demonstrated that at physiological pH it was a monotertiary, monoquaternary benzylisoquinolinium compound and a positively charged, lipophobic molecule. Prolonged action has been reported in patients with impaired renal function and metabolic acidosis. It is also a weak ganglion blocking agent and causes release of histamine. Transient hypotension and fall in peripheral vascular resistance have been observed.

Metocurine iodide (134) has the same pharmacologic action as that of tubocurarine chloride, however, it is considerably more potent than d-tubocurarine and has much less effect on the respiration. β-Erythroidine, and alkaloid, obtained from the seeds of the trees and shrubs of the genus Erythrina. This has been studied carefully and subjected to clinical trial.

Gallamine triethiodide (136) is used for muscular relaxation in conjunction with surgical anaesthesia. It binds with greater affinity to the M₂ receptors rather than the M₁ muscarinic receptor which is the cause of its strong vagolytic action. It also gives rise to tachycardia. The semisynthetic derivative alcuronium chloride is obtained from the most potent of all curare alkaloids toxiferines in Strychnos toxifera. The interonium distance in the flexible depolarising agents varies up to the maximal bond distance (1.45 nm for decamethonium), whereas for rigid competitive blockers is usually 1.0 ± 0.1 nm.

Among the benzylisoquinolinium drugs as neuromuscular blockers, of prime importance are atracurium besylate (138), doxacurium chloride (139) and mivacurium chloride (140). Atracurium (Tracrium®) with a reverse ester link in
the bridge between the two quaternary groupings was developed by Stenlake\textsuperscript{314} through a collaborative work at the University of Strathclyde and the Wellcome Research Laboratories (U.K.). The compound undergoes ester hydrolysis in the

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{N} \quad \text{CH}_3 \\
\text{CH}_3\text{O} & \quad \text{N} \quad \text{CH}_3 \\
\text{CH}_3\text{O} & \quad \text{N} \quad \text{CH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{N} \quad \text{CH}_3 \\
\text{CH}_3\text{O} & \quad \text{N} \quad \text{CH}_3 \\
\text{CH}_3\text{O} & \quad \text{N} \quad \text{CH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{C}_6\text{H}_5\text{SO}_3^- & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\end{align*}
\]

(138)

plasma, and is also degraded by Hofmann process.\textsuperscript{315,316} It possesses intermediate duration and slow onset of neuromuscular block. Its action is antagonised by neostigmine and edrophonium. Atracurium does cause histamine release and may cause a modest fall in peripheral vascular resistance due to histamine release.\textsuperscript{317} Cisatracurium, the $R-R'$ optical isomer in the cis-cis configuration, representing about 15% of the mixture was shown to be 1.5 times more potent than atracurium and devoid of the side effects of the latter.\textsuperscript{318}

Wellcome introduced doxacurium chloride (Nuromax\textsuperscript{30}) (139) as a very potent, slow onset, long-acting bisquaternary, nondepolarising neuromuscular
blocking agent with insignificant cardiovascular effects. It is a mixture of 3 isomers and is largely excreted unchanged in the urine.

Mivacurium chloride (Mivacron®) (140), also introduced by Wellcome group, is a bisquaternary benzylisoquinolinium compound with an ester bridge susceptible to hydrolysis by plasma cholinesterase and is a mixture of three...
isomers, pertaining to cis-cis isomeric structure. Its significant short-acting profile provides conditions suitable for tracheal intubation. Mivacurium has a rapid plasma clearance and the neuromuscular block is reversed by neostigmine. Clinical trials report that it is well tolerated with few side effects.

Fazadinium bromide (141), a product of Allen and Hanburys Limited (U.K.), is curariform in action, readily reversed by anticholinesterases and somewhat longer acting in human beings. Its metabolism involves the reduction of the diazo linkage in the liver. The drug may be used in short surgical or diagnostic procedures or in patients whose renal function is compromised.

Neuromuscular blockers have been reviewed periodically. Savrese and Kitz highlighted the desirable properties in an ideal short-acting nondepolarising neuromuscular blocking drug, i.e., rapid onset of paralysis, optimum duration of action for the required use, total absence of cardiovascular effects, lack of cumulative effects, ready reversibility by anticholinesterases or other appropriate agents, freedom from undesirable interaction with other anaesthetics and adjuncts to anaesthesia or surgery, high potency and specificity and lack of histamine release. For use in intubation and
general surgery, agents should preferably be rapidly metabolized, giving speedy recovery, thus reducing the hazards of postoperative recurarization. To prevent bradycardia secondary to vagal reflexes and hypertension due to the stimulus of intubation, a mild degree of vagal and/or ganglion block may be advantageous.

**QUATERNARY AMINOSTEROID DRUGS AS NEUROMUSCULAR BLOCKING AGENTS**

Taylor\textsuperscript{326} has surveyed in general the status of clinically used neuromuscular blockers. The work on azasteroidal neuromuscular blocking agents has been reviewed.\textsuperscript{327,328,324,329-331} The prominent features are discussed under the heads: Malouetine and Other Earlier Studies, Pancuronium and Related Investigations, and Chandonium and Allied Aspects.

**Malouetine and Other Earlier Studies**

The steroid nucleus with two cationic heads has been proposed to be of appreciable interest. The relative rigidity of the nucleus and somewhat fixed

![Chemical Structure](142)
spatial relationship between two onium centres provides room for limited flexibility through conformational variations. The hydrophilic to lipophilic ratio was apparently comparable to that of $d$-tubocurarine (133) in the steroidal alkaloid malouetine (142) and its C-3 and C-20 configurational isomers on the discovery of neuromuscular blocking potency. Bisquaternary ammonium steroids with interonium distance varying between 1.1 to 1.25 nm due to free rotation of the side chain was worth considering.

A series of $3\alpha,17\alpha$-bisquaternary ammonium-5$\alpha$-androstanes (143) with favourable interonium distance (0.92-1.06 nm) and devoid of steric hindrance to postjunctional binding by $\beta$-face angular methyl groups on C-10 and C-13 were
synthesised\textsuperscript{337} which were less active than \textit{d}-tubocurarine. Studies and tests performed \textit{in vivo} on the cat or monkey sciatic nerve tibialis muscle preparation on dipyridamidium chloride (144)\textsuperscript{338-340} and all the eight of its isomers\textsuperscript{340,341} have shown that 3\(\beta\) isomers were in general more potent than the corresponding 3\(\alpha\) compounds with nonexisting relationship between potency and interonium distances.

Bamford \textit{et al.},\textsuperscript{340} supported the adumbration theory of Loewe and Harvey,\textsuperscript{342} postulating an one-point attachment theory where the steroid nucleus shields the receptor, rather than a two-point receptor complex suggestion given by Cavallito and Gray\textsuperscript{343} and Waser.\textsuperscript{344} Steroidal compounds of 5\(\alpha\) series were more potent than the corresponding 5\(\beta\) analogues due to relatively flat steroid nucleus and more effective shield in the former case. Bamford \textit{et al.},\textsuperscript{345} resupporting the one-point attachment theory concluded that a quaternary centre is indispensable at C-3 rather than at C-17 eliciting potency of nondepolarising type.

Most of the quaternary salts (145) derived from the alkaloid conessine\textsuperscript{346} at Glaxo Laboratories (U.K.) showed in the cat short-acting muscle relaxant
properties, shorter block duration and less cumulation than gallamine or \(d\)\-tubocurarine. The bisquaternary compounds reported possess the interonium distance of 1.01 nm. Seven of the eight 3-monoquaternary compounds tested were also potent neuromuscular blockers. \(N,N'\)-Dimethyl conessine (145) was of comparable potency to \(d\)-tubocurarine and duration of action comparable to that of suxamethonium in cat. In humans the rate of recovery is slow.\(^{347}\)

The related monoquaternary drug stercuronium iodide (146) is devoid of histamine release property and has the duration of action lying between gallamine and suxamethonium.\(^{348}\) It has a rapid onset of action and a fairly large ratio between neuromuscular and ganglion blocking doses.\(^{349}\) The short duration of action was due to hepatic and renal uptake\(^{350}\) without any biotransformation of the drug being excreted unchanged in urine. Stercuronium is withdrawn from clinical practice for its high degree of cardiac and vagolytic action. It possesses significant affinity for cardiac receptors and the inhibitory muscarinic receptors on sympathetic nerve endings.\(^{351}\)
Pancuronium and Related Investigations

Pancuronium bromide (Pavulon®)\(^{352-356}\) (147), discovered at Organon Laboratories Limited (U.K.), is a potent nondepolarising neuromuscular blocking agents which was introduced in 1967 as an alternative to \(d\)-tubocurarine. It is a bisquaternary aminosteroid with an acetylcholine-like group at the 2-3 position and at 16-17 position. The 2β-piperidino and 3α-acetoxy groups are both considered to be pseudoequatorial due to the twisted boat conformation of ring A.\(^{357}\) In this preferred conformation which may be rigid due to steric compression,\(^{356}\) ring A substituents are in specific molecular conformation akin to the neurotransmitter acetylcholine (130), and may expectedly occupy the transmitter’s site of action thus affecting neuromuscular transmission. X-ray crystallographic studies\(^{358}\) reveal the actual interonium distance in the solid state to be 1.108 nm as against 1.06 nm calculated from Dreiding models. A chair conformation of ring A with O-C-C-N\(^+\) torsion angle approaching 180° has been proposed against 53° observed by X-ray crystallography.\(^{358}\)

Pancuronium is approximately 5 times more active than \(d\)-tubocurarine with rapid onset of action. Because it did not cause a fall in venous return to the
heart,\textsuperscript{356} it was especially suited to be the requirements of cardiac surgery anaesthesia.\textsuperscript{352,359} Although pancuronium is lipophobic, it is rapidly distributed to the liver and kidney. Only the 3-OH metabolite has neuromuscular blocking activity which is about 50% that of the parent compound.\textsuperscript{360,361} Renal elimination appears to be the major excretory pathway,\textsuperscript{362-367} however variable amount of drug is excreted in the bile. Although its block is usually easily reversed by neostigmine, there are reports of failure of reversal, especially in patients with renal disease.

Pancuronium produces vagolysis causing a dose-related tachycardia.\textsuperscript{359} It also depresses the re-uptake of noradrenaline at nerve endings causing a rise in blood pressure which is potentially dangerous in patients with myocardial ischemia and coronary artery disease.\textsuperscript{368-370} There is a very little placental transfer of the drug.\textsuperscript{371}

Dacuronium bromide (148), an analogue of pancuronium bromide was three to four times shorter in duration of action in cats, than pancuronium,\textsuperscript{356} but

\[
\text{(148)} \quad R = \text{OH} \\
\text{(149)} \quad R = \text{H}
\]

the potency and short time-course of action were not borne out in
humans. Org 6368 (149) was also short-acting, lacking cumulation in cats and five-fold less potent than pancuronium; the potency ratio also being borne out in man. The short-acting Org 6368 suffers early hepatic uptake.

Organon Laboratories discovered vecuronium bromide (Org NC 45; Norcuran\textsuperscript{(9)}) as a potent monoquaternary, montertiary nondepolarising neuromuscular blocking agent possessing short duration, rapid onset of action and little cumulative effect. The quaternary ring D acetylcholine fragment is relatively suited to skeletal muscle nicotinic receptors than cardiac muscarinic receptors. It is highly specific for the neuromuscular junction and is free of vagolytic or ganglion blocking effects. The removal of one or both of the acetylcholine moieties resulted in loss of neuromuscular blocking potency of the desacetoxy analogues of pancuronium and vecuronium.

The clinical pharmacology of vecuronium has been reviewed. The potency of vecuronium appears to be slightly greater than pancuronium. The usual clinical dose varies from 80 to 100 \textmu g kg\textsuperscript{-1}. No significant cardiovascular adverse effects are reported but vecuronium may increase the risk of
bradyarrhythmias. It is distributed and metabolized in a manner similar to pancuronium. The 3-OH derivative has approximately 50% the blocking potency of the parent compound. Vecuronium seems to exert a prolonged neuromuscular blockade in patients with cirrhosis.

At Organon, modifications in the 17-ester group of vecuronium bromide led to the development of Org 9453 (151) Org 9489 (152) and Org 9487 (153). Animal studies have proved these analogues to be short-acting with short duration of action. Org 9487 was selected as an alternative to suxamethonium chloride in conditions of short-lasting relaxation.

Rocuronium (Org 9426) (154) is devoid of an ester group at the 3-position and is, therefore, less likely to undergo metabolism in the body and no metabolites have thus far been identified in any quantity. It was developed in an attempt to achieve a nondepolarising drug with an onset time as short as...
suxamethonium. The dose requirements are about 5-6 times that of pancuronium or vecuronium. Organon has launched rocuronium bromide in United States (Zemuron<sup>®</sup>) and Great Britain (Esmeron<sup>®</sup>) as an adjunct to general anaesthesia to facilitate tracheal intubation and provide skeletal muscle relaxation or mechanical ventilation. The rapid onset of action may be related to reduced plasma protein binding or to an early major presynaptic action. In doses in excess of 4 x ED<sub>95</sub> some minor vagolytic action can be detected. Rocuronium is rapidly distributed to the liver and 30% of the drug can be recovered from the urine in 12 hr.

Pipecuronium bromide (RGH-1106, Arduan<sup>®</sup>) (155), a bisquaternary aminosteroid which was designed at the laboratories of the Gedeon Richter Limited (Budapest, Hungary) has a greater interonium distance as that in pancuronium bromide. This nondepolarising blocker has shown activity 2-4 times as that of pancuronium <em>in vitro</em>. The duration of action is twice as long as that of pancuronium bromide in equiactive doses and produces no histamine release along with insignificant cardiovascular effect. Because it is largely
excreted in the urine, prolongation of paralysis occurs in patients with low renal blood flow. Safety tests were carried out with pipecuronium. It causes a mild bradycardia in contrast to the heart rate increasing effect of pancuronium. Administration of pipecuronium is accompanied by hemodynamic stability.

Chandonium and Allied Aspects

The University Institute of Pharmaceutical Sciences, Panjab University is engaged in an ongoing programme of study and synthesis of bisonium steroids as potential neuromuscular blocking agents, with one or both of the cationic systems present as part of the steroid ring skeleton at different interonium
distance and the first compound designed was 4,17α-dimethyl-4,17α-diaza-D-
homo-5α-androstane dimethiodide (156). In the anaesthetised cat exhibited nondepolarising neuromuscular blocking activity with rapid onset of action. The activity is equal to that of d-tubocurarine and the duration of action one third of that of the latter. 157 was approximately equipotent to d-tubocurarine.

In the series, the compound 17α-methyl-3β-pyrrolidino-17α-aza-D-
homo-5-androstene dimethiodide (158) christened as chandonium iodide (Candocuronium, Candonium) after the name of Chandigarh (India), the city beautiful, proved to be of particular interest. It possesses a powerful nondepolarising neuromuscular blocking activity of short duration and rapid onset, being only slightly less active than pancuronium with little or no ganglion blocking activity.

Further chemical modifications were performed using chandonium as a prototype. The saturated congener dihydrochandonium iodide (159) and the analogues possessing bulkier cationic heads have been synthesised. It has been observed that saturation of 5,6-double bond in chandonium and increase in
the onium bulk in 158 or 159 decrease the potency. 159 have high potency (half of chandonium), brief neuromuscular block, no ganglion block and the least vagolytic activity in anaesthetised cat. Keeping in track of the observation that there is decrease in potency with increase in the onium bulk in chandonium, 160 was prepared. The analogues, (160) and (161), possessing acetylcholine-like and choline-like moieties are equipotent to chandonium iodide as neuromuscular blocking agents in anaesthetised cat.

Chandonium enjoys an unprecedented superiority despite several efforts to synthesise other chandonium-related compounds. Most of these compounds are devoid of 17a-aza-D-homo system but possess an extranuclear
quaternary ammonium head with nearly equivalent steric placement. The activity of these compounds has been evaluated in the rat phrenic nerve diaphragm preparation.

Amongst various compounds synthesised at the Panjab University, chandonium iodide (158) remains the most promising compound of the series.427,428 The interonium distance in chandonium iodide (by Dreiding models) was found to be 1.02 nm. Crystallographic analysis429 at the Department of Crystallography, Birbeck College, University of London, has shown the interonium distance to be 1.029 nm. Chandonium iodide has been analysed both spectrofluorimetrically430 and spectrophotometrically.431 The parenteral preparation of chandonium in normal saline withstands autoclaving.431 There is no change in potency on usual and accelerated shelf storage and is not photosensitive. In vitro protein binding studies carried out in rat433 and monkey434 have revealed some binding of the drug to plasma proteins and red blood cells, and a limited distribution of the drug to selective tissues.432 Unaltered renal clearance is the major excretory route of the drug.

The toxicity studies of chandonium iodide were performed at the Central Drug Research Institute, Lucknow, on rats and monkeys which revealed the absence of any adverse effects. Phase III multicentric comparative clinical trials in 816 cases have been completed at 4 centres which indicated that chandonium iodide is a safe and effective nondepolarising neuromuscular blocking agent with better cardiovascular stability than pancuronium.
Neither the onset nor duration of action of neuromuscular blockade of the drug is influenced by intravenous anaesthetics. Halothane, diethyl ether and trichloroethylene potentiated the neuromuscular block. Antimicrobials (streptomycin, tetracycline and metronidazole) except ampicillin increased the neuromuscular block and prolonged the recovery time. Lignocaine produced least potentiation compared to bupivacaine in degree and duration of neuromuscular block. Preoperative drugs (chlorpromazine, diazepam and fentanyl-droperidol combination) produced mild to moderate dose-dependent rise in degree and duration of neuromuscular blockade of the drug. Chandonium iodide is a stable formulation and clinically safe in humans.

Chandonium possesses cardiostimulatory response which is desirable to overcome the bradycardia produced by anaesthetic agents and preoperative cardioactive drugs. Its duration of action is 20-30 min. Higher doses of chandonium iodide (0.20 - 0.24 mg kg\(^{-1}\)) produce rapid onset (< 120 sec) and prolong the duration of action to 30-40 min.

Chandonium iodide in low doses administered along with N\(_2\)O : O\(_2\) (70 : 30) may not require reversal with anticholinesterases. In patients requiring long term muscle relaxation, pancuronium, vecuronium and chandonium do not produce difficulty in reversal of neuromuscular block.

Chandonium iodide has been approved by the World Health Organisation (W.H.O.), Geneva and is likely to be released commercially for clinical use in India.
The Gedeon Richter scientists have designed RGH-4201 (Duador®) (162), 3α-isomer of dihydrochandonium and found it to be equipotent to chandonium in conscious dog but 2-3 times less active in the anaesthetised cat.\(^{435}\)

Organon group has reported 19-norchandonium iodide (163).\(^{436}\) This was found to be 3-4 times less active than chandonium iodide. Amazingly, the enantiomer (164)\(^{436}\) has virtually the same potency as 163. It is evident that the effect of complete change in steric configuration is insignificant compared to that resulting from the change in lipophilicity caused by the removal of the 10-angular methyl group of chandonium.
Before closing this account, a mention may be made of the inferences evident from the molecular orbital calculations which have been carried out on structural models corresponding to chandonium iodide (158) and other azasteroidal neuromuscular blockers. The conformations evident from the calculations are similar to those apparent on crystallographic analysis of the azasteroids. Further, the calculations show high degree of delocalisation of positive charge.