

## SELECTIVE ESTROGEN RECEPTOR MODULATORS; ROLE OF SIDE CHAIN IN ACTIVITY MODULATION

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### ABSTRACT

Selective estrogen receptor modulators (SERMs) are a class of molecules that activate estrogen receptors (ER), impacting differently on different tissues. Upon binding to ER, the ligand-receptor complex may present various conformations due to the presence of two different kinds of ERs. Few of these ligands show estrogenic effects, whereas others will inhibit the action of estrogens. Researchers are working in the direction to generate the SERMs that have a desirable estrogen-like effects on the various sites i.e., bones, improving lipid profile, reduce hot flushes, but do not act like estrogens in unwanted ways i.e., causing breast cancer, uterine endometrial proliferation. Given the comprehensive nature of this article, it is not our intention to revisit many of the issues relating to SERMs, which have already been covered in detail. Rather this article focuses on the aspect that ligand-mediated structural perturbations in and around the ligand binding pocket, contributed by the side chain effects lead to receptor antagonism. Adjusting the balance of these effects may provide a novel strategy for designing of improved SERMs. In the light of this, the article will provide an overview of the SERMs and their structural diversity.

**Keywords:** Ligand and estrogen receptor; Side chain of selective estrogen receptor modulators, Selective estrogen receptor modulators, Mechanism of action.

### INTRODUCTION

Over the years, anti-estrogens have been used as a potential treatment of hormone dependent neoplasm and also the object of increasing intensive study. Estrogens are the components crucial to female physiology. They have been sadly neglected because they have rather unjustly earned a bad name, continuously linking estrogen use with breast cancer and endometrial cancer. Despite all these scares, they are able to prevent postmenopausal osteoporosis and to successfully palliate the symptoms of perimenopause i.e., hot flushes, fatigue, insomnia and headache, incontinence, changes in blood lipid profile (i.e., increased low density lipoprotein [LDL], cholesterol) and various abnormalities consequent upon vasomotor instability [1-4]. Estrogens, a classic structural array of compounds that are involved in growth development and homeostasis of a number of tissues [5]. They function via binding to two ligand inducible nuclear transcription factor, Estrogen receptor (ER)  $\alpha$  and  $\beta$ . Ligands initiate a series of molecular events, upon binding to ligand binding domain (LBD) of ER, culminating in the activation or repression of target gene, subsequently producing some response [5]. There are various representative of this estrogen group like 17  $\beta$ -estradiol, estrone, estrioles, phytoestrogens such as coumestrol and genistein, synthetic estrogens like diethylstilbestrol (DES), and various other xenobiotics [6-11]. As an alternative to estrogen replacement therapy, selective ER modulators (SERMs) are now also the members of this estrogen family [12]. SERMs work in a manner, acting as estrogen agonist in extracellular tissues (liver, bone, brain) while having no effect or antagonizing the estrogenic response in reproductive tissues (uterus, breast) [13]. This tissue selective spectrum of SERMs was explored out via studies with various deletion and point mutated receptors. Studies reveal two independent transcription activation domains activation function 1 (AF1) and AF2 within the receptor that allow the expression of cell and promoter specific agonist activity [14]. SERMs act in a very selective way causing induction of unique receptor conformation in which the antagonist activity in some tissues is a result of functional AF1 domains [15,16]. Crystal structure studies conducted on LBD of ER in complex with the endogenous estrogens 17  $\beta$ -estradiol, synthetic non-steroidal estrogens, DES and

selective antagonist raloxifene (RAL) [6,7] show that stilbene portion of tamoxifene (first SERM to be clinically used) was required for F1 activity leading to agonist response in bone and ethanol amine side chain was responsible for blocking the AF2 activity leading to antagonism in the uterus [17]. It is now apparent that antagonist activity of anti-estrogens is due to the presence of a long carbon chain at position 7  $\alpha$  or 11  $\beta$  or any equal location in the steroids and steroids like skeleton. Side chain makes hydrophobic interactions with amino acids of the ER's LBD [18,19]. The growing realization of the significant role of the side chain in the modulating activity of SERMs in various physiological processes resulted in extensive research in this area. Several excellent publications over the recent years, describe the crucial role played by the side chain in maintaining the tissue selective agonist and antagonist activity. The contributions by Brzozowski et al. [20], Pike [21], Walker et al. [22], together with the most recent work are the excellent starting point for anyone intending to enter this field.

### STRUCTURAL BASIS OF AGONISM AND ANTAGONISM

All ER ligands bind exclusively to the C-terminal LBD and this LBD recognizes a variety of compounds diverse in their shape, size and chemical properties. Most non-steroidal selective estrogens so far available, share a common pharmacophore which consists of two aryl groups separated by two atoms, often in stilbene framework. Beside all these, the SERMs typically bear a third aryl group containing a 4-amino ethoxy substitution. Transcriptional activity manifested by ER, is mediated by at least two activation functions AF-1 at N-terminus and AF-2 in LBD [7] (Fig. 1). Mitogen-activated protein kinase pathway is involved in regulating AF-1 and AF-2 binding is responsive to ligand binding [23,24]. Recent X-ray co-crystallographic studies of structures of LBD complexed with  $E_2$ , DES, RAL and 4-OH tamoxifen (or OH-T, a high affinity metabolite of tamoxifen) show that although all these ligands bind at the same site within the core of LBD but these ligands induce differential conformation of helix-12 of ER. Helix-12 in  $E_2$ -LBD complex packs against helices 3, 5/6, 11 in a conformation that is common to the agonist bound NR-LBD structure. While in RAL-LBD complex, helix 12 lies in a hydrophobic groove composed of residues from helices 3 and 5.

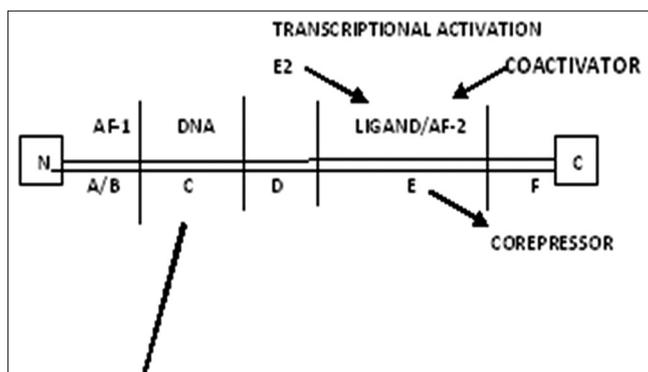


Fig. 1: Estrogen receptor and its domains [30]

This specialized orientation of helix-12, partially buries residues in the groove that are necessary for AF-2 functions suggesting that RAL and other antagonist block AF-2 functions by disrupting the topography of the AF2 surface [22]. Studies showed that OH-T is also bound within the same pocket that retains the DES, E<sub>2</sub> and RAL. The orientation of OH-T within the binding pocket appears to be established by two structural features of the ligand, the phenolic A ring and bulky side chain [25] (Fig. 2a and b). The A ring of OH-T is bounded in nearly the same way as the ring A of DES and E<sub>2</sub> near the helices 3 and 6. Its phenolic hydroxyl hydrogen bonding to structurally conserved water molecule and to the side chain of Glu-353 and Arg-394 [26]. The side chain of OH-T exits the binding pocket between helices 3 and 11 (like in RAL) [25] while its C ring makes van der waals contact with the side chain of Met-343, Leu-346, Thr-347, Ala-350, Trp-383 and salt bridge between the dimethyl amino group of the side chain and  $\beta$ -carboxylate of Asp 351. The positioning of the A ring and the side chain in the context of rigid triphenylethylene framework of the OH-T requires that the ethylene group of OH-T in an orientation nearly orthogonal to that of ethylene group of DES [26]. As a result, the B ring is driven more deeply into the binding pocket than A ring of DES. As a consequence of this side chain induced conformation, many inter-residue Vander waal contacts present in the DES and E<sub>2</sub> complex, are now lacking in OH-T complex [25,26]. The binding of OH-T to ER induces a helix 12 conformation that inhibits the binding of coactivator. That leads helix 12 being prevented from being positioned over the ligand binding pocket by the OH-T side chain [26]. The side chain of RAL and other SERMs, like that of OH-T, sterically hinders the agonist bound conformation of helix-12, thereby interference of coactivator recruitment can interferes with the cellular transcription and thus allow SERM to behave as an antagonist on the target gene with a given cell type [27]. It makes clear that the tissue selectivity of various SERMs may be due to its ability to block particular coactivator recruitment site on the surface of the ER-LBD. Selective antagonism of this kind exhibited by various prototypes is a complicated phenomenon that arises through interplay of a number of factors including differential ligand effect (mainly caused by side chain) on the trans activation functionalities of the ER, the type of coactivator recruited and the cell and promoter context. From these mechanistic studies, it is clear that certain well-characterized modulators such as heat shock proteins and a number of others maintain the inactive state of the receptor. Binding of the ligand leads to the dissociation of these chaperones and convert the receptor into a DNA binding form [11] and activated ligand-receptor complex then interacts with specific DNA sequences. However, coactivators mediate this ligand (agonist)dependent activation of transcription. Several proteins including steroid receptor coactivator-1 (SRC-1)/N-CoA1, GR1P1/TIF2/N-CoA2 and CBP/P300 act as a coactivator by associating in the ligand-dependent manner with ER and several NRs. Coactivators SRC-1 and GR1P1, being the member of P160 family of coactivators, recognize the agonist bound NRs through a short signature motif L is leucine and X is an amino acid known as NR box. And the antagonist mediates its response by blocking coactivator binding [28]. Activated complex interacts with specific DNA sequence and is, usually, located a

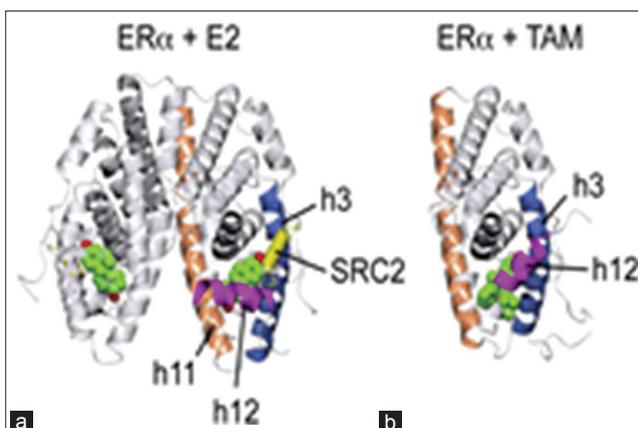
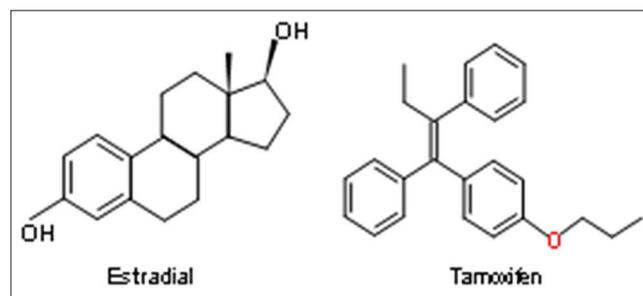


Fig. 2: (a and b) Receptor binding with E2 and TAM [30]

couple of hundred base pair upstream the regulated gene. It modulate the genomic expression by influencing the synthesis of m-RNA and thereby respective protein thus producing cellular response [28]. These mechanistic studies discussed above give valuable insight into the binding of the ligand to the receptor thus can provide a sound basis for structure based design of improved SERMs.

#### VARIOUS GENERATIONS AND CLASSES OF SERMS

Historically a no. of nonsteroidal compounds, capable of interacting ERs, have been found as contraceptive and for the treatment of breast cancer, uterine dysfunction and other complications concerning female reproductive system. A frequently used strategy designing most of the SERMs, was the attachment of a basic side chain chemically, to a molecule or resembling molecules that interact with the ER such as 17  $\beta$ -estradiol or DES. The generation of tamoxifen from DES can be taken as prime example. Tamoxifen being the first SERM characterized as an anti-estrogen in the treatment of breast cancer. Subsequent to its development for the same, it was observed that tamoxifen exhibited estrogen agonist effect on the skeleton and the liver. However, unfortunate finding with this was its stimulatory effect on uterine endometrium, thereby increasing the risk of uterine cancer. The second generation of SERMs includes the compounds RAL [27], droloxifen [29], idoxifen [29] and levormetoxifen [29]. RAL was developed and marketed mainly for osteoporotic disorders. RAL efficiently reduces LDL cholesterol. However, it has been shown to exacerbate vasomotor instability as well. It has no proliferative effects on uterus and breast thereby preventing from any risk of neoplastic breast and endometrium [27]. Third generation of SERMs lasoxifene, TSE-424 have improved profile over RAL. Though these candidate drugs lack any uterine or breast stimulation, but also lack relief from vasomotor instability. For years, a considerable effort has gone into synthesizing an ideal SERMs having efficacy as an equivalent replacement for hormone replacement therapy.



#### CONCLUSION

Knowledge of the role of the side chain of SERMs in activity modulation is becoming increasingly well defined. Now in present scenario the

development of SERMs for various bone diseases, breast and uterine disorders can be more apparently understood in context of two different ER $\alpha$  and ER $\beta$ , their interaction with ligand with variable affinities, orientation of side chain of SERMs in receptor core and cellular, biochemical and structural aspects of ER and ligand. The tissue-selective conformation of ER-ligand complex regulates the phosphorylation and recruitment of a variety of coactivators and corepressors, which in turn decides upon the transcriptional response. Understanding various critical aspects ER-ligand complex and more specific tissue selective role of side chain of SERMs will definitely provide sound basis for developing novel SERMs with target based optimal activity and minimal side effects and that will serve society as therapeutic agent.

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