Non-steroidal steroid receptor modulators

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The discovery and launch of non-steroidal ligands for estrogen receptors (ERs) and for androgen receptors (ARs) demonstrated the potential of these ligands as therapeutic agents. Based on these successes, substantial attention in the past ten years has been focused on identifying non-steroidal ligands for all of the classic steroid receptors. Non-steroidal ligands are currently in the discovery phase or in early clinical development for glucocorticoid, mineralocorticoid and progesterone receptors, and therefore must still provide evidence of their beneficial features over their steroidal counterparts. Although many new compounds for ERs and ARs are also undergoing discovery phase investigation or (early) development, none have been launched in the past ten years. The complexity of steering functional selectivity remains an ongoing challenge in the development on non-steroidal ligands.

Keywords Androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), non-steroidal, nuclear receptor (NR) modulators, progesterone receptor (PR)

Introduction

Steroid hormones control a wide variety of cellular functions that are important for cell homeostasis, proliferation, differentiation and apoptosis. Although the classic steroid hormones (androgen, estrogen, glucocorticoid, mineralocorticoid and progesterone) were first isolated early in the 20th century, their receptors remained elusive until the 1960s. The receptors for estrogen (ERs), androgen (ARs), glucocorticoid (GRs), mineralocorticoid (MRs) and progesterone (PRs) are now known to be ligand-inducible transcription factors. Their primary site of action is the nucleus, where they bind either directly or through adaptor proteins to specific response elements within the regulatory regions of target genes. The formation of a new complex of the nuclear receptor (NR) and various co-regulator proteins on DNA yields the required chromatin remodeling and ATP-dependent chromatin modification complexes. These events facilitate the recruitment of transcription machinery, leading to RNA activation and protein biosynthesis.

Biology of steroid receptors

Historically, an understanding of the mechanism of action of steroid hormones has coincided with the development of technologies and scientific methodologies across many different disciplines. An elucidation of such mechanisms of action has also been intertwined with the development of assays to screen chemical libraries of steroidal and, more recently, non-steroidal molecules to discover both receptor agonists and antagonists. In the 1960s, steroids were first demonstrated to cause an increase in the incorporation of radiolabeled precursor nucleotides into RNA. Researchers then hypothesized and demonstrated that steroid hormones induce the synthesis of proteins by first increasing the levels of their specific mitochondrial (m)RNA. These discoveries provided the first indications to the existence of steroid NRs, but further advancements in molecular biology were needed to generate the tools that were necessary to identify specific ligands for these receptors. This identification finally occurred in the 1970s, when the first steroid receptor, GR, was cloned. Researchers also demonstrated that glucocorticoids induced the radiolabeled mRNA synthesis of the viral gene mouse mammary tumor virus, leading to the discovery of hormone DNA response elements – the binding sites for steroid receptors on DNA. From this discovery, constructs could now be prepared that were capable of mimicking the switching on or off of gene expression in response to a receptor ligand. In addition, the advent of co-transfection techniques resulted in the development of transactivation assays that could characterize receptor function in drug discovery for the pharmaceutical industry; these assays remain the dominant method for measuring steroid receptor function.

The precise mechanism of hormone and anti-hormone receptor binding and the conversion from inactive to active forms of receptors were not revealed until the 1990s; these mechanisms were supported by the resolution of the first steroid receptor crystal structures later in the decade. The understanding of the receptor was then further clarified by the discovery of regulatory molecules that did not bind directly to the DNA, but rather to the steroid receptor coactivator (SRC). SRC-1 was identified in 1995 as the first selective steroid receptor co-regulator complementary DNA with a clear biological activity. This research led to an understanding of the ligand-induced co-activator exchange with the co-repressor at the C-terminus of the steroid receptor, resulting in the ligand regulation of gene expression. The discovery of the role of co-regulators has also fuelled recent advances in *in vitro* methods for the screening of compound libraries, enlabling ligand binding to isolated receptors in the presence of co-regulator peptides to be identified and characterized. The recruitment and displacement of different co-regulators in various cellular settings can now be investigated with various ligand molecules, providing new possibilities for the development of drugs with functional selectivity.

The search for NR modulators has been focused on the identification and optimization of compounds that can fit into the canonical ligand-binding pocket of the NRs. However, some recent research suggests the existence of ancillary pockets in certain NR-LBDs (ligand binding domains). Novel regulatory surfaces that bind proteins from the transcription complex or other interacting proteins have also been identified on several NR-LBDs. Such regulatory surfaces and

alternative ligand-binding pockets may offer novel drug discovery opportunities, and ensure that steroid receptors remain highly featured as druggable targets in the future.

Non-steroidal versus steroidal ligands

Since the discovery of steroid hormones, a substantial amount of research has been focused on their modification, with the goal of designing compounds with desired profiles (ie, reduced cross-reactivity, improved absorption, distribution metabolism, excretion and toxicity properties, improved pharmacokinetic/pharmacodynamic profiles, fewer adverse effects, and desired product advantages). Although several steroids are currently on the market, an ongoing need still exists for ligands that can function with a high degree of tissue selectivity and can demonstrate an improved safety profile. The discovery of non-steroidal ligands for ERs (eg, tamoxifen and raloxifene; Figure 1) and ARs (eg, bicalutamide; Figure 1) has demonstrated the potential of non-steroidal compounds as therapeutic agents. For several reasons, much attention in the past ten years has been focused on identifying non-steroidal ligands for these steroid receptors. First, non-steroidal compounds are believed to be less prone to cross-reactivity, and may therefore be more selective (resulting in fewer adverse effects). Second, different chemotypes have specific physicochemical properties that may cause distinct tissue distribution profiles and pharmacokinetics, with resultant differences in functionality with respect to steroidal ligands. Third, the binding of different chemotypes to LBDs has been demonstrated to cause differences in the orientations of key structural elements that are involved in the binding of cofactors, and may therefore change the relative equilibrium among the affinities of proteins (eg, selective ER modulators (SERMs), selective AR modulators (SARMs), selective GR agonists (SEGRAs) and selective PR modulators (SPRMs)). Finally, non-steroidal ligands are anticipated to have lower associated costs, because of much shorter synthesis routes compared with their steroidal counterparts.

Table 1 provides an overview of the current development status of selected non-steroidal ligands for ERs, ARs, GRs, MRs and PRs, and includes some potential clinical indications for these ligands. Some representative structures of the ligands are depicted in Figure 1.

A structural perspective of steroid receptors

The shared domain structure of steroid receptors incorporates a variable N-terminal domain, a highly conserved DNA-binding domain and, most importantly for drug design, a moderately conserved LBD. The LBD combines a number of functions, including hormone-binding, receptor dimerization and binding to other transcription co-modulating proteins. These functions can have influences on one another; for example, ligand-binding can influence the pattern of co-modulator recruitment.

Crystal structures have been elucidated for the LBDs of each of the steroid receptors, with almost 100 structures available in the public domain. Despite this apparent wealth of data, an insufficient diversity in co-ligands remains a concern. While a significant number of crystal structures of non-steroidal compounds complexed to ERs are available, only a handful of non-steroidal structures have been released for the other receptors. This uneven availability of public data is unfortunate, but it likely does not reflect the diversity of structural data that are available within corporate resources.

Receptor affinity

Despite the moderate to low sequence identity across the LBDs of steroid receptors (eg, ~ 25% between AR and ERα), the receptors remain structurally well conserved, and all adopt a three-layered antiparallel α-helical canonical sandwich. The steroid receptor binding cavities, which are completely partitioned from the external environment in the classic active conformation, vary in volume between 400 and 600 $Å^3$ and are predominately hydrophobic in nature, with polar functions at either end. The structure of the steroid receptor binding cavity is complementary to the general pharmacophore that is observed for the endogenous ligands of steroid receptors, which is characterized by the presence of polar groups on the 3 and 17 positions of the hydrophobic steroidal scaffolds. Therefore, it is unsurprising that the pharmacophore of steroidal ligands is also shared by many non-steroidal ligands; this similarity is illustrated by a complex of tanaproget (Ligand Pharmaceuticals Inc/Wyeth; Figure 1) and PR, in which the ligand adopts many of the characteristics of steroid binding. As a result of the similarity of the pharmacophore of steroidal and non-steroidal ligands, the optimization of hydrophobic interactions between ligands and receptors, while maintaining drug-like properties, continues to be a recurring theme in the design of non-steroidal modulators.

Binding pocket plasticity of steroid receptors is of growing interest to drug designers. The flexibility that is now observed with these ligands is less apparent when only steroid-bound structures are studied, but as more diverse ligand co-crystals are solved, it will be possible to exploit this plasticity when designing new chemical entities (NCEs). For instance, the wide range of steroidal and non-steroidal compounds that are tolerated by ERs can partly be explained by the flexibility that is observed at one of the crucial polar residues (His^{524} in human ERα); the residue adopts different conformations depending on the bound ligand, and is thereby able to retain polar interactions that would otherwise be lost.

Selectivity

A substitution of the polar residues at either end of the steroid receptor binding pockets allows individual receptors to recognize and differentiate their particular endogenous ligands. While it may be tempting to focus only on these polar interactions when considering the molecular basis for selectivity, this elegant system does not fully explain the selectivity of endogenous steroids or of the various non-steroidal ligands. The complete picture requires a consideration of more subtle differences in binding pockets, resulting from relatively conservative substitutions among hydrophobic residues. Because of its dependency on these delicate variances, the factor of selectivity generally tends to be a less tractable problem than potency, as illustrated by the number of potent but non-selective NCEs that are described in the literature.

Table 1. Selection of non-steroidal compounds launched or in preclinical/clinical phases of development.

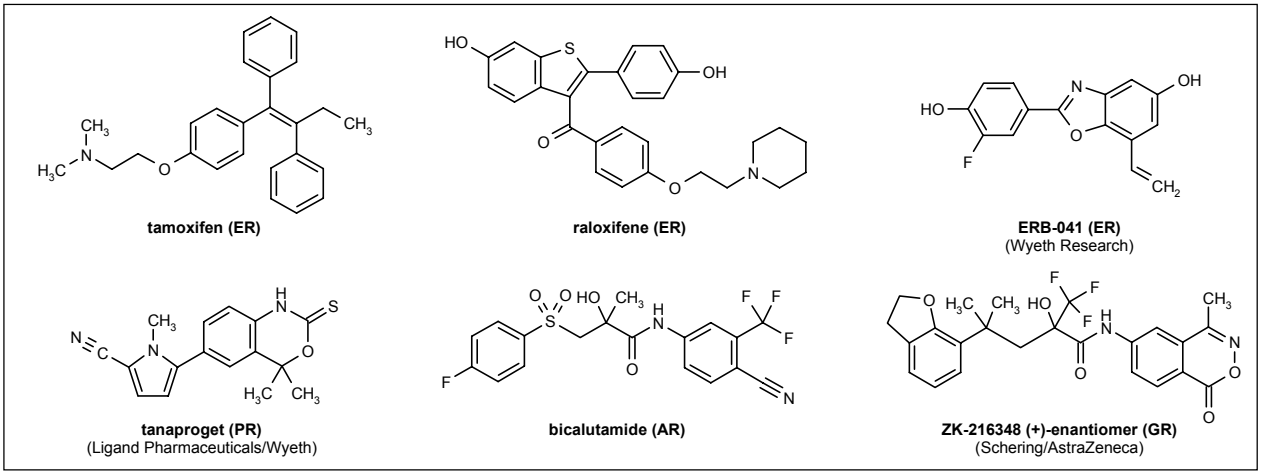
Estrogens			
Name	**Company**	**Indication(s)**	**Phase**
(Selective) estrogen receptor modulators			
Several	For example: Akzo Nobel NV, Eli Lilly & Co, GlaxoSmithKline plc, Merck & Co Inc, Novartis AG, Schering AG, Wyeth	For example: osteoporosis, breast cancer	Preclinical
Acolbifene	Université Laval	Breast cancer	Phase II, then no further development reported
Enclomiphene	Repros Therapeutics Inc	Testosterone deficiency	Phase III
Arzoxifene	Eli Lilly & Co	Osteoporosis, breast cancer	Phase III
Bazedoxifene	Wyeth Pharmaceuticals	Osteoporosis	Phase III
Clomifene	sanofi-aventis	Ovulation induction	Launched (1967)
Tamoxifen	AstraZeneca plc	Breast cancer	Launched (1973)
Toremifene	Orion Corp	Breast cancer	Launched (1988)
Raloxifene	Eli Lilly & Co/Chugai Pharmaceutical Co Ltd	Osteoporosis	Launched (1998)
ERβ modulators			
Several	For example: Akzo Nobel NV, AstraZeneca plc, Bristol-Myers Squibb Co, Eli Lilly & Co	For example: inflammation, bowel disease, infertility, BPH, prostate cancer	Preclinical
SERBA-1	Eli Lilly & Co	BPH	Preclinical
ERB-041	Wyeth Research	Rheumatoid arthritis, endometriosis	Phase II
ERB-196	Wyeth Research	Inflammation	Phase I
Androgens			
Name	**Company**	**Indication(s)**	**Phase**
Androgen receptor antagonists			
Several	For example: Bristol-Myers Squibb Co, Chugai Pharmaceutical Co Ltd, Schering AG, Warner Lambert Co	For example: prostate tumor, acne, hirsutism, alopecia, BPH	Preclinical
PSK-3841	ProStrakan Group plc	Alopecia (topical treatment), acne	Phase II
Flutamide	Schering-Plough Corp	Prostate tumor	Launched (1983)
Nilutamide	Aventis Pharma AG	Prostate tumor	Launched (1987)
Bicalutamide	AstraZeneca plc	Prostate tumor	Launched (1995)
Topilutamide	Interpharma Praha AS	Alopecia (topical treatment)	Launched (2003)
(Selective) androgen receptor modulators			
Several	For example: Akzo Nobel NV, Bristol-Myers Squibb Co, GlaxoSmithKline plc, Janssen Pharmaceutica NV, Kaken Pharmaceutical Co Ltd, Karo Bio AB, Ligand Pharmaceuticals Inc, Merck & Co Inc, Warner Lambert Co	For example: osteoporosis, female sexual dysfunction, hypogonadism, cachexia/muscle wasting disease, hormone deficiency, lower urinary tract symptoms	Preclinical
Andromustine	GTx Inc	Prostate tumor	Preclinical
Ostarine	GTx Inc	Muscle wasting disease, testosterone deficiency	Phase II
LGD-2226	Ligand Pharmaceuticals Inc/TAP Pharmaceutical Products Inc	Endocrine disease	Phase I
BMS-564929	Bristol-Myers Squibb Co	Hypogonadism, osteoporosis, female sexual dysfunction	Phase I, then no further development reported
Andarine	GTx Inc/Johnson & Johnson Pharmaceutical Research & Development LLC	Cachexia	Phase I
Glucocorticoids			
Name	**Company**	**Indication(s)**	**Phase**
(Selective) glucocorticoid receptor modulators			
Several	For example: AstraZeneca plc, Bristol-Myers Squibb Co, GlaxoSmithKline plc, Ligand Pharmaceuticals Inc, Merck & Co Inc, Schering AG	For example: autoimmune diseases	Preclinical

Table 1. Selection of non-steroidal compounds launched or in preclinical/clinical phases of development (*continued*).

(Selective) glucocorticoid receptor modulators (*continued*)			
AL-438	Ligand Pharmaceuticals Inc	Inflammation, cancer	Preclinical
ZK-216348	Schering AG/AstraZeneca plc	Respiratory disease, rheumatoid arthritis, dermatitis	Preclinical
Mineralocorticoids			
Name	**Company**	**Indication(s)**	**Phase**
(Selective) mineralocorticoid receptor modulators			
Several	For example: Bayer AG, Eli Lilly & Co, Incyte Corp	For example: cardiac failure, hypertension	Preclinical
Progestins			
Name	**Company**	**Indication(s)**	**Phase**
(Selective) progesterone receptor modulators			
Several	For example: Akzo Nobel NV, Janssen Pharmaceutica NV, Schering AG, Wyeth	For example: gynecological indications	Preclinical
Tanaproget	Ligand Pharmaceuticals Inc/Wyeth	Oral contraception	Phase II

BPH benign prostatic hyperplasia, **ER** estrogen receptor.

Figure 1. The structures of representative non-steroidal ligands.



AR androgen receptor, **ER** estrogen receptor, **GR** glucocorticoid receptor, **PR** progesterone receptor.

Despite the challenges involved in designing selective non-steroidal ligands, the generation of selective compounds has been possible – even between ERα and ERβ, whose binding pockets differ by only two conservative hydrophobic residue substitutions. Several highly selective compounds have been identified for both receptors, including ERB-041 (Wyeth Research; Figure 1), which exhibited a 200-fold selectivity for ERβ over ERα. The crystal structure of the ERB-041/ERβ-LBD complex has been solved, allowing researchers to conclude that the observed selectivity was caused predominately by the presence of an isoleucine, rather than a methionine, in the ERβ pocket. However, designing compounds to exploit such small changes remains a challenge, even when structural information is available.

Functional activity

The molecular basis for antagonism in steroid receptors is perhaps the most important and intriguing information that structural biology has provided for this family of receptors. As first described for ERα, the specific addition of bulky groups to typically estrogenic scaffolds can induce a dramatic conformational change in the C-terminal α-helix of the LBD (often referred to as the AF2 helix), resulting in a disruption in the pattern of co-modulating protein binding. As a consequence, a divergence in functional activity occurs, giving rise to full agonists, full antagonists, and partial (ant)agonists, all of which have unique and potentially desirable biological activities. The ability to select compounds with these activities provides an opportunity to design increasingly function-specific steroid-receptor ligands. Raloxifene and tamoxifen, both prototypical ER antagonists, have receptor co-crystal structures that exemplify the importance of increased bulk for disturbing the AF2 helix, but also demonstrate how other, more specific interactions are important. For example, the presence of a basic nitrogen on the ligand allows for the formation of a

salt-bridge with an aspartate; this interaction is critical for SERM-type antagonism.

In addition to a direct steric interference of the AF2 helix, structural biology research has revealed that certain interactions with the loop and helix that precede the AF2 helix can induce antagonism, as can the abrogation of stabilizing interactions between agonistic ligands and the steroid receptor. Such mechanisms are illustrated by the binding of the antagonist hydroxyflutamide to AR. Hydroxyflutamide is small enough to be accommodated within the agonist conformation of the receptor, and its antagonism appears to be caused by a loss of the stabilizing interactions that are made by agonistic ligands with the receptor.

Interestingly, the steroid receptors do not have equal sensitivity toward antagonism. For example, ERβ (discussed below) generally displays more sensitivity than ERα toward antagonists. This difference in sensitivity can be explained at the structural level by the absence of stabilizing interactions within the ERβ protein. As a result, ligands such as tetrahydrochrysene induce an agonistic conformation when bound to ERα, but induce an antagonistic conformation when bound to ERβ.

Further elucidation of the interactions of non-steroidal ligands and steroid receptors, as well as the discovery of additional mechanisms of activity, will be critical for the continued rational design of function-specific NCEs.

Estrogen receptors

The classic ERα mediates the activity of estrogens in the regulation of a number of important physiological processes, including the development and function of the female reproductive system and the maintenance of bone mineral density and architecture. While the endogenous ligand 17β-estradiol has demonstrated poor oral pharmacokinetics, the more stable 17α-ethynyl-17β-estradiol has been widely applied in the development of oral contraceptives (OCs) and in hormone therapy as a supplement to alleviate menopause-related disorders, such as climacteric symptoms, urogenital atrophy and osteoporosis. While the stimulation of ERα-regulated signaling processes in these estrogen-deficient tissues (by exogenous supplements of endogenous ovarian hormones) has important health benefits, adverse effects such as the development of cancer in breast and uterine tissue, and cardiovascular safety risks, are well recognized. A better understanding of the mechanisms through which estrogens exert their activity, and the factors that guide tissue selectivity, is pivotal to the future development of new generations of estrogens with superior tissue selectivity. The requirement for novel estrogens with increased levels of tissue selectivity, as well as the known complexity and lengthy synthesis that is involved in the preparation of steroidal estrogens, has encouraged extensive research toward non-steroidal alternatives. Substantial research efforts in the past 40 years have resulted in the disclosure of hundreds of different non-steroidal ligands. A special category of these ligands are SERMs, which comprise a class of non-steroidal estrogens that mimic estrogen in some tissues, while antagonizing the action of estrogen in others.

On the whole, the clinical application of non-steroidal estrogens has met with limited success. Only five ER ligands are currently approved for clinical use, while a few SERM-type compounds are in advanced clinical trials (for osteoporosis and breast cancer). Although a variety of related non-steroidal estrogens have been subjected to clinical evaluation, most of these compounds were not successful because of demonstrated ineffectiveness, lack of improvement with respect to existing therapies, or the occurrence of adverse side effects (eg, endometrial stimulation or uterine prolapse). These disappointing results illustrate the difficulty of developing effective estrogens with an acceptable safety profile, as reflected in the small number of drugs that have been launched in the past 40 years. In 1967, the stilbene-derived ER ligand clomifene was launched as a medication to improve ovulation induction; the drug is also currently in clinical development for the treatment of testosterone deficiency by Repros Therapeutics Inc. Two other stilbene-type compounds of this class, tamoxifen and toremifen, were launched for the treatment of hormone-dependent breast cancer, while the benzo-thiophene-type SERM raloxifene is available for the prevention and treatment of osteoporosis.

In 1996, the new receptor ERβ was identified. The occurrence of two ER receptors and their respective tissue distribution suggest that the design of subtype-selective estrogens could be a viable approach to dissect the positive and negative aspects associated with estrogen therapy and to increase tissue selectivity. Tissue expression analysis and studies with ERα and ERβ knockout animals have indicated that the subtype receptors have distinct biological activities. However, a full assessment of the therapeutic prospects of the individual subtypes requires a more detailed understanding of their physiological role, which can only be achieved by evaluating sufficiently selective ligands in appropriate animal models. Despite a high homology in the ERα and ERβ ligand-binding regions, significant progress has occurred in the past four years in the design of subtype-selective ER ligands, resulting in the identification of various ligands that express excellent ER affinities and over 100-fold subtype selectivities. ERB-041, the most advanced ERβ agonist, is currently in phase II clinical trials for the treatment of rheumatoid arthritis and endometriosis.

The use of selective ligands to clarify the biological role of the ERα and ERβ subtypes is clearly associated with the use of biological approaches, such as tissue distribution and knockout studies. The evidence that has been generated thus far indicates that ERβ agonists can reduce inflammatory processes and promote female fertility without the typical estrogenic effects on uterine and breast tissue that occur with other non-steroidal estrogen compounds. In males, ERβ agonists may be used to alleviate benign prostate hyperplasia and prostate cancer. For the treatment of disorders associated with menopause, ERα stimulation is mandatory. Although ERα agonists can be useful as a remedy for climacteric complaints and osteoporosis, these compounds are also associated with uterotrophic and mammotrophic activity. Conversely, ERα-selective antagonists might be useful in preventing bone loss and in

reducing proliferative effects in the breast, although researchers have not yet established whether ERα selectivity offers benefits over non-selective anti-estrogens. However, SERM-type antagonists are unlikely to provide a useful benefit for the treatment of climacteric disorders. A 'super SERM' that can treat all symptoms associated with menopause without exhibiting adverse effects in uterine and breast tissue is not yet within reach.

The biological effects of estrogens can be mediated through several pathways. Apart from the direct binding of a liganded ER homodimer to well-established estrogen responsive elements on DNA, binding can also occur through adaptor proteins, such as AP1 or Sp1, at different regulatory regions on the DNA. The binding of liganded ERs to different response elements may lead to an allosteric modulation of the receptor conformation, thereby influencing the recruitment of specific co-activator proteins. Although both liganded ERα and ERβ readily form homodimers, the subtypes are also known to form functionally active heterodimers that may give rise to unique patterns of gene regulation. The ER monomers may also interact with other transcription factors, such as nuclear factor (NF)κB, resulting in the repression of gene transcription. Recently, a G_q-protein coupled ER was identified that can activate protein kinase C in hypothalamic neurons; this membrane receptor is activated by ligands that do not bind to intracellular ERs.

The interplay between ERs, their splice variants and membrane counterparts versus the interplay of ligands, responsive elements, co-activators, co-repressors, as well as various transcription factors, clearly depict the highly complex mechanisms that lie behind estrogen signaling. Ligands are well recognized to have an important influence on the ultimate selection of a signaling pathway in a given tissue. However, an extended profiling of ER ligands in predictive *in vitro* models and in relevant animal models is needed to further clarify the biological role of ERα, ERβ and ER-related proteins, as well as the underlying signaling mechanisms; such research will provide new opportunities to design ligands with further improved tissue selectivity.

Androgen receptors

Androgens play an important role in many physiological processes in males and females, including postnatal development, musculoskeletal anabolism and development, and differentiation and maintenance of sexual characteristics. The endogenous ligands for ARs are testosterone and dihydrotestosterone (DHT). Testosterone is unsuitable for oral treatment because it has poor oral pharmacokinetics and is rapidly metabolized to DHT by 5α-reductase and to estrogen by aromatase, resulting in undesirable side effects. Carbon 17 methylated steroids such as 17-methyltestosterone and fluoxymesterone exhibit slow hepatic metabolism, allowing oral administration, but also exhibit liver toxicity, which limits the use of these compounds for chronic administration. Since the mid 1990s, substantial research has been performed on the development of non-steroidal androgens. This research was mainly triggered by the success in identifying non-steroidal AR antagonists such as flutamide and bicalutamide, both of which had a primary indication of prostate cancer upon entering the market. Other formulations of androgen antagonists have focused on the treatment of acne, hirsutism, benign prostatic hyperplasia and alopecia. Currently, the non-steroidal anti-androgen compound bicalutamide (Casodex; sales of US $1.123 billion in 2005) is most frequently prescribed for prostate cancer, because of its good efficacy and side-effect profile, although the non-steroidal compounds flutamide and nilutamide are also commonly used. Approximately 80% of patients in an advanced disease state responded to complete androgen blockade therapy (combination of LHRH agonist and AR antagonist), but many patients relapsed into an antagonist-resistant prostate cancer (also termed hormone-refractory or androgen-independent prostate cancer) within two years. Despite the name 'hormone-refractory', which implies that further hormonal treatments would be of limited clinical value, recent research has demonstrated that AR signaling continues to play an important role in androgen-independent cancers. Therefore, many companies are pursuing active programs to identify second-generation androgen non-steroidal antagonists with higher potency, and are focusing on strategies to bypass the hormone-refractory state. However, most of these compounds are still in the discovery phase of research, and their value remains to be proven.

AR agonists may be useful for hormone replacement therapy or for the treatment of conditions such as muscle wasting disease, hypogonadism, osteoporosis, male and female sexual dysfunction, male contraception, cachexia, hormone deficiency and lower urinary tract symptoms. The focus in developing such treatments is functional selectivity, as AR agonist compounds should protect prostate cells from proliferation. Many companies currently have AR agonist compounds in the discovery phase of research, and it will be interesting to determine the therapeutic value of these compounds. Tissue selectivity (muscle over prostate) has been claimed for several SARM compounds. However, it is unclear if this functional selectivity is caused by a difference in tissue distribution or by a different profile in co-activator/co-repressor activity in different tissues. Consequently, no bridge has yet been built between observed pharmacological data and relevant biochemical models, and more investigational research is evidently needed to clarify the tissue selectivities that have been observed. Only two compounds, ostarine (GTx Inc) and LGD-2226 (Ligand Pharmaceuticals Inc/TAP Pharmaceuticals Products Inc), have entered phase I clinical trials. Researchers in the androgen receptor field will likely follow the progress of these compounds with high interest to see if they fulfill their described functional selectivity profile in the clinical phase.

Glucocorticoid receptors

Glucocorticoids (GCs) represent a widely used class of drugs that are effective in the treatment of inflammatory diseases such as multiple sclerosis, inflammatory bowel disease, asthma and rheumatoid arthritis. Unfortunately, the duration of use of marketed steroidal GCs is limited because

of their adverse systemic effects, which can potentially result in osteoporosis, hyperglycemia, hypertension, growth retardation and skin atrophy. The pharmaceutical industry is aiming to develop novel GCs that exhibit significantly fewer side effects without losing their anti-inflammatory properties. The key toward this goal must be found in the molecular mechanism of GC-mediated activities. Many of the undesired metabolic side effects of GCs are associated with a classic GR-mediated transcription of genes, whereas the anti-inflammatory effects of GCs are caused by a GR-mediated transrepression of transcription factors such as AP1 and NFκB. SEGRAs or selective GR modulators (SGRMs) are expected to be able to dissociate transactivation activity from transrepression activity, and the pharmaceutical industry has focused mainly on identifying non-steroidal SEGRAs or SGRMs to reach this goal. Most of these compounds are currently in the discovery phase, and many have exhibited good dissociating profiles *in vitro* (ie, transrepression over transactivation). Some of the compounds have also demonstrated activity in a relevant inflammation model *in vivo*. Only AL-438 (Ligand Pharmaceuticals Inc) has demonstrated that a distinct pharmacological profile is related to a specific peptide recruitment profile (ie, providing a bridge between a relevant biochemical model and a pharmacology model). In July 2005, AstraZeneca plc and Schering AG began a three-year collaboration to jointly develop SEGRAs, including ZK-216348, up to the end of phase I clinical testing. The development of one of these compounds will likely be viewed as a test case to demonstrate proof-of-concept for SEGRAs in general.

Mineralocorticoid receptors

The mineralocorticoid aldosterone is of growing interest because of its profile as a key cardiovascular hormone. MR antagonists based on this natural steroid have been evaluated in clinical trials for their potential in treating hypertension and cardiac failure. While the beneficial effects of mineralocorticoids have been demonstrated, these steroids have also exhibited significant adverse effects that appear to be caused by the low selectivity of the compounds to other steroid hormone receptors. As a result, an increased interest in non-steroidal MR antagonists has arisen. The limited information that is currently available for non-steroidal MR antagonists has been mainly focused on *in vitro* data demonstrating potency and selectivity. While Eli Lilly & Co have described the *in vivo* activity for one compound in a relevant hypertension model, the lack of further detailed information prevents a current judgment on the potential of these compounds as MR antagonists.

Progesterone receptors

Progestin ligands have been applied mainly in the development of OCs and as treatments for a wide variety of cancer and gynecological indications. The OC field has undergone notable evolutionary changes in recent decades. Four generations of progestin agents have been developed under the driving force of diminishing the adverse effects associated with these drugs. The main challenge in the development of progestin agents has been the identification of ligands that demonstrate no cross-reactivity and therefore have no androgenic or estrogenic activity. Non-steroidal progestins may have diminished cross-reactivity, and would thereby demonstrate the desired functional selectivity. Many pharmaceutical companies have entered the research area of identifying non-steroidal progestins to substitute steroidal progestins; most of these compounds are currently in the discovery phase. Researchers have focused on identifying potent and selective steroid surrogates *in vitro* that are also active in relevant *in vivo* contraceptive models (eg, ovulation inhibition and McPhail). Minimal information is currently available on the functional selectivity or other beneficial effects (pharmacological and/or biochemical) of these compounds in comparison with their steroidal counterparts. Currently, the main question is whether these compounds will function as real steroid mimics or will have beneficial effects as a result of functional selectivity issues or of their distinct physicochemical properties. Tanaproget is the only PR compound to have entered the clinical phase (currently in phase II clinical trials). The results from these trials will indicate whether the compound fulfills its expectation of being launched as part of the next generation of OCs.

Conclusion

In the field of steroid receptors, the main challenge lies in attaining complete control in the generation of functional selective ligands. Developing reliable information (based on validated systems) that allows for the rational design of ligands with the required selectivity and functional profiles is essential. Therefore, new technologies (eg, peptide recruitment assays) that allow for the screening of desired profiles that correlate with required functional selectivities will continue to be important. The co-crystallization of diverse sets of ligands and LBDs of steroid receptors will provide more insight into the correlation between the plasticity of these LBDs and resulting functional selectivity (eg, selective modulators for steroid receptors). While knowledge in the field continues to accumulate, a complete rationalization in the generation of selective modulators for steroid receptors is not yet possible. Although many non-steroidal compounds are approaching or have entered clinical development, further research is required to determine whether these compounds exhibit distinct functional selectivity or are simply steroid mimics.

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