Progesterone Receptor Agonists and Antagonists as Anticancer Agents

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Abstract: Progesterone is a major female steroid hormone produced by the ovarian corpus luteum and by the placental syncyiotrophoblast during the second trimester. The biological effects of this steroid hormone are mediated by the ubiquitously expressed progesterone receptor. The exact link between progesterone and female reproductive organ cancer is a controversial issue with various cross-talks. The present review summarizes recent trends in the development of some (anti)progestagen in the cure and management of breast and uterine cancers.

Keywords: Progesterone receptor, progestagens, agonists, antagonists, anti-cancer.

1. INTRODUCTION

Progesterone, a major steroid hormone produced by the ovarian corpus luteum and by the placental syncyiotrophoblast during the second trimester, is considered to be essential for the successful maintenance of pregnancy. This hormone regulates the characteristic transformation of uterine epithelium from proliferative to the secretary state. A disturbance in progesterone biosynthesis and secretion results in abortion and preterm birth [1-3]. In addition, progesterone plays an important role in bone metabolism and neurotrophic functions as well [4]. Synthetic steroidal progestins are widely used as the therapeutic agents in the control of fertility, combination hormone replacement therapy and a variety of endocrine related disorders [5]. Estrogen and progesterone are the two key regulators of proliferation and differentiation in reproductive tissues and the latter are known to oppose the biological effects of the former in many systems [6-8]. Such functional interplay between estrogen and progesterone is fundamental to maintain a significant physiological process [9].

The biological effects of progesterone are mediated by the ubiquitously expressed progesterone receptor (PR). PR belongs to the nuclear receptor super family comprising receptors for steroid hormones, vitamins D3, thyroid hormones and retinoids. These receptors have conserved DNA- and ligand-binding domains (DBD and LBD, respectively) and variable hinge and N-terminal regions [10] (Fig. **1**). Upon ligand binding the ligand-receptor complex binds to the regulatory regions of progesterone-responsive genes and subsequently stimulates their transcriptions (Fig. **2**). Antiprogestative compounds may also bind to PR, but blocks its transcriptional activity. PR is unique within the family of steroid hormone receptors since it exists as two isoforms, PR-A (~94 kDa) and PR-B (~116 kDa). Basically, PR-A is truncated form of PR-B lacking the first 164 N-terminal amino acids. Two distinct promoters within the single copy gene for PR have been shown to independently regulate the expression of PR isoforms [11,12]. The expression of pure homodimers of PR-A and PR-B has shown that they act as repressors and activators of transcriptions respectively [13]. Further, the DNA binding domain contains the sites for phosphorylation, sumoylation, ubiquitination and acetylation [14-16]. In case of PR-B, 14 residues can remain phosphorylated while 8 residues in case of PR-A, of which few sites are basally phosphorylated and few gets activated upon ligand binding [17]. Protein kinases like casein kinase, mitogen-activated protein kinase, cyclin dependent kinase can also cause PR phosphorylation upon activation by growth factors [14,18,19]. Moreover, progesterone can be linked to single nucleotide polymorphism (SNP) of PR since it has been reported that valine to leucine polymorphism of PR (V660L) increases the risk of breast cancer [20,21]. Further, an inhibitor function region (IF) has also been characterized at 292 amino acids upstream of activation function element 1 (AFI) which is known to inhibit the function of both activation function elements (AFI and AFII). Since PR-B contains an additional activation function element (AFIII) which is not inhibited by IF, that could explain the functional difference between these receptor isoforms [22] (Fig. **1**).

In recent times hormone replacement therapy (HRT) is one of major choice for the cure and management of several diseases which utilizes a combination of estrogen and progesterone as therapy. In spite of the widespread use of progestins (also progesterone) in various clinical applications, there are several reports linking the effect of this hormone with breast, uterine and ovarian cancers [23-26]. Further, synthetic progestins have been found to intensify cancerous conditions more than their natural analogues [27]. This has led to a strong debate among the clinicians and the researchers for the use of progesterone or its synthetic forms (progestins) in HRT. In the present review we summarize the recent developments of some natural and synthetic PR agonists and antagonists having potential to control uterine and breast cancers.

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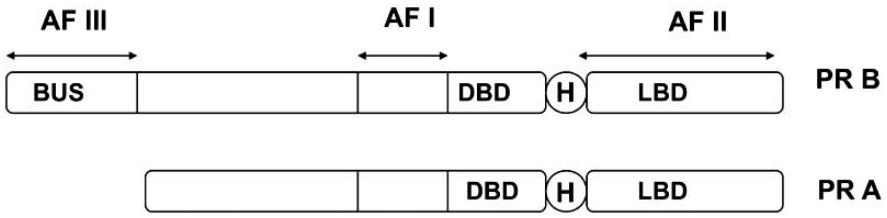


Fig. (1). General structure of progesterone receptor **A** and **B**. DBD, DNA binding domain; LBD, Ligand binding domain; AF, Activation function; H, Hinge region; BUS, B-upstream segment.

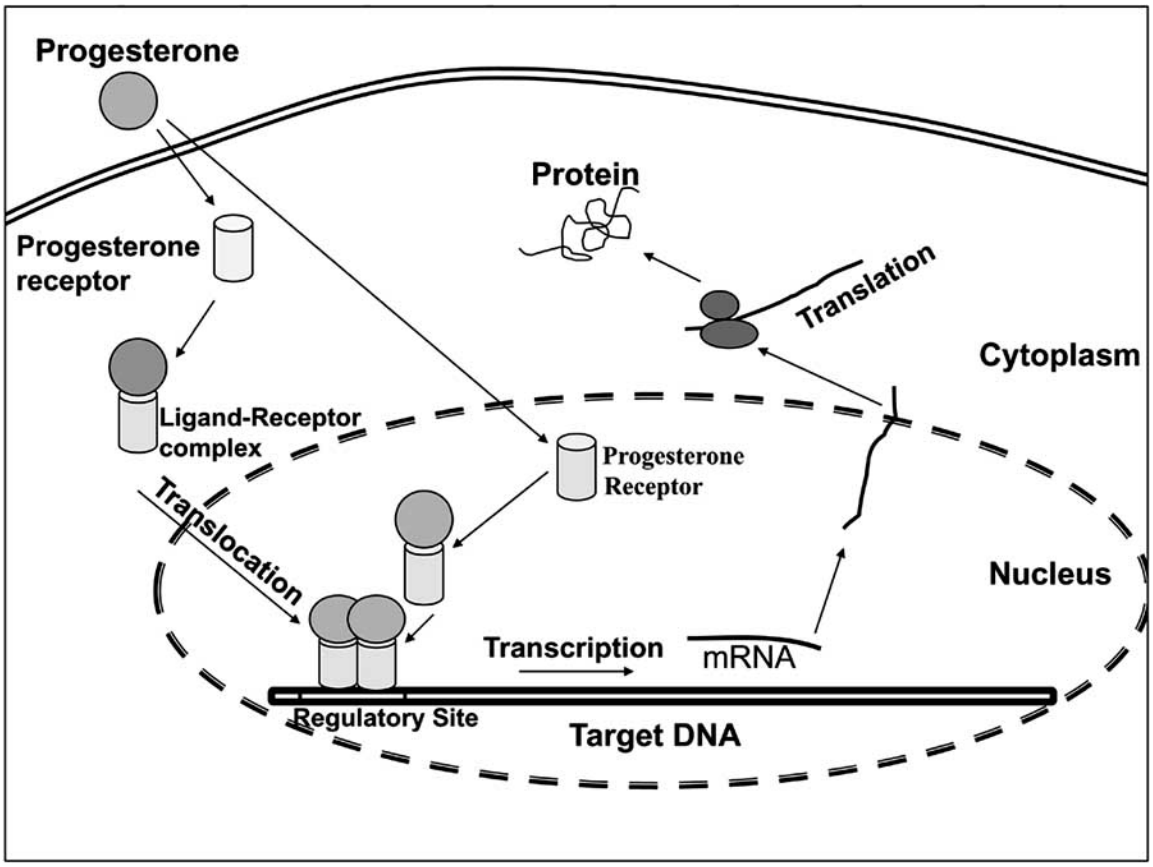


Fig. (2). Schematic representation of action of progesterone in a target cell.

2. PROGESTERONE RECEPTOR AND REPRODUCTIVE ORGAN CANCERS

For reproductive organs, hormonal stimulations are considered to be the critical factor for carcinogenesis. In females the sex steroid hormones, estrogen and progesterone, play an important role in normal mammary gland development, and it is believed that the breast cancer progression is influenced by them and/or their receptors [28-30]. A number of studies have reported the antagonistic nature of PR for the estrogen receptor (ER) functions [31]. This fact could be complementary to the role of PR as an antiproliferative protein, atleast in ER responsive cancers. PR binds to non-liganded ERα and modifies its function as a proliferative transcription factor [32]. Further, it has also been reported that the PR isoform in breast and endometriod cancers are differentially expressed. Other than FIGO grade I tumors (in which the tumors are graded according to the histological nuclear chromatin structure by International Federation of Gynecology and Obstretics- FIGO) other tumors expressing PR do not bear 1:1 ratio of PR-A:PR-B which aids to its proliferative effects [33]. This may be due to the functional antagonism of the effecter genes to PR. A major difference exists in the differential sub cellular localization of PR which results in its associations with MAPK for its extra nuclear proliferative effects [34]. PR-B is present both in the nucleus and cytoplasm whereas PR-A is strictly nuclear [35] and PR-A and PR-B respectively has 2 and 3 MAPK induced phosphorylation sites. Thus PR not only acts on the genes bearing progesterone response elements, but it also interacts with other genes bearing estrogen response element or c-src, c-myc, ERK1/2, MEK or ras binding sites [36]. Further, PR isoforms also has differences in the transcriptional activities [19,37-39], turnover rates [37,40], protein complex formation and target gene specificity [31,34-37]. Hence PR is found to exhibit both proliferative and anti-proliferative activities. Fig. (**2**) summarizes the mode of action of progesterone in a target cell.

3. PROGESTERONE AND PROGESTERONE RECEPTOR INTERACTION

Progesterone-PR complexes have been analyzed to understand the mode interaction of this steroid to its receptor. From the crystal structures it seems that the 3-keto oxygen molecule of the A-ring of progesterone (Fig. **3a**) is responsible for hydrogen bonding with the Glutamine 725 of helix 3 of PR and it is conserved in all the steroid receptors except ER [41]. Another residue required for the progesterone interaction with its receptor is the presence of a methyl-ketone substituent at C-17 position. Threonine 894 of helix 11 seems to bind hormones containing a 17-alkyl-keto group (progesterone, glucocorticoid and mineralocorticoid receptors), and is replaced by a large hydrophobic residue in the androgen and estrogen receptors, both of which bind hormones with a much simple 17-hydroxyl group [41]. Progesterone contacts residues from helices 3, 5, 7, 11 and 12, and the β-turn of the receptor. The most important aspect of progesterone binding to PR is that it stabilizes the C-terminal extension of helix 12 of PR which is responsible for the receptor-dimer stability and its protection from general protease degradation [41]. The core zinc modules of the PR DNA binding domain and the intrinsically disordered carboxyl-terminal extension (CTE) of the DNA binding domain interact with recently discovered basic region plus leucine zipper (bZIP) domain of jun dimerization protein 2 (JDP2). Chemical shift changes in PR upon titration with JDP2 revealed that most of the residues involved in binding of JDP2 reside within CTE. Point mutations within CTE sites identified by NMR and a CTE domain swapping experiment also confirmed the functional importance of JDP2 interaction with the CTE for enhancement of PR transcriptional activity [42]. These features of the ligand receptor interactions are taken care of while designing the agonist or antagonist molecules. After the ligand bound receptor undergoes conformational change it activates the progesterone responsive genes in a classical mode of action (Fig. **2**).

Other than the transcriptional activity relationship of progesterone-PR complex, the non-transcriptional role of PR is also worth mentioning. Progestagens can reinitiate the cell cycle progression in anti-estrogen arrested breast cancer cells [23]. These molecules have been reported to activate PI3K/Akt/NFκB pathway to initiate cell cycle [43]. Such an effect is due to the conventional N-terminal DNA binding domain or the SH3 domain interaction but occurs through the interaction of phosphorylation sites of PR-B with cyclin D1 [23]. Progesterone is also found to activate IP3 receptor which is in turn involved in the nuclear translocation of p-Akt in neuronal cells [44]. These genomic actions of progesterone have been shown even in breast and endometrial tissues [45]. Thus anti-progestagens in PR positive breast cancer cells may act effectively by blocking these activities. However, prolonged progestagen exposure first resulted in increased expression of cyclin dependent kinase (CDK) inhibitor $p21^{Cip1}$ followed by increase in $p21^{Kip1}$ which may nucleate cyclin/CDK complexes and block cell cycle progression. Overexpression of cyclin D, E or A or the loss of $p21^{Cip1}$ in PR positive breast cancer cells is predicted to bypass these controls [46]. Arnette-Mansfield *et al.* [33] has reported that there has been a reduced expression of PR in endometrial tumors as compared to normal tissue due to epigenetic control. However, DNA methyl transferase inhibitors (5-aza-20-deoxycytidine) and histone deacetylase inhibitors (trichostatin A) leads to restoration of the receptor expression in these tissues [47] causing apoptosis of endometrial tumors. Progestagens are also reported to induce Fas/FasL pathway leading to apoptosis and deregulation leads to induced resistance [48]. Thus depending on the pattern of the interaction between progesterone and PR new molecules could be discovered for the cure of breast and uterine cancers since these interactions make PR to function either as proliferative or anti-proliferative protein.

4. ANTICANCER MOLECULES

4.1. Synthetic Progesterone (Progestins) :

4.1.1. Dydrogesterone

Almost 200 progestins have been synthesized till date. According to a recent review a combination of progestins and estrogens at a low dose and for a limited period (less than 5 years) may have beneficial effects in peri- and postmenopausal women [49]. It has been reported to block the enzymes involved in estradiol bioformation (sulfatase, aromatase, 17β-hydroxysteroid dehydrogenase) responsible in progression of breast cancer [50]. Dydrogesterone and its 20-dihydroderivatives have been shown to be potent inhibitors of estrone sulfatase in MCF-7 breast cancer cells [50]. Experimental evidence indicates that the use of natural progesterone (Fig. **3a**) and its retro isomer, dydrogesterone (Fig. **3b**), elicit different, or in other words, opposite effects as compared to synthetic progestins like medroxyprogesterone acetate (MPA) or norethisterone acetate (NETA) [51,52]. According to a previous report by Madjerek and Smit-Vis [53,54] the progestational activity of dydrogesterone was found to be about half as that of natural progesterone whereas MPA was almost 5-folds more active than the parent hormone. The assay was based on the ability of the progestins on the production of a decidual reaction in the uterus

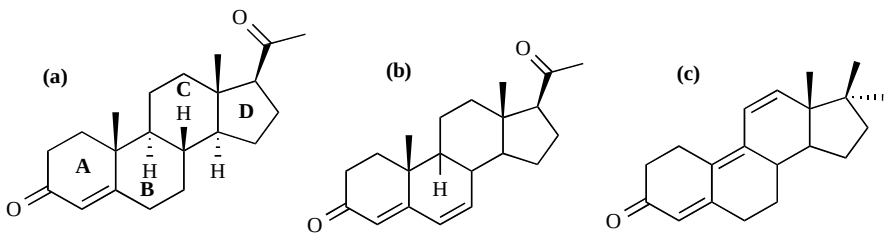


Fig. (3). Various types of progestagens (**a**) Progesterone (**b**) Dydrogesterone (**c**) Tribolone

in immature rats following subcutaneous application of hormone [53,54]. MCF-7 and T47D cells when exposed to dydrogesterone and its main metabolite 20α-dihydro-dydrogesterone, was found to reduce estradiol formation by estrone sulfatase. It is also found to inhibit 17β-hydroxysteroid dehydrogenase [50]. This retroisomer of progesterone acts directly on the metabolism of estrogen and does not antagonize the positive estrogenic effect unlike progesterone itself. It is even beneficial in increased blood pressure [55,56] and thromboembolism [57-59].

4.1.2 Tibolone

Tibolone (Org OD-14) is a synthetic steroid (Fig. **3c**) that has estrogenic (in bone and vaginal tissue), androgenic (in brain and liver) and progestagenic (in endometrium) activities [60-62]. Studies have shown that tibolone are potent sulfatase inhibitors at low concentrations in hormone dependent breast cancer cells [63]. These sulfatases are produced in higher amounts in breast cancer tissues as compared to normal tissues and also in the postmenopausal breast than in premenopausal women [63].

4.2. Natural Progesterone

In its natural form micronized progesterone which is used in HRT along with estrogen is considered to be safe [64]. The progesterone molecule has anti-estrogenic effect due to transcriptional down regulation of estrogen receptors and stimulation of pathways for estrogen metabolism [50]. Progesterone significantly decreased secretion of pro-MMP-2 and MMP-2 transcript expression levels in a dose-dependent manner in JAR human choriocarcinoma cell lines [65]. These matrix metalloproteinases (MMPs) are responsible for invasion, angiogenesis and tissue remodeling which is required for the generation of malignancy in a tumor. Recent findings show that micronized progesterone used along with estrogen is safe so far its carcinogenic activity is considered [64]. But long term HRT is anyway related to progression of breast cancer and hence search for anti-progestagens with reduced cross-reactivity for other steroidal receptors are thus warranted. Recent discoveries by Sarkar *et al.* revealed that isothiocyanate-progesterone conjugates act as a potent anticancer compound on breast cancer cells [66].

4.3 Anti-Progestagens as Anticancer Agents

4.3.1. Anti-Progestins

4.3.1.1. ZK 230211

Mifepristone **(**RU-486) (Fig. **4a**) is a potent antiprogestin but has cross reactivity for GR also. Hence there was an urgent need for the development of new PR antagonists without other steroid hormone binding activities. In 2000 Fuhrmann *et al.* [67] identified a novel, highly potent progesterone receptor antagonist, ZK 230211. This pure, highly potent compound with anti-progestagenic endocrinological profile has been developed by introducing a-pentafluoroethyl side chain into the D-ring of the steroidal skeleton. Substitution of D ring at C-17 position gives rise to the flexibility in structure to induce stable binding to the receptor. On the basis of the structure activity relationship this profile of ZK 230211 was predominantly attributed to the pentafluoroethyl side chain. ZK 230211 showed the best receptor selectivity and a potent anti-progestin activity [68]. In clinical phase I study, ZK 230211 showed favorable pharmacokinetics after oral administration and was well tolerated at all dose levels. This compound is currently under development for treatment of progesterone dependent breast cancer.

4.3.1.2. Pyrazoline Based PR Antagonists

Docking studies of both mifepristone into a PR antagonistic homology model generated from the crystal structure of PR complexed to the endogenous progesterone [41] and tamoxifen to ERα using the computer-aided drug design program MVP [69] reveled that the N,N-dimethylaniline moiety of mifepristone compound was responsible for driving the receptor into an antagonist conformation through displacement of the hormone binding domain (AFII helix) of the PR. This approach resulted in the design of diarylpyrazolines, a previously unexplored PR chemotype. Based on this, Jones *et al.*, 2005 [70] for the first time introduced pyrazoline based PR antagonists (Fig. **4b**). In silico structure resulted in selection of derivatives with the 3-aryl ring of the pyrazoline which suitably overlay with the A-ring of mifepristone. The pyrazoline ring acts as a C-ring moiety, and the benzenesulfonamide portion extends into a 17α -pocket previously observed in the crystal structure of PR bound with mometasone furoate, a progesterone agonist. A series of compounds designated as 7a–11 were representatives of an array of 4- and 5-substituted positions of pyrazoline sulfonamides (R_2 and R_3) were synthesized (Fig. **4b**) and tested for receptor binding as well as functional activity in CV-1 cells in their study. The study revealed that the binding affinity constant of the compound 7b, which is a mifepristone mimetic, is 96% as compared to 100% in mifepriston for PR and the functional profile is also comparable to those of the steroidal PR antagonist. Henceforth, 4-Aryl-pyrazolines were proposed to mimic the antagonistic interaction of mifepristone's N,N-dimethylaniline in the PR ligand binding pocket.

4.3.1.3. C17 Phosphorus Derivatives as PR Antagonists

Mifepristone, a potent PR antagonist, is also known to inhibit GR in a similar manner as it is with the former. This fact could be attributed to the similarity in the structure and mode of actions of various steroid receptors. Due to the lack of receptor selectivity it was proposed to modify the C-17α position of mifepristone around the propynyl group to increase receptor selectivity without altering the antagonistic properties [71]. Several literature reports exists depicting phosphinic acid as bioisosteres of the carboxylic acid group [72]. Jiang *et al.* (2006) proposed the replacement of the CH_3 on the propynyl group with a phosphonyl group in order to increase the possibility of both hydrophobic and hydrophilic inter-actions between steroid and PR [73]. These subtle changes might impact differential binding to progesterone and glucocorticoid receptors, and thereby altering the selectivity profile of the target molecules. Several compounds, viz., 17a-h have been synthesized in this context by them (Fig. **4c**) [73]. In the next stage, those compounds were evaluated for PR antagonist activity based on their ability to block progesterone-induced alkaline phosphatase activity in the human breast cancer cell line T47D. Simultaneously, these compounds also inhibited corticoid- induced transcription from a GRE-linked luciferase reporter gene as evaluated

in human lung carcinoma cell line. In this context, compounds 17a, 17c, 17e, 17g and 17h showed better selectivity to PR than GR as compared to mifepristone. Amongst them, compounds 17a and 17c were equi-potent and more selective than mifepristone. Out of these, three most potent and selective compounds (17a, 17c, 17e) were tested *in vivo* in ovariectomized Sprague Dawley rats by rat complement C3 assay. Upon administration of the compounds *via* the oral route along with ethinyl estradiol (EE) and progesterone, 17a was as potent and efficacious as mifepristone, with an ID_{50} of 3 mg/kg. Compound 17a was more potent *in vivo* than compound 17c or 17e and was consistent with the *in vitro* T47D data. In summary, out of the series of novel phosphorus-containing C-17 side chain mifepristone analogues developed by Jiang *et al*. [73], compounds 17a and 17c showed 30- and 10-folds more selectivity, respectively, as compared to mifepristone, but with similar PR potencies.

4.3.1.4. Oxazoles as PR Antagonists

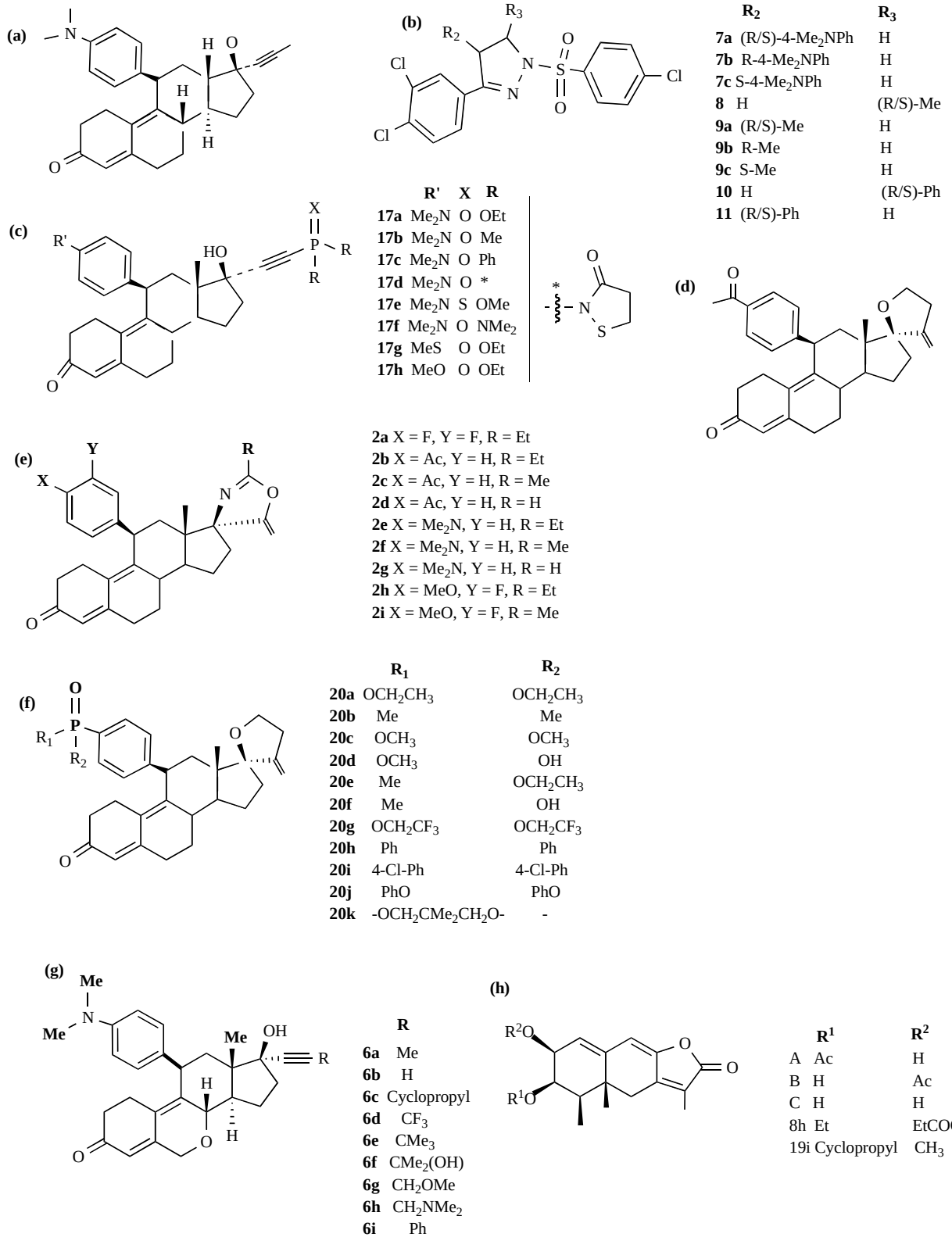
Recently Jin *et al*., 2007 [74] has developed a convenient method of synthesis of novel 11β-aryl-4′,5′-dihydrospiro [estra-4,9-diene-17β,4′-oxazole]s (e.g., 2a; X = F; Y = F; R = Et) (Fig. **4e**) *via* copper (I)-catalyzed cyclization of the corresponding acylaminoacetylenes as described earlier [75] to develop a highly potent anti-progestin with reduced endocrine side effects. These novel spiro-oxazoles are structurally similar to the reported 17,17-spirocyclic steroids (e.g., ORG 33628) (Fig. **4d**). These novel compounds have been reported to show enhanced anti-progestational effects with considerably reduced anti-glucocorticoid activities. Jin *et al*. (2007) reported a series of new 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs, viz., (2b–i) and their anti-hormonal properties [74] (Fig. **4e**). Subsequently, compounds 2b–i were evaluated for PR antagonist activity based on their ability to block promegestone (R5020), a PR agonist, induced alkaline phosphatase activity in human breast cancer cell line, T47D. GR antagonist activity was also tested by its ability to inhibit corticoid-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549 for those compounds. Amongst them, some of the compounds (2b, 2c, and 2h) showed high potency and a better selectivity profile in the separation of anti-progestational and anti-glucocorticoid activity than mifepristone. In the T47D assay, compounds 2b and 2c exhibited subnanomolar IC_{50} values indicating potent PR antagonism. In a supportive manner in A549 assay, compounds 2b and 2c were 6- and 7-folds, respectively, more selective than mifepristone with subnanomolar potencies for PR. Based on these results it could be conceived that the novel steroidal spiro-oxazoles could be a promising drug target due to their potent anti-progestagenic activity.

4.3.1.5. Phosphorus-Containing 11b-Aryl-Substituted Compounds as PR Antagonists

Kehler reported that phosphinic acids act as bioisosteres of the carboxylic acid group [72]. Org 33628 (Fig. **4d**) with a spiral cyclic chain at the C-17 position has already been shown to exhibit higher PR antagonist potency with significantly lower GR activity [72, 76] as compared to mifepristone. Thus, Jiang *et al*., 2006 [77] envisioned that phosphonyl groups can serve as bioisosteres for the carbonyl group on the 11-β-aryl group of Org-33628 (Fig. **4d**). This helped them to use Org 33628 as a template to make a chemically novel steroidal series with similar PR potency and higher selectivity against GR, when compared to mifepristone. As shown in Fig. (**4f**), a novel series of phosphorus-containing C11 aryl-substituted steroids were synthesized by utilizing Pd-catalyzed phosphination reaction of triflate by Jiang *et al*. [77]. These compounds (20a-k) were tested in cell-based *in vitro* bioassays for progestin and glucocorticoid antagonistic activities. Most of the compounds were potent PR antagonists at nanomolar range, with some (20b and 20e) demonstrating better selectivity (~13-folds) as compared to mifepristone. The authors attributed the probable cause of poor antagonistic activities of 20d and 20f as to the negative charge of the compounds which might prevent them from penetrating the membrane and enter the cell. Selected compounds also showed modest oral anti-progestin activity in rat uterus complement C3 assay (20a, 20b and 20e) which was also consistent with the T47D alkaline phosphatase assay data [77].

4.3.1.6. Oxa-Steroids as PR Antagonists

Recently, a novel series of oxa-steroids (Fig. **4g**) (series 6a-i) have been synthesized and identified as potent and selective PR antagonists by Kang *et al*., 2007 [78]. The basic feature of this series of novel PR modulators is the structural similarities with mifepristone, altered C-17α position and introduction of an oxygen atom in the steroidal skeleton. In this context, the first enantioselective synthesis of (8S, 13S, 14R)-7-oxa-estra-4,9-diene-3,17-dione 1 with the unambiguous trans-C/D ring junction was achieved by the authors previously [79]. With the step-wise synthesis of series of compounds, oxa-steroids compound 6a (R = CH_3) was examined for PR antagonist activity based on its ability to block progesterone induction of alkaline phosphatase activity in the human breast cancer cell line T47D. Subsequently, GR antagonist activity was also tested based on its ability to inhibit corticoid-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549. These results demonstrated that the compound 6a was a potent PR antagonist with an IC_{50} value of 7.5 nM since it inhibited T47D alkaline phosphatase activity and found to be about 10-folds more selective to PR over GR, thus exhibiting a slightly better selective profile to that of mifepristone. Thus the group synthesized a series of oxa-steroids (6a-i) (Fig. **4g**) with various substitutions [78]. Although compound 6a was somewhat less potent than mifepristone, their similar PR activity was suggested by this group's computational analysis. The computational model was built on the basis of X-ray crystal structures of hPR-norenthindrone [80] and hGR-mifepristone [71] complexes. While these models were constructed, helix 12 of the hPR-norenthindrone crystal structure was removed in order to open the ligand- binding site. Using ligand-receptor docking program Glide which is extremely accurate in binding mode predictions [81], mifepristone and compound 6a were docked into the ligand-binding site and subsequently re-packing of helix 12 back to the antagonism position in reference to the hGR-mifepristone crystal structure. Widely used accurate protein structure prediction program



	R_2	R_3
7a	(R/S)-4-Me_2NPh	H
7b	R-4-Me_2NPh	H
7c	S-4-Me_2NPh	H
8	H	(R/S)-Me
9a	(R/S)-Me	H
9b	R-Me	H
9c	S-Me	H
10	H	(R/S)-Ph
11	(R/S)-Ph	H

	R'	X	R
17a	Me_2N	O	OEt
17b	Me_2N	O	Me
17c	Me_2N	O	Ph
17d	Me_2N	O	*
17e	Me_2N	S	OMe
17f	Me_2N	O	NMe_2
17g	MeS	O	OEt
17h	MeO	O	OEt

2a X = F, Y = F, R = Et
2b X = Ac, Y = H, R = Et
2c X = Ac, Y = H, R = Me
2d X = Ac, Y = H, R = H
2e X = Me_2N, Y = H, R = Et
2f X = Me_2N, Y = H, R = Me
2g X = Me_2N, Y = H, R = H
2h X = MeO, Y = F, R = Et
2i X = MeO, Y = F, R = Me

	R_1	R_2
20a	OCH_2CH_3	OCH_2CH_3
20b	Me	Me
20c	OCH_3	OCH_3
20d	OCH_3	OH
20e	Me	OCH_2CH_3
20f	Me	OH
20g	OCH_2CF_3	OCH_2CF_3
20h	Ph	Ph
20i	4-Cl-Ph	4-Cl-Ph
20j	PhO	PhO
20k	-$OCH_2CMe_2CH_2O$-	-

	R
6a	Me
6b	H
6c	Cyclopropyl
6d	CF_3
6e	CMe_3
6f	$CMe_2(OH)$
6g	CH_2OMe
6h	CH_2NMe_2
6i	Ph

	R^1	R^2
A	Ac	H
B	H	Ac
C	H	H
8h	Et	$EtCOO^-$
19i	Cyclopropyl	CH_3

Fig. (4). Progesterone receptor antagonists (**a**) Mifepristone, (**b**) Pyrazoline-based PR antagonists, (**c**) C-17 phosphorous derivatives, (**d**) ORG 33628, (**e**) Structure of steroidal spiro-oxazoles, (**f**) Phosphorus-containing 11b-aryl-substituted compounds, (**g**) The structure of oxa-steroid compounds, (**h**) Structure of PF1092 A, B, C and non-steroidal tetrahydronaphthofuranone derivatives (The figures are reproduced with the permission from the respective authors as: 4b [70], 4c [73], 4e [74], 4f [77], 4g [78], 4h [82] copyright Elsevier).

Prime was then used to re-built the loop between helices 12 and 11 [81]. The final complex structures were optimized by energy minimization with the aid of force field-based molecular modeling program MacroModel [81]. In those models, mifepristone and compound 6a were predicted to bind in a similar way to PR, with a water molecule bridging the D-ring hydroxyl group and asparagine at 719 positions through hydrogen bond. Another hydrogen bond may also exist between the A-ring carbonyl group and glutamine at 725 at the other end. Though the data has not been shown by the authors, but it could be presumed that in comparison to the 7-methylene group in mifepristone, the polar and eletronegative 7-oxygen atom in oxa-steroid 6a may play a marginally different role in the ligand–protein interactions with the nearby methionine at 756. On the other hand, preliminary structure–activity relationship (SAR) study revealed replacement of the methyl group in compound 6a with the smaller hydrogen atom in compound 6b lowered the PR activity. It was found that substitution of the methyl group in compound 6a to the phenyl group yielded the most potent and selective PR antagonist, compound 6i, having an IC_{50} value of 1.4 nM and was over 200-folds more selectivity for PR over GR. It was for the first time that the 7-oxa-steroids were reported as potent and selective PR antagonists by this group. These novel oxa-steroids seem to have similar or comparable potency to that of mifepristone. In brief, preliminary structure–activity relationship (SAR) study resulted in the discovery of the most potent 17-phenylethynyl oxa-steroid, 6i, as a new potent selective PR antagonist and in contrast to non-selective mifepristone, compound 6i had over 200-folds selectivity for PR over GR which has been unleashed by T47D alkaline phosphatase and A549 assays respectively.

4.3.2. Nonsteroidal Fungal Metabolites as PR Antagonists

Following the microbial screening studies by Shinei *et al*., 2006 [82] to find novel nonsteroidal PR ligands, the substituents at positions 6- or 7 of tetrahydronaphthofuranone from the rare fungus *Penicillium oblatum* PF1092 was found to be critical for binding affinity to PR *in vitro*. Fungal metabolites PF1092 A, B and C (Fig. **4h**) have been isolated from extracts of cultures of the rare fungus *Penicillium oblatum* PF1092 [83,84]. Thereby the Shinei group has reported the synthesis of a novel series of PR ligands in which the hydroxyl group(s) at the 6- and/or 7-positions of PF1092C were modified. All the compounds of the series were then evaluated for their ability to inhibit the binding of [^{3}H] progesterone to human PR in human breast carcinoma (T47D) cells. Finally, the relative binding affinity (RBA) was calculated as per following equation:

RBA = (IC_{50} of progesterone/IC_{50} of test compound) x 100.

Further assessment of the compounds for functional activity was carried out using the progesterone- dependent exogenous luciferase expression assay in T47D cells. During this assay, T47D cells were transfected with the exogenous reporter gene, using plasmid pMANneo-LUC for assessment. The compounds were evaluated in the presence or absence of progesterone. Subsequently, the structure–activity relationship (SAR) of tetrahydronaphthofuranones as human PR ligands was also characterized. As a result of those studies, they identified two compounds, the 6,7-syn dipropionate (8b) (R^1=Et, R^2=$EtCOO^-$) and 6,7-anti derivative (19i) (R^1=Cyclopropyl, R^2=Me=CH_3) (Fig. **4h**) which showed remarkable selectivity for PR over other related steroid hormone receptors. Both compounds were identified as PR antagonists in the PR binding assay. Steroid receptor selectivity assay was carried out for both the compounds and they exhibited a high selectivity of at least 100-folds for PR over the androgen receptor [82]. Furthermore, no binding interaction with the glucocorticoid and estrogen receptors at a concentration till 10 μM was observed. On the contrary, RU486, a potent PR antagonist has shown cross reactivity with the androgen as well as the glucocorticoid receptor. The carbamate 19i was subsequently evaluated *in vivo* and confirmed to show antagonistic activity. Further, structural modification studies of tetrahydronaphthofuranones are in progress in order to find compounds with more potent activity *in vivo*.

4.3.3. Natural Anti-Progestagen

4.3.3.1. N-Butylbenzene-Sulfonamide (NBBS)

This is a recently discovered natural anti-progestagen from extracts of *Pygeum africanum*. Even at a concentration of 10-100μM it is effective in inhibiting the transcriptional activities of PR-B in presence of 0.01-1 nM progesterone [85]. The exact mechanism of action of this compound to inhibit PR is yet to be understood.

4.4. Progesterone Metabolites as Anti-Cancer Agents

Progesterone is metabolized in the target tissue like breast to form 5α-dihydroprogesterone (5αP) and 3α-dihydroprogesterone (3 αHP) (Fig. **5**) by 5 α-reductase and 3 α-hydroxysteroid oxidoreductase enzymes, respectively [23]. But these two metabolites are completely antagonist in nature. While 5αP is tumorigenic the other metabolite i.e. 3αHP is an apoptotic molecule. Wiebe *et al*. (2009) [86] recently showed that the proliferation of breast cancer cells, both estrogen responsive and unresponsive, can be suppressed by blocking the former and/or increasing the later. The differential presence of progesterone metabolites have been reported among various tumorous and normal breast tissues irrespective of age, subtypes, grades of carcinoma, estrogen or progesterone positive and/or negative [23]. The most important feature of these metabolites is their receptor specificity. The binding of 5αP to the membrane 5α-pregnane-3,20-dione receptors and 3αHP towards 3α-hydroxy-4-pregnen-20-one receptor in the plasma membrane can be targeted as an unique feature in breast cancer cells [87-89]. These receptors have shown only negligible affinity for other steroids like estradiol-17β, the parent molecule

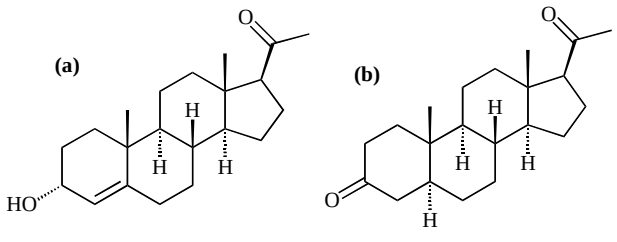


Fig. (5). Progesterone metabolites (**a**) 3α-dihydroprogesterone, (**b**) 5α-dihydroprogesterone.

progesterone and its other metabolites, androgens and corticosteroids [87]. Till date neither any study has been proposed using these metabolites as drugs, nor their analogues targeting their receptors which act as the key modulators for the downstream effecter signaling cascade to cause cell proliferation or apoptosis. Wiebe *et al.* [86] have termed 5αP and 3αHP as regulatory hormones in the breast microenvironment which distinguishes normal breast from a cancerous one.

5. CLINICAL IMPLICATIONS

Hormone replacement therapy is an important clinical aspect with an aim to affect a particular reproductive organ. However, when applied, the hormones act not only on a particular organ but have wide aspects on all the physiological organs having the respective receptors. A balance between estrogen and progesterone is required for the proper endometrial function and to maintain the reproductive health in a woman at pre-menopause, menopause and post-menopause. Progesterone at low doses and short term period has beneficial effects on the endometrial milieu and even prevents it from endometriosis or tumor formation. But the effect of progestagens, either synthetic or natural, on the breast tissue of these patients are never taken into consideration while treatments. The PR-A and PR-B status in uterus and breast plays an important role in the action of the hormone replacement therapies. Although progestagens are currently used for the management of advanced breast cancer [90], their effect on the malignant phenotype is still controversial [28]. Depending on the tissue, progesterone is classified as proliferative or differentiative sex steroid hormone [91,92]. In breast cancer cells, progesterone often acts as an inhibitor of cell growth, but some other reports showed its growth stimulator activity [28]. The biological functions of progesterone are mediated by PR, which functions as ligand responsive transcription factor in the nucleus [93]. It has been shown that progesterone acts through progesterone receptor and activates a number of genes [94]. The signal transduction of progesterone is further complicated by its cross-talk with other signaling pathways like growth factors [15], cytokines [95], steroidogenic enzymes [50] and through direct interactions with protein kinases [96] (Fig. **6**). Not only the binding of progestagens and/ or anti-progestagens to a particular site (LBD) of PR modulates its functions, also the presence of the particular form of PR (mutant or wild type) and the ratio between PR-A and PR-B is to be considered before the implication of hormonal therapy and development of anti-cancer drugs. As shown in Fig. (**6**), there exists a complex interplay between progesterone and estrogen which is mainly mediated by PR.

6. CONCLUSIONS

The effects of (anti)progestagens on breast and uterine cancer cell proliferation is an area of great importance which is as yet not very well understood like that of estrogen. Both *in vitro* and *in vivo* reports demonstrated either no effect or stimulation or inhibition of cancer cells. In human it is presumed that the loss of the coordinated expression of progesterone receptor isoforms leads to gynecological cancers atleast in case of endometrium. This could indicate that expressions of both isoforms are critical for prevention and therapy of cancer by (anti)progestagens. Simultaneously there are several reports demonstrating the roles of progesterone receptor agonists and antagonists in the management of hormone dependent cancers as has been depicted in this review and summarized in Table **1**. The female gynecological cancers expressing estrogen or progesterone receptors can be targeted by such PR binding agonist or antagonists to act not only towards the receptor mediated growth arrest but also towards more sophisticated apoptotic enhancers. Molecules like isothiocyanate-progesterone conjugates and 3α-dihydroprogesterone has opened up new dimensions in this

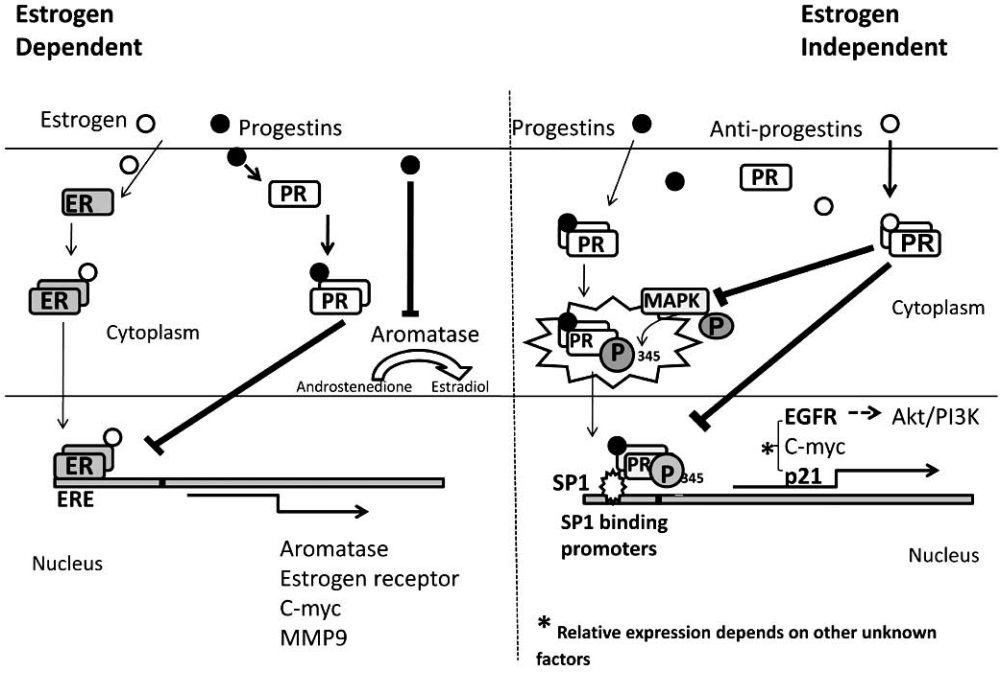


Fig. (6). Probable mode of action of progesterone receptor agonists and antagonists on target cells and its cross-talk with estrogen.

Table 1. Summary of Progesterone Receptor Agonists and Antagonists as Anticancer Molecules

Progestins	**Mode of action**			**References**
A) Dydrogesterone	Blocks activities of estrone sulfatase, aromatase and 17β-hydroxysteroid dehydrogenase			[50]
B) Tibolone	Estrone sulfatase inhibitor			[63]
C) Natural Progesterone	Antagonizes the proliferative action of estrogen and promotes estrogen metabolism			[50]
Progesterone metabolite				
3α-dihydroprogesterone	Apoptotic in both estrogen responsive and non-responsive breast cancer cell lines			[86]
(I) Antiprogestins	**Reporter based transactivation assay (IC_{50})**	**T47D Alkaline phosphatase assay(IC_{50})**	**Progesterone binding assay (RBA)**	
A) ZK 230211	0.0036 nM antagonistic PR-A 0.0025 nM antagonistic PR-B	d.n.a.	d.n.a.	[67]
B) Pyrazoline : 7b	1.5μM antagonistic PR-B	d.n.a.	d.n.a.	[70]
C) C17 phosphorus derivatives: 17 a 17c	d.n.a.	0.28 nM 0.33 nm	d.n.a.	[73]
D) Oxazole: 2b 2c	d.n.a.	0.34 nM 0.59 nM	d.n.a.	[74]
E) Phosphorus-containing 11b-aryl-substituents: 20a 20b 20e	d.n.a.	9.9 nM 3.58 nM 1.6 nM	d.n.a.	[77]
F) Oxa-steroids: 6i	d.n.a.	1.4 nM	d.n.a.	[78]
G) Non-steroidal fungal metabolites: 8b 19i	d.n.a.	d.n.a.	20 24	[82]
(II) Natural anti-progestagen				
A) N-butylbenzene-sulfonamide	10-100 μM	d.n.a.	d.n.a.	[85]

d.n.a = Data Not Available.

regard which have to be further investigated. Hence all these data warrants further study towards the development of newer synthetic molecules with specific PR binding affinity either as agonists or antagonists and understand their exact crosstalk with other signaling pathways.

ACKNOWLEDGEMENTS

The study was supported by the Department of Biotechnology (DBT, no. BT/BCE/08/355/04), Government of India and Council of Scientific and Industrial Research (CSIR, no. 37(1200)/04/EMR II), Government of India as funded projects to P.R and fellowship to S.C. from CSIR. A.C. was supported by fellowship from University of Grants Commission, Government of India. The authors would thus like to thank all these funding agencies. We would also like to convey our sincere thanks to Elsevier for providing permissions for using some of the figures (Figs. **4b**, **4c**, **4e**, **4f**, **4g** and **4h**) as detailed in corresponding references in the reference list.

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Received: December 12, 2009 Revised: March 06, 2010 Accepted: March 08, 2010