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Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

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"PREPRATON AND CHARACTERIZATION OF STEROID-LINKED DERIVATIVES AS ANTICANCER AGENTS"

M. D. Sangale and A. S. Daptare

Head Department of Chemistry, A.A.College, Manchar, Dist-pune(M.S.)
Department of Chemistry, A.A.CollegeManchar, Dist- Pune (M.S.)

Abstract:-Progesterone is an important steroidal hormone for women since it is involved in the control of a variety of regulatory function such as ovulation, development of corpusluteum, and maintenance of uterine quiescence during pregnancy. It is also involved in proliferation and differentiation of mammary glands. The biological activity of progesterone is mediated by the progesterone receptor [PR], a member of the large gene family of nuclear receptors, which induces a cascade of transcriptional events after binding with the hormone. PR contains three functional domains including the N-terminus, a centrally located DNA binding domain (DBD) and C-terminal ligand binging domain (LBD) respectively. The binding of progesterone induces of conformational change in PR that promotes dissociation from a multi-protein complex followed by homo dimerization and binding to specific progesterone response elements (PRE) within the promoter genes. The hormone activated PR recruits co-activators through protein-protein interactions, which serve as intermediates for the initiation of transcriptional processes.

Keywords: Progesteron, steroidal hormone, promoter genes, transcriptional process.

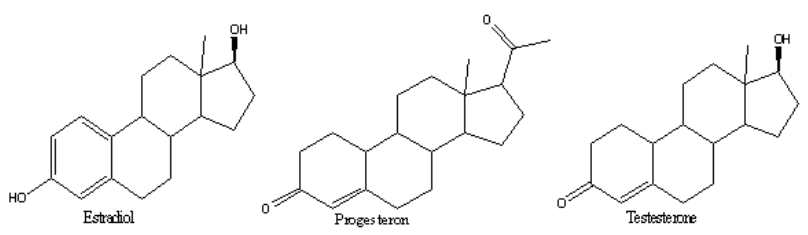
INTRODUCTION:

Breast cancer is the most common cancer of woman in the western world. In most cases, breast cancer isestrogen dependent, and treatment with estrogen antagonists that inhibit estrogen receptor (ER) action, particularly tamoxifen, has contributed to a dramatic reduction in breast mortality. Statistically it is known that 1 in 8 American woman and 1 in 12 woman in UK are susceptible develop Breast Cancer in their lifetime. This is the most common malignancy in Northwestern European woman and American woman. [1a] It was estimated that in 2006, approximately 40,410 deaths occurred due to Breast Cancer. [1b,28,29] Breast Cancer incidence and death rates generally increase with age.

The sex steroids present in the humans are of the following 3 types:

Androgens (Males) SEX STEROIDS Progestin (Females)
↓
Estrogens (Females)
Estradiol

Of these, Estradiol is the endogenous estrogen and Progesterone is the endogenous Progestin.



Estrogens act on many tissues such as those of the reproductive tract, breast and CNS. The primary physiologic action is to stimulate the development of secondary sex tissues. The Estrogen receptors are of 2 types: ERα and ERβ. Both differ in their sizes. The ERα has 595 amino acids and is a prominent estrogen receptor in female reproductive tract and mammary glands while the β receptor is the smaller one, has 530 amino acids and predominant ER receptor in vascular endothelial cells, bone and male prostate tissues. There is 56%homology between the 2 structures. [2,3,30,31] The Estradiol has similar affinities forboth ERα and ERβ. ERβ is highly homologous to ERα in its DNA and ligand-Binding domains. It is activated by E2 and usually inhibited by anti-estrogens such as Tamoxifen. Despite the 56% sequence identity between both the receptors in the LBD, both the receptors share subtle differences in the ligand binding specificity.

Estrogen-Receptor Mechanism:

The Estradiol binds to ER in the Ligand Binding Domain (LBD) and leadsto a conformational change yielding the ER-receptor complex through the Estrogen responsive elements (EREs). This leads to initiation of transcription of DNA sequence to produce mRNA and finally the elevated levels of mRNA lead to an increase in the protein synthesis in the ER.

Another target of estrogens is the breast tissue. Estrogen can stimulate the proliferation of breast cells and promote the growth of hormone-dependent mammary carcinoma. The selective action of estrogens on different tissues depends on the binding of estrogen to specific proteins that constitute the Estrogen receptor (ER) mechanism within those tissues. [4,5,32] Thus, although the Effects of estrogen actions are beneficial to some extent, experiments on the Clinical level have shown that most the breast cancers depend on these Estrogens for their development.[6]

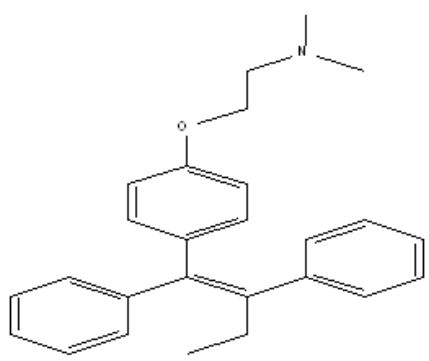
Risk Factors for Breast Cancer:

Many factors are predictive of the breast cancer risk, which includes age [7], family history [8,9], early menarche [10], late menopause [11] , younger age at First live birth [12], previous benign breast biopsy etc. Some of these are a cause of high estrogenic activity while some can also be classified as inherited risk; these include breast density, high bone density and high circulating estrogens.[13] The findings from research on the risk factors suggest that breast density might be useful as a surrogate marker of risk with endocrine intervention. Also, most of the risk factors appear to be interrelated.

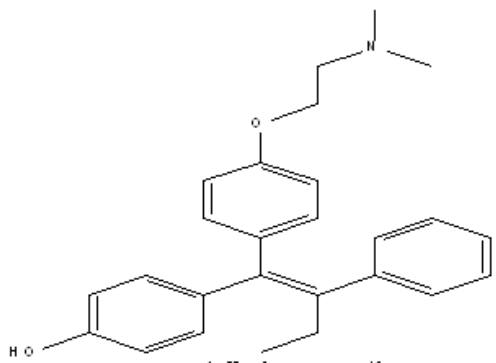
Estrogen Receptors as Therapeutic Targets in Breast Cancer:

These therapeutic agents are of the following 3 types:

SERMs: Selective Estrogen Receptor Modulators or SERMs as they are popularly knows are used for their importance of selectivity in the modulation of ER for the treatment of breast cancer. Tamoxifen, a triphenylethylene was first discovered as a possible contraceptive in 1967. Later it received FDA approval
by the US government as an adjuvant treatment for node-positive breast cancerin postmenopausal woman with chemotherapy.

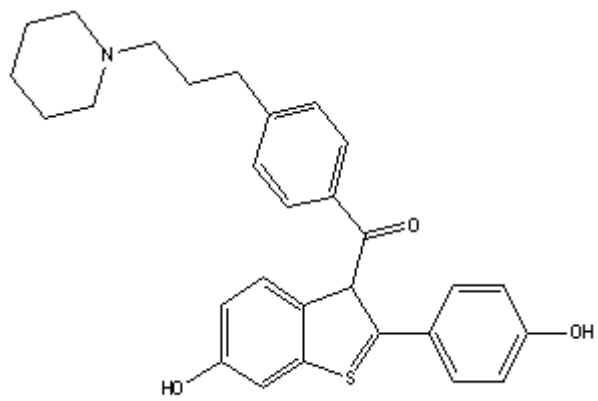


Tamoxifen



4-Hydroxymoxifen

Tamoxifen is metabolized by cytochrome P450 enzyme to the potent active metabolite 4OHT (show in the figure above) and by cytochrome P450 3A to the week antiestrogen N-desmethyl-tamoxifen. The major metabolite in humans [14, 15]. It is known to function as a partial agonist depending on the tissue and promoter context. Raloxifene is another SERM. Abenzothiopene derivative approved by the FDA prevention of osteoporosis in postmenopausal women in1997. It is known to prevent bone loss and reduce the risk of vertebral fractures. [16]

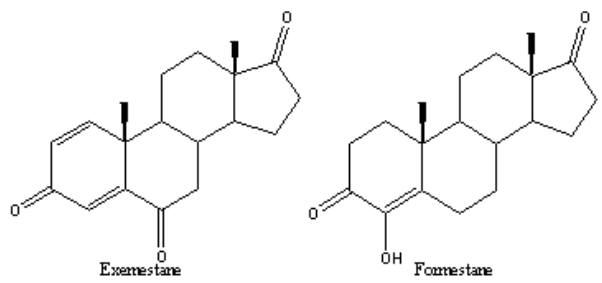


Raloxifene

In addition, like Tamoxifen, Raloxifene apparently prevents breast cancer in high-risk women, but unlike Tamoxifen has not been noted to increase the incidence of endometrial cancer. Thus, although Raloxifene comes close to being an ideal SERM.it is not so. An ideal SERM would be one that decreases the incidences on hot flashes, vaginal discharge, blood clots and endometrial cancer [17]. Thus over the last decade multiple other SERMs were identified, developed and discarded, while many are still under development. Some of these include, Toremifine, Droloxifene, GW5638 and GW7604. Just as Raloxifene showed improved therapeutics compared to Tamoxifen. The new SERMs show interesting properties.

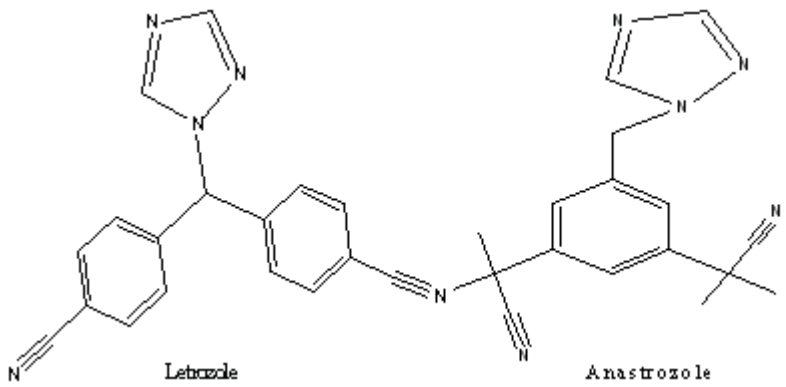
Aromatase Inhibitors: Aromatase is an enzyme responsible for the synthesis of Estrogen from androgenic substrates. This enzyme, the product of CYP19Gene, belongs to the cytochrome P450 superfamily. These Aromatase Inhibitors are described as first-, second- and third-generation inhibitors to the chronological order of their clinical development and as Type 1 or 2 based on their mode of action. The Type 1 inhibitors are the steroidal motifs whereas the Type 2 are of the non-steroidal motifs.

Steroidal Aromatase Inhibitors: The steroidal Aromatase inhibitors (Als) like the ones shows below: Formestane and Exemestane inhibit Aromatase by binding to the enzyme's substrate binding site, causing irreversible inhibition.



Formestaneis administered biweekly in order to achieve 85-90% inhibition of Aromatase activity, which is not recommended. Hence, the third-generation Als came into existence, which do not require such high dosing. Exemestane is orally active and because of its steroidal structure has a better safety profile than the other Als. Although, the Als appear to be slightly more effective than Tamoxifen and show significant advantage in terms of disease-free survival, this has not translated into increased overall survival in most cases.

Non-steroidal Aromatase Inhibitors: The non-steroidal Aromatase inhibitors are of the third-generation. These were also compared with the standard Tamoxifen for their setting them as adjuvant therapy for breast cancers. In a trial conducted in 2001, Letrozole was administered to nearly 977 patients with advanced breast cancer. The statistical results of the differences between its therapeutic use was not significantly from that of Tamoxifen.



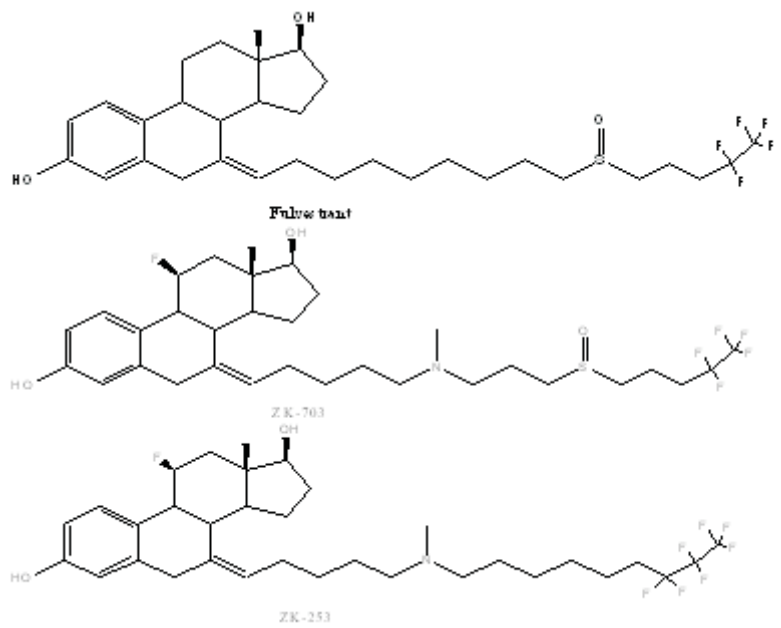
Anastrozole was evaluated against Tamoxifen in the advanced breast cancer cases with adjuvant setting. Although, more advents were recorded in the anastrozole group, these were less serious than seen with Tamoxifen [19].

Pure Antiestrogens:

Fulvestrant: This is an analogue of 17β Estradiol has chemical structure completely different from the non-steroidal structures and other SERMs. Also, unlike other antihormonal therapies, which can be given orally, Fulvestrant requires intramuscular administration. It is a second-generation pure antiestrogen with very high affinity for the ER. It causes destruction of ER and removal of the ER signal transduction in ant-hormonal resistant breast cancers. Although Fulvestrant did not prove to be better than other therapies; it clearly represents an additional treatment option for woman with breast who fails to respond to any other therapy.

ZK-703 and ZK-253: The low-bioavailability problem associated with Fulvestrant often is accompanied by discomfort. Therefore in order to overcome this novel pure antiestrogenic compounds namely, the ZK-703 and ZK-253 weredeveloped. Both can be administered subcutaneously. ZK-253 was found to Inhibit tumor growth better than either Tamoxifen or Fulvestrant. The clinicalstudies in this direction are currently going on.

Endocrine therapies of breast cancer



I. Single treatment
Surgical: - castration
-hypophysectomy

-adrenalectomy
Medical: - estrogens: antiestrogens
-progestins: antiprogestins
-androgens: antiandrogens
-corticosteroids: anticorticoroids
-aromatase inhibitors
-LHRH analogues
-somatostatin analogues
-prolactin analogues
-compounds blocking growth factor binding or signal transduction
-inhibitors of specific factors involved in metastatis

Combined hormonal treatment
Alternating hormonal treatment

II. Combined hormone-chemotherapy

Alternating hormonal [inhibitory or stimulatory and chemotherapy]
Cytotoxic metal complexes incorporated into hormonal agents

III. Radiolabelled (anti) hormones or growth factors

Molecular Targets:

Proteasome inhibition: Within all the breast cancer trials, new and promising classes of compounds fast emerging on the scene are the proteasome inhibitors, which act by a completely different mechanism. They happen to target the proteasome ,a multi protease enzyme complex, present in all cells, and responsible for the degradation of various protein involved in inflammation, cell cycle regulation, gene expression, cellular growth and differentiation [20]. The proteasome inhibitors mechanisms of actions can several and can be summarized as follows:

Inhibition of NF-kB activation (NF-kB: intracellular redox active signaling Factor)
Induction of apoptosis, by increasing the levels of key proteins, such as p21 and bax (anti-apoptotic proteins), and consequently leading to cell cycle arrest atG1 or G2- M Phase
inhibition of cell growth signaling pathways
Anti-angiogenesis effects

PS=341 (Velcade®),a boronic acid dipeptide is a potent, selective and reversible Proteasome inhibitor.
COX-2 inhibition: The COX-2 inhibitors are also a new class of compounds Included in the ongoing investigations in breast cancer. These enzymes catalyze the synthesis of prostaglandins from arachidonic acid. These enzymes are Basically of two: COX-1 (which is constitutively expressed in many tissues including stomach, kidney, colon, and platelets is responsible for a number of physiologic functions and COX-2 (which is induced in response to growth factors, cytokines, oncogenes and tumor promoters in macrophages and tumor tissues). The mechanism of action of these enzymes is believed to be blocking the development of carcinogen-induced mammary tumors, through inhibition of tumor cell migration, cell invasiveness and angigogenesis, in animal models [21].Although the exact mechanism is not yet clear, in the light of all the findings of the experimental data hopefully link the non-steroidal anti-inflammatory drugs (NSAIDs) to decreased breast cancer incidences.
Cell Cycle inhibition: The cyclin dependent kinases viz. Serine /threonine kinases that regulate cell cycle progression by leading to phosphorylation of the 33333Rb protein. In turn, they are regulated by cyclins and endogenous cdk inhibitors such as p16,p 21 and p27 [22]. Since most of the breast carcinomas have a deregulated cell cycle progression due to altered expression or function ofcdks, cyclins or endogenous cdk inhibitors renders these molecules attractive as potential targets for anticancer therapy. Flavopiridol, UCN-01 are currently the drugs used for inhibition of cdk and induction of apoptosis through abrogation ofG2 checkpoint following exposure to DNA damaging agents.
Agents targeting the HER-family receptors: These are the agents targeting the HER family of receptors and their subsequent downstream pathways. This forms a multiple step-targeted approach and involves inhibition of the ligand binding to the extracellular part of the receptor [23]. Breast cancer is often characterized by EGFR over expression, absence of ER and endocrine resistance in breast cancer usually with the advanced stage of disease [24] and therefore represents a potential target in such cancers. Amongst the latest drugs on block are the Iressa (ZD-1839) and Tarceva (OSI-774), both selective EFFR-tyrosine kinase inhibitors. Pre-clinical data suggests that these drugs can inhibitHER-2 mediated signal transduction, but with a modest activity. Hence, a combination therapy with other drugs might work for such compounds. This approach would prove particularly useful as HER-family signaling pathway might be involved in hormone resistance.
Targeting the Ras-Raf-MAPK pathway: The HER-family receptors signal mainly through two key pathways, one of them

being the Ras-Raf-MAPK pathway. Farnesylation, performed by the enzyme forms a key step process in the activation of some downstream proteins involved in intracellular signaling pathways. Thus, the use of farnesyltransferase inhibitors such as the R115777 (Zarnestra) has been in the forefront. This is the unique molecule, which is in the Phase III clinical trials. Targeting the Pi3K-Akt pathway: This is the second pathway for the transmission of HER mediated growth signal, which mediates signaling from growth-factor receptors, estrogens and IGF-1 and controls cell proliferation, cells survival and growth and metastization. One of the downstream effectors of thePi3K pathway is the mammalian target of rapamycin (mTOR) and is known to be involved in a variety of cellular processes including protein translation, RNA transcription, protein kinase C (PKC) signaling and other biological function [25]The eventual effect of inhibition of mTOR is an arrest of tumor cells in the G1phase of the cell cycle, resulting in inhibition of cell replication and tumor growth.[26] CCI-779 is an mTOR inhibitor currently in phase II evaluation in metastastic breast cancer, both as single agent and in combination with other endocrine agents.

Given below is a table which showing various agents targeting breast cancers. Their mechanism of action and clinical developmental status.

Agent	**Mechanism of action (Mode of administration)**	**Clinical development status in breast cancer**
R115777 of Zamestra	Farnesyl transferase inhibitor (oral)	Phase II/III
CCI-779	mTOR inhibitor (weekly i.v and oral)	Phase II single agent and combination studies.
ZD-1839 of Iressa	Tyrosine kinase inhibitor (Oral)	Tyrosine kinase inhibitor
OSI-774 or Tarceva, CI-1033, PK-166	Tyrosine kinase inhibitor (Oral)	Phase I/II
PS-341 or Velcade	Proteasome inhibitor (i.v)	Phase I Combination studies (docetaxel;Trastuzumab)
BAY-43-9006	Raf Kinase inhibitor (Oral)	Phase I/II alone and in combination with cytotoxic agents
17-Allyaminogeldanamycin (17-AAG)	HSP inhibitor (i.v)	Phase I
rhuMAb VEGF	Monoclonal antibody targeting VEGF (i.v)	Phase II-III combination trails (with capecitabine, vinorelbine, paclitaxel and docetaxel)
Marimastat (BB-2516)	Matrix metalloproteinase inhibitor (Oral)	Phase III
Flavopiridol	Cell cycle inhibitor (i.v)	Phase I combination with trastuzumab
COX -2 inhibitors	COX-2 inhibitors (Oral)	Phase II combination trails (with trastuzumab, exemestane): chemopreventione studies
Genasense	Bcl-2 antisense (i.v)	Phase I

ER negative Breast Cancers:

The ER can also mediate via. a different pathway through protein-protein interactions at AP1 sites which is ligand independent. The inherent structural differences between the ER α and ER β where ER β lacks a functional AF1 domain, results in a large effect on the activation profiles of the target genes. Thus, the activity of ER which are ligand-independent or non-genomic effects may be mediated by part by plasma membrane associated forms of ER[27]. Approximately, one-third of all invasive cancers are ER-ve and women with a family history of breast and ovarian cancers have a high risk of developing ER-ve breast cancers compared with the general population. The chemo preventive agents that are currently being evaluated in the ER-ve breast cancers include tyrosine kinase inhibitors, CDK inhibitors, Rexinoids (RXR selective ligands) and COX-2 selective agents.

Problem areas:

Majority of the drugs target ER +ve breast cancers

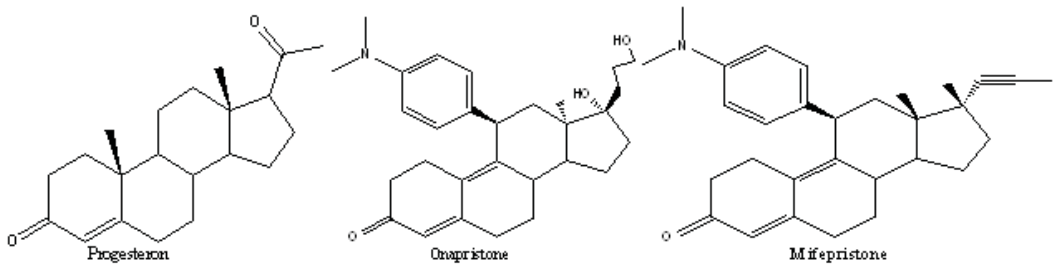
Resistance to most of the current drugs
High therapeutic dosage required for desired action
Toxicity problems over prolonged use

Nature and Scope of present investigation:

The hypothetical role of progesterone-initiated intracellular signaling in tumorigenesis of mammary gland has received corroboration from the marked reduction in mammary tumor incidence in PR gene knock-out mice (PRKO) as compared to isogenic wild types. It has, therefore, been suggested that in absence of the PR function, prolactin alone is not sufficient to induce the neoplasmic transformation and that PR may activate mitogenic mediators of the prolactin pathway. The epithelial cells under such conditions might exhibit lower proliferative index and thus are poor candidates for malignant transformation upon administration of carcinogen. These results give strong support for the use of PR anatgonists commonly referred as anitprogestins, in breast cancers as they might inhibit the prolactin mitogenic action on the luminal epithelium.

The quantitative light and electron microscopic data has indicated that antitumor action of antiprogenstins is accompanied by the initiation of terminal differentiation leading to apoptotic cell death. It has been suggested that the antitumor activity of the antiprogestins in breast cancers does not depend on its antihormonal nature as in case of Tamoxifen but also on their other properties as well as the accumulation of tumor cell in the G0-G1 phase of the cell cycle, terminal differentiation and appearance of apoptotic cell death. The results of recent Phase II clinical trial on a pristone have confirmed an overall tumor remission rate of 67% confirming that such progesterone antagonists are capable of inducing tumor responses in human breast cancers and can offer potential benefit by extending the therapeutic options even to antiestrogen refractory disease.

Several synthetic ligands of steroidal and non-steroidal origin have been developed as antiprogestin compounds which are capable of inhibiting progesterone receptor activity. Mifepristone was the first of these PG antagonists reported in 1981 and has been used in numerous clinical studies. It is a derivative of 19-nortestosterone, which has an additional 4-(Dimethlyamine)- phenyl group at the 11β position, a double bond at C-9 and 1-propynyl chain at the 11 α position respectively. In general positions C-11 and C-17 in the progesterone scaffold have been chosen for structural modification and various potent antiprogestin compounds have been evolved with considerably reduced endocrine side effects.



hydrazones having side chain at C-3 position which would exhibit potent entiproliferative activities against hormone dependent and independent breast cancer cell lines.

Current Approach:

In women and nonhuman primates, treatment with progesterone antagonists suppresses estrogen-dependent mitotic activity in the endometrial glands. The current study was aimed at developing some novel progesterone antagonists using the steroidal scaffold with substitution at C-3 position. Progesterone was conjugated with cytotoxic pharmacophores like thiosemicarbazone, semicarbazone and hydrazones. This steroid-pharmacophore combination is anticipated to exert multi-target effects on breast cancer cells thereby being effective on hormone dependent as well as hormone independent cells. The synthesis and characterization of the synthesized compounds has been described further.

Experimental:

Materials used:

All chemicals used for synthesis of ligands their metal conjugated were of analytical grade. Solvents were distilled prior to their use. Progesterone was purchased from Sigma-Aldrich while all the amines were purchased from Loba, Chemicals, India.

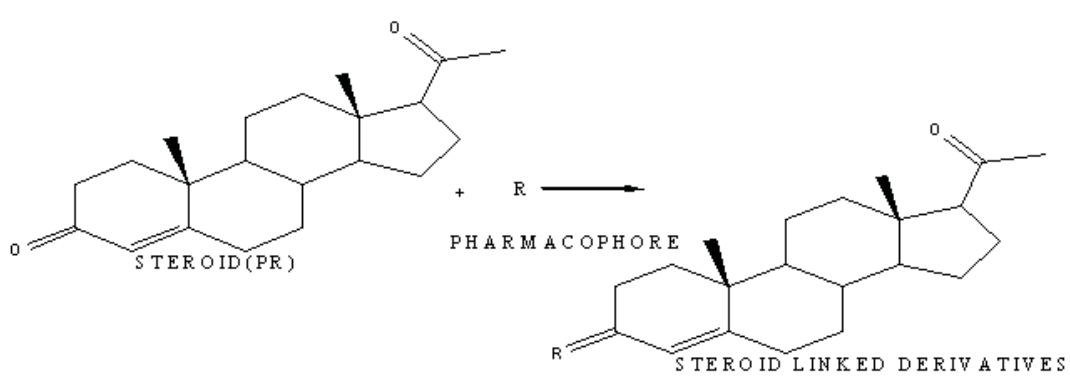
Infra-Red spectroscopy: IR Spectra were recorded in KBr discs in the range of 4000-200 cm^{-1} on the Shimadzu FTIR

spectrometer (Model no. 8400) at Department of Chemistry, University of Pune, Pune.

Electronic spectroscopy: The electronic spectral measurements of the synthesized compounds in DMSO solvent were carried out on SpectronicGenesys -2 Spectrophotometer using 10mm rectangular quartz cuvettes in the range of 250-1100nm at the Department of Chemistry, University of Pune, Pune.

^{1}H NMR spectroscopy: The^1 H NMR data spectra were obtained from 300 MHz Varian instrument.
CLog P calculations: The clog P calculations were performed using log P predictor software.

Synthetic scheme of Progesterone derivatives:



1. R= (PRTSC)

2.R= (PRSC)

3.R= (PRNH)

4.R= (PRNH)

5.R= (PRAI)

6.R= (PRSH)

7.R= NH_2OH. (PRNOH)

Synthetic scheme of Progesterone derivatives:

PRTSC: Progesterone acetate (PR) (100mg. 0.317 mM) was initially dissolved in about 5ml of distilled methanol. To this (40.38 mg. 0.317 mM) of Thiosemicarbazide (TSC) was added in a 1:1 ratio. This clear solution upon stirring for 4 hours yielded a pale yellow coloured precipitate. After confirming the product formation from TLC. The precipitate was filtered and dried in vacuo. An 85% yield was obtained.

PRSC: A similar procedure mentioned above was carried out using PR (100mg 0.317mM) and Semicarbazide hydrochloride (SC) (35.46mg. 0.317 mM) ina 1:1 molar ratio. The Product was obtained in 75% yield.

PRNH: A similar Procedure mentioned above was carried out using PR (35.46mg. 0.317 mM) and Nicotinichydrazide (NH) (43.65mg\0.317 mM) in a 1:1 molar ratio. The Product was obtained in 75% yield.

PRINH: A similar Procedure mentioned above was carried out using PR (35.46mg. 0.317 mM) and Isonicotinic hydrazide (NH) (43.65mg, 0.317 mM) in a 1:1 molar ratio. The Product was obtained in 75% yield.
PRAI: PR (35.46mg. 0.317 mM) was added to amino indole (Al) (42.025mg, 0.317 mM). A light brown coloured solution was obtained upon stirring. The reaction was stirred for 24 hrs. after which Piperidine (27µl) was added in a 1:1 molar ratio of the ligand, Progesterone. This yielded a brown coloured product which was later dried in cacuo. The product was obtained was confirmed by TLC and the 55% yield was recorded.

PRSH: A similar Procedure mentioned above using PR (100mg. 0.317 mM) and Salicyclic hydrazide (SH) (48.368mg, 0.317 mM). A 65% yield was recorded.

PRNOH: Procedure similar to above using PR (100mg. 0.317 mM) and Hydroxylamine Hydrochloride (HA) (22.09mg, 0.317 mM). A 60% yield was recorded.

RESULTS AND DISCUSSION:

a)Compositional studies: The compositional data on all synthesized ligands are summarized in Table 1

.Table 1: Analytical data on progesterone Schiff bases

Compound	Molecular Formula	Degradation Temperature Range^{0}C	C log P	Compositional Data			
				%C	%H	%N	%S
PR	$C_{21}H_{30}O_2$	128-132	3.74	(80.21)	(9.62)	(10.18)	---
PRTSC	$C_{22}H_{33}N_3OS$	225-228	5.18	(68.22)	(8.53)	(10.85)	(8.27)
PRSC	$C_{22}H_{33}N_3O_2$	105-110	3.75	(71.15)	(8.89)	(11.32)	---
PRNH	$C_{27}H_{35}N_3O_2$	95-100	4.41	(74.83)	(8.08)	(9.69)	---
PRINH	$C_{29}H_{43}N_3O_2$	117	4.39	(74.83)	(8.08)	(9.69)	---
PRAI	$C_{29}H_{36}N_2O$	205-220	5.18	(81.31)	(8.41)	(6.54)	---
PRSH	$C_{28}H_{36}N_2O_3$	200-205	3.15	(75.00)	(8.08)	(6.25)	---
PRNOH	$C_{21}H_{31}NO_2$	125-135	4.35	(76.60)	(9.42)	(4.26)	---

*values in parenthesis are calculated ones

b) Infrared Spectroscopy:

The spectral profiles of the significant Infrared bands of the compounds synthesized are shown in table 2. The Infrared spectrum of Progesterone (PR) exhibits 2 significant frequency at 1664.5 (3C) and 1697.2 (18C). Upon condensation the carbonyl frequency at the C-3 position is replaced by the imino stretches observed at 1606-1517 cm-1, while the C-18 carbonyl frequency is observed at≈1697 cm-1 for the synthesized compounds.

Table 2: Selected IR frequencies (cm-1) for progesterone Schiff base ligands

COMPOUNDS	ν(C=O)	ν(N=C)
PR	1664.5,1697.2(free)	---
PRTSC	1693.4	1587.3
PRSC	1697.2	1517.9
PRNH	1664.5	1525.6
PRINH	1670.2	1525.6
PRAI	1695.3	1596.9
PRSH	1699.2	1539.1
PRNOH	1697.2	1606.6

c) Electronic Spectroscopy:

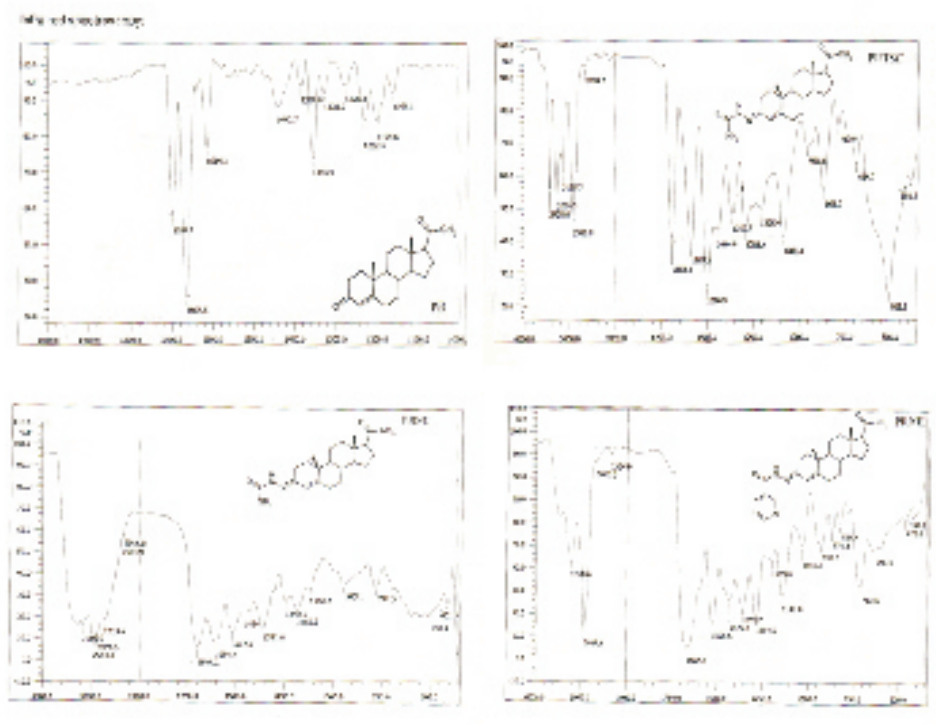
Electronic spectra of the synthesized compound along with their probable assignment of transition is shown in table 3. PR (starting compound) shows a ππ* at a 256 nm to lower values. This is because C=O bond is weaker (ionic type) as compared to C=N (as N is less electronegative than O) Thus more energy is required for the electronic excitation in C=N hence lower value.

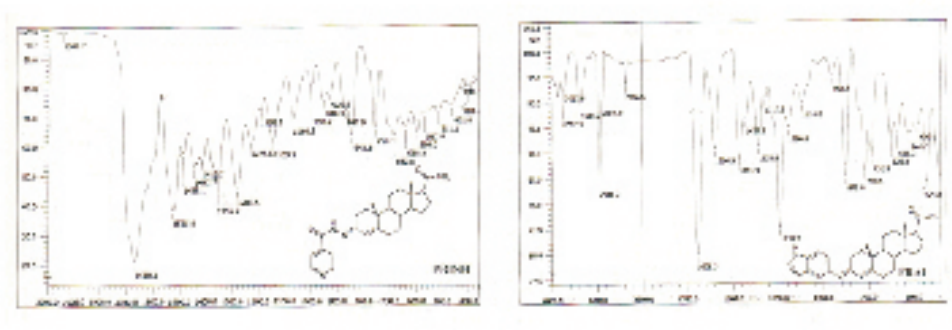
Table 3: Electronic data of Progesterone Schiff bases

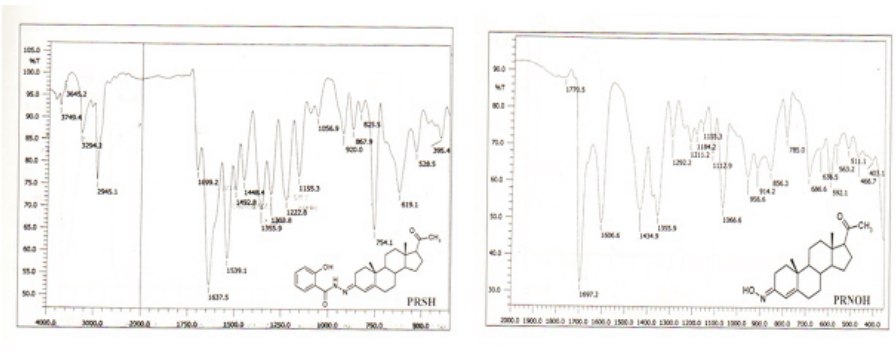
COMPOUNDS	π→π*(nm)	n→π*(nm)
PR	256	301
PRTSC	220	307
PRSC	235	271
PRNH	247	301
PRINH	240	285
PRAI	214	379
PRSH	216	312
PRNOH	237	---

d) Nuclear Magnetic Resonance Spectroscopy:

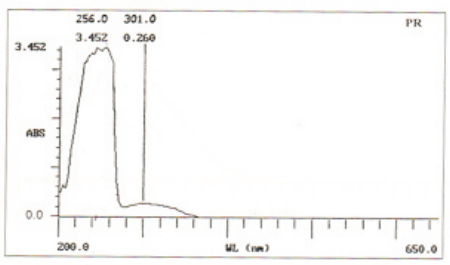
The NMR data for PR shows 3 peaks for the different methyl (C-18,C-19&C-21) at 0.53δ,1.10δ and 2.02δ. The =C-H proton at C-4 position shows a peak at 5.60δ. On formation of PRNH, PRINH,PRAI and PRSH these methyl peaks and =C-H peak is seen along with the peak for aromatic protons. For PRSC & PRTSC peak for N-H protons along with methyl protons and =C-H proton of PR. For PRNOH we get H peak along with the signals for PR.

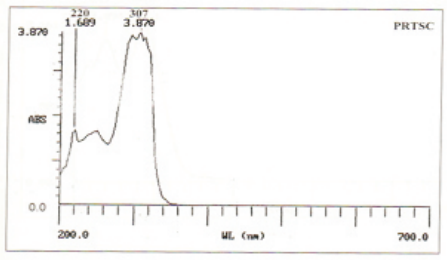


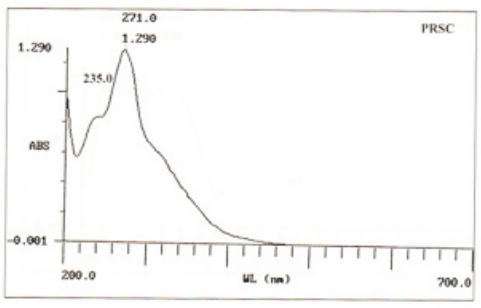


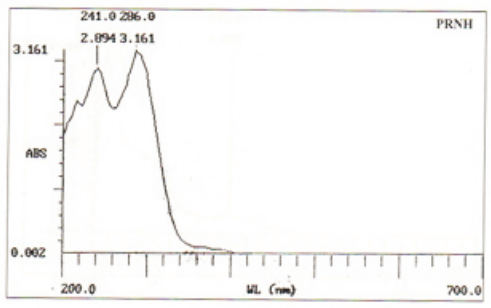


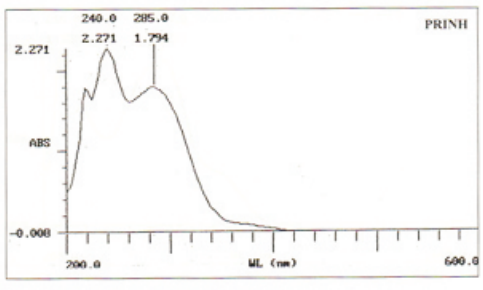
Electronic spectras:

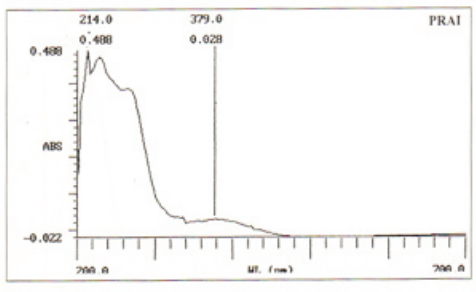


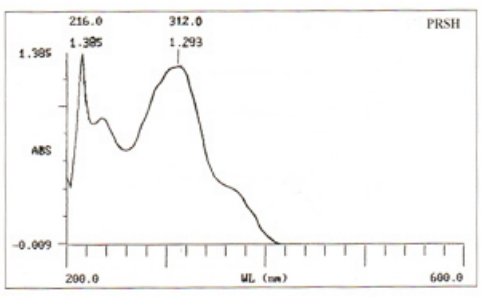


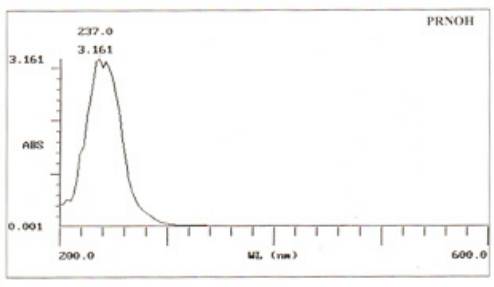


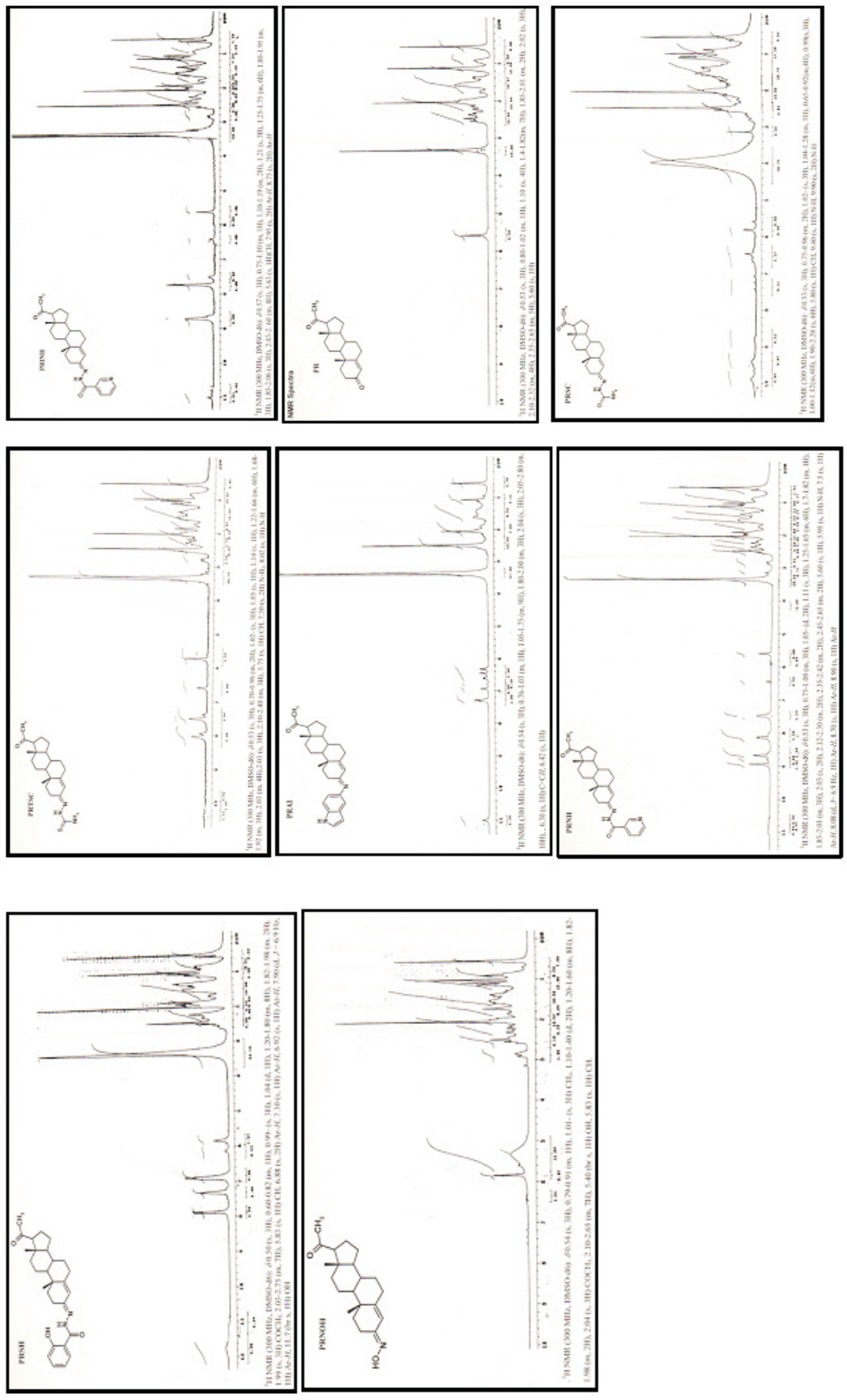












CONCLUSIONS:

The present study described a facial synthesis of novel promising anticancer steroid derivatives. we have synthesized new class of estrogen linked cytotoxic agents breast cancers. The synthesized compounds were characterized using spectroscopic techniques like Infrared, electronic and nuclear magnetic resonance (NMR). We hypothesize that the synthesized ligands containing the carrier steroidal moieties by appending cytotoxic linkers to them will show anti proliferative activity.

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