

# Selective androgen receptor modulators in preclinical and clinical development

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**Androgen receptor (AR) plays a critical role in the function of several organs including primary and accessory sexual organs, skeletal muscle, and bone, making it a desirable therapeutic target. Selective androgen receptor modulators (SARMs) bind to the AR and demonstrate osteo- and myo-anabolic activity; however, unlike testosterone and other anabolic steroids, these nonsteroidal agents produce less of a growth effect on prostate and other secondary sexual organs. SARMs provide therapeutic opportunities in a variety of diseases, including muscle wasting associated with burns, cancer, or end-stage renal disease, osteoporosis, frailty, and hypogonadism. This review summarizes the current standing of research and development of SARMs, crystallography of AR with SARMs, plausible mechanisms for their action and the potential therapeutic indications for this emerging class of drugs.**

Received March 28th, 2008; Accepted November 12th, 2008; Published November 26th, 2008 | **Abbreviations:** ACS: American Chemical Society; ADT: androgen deprivation therapy; AF-1: activation function-1; AF-2: activation function-2; AIS: androgen insensitivity syndrome; Akt: protein kinase B; AR: androgen receptor; ARE: androgen responsive element; ASBMR: American Society for Bone and Mineral Research; BC: bulbo cavernosus; BFR: bone formation rate; BMC: bone mineral content; BMD: bone mineral density; BMS: Bristol-Myers Squibb & Co., Inc.; CaP: cancer of the prostate; CHIP: chromatin immunoprecipitation; DBD: DNA binding domain; DHT: dihydrotestosterone; ER: estrogen receptor; FBM: fat body mass; FSH: follicle stimulating hormone; GnRH: gonadotropin releasing hormone; GR: glucocorticoid receptor; GSK: GlaxoSmithKline; HRT: hormone replacement therapy; HSP: heat shock protein; IC: intact control; IND: independent new drug application; J&J: Johnson & Johnson; LA: levator ani; LBD: ligand binding domain; LBM: lean body mass; LH: luteinizing hormone; MAPK: mitogen-activated protein kinase; MR: mineralocorticoid receptor; NCoR: nuclear receptor corepressor; NLS: nuclear localization sequence; NTD: N-terminal domain; ORX: orchidectomy; PD: pharmacodynamics; PI3K: phosphatidylinositol-3-kinase; PK: pharmacokinetics; PR: progesterone receptor; PSA: prostate specific antigen; SAR: structure activity relationship; SARM: selective androgen receptor modulator; SBMA: spinal bulbar muscular atrophy; SERM: selective estrogen receptor modulator; SGRM: selective glucocorticoid receptor modulator; SMRT: silencing mediator of retinoid and thyroid receptors; SPRM: selective progesterone receptor modulator; SRM: selective receptor modulator; SV: seminal vesicles; T: testosterone; Tfm: testicular feminization; THQ: tetrahydroquinoline; TP: testosterone propionate; VP: ventral prostate | Copyright © 2008, Narayanan et al. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited.

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## Introduction

Androgens, the major circulating sex hormone in males, regulate a broad spectrum of physiological processes through an intracellular androgen receptor (AR) [Bocklandt and Vilain, 2007; Leder, 2007]. Alteration in the circulating level of androgens, modulation in AR function through mutations or a change in the dynamic intracellular AR complex leads to multiple disorders such as hypogonadism, muscle wasting, cachexia, osteoporosis, loss of reproductive function, prostate cancer and others [Araujo et al., 2007; Brooke et al., 2008; Leder, 2007; Morley et al., 2005].

The ubiquitous expression of AR and the steroidal backbone of the natural ligands limit their use for therapeutic purposes, factors which encourage the pursuit of nonsteroidal tissue-selective androgen receptor modulators (SARMs). The first SARMs were reported in 1998 [Dalton et al., 1998; Edwards et al., 1998]. Since then, SARMs with a variety of structural scaffolds and a range of tissue selectivity and specificity have been discovered [Allan et al., 2007b; Manfredi et al., 2007]. The concept of tissue selective receptor modulators (SRMs) evolved from selective estrogen receptor modulators (SERMs), which have been clinically used for over two decades to replenish the diminishing circulating estrogens in postmenopausal conditions [Ward, 1973]. Efforts among the pharmaceutical and academic

communities to discover SRMs for other receptors such as glucocorticoid receptor (SGRM) [Link et al., 2005; Mohler, 2007a; Mohler, 2007b], progesterone receptor (SPRM) [Tabata et al., 2003] and others are in progress.

## Structure and function of androgen receptor (AR)

AR belongs to the largest class of DNA binding transcription factors, called nuclear receptors, comprised of 48 members [Evans, 1988; Tsai and O'Malley, 1994]. Each member of this family has a crucial non-redundant role and regulates critical biological functions in vertebrates and non-vertebrates [Escriva et al., 1997; Lu et al., 2006; Owen and Zelent, 2000]. Phylogenetic studies indicate that the members are highly conserved from the earliest metazoan [Owen and Zelent, 2000]. Of the 48 receptors, 24-27 bind ligand with a characterized ligand binding domain (LBD). The members of this family are divided into three classes, with class I containing receptors for estrogen, progesterone, mineralocorticoids, glucocorticoids and androgens. Receptors for vitamin D, retinoids and thyroids are categorized into class II. Class III receptors are those for which ligands have not yet been identified, and are hence classified as "orphans". However, in recent years, natural and synthetic ligands for many of these orphan receptors have been uncovered [Blumberg and Evans, 1998; Tsai and O'Malley, 1994].

The AR DNA sequence is present in the X chromosome, which has 8 exons spanning 90 kb sequences encoding a 919 A.A. protein [Lu et al., 2006]. Like the PR and ER, AR exists as 2 isoforms, AR and AR-A, formed by an alternate translation start. Similar to PR-A, which lacks the first 164 amino acids of PR-B, AR-A lacks the first 187 amino acids of AR. However, this isoform is not as well characterized as PR or ER isoforms and its functions remain unknown [Wilson and McPhaul, 1994; Wilson and McPhaul, 1996]. AR, similar to the other members of the class, contains four structural domains, with each performing exclusive functions. The complete function of the N-terminal domain (NTD) is still under investigation. However, the activation function (AF-1) located in the NTD plays a pivotal role in AR function. Unlike other receptors, the NTD of AR is the major transactivation domain and deletion of AF-1 leads to a significant loss of AR function [Alen et al., 1999; Bevan et al., 1999; Gao et al., 1996; Jenster et al., 1991; Simental et al., 1991]. In addition, the NTD is the major coactivator interaction surface for AR and mediates the growth factor- and cell signaling-dependent transactivation [Heinlein and Chang, 2002]. The NTD of AR and other receptors have not yet been crystallized. Structural knowledge of the NTD would lead to better understanding of the function of this domain.

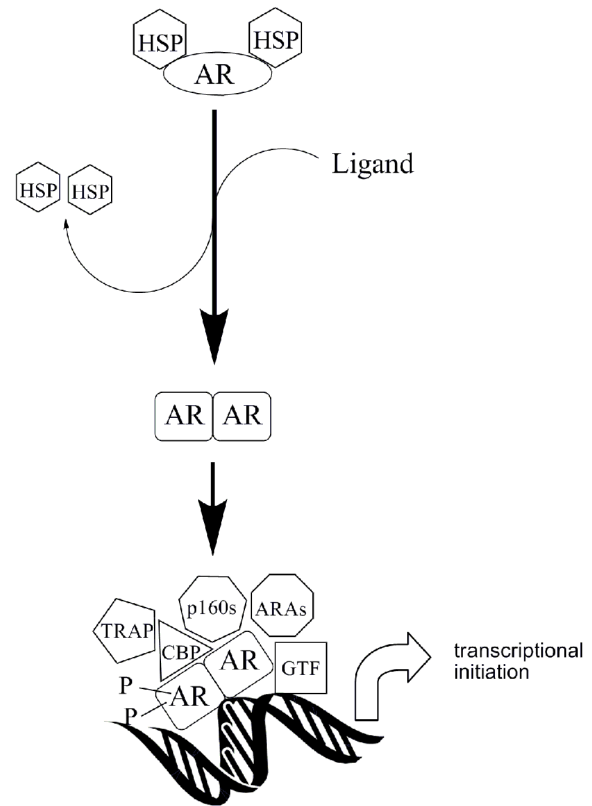
The DNA binding domain (DBD) is the most highly conserved domain and, as the name indicates, is responsible for the binding of AR to the promiscuous androgen responsive element (ARE), 5'-AGAACANNNTGTTCT-3', on the promoter of androgen responsive genes [Verrijdt et al., 2003]. The DBD has 2 classical cysteine zinc finger motifs that are responsible for DNA recognition and dimerization [MacLean et al., 1997]. Each finger has 4 cysteines, 1 zinc atom,  $\alpha$ -helices and a carboxyl extension. This region has a highly conserved 66-amino acid residue core referred to as P, D, T and A boxes. The DBD crystal structure has been solved both in the presence and absence of the DNA elements [Shaffer et al., 2004].

The hinge region that lies between the DBD and the ligand binding domain (LBD) is a lysine-rich region that is important for the nuclear localization signal (NLS) of the receptor [Gao et al., 1996; Ylikomi et al., 1992]. Deletion of this domain eliminates nuclear localization of AR in the presence of ligand and, hence, loss of transcriptional activity. Particularly, one amino acid residue, Ser<sup>650</sup>, is critical for nuclear localization [Gioeli et al., 2006]. Phosphorylation of Ser<sup>650</sup> by p38 MAPK prevents the nuclear localization of AR and subsequently inhibits AR-mediated gene expression. Some prostate cancer-specific mutations in the hinge suggest that the hinge region plays a role in DNA binding and coactivator recruitment [Tilley et al., 1996; Wang and Uchida, 1997]. The LBD of the receptor is responsible for ligand binding and is not well conserved among the receptors. As opposed to its family members, the LBD of AR is comprised of only 11 helices, due to the absence of helix 2 [Dehm and Tindall, 2007]. The LBD forms a pocket to capture the cognate ligand. In addition to the ligand binding function, the LBD also contains a second

activation function (AF-2) that is important for the ligand-dependent activation of the receptor. Further details of the LBD and crystal structure are described later.

### Mechanism of AR action

In the absence of ligand, AR is maintained in an inactive conformation by heat shock proteins HSP 70 and HSP 90 in the cytoplasm (Figure 1). Upon ligand binding, the C-terminal helix 12 in the LBD shifts position to close the ligand binding pocket. HSPs dissociate from the receptor, leading to homo-dimerization. This facilitates a series of conformational changes, with helices 3 and 5 serving as the key interfaces following the dissociation of corepressor complexes, leading to nuclear entry and binding to AREs in the promoter of androgen-responsive genes.



**Figure 1. Mechanism of action.** AR is maintained in an inactive complex by HSP 70 and HSP 90 and corepressors (CoR). Upon ligand binding, the receptor homodimerizes and enters the nucleus. The receptor is basally phosphorylated in the absence of hormone and hormone binding increases the phosphorylation status of the receptor (P). The AR binds to the ARE on the promoter of androgen responsive genes, leading to the recruitment of coactivators (p160s, CBP, TRAP, ARAs) and general transcription factors (GTF), leading to gene transcription.

Activated AR has been shown to recruit coregulators and general transcription factors, subsequently leading to the enhancement or repression of transcription of target genes. Recent chromatin immunoprecipitation (ChIP) studies indicated that ER and AR agonists recruit coregulators cyclically in a time-dependent manner to the respective response elements, indicating the complexity

and dynamicity of events occurring around these response elements [Shang et al., 2000; Shang et al., 2002; Wang et al., 2005]. After initiation of transcription, AR leaves the DNA, and is targeted for ubiquitination, proteolytic cleavage, and export out of the nucleus to the cytoplasm. Another round of transcriptional activation occurs with newly-synthesized receptor [Cardozo et al., 2003; Fang et al., 1996; Pajonk et al., 2005].

To date, a number of clinical disorders of the AR have been reported. Several mutations of AR LBD occur in prostate cancer. A comprehensive list of mutations of AR in prostate cancer can be found at the McGill Androgen Receptor Gene Mutations Database World Wide Web Server website [McGill\_University, 2008]. Classical AR functional abnormalities cause a spectrum of disorders such as androgen insensitivity syndrome (AIS), testicular feminization (Tfm), ablation of masculinization of reproductive organs, osteoporosis and cachexia.

Another disorder that has been attributed to androgen sensitivity due to an AR polymorphism is Kennedy's disease or spinal bulbar muscular atrophy (SBMA) [Palazzolo et al., 2008]. AR has a stretch of polyglutamine (CAG) repeats in the NTD varying in length between 12 and 25 amino acids, with an average between 21 and 22 [Palazzolo et al., 2008]. The length of the CAG is inversely proportional to AR activity. In Kennedy's disease, the number of CAG repeats range from 36 to 70, leading to androgen insensitivity [Palazzolo et al., 2008]. In addition, the inactive AR forms accumulate in the nucleus, leading to cellular toxicity and neuro- and musculo-pathological conditions [Palazzolo et al., 2007].

## SARMs in clinical development

The field of nonsteroidal androgens, and especially selective androgen receptor modulators (SARMs), has grown tremendously since the first report in 1998. Many of the major pharmaceutical companies have now published *in vivo* characterizations of tissue selective AR agonists, and the rate of new contributions to this field continues to accelerate. The expansion of the field has resulted in a broadening of the chemical space originally occupied by the traditional steroidal agonists (not shown) and nonsteroidal antagonists (compounds (1), (2), (3), and (4); Figure 2), whose use is limited by prostate liability and lack of tissue-selectivity, respectively.

Many chemically distinct putative AR agonist templates have been reported, with fewer having demonstrated *in vivo* tissue-selectivity for anabolic tissues (i.e., SARMs), as summarized in Supplementary File 1. This pharmacophoric diversity portends pharmacokinetic (PK) and pharmacodynamic (PD) diversity across many chemotypes, suggesting the potential for broad therapeutic application for SARMs. The field of SARMs will be reviewed with emphasis given to groups with the most complete preclinical PK/PD characterizations, or clinical data. Other excellent SARM reviews are available [Allan and Sui, 2003; Buijsman et al., 2005; Cadilla and Turnbull, 2006; Mohler et al., 2008; Mohler et al., 2005].

## Clinical indications under investigation

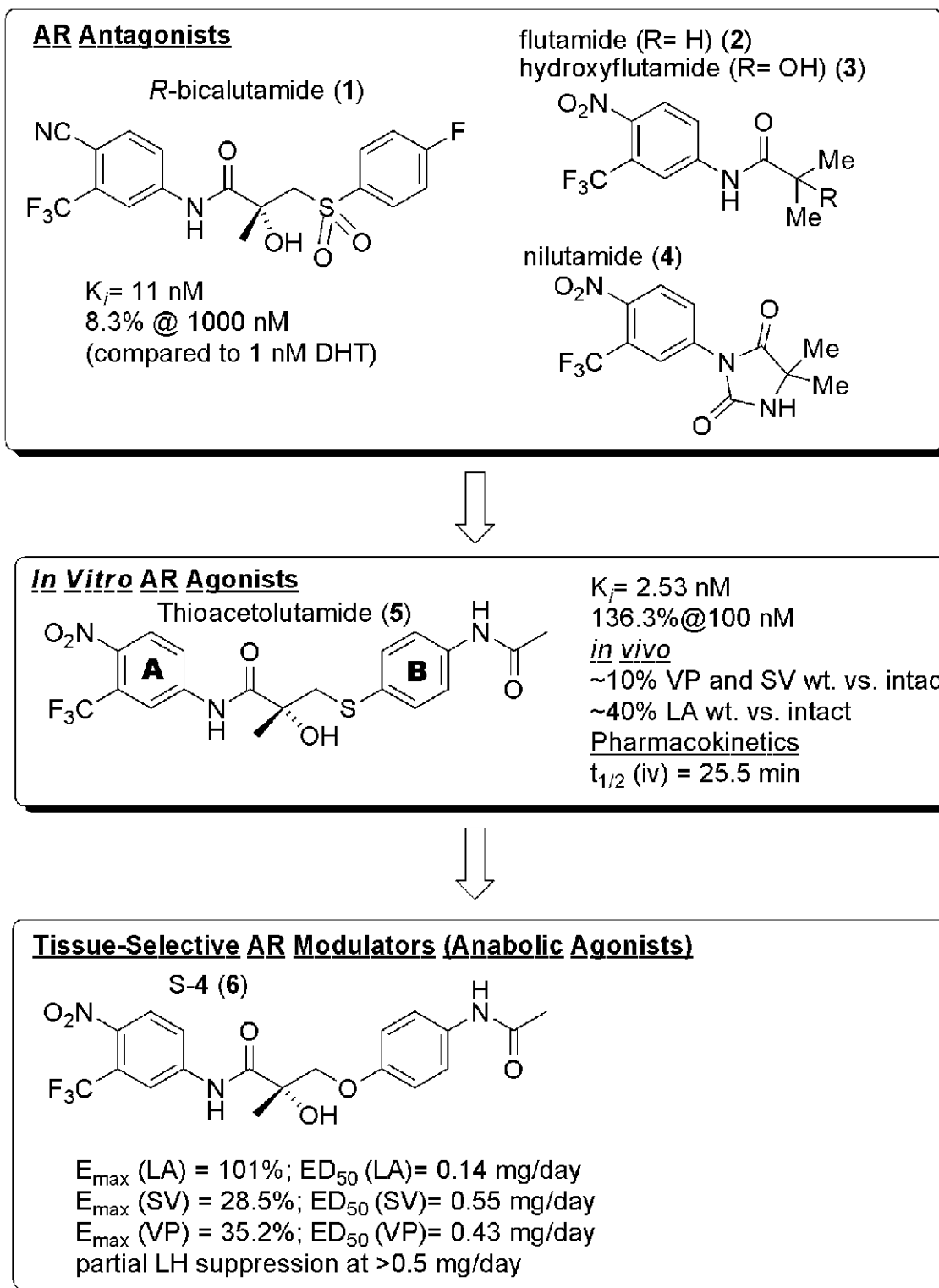
Several groups have advanced SARMs to the clinic for specific indications. The current clinical practices, followed by the rationale and results (where available) of these trials, are briefly discussed below.

### Sarcopenia (GTx/Merck; Pharmacopeia/BMS; Ligand/TAP)

Age-related decline in lean body mass results in the clinical condition known as sarcopenia in older individuals [Marzetti and Leeuwenburgh, 2006]. An increase in the elderly population has contributed to the growing number of frail men and women that are unable to carry out activities of daily living and are thus in need of assisted-care. While enhancing protein intake and exercise programs offer means to combat the muscle loss that occurs with aging, hormonal therapy is likely to show more drastic effects. An agent capable of selectively increasing muscle performance without androgenic side effects such as prostate growth in men and virilization in women (side effects of steroidal androgens) is desirable for the treatment of sarcopenia. A Phase IIa study with the drug Ostarine™ has shown significant improvement in the ability of healthy, elderly men and women to climb stairs, accompanied by significant increases in lean body mass and decreases in fat mass after only 86 days [Dalton, 2007a; Dalton, 2007b]. Lack of PSA increases in men and hair growth in women further corroborated selective anabolic effects of Ostarine™. Reductions in serum lipids were observed. However, LDL/HDL ratios remain in the low cardiovascular risk category. The occurrence of adverse events were otherwise similar in the placebo and treatment groups. Thus, clinical proof of the benefits of SARM treatment for improving strength exists and shows promise for treating age-related decline in muscle strength, as well as other related indications being pursued by Pharmacopeia (age-related functional decline) and Ligand Pharmaceuticals (frailty), both having completed Phase I trials.

### Cancer cachexia (GTx/Merck)

Disease states that result in rapid loss of muscle are likely to show significant benefit from SARM treatment. Cachexia often occurs in patients with AIDS, cancer, kidney disease, sepsis, and burns and is characterized by weight loss, muscle wasting, and decrease in appetite. Elevated levels of cytokines, namely IL-6, TNF $\alpha$ , INF 1 $\beta$ , INF $\gamma$ , and proteolysis inducing factor [Melstrom et al., 2007] are thought to be the main contributors. At least 30% of cancer-related deaths result from cachexia due to wasting of the respiratory muscles, which eventually causes pneumonia [Palesty and Dudrick, 2003; Windsor and Hill, 1988]. While antibodies and inhibitors of cytokines have shown benefit in chronic inflammatory conditions such as rheumatoid arthritis and Crohn's disease, minimal efficacy has been shown in cachexia treatment [Goldberg et al., 1995; Inui, 2002]. Appetite stimulants including the synthetic progesterone derivative, megestrol, and the synthetic cannabinoid, dronabinol, are currently available in the United States, however side effects of these drugs limit their benefit [Yeh et al., 2007; Yeh and Schuster, 2006]. Though megestrol causes



**Figure 2. Discovery of propionamide AR agonists *in vitro* and *in vivo*.** The nonsteroidal antiandrogens (1-4) demonstrate therapeutic utility in prostate cancer, and are structurally similar to some nonsteroidal tissue-selective agonists (i.e., SARMs). An early example of which was (5), which was vastly improved by together to ether conversion, resulting in the prototypic SARM, S-4 (6). Preclinical characterizations of this molecule catalyzed the development of the SARM field, as discussed herein and in the literature in general.

weight gain, its antianabolic properties result in decrease in lean body mass despite weight gain [Lambert et al., 2002]. Furthermore, the compound increases the risk of thromboembolic events and suppresses adrenal function. Likewise, problems with sedation and confusion in the elderly limit the use of dronabinol [Volicer et al., 1997].

Studies with anabolic agents such as nandrolone for cachexia have shown improvements in lean body mass and bone density [Batterham and Garsia, 2001; Frisoli et al., 2005], however side effects such as liver toxicity and masculinization in women occur. Increases in lean mass and muscle performance in HIV-infected men with wasting

disorders upon testosterone treatment have also been shown [Dolan et al., 2007; Grinspoon et al., 2000]. The proliferative effects on the prostate and elevation of hematocrit remain drawbacks, however. An ideal SARM, as first described by Negro-Vilar [Negro-Vilar, 1999], that selectively increases muscle mass and lacks the growth effect on prostate and increase in hematocrit, would make androgen treatment a more beneficial option. Improvement of muscle strength will conceivably result in decreased morbidity and mortality in cachectic individuals and may allow patients to undergo more intensive treatments (e.g., radiation and chemotherapy regimens). Currently, Ostarine™ is in a Phase IIb clinical trial for cachexia in cancer patients, with results expected in 2008.

#### Osteoporosis (Ligand/TAP, GTx/Merck)

Preventing bone loss and increasing bone formation are two mechanisms of protecting against osteoporosis. In addition to calcium and vitamin D supplementation, bisphosphonates are agents available to both men and women. These drugs increase bone mineral density (BMD) by inhibiting osteoclast activity [Fisher et al., 1999]. The role of estrogens in maintaining bone mass in women is also crucial, as shown by the rapid decline in BMD in postmenopausal symptoms. Hormone replacement therapy (HRT) is commonly utilized to treat menopausal symptoms, but is no longer recommended for long-term treatment, due to increases in the risk of breast and endometrial cancer, gallbladder disease, and thromboembolism. SERMs such as tamoxifen and raloxifene have thus replaced HRT treatment for osteoporosis in women, as these agents selectively maintain bone mass without proliferative effects in the breast and uterus, but are associated with a higher risk of venous thromboembolic events [Epstein, 2006; Ettinger et al., 1999; Song et al., 2006]. Likewise, androgens are known to have a positive effect on BMD through increase in periosteal bone formation [Hanada et al., 2003]. Their importance in maintaining bone mass is further exemplified by the occurrence of osteopenia in male AR knockout mice [Kawano and Kawaguchi, 2006; Kawano et al., 2003] and evidence that men undergoing androgen deprivation therapy (ADT) for a prolonged period suffer from decreases in BMD. The use of SARMs for osteoporosis is likely to provide benefit in both men and women, as they lack the side effects of virilization seen with steroidal androgens. A 120-day study comparing SARM S-4 and dihydrotestosterone (DHT) treatment in ovariectomized rats demonstrated that S-4 was able to maintain bone mass and bone strength to the levels of intact controls and exhibited greater efficacy than DHT [Kearbey et al., 2007]. Studies by Ligand Pharmaceuticals with LGD2226 [Rosen and Negro-Vilar, 2002] demonstrated increased bone mass and strength in rats after 16 weeks of treatment. These effects are thought to be mediated through AR in osteoblasts, thus increasing the rate of bone formation. Therefore, combination regimens with SARMs and currently available bisphosphonates should provide a more efficacious treatment option for osteoporosis. Currently, LGD2941 is in Phase I trials for osteoporosis (and frailty).

## Possible future clinical indications

### Prostate cancer

The first-line pharmacologic treatment option for patients with androgen-dependent prostate cancer is ADT, which includes a GnRH superagonist such as leuprolide to shut down endogenous synthesis of testosterone with or without an AR antagonist such as bicalutamide [Furr and Tucker, 1996; Iversen et al., 2004; Wirth et al., 2004]. While the treatment is effective for slowing the cancer growth, patients experience a number of side effects including hot flashes, loss of libido, loss of lean body mass, osteoporosis and a decrease in physical performance [Clay et al., 2007; Malcolm et al., 2007; Perlmutter and Lepor, 2007]. SARMs of varying agonist activity have been discovered and shown to have a wide-range of efficacy in animal models. While the more potent agonists restore castrated rat prostate size back to the intact control levels and muscle well beyond 100% of the weight of the intact animals [Chen et al., 2005b; Kim et al., 2005], other SARMs have been discovered that restore muscle to nearly 100% with little increase in prostate size compared with castrates [Gao et al., 2004]. Thus, a patient receiving leuprolide may benefit from adjuvant SARM treatment to combat the side effects on muscle and bone [Bahnsen, 2007].

### Male hormonal contraception

Despite prevalent use of oral contraceptives for women, no oral pharmacologic option has been approved for men. Hair et al. [Hair et al., 2001] found that desogestrel, an oral synthetic progestin, in combination with a transdermal testosterone patch, reversibly suppressed spermatogenesis, but was not as efficacious as combination testosterone injection regimens. Preclinical studies in our laboratory have shown that propionamide SARMs suppress luteinizing hormone (LH) and follicle stimulating hormone (FSH) through the hypothalamus-pituitary-testis axis in rats, thus decreasing testosterone in a dose-dependent manner [Chen et al., 2005a]. Furthermore, spermatogenesis was found to be significantly decreased with 1 mg/day treatment for 10 weeks in these animals with the SARM, C-6 (see literature for structure). Studies with the SARM, LGD2226, assessing the effects on mating behavior of rats, show maintenance of libido and sexual function in rats [Miner et al., 2007]. As a whole, these data are encouraging towards the development of a SARM as a male contraceptive pill.

## SARMs in preclinical development

### GTx, Inc. – propionamide SARMs

#### Discovery of nonsteroidal SARMs (University of Tennessee Health Science Center (UTHSC))

Dalton *et al.* unexpectedly discovered several nonsteroidal ligands with the ability to fully stimulate *in vitro* AR-dependent transcriptional activation. This unprecedented activity was observed for propionamides that differed slightly from hydroxyflutamide and bicalutamide [Dalton et al., 1998; He et al., 2002b]. Compound (5), in which the sulfonyl-linkage and

*para*-fluoro substituent of bicalutamide were replaced with a thioether linkage and *para*-acetamide substituent, respectively, emerged as an early lead from structure-activity relationship (SAR) inquiries (Figure 2). Compounds such as (5) demonstrated improved *in vitro* agonist activity and avoided concerns related to the chemical reactivity of the haloacetamides that were first reported (Figure 2) [He et al., 2002b; Yin et al., 2003b]. Hydroxyflutamide analogs [Marhefka et al., 2001] and non-propionamide templates were also explored with less success [Yin et al., 2003b] (data not shown).

These thio-ether linked propionamides seemed very promising, but suffered from a lack of the expected pharmacologic activity *in vivo*, due to metabolic oxidation of the thioether to sulfoxides or sulfones with little to no agonistic activity [Yin et al., 2003c]. Elimination of metabolic liability inherent in the sulfur group was achieved with replacement of the thioether with an ether. An *in vivo* pilot study in castrated rats demonstrated that these AR ligands were capable of AR-dependent full agonist activity *in vivo*, but also showed unprecedented tissue-selective pharmacologic activity [Marhefka et al., 2004; Yin et al., 2003a] in a typical Hershberger assay (castration of male rats, followed by an androgen treatment regimen and analysis of organs weight for levator ani (LA) or other skeletal muscle as an indicator of anabolic activity and seminal vesicles (SV) and/or ventral prostate (VP) weight as indicators of androgenic activity) [Hershberger et al., 1953]. Subsequently, the Hershberger assay has become the assay of choice to demonstrate tissue-selectivity in preclinical characterizations of SARMs. Hershberger assays can be performed in a maintenance mode (androgen treatment immediately after castration) or restorative mode (waiting period to allow atrophy prior to androgen treatment). The ether-linked propionamides were selective, full anabolic agonists with the ability to fully support the weight of levator ani (LA) muscle, but weak partial agonists (or antagonists) in androgenic tissues such as ventral prostate (VP) and seminal vesicles (SV) (S-4 (6) in Figure 2). Molecules such as these were termed selective androgen receptor modulators (SARMs) in analogy to selective estrogen receptor modulators (SERMs), where tissue-specific anabolic (bone maintenance) and estrogenic (breast and/or uterine maintenance) activities have been separated.

A potent SARM, S-4 (6), was identified that demonstrated rapid and complete oral absorption at low doses and reasonable elimination half-life ( $t_{1/2}$  = 2.6 h to 5.3 h) in rats, suggesting compounds such as this would be excellent candidates for clinical development [Kearbey et al., 2004]. S-4 (6) also demonstrated the ability to improve skeletal (soleus) muscle strength, increase lean body mass (LBM), reduce body fat, and prevent bone loss in rats, in addition to promising pharmacologic activity in animal models of benign prostatic hypertrophy and male fertility [Chen et al., 2005a; Gao et al., 2004; Gao et al., 2005; Kearbey et al., 2007]. These successes encouraged us to expand our efforts toward exploration of the diaryl propionamide class of SARMs, many of which

have been published [Bohl et al., 2004; Chen et al., 2005a; Chen et al., 2005b; Kim et al., 2005].

#### Clinical development (GTx, Inc.)

Ostarine™ is an aryl propionamide SARM and the most advanced clinical candidate. Ostarine™ demonstrated exciting data in an initial proof-of-concept Phase IIa clinical trial. GTx, Inc. reported in December 2006 the results of this clinical trial, which was a double blind, randomized, placebo-controlled trial in sixty elderly men and sixty postmenopausal women [Dalton, 2007a; Dalton, 2007b]. Without a prescribed diet or exercise regimen, all subjects treated with Ostarine™ had a dose-dependent increase in total LBM, with the 3 mg/day cohort achieving an increase of 1.3 kg compared to baseline and 1.4 kg compared to placebo after 3 months of treatment. Treatment with Ostarine™ also resulted in a dose-dependent improvement in functional performance measured by a stair climb test, with the 3 mg/day cohort achieving clinically significant improvement in speed and power. Interestingly, subjects treated with 3 mg/d of Ostarine™ had on average an 11% decline in fasting blood glucose, a 17% reduction in insulin levels, and a 27% reduction in insulin resistance (homeostasis model assessment) as compared to baseline, suggesting that SARMs might have therapeutic potential in diabetics or people at risk for diabetes. Phase I clinical studies with Ostarine™ showed that it was rapidly absorbed after oral administration with a half-life of about 1 day (unpublished data). An additional Phase II study in muscle wasting associated with cancer cachexia began in 2008 as an early objective of clinical development. Ostarine™ also resulted in a dose-dependent decrease in LDL and HDL cholesterol levels, with the average LDL/HDL ratio for all doses remaining in the low cardiovascular risk category.

GTx, Inc. and Merck & Co., Inc. announced an agreement providing for research and development, and global strategic collaboration for their respective SARMs programs.

#### Ligand Pharmaceuticals, Inc. – quinolinones (pyridones) and derivatives

Ligand was an early leader in the development of nonsteroidal AR ligands with their series of tricyclic quinolinones [Edwards et al., 1999; Edwards et al., 1998; Hamann et al., 1999; Higuchi et al., 1999]. Ligand has published and patented an extensive array of bi-, tri-, and tetracyclic (not shown) quinolinone templates, with bi- and tricyclics demonstrating high affinity and potent tissue-selective anabolic agonist activities. The structural core of this series is a quinolinone (also known as pyridone) A-ring, which occupies a space in the receptor similar to the steroidal A-ring (discussed *infra*). The earliest members of this class were antagonists such as LG-120907 (7), which bound with a  $K_i$  value of 26 nM (Figure 3) (US Patent 6,017,924 [Edwards et al., 2000]) and inhibited testosterone-induced increases in VP and SV tissue weights ( $ED_{50}$  [VP] = 18.3 mg/kg;  $ED_{50}$  [SV] = 19.2 mg/kg). Changing the A-ring from the  $\alpha,\beta$ -unsaturated quinolinone to a coumarin (representative

example (8)) within a 2,2-dimethyl substituted pyridone template retained antagonist activity; however, changing the alkylation pattern from 2,2-dimethyl to 4-ethyl such as in LGD121071 (9) produced an early high affinity, potent AR full agonist in *in vitro* transcriptional studies ( $K_i = 17$  nM; *in vitro*  $EC_{50} = 4$  nM; 100%) [Hamann et al., 1999]. This  $\alpha,\beta$ -unsaturated quinolinone became a conserved feature in subsequent Ligand agonist series.

Higuchi *et al.* has explored two distinct oxazino variant templates exemplified by (10-11) and (12), respectively [Higuchi et al., 2007b]. In their initial efforts, they characterized their 7H-[1,4]oxazino(3,2-g)quinolin-7-ones (an anthracene-like fused ring system) and showed tissue-selective myoanabolic activity (see compound (10) in Figure 3) (US Patent 6,462,038 [Higuchi et al., 2002]). Recently, this group published an *in vitro* SAR of this template series and an *in vivo* characterization of (11), (as outlined in Figure 3) which differs from (10) by the addition of an (*R*)-methyl group  $\alpha$  to the oxazino nitrogen. This work demonstrated some tolerance *in vitro* to various substitutions at the N, and carbons  $\alpha$  and  $\beta$  to the nitrogen of this oxazino ring system [Higuchi et al., 2007a].

Separately, Higuchi *et al.* characterized a template of constitutional isomers, the 8H-(1,4)oxazino(2,3-f)quinolin-8-ones, which were incidentally formed as a minor product in the synthesis of (11) [Higuchi et al., 2007a]. Serendipitously, these new phenanthracene-like oxazino isomers also demonstrated AR agonist activity. Similar to the anthracene configuration oxazines (i.e., (10-11)), these new oxazino isomers were antagonists if the oxazino nitrogen substituent was removed. Likewise, these phenanthrenoids were sensitive to the substituent present at the oxazino nitrogen ( $R_1 =$  allyl and  $CH_2CF_3$  are optimal) and the carbon  $\alpha$  to it ( $R_2 =$  Et and *i*Pr are optimal). *In vitro* characterization identified (12) (i.e.,  $R_1 = CH_2CF_3$ ,  $R_2 = (R)$ -Et) as a lead for *in vivo* proof-of-concept studies for this template in which (12) demonstrated selective myoanabolism in a maintenance Hershberger assay (i.e., treatment immediately after castration), as shown in Figure 3.

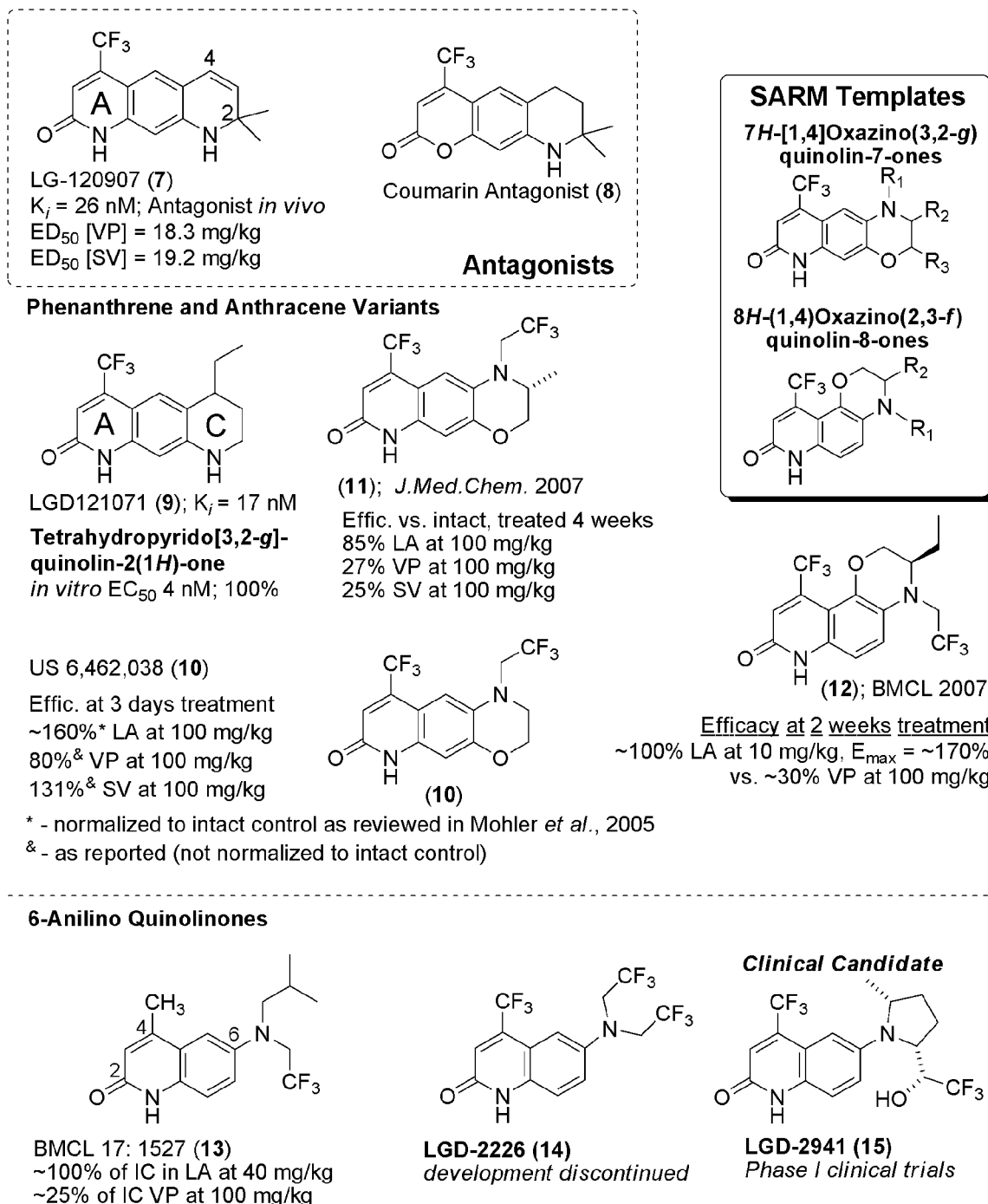
Clinical candidates thus far from this group have been bicyclic 6-anilino quinolinones in which the aniline was generally disubstituted such as in (13), that demonstrated tissue-selective full myoanabolic activity [van Oeveren et al., 2007a]. Ligand chose LGD2226 (14) as its first pre-clinical lead compound. Although development of LGD2226 (14) was later discontinued, Ligand scientists published characterizations of LGD2226 (14) with regard to the SAR for similar compounds [van Oeveren et al., 2007a; van Oeveren et al., 2007b], discovery/organic synthesis [van Oeveren et al., 2006], co-crystal structure with AR [Wang et al., 2006] (discussed *infra*), myo- and osteoanabolic activity, and maintenance of sexual function in castrated rats [Miner et al., 2007]. LGD2226 (14) demonstrated myoanabolism weaker than testosterone and osteoanabolism which was shown to increase bone mineral density (BMD), improve bone structure and strength, and positively affect bone biomarkers.

In 2005, Ligand filed an investigational new drug application (IND) for LGD2941 (15), which is currently in Phase I clinical trials for frailty and osteoporosis in collaboration with TAP Pharmaceuticals (an Abbott subsidiary). A recent publication characterized the pre-clinical osteo- and myoanabolic properties of LGD2941 in rats (15) [Martinborough et al., 2007; Wang et al., 2006]. LGD2941 (15) demonstrated improved bioavailability relative to LGD2226 (14), while maintaining hypermyoanabolic and hyperosteoanabolic properties in male and female *in vivo* maintenance models. The myoanabolism was seen as 180% and 100% of LA weight retention at 10 and 1 mg/kg, respectively, compared with 100% and 50% of VP weight retention at the same doses. The osteoanabolism was seen as a small increase in lumbar space compression strength (46 N vs. 43 N for intact control) and larger increases in femur bending strength (230 N vs. 175 N for intact control), indicating effectiveness in cancellous and cortical bone, respectively. In each case, these bone effects were in excess of those seen for estradiol and DHT.

A third compound in preparation for clinical testing, LGD-3303 (structure not disclosed), was recently reported at the 2007 American Society for Bone and Mineral Research (ASBMR) Meeting (unpublished data). LGD-3303 is a hypermyoanabolic and osteoanabolic agonist in rats with an LA  $E_{max}$  of 220%, but also supports 100% of prostate at this dose. The dose for 100% LA support is 1 mg/kg per day and prostate support at this dose is only ~20%. Ligand performed a pre-clinical bone characterization in a postmenopausal rat model (ovaries removed, followed by 8 week waiting period, before a 12 week treatment period) that demonstrated improvements in BMD (0.19 g/cm<sup>2</sup> for LGD-3303 vs. 0.175 g/cm<sup>2</sup> for control), femur mechanical strength (230 N for LGD-3303 vs. 190 N for control), and trabecular bone volume (14% for LGD-3303 vs. 10% for control) compared to untreated ovariectomized control. LGD-3303 alone did not fully recover BMD or trabecular volume as compared with sham operated intact females.

### Kaken Pharmaceutical Co., Ltd – tetrahydroquinolines (THQ)

Kaken built their compounds around the bicyclic THQ and tricyclic 3,4-cyclopentano THQ scaffolds (Figure 4) and disclosed structure-activity relationships for the binding to AR based on THQ substitution patterns (US Patent 6,777,427 [Miyakawa et al., 2004a]). Substitution at the 2- and 4-positions of the THQ ring with a variety of groups led to the identification of a series of analogs with 7-fold higher affinity than hydroxyflutamide. S-40503 (16) was selected for their initial *in vivo* studies. S-40503 (16), when administered for 4 weeks to castrated rats beginning immediately after surgery exerted androgenic effects, and partially restored the prostate back to a normal level (78 mg/100 g of body weight for S-40503 (16) treated, castrated rats versus 9 mg/100 g of body weight for untreated castrated rats). This compound was also reported to maintain BMD in males at a comparable level with control animals (Figure 4). A similar study was



**6-Anilino Quinolinones**



BMCL 17: 1527 (13)  
~100% of IC in LA at 40 mg/kg  
~25% of IC VP at 100 mg/kg



LGD-2226 (14)  
*development discontinued*



LGD-2941 (15)  
*Phase I clinical trials*

**Figure 3. Quinolinone (pyridone) fused-ring SARMs.** Ligand Pharmaceuticals, Inc. thoroughly explored the structural space surrounding their core quinolinone motif (ring A). SARM activity was achieved using several related templates including: tetrahydropyrido[3,2-g]-quinolin-2(1H)-one (9); anthracenoid (7H-[1,4]oxazino(3,2-g)quinolin-7-ones) (10-11) and phenanthroid (8H-(1,4)oxazino(2,3-f)quinoline-8-ones) (12) oxazino variants; and c) 6-anilino quinolinones (13-15). This latter class produced two clinical candidates in collaboration with TAP Pharmaceuticals. Data shown for (7) and (10) are derived from US Patents US 6,017,924 and 6,462,038, respectively. IC is an abbreviation for intact control. Other abbreviations are as described in the text.

performed in ovariectomized (i.e., removal of the ovaries, which are the primary estrogen producing gland in females) female rats, but a waiting period of four weeks was added before they were treated with S-40503 (16) for 8 weeks (i.e., a restorative or postmenopausal model). DHT was used as a positive control. S-40503 (16) also increased BMD in female ovariectomized rats, indicative of osteoanabolic activity, and had the same or better anabolic effects as DHT (Figure 4). Hanada *et al.* [Hanada

*et al.*, 2003] further characterized the osteoanabolic activity of S-40503 (16) by showing it increased BMD and biomechanical strength of femoral cortical bone compared to estrogen, an antiresorptive agent that does not increase these parameters (not shown). They also demonstrated the expected myoanabolic activity in LA at 30 mg/kg to be greater than intact control, but less than DHT at 10 mg/kg. Unfortunately, when S-40503 (16) was administered for an 8-week period to castrated rats, the



prostate weight was restored to the level of the control, illustrating it also showed full androgenic agonist activity. To our knowledge, S-40503 (16) was not advanced to clinical trials.

Kaken also explored a variety of tricyclic THQ derivatives similar to some of Ligand's tricyclic quinolinone templates. However, the templates from Kaken have the nitrogen in the B-ring, which is always saturated (i.e., tetrahydroquinoline (THQ); Figure 4). Early patents demonstrated *in vivo* osteo- and myoanabolic activities for molecules with 3 distinct C-rings: 1.) cyclopentene (World patent application WO2001 027086 [Hanada et al., 2001]), 2.) cyclopentane (World patent application WO2004 013104 [Miyakawa et al., 2004b]), and 3.) tetrahydrofuran (World patent application WO2004 000816 [Miyakawa et al., 2004c]).

An exemplary compound (17) of the cyclopentene template (template 1 in Figure 4), when administered at 5 days post-castration at 60 mg/kg for 4 weeks increased VP weights (56 mg/100 g body wt.) relative to vehicle-treated, castrated (9 mg/100 g body wt.), but did not approach the positive control of 10 mg/kg DHT (150 mg/100 g) or sham (i.e., intact) control (104 mg/100 g), demonstrating partial agonist activity in VP. Although BMD for (17) was comparable to DHT and sham controls, the difference between vehicle-treated castrated and sham was small (124 mg/cm<sup>2</sup> vs. 132 mg/cm<sup>2</sup>). Compounds (18) and (19) were shown to partially prevent testosterone-induced increases in prostate size in castrated rats treated with testosterone, suggesting that these compounds may be useful in the treatment of prostate cancer (CaP), prostatic pre-malignancies such as prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA) [De Marzo et al., 2007], and benign prostatic hyperplasia (BPH), or other androgenic prostatic maladies.

Compound (20) (template 2 in Figure 4) is an example of the Kaken template with the cyclopentane ring fused to the 3,4 positions of the THQ ring system. Compound (20) (30 mg/kg) partially increased VP weight as compared to intact controls (70 mg/100 g vs. 94 mg/100 g), but demonstrated full osteoanabolic activity and hypermyoanabolic activity. Compounds (21), (22), and (23) demonstrated full myoanabolic activity, with androgenic effects in the prostate varying from partial to full, demonstrative of the unique characteristics of these and other SARM pharmacophores on a molecule-by-molecule basis. The tetrahydrofuran variants with oxygen at the 4-THQ position of the THQ nucleus such as in (24) (template 3 in Figure 4) also demonstrated full myo- and osteoanabolic SARM activity (World patent application WO2004 000816 [Miyakawa et al., 2004c]).

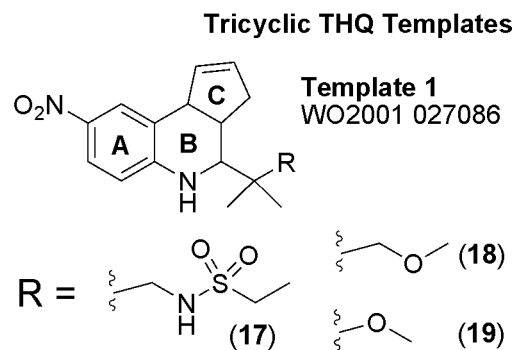
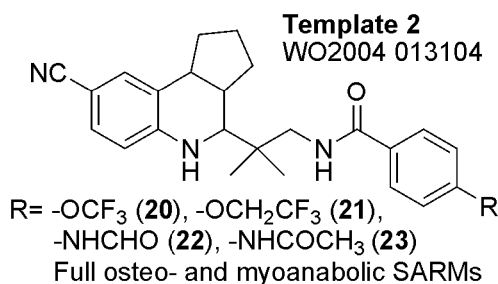
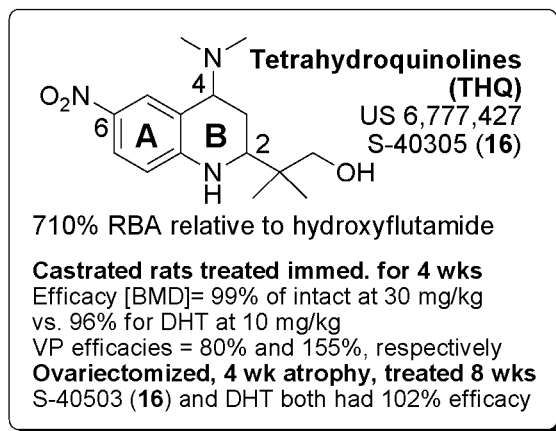
Despite multiple early and thorough demonstrations of tissue-selective hypermyoanabolic and osteoanabolic activities from several related structural templates, no clinical candidates are known from Kaken.

## Bristol-Myers Squibb & Co., Inc. (BMS) SARMS – hydantoins and variants thereof

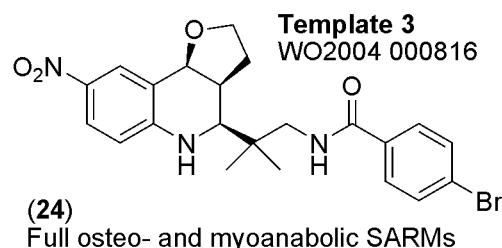
BMS has a very broad portfolio of AR ligands, many of which are antagonists [Balog et al., 2004; Salvati et al., 2005a; Salvati et al., 2005b]. Examples of the diversity within the patented AR ligand template portfolio have been recently reviewed [Mohler et al., 2008]. The A-rings are typically naphthyl or trisubstituted phenyl aniline derivatives (representative examples in Figure 5). BMS reported mutagenicity associated with the naphthyl aniline hydrolytic metabolites and the ability to design out these problems using trisubstituted phenyl A-rings which are *para* cyano/nitro, meta halogen and ortho methyl anilines [Hamann et al., 2007]. Separately, BMS explained how to convert certain of their antagonists into agonists with SARM activity [Sun et al., 2006]. Figure 5 illustrates how BMS obtained potent and selective SARM activity by simplifying the B-ring to a [5.5] bicyclic hydantoin, which has a hydroxyl substituent properly located to interact with N705 (contrast (25b) and (26)).

In addition to optimizing the aryl aniline (i.e., A-ring) portion of their SARMS, BMS has also explored variants of the [5.5] hydantoin ring system (i.e., B-ring), as illustrated in Figure 5 (middle). The optimal molecule in each case has the same *p*-CN, *m*-Cl, and *o*-methyl substituted aniline A-ring (i.e., optimized to be non-mutagenic), which provides the desired 58-62° aryl ring to hydantoin ring dihedral angle [Manfredi et al., 2007]. The first hydantoin variant replaces the 3-oxo group of (26) with a sulfonyl group, a change which was postulated to reduce aniline release *in vivo* [Manfredi et al., 2007]. Also explored within the sulfone template, was the reduction of the other hydantoin C=O (not shown). *In vitro* results suggest the reduced template retains tight binding, but has poor transactivational ability. Moreover, the stereochemistry of the hydroxyl group is very important for binding affinity and *in vitro* activity. The most potent and selective compound, (27) in Figure 5, was characterized as a tissue-selective partial myoanabolic agonist in a restorative *in vivo* assay (i.e., waiting period to allow diminution of tissues between castration and treatment).

A second hydantoin variant (represented by (28) in Figure 5) involves the replacement of one of the C=O groups of the hydantoin moiety with small alkyl groups, forming an imidazolin-2-one-containing [5.5] ring system. This design concept emerged from crystallographic studies which demonstrated that this C=O makes no direct contacts with the AR, and may be replaced with a hydrophobic group [Li et al., 2007]. Also explored in this work were  $\alpha$ ,  $\beta$ -unsaturated and saturated cyclic amide variations on the hydantoin theme (not shown) which retained significant affinity and *in vitro* activity, but demonstrated poor *in vivo* activity. Compound (28) was the only molecule that demonstrated full myoanabolic efficacy in the same restorative assay as for (27). Cumulatively, these results (and similar templates from Johnson & Johnson, as discussed *infra*) suggest that the AR is tolerant to a wide variety of rigidified [5.5] bicyclo ring



(17) in castrated rats treated immed. for 4 wks  
Efficacy[BMD]= full anabolic activity at 60 mg/kg  
VP efficacies = 56% vs. 150% for DHT  
(18) & (19) inhibit T-induced VP growth in  
castrated rats



**Figure 4. Tetrahydroquinoline (THQ) SARMs.** S-40305 (16) was extensively characterized for its osteoanabolic activity by Hanada *et al.* Additionally, Kaken scientists patented SARM activity for tetrahydroisoquinoline (THQ) templates 1, 2, and 3. Note that some or all of the data presented for (16-24) was derived from the indicated patents. RBA is an abbreviation for relative binding affinity. Other abbreviations are as described in the text.

systems with variable sensitivity to the stereochemistry of the hydroxyl carbon across the different templates.

BMS has published the preclinical characterization of their only clinical candidate, BMS-564929 (29), which combines the low mutagenicity A-ring already discussed with a [5.5] hydantoin B-ring, as exemplified by (26) [Ostrowski *et al.*, 2007; Wilson, 2007]. BMS-564929 (29) is a potent and hyperanabolic agonist compared to testosterone in skeletal muscle (LA) with an efficacy of 125% (comparable to other SARMs) and high potency ( $ED_{50} = 0.0009$  mg/kg), with hypostimulation of the prostate relative to testosterone ( $ED_{50} = 0.14$  mg/kg). As illustrated in Figure 5, these experiments in castrated rats demonstrated a 160-fold selectivity for LA compared to prostate, which they characterized as 'unprecedented muscle vs. prostate selectivity.' However, BMS may have over-estimated the selectivity of their compound, as evidenced by irregularities in the dose response curves and size of the prostate and LA muscle in castrated rats. Further, the limiting factor for this compound is the 9-fold selectivity between muscle action (i.e., myoanabolic activity) and LH suppression ( $ED_{50} = 0.008$  mg/kg). LH suppression may cause side effects, especially in elderly men, due to suppression of endogenous testosterone and subsequently estrogen levels, leading to detrimental effects on multiple organs systems including pro-osteoporotic changes in bone. BMS-564929 (29) is reported to be in Phase I clinical trials for age-related functional decline. In October 2007, BMS licensed their SARM program including BMS-564929 (29) (now

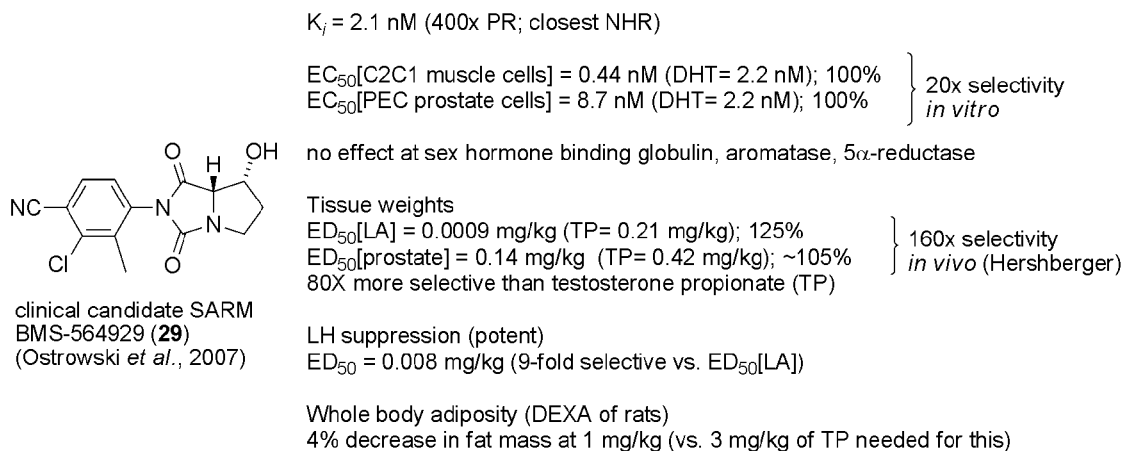
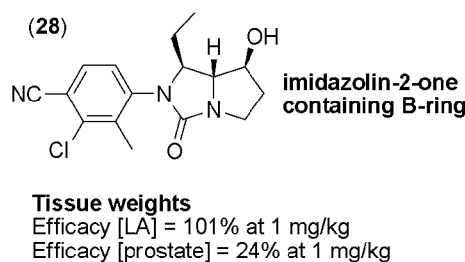
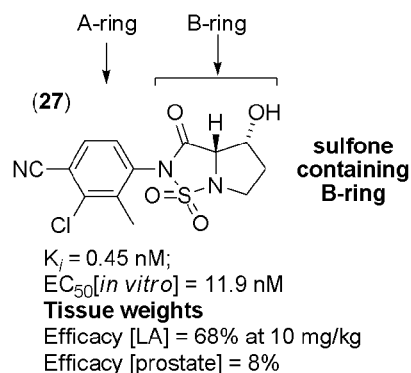
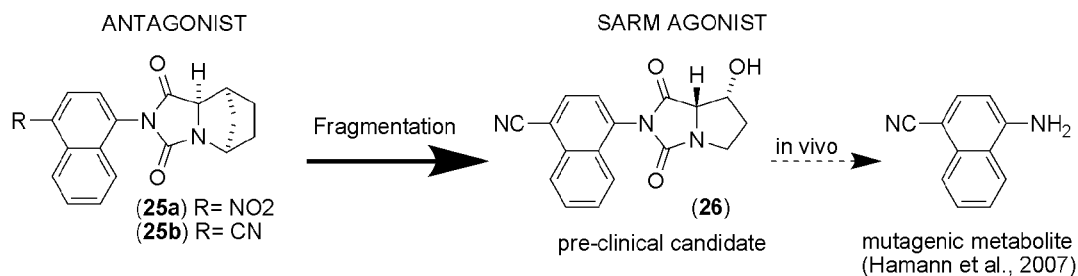
PS178990) and various back-up compounds to Pharmacoepia Drug Discovery.

### Johnson & Johnson (J&J)

#### Benzimidazole, imidazolopyrazole, indole, hydantoin and pyrazole SARMs

J&J and its subsidiaries initiated comprehensive SARM and AR antagonist programs and demonstrated tremendous pharmacophore diversity, several for which tissue-selectivity has been demonstrated. In addition to multiple patent series, J&J has published *in vivo* SARM characterizations belonging to five chemically distinct templates (Figure 6): 1.) benzimidazoles [Ng *et al.*, 2007a; Ng *et al.*, 2007b; Ng *et al.*, 2007c]; 2.) imidazolopyrazoles [Zhang *et al.*, 2007b]; 3.) indoles [Allan *et al.*, 2007a; Lanter *et al.*, 2006; Lanter *et al.*, 2007]; 4.) hydantoin variants [Zhang *et al.*, 2006a]; and 5.) pyrazoles [Zhang *et al.*, 2007a].

Some of the published benzimidazole compounds were characterized as potent and efficacious myoanabolic SARMs. For instance, (30) is a 2-(2,2,2)-trifluoroethyl-benzimidazole which when dosed at 2 mg/kg supported 126% LA weight (compared to 1 mg/kg testosterone) with an  $ED_{50}$  of 0.03 mg/d, but with little stimulation of the prostate [Ng *et al.*, 2007a], demonstrating an activity profile comparable to other hyperanabolic SARMs. Other molecules in the benzimidazole class were recently characterized as AR antagonists [Ng *et al.*, 2007a; Ng *et al.*, 2007b]. Compound (32) of template 2 (Figure 6), the imidazolopyrazoles, was characterized as a potent ( $ED_{50}$



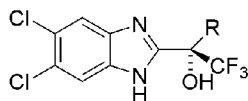
### Phase I for age-related functional decline reportedly ongoing

**Figure 5. Conversion of antagonist to agonist, elimination of mutagenic potential, and various hydantoin replacements.** Bristol-Myers Squibb (BMS) extensively explored antagonist templates and demonstrated the conversion of antagonist templates into agonist templates using a fragmentation approach. This group also explored several [5.5] bicyclic templates as alternatives to their [5.5] bicyclic hydantoin template of BMS-564929 (29). BMS-564929 (29) was characterized as a high potency myoanabolic SARM with high *in vivo* selectivity as related to the prostate, but a relatively narrow therapeutic index with regard to LH suppression. BMS licensed their SARM program to Pharmacopeia Drug Discovery, including BMS-564929 (29) (now PS178990).

= 0.07 mg/d) but a relatively less efficacious (91% at 3 mg/kg) myoanabolic SARM, and also demonstrated less tissue-selectivity than (30). Several articles characterizing compounds of template 3 (Figure 6), the indoles, were recently published, with only JNJ26146900 (33) demonstrating tissue-selective activity [Allan et al., 2007a]. JNJ26146900 (33) retains LA weight but reduces prostate weight in intact animals, and partially offsets castration-induced losses in BMD and LBM. JNJ26146900 (33) blocked testosterone-induced prostate cancer growth and demonstrated favorable activity in a prostate cancer xenograft model using CWR22-LD1 prostate cancer cells (Figure 6). Template 4, the hydantoin derivatives, are structurally similar to BMS-564929 (29) and other [5.5]

templates reviewed *supra*, and J&J template 2, the imidazolopyrazoles. Based on (34) ( $ED_{50} = 2.9$  mg/d), they have SARM activity with lower myoanabolic potency than (30) and (32) above, and variable myoanabolic efficacy for (34) (75%) and (35) (117%) (Figure 6). Template 5, the pyrazoles, were recently published, with the lead molecule (36) shown in Figure 6 demonstrating relatively weak potency ( $ED_{50}$ [LA] = 3.8 mg/kg;  $E_{max} = 120\%$ ) myoanabolism. Compound (36) demonstrated mixed agonist/antagonist effects in the prostate (28% at 2 mg/d for 5 days in castrated rats and 33% prostate weight inhibition at 30 mg/d in intact rats), suggesting possible utility in prostatic maladies.

**Template 1: Benzimidazoles**



**Potent and high efficacy SARM**

**(30)** (R= CH<sub>2</sub>CN)

*In vivo organ weights*<sup>&</sup>

(castrated rats dosed 2 mg/kg/d x5)

VP weight = 9% of 0.1 mg/d T

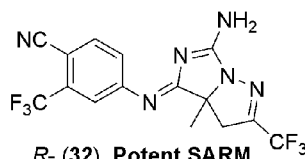
LA weight = 126% of 1 mg/d T

ED<sub>50</sub> = 0.03 mg/d

**TOXIC;** **(31)** (R= CH<sub>2</sub>CH<sub>3</sub>)

*Bioorg.Med.Chem.Lett.* 17(6): 1784-7.

**Template 2: Imidazolopyrazole**



**R- (32) Potent SARM**

*In vivo organ weights*<sup>&</sup>

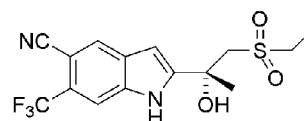
ED<sub>50</sub> (LA) = 0.07 mg/d

efficacy = 91% at 3 mg/d

Prostate efficacy = 36% at 3mg/d

30% inhib of 2 mg/d T

**Template 3: Indoles**



JNJ-26146900 **(33)** - **Weak SARM**

K<sub>i</sub> = 400 nM

IC<sub>50</sub> = 2200 nM (weak inhib)

retention of LA weight (intact)

reduces prostate weight (intact)

BMD: reduces loss in ORX rats

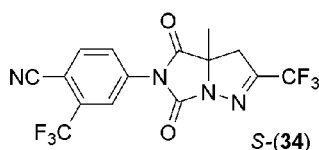
LBM: reduced loss in ORX rats

block T-induced CaP growth

100mg/kg suppressed CWR22-LD1

CaP xenograft growth

**Template 4: Hydantoin Variants**



**S-(34)**

**Modest potency but efficacious**

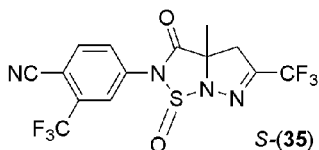
*In vivo organ weights*<sup>&</sup>

(castrated rats dosed 2mg/d x5)

11% VP weight increase (at 3mg/d)

75% LA stimulation

ED<sub>50</sub> = 2.9 mg/d

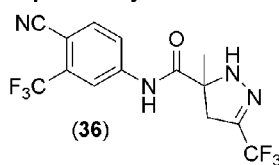


**S-(35)**

58% prostate weight stimulation

117% LA weight stimulation

**Template 5: Pyrazolines**



**(36)**

**Modest potency but efficacious**

*In vivo organ weights*<sup>&</sup>

(castrated rats dosed at 2mg/d x5)

28% VP weight increase (agonist)

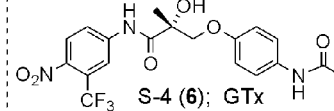
33% VP weight decrease

in intact rats dosed at 30 mg/kg

120% LA stimulation (2mg/d x5)

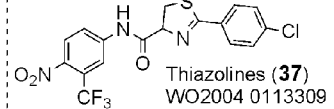
ED<sub>50</sub> = 3.8 mg/d

**Cyclic Propionamide Mimetics**



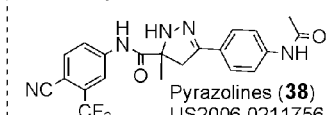
**S-4 (6);**

GTx



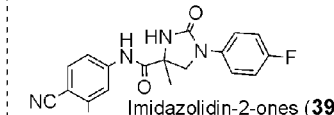
Thiazolines **(37)**

WO2004 0113309



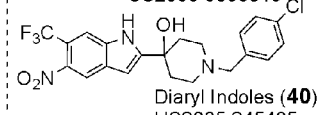
Pyrazolines **(38)**

US2006 0211756



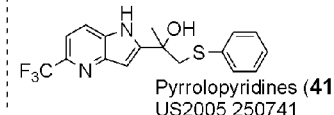
Imidazolidin-2-ones **(39)**

US2006 0063819



Diaryl Indoles **(40)**

US2005 245485



Pyrrolopyridines **(41)**

US2005 250741

& - Modified Hershberger- test compound + TP (0.1 mg/d) for 5 days in castrated immature rats

**Figure 6. Peer-reviewed SARMs from Johnson & Johnson (J&J) and subsidiaries.** J&J published a wide variety of AR ligand templates, many of which have demonstrated SARM activity (Templates 1-5). These compounds covered a broad range of pharmacological profiles and chemotype diversity. Additionally, J&J patented a wide variety of propionamide bioisosteres (37-41), some of which have not been published. ORX, T, TP, and CaP are abbreviations for orchidectomy, testosterone, testosterone propionate, and prostate cancer, respectively. Other abbreviations are as described in the text.

**Johnson & Johnson Co. and subsidiaries – cyclic bioisosteres of propionamides**

Johnson & Johnson scientists patented a wide variety of diaryl templates in which the propionamide linker segment has been replaced by a variety of cyclic elements to include: thiazolines (37) (World patent application WO2004 113309 [Ng and Sui, 2004]), pyrazoles (38) (US patent application US2006 0211756 [Zhang et al., 2006b]), imidazolin-2-ones (39) (US patent application US2006 0063819 [Lanter and Sui, 2006]), diaryl indoles

(40) (US patent application US2005 245485 [Lanter et al., 2005b]), and pyrrolopyridines (41) (US patent application US2005 250741 [Lanter et al., 2005a]). Representative examples of each bioisosteric template are shown in Figure 6 (bottom right).

Although the pharmacophoric diversity embodied in the AR ligand portfolio advanced by J&J and subsidiaries is impressive, the patents only disclose tissue-selectivity in qualitative terms, with no quantitative measures of

pharmacologic activity or structure-activity relationship. The published literature does outline a number of significant advances in tissue-selective modulation, as discussed *supra*. Nonetheless, J&J does not appear to be pursuing clinical development of a SARM at this time.

### Merck & Co., Inc.

#### Azasteroidal SARMs

Merck scientists patented a variety of steroidal SARMs which were variations of the 4-azasteroidal template of finasteride, a 5 $\alpha$ -reductase inhibitor. Modifications at several positions reportedly produce tissue-selective activity, with agonist activity in bone and muscle and antagonist activity in prostate or uterus. Some of the points of variation included fluorination of the A-ring (World patent application WO2003 077919 [Meissner and Perkins, 2003]), substitution at the 4-[all azasteroidal patents discussed herein], 6- (US patent application US2004 0235808 [Wang, 2004]), and 7- (World patent application WO2004 0100874 [Meissner and Perkins, 2004]) positions, and addition of an imidazole ring fused to the 3 and 4 positions (World patent application WO2006 0026196 [Wang and Close, 2006]). The most conspicuous changes occur at the 17 $\beta$  position. These 17 $\beta$  substituents are a wide variety of nitrogen-linked aryl groups which includes carboxamides and acetamides (World patent application WO2005 0005606 [Dankulich et al., 2005a]; World patent application WO2005 009949 [Dankulich et al., 2005]; World patent application WO2005 099707 [Wang and McVean, 2005]; US patent application US2006 0258661 [Dankulich et al., 2006]), amines (US patent application US2006 0241107 [Meissner and Perkins, 2006]), C17 heterocyclics (World patent application WO2005 0025579 [Meissner and Mitchell, 2005]), and C20 heterocyclics (World patent application WO2005 004807 [Dankulich et al., 2005b]) (see Figure 7, top left for atom numbering). Merck reported data at the 2007 National American Chemical Society (ACS) Meeting to substantiate their claims of SARM activity with an azasteroidal template. A series of 17-hydroxy-4-azasteroids was analyzed in an *in vitro* transactivation assay. This SAR information was used to select the azasteroid (42) shown in Figure 7 which was demonstrated to be osteoanabolic with an *in vivo* bone formation rate (BFR) of 82% of DHT at 3 mg/kg, but little to no ability to stimulate uterine weight in a 24-day *in vivo* ovariectomized rat model (only 1% of uterine weight [Nantermet et al., 2005]) in a 24-day *in vivo* ovariectomized rat model (unpublished data).

#### Diaryl butanamides and carbonylamino-benzimidazoles

Merck scientists also patented two distinct diaryl SARM templates, as shown in Figure 7 (43-45). The diaryl butanamides (representative examples (43) and (44)) closely resemble the propionamides, but differ by the quarternary carbon being ethyl substituted (making them butanamides) and the insertion of a methylene group between the A-ring and the amide. Points of variation include the A-ring which can be pyridin-4-yl (World patent application WO2007 016358 [Perkins et al., 2007]), pyridin-3-yl (World patent application WO2006 060108

[Kim et al., 2006]), or benzyl (US patent application US2005 277681 [Hanney et al., 2005]).

The tertiary carbon alkyl substituent can be methyl or ethyl and perfluorinated or not. Also various A- and B-ring substituents and substitution patterns have been explored. The carbonylamino-benzimidazole (World patent application WO2004 041277 [Kim et al., 2004]) template has three basic variations: 1) diaryl benzimidazoles with no linker (not shown), 2) urea+benzimidazole linked triaryl compounds [representative example (45) shown in Figure 7], and 3) amide+benzimidazole linked triaryl compounds (not shown).

#### Chromeno and quinolinyl benzazepines

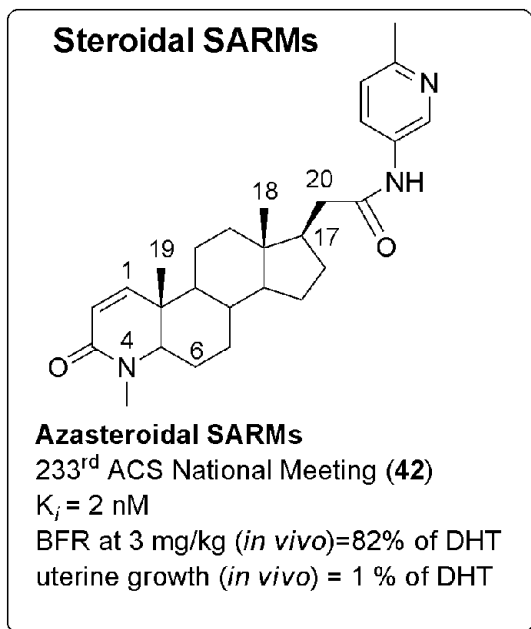
Merck scientists patented a handful of anthracene-based chromeno and quinolinyl benzazepines (representative example (46) in Figure 7). These compounds are reportedly SARMs that were tested by the same panel of assays as for other Merck patents (*supra*); however no activity is reported (US patent US 7,196,076 [Coleman and Neilson, 2007]).

### GlaxoSmithKline (GSK)

#### Disubstituted and diaryl anilines

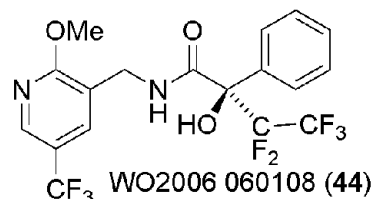
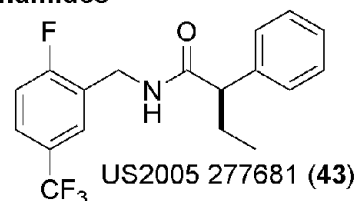
GSK patents outline a wide variety of disubstituted aniline templates to include *para* nitro/cyano and *ortho/meta* electron withdrawing A-ring substituents on a phenyl A-ring. Examples of the structural diversity of this series are given in Figure 8 with the following compounds: (47-48) (US patent application US2006 0148893 [Blanc et al., 2006]), (49) (World patent application WO2005 000795 [Blanc et al., 2005]), (50) (World patent application WO2005 085185 [Turnbull et al., 2005]), (51-52) (World patent application WO2006 133216 [Turnbull et al., 2006]). Alternatively, these compounds have *para* nitro/cyano naphthyl A-rings such as (53-54) (US patent application US2006 0142387 [Cadilla et al., 2006]). The aniline substituents of these templates include alkyl, haloalkyl, alkenyl, cycloalkyl, alkanol, alkylamino, and carboxylate and derivatives (Figure 8). The patents describe GR, PR, MR, and AR binding affinity and AR-luciferase transactivation *in vitro* assays and *in vivo* studies in castrated rats analyzing VP and SV, LA and bulbocavernosus (BC) muscles as androgenic and anabolic indicators, respectively. However, the only GSK disubstituted aniline for which biological data is disclosed is a nilutamide-like cyclic aniline template [Trump et al., 2007]. A virtual screening-guided combinatorial chemistry approach was used to find AR agonists with various substitutions of the left ring and various replacements of the right ring, as shown, of compound (55). This yielded 352 submicromolar and 17 subnanomolar AR agonists, as measured by a cell-based reporter gene functional assay.

GSK scientists patented a series of diarylanilines which are described as AR modulators, but did not disclose biological activities other than 'favorable' compounds have pIC<sub>50</sub> (binding) <5 and LA hypertrophy with little prostate stimulation (i.e., SARMs) (World patent application WO2006 044707 [Turnbull et al., 2006]). A



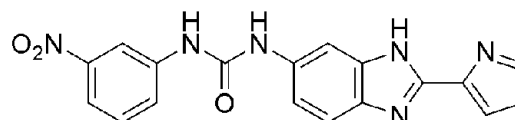
## Nonsteroidal AR Ligands

### Butanamides



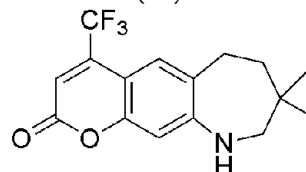
### Carbonylamino-benzimidazoles

WO2004 041277 (45)



### Chromeno- and Quinolinyl-Benzazepines

WO2003 034987 (46)



**Figure 7. SARM templates from Merck.** Merck explored several templates, but focused the most effort on the 4-azasteroidal and the butanamide scaffolds, for which they have multiple patents. Merck recently disclosed SARM activity for the first time for (42) at an ACS meeting. (42) was characterized as osteoanabolic with low virilization potential in *in vivo* rat uterine growth assays (Nantermet et al., 2005). The structures for (43-46) were derived from the indicated patent applications. BFR is an abbreviation for bone formation rate.

representative example (56) is given in Figure 8. Structural variation included diaryl compounds similar to bicalutamide, but separated by 3, 4, 5 or 6 atoms. Often times, the anilido nitrogen was tertiary with a substituted aliphatic side chain. Also, the linker chiral alcohol of propionamides was replaced by a central amide separate from the aniline.

### Benzoxazepinones

At the 234<sup>th</sup> ACS National Meeting (Fall, 2007), Rafferty *et al.* disclosed a structurally dissimilar template of putative SARMs to include GSK8698 (57) and GSK4336A (58) shown in Figure 8 which are benzoxazepinones with an electron-deficient anilide side chain. In *in vitro* analyses, these two representative examples are potent agonists with variable half-lives (unpublished data). *In vivo* activity is not known.

### Preclinical and clinical SARMs (undisclosed structures)

At the 89<sup>th</sup> Annual Endocrine Society Meeting (June, 2007), Han *et al.* disclosed their first *in vivo* SARM characterization. GSK2420A (structure not given) demonstrated an ED<sub>50</sub> (LA) of 0.026 mg/kg in a seven day castrated rat model and restored castration-induced

LA muscle atrophy in a 28-day treatment. The effects of GSK2420A on the prostate are consistent with partial agonist activity, eliciting a 2-fold increase over vehicle (versus 7-fold stimulation for DHT (3 mg/kg)), and decreased prostate weight in intact rats (unpublished data).

As reported on ClinicalTrials.gov (A service of the U.S. National Institutes of Health) [NIH, 2008], the first clinical candidate from GSK is GSK971086 (structure not published) for which they are currently enrolling a Phase I clinical trial to test the safety, tolerability, and blood levels after 1 dose & 7 days of dosing in healthy adult males.

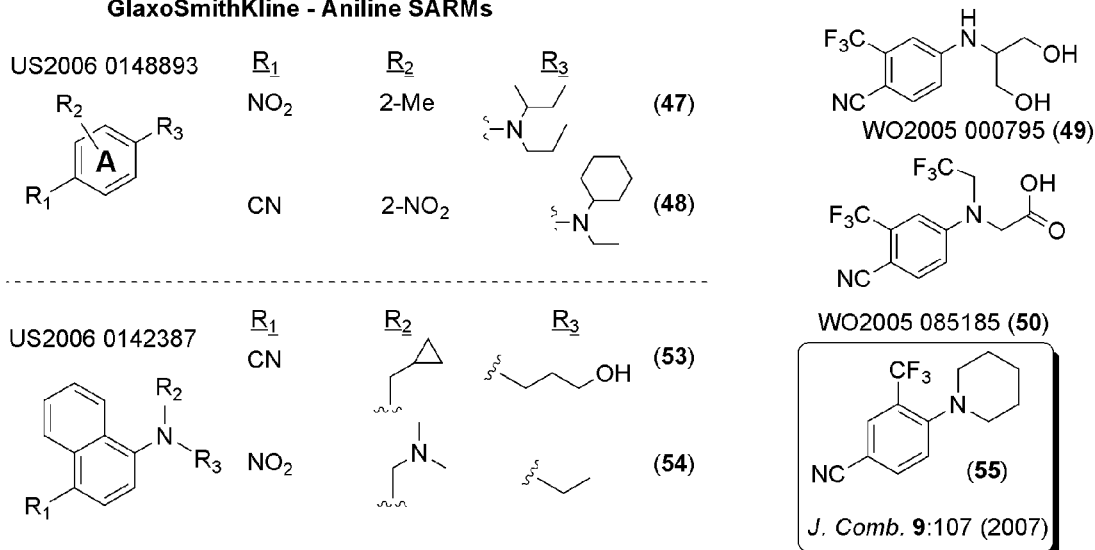
### Miscellaneous others

#### Eli Lilly and Company, Inc.

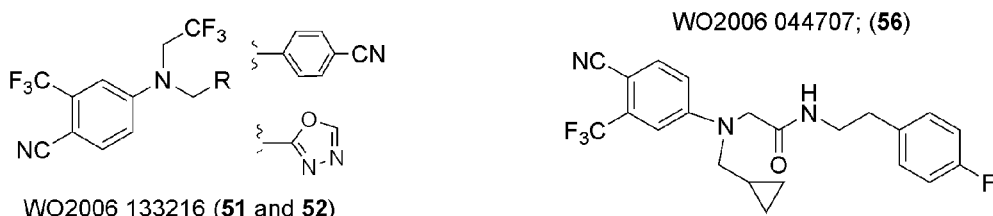
Lilly scientists patented two distinct SARM templates, the *N*-arylpyrrolidines and the tetrahydrocarbazoles, along with *in vivo* demonstrations of tissue-selective myoanabolic activity.

Substituted *N*-arylpyrrolidines: Lilly scientists patented a series of substituted *N*-arylpyrrolidines as a SARM

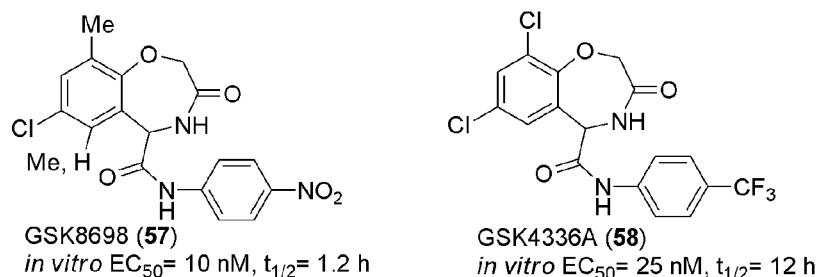
GlaxoSmithKline - Aniline SARMs



GlaxoSmithKline - Diaryl Anilines



GlaxoSmithKline - Benzoxazepinones



**Figure 8. SARM templates from GlaxoSmithKline (GSK).** GSK patented an assortment of aniline SARMs (47-54) without specific SARM characterization, but rather just *in vitro* data. The aniline (55) was characterized *in vitro* as an AR agonist. Separately, GSK reported in conference abstracts *in vitro* characterizations of benzoxazepines as AR agonists. GSK has reported their first public disclosure of SARM activity in a conference abstract for GSK2420A (structure not known), and is pursuing GSK971086 (structure not known) as a clinical candidate. Although there is not much public information from GSK, the breadth of their patents and presentations suggests that they have an active SARM program.

template (World patent application WO2006 124447 [Gavardinas et al., 2006]). *In vitro* activity was reported for numerous compounds to achieve low nM AR binding with several potent transcriptional activators that approach full agonist efficacy in C2C12 cells as an indicator of agonist activity in muscle tissue. The *in vivo* activity was reported for two compounds (59) (reportedly commercially available) and (60), which were tested in castrated (at 8 weeks) mice after 8 weeks of wasting (Figure 9). Test animals were dosed over a two week timeframe at 0.3, 1, 3, 10, and 30 mg/kg per day by mouth (i.e., per os (po)) or subcutaneously (s.c.). Positive control animals were dosed at 10 mg/kg daily with T enanthate. LA muscle was used as the indicator of efficacy with % efficacy (treated

vs. vehicle-treated castrated animals) for (59) and (60) of 186% (s.c.) and 164% (po) at 10 mg/kg per day, respectively. Although no data was disclosed, these compounds reportedly did not increase weights of SV or prostate. The most active compounds are highly similar in structure. Apparently, *p*-CN, *m*-Cl, *o*-Me substitution of the A-ring (similar to BMS-564929 (29)) with an aryl R substituent off an otherwise unsubstituted pyrrolidine produces the most potent *in vitro* agonists, some of which also have *in vivo* agonist activity in LA. Pfizer has also reported SARM activity with a similar molecule ((62) in Figure 9), an *N*-arylpiperidine described *supra*. Likewise, GlaxoSmithKline has also reported similar compounds

such as (55) with reported *in vitro* agonist activity [Trump et al., 2007] (Figure 8).

**Tetrahydrocarbazole SARMs:** Lilly scientists patented the synthesis, binding affinity and transcriptional activation (AR/ARE in C2C1 cells) of a large series of tetrahydrocarbazoles, many of which were high affinity, *in vitro* agonists (World patent application WO2007 002181 [Fales et al., 2007]). Additionally, several compounds were characterized in the same *in vivo* mouse model of efficacy in SV, VP, and LA tissues as discussed above (i.e., % efficacy expressed as percent of castrated, vehicle-treated control). The myoanabolic compound, (61), supported 306% of LA in this model, and reportedly showed no statistical weight change in SV or VP, as compared to vehicle-treated castrated controls (data not shown). This efficacy is in excess of that demonstrated with the *N*-arylpiperidine template, but unfortunately these values are hard to compare to SARMs from other groups, due to a lack % efficacy versus intact control or testosterone.

#### Pfizer – cyclic or disubstituted anilines

Pfizer scientists patented a series of high AR affinity (low nM range) cyclic (62) or disubstituted (63) anilines (World patent application WO2005 108351 [Gant et al., 2005]). Compounds (62) and (63) displayed IC<sub>50</sub> values of 5.2 nM and 1.1 nM in binding assays (Figure 9), respectively. Compound (62) (10 mg/kg/d, s.c.) effectively reduced fat mass (121 g vs. 121 g sham and 149 g for ovariectomized) and increased LBM (319 g vs. 299 g sham and 291 g ovariectomized). Similar results were achieved with these compounds in aged (11 month old) rats. Compound (62) increased BMC (11.9 mg/mm vs. 11.9 mg/mm for sham and 11.0 mg/mm for ovariectomized), but had lesser effect on BMD (602 mg/cm<sup>3</sup> vs. 672 mg/cm<sup>3</sup> for sham and 593 mg/cm<sup>3</sup> for ovariectomized). Compounds (62) and (63) showed tissue-selectivity in castrated immature male rats (25 days old). Daily subcutaneous administration (30 mg/kg) of (62) and (63) for 4 days retained 100% and 89% of LA weight, respectively, but only 33% and 39% of VP weight, as compared to DHT (10 mg/kg).

At the 27th Annual Meeting of the ASBMR (2005), Ke et al. reported osteoanabolic SARM activity for CE-590 (structure unknown), (unpublished data). CE-590 is a high affinity (IC<sub>50</sub> = 16 nM) SARM *in vivo*. An 8-week treatment schedule of CE-590 (30 mg/kg orally, twice per day) significantly decreased prostate weight by 26% in sham rats (acting as an AR antagonist), as compared to 66% increase for DHT-treated sham rats (agonist activity). CE-590 completely prevented castration-induced decreases in trabecular content, trabecular density, cortical content, cortical area and cortical thickness and increases in bone resorption and turnover. No SARM clinical candidates are known for Pfizer.

#### Acadia Pharmaceuticals, Inc. – aminophenyl derivatives

Acadia patented a novel template for SARMs involving typical A-rings, but the aniline component is a [3.2.1] tricyclic ring system, similar to some of the BMS

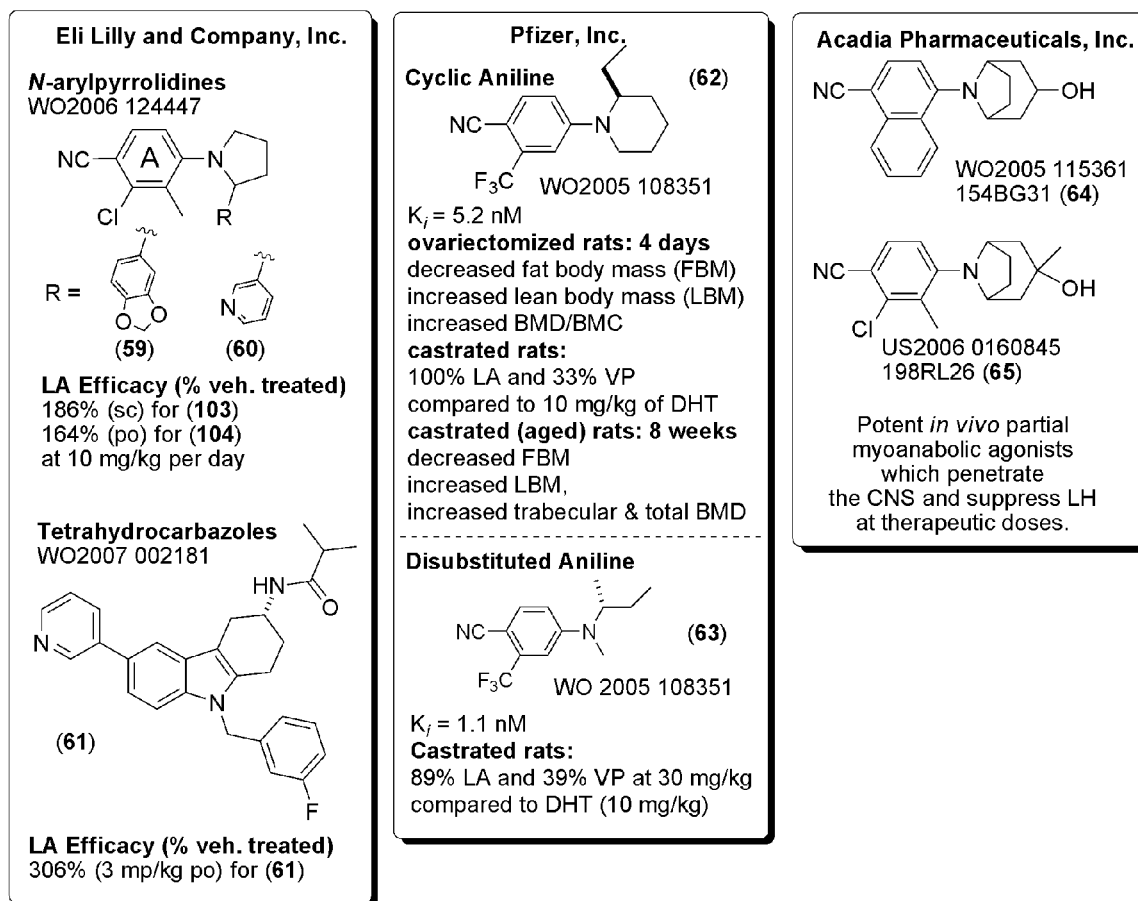
templates. Acadia reported compounds with modest potency in terms of *in vitro* transcriptional agonist activity (mid to high nM range) with efficacies ranging from 41% to 94% (World patent application WO2005 115361 [Schlienger et al., 2005]). Compound 154BG31 ((64) in Figure 9) produced significant increases in VP, SV, and LA as compared to vehicle. LA weight was approximately 60% at a dose of 30 mg/kg, as compared to testosterone propionate (1 mg/kg), whereas VP was approximately 20%. This represents ~3-fold tissue-selectivity, but only partial myoanabolic agonism. 154BG31 (64) also fully suppressed LH at a dose of 10 mg/kg, which is in the same range as myoanabolic activity, possibly limiting the utility of these compounds for muscle indications. Compound 198RL26 (65) was separately reported to be a high affinity ligand (79% with an *in vitro* potency of pEC<sub>50</sub> = 8.8) and was selected for *in vivo* experimentation. Like 154BG31, 198RL26 (65) is an *in vivo* partial myoanabolic agonist of similar potency and efficacy, and produced a dose-dependent suppression of plasma LH levels such that a complete reversal was evident at 10 mg/kg, suggesting CNS penetration (US patent application US2006 0160845 [Schlienger et al., 2006]). Acadia also reported ACP-105 (structure unknown) as a SARM development candidate that has reversed endocrine and bone-related markers of testosterone deficiency in preclinical animal testing, with little effect on the prostrate (unpublished data).

#### Summary of the SARM field

The development space for SARMs is becoming more crowded with more *in vivo* characterizations of diverse structural templates emerging at an accelerating pace. Several groups have produced clinical candidate SARMs to include: GTx, Inc. (Ostarine™ for cachexia in Phase II, structures not published), Bristol-Myers Squibb (BMS-564929 (29) for age-related functional decline in Phase I), and Ligand Pharmaceuticals, Inc. (LGD2941 (15) for frailty and osteoporosis in Phase I, and LGD2226 (14), which has been discontinued), and GlaxoSmithKline (GSK971086) in dose finding Phase I studies, with more clinical candidates likely to emerge in the near future. Not surprisingly, some of these groups have published the most detailed characterizations of their SARMs to include S-1 (see literature for structure) and S-4 (6) (and many others) from GTx, Inc.; BMS-564929 (29) from BMS; LGD2226 (14) and LGD2941 (15) and others from Ligand.

The salient features for promising clinical and preclinical SARMs include hypermyoanabolic and hyperosteoanabolic efficacy (hyper-defined as in excess of intact control) at doses associated with decreased prostate size and little to no suppression of pituitary gonadotropins. Others, such as (18-19) from Kaken and S-1 from GTx, have demonstrated partial agonist activity in prostate with potential in retarding growth of the prostate, while retaining agonist effects in anabolic tissues. The utility of the various SARMs in patients is yet to be proven, but indeed seems very promising, given the multitude of *in vivo* pre-clinical characterizations by many groups, and the auspicious proof of concept results for Ostarine™ in Phase II clinical trials (discussed *supra*).





**Figure 9. SARMs patented by Lilly, Pfizer, and Acadia.** Lilly patented two SARM templates, the *N*-arylpyrrolidines and tetrahydrocarbazoles, which they characterize as tissue-selective. Unfortunately, their comparisons are to vehicle-treated animals, making it hard to assess the relative activity compared to other templates. Pfizer likewise has patented an aniline series of SARMs, which they characterize as high affinity and tissue-selective full agonists. Acadia too has patented a novel SARM template of [3.2.1] tricyclic anilines, which they characterize as weak anabolic agents that suppress LH at therapeutic doses.

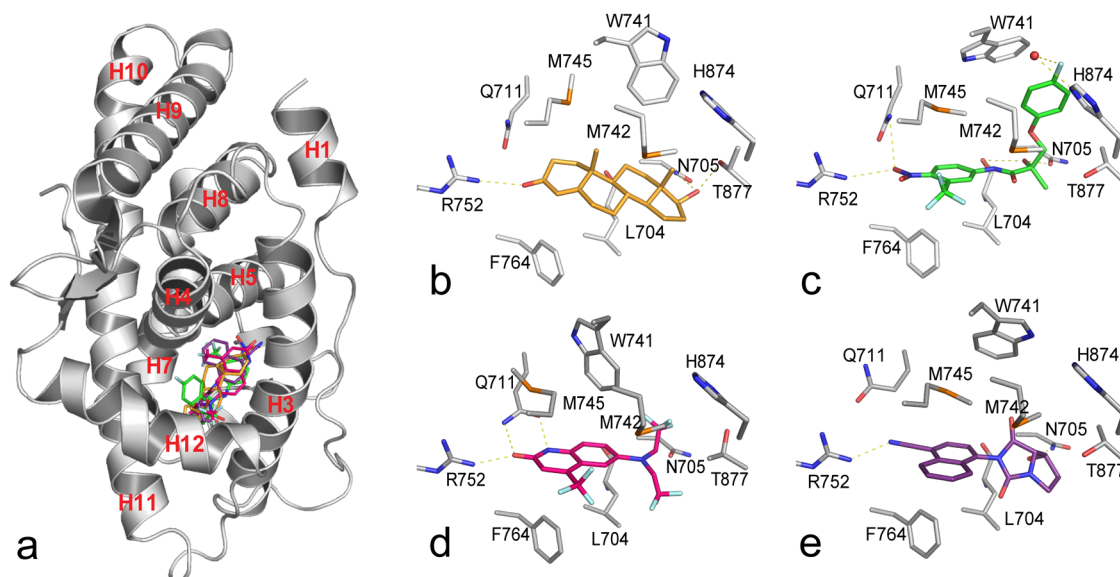
These and other *in vivo* characterizations of SARMs are summarized in Supplementary File 1.

## Knowledge of SARM interactions with the AR

X-ray crystallography has elucidated binding modes of a number of the above-mentioned SARMs. The crystal structure of the AR LBD was first described by Matias *et al.* in 2000 in a complex with the synthetic steroid, R1881 [Matias *et al.*, 2000]. The first SARM-bound AR crystal structure, reported in 2005, was with the bicalutamide derivative, S-1 [Bohl *et al.*, 2005b]. Whereas helix 12 of the ER $\alpha$  LBD adopts an alternate conformation when comparing estradiol and SERM (i.e., tamoxifen and raloxifene)-bound structures, the protein fold of the SARM-bound AR LBD and steroidal-agonist bound (e.g., R1881, DHT) are the same [Bohl *et al.*, 2005b; Brzozowski *et al.*, 1997; Matias *et al.*, 2000; Sack *et al.*, 2001; Tanenbaum *et al.*, 1998; Wang *et al.*, 2006]. This finding has held true for all SARM-bound AR LBD x-ray crystal structures published to date (Figure 10a). Thus, a structural basis for the mechanism of SARM activity was not made apparent through the use of x-ray crystallography. However, information regarding the binding modes of the various nonsteroidal

pharmacophores complexed to the AR provides information for rational drug design (Figure 10). Additionally, the different receptor interactions when comparing steroidal agonists and nonsteroidal SARMs may play a role in altering the protein conformation in solution.

Until S-1 binding mode was solved, it was unknown how the AR could accommodate aryl propionamide SARMs. Various modeling studies proposed alternate areas in the AR binding pocket where the B-ring was positioned [Bohl *et al.*, 2004; Salvati *et al.*, 2005a; Soderholm *et al.*, 2005]. Each of these molecular models was based on theoretical flexible regions of the AR, as the binding pocket in the steroidal-bound crystal structures was not of adequate size to accommodate the bulk of such analogs. The x-ray crystal structure of the S-1-AR LBD complex elucidated that the W741 side chain is displaced by the B-ring to expand the binding pocket such that the compound bends 90° and orients towards the AF-2 region [Bohl *et al.*, 2005b] (Figure 10c). The fluorine on the *para* position of the B-ring appears to act as a hydrogen bond acceptor to a conserved water molecule that is stabilized by the H874 side chain and backbone of helices 4 and 5, explaining the increased potency of compounds with cyano and nitro substitutions at this position [Kim *et al.*,



**Figure 10. X-ray crystal structures of the AR LBD with DHT and SARMS.** (a) The general protein fold of the AR LBD-DHT complex (PDB code 1i37) superimposed with binding conformations of nonsteroidal SARMS including S-1 from GTX (green, PDB code 2axa), LGD2226 from Ligand Pharmaceuticals (pink, PDB code 2hvc), and '10b' from BMS (purple, PDB code 2ihq) shows how the compounds are accommodated within the agonist form of the AR LBD. Oxygen-red; nitrogen-blue, sulfur-orange; fluorine-cyan. (b) Bound conformation of DHT shows hydrogen bonds between the 3-keto group and R752, as well as the 17 $\alpha$ -hydroxyl group with N705 and T877. (c) The A-ring nitro group of S-1 interacts with R752 similar to the 3-keto group of DHT, while the amide NH and hydroxyl groups form hydrogen bonds to L704 and N705, respectively. The B-ring of S-1 orients towards the AF-2 by displacing W741 and the p-fluorine of the B-ring forms a water-mediated hydrogen bond to H874. (d) LGD2226 binds similar to DHT with the ketone on the A-ring hydrogen bonding to R752, but contains an additional hydrogen bond to Q711 through its heterocyclic A-ring. (e) 10b forms hydrogen bonds to R752 and N705 with increased hydrophobic contacts as a result of its bicyclic ring systems.

2005]. Similar to the steroidal androgens R1881 and DHT (Figure 10b), hydrogen bonding occurs with R752 and Q711 to the A-ring nitro group, and N705 to the hydroxyl group of S-1. However, unlike steroidal binding, no hydrogen bond to T877 occurs. Given the close chemical structures of S-1 and *R*-bicalutamide, it became clear from the S-1-bound AR LBD structure as to why the two compounds exhibited different activities (i.e., agonist vs. antagonist). A sulfonyl linkage on *R*-bicalutamide in place of the ether linkage of S-1 would be poorly accommodated in the agonist fold of the AR LBD. While it had been shown that *R*-bicalutamide induces an agonist fold in the W741L mutant AR LBD through x-ray crystallography [Bohl et al., 2005a], the sulfonyl group of *R*-bicalutamide would result in steric clash between W741 and M895 in the wt AR, thus precluding the conformation seen in the S-1- and steroid-bound structures. To date, no structure of an antagonist-bound wt AR LBD has been reported.

Ligand Pharmaceuticals published a crystal structure of LGD2226, a bicyclic hydantoin SARM bound to the AR LBD in 2006 [Wang et al., 2006]. This structure exhibited interesting interactions including a repositioning of Q711 to form a hydrogen bond with both the carbonyl and secondary amine of the A ring, as well as a hydrogen bond to R752 (Figure 10d). Favorable contacts with the two branched trifluoromethyl groups were observed. While there were no apparent hydrogen bonds to N705 or T877, van der Waals interactions with the trifluoromethyl groups and these residues may play a role in this compound's high affinity. Unlike aryl propionamide SARMS, this compound does not expand to the binding pocket relative to R1881- and DHT-bound AR.

Bristol-Myers Squibb (BMS) published their first crystal structure of a SARM bound to the wt AR LBD in 2006 [Sun et al., 2006] (Figure 10e). It demonstrated how a compound similar to BMS-564929 (29), a derivative of the antiandrogen nilutamide containing two fused five-membered rings binds the AR. Similar to aryl propionamide SARMS, this compound forms a hydrogen bond to N705 and not T877.

### Plausible mechanisms for tissue selectivity of SARMS

The importance of androgens is not appreciated until post-andropause diseases such as osteoporosis, cachexia and others develop. Though administration of steroidal androgens improves muscle mass and bone mineral density, they also have undesired effects leading to increased prostate size, acne, effects on serum lipids and others. The greatest challenge in the discovery of SARMS was to separate the androgenic (effect on secondary sexual organs) and anabolic effects (effects on muscle and bone). Many have now shown successfully in preclinical models and in clinical trials that the SARMS efficiently separate the androgenic and anabolic effects [Chen et al., 2005c; Gao and Dalton, 2007; Kearbey et al., 2007]. How this separation is achieved by SARMS, almost identical to SERMS, is complex and still under investigation. Though 10 years have elapsed since the discovery of the first SARM, the proposed mechanisms for SARM action have been adopted from SERMS.

The proposed mechanisms for the tissue selectivity of SARMS include the role of 5 $\alpha$ -reductase, tissue-specific expression of coregulators, differences in the complexes

formed by AR in anabolic and androgenic tissues, and the tissue-specific role of intracellular signaling cascades.

### 5 $\alpha$ -reductase and aromatase

SARMs developed to date are resistant to 5 $\alpha$ -reduction or aromatization. This is considered as at least one plausible contributing factor for the tissue selectivity of SARMs [Buijsman et al., 2005]. The most active androgen in prostate is DHT, which is formed by the 5 $\alpha$ -reduction (5 $\alpha$ -reductase is expressed in prostate and skin) of testosterone, the most abundant circulating androgen. Inhibition of 5 $\alpha$ -reductase by finasteride leads to inhibition of prostate size without any effect on muscle or bone mass, indicating that the lack of 5 $\alpha$ -reduction of testosterone separates the prostate from muscle or bone effects [Wright et al., 1999]. More importantly, 5 $\alpha$ -reductase is expressed in high levels in prostate, but at very low levels in bone or muscle, which explains the significance of DHT in prostate and testosterone in muscle and bone function. As SARMs lack interaction with 5 $\alpha$ -reductase, this is considered a logical explanation for at least some of their tissue selectivity.

Another enzyme that plays a pivotal role in androgen metabolism is aromatase, the enzyme that converts testosterone to estradiol. This enzymatic reaction has been shown to be very critical for several physiological and pathological processes. Aromatase is ubiquitously expressed throughout the male reproductive tract, indicating that local conversion of testosterone to estradiol increases the prostate growth [Matzkin and Soloway, 1992; Tsugaya et al., 1996]. Estradiol increases the prostate size and predisposes men to prostate cancer. Testosterone, the aromatizable androgen, increases the prostate size, both through conversion to estradiol and DHT. SARMs cannot be aromatized, conferring all their effects to AR binding and not to metabolic conversion to active androgens/estrogens in prostate.

### Coregulator function

The functions of AR and its family members are dependent on the expression of associated proteins called coregulators. These coregulators do not bind to the DNA, but are recruited to the DNA by hormone bound receptors, enhancing (coactivators) or reducing (corepressor) the AR transactivation. In total, around 300 coregulators have been identified as belonging to several classes that play a pivotal role in AR function. Readers are referred to other excellent reviews on coregulators for more information [Smith and O'Malley, 2004].

AR differs from other receptors in its interactions with coregulators. The LBD of AR and other nuclear receptors have 12 anti-parallel helices that undergo significant rearrangement upon ligand binding, creating a shallow hydrophobic pocket containing LxxLL motif to facilitate association with coactivators [Heery et al., 1997; Shiau et al., 1998]. However, in AR most of the coactivators bind to a LxxLL motif in AF-1 domain and some bind to the LxxLL motifs in AF-2 domain [He et al., 2002a; Heinlein and Chang, 2002]. Activated AR also appears

to bind strongly and specifically to unusual FxxFF and FxxFM motifs in a subgroup of LBD-binding cofactors such as gelsolin and PAK6 [van de Wijngaart et al., 2006]. Moreover, there are several proteins that exclusively coactivate AR (ARA family of coactivators) and are not shared by other receptors [Fujimoto et al., 1999; Kang et al., 1999].

Conformational differences induced by SARMs lead to association and recruitment of different coregulator complexes [Chang and McDonnell, 2002]. Using combinatorial peptide-phage display, McDonnell and his colleagues showed that different ligands induce distinct AR and ER conformations leading to their association with different coactivator peptides [Chang et al., 1999; Chang and McDonnell, 2002]. The SARMs RTI-018 and RTI-001 possessed a spectrum of agonist activities and altered kinetics of response and these differences were attributed to SARM-mediated structural differences leading to the association of SARM-AR with coactivator peptides distinct from the DHT-AR complex [Kazmin et al., 2006].

Another study in support of the above conclusion was published recently by Rosenfeld and his colleagues [Baek et al., 2006]. This study was performed to provide a mechanism for the agonist effect of bicalutamide in the presence of an activated IL-8 pathway. As an antagonist, bicalutamide recruited corepressors NCoR and SMRT and as an agonist, in the presence of IL-8, the same ligand recruited p160 coactivators. This study also demonstrates that of the 3 LxxLL helices (LXD<sub>s</sub>) in the receptor interacting domain of SRC-1, DHT required LXD<sub>1</sub> and LXD<sub>2</sub>, whereas SARM-mediated action required LXD<sub>2</sub> and LXD<sub>3</sub> [McInerney et al., 1998].

Based on the above-provided and other literature information, SARMs possibly recruit coactivator complexes similar to DHT in anabolic tissues and corepressor complexes in androgenic tissues. Another possibility is that the SARMs, in androgenic tissues, might recruit a complex containing both coactivators and corepressors, leading to weaker agonist properties. All of the above-proposed mechanisms need to be addressed in appropriate animal models.

### Intracellular signaling cascades

The identity of the pathways impacted by androgen in a given cell is a function of both AR-dependent and AR-independent criteria. AR ligands affect different signaling pathways in different cells to elicit their effects. A classical example is that testosterone signals through inhibition of p38 MAPK, Notch-1, Notch-2 and Jagged-1 signaling pathways in macrophages. Whereas testosterone signals through activation of PI3K-Akt pathways in bone cells [Guo et al., 2004; Kang et al., 2004; Liu et al., 2006]. However, androgens did not inhibit p38 MAPK in bone cells, corroborating the fact that the same ligand impacts diverse pathways, depending on cell and tissue type, to mediate the physiological response [Huber et al., 2001].

Binding of ligand to AR leads to posttranslational modifications of receptors and their associated proteins, which occur in a pathway-specific manner. One of the important posttranslational modifications that plays a critical role in receptor and coregulator function is phosphorylation. Depending on the cell type, upon tamoxifen binding to ER, the receptor is specifically phosphorylated, which in turn alters the ligand and DNA binding functions of ER and coregulators [Likhite et al., 2006]. AR phosphorylation is also affected ligand-dependently and -independently through growth factor alteration, leading to divergent physiological responses [Dehm and Tindall, 2006].

The role of non-genomic effects (an evolving field of study) in androgen and estrogen signaling is still conflicting. Manolagas and his colleagues demonstrated that non-genomic signaling is important for the bone protective effects of androgens and estrogens, whereas genomic effects are critical for the development of sexual organs [Kousteni et al., 2001]. Separation of these two pathways by SARMs leads to increase in bone mass, with no effect on sexual organs. Testosterone and androstenedione, but not DHT and the synthetic androgen R1881, mediate non-genomic effects in mature *Xenopus laevis* oocytes. However, evidence from other laboratories has also implicated a role for this non-genomic signaling in the development and pathology of sexual organs. Lutz et al. suggested the development of SARMs that selectively impact non-genomic pathways could be used therapeutically for polycystic ovarian syndrome [Lutz et al., 2003]. More studies are required to evaluate the role of non-genomic signaling in androgen and estrogen biology.

Unlike the role of coactivators, several animal studies validated the role of genomic and non-genomic signaling in physiological responses and correlated with responses obtained with different ligands. However, all these validations were done with native ligands and hence have to be extended to SARMs.

## Conclusions

The AR has recently undergone a renaissance as a therapeutic target with the emergence of a new class of potential therapeutic agents (i.e., SARMs). Major milestones along the road include the discovery of nonsteroidal AR ligands (antiandrogens) in the 1970s with flutamide, improvement of the pharmacokinetic characteristics resulting in bicalutamide in the 1980s, and the conversion of antagonist to agonist templates in the late 1990s serving as seminal events that defined the field of SARMs. These events served as a tremendous catalyst for the exploration of AR ligands with SARM activity; a field that expanded from two small groups (University of Tennessee Health Science Center and Ligand Pharmaceuticals, Inc.) to encompass many of the major players (BMS, GSK, J&J, Lilly, Merck, etc.) in the pharmaceutical industry.

Deeper analysis of the *in vivo* activity profiles achieved by SARMs suggests a promising outlook. AR is the only

target which concurrently addresses bone and muscle weakness, and the improved PK/PD profiles of SARMs, as presented herein relative to FDA-approved steroidal agonists, bodes well for this class as the next generation of androgen therapy. Also SARMs, as osteo- and myoanabolic agents, have the potential to achieve the status of anabolic-agent-of-choice for many conditions that only require osteo- or myoanabolic effects, since the (side) effect in the untreated tissue is beneficial and synergistic.

The relatively recent proof-of-principle clinical trials demonstrating potent anabolic effects in humans was a valuable observation that sets the stage for exploration of the many clinical applications of an agent with unprecedented osteo- and myoanabolic activity. The rapid advancement of the SARM field in terms of chemotype diversity, mechanistic understanding, and information gleaned from the multiple SARMs in the clinic will help to define the ideal pharmacologic profiles for various potential indications under investigation. Already, auspicious preliminary clinical reports suggest that SARMs are a class of promising preclinical and clinical candidates. As this trend continues, many of the clinical and regulatory challenges with SARMs will be addressed and overcome, and hopefully, the full potential of SARMs as a class of promising preclinical and clinical candidates will be realized. If the full potential that is embodied in this class is realized, SARMs will force a paradigm shift in the treatment of patients requiring anabolic therapy.

## Supplementary Material

**Supplementary File 1:** *In vivo* characterizations of tissue-selective androgen receptor modulators.

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