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2008 Medicinal Chemistry Division Award Address

Nonsteroidal Selective Androgen Receptor Modulators (SARMs): Dissociating the Anabolic and Androgenic Activities of the Androgen Receptor for Therapeutic Benefit

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1. Introduction

Interest in the development and therapeutic potential of nonsteroidal tissue-selective androgen receptor modulators (SARMs)[a] has increased dramatically within the past decade. Rapidly expanding knowledge of nuclear hormone receptor structure and function and successful proof-of-principle clinical trials with SARMs have revived an almost dormant search for improved androgens. This Award Address attempts to chronicle the landmark discoveries (with emphasis on our work), organize the SARM landscape into clinically relevant bins, and provide insight into the clinical prospects for SARMs.

1.1. Origins of Androgen Use. An early (1889) and unusual experiment in androgen therapy was performed by Charles Edouard Brown-Séquard, age 72.[1] He administered a testicular extract to himself and reported that he felt "increased vigor and capacity for work".[2] Despite retrospective suggestions that any effect was purely placebo, this report resulted in widespread use of testicular extracts throughout Europe and North America for several decades.[3] Attempts to isolate the active components of testicular extract failed until 1935 when testosterone (17β-hydroxy-4-androsten-3-one) was isolated from bull testes.[4] Shortly thereafter, its synthesis was reported.[5] In the same year, extracts of urine from males were shown to cause nitrogen retention, an indicator of anabolic metabolism.[6] Testosterone was the first anabolic androgen to be used clinically,[7] but its use is limited by its androgenicity and pharmacokinetic (PK) issues. [1]

In the latter half of the 20th century, the chemical scaffold of testosterone was modified extensively, producing many

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[a] Abbreviations: 5α-R, 5α-reductase; 3D-QSAR, three-dimensional quantitative structure−activity relationship; AAS, androgenic-anabolic steroids; AF2, activation function-2; ALT, alanine amino transferase; AR, androgen receptor; ARE, androgen receptor responsive element; ARKO, androgen receptor knockout; BMC, bone mineral content; BMD, bone mineral density; BMS, Bristol-Myers Squibb; BPH, benign prostatic hyperplasia; CETP, cholesteryl ester transfer protein; CL, clearance; CoA, coactivator; CPA, cyproterone acetate; COPD, chronic obstructive pulmonary disease; CSA, cross-sectional area; CVD, cardiovascular disease; DHT, 5α-dihydrotestosterone; DMPK, drug metabolism and pharmacokinetics; E_2, estradiol; EB, estrogen benzoate; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; FBM, fat body mass; FSH, follicle-stimulating hormone; $G_{\alpha\beta\gamma}$, heterotrimeric G-protein-coupled receptor; HPG, hypothalamic−pituitary−gonadal; HSPs, heat shock proteins; IGF-1, insulin-like growth factor-1; IOC, International Olympic Committee; IP_3, inositol triphosphate; iv, intravenous; JNK, Jun N-terminal kinase; JNJ, Johnson & Johnson; LA, levator ani; LBD, ligand binding domain; LBM, lean body mass; LH, luteinizing hormone; M2R, muscarinic receptor subtype 2; MAPK, mitogen-activated protein kinase; MEK, MAP/ERK kinase; MEKK, MAP/ERK kinase kinase; MS, mass spectrometry; N, Newton; NMJ, neuromuscular junction; P_0, peak tetanus pressure; p38, p38 MAPK; PD, pharmacodynamics; PI3K, phosphatidylinositide 3-kinase; PIC, preinitiation complex; PK, pharmacokinetics; PKC, protein kinase C; PLC, phospholipase C; pQCT, peripheral quantitative computed tomography; PR, progesterone receptor; PSA, prostate specific antigen; RBA, relative binding affinity; SAR, structure−activity relationship; SARM, selective androgen receptor modulator; SERM, selective estrogen receptor modulator; SHBG, steroid hormone binding globulin; Src kin, Src kinase; SV, seminal vesicles; TP, testosterone propionate; tP_t, twitch time to peak tension; $t_{1/2}R$, twitch recovery time; TRT, testosterone replacement therapy; VP, ventral prostate; V_{ss}, volume of distribution at steady state; wt, wild type.

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steroidal androgens.[7] Although some enhancements of the anabolic effects were obtained relative to testosterone,[8] it was never possible to adequately dissociate the anabolic and androgenic activities of these compounds, and they came to be referred to collectively as anabolic-androgenic steroids (AAS). Because of significant pharmacokinetic (PK) and pharmacodynamic (PD) problems associated with their use, many of the AAS have been withdrawn as licensed products.[7] Only relatively recently, as a result of the discovery of SARMs, has the potential of the androgens as anabolic agents been revisited.[9−11] This Award Address will focus on our discovery and development of the prototypical propionamide class of SARMs, the development of diverse SARM chemotypes in general, and the prospects of SARMs as the next generation of androgen therapy.

1.2. Testosterone Biosynthesis and Prohormone Status. The predominant circulating androgen, testosterone, is primarily (95%)[12] synthesized by the Leydig cells of the testes (males) and adrenal cortex (females and castrated males) under the control of the hypothalamic−pituitary−gonadal (HPG) axis. Testosterone is synthesized in response to luteinizing hormone (LH), which is secreted by the anterior pituitary gland. High levels of testosterone or exogenous androgens bind to androgen receptor (AR) in the CNS and exert feedback inhibition of testosterone synthesis.[13] Consequently, peripherally selective (i.e., no LH suppression), nonsteroidal androgens are needed for optimal androgen therapy.

During androgen therapy, it is critically important to maintain endogenous testosterone levels in order to allow the androgen to function as a prohormone. For instance, 5α-reductase amplifies the AR signal[14] in the external genitalia, prostate, and skin[15] via conversion of testosterone to 5α-dihydrotestosterone (DHT). Another prohormone action occurs via aromatase-dependent conversion of testosterone into 17β-estradiol (E_2, (8*R*,9*S*,13*S*,14*S*,17*S*)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthrene-3,17-diol), the most potent estrogen, providing the major source of estrogens in males (and an extragonadal source for females). Therefore, suppression of the HPG axis (i.e., LH suppression) by exogenous androgen administration has the potential to lower DHT and 17β-estradiol levels and result in hypoandrogenicity and bone resorption,[16] respectively.

Typically for anabolic indications, exogenous androgens that suppress LH (and FSH) and testosterone should be avoided. The AAS and testosterone replacement therapy[17] suppress LH and are substrates for 5α-reductase and aromatase, causing a variety of untoward side effects. Consequently, anabolic enrichment (e.g., significantly reduced prostatic liability) is not the only criterion for SARM status (steroidal or nonsteroidal).[7,18]

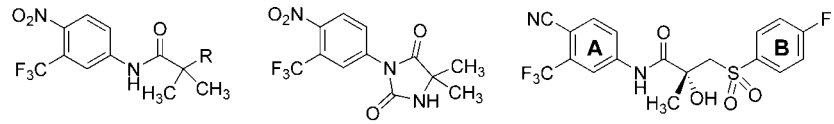
1.3. Targeting Myo- and Osteoanabolism. Regardless of its source or disposition, testosterone exerts its nonselective anabolic and androgenic effects via the AR. The levels of testosterone, DHT, and other androgens are highly influential in the extent of masculinization that occurs throughout life. These endogenous androgens are necessary to support gender dimorphic features, key among which are stronger muscles and bones which are derived via AR-dependent anabolic processes (i.e., myoanabolism and osteoanabolism, respectively). Most of the effort in developing the next generation of anabolic androgens has been directed toward in vivo anabolic selectivity. The term anabolic relates to the synthetic phase of metabolism that is characterized by the promotion of constructive processes. These hypertrophic processes at the tissue level are supported at the biochemical level by protein accretion processes such as nitrogen retention and net protein synthesis (i.e., protein anabolism), as well as utilization (or disposition) of calories from fat stores or blood glucose for synthetic metabolism rather than storage in adipose. Alternatively, anabolism can be measured at the whole body level as increased lean body mass (LBM) typically with concomitantly decreased fat body mass (FBM).

1.4. Androgen Assays. The SARM discovery paradigm, similar to other selective nuclear receptor modulator discovery, utilizes a battery of in vitro and in vivo tests but ultimately relies on phenotypic screening in vivo. In vitro binding affinity and transcriptional activation (trans-activation) assays serve as tools to profile ligands, but in vivo tissue-selectivity remains the scientific standard by which a SARM is defined.

1.4.1. Binding Affinity and in Vitro Trans-Activation. The ligand binding affinity to AR is often measured by competitive displacement of a radiolabeled high affinity ligand such as [^{3}H]mibolerone from AR. AR may be obtained from rat cytosol,[19] purified recombinant glutathione-*S*-transferase fused ligand binding domain,[20] or whole cells.[21] For useful comparison between protein sources, novel AR ligand binding affinity data are reported in terms of relative binding affinity (RBA), for instance, compared to DHT. Functional activity can be determined in vitro by transient transfection of expression vectors containing AR, an AR response element (ARE) tagged to a luciferase reporter, and a constitutively active internal control for normalization. Novel androgens are then classified by their ability to activate (agonist) or repress (antagonist, co-treated with DHT) AR-mediated in vitro transcriptional activation. If the novel androgen has high AR affinity and substantial in vitro agonist activity, then it may be considered a candidate for in vivo testing for tissue-selectivity, the hallmark of a SARM.

1.4.2. Hershberger in Vivo Assays. The Hershberger assay and variations thereof are the method-of-choice for indentifying for AR-dependent myoanabolic tissue-selective activity.[22] The definition of androgenic vs anabolic activity is derived from the Hershberger[23] assay. The foundational observation for the assay was that the levator ani (LA) muscle in rats atrophies rapidly in response to castration in immature rats and subsequently hypertrophies rapidly and robustly upon administration of exogenous androgens. This reflects anabolic activity not just in LA but throughout the musculoskeletal system and has also been correlated with protein anabolism (e.g., nitrogen retention), anabolism in other AR target tissues (e.g., increased RBC), and whole body (e.g., increases in LBM) anabolic activity. Optimal anabolic androgens demonstrate robust LA hypertrophy but only sparingly affect the weight(s) of androgenic tissues such as ventral prostate (VP) (i.e., the Hershberger et al. modification[23]) and seminal vesicles (SV) (the original androgenic comparator tissue) compared to vehicle-treated castrated rats. Hence, favorable compounds have a high myotrophic-androgenic dissociation index defined as [(experimental LA wt) − (vehicle-treated control LA wt)]/[(experimental VP wt) − (vehicle-treated control VP wt)] (normalized to body weight), which can be achieved via differential efficacy (E_{max}) and/or differential potency (ED_{50}), with the former being preferable as it would persist at all doses. For simplicity, this index is referred to herein as myotrophic index. Any enrichment of the LA/VP organ weight ratio compared to testosterone is indicative of tissue selectivity. Because of the higher concentration of AR in LA relative to other skeletal muscles in rat, the use of these assays to reflect anabolism was the source of some controversy early on. However, recent studies support LA hypertrophy as a predictive model for skeletal muscle anabolism and anabolic tone in general.[7,24]

Chart 1



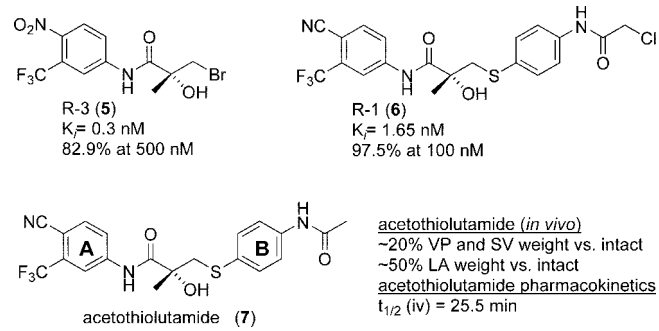
Often the results of these assays are reported in terms of the percent efficacy of test compound with regard to LA relative to intact control: [(weight of LA in treated castrated rats)/(weight of LA in vehicle-treated intact rats)] × 100. This intrinsically compares the exogenous androgen to endogenous androgenic tone and allows the rapid segregation of compounds into partial, full, or hyper (superagonist) myoanabolic categories based on <100%, ~100%, or >100% LA hypertrophy. For general screening purposes, meaningful results can be obtained within a short time frame (typically 14 days, but variations from 3 days to many months have been reported). Typically the Hershberger assay and modifications thereof are performed in maintenance (androgen treatment immediately after castration) mode to reflect the ability to maintain tissue weight. A more stringent alternative is the restorative mode (androgen treatment after a waiting period) which reflects the ability to regrow atrophied tissue. Importantly, CNS penetration can be assessed using the Hershberger assay by determining the levels of HPG-related serum hormones, particularly serum LH and testosterone, at or immediately prior to necropsy.

The simplicity of the Hershberger assay makes it easily amenable to analysis of many tissues in addition to LA and VP. For instance, other variations include coadministration with a reference androgen (i.e., testosterone propionate (TP) or DHT) to reflect antiandrogenic activity, which might be favorable in VP but not LA.[25] Osteoanabolic activity can also be observed in castrated or ovariectomized rats. However, it requires longer treatment times (often >4 weeks). Collection of bones from these rats allows analysis for increases in bone mineral density (BMD), biomechanical strengthening, or histomorphometry changes indicative of osteoanabolic and/or antiresorptive activity.[26] A variant of the Hershberger assay in ovariectomized female rats is used to analyze uterotrophic effects. Uterine hypertrophy is considered by some as an indication of a deleterious virilizing influence exerted by the androgen which can provide insight into the potential for use of a SARM candidate in women.[27] However, differences in androgen biosynthesis between species and the predominant influence of androgens on myometrial as opposed to endometrial cells confound the predictive ability of this measure.

1.5. Nonsteroidal AR Ligands. Although endogenous androgens are androstane (i.e., steroidal) derivatives, numerous classes of nonsteroidal AR ligands have been discovered, including the antiandrogens and nonsteroidal agonists reported below.

1.5.1. The Antiandrogens. Androgen receptor antagonists, also referred to as antiandrogens or "pure" antagonists, are compounds that competitively antagonize the biological responses of androgens in all tissues. Antiandrogens can be steroidal, such as cyproterone acetate ((2a*R*,3a*S*,3b*S*,3c*S*,5a*S*,6*R*,8a*S*,8b*R*)-6-acetyl-10-chloro-3b,5a-dimethyl-2-oxo-2,2a,3,3a,3b,3c,4,5,5a,6,7,8,8a,8b-tetradecahydrocyclopenta[*a*]cyclopropa[*g*]phenanthren-6-yl acetate).[28] However, clinically relevant antiandrogens currently are nonsteroidal anilide derivatives. Antiandrogens used for prostate cancer include the monoarylpropionamide flutamide (**1**) (a prodrug of hydroxyflutamide (**2**)),[29−31] the hydantoin nilutamide (**3**),[32−34] and the diarylpropionamide bicalutamide (**4**) (Chart 1).[35−37]

Chart 2



Compound **4**, launched as Casodex in 1995, is considered a second generation nonsteroidal antiandrogen that replaces one of the methyl groups in **2** with a 4-fluorophenylsulfonyl ring (denoted as the B-ring in this manuscript). Binding affinity was enhanced 2- to 4-fold higher relative to **1** and **3**,[38] while hepatotoxicity was decreased because of reduced amide hydrolysis afforded by B-ring addition. Further, the half-life was increased ($t_{1/2}$ = 140 h in man) because of the elimination of the bioreductively active NO_2.[30,39−43] Cumulatively, this produced much more efficient androgen blockage.[35,38,41,44] These advantages of **4** over the other antiandrogens in terms of pharmacokinetic and pharmacodynamic profiles suggested that diarylpropionamides, hereafter referred to as propionamides, were an attractive starting point for further exploration of nonsteroidal AR ligands.

1.5.2. Nonsteroidal AR Agonists. The Propionamides. Our group at the University of Tennessee Health Science Center (UTHSC) College of Pharmacy collaborated in the 1990s on the development of nonsteroidal AR ligands intended to irreversibly alkylate the AR and thereby permanently inactivate AR-dependent growth in late-stage prostate cancer.[19,45,46] As part of this project, the enantioselective binding of **4** was elucidated, demonstrating a 30-fold *R*-stereoselectivity[47] (in agreement with earlier in vivo work from Tucker et al.)[48] and refuting arguments that the *S*-isomer would have activity if it did not undergo extensive first pass metabolism.[49] This stereoselective binding and metabolism suggested that propionamide explorations using asymmetric synthesis would afford many advantages over racemic mixtures.

En route to putative AR irreversible inhibitors, a novel observation was made that certain propionamide synthetic intermediates (R-3 (**5**))[9] and thioether variants (R-1 (**6**))[9] possessed substantial AR agonist efficacy albeit at low potency during in vitro transactivation assays (Chart 2).[9] This observation was unexpected and more importantly unprecedented, and the implications of AR agonism in a synthetically amenable nonsteroidal template were recognized (e.g., concurrent osteo- and myoanabolism but without the virilizing and feminizing side effects of 5α-reductase or aromatase substrates, respectively).

Chart 3

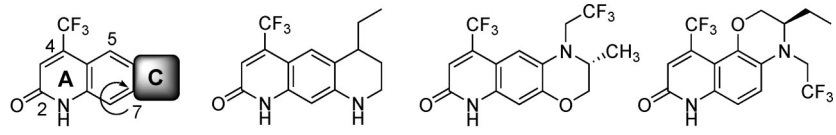
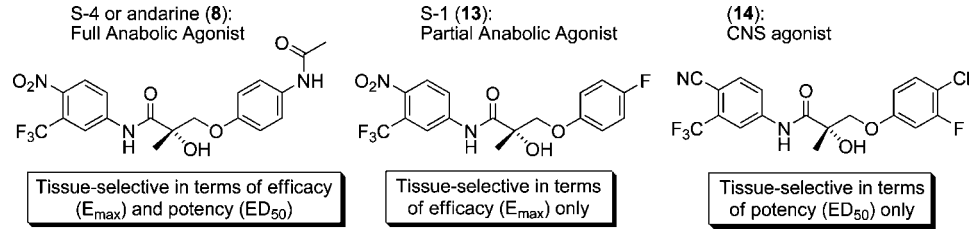


Chart 4



Further it was recognized that most nonsteroidal estrogens were tissue-selective, and thus, this was likely true for these nonsteroidal androgens. But alas no significant in vivo activity was observed with the thioether propionamides (data not shown) owing to hepatic metabolism and poor tissue exposure.[50]

Efforts to expand androgen use in a fashion parallel to estrogens vis-à-vis SERMs were immediately initiated. For instance, B-ring reactivity was eliminated (e.g., replaced *p*-chloroacetamide with *p*-acetamide), sites of potential aryl hydroxylation were blocked (e.g., halogenated B-ring), and AR binding was improved (e.g., methyl converted to CF_3)[51,52] but to no avail in terms of improving the in vivo activity.[50] The in vitro SAR led us to acetothiolutamide (**7**),[50] a potent in vitro agonist that lacked commensurate in vivo activity (Chart 2).[50] Mass spectrometry (MS) studies of the metabolites from **7** in vivo demonstrated that the main point of metabolic lability was the heteroatom linked to the B-ring, namely, the thioether that was rapidly converted to the sulfoxides and sulfone such as present in **4**, a pure antagonist.[50] We too have observed antagonist activity in our SAR studies with linker sulfoxides[53] and sulfones.[51]

So the question became how do we replace the thioether with an intrinsically nonoxidizable heteroatom? The idea of an ether seemed plausible and convenient, although there were some concerns of possible ether cleavage. These fears were limited because the phenol produced by this cleavage would be acetaminophen (i.e., B-ring *p*-acetamide), a well characterized molecule and a relatively innocuous metabolite. Thus, melding this ether to the A-ring/chiral center of **7** seemed like a logical extension of our thioether SAR. The resulting compound known in the literature as S-4[54,55] and in press releases as andarine (**8**, *S*-3-(4-acetylaminophenoxy)-2-hydroxy-2-methyl-*N*-(4-nitro-3-trifluoromethylphenyl)propionamide) (discussed below) was shown to possess SARM activity and allowed the pharmacodynamic exploration of this novel class of drugs, as discussed in section 2.[54,55]

1.5.3. Nonsteroidal AR Agonists. The Quinolinones. Ligand Pharmaceuticals, Inc., was also an early leader in nonsteroidal agonists with their series of bi-, tri- or tetracyclic quinolinones of general formula **9**[20,56−62] (Chart 3, where the boxed C indicates an appended ring). Initially these nonsteroidal ligands were also antagonists (not shown).[20,60,63] However, in the late 1990s, they reported several templates with in vitro agonist activity.[56,60,64] The quinolinone A/B-ring with a 4-CF_3 and 3,4-unsaturation[56,57] was a conserved feature. Their SAR studies demonstrated that the B/C ring fusion geometry (i.e., 6,7- or 5,6-), C/D ring heteroatoms (piperidino or oxazino), and C/D ring alkylation patterns[60] were important for potent agonist activity (Chart 3).[58,64] For instance, the C-ring piperidine LGD121071 (**10**)[64] (Chart 3), was a high affinity, potent full agonist in cotransfection assays ($EC_{50} = 3$ nM, 107%).[64] Also, C-ring morpholines (aka oxazinoquinolinones) such as **11** and **12** demonstrated in vitro agonist activity[56,60−62,65] and were recently revealed as tissue-selective in vivo (i.e., SARMs), as discussed below.[59,65,66]

2. Dissociating Anabolic and Androgenic Activities of the AR. The Propionamides

Concurrent successful marketing of SERMs and demonstrations of nonsteroidal androgens[9,56,57,64] raised hopes of developing the AR counterpart of SERMs (i.e., SARMs). After the nearly simultaneous reporting by our group and Ligand Pharmaceuticals of the first nonsteroidal AR agonists in early 1998,[9,57] Negro-Vilar of Ligand Pharmaceuticals, Inc., published a "Commentary" article in which he outlined the desirable characteristics of candidate SARMs and suggested that tissue selectivity could be achieved for AR, as it had been for ER and other nuclear receptors.[10] In his opinion, "androgen therapy was about to experience a fundamental change, both in extent of use and in the range of applications".[10] It is now apparent that the SARMs, whose discovery was imminent then, have diverse pharmacology ratios across different tissues (i.e., distinct intrinsic full and partial agonist profiles), allowing segregation into distinct therapeutic bins.

We and others have subsequently discovered and reported SARM compounds of at least three distinct pharmacological ratios: (1) anabolic agonists with full efficacy in bone/muscle as will be exemplied by **8** (i.e., high E_{max}[LA]) (Chart 4); (2) anabolic agonists with partial efficacy in bone/muscle but with even further reduced sexual accessory tissue efficacy or potency as will be exemplied by S-1[67] (**13**) (i.e., low E_{max}[VP]) (Chart 4); and (3) central agonists (e.g., low ED_{50}[HPG]) that retain peripheral anabolic agonism as will be exemplied by **14**[68] (Chart 4). The preclinical characterizations of **8**, **13**, and **14** will be discussed in some detail and serve as examples that typify the different therapeutic bins for SARMs. Subsequently, representative examples of diverse chemotypes will be similarly segregated and briefly discussed (section 3). The existence of these variables between SARM compounds expands the gamut of putative clinical applications available to SARMs.

Table 1. Pharmacokinetics and Pharmacodynamics of Propionamides in Rats

	8, full anabolic agonist	**13**, partial anabolic agonist	**14**, CNS agonist
	Pharmacokinetics		
iv CL (mL min^{-1} kg^{-1})	1.0−2.1	3.6−5.2	0.87
iv V_{ss} (L/kg)	0.42−0.48	1.5	0.66
terminal $t_{1/2}$ (h)	2.6−5.3	3.6−5.2	11.9
% *F* (rat)	100% (rat), 91% (dog)	55−60	96
	HPG Axis		
	suppresses LH at high doses (>0.5 mg/d)	suppresses LH and FSH by 80% and 30% at >0.5 mg/d	0.1 mg/kg reduces LH >50%[b]
	Pharmacodynamics		
K_i (nM)	4	6.1	1.7
in vitro functional assay	93%	43%	96%
cross-reactivity	none	none	none
	Myoanabolism in Hershberger (14 Day Maintenance) and Strength Assays		
E_{max}[LA]	101% (104% for TP)	74.3%	139%
ED_{50}[VP] (mg/d)	0.43 (0.13 for TP)	0.42	0.43
E_{max}[VP]	35.2% (120% for TP)	14.9%	138%
ED_{50}[SV] (mg/d)	0.55 (0.12 for TP)	0.38	0.41
E_{max}[SV]	28.5% (70% for TP)	13.4%	144%
	Restored Soleus Muscle Strength (P_0/CSA)		
	156 kN/m^2 (10 mg/kg S-4) vs 83 kN/m^2 (vehicle)	n/a	n/a
	Osteoanabolism		
osteocalcin	70% of pretreatment levels	n/a	n/a
$BMD_{whole\ body,femoral}$	see below[a]	n/a	increased (whole body)
biomechanical strength	maintained in ovariectomized females	n/a	n/a
	Whole Body Anabolic Effects		
8 maintained and restored favorable body composition in hormone-ablated male and female rats			anabolic changes in LBM and FBM

[a] Maintained BMD in hormone ablated male or female rats; partially restored whole body BMD in castrated rats. [b] In combination with estrogen benzoate to support sexual activity, **14** suppressed sperm count and attained hormonal male contraception at 0.1 mg/d in rats. See text for details.

2.1. 8, a Full Efficacy Anabolic SARM. As discussed above, SAR-guided structural modifications of the propionamide template led to a series of thioethers with surprising agonist activity in vitro but disappointing in vivo activity. The lead thioether compound **7** was converted to an ether, **8** (Chart 4), which was intrinsically not oxidizable, producing a PK profile consistent with Negro-Vilar's "ideal" SARM,[10] as described below.

2.1.1. "Ideal" Pharmacokinetics (PK) of 8. The ideal anabolic SARM was defined by Negro-Vilar[10] as an orally active agent with once daily dosing and anabolic effects on muscle and bone, but no or lesser activity in the prostate. Kearbey et al.[69] demonstrated that the iv (intravenous) and oral PK profile of **8** in rats (Table 1) was dramatically improved compared to acetothiolutamide (**7**) (Chart 2).[50] **8** had a reasonable iv clearance (CL) which ranged between 1.0 and 2.1 mL min^{-1} kg^{-1} (varied with dose),[69] suggesting that this ether was not rapidly degraded or excreted relative to previous thioethers. Further, the volume of distribution at steady state (V_{ss}) value of 0.42 and 0.48 L kg^{-1} in all treatment groups[69] was consistent with distribution of the drug to the peripheral tissues without deposition into the fat. This produced an iv half-life that was consistent with once daily dosing (between 2.6 and 5.3 h in rats, Table 1) which was greater than 6-fold longer than **7**.[69] Importantly, **8** was completely orally bioavailable (% *F*) (Table 1) at pharmacologically active doses (% $F = 100\%$ at 10 mg/kg) in rats.[69] Perera et al. found that the oral bioavailability was maintained in dogs (91%), despite some species differences in metabolic pathways. For example, dogs demonstrated B-ring deacetylation vs amide hydrolysis in rat as the major path.[70,71] All these PK improvements also came with retained AR affinity and improved in vitro potency (Table 1) relative to thioethers (Chart 2).[50,55] As a result, tissue exposure to this compound was radically improved, revealing the in vivo pharmacodynamic character of **8** and SARMs in general for the first time.[54,55]

2.1.2. Peripheral Selectivity of 8. The target population for SARMs will generally be intact (i.e., not castrated) adults or postmenopausal women. As such, maintaining the endogenous androgenic and estrogenic tone by not suppressing the HPG axis is crucially important. Yin et al.[50] examined the effects of **8** on serum LH and FSH levels to investigate whether **8** acts as an AR agonist in the central nervous system (i.e., HPG suppression detected as LH and FSH suppression). As expected, castrated rats had a significant elevation in plasma LH and FSH levels compared with intact controls. **8** partially suppressed LH and FSH production but only at supratherapeutic doses (i.e., >0.5 mg/day vs ED_{50}[LA] = 0.14 mg/d) (Table 1), suggesting anabolic effects can be exerted without suppressing the HPG axis. Interestingly, FSH was less sensitive to suppression by **8** than LH, an observation also seen with other SARMs.

2.1.3. Tissue-Selective Maintenance and Restoration of Myoanabolism by 8. Yin et al. reported **8** as a high affinity ($K_i = 4$ nM) and potent in vitro agonist (10 nM **8** demonstrated 93% of the transactivation activity of DHT at 1 nM).[55] Unlike testosterone (TP (testosterone propionate) in Table 1), **8** demonstrated weak partial agonism in VP and SV but potent full agonism in LA muscle[55] in maintenance Hershberger assays. This produced the long-sought anabolic tissue selectivity in terms of efficacy (E_{max}[LA/VP or SV] ratio of >1) and potency (ED_{50}[LA/VP or SV] ratio of <1) (Table 1) establishing **8** as a promising preclinical SARM with unprecedentedly favorable myotrophic index in a nonsteroidal compound. Further, **8** (3 mg/kg) fully restored LA to 100% compared to 41% for vehicle in a protocol involving a 12 week atrophy followed by 8 weeks of treatment (**8**, DHT, or vehicle) while retaining tissue

selectivity (20% VP weight for **8** (3 mg/kg) vs ~200% for DHT (3 mg/kg) and 3.6% for vehicle) (data not shown).[72]

2.1.4. Anabolic Effects on Muscle Strength. Gao et al. extended the investigation of myoanabolic effects of **8** to muscle strength effects using rat soleus muscle as a model.[72] Rats were castrated and allowed to atrophy for 12 weeks, then treated for 8 weeks with **8**, DHT, or vehicle via subcutaneous injection. Muscle strength restoration was then measured using the soleus muscle isolated from sacrificed rats. As expected, total body weight of the castrated rats treated with vehicle decreased. The soleus muscle, an antigravitational muscle of the hind limb, also atrophied in a commensurate manner in vehicle-treated animals. The kinetics and strength of the soleus muscle contraction were investigated using the waveform descriptors for single twitch and tetanic contractions. The primary metric for soleus strength was the peak tetanus pressure (P_0) in newtons (N), which was normalized to muscle cross-sectional area (CSA in Table 1). Castration produced statistically significant decreases in P_0/CSA (83 kN/m^2 (castrated) vs 124 kN/m^2 (vehicle treated intact control)). **8** and DHT restored soleus muscle strength to intact or better levels, despite only partial recovery in muscle mass (120 and 156 kN/m^2 for 3 and 10 mg/kg of **8** vs 138 kN/m^2 for 3 mg/kg of DHT). The twitch waveform was largely unaffected by treatment group (i.e., unchanged twitch time to peak tension (tP_t) and twitch recovery time ($t_{1/2}R$)), indicating that the kinetics of contraction were not affected. The results demonstrated that **8** exerts an anabolic influence on skeletal muscle strength and suggested that myoanabolic effects extend beyond simple hypertrophy of an arguably androgenic muscle (LA) to metrics of musculoskeletal performance. The data also support the argument that the LA weight (i.e., hypertrophy) is reflective of anabolic effects in skeletal muscle, addressing the concerns of some critics of the field. The mechanism(s) by which androgens produce these anabolic changes in skeletal muscle strength without hypertrophy is poorly understood and remains a topic of active investigation[73] but may be due to remodeling of the neuromuscular junction (NMJ) or modulation of growth hormone signaling.[72] Cumulatively the myoanabolic effects of **8** suggest that SARMs can be used in muscle wasting disorders (i.e., sarcopenia, discussed in detail in section 4.4) of diverse origins such as amyotrophic lateral sclerosis and cystic fibrosis (i.e., NMJ diseases), sports and burn injuries (i.e., trauma), cancer cachexia and HIV wasting (i.e., hypercatabolic diseases), and geriatric frailty (i.e., disuse and senescence).

2.1.5. Osteoanabolic Effects of 8. Gao et al. also investigated the effect of **8** and DHT on markers of bone turnover in castrated rats.[72] After 20 weeks of androgen deprivation, osteocalcin levels in castrated rats were similar to those observed in intact rats, indicating that castration-induced bone turnover had reached equilibrium. **8** or DHT reduced osteocalcin levels to 70% (Table 1) and 50% of this level, respectively, suggesting that they exert an antiresorptive mechanism effect on bone turnover (i.e., inhibition of osteoclast activity).[72] Whole body bone mineral density (BMD) and bone mineral content (BMC), global indicators of the activity in the skeleton, measured at 12 weeks of androgen deprivation were significantly reduced. Interestingly, **8** (3 or 10 mg/kg for 8 weeks), but not DHT, partially restored BMD and BMC relative to vehicle-treated castrated rats, suggesting bone protective effects with **8** but not DHT (Table 1).

Treatment of female rats with **8** also demonstrated bone maintenance in terms of BMD and bone strength.[74] In ovariectomized rats 120 days after surgery, the whole body BMD was significantly lower than that observed in intact controls (0.197 vs 0.215 g/cm^2).[74] **8** treatment for 120 days prevented the loss of whole body and lumbar (L5−L6) vertebrae BMD in ovariectomized rats. In intact rats, **8** maintained BMD at the same level as vehicle-treated intact controls, while DHT caused a significant decrease in BMD in intact rats as determined by DEXA. Regional analysis of BMD (pQCT) showed increased cortical thickness, cortical content, and cortical (and trabecular) BMD in ovariectomized rats, in some cases in excess of intact controls.[74] Consistent with these results, **8** maintained biomechanical strength in ovariectomized rats.[74] Thus, **8** prevents bone loss and improves bone quality in this postmenopausal model of osteoporosis. In vitro experiments with bone marrow osteoprogenitor cells suggested that **8** and DHT dose-dependently increased differentiation toward the osteoblast lineage and inhibited osteoclastogenesis, possibly explaining the presence of both osteoanabolic and antiresorptive effects of **8**.[74] Recent evidence suggests that SARMs can be combined with antiresorptive agents currently marketed to produce additive skeletal protective effects.[26]

Cumulatively, these results suggest that SARMs have great potential for the treatment of osteoporosis in both men and women alone or in combination with conventional antiresorptive therapies. In addition to direct bone effects, SARMs such as **8** would be expected to reduce fracture incidence via reduced falls secondary to musculoskeletal performance enhancement. Hence, the geriatric frail may benefit from **8** treatment by lowering the incidence of disability.

2.1.6. Whole Body (i.e., Protein Anabolic) Anabolic Effects of 8. Kearbey et al.[74] studied the effects of **8** on body weight and body composition in female rats. **8** in ovariectomized rats maintained total body weight and dose-dependently improved body composition. It decreased the percentage of fat body mass (FBM) and increased the percentage of lean body mass (LBM). The highest dose (3 mg/d for 120 days) returned these body composition values to intact control values despite increased total body weight. In intact female rats, **8** (1 mg/d) also significantly improved body composition but decreased total body weight. In castrated rats, Gao et al.[72] demonstrated that **8** (3 and 10 mg/kg) was able to fully *restore* anabolic growth, returning changes in total body mass, LBM, and FBM back to intact control levels.

2.1.7. 8, the Prototypical Full Efficacy SARM. 8 was a SARM that served as the predominant model compound early in the development of the SARM field. Many of the landmark studies with **8** served as proofs-of-concept in the SARM field (e.g., concomitant myo- and osteoanabolism in the absence of VP proliferation, musculoskeletal performance enhancement, etc.). Preclinical characterization of **8** demonstrated high binding affinity for AR (K_i = 4 nM) and ideal pharmacokinetics (complete oral bioavailability, plasma half-life consistent with daily oral dosing in rats and dogs) with no cross-reactivity with the other nuclear receptors. Myoanabolism was demonstrated in terms of maintenance and restoration of LA weight and restoration of soleus muscle strength in castrated rats. Likewise, osteoanabolism was observed in maintenance and restorative modes in male and female rats with improvements in biomechanical strength, cumulatively demonstrating musculoskeletal performance enhancement. The anabolic effects were also observed at the level of the entire organism as revealed by favorable body composition changes. Importantly, these anabolic effects were tissue-selective when compared to androgenic tissue and HPG axis effects, establishing **8** as a prototypical preclinical SARM. The peripheral and selective anabolic preclinical pharmacodynamic profile of **8** seemed highly promising and

stimulated us to pursue landmark clinical trials of the SARMs, andarine **8** and Ostarine.[75] Although phase I studies with **8** were successful with no deficiencies noted (March 17, 2004, press release), Ostarine was selected for advanced clinical development based on corporate strategy. Readers are cautioned to note that the name Ostarine is often mistakenly linked to the chemical structure of **8**, which is also known as andarine. The chemical structure of Ostarine has not been publicly disclosed. The authors are unable to provide additional information.

Collectively, these preclinical and clinical studies have provided the foundation for the massive body of SARM characterizations that are now published and patented (discussed below). Importantly, many of these pharmacodynamic observations have proven to be typical of subsequently published chemodiverse SARMs, as discussed in section 3.

2.1.8. Clinical Implications of 8 Discovery. Cumulatively, these data are consistent with the anabolic subset of AR activities (osteo- and myoanabolic, protein anabolism, etc.) as has been observed with testosterone therapy but without the major concern of increasing risk of benign prostatic hypertrophy, prostate cancer, acne, or virilization of women. Fifty percent of men older than 50 years of age harbor occult prostate cancers that are known to be reactivated by exogenous androgens. Hence, elimination of this prostate liability represents a quantum leap in androgen therapy.

The ability of SARMs to increase LBM and decrease FBM would be beneficial to an elderly population. Aging in all species proceeds with the loss of muscle and its replacement with fat and fibrous tissue.[76] Correspondingly, sarcopenia incidence increases with age but accelerates in the elderly (~70+ years old), as discussed further in section 4.4. Common detrimental influences on myoanabolism in the elderly include decreased appetite, decreased protein intake (lower ghrelin response), peripheral insulin resistance, decreased anabolic hormones (IGF-1, growth hormone, insulin, testosterone), weakness, fear of falling, reduced activity, depression, hospitalization, etc.[77] SARM therapy, alone or in conjunction with resistance training and increased dietary protein,[76] may attenuate many of these detrimental influences and improve the quality of life of the sarcopenic elderly. Younger patrons could also benefit from the increased muscle mass reflected by LBM increases, as muscle is a component of blood glucose disposal and energy expenditure. Hence, sarcopenic obese and glucose intolerant populations (i.e., metabolic X syndrome and diabetics) may benefit from improved disposition of dietary calories, an observation supported by clinical trials (unpublished data).[75]

2.2. 13, a Partial Efficacy SARM. Another early SARM characterized in our laboratories was **13**. **13** is a close analogue to **8** that only differs at the para B-ring position (*p*-fluoro instead of *p*-acetamido) (Chart 4). This slight structural difference produced pharmacokinetics comparable to **8** (Table 1) but significantly reduces its AR agonist efficacy in vitro and in vivo (E_{max}[LA] = 74.3% vs 101% for **8**) (Table 1). However, in vivo tissue selectivity was maintained or slightly increased albeit with partial agonist E_{max} in anabolic tissues (E_{max}[LA] and E_{max}[VP] tend to trend together, so many low E_{max}[VP] compounds are also partial myoanabolic agonists). Importantly, **13** has similar agonist properties as **8** in the pituitary (i.e., maintained peripheral selectivity).

This low E_{max}[VP] profile represents a second therapeutic bin that may allow therapeutic antagonism of androgenic tissues (as revealed by Hershberger assays in the presence of an agonist) while maintaining anabolic tissues in intact animals. For instance, this selective prostatic antagonism would be therapeutic in common prostatic diseases such as benign prostatic hyperplasia (BPH) or prostate neoplasms. Alternatively, this profile allows the use of **13** and similar compounds in anabolic indications similar to **8** (i.e., sarcopenia, osteopenia, or combinations thereof) but with reduced prostatic liability. This approach may require higher doses to produce comparable anabolic effects, and hence, these SARMs would require very clean pharmacokinetic and side effect profiles.

The reduced E_{max}[VP] (Table 1) led to the investigation of **13** in intact rats with regard to potential to treat BPH. **13** was compared to two common BPH treatments, **2** (an antiandrogen) and finasteride (a 5α-reductase type II inhibitor).[78] All three agents produced similar reductions in VP weight, suggesting similar efficacy in BPH. However, only **13** did not increase (or decrease) the T, LH, or FSH levels after 9 days of treatment at therapeutic doses. **2** elevated all three, suggesting central AR antagonism and finasteride elevated testosterone (i.e., accumulation of substrate). Additionally, **2** also decreased LA weight, demonstrating a detrimental antianabolic activity. Consequently SARMs with reduced prostate efficacy (i.e., E_{max}[VP] values in castrated rats comparable to vehicle treated castrated rats) could be candidates for the treatment of BPH with approximately similar effectiveness to approved agents but reduced side effects (i.e., no androgen withdrawal-related hot flashes induced by **2** or estrogen-related prostatic stromal growth or gynecomastia induced by finasteride).

2.3. 14, an Androgenic SARM. Certain SARMs do not achieve peripheral selectivity, while others such as **14** maintain anabolic selectivity (in terms of potency in this case; Chart 4, Table 1) but can be dosed above their ED_{50} for HPG suppression.[68] In both cases, LH and FSH levels fall, reducing testosterone and sperm production, respectively. The reduced intratesticular testosterone causes low testicular tissue weight as well as reduced spermatogenic capacity relative to vehicle-treated intact controls. Correspondingly, **14** in combination with a low level of estradiol benzoate (EB, 5 μg/d, necessary to maintain normal sexual behavior in rats) was recently characterized for its ability to induce infertility in intact male rats. This level of EB alone was sufficient to suppress LH but did not reduce testes weight (94% vehicle) nor induce infertility (12/12 pregnancies in mating trials), but anabolic (LA) and androgenic (SV, VP) weights were reduced. Addition of **14** (0.05 to 0.75 mg/d) to EB further suppressed the HPG, resulting in decreased testicular weights. However, **14** caused dose-dependent increases in anabolic tissues (e.g., LA and whole body LBM). As expected, the testicular/spermatogenic antagonism was biphasic with the zenith at 0.1 mg/d of **14** (i.e., higher testicular weights at 0.05 and ≥0.3 mg/d). Immediately after the treatment period of 10 weeks, the 0.1 mg/d group demonstrated azoospermia and infertility in mating trials (0/12 pregnancies) whereas 0.05, 0.3, and 0.5 mg/d produced unacceptable failure rates (each producing 2/12 pregnancies). At high dose (0.75 mg/d), **14** is capable of supporting spermatogenesis and fertility (6/12 pregnancies). This can be rationalized as the initial suppression of endogenous testosterone production with commensurate tissue weight losses (i.e., an indirect antagonist effect at 0.1 mg/d of **14**), giving way to direct **14** agonist effects in the testes at higher doses.

Although the therapeutic window in rats was narrow, the addition of progestin or HPG hormones to rapidly suppress LH and FSH may broaden the therapeutic window in humans. Nonetheless, the demonstration of an orally bioavailable (Table 1) nonsteroidal androgen that can reversibly (all treatment groups were completely fertile at 14 weeks after treatment, and all tissue

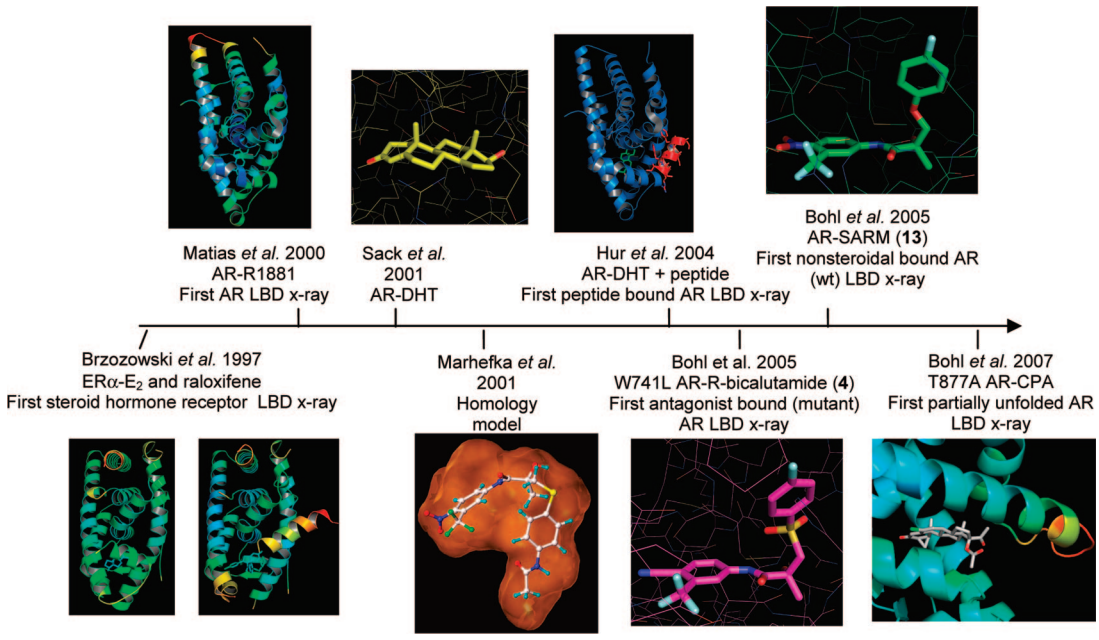


Figure 1. This AR ligand binding domain (LBD) X-ray crystallography timeline chronicles the development of the AR structural biology field. Within the past decade, numerous crystal structures of the AR LBD have been reported elucidating ligand binding conformations and progressing knowledge of AR structure and function. Brzozowski et al.[85] in 1997 used ERα structures to establish the importance of helix 12 position with regard to ligand pharmacology. In 2000 and 2001, Matias et al.[83] and Sack et al.[84] reported the first AR LBD crystal structures that were steroid-bound (R1881 and DHT, respectively) agonist conformations that supported the earlier ERα work. Hur et al. and He et al. reported crystals that helped identify how F and W rich motifs interact with the AF2.[86,87] Prior to these crystal structures, our laboratories formulated[79] and utilized[80] an AR homology model to understand the propionamide SAR. We later discovered through AR cocrystallography with **13** that such molecules expand the pocket to accommodate the B-ring by displacing W741[81] (homology model predicted M780 displacement). Our laboratories have also crystallized a number of clinically approved antiandrogens bound to resistance-conferring mutants (i.e., antiandrogens act as agonists) of AR. These included *R*-**4** in W741L[21] and cyproterone acetate (CPA) and **2** in T877A.[81,88] These structures all demonstrated the same overall global fold (i.e., agonist conformation) with no substantial differences in AR LBD conformation. An exception is CPA in which the C-terminal of helix 11 is partially unwound and helix 11−12 loop is disordered.

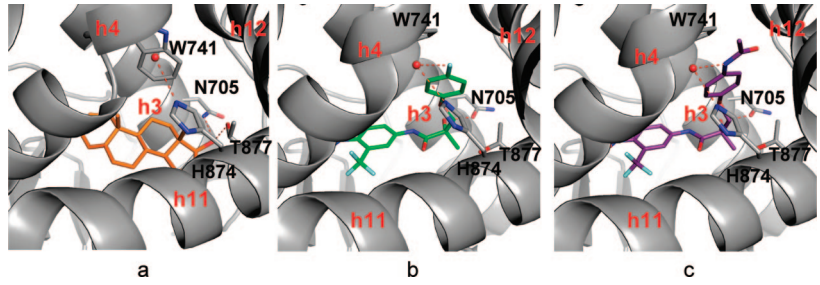


Figure 2. Comparison of DHT (PDB code 1i37), **13** (PDB code 2axa), and **8** (PDB code 3b68) binding conformations. (a) DHT forms a hydrogen bond with the helix 11 (h11) residue, T877. (b) Propionamides such as **13** and **8** occupy an additional region in the AR compared to steroids located between helices 4 and 12. The para fluorine on the B ring of **13** forms a weak hydrogen bond to H874 through a conserved water molecule but lacks hydrophilic interaction with T877. (c) The substitution of an acetamide on **8** for this fluorine results in stronger hydrogen bonding through the nitrogen atom.

weights except VP (79.4% for the 0.3 mg/d group) returned to pretreatment weights) induce infertility in intact male rats while maintaining muscle mass (Table 1) and anabolic activities of the AR is a substantial advance toward male contraception. These studies also demonstrate that SARMs with high E_{max}[VP] can be used to target androgenic tissues.

2.4. Structural Biology of Propionamides. The first crystal structures of the AR contained DHT in the ligand binding pocket. DHT forms tight hydrophobic interactions with the receptor, leaving very little unoccupied space. Attempts to dock propionamide SARMs into this structure were unsuccessful. This presented us with the problem of explaining how our large propionamides (relative to DHT) could bind to the AR. In order to address this issue, we formulated[79] an AR homology model (Figure 1) created from PR in which M780 was displaced, forming a B-ring pocket and providing an alignment rule for 3D-QSAR studies.[80] (As discussed below, we subsequently found that W741 and not M780 was displaced to accommodate the B-ring.)[81]

As outlined in Figure 1, our laboratories have resolved a number of AR LBD structures complexed with propionamide SARMs, revealing unique interactions for such nonsteroidal ligands compared with steroidal compounds.[21,82] Conserved interactions include the hydrogen bonds of the 3-keto group (DHT) to R752 and Q711 of AR as represented by the cyano or nitro group on the A-ring of propionamides. Also, the hydrogen bond between the hydroxyl group of steroidal agonists or propionamide SARMs and N705 is conserved (Figure 2).[21,81,83,84] The aromatic amine of the propionamide scaffold hydrogen-bonds to form a novel interaction with the carbonyl of L704 (Figure 2), which is not seen with steroidal AR ligands. Conversely, no hydrogen bond to T877 is formed with the

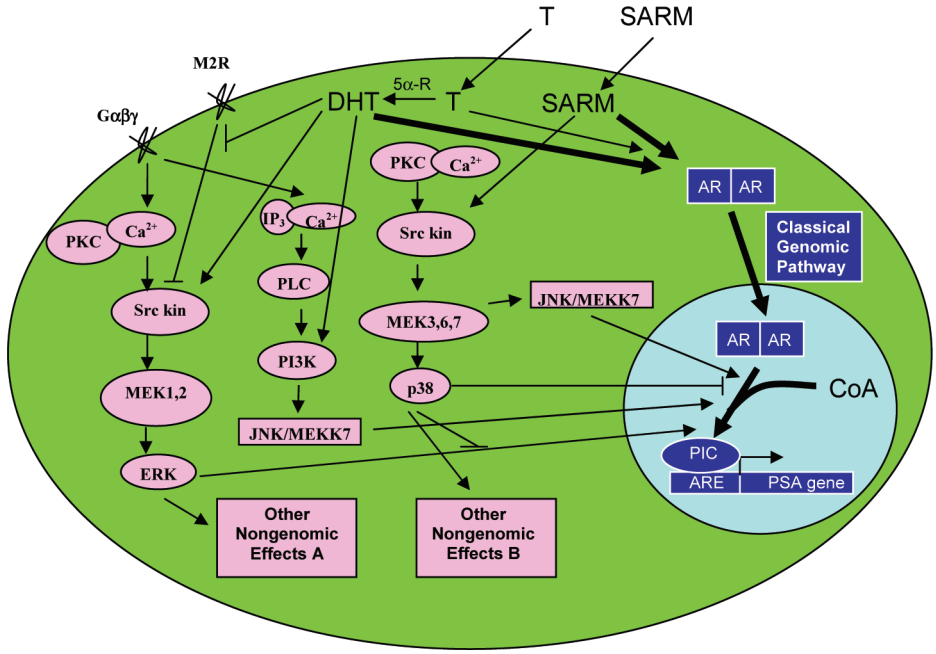


Figure 3. Differential intracellular signaling in prostate. This model is an extension of the published work by Narayanan et al.[89] in which small molecule inhibitors of a panel of kinases (and muscarinic agonists or antagonists) were used to identify the players in the intracellular signaling of DHT and a representative SARM, S-22[97] (*S*-3-(4-cyanophenoxy)-2-hydroxy-2-methyl-*N*-(4-cyano-3-trifluoromethylphenyl)propionamide). Cumulatively, these studies suggested that DHT and SARMs operate via distinct mitogen-activated protein kinase (MAPK) cascades and distinct upstream signaling pathways.[98] For instance, DHT activates the proliferative ERK and JNK MAPKs, whereas S-22 activates the antiproliferative p38 (and JNK) MAPKs. Coactivator and AR recruitment assays were performed in the presence of these kinase inhibitors, allowing the integration of the nongenomic and genomic effects of DHT vs S-22. The proliferative MAPKs downstream of DHT allowed coactivator and AR recruitment, whereas p38 prevented recruitment. Further, p38 attenuated ERK mediated recruitment. This model is consistent with phenotypic observations for SARMs vs DHT in which SARMs reduced prostate size in intact and castrated animals but needs to be validated in animal studies. Abbreviations: muscarinic receptor subtype 2 (M2R), heterotrimer G-protein-coupled receptor ($G_{\alpha\beta\gamma}$), testosterone (T), 5α-dihydrotestosterone (DHT), 5α-reductase (5α-R), protein kinase C (PKC), Src kinase (Src kin), MAP/ERK kinase (MEK), extracellular signal-regulated kinase (ERK), inositol triphosphate (IP_3), phospholipase C (PLC), phosphoinositide 3-kinase (PI3K), p38 (p38 MAPK), Jun N-terminal kinase (JNK), MAPK kinase kinase (MEKK), androgen receptor (AR), preinitiation complex (PIC), (ARE), and prostate-specific antigen (PSA).

propionamides, which is present upon DHT and R1881 binding. More interestingly, the propionamides adopt a bent conformation with the B- ring folding about 90° from the steroidal plane toward the AF2 by displacing W741. This ligand conformation allows for a hydrogen bond to a conserved water molecule that interacts with H874 of helix 11 and backbone residues of helixes 4 and 5.[81] Combining knowledge of the propionamide SARMs in the wild-type (wt) AR and *R*-bicalutamide (**4**) in the W741L AR shows that *R*-**4** binding to the wt AR would clearly put strain on helix 12 explaining its antagonist effects. Further we were able to rationalize the full (**8**) vs partial (**13**) efficacy of propionamides based on the presence of a more hydrophilic group on the para B-ring position.[82] DHT stabilizes the active AR LBD conformation via hydrogen bond with T877 on helix 11, whereas the fluorine of **13**[81,82] and the acetamide of **8** hydrogen-bond to H874 via a water molecule (Figure 2).[82]

2.5. Putative Tissue Selectivity Mechanism(s). The mechanism by which SARMs dissociate the anabolic and androgenic actions has been debated since the discovery of the first nonsteroidal androgens in 1998.[9,56] Some of the speculated mechanisms have been adapted from the field of SERMs such as the hypothesis of tissue-specific expression and function of coregulators. Others are based on the recent observation from our laboratories of tissue selective intracellular signaling for SARMs.[89] Lastly, the simple lack of SARM prohormone activity (i.e., not converted by 5α-reductase or aromatase) may confer some tissue selectivity. These mechanisms are not mutually exclusive and may all contribute to some extent. We will review these few mechanistic models here with emphasis on some recently published and unpublished results from our group. For detailed understanding of these mechanisms, the readers are referred to other reviews.[11,90,91]

2.5.1. Differential Cofactor Recruitment. AR is maintained in an inactive conformation in the cytoplasm by heat shock proteins (HSPs). Upon ligand binding, HSPs dissociate from AR, resulting in a series of conformational changes, leading to homodimerization and translocation to the nucleus. The receptor then binds to androgen response elements (AREs) located in the promoter or regulatory elements of AR target genes and recruits coregulators to facilitate the activation or repression of these genes. Earlier studies have shown that SARMs induce a conformational change distinct from DHT, thus recruiting different coregulator complexes.[92,93] Moreover, unpublished chromatin immunoprecipitation assay results from our group also indicate that SARMs recruit both coactivators (CoA) and corepressors to the PSA promoter in LNCaP prostate cancer cells. These results raise the possibility that SARMs and DHT recruit similar coactivator complexes in anabolic tissues but distinct multifarious complexes in androgenic tissues.[89] Accordingly, SARM vs DHT nucleated complexes demonstrate differential recruitment of members of the preinitiation complex (PIC) resulting in variable rates of transcriptional initiation.

2.5.2. Differential Intracellular Signaling. In addition to the genomic effects (i.e., effects requiring DNA binding and protein expression), AR ligands also mediate rapid effects that do not require DNA binding. These are classified as nongenomic effects.[94] Both the genomic and nongenomic effects are medi-

ated through the activation of intracellular signaling pathways. These signaling pathways regulate protein function through post-translational modifications of AR and its coregulators. Recent results from our laboratories indicate that SARMs mediate distinct genomic and nongenomic effects in cell lines derived from different tissues.[89] In bone cells, DHT and SARMs both activated proliferative extracellular signal-regulated kinase (ERK) signaling. However, in prostate cells, DHT stimulated ERK whereas SARMs activated the antiproliferative p38 mitogen-activated protein kinase (MAPK) (Figure 3). As p38 MAPK activity has been implicated in the export of AR back to the cytoplasm,[95] we followed the intracellular dynamics of the receptor. Some of the SARMs translocated AR into the nucleus at much higher concentration than DHT/R1881, indicating that possible activation of p38 MAPK is slowing the translocation into the nucleus and attenuating the otherwise robust biological effects. These results suggest that the conformation changes of AR in the presence of different ligands activate distinct combinations of intracellular signaling pathways leading to completely different biological effects in some AR target tissues such as prostate. Moreover, the nongenomic pathways impinge on the genomic pathways via phosporylation of AR or coactivators, altering their recruitment to target genes (Figure 3). These results were collected in vitro and need to be validated in animal models.

2.5.3. Antagonism of Endogenous DHT. Although testosterone is the major circulating androgen in muscle and bone, it is converted to DHT in the prostate and skin by 5α-reductase. This enzyme is highly expressed in the prostate and skin, and studies have shown that inhibition by finasteride leads to a reduction in prostate size.[96]

Therefore, DHT is a more active metabolite in the prostate, producing an amplified effect, rendering testosterone extremely potent in both androgenic and anabolic tissues. Nonsteroidal SARMs are not substrates for reduction by 5α-reductase which is likely a contributing factor to their tissue selectivity. Thus, in addition to the differential activities in terms of intracellular signaling, availability/recruitment of cofactors, and AR intracellular trafficking effects as mentioned above, competition between SARM and DHT for binding to the AR could contribute to attenuation of AR-mediated effects in the prostate and other androgenic tissues.

3. Diverse SARM Chemotypes and Their Development

Following the discovery of the nonsteroidal androgens (section 1.5) and their characterization as SARMs (section 2), many diverse SARM chemotypes rapidly emerged.[99,100] Most of these novel chemotypes have demonstrated full myoanabolic efficacy (relative to intact control). Nonetheless as seen for propionamides, small changes in chemical structure of the diverse chemotypes can have dramatic effects on in vivo pharmacology and pharmacokinetics.[101] Representative examples of diverse SARMs are segregated below by pharmacology into full and partial myoanabolic agonists (Tables 2 and 3) and sexually active or androgenic SARMs (Table 3). SARMs herein are identified by their heterocyclic ring systems (i.e., quinolinones vs imidazolopyrazoles, etc.). Comprehensive analysis of the breadth and depth of SARM chemotypes is the subject of our other recent SARM reviews.[90,91] Consequently only representative examples of well characterized and structurally diverse SARMs are discussed briefly below.

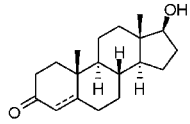
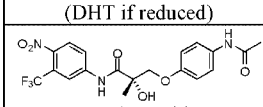
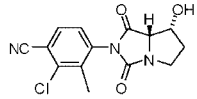
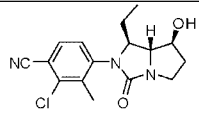
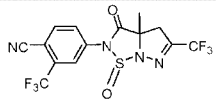
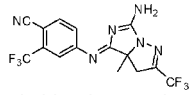
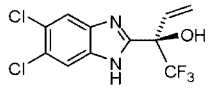
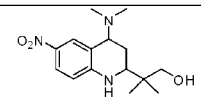
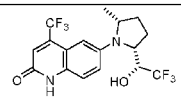
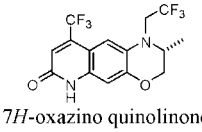
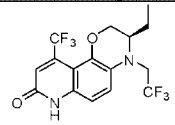
3.1. Preclinical Characterizations of SARM Anabolism. The three most salient features of a SARM are the *anabolic efficacy* (full vs partial E_{max}[LA]), the *myotrophic index* (anabolic to androgenic efficacy ratio (i.e., E[LA]/E[VP]} at E_{max} (preferable) or within a therapeutic range), and the *androgenic activities* as reflected by E_{max}[VP] and/or CNS penetration/activation (i.e., LH suppression). Depending on the intended use, each of these features can be the targeted activity. For typical anabolic indications such as sarcopenia or osteoporosis, the anabolic efficacy and myotrophic index are important. In hypercatabolic subcategories of sarcopenia such as cancer cachexia, hypermyoanabolic efficacy may be very advantageous or even necessary in order to reverse the out-of-control pathologic catabolism that kills approximately 20% of this population before they succumb to the cancer.[102] However, a younger hypogonadal man may prefer an agent that is a partial myoanabolic agent (to augment his endogenous androgen production) with a wide myotrophic index (to limit prostate liability with long-term therapy).

Indications also exist in which the target organ is a sexual organ or behavior, requiring androgenic activity to manipulate it. Examples include male contraception and various male or female sexual dysfunctions including disorders in motivation (i.e., lack of arousal) or response (inability to carry out sexual act). The goal in male contraception is to suppress endogenous testosterone and replace it with an androgenic SARM that can support the anabolic activities of AR[103] while not sacrificing the myotrophic index.

For sexual motivation and response disorders the target tissue is the brain and sexual organs,[104] respectively. In certain of these situations, CNS penetration and/or high E_{max}[VP] may actually be favorable and more important than myoanabolic efficacy. The SARMs with reported androgenic effects (i.e., **14**, LGD2226[105] (**15**), and JNJ-29330835[106] (**16**) as shown in Table 3) all have relatively high E_{max}[VP] values suggesting that this parameter may reflect the ability of SARMs to affect sexual behavior or performance. However, more preclinical and clinical studies are needed to establish this as a surrogate marker for any of these indications.

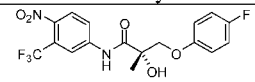
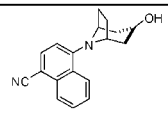
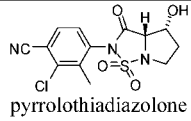
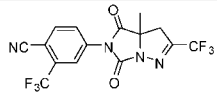
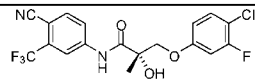
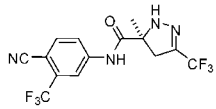
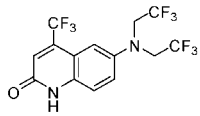
3.1.1. Tricyclic Aniline. Acadia. Piu et al. of Acadia Pharmaceuticals recently reported their preclinical characterization of AC-262536[107] (**17**) (Table 3), an aminophenyl A-ring derivative in which the aniline is part of a tricyclic B-ring, which is a low potency partial myoanabolic agonist (ED_{50}[LA] = 17.3 mg/kg, 66%). Additionally, this compound potently and fully suppresses LH in castrated rats at myoanabolic doses (ED_{50}[LH] = 2.8 mg/kg, 117%). Importantly, reduced efficacy in prostate suggests to some in the field that there is an increased margin of safety with regard to targeting anabolic indications. They have argued that this profile is advantageous to the full agonist model. The argument is that for partial agonists, activity makes dissociation of anabolic and androgenic activities more feasible. In this report Piu et al. discuss certain beneficial functional implications of partial agonist SARMs which include the following: (1) "because receptor reserve may vary between tissues depending on the number of receptors present in a particular tissue and the efficiency of coupling, differences in tissue-selective actions could be enhanced"; (2) "in tissues with high natural androgen contents, such as the prostate, a partial AR agonist could act as a functional antagonist of endogenous androgen action by effectively competing with testosterone and DHT"; (3) "as partial agonists tend to induce less desensitization (loss of responsiveness) than full agonists, a partial agonist SARM would be more effective and safer than a full agonist".[107] Although the evidence from the field of SARMs research is far from proving these postulates, the enhancements in tissue selectivity for several partial agonists relative to full agonists

Table 2. Hypermyoanabolic and Myoanabolic SARMs

SARM	K_i (nM)	LA [a]	VP [a]	HPG [a]	Other	Ref.
 testosterone (DHT if reduced)		0.15 (104%)	0.13 (120%)		Endogenous androgen	55
 propionamide **8** from GTx	4	0.14 mg/d (101%)	0.43 mg/d (35%)	LH suppr. at >0.5mg/d	discussed extensively in Section 2.1	54, 55
 bicyclic hydantoin **18** from BMS	2.1	0.009 mg/kg (125%)	0.14 mg/kg (105%)	0.008 mg/kg	Phase I for ARFD	108
 imidazolin-2-one **19** from BMS	0.9	0.09 mg/kg (~120%)	4.6 mg/kg (~65%)	nr [b]	Acceptable F, $t_{1/2}$, cyp inh, and genotox screens	110
 bicyclic thiohydantoin *S*-**21** from JNJ	nr	117% at 3 mg/d [c]	58% at 3 mg/d [c]	nr		112
 imidazolopyrazole *R*-**22** from JNJ	nr	2.8 mg/kg (91%) [c]	>30 mg/kg (31%) [c]	nr	26% VP reduction in intact vs. none for LA	113
 benzimidazole **24** from JNJ	1.1	0.8 mg/kg (101%) [c]	21% at 3 mg/kg [c]	suppressed FSH; decreased testes size	46% VP reduction in intact; Restored LBM by 20%	25
 tetrahydroquinoline **25** from Kaken	14.9	~115% at 30 mg/kg	~80% at 30 mg/kg	nr	Preclinical proof-of-concept of SARM osteoanabolism	115
 bicyclic quinolinone **26** from Ligand	1.5	~180% at 10 mg/kg	~100% at 10 mg/kg	nr	Improved bone strength in OVX rats at 1 mg/kg	116
 7*H*-oxazino quinolinone **11** from Ligand	7.1	85% at 100 mg/kg	27% at 100 mg/kg	Suppressed LH (no specifics disclosed)		65
 8*H*-oxazino quinolinone **12** from Ligand	nr	100% at 10 mg/kg; 170%	80% at 100 mg/kg	nr		59

[a] Reported as ED_{50} (maximal % efficacy) or % efficacy at stated dose in maintenance Hershberger assays. [b] nr indicates not reported. [c] Modified Hershberger assay in which testosterone propionate (TP) is replaced subdermally.

Table 3. Partially Myoanabolic or CNS-Active SARMs[a]

SARM	K_i (nM)	LA [a]	VP [a]	HPG [a]	Other	Ref.
Partial Efficacy SARMs						
 propionamide **13** from GTx	6.1	0.44 mg/d (75%)	0.42 mg/d (14.5%)	LH supp. at >0.5 mg/d	See Section 2.2	67
 tricyclic aniline **17** from Acadia	5	17.3 mg/kg (66%)	27% at 30 mg/kg	2.8 mg/kg (117%)	Potent HPG agonist	107
 pyrrolothiadiazolone **20** from BMS	0.45	68% at 10 mg/kg	8% at 10 mg/kg	nr[b]		111
 imidazopyrazole *S*-**23** from JNJ	nr	2.9 mg/d (75%) [c]	11% at 3 mg/d [c]	nr		112
CNS Active SARMs						
 propionamide **14** from GTx	1.1	See Table 1	See Table 1	See Table 1	Male contraceptive effects	68
 pyrazoline **16** from JNJ	630	3.8 mg/kg (100% at 10 mg/kg) [c]	26% at 10 mg/kg [c]	FSH supp. by ~40% at 30 mg/kg	Partially restored LBM and TBW in castrated rats; favorable effects on bone turnover markers; Supports precopulatory sex beh. in OVX rats	106, 114
 bicyclic quinolinone **15** from Ligand	1.5	100% at 3 mg/kg; E_{max} = 150%	100% at 100 mg/kg	100% supp. at 3 mg/kg	*In vitro* evidence for reduced N-C interaction; Tc activity not GRIP-1 dep.; Hyperosteoanabolic in tibiae at 1 mg/kg; Supports mating beh. in castrated rats	105, 118

[a] Reported as ED_{50} (maximal % efficacy) or % efficacy at stated dose in maintenance Hershberger assays. [b] nr indicates not reported. [c] Modified Hershberger assay in which testosterone propionate (TP) is replaced subdermally.

of the same or similar chemotypes (e.g., **13** relative to **8**) do seem to support this philosophy. Still, many have achieved adequate tissue selectivity in a hypermyoanabolic agonist with the most extreme example being the LA vs VP tissue selectivity of PS178990 or BMS564929[108] (**18**) (discussed below) (Table 2), arguing against these postulates. Moreover, in order to compensate for lack of full efficacy, partial agonist SARM doses would need to be increased to achieve the desired clinical end point, amplifying any toxicity, DMPK, or side effect related concerns for the candidate SARM.

3.1.2. Bicyclic Hydantoins and Variations. Bristol-Myers Squibb (BMS). The bicyclic hydantoin **18** (Table 2) is the most extensively characterized SARM from Bristol-Myers Squibb (BMS). It is among the most potent myoanabolic SARMs with a wide myotrophic index but also potently suppresses the HPG axis. Whole body anabolic activity has been observed as a 4% reduction in whole body adiposity via DEXA in rats. Functionally this template serves as an extension of the nilutamide (**3**) scaffold. It exhibits increased bulkiness by replacing the disubstituted phenyl A-ring with a trisubstituted phenyl ring. Like the propionamides, the A-ring occupies a similar binding pocket as steroidal agonists and the p-CN interacts with R752 and Q711 (see below).[109] Agonist activity of **18** over the antiandrogen **3** likely gained from the lack of steric interaction with T877 by replacing the dimethyl substitution with a bicyclic hydantoin system with an asymmetric hydroxyl that forms a hydrogen bond to N705. **18** has been studied in phase I trials for age-related functional decline. BMS expanded their bicyclic hydantoin motif into several related chemotypes as backups to **18**. These include an imidazolin-2-one (**19**)[110] (Table 2) which is a full myoanabolic agonist and a pyrrolothiadiazolone derivative (**20**)[111] (Table 3) which is a partial myoanabolic agonist. These compounds were licensed to Pharmacopeia in October 2007, and Pharmacopeia was acquired by Ligand Pharmaceuticals in September 2008.

3.1.3. Bicyclic Hydantoins and Propionamide Analogues. Johnson & Johnson (JNJ). A number of variants on the bicyclic hydantoin theme were also explored by JNJ to include

the bicyclic thiohydantoin *S*-**21**[112] (Table 2) and the imidazolopyrazole *R*-**22**[113] (Table 2), which were full agonists at 3 mg/d, and the imidazopyrazole *S*-**23**[112] (Table 3), which was a partial agonist. *R*-**22** and *S*-**23** B-rings share the pyrazole moiety but differ in their connection to the A-ring. Unlike the BMS bicyclic hydantoin series, these bicyclic ring systems are relatively planar and CF_3 substitution replaces the asymmetric OH. Although no crystallography has been reported, it can be hypothesized that the CF_3 acts as a hydrogen bond acceptor from T877 or N705.

A recently reported benzimidazole A-ring chemotype contained a tertiary alcohol (presumably to interact with N705) reminiscent of the propionamide scaffold but lacks a B-ring system. The lead molecule JNJ-37654032[25] (**24**) (Table 2) was reportedly a full myoanaobolic agonist that restored LBM in castrated rats by 20% and suppressed FSH.[25] In intact rats, androgenic antagonism was observed as decreased testes size and 47% reduction in VP weight (i.e., VP antagonism) while maintaining 100% LA.

Another variation on the propionamide theme was the pyrazoline chemotype of **16**[106,114] (Table 3) in which the tertiary alcohol was replaced with a cyclic pyrazoline NH, thereby maintaining hydrogen bond donation to the N705. Otherwise, the binding interactions of these templates can be rationally hypothesized to mirror the A-ring portion of the propionamide scaffold. **16** demonstrated modest potency (ED_{50}[LA] = 3.8 mg/d) but full efficacy (120% at 2 mg/d for 5 days in castrated rats) with respectable myotrophic index (28% VP efficacy at 2 mg/d for 5 days in castrated rats). Also, a 33% decrease in VP weight was observed in intact rats dosed at 30 mg/kg without reducing LA weights, demonstrating a mixed agonist/antagonist profile in VP but pure LA agonism. Further, **16** demonstrated maintenance of sexual behaviors in ovariectomized rats. It dose-dependently increased precopulatory behaviors associated with sexual motivation and desire. For instance, **16** dose-dependently supported the preferential association of ovariectomized females with intact males and increased sexual solicitations (hop darts, ear wiggles, and positional orientation) from these females.[106]

3.1.4. Tetrahydroquinoline. Kaken. An early contribution to the SARM field was the tetrahydroquinoline S-40503[115] (**25**) (Table 2) from Kaken Pharmaceutical Company, Ltd., which was characterized in castrated rats to increase femur BMD and LA weight.[115] **25** was shown to also increase femur BMD and biomechanical strength in ovariectomized mature rats in excess of that seen with estrogen replacement. As a proof-of-concept that SARMs are osteoanabolic independent of myoanabolic activities, castrated rats were immobilized via sciatic neurectomy and treated with **25**, resulting in a marked increase in cortical tibial BMD, despite significant gastrocnemius atrophy that was not improved by SARM treatment.

3.1.5. Quinolinones. Ligand Pharmaceuticals. Ligand Pharmaceuticals, Inc., has published and patented a very broad set of putative SARMs (reviewed elsewhere)[90,91] in which the conserved structural feature is the quinolinone moiety. SARM clinical candidates from Ligand Pharmaceuticals have included the bicyclic quinolinones **15** and LGD2941[116] (**26**). **15**[117] (Table 3) was an early clinical candidate that was discontinued because of preclinical toxicity. Subsequently it has been extensively characterized with regard to binding mode (see below),[117] myo- and osteoanabolic activity, tissue-selectivity mechanism, and support of sexual activity.[118] **15** demonstrated hypermyoanabolic efficacy with acceptable myotrophic index at therapeutic doses (~3 mg/kg) but potently suppressed LH (100% at 3 mg/kg) in this range. Potent hyperosteoanabolism has been demonstrated in rat tibiae in terms of BMD and strength. **15** demonstrates full androgenic efficacy at high doses (E_{max}[VP] = 100% at 100 mg/kg). In vitro peptide interaction profiles demonstrated differences between **15** and steroidal agonists which may reflect their relative tissue selectivities. For instance, **15** poorly recruited an F-peptide derived from the AR N-terminus, suggesting a reduced ability to induce the N-to-C AR conformation. **15**, unlike steroidal agonists, does not recruit the coactivator GRIP-1 during transcriptional activation. This possibly reflects subtle conformational differences between SARM and steroid bound AR conformation. Differential GRIP-1 recruitment may explain differential binding to AR-dependent promoters, which is a postulated basis for distinct gene activation programs for SARMs vs steroidal AR agonists.

CNS penetration as reflected by LH suppression may explain some of the androgenic behavior effects of **15**. Reliable males (i.e., sexually motivated and with prior successful copulation) were castrated and dosed with 17β-estradiol via a silastic subdermal implant. The SARM was fully efficacious in preventing castration-induced loss of sexual function in male rats as measured by the number of mounts, intromissions, and ejaculations.[118]

26 (Table 2) is another bicyclic quinolinone clinical candidate that differs from **15** in that the aniline nitrogen is part of a pyrrolidine ring that is substituted with an asymmetric hydroxyl in addition to a CF_3. This compound has a similar activity profile as **15** (hypermyo- and hyperosteoanabolic with full androgenic E_{max}; LH suppression data are not reported). Another compound in their development pipeline, LGD3303 (9-chloro-2-ethyl-1-methyl-3-(2,2,2-trifluoroethyl)-3*H*-pyrrolo[3,2-*f*]quinolin-7(6*H*)-one]),[119] has recently been characterized.[26]

A couple of their early tricyclic nonsteroidal androgen templates (discussed in section 1.5.3) were recently published as SARMs. The 7*H*-oxazinoquinolinone (**11**) (Table 2) had an acceptable myotrophic index (E_{max}[LA] and E_{max}[VP] values of 85% and 28%, respectively). LH suppression was reported without specifying its potency relative to myoanabolism. The 8*H*-oxazinoquinolinone (**12**) (Table 2) exhibited greater E_{max} values and potentially reduced myotrophic index. On the basis of modeling, the C-ring CF_3 group is postulated to accept a hydrogen bond from T877.[59]

In terms of binding mode, this series of compounds (bi- and tricyclic quinolinones) occupies a similar binding pocket as steroidal agonists. The conserved quinolinone moiety forms a direct tripartite interaction between the R752 and Q711 which firmly anchors the SARM in the binding pocket. On the end of these molecules opposite the A-ring, there are generally hydrogen bond acceptor (i.e., CF_3 or ether oxygen) and sometimes a hydrogen bond donor (asymmetric OH, **26**) substituents that could interact with either N705 or T877 or both.

Several other groups are active in the SARM area as evidenced by abstract and patent activity as well as ongoing clinical trials in some cases but have not published any of their preclinical characterizations in peer-reviewed journals. These groups include GlaxoSmithKline, Lilly, Merck, and others, as reviewed elsewhere.[91]

3.1.6. Structural Basis for SARM Binding. Although diverse, some common themes are observed across all chemotypes. For instance, most SARMs retain the A-ring from the propionamide template which is an electron-deficient phenyl ring that occupies the same or similar space within the AR ligand binding pocket as the DHT A-ring. The SARM A-ring interacts favorably with F764 (not shown), and its electron withdrawing substituents (NO_2, CN, α,β-unsaturated C(O)NH (quinolinone))

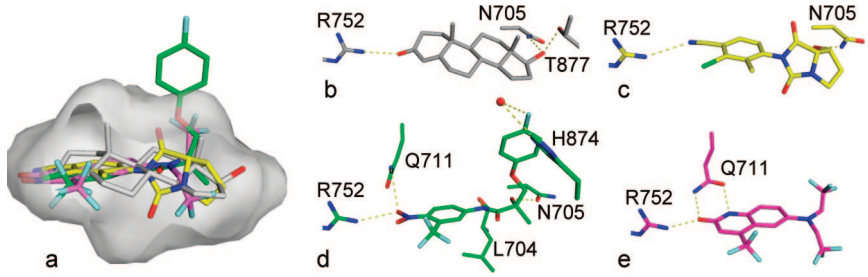


Figure 4. Binding modes for diverse chemotypes. In panel a, the ligand binding pocket for DHT is shown in gray. The binding mode of propionamides cannot be rationalized by the DHT crystal structure and is unique among the SARMs crystallized to date. Unlike other SARMs, **13** binding induces the formation of a B-ring pocket, as demonstrated by the superimposition of **13** with ligands of the other available crystallized SARM/AR scaffolds. These diverse scaffolds are also shown separately in panels b−e, which are DHT (endogenous steroid), **18** (synthetic bicyclic hydantoin), **13** (propionamide), and **15** (bicyclic quinolinone), respectively.

interact with R752 and/or Q711 (like the 3-keto of DHT), sometimes involving a water molecule (Figure 4). Also, an aniline nitrogen atom is retained that connects the A-ring to the rest of the compound. Typically the rest of the molecule contains a hydrogen bond donor (OH or NH) that interacts with N705, but the B-ring seems dispensable for some chemotypes.

The diverse chemotypes also have important structural differences from the propionamides. Unlike propionamides such as **8**, the diverse chemotypes (thus far) are typically shorter. This results from the absence of a B-ring system or the presence of an abridged intermediate chain between ring systems. They are also more rigid because of the presence of a rigid and often complex heterocyclic B-ring system (Tables 2 and 3). These dissimilarities between propionamides and other SARMs are reflected in the unique binding mode of propionamides (Figure 4a) in which H874 makes a water-mediated direct interaction with the para B-ring substituent (Figure 4d). SARMs with diverse chemical scaffolds have been cocrystallized with the AR LBD explaining receptor interactions important for binding (Figure 4).

4. SARMs as the Next Generation of Androgen Therapy

The AR has been successfully targeted previously using steroidal agonists such as testosterone and synthetic anabolic-androgenic steroids (AAS). Despite some anabolic enrichment achieved with AAS, none of them are considered acceptable for long-term therapy.[120−122] Most of the AAS that have been approved or used illicitly (extensively reviewed by Kicman et al.)[7] have been withdrawn as registered products in numerous countries worldwide. This leaves a variety of testosterone injections and transdermal patches and gels as the most widely used androgen (i.e., testosterone replacement therapy (TRT)),[123] whose use for hypogonadism[124] and hypoandrogenic metabolic syndromes[125] has increased in recent years.

4.1. Pharmacokinetic Advantages. The criteria that Negro-Vilar[10] outlined in order to capitalize on the therapeutic potential of SARMs have largely been met. The absorption characteristics of SARMs of diverse templates allow oral bioavailability and half-lives consistent with daily dosing (Tables 1−3).[101] In contrast, formulation issues with testosterone replacement therapy cause half-life variability, and AAS bioavailability (conferred via 17α-methylation) is associated with hepatic toxicity. Distribution of SARMs is not impeded by steroid hormone binding globulin (SHBG),[108,126] a plasma protein that significantly suppresses bioavailability of steroidal androgens when present in the plasma. Unlike testosterone replacement therapy and AAS, metabolites of SARMs are neither virilizing in women nor feminizing in men because of the phenyl A-ring that is neither aromatizable or 5α-reducible,[67,108] and most SARMs do not suppress endogenous testosterone or prohormone activities.

4.2. Pharmacodynamic Advantages. By definition all SARMs have achieved a favorable myotrophic index relative to testosterone. However therapeutically relevant in vivo separation of the anabolic and androgenic activities of the AR has been reported in animal models (sections 2 and 3) and human clinical trials (see below). Additionally, peripheral selectivity has been achieved for most of the SARM templates (Tables 1−3). By contrast, superphysiologic doses of testosterone (or any dose of AAS) produce an intrinsic prostatic liability[127] and other androgenic side effects, which is further complicated by HPG suppression and prohormone perturbation, cumulatively limiting the clinical indications available to them.[128] Moreover, achieving steroid receptor selectivity has not been problematic for SARMs, unlike steroidal AR ligands. Further, various pharmacologic ratios have been reduced to practice by several in the field (Tables 1−3), expanding the possible uses of SARMs. These PK/PD advantages of SARMs compared to steroidal agonists bode well for the future of selectively anabolic nonsteroidal SARMs as the next generation of androgen therapy.

4.3. Clinical Investigations into SARMs. Although we are still learning about how SARMs work at a molecular level, the well established body of evidence supporting their in vivo tissue selectivity has stimulated immense interest and speculation regarding the therapeutic potential of SARMs in humans. SARM development has exploded over the past decade with several programs culminating in clinical trials.[18] Tissue selective pharmacology extends the potential therapeutic use of androgens beyond traditional indications, such as in hypogonadal men,[123] and into disease states requiring long-term administration in otherwise healthy individuals. The reduced androgenic liability and improved pharmacokinetic properties of SARMs may afford lengthy treatment paradigms required in age-related disorders, such as osteoporosis and frailty, in both sexes.[129,130] Despite burgeoning development, most clinical investigations to date are limited to phase I studies.

Ligand Pharmaceuticals, an early pioneer in SARM development, entered **26** into phase I trials in collaboration with Abbott (formerly TAP) with the hopes of meeting a therapeutic need for both geriatric frailty and osteoporosis in men and women alike.[116] Osteoporosis is a common skeletal disease, with myriad etiologies, characterized by reduced bone strength and an increased risk for fracture. While typically ascribed to post-menopausal women,[131] osteoporosis in men is growing in recognition.[132] Androgens have shown direct anabolic and antiresorptive effects on bone, making SARMs a viable therapeutic option.[133] SARMs also offer potential synergy in

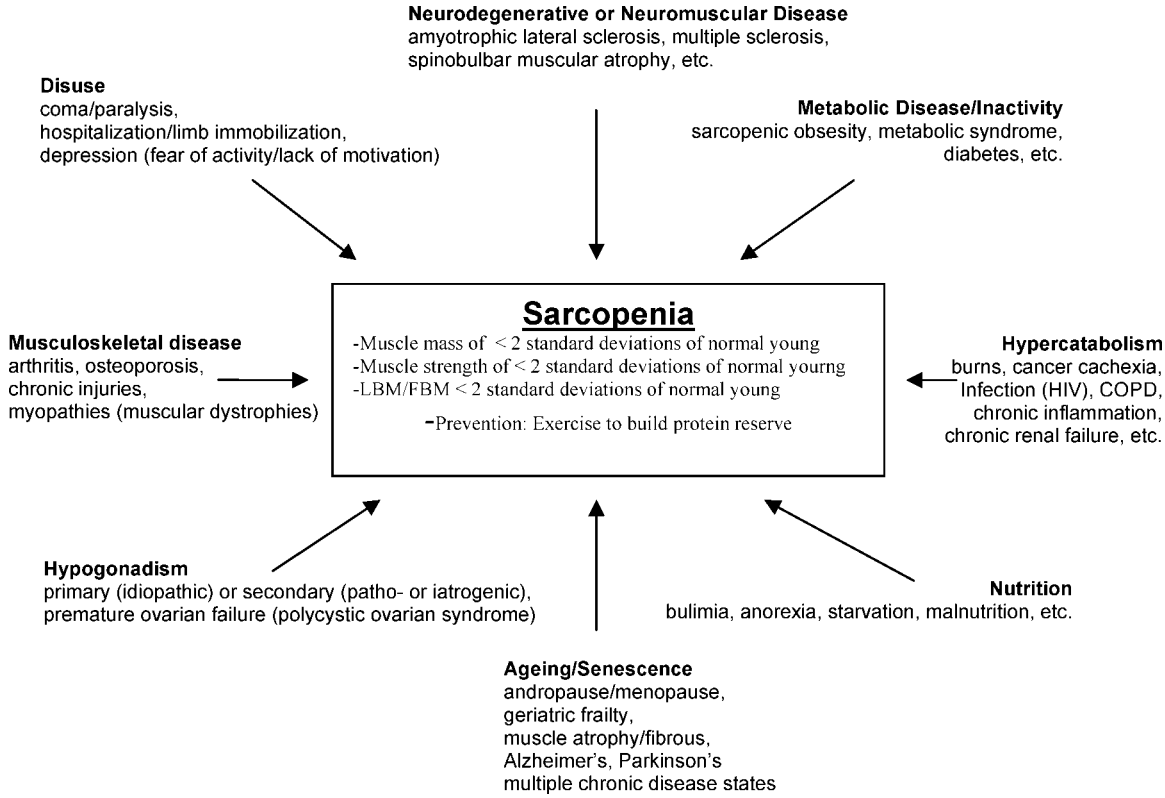


Figure 5. Etiologies of sarcopenia. Some of the definitions of sarcopenia[142,143] and factors contributing to its development are outlined above. The pathophysiology underlying the sarcopenic influence of these conditions can be found in the literature.[143,152−156] The best protection against these degenerative processes is the accumulation of a protein reserve as early in life as possible. Failing adequate protein reserves, successful development of SARMs may currently be the best hope for reversing sarcopenia[149−151] and preventing its degeneration into frailty[147] in these populations.

treatment of osteoporosis as increased muscle mass and strength could lead to increased stimulatory mechanical bone stress and reduced falls, a major morbidity in diseases of bone frailty.[134] Current osteoporosis therapies are unsatisfactory for a number of reasons including parenteral dosing, increased risk of osteosarcoma or venous thromboembolism, and singular mechanism of action.[135] An orally available, anabolic SARM could offer improved therapy for the treatment of both primary (age related) and secondary (i.e., xenobiotic induced) osteoporosis.

Bristol-Myers-Squibb (BMS) entered its first clinical candidate **18** into a phase I trial in 2007 for treatment of age related functional decline or geriatric frailty in men.[108] Pharmacopeia expected to start phase II trials with **18** in the first quarter of 2009, but Pharmacopeia was subsequently acquired by Ligand Pharmaceuticals in September 2008. Ligand Pharmaceuticals lists **18** as one of their internal SARMs, but they do not outline a timeline to progression to phase II. Geriatric frailty, common to both men and women, is defined as a status of global impairment of physiological reserves involving multiple organ systems where patients realize impaired response to both internal and external stressors.[136] Several studies have shown benefits of testosterone therapy in aging men,[137−139] though the risks associated with long-term traditional androgen treatment are considered unacceptable in a population already prone to prostatic neoplasia. SARM therapies may provide a preferred alternative for treatment of geriatric frailty in men.

GTx, Inc., and Merck have the most advanced clinical candidates that are currently under phase II evaluation. Ostarine (structure not publicly disclosed, MK-2866) completed a successful 2006 phase II study where dose dependent increases in lean body mass couple with decreases in fat mass were reported in healthy elderly men and women.[75] A follow-up phase IIb study was recently reported that evaluated Ostarine in the treatment of cancer cachexia which demonstrated comparable results in a morbid population (unpublished results). The most common side effects reported among all subjects in the trial were fatigue, anemia, nausea, and diarrhea. Some changes in alanine amino transferase (ALT) were observed. However, no subjects discontinued treatment as a result of ALT changes. Cachexia encompasses hypercatabolic states resulting in significant loss in lean body mass with consummate functional impairment, fatigue, and respiratory complications and is common in HIV/AIDS, late stage renal failure, and neoplastic disease.[102,140] Steroidal androgens have shown efficacy in the treatment of muscle wasting, though serious side effects have been reported.[141] SARM therapy is expected to show myoanabolic efficacy with reduced side effects filling yet another unmet therapeutic need. Another compound under this collaboration, MK-0773 (structure not publicly disclosed), is currently in a phase II clinical trial in women with sarcopenia (NCT00529659).

4.4. Sarcopenia. Perhaps the most compelling putative use of a SARM is sarcopenia. There is no clear singular definition of sarcopenia at present,[142,143] and it is currently underrecognized as a significant contributor to the morbidity and mortality of many disease states.[102,144−146] Figure 5 summarizes the diverse and myriad etiologies of sarcopenia (broadly defined to include cachexia and other types of muscle wasting including younger populations).[142] Cumulatively, these disease states represent a very large potential target population in which SARMs can address an unmet clinical need for myoanabolism. Proof-of-concept phase II clinical trials in healthy elderly (2006)[75] and cancer cachexia (2008) (unpublished data) populations demonstrate the clinical relevance of SARMs and provide

incentive to the medical community to reconsider the importance of muscle mass/strength assessment in their routine clinical evaluations.[147] Hopefully, the trends within the general public and medical community toward understanding the necessity of having a healthy protein reservoir (best acquired through exercise and proper nutrition)[148] and the ability to protect this reservoir using SARMs[149−151] will continue.

4.5. Anabolic Drug Abuse. The cosmetic and performance enhancing ability of anabolic agents produces the likelihood of their abuse.[157] Anabolic steroids (testosterone and AAS) are controlled substances in the U.S. The Anabolic Steroids Control Act of 1990 places steroids in the schedule III legal class. The possession of schedule III drugs without a prescription is a federal offense and can be enforced in every state. The International Olympic Committee (IOC) had earlier banned the use of anabolic steroids in the Olympics in 1974 and then enhanced the ban of performance enhancing drugs with an additional amendment in the 1990s to include all anabolic agents such as insulin and growth hormone.[158] In January 2008, the IOC added SARMs to the list of prohibited anabolic substances.[158] Extensive effort has already been expended toward development of MS-based doping screens for SARMs.[159−161]

5. Prospects and Outlooks

5.1. Landmarks in SARM Development: Past and Future. The concept of SARMs and their added potential to androgen therapy was recognized in the late 1990s following the discovery of propionamide and quinolinone nonsteroidal androgen templates. The first preclinical proof-of-principle of concurrent myo- and osteoanabolism in vivo came in 2003[55,115] along with the demonstration of the postulated diverse pharmacologic ratios (i.e., **13** as partial myoanbolic agent). These early molecules (**8**, **13**, **25**) became prototypes for the pharmaceutical industry, allowing an explosion of activities in the SARM field. Clinical proof-of-principle experiments in healthy elderly (2006) and cancer cachectic (2008) patients have validated the relevance of SARMs for the treatment of sarcopenic populations. The next major landmark will be the successful completion of a phase II or III sarcopenia or cachexia trial(s) demonstrating the safety and efficacy of a SARM in the target population. Given the more acute nature of clinical trials, the first successful foray into clinically use of SARMs is most likely in cancer cachexia and other disease- or injury-induced conditions associated with muscle atrophy.

5.2. SARMs as Anabolic Agent of Choice. SARMs uniquely produce concurrent osteoanabolic and myoanabolic activity (Tables 1−3) without the PK/PD problems of steroidal agonists.[162] This allows the extension of androgen therapy to many disease states requiring this dual anabolism such as geriatric frailty or age-related functional decline with auxiliary benefits from the observed androgenic antagonism. SARMs also 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. This includes many unmet clinical needs resulting from sarcopenia such as cachexia, chronic renal disease, chronic obstructive pulmonary disease, geriatric frailty, and other causes of muscle wasting as reviewed extensively elsewhere.[11,18,91] Likewise, the unique ability of SARMs to restore bone strength (i.e., osteoanabolic) while improving musculoskeletal performance (i.e., prevent debilitating falls)[72,75,162] and the recent demonstration of additivity with antiresorptives[26] cumulatively suggest SARMs can penetrate the competitive osteoporosis market. Although efforts toward myostatin inhibitors, ghrelin agonists, and recombinant growth hormone will undoubtedly continue, the unique oral bioavailability, pleiotropic anabolic and metabolic effects, and diverse chemotypes of SARMs position them favorably to assume status as the therapy of choice for many diseases requiring anabolic intervention.

Biographies

Michael L. Mohler received a B.S. in Chemistry and Biology (double major) from the University of Tennessee at Martin in 1994 and an M.S. in Biochemistry in 1998 at the University of Tennessee, Knoxville, studying enzyme-mediated antibiotic resistance using NMR techniques. He received Pharm.D. (2002) and Ph.D. (2005) degrees from the University of Tennessee Health Science Center in Memphis, all under the direction of Prof. Duane D. Miller. He joined GTx, Inc., in 2004 as a Research Scientist (Medicinal Chemistry Department) and Legal Liaison. His interests include the design of endocrine-related ligands that directly (i.e., nuclear hormone receptor ligands) or indirectly (i.e., metabolic enzymes, cross-talk receptors) affect androgenic or estrogenic signaling.

Casey E. Bohl received his undergraduate degree in Biochemistry from The Ohio State University in 2001. He then completed a Ph.D. at The Ohio State University in 2005 under the direction of Prof. James T. Dalton in Pharmaceutics, focusing on structural interactions of nonsteroidal ligands with the androgen receptor and mechanisms of antiandrogen withdrawal syndrome. He stayed at The Ohio State University for an additional 2 years to finish clinical requirements for his M.D. degree while working as a part-time postdoctoral fellow on nuclear hormone receptor crystallography and structure-based drug design. Following graduation, he joined GTx, Inc., as a Research Scientist where he heads a structural biology and structure-based drug design laboratory.

Amanda Jones received her Bachelor's degree in Pharmaceutical Sciences from the University of Toledo in 2003 and remained there to receive her Master's degree in Pharmaceutical Sciences, with an emphasis in preformulation. She is currently working on her Ph.D. under the direction of James T. Dalton, Ph.D., at The Ohio State University, focusing on SARMs for hormonal male contraception, female sexual behavior, and muscle wasting.

Christopher C. Coss received dual Bachelor's degrees in Molecular Genetics and Computer Science from The Ohio State University in 2003. In December 2008 he completed his thesis on SARM mechanisms and received his Ph.D. in Pharmaceutics under the guidance of Prof. James T. Dalton, Ph.D., also at The Ohio State University. He has accepted a Research Scientist position in Preclinical Research and Development at GTx, Inc., in Memphis, TN, where he continues to work on novel hormone therapies.

Ramesh Narayanan received his Ph.D. in Biochemistry from the University of Madras, India. He did his postdoctoral research in The Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX. His postdoctoral research included the post-translational modifications of human progesterone receptor, the role of intracellular signaling and cell cycle kinases in progesterone receptor and vitamin D receptor functions, and the role of novel vitamin D receptor ligands to counteract the microgravity induced weightloss. He has authored more than 20 peer reviewed high impact journal articles, book chapters, and reviews and has more than 11 years of research experience in the field of nuclear receptor biology and signaling. Currently he is a Senior Scientist in Drug Discovery at GTx, Inc.

Yali He received his B.S. degree from the Inner Mongolia University (1986) and his M.S. degree in Medicinal Chemistry (1992) at Peking Union Medical College in China. After graduation, he worked for the National Institution for the Control of Pharmaceutical and Biological Products (NICPBP) in Beijing, China. He received his Ph.D. degree in Medicinal Chemistry from University of Tennessee Health Science Center in 2000, under the direction of the Prof. Duane D. Miller. In the same year he worked for IRIX

Pharmaceuticals, Inc., as a Principal Scientist. Since 2003, he has been a Senior Scientist at GTx, Inc. Research interests include drug design and synthesis, especially in the nuclear receptor area.

Dong Jin Hwang was born in 1968 in Seoul, Korea. He received a B.S. (1992) at Chongju University and an M.S. (1995) at the Department of Chemistry of Chungbuk National University. He obtained his Ph.D. (2002) degree from Korea Institute of Science and Technology and Hanyang University in Seoul, Korea. From 2002, he did postdoctoral work on SARMs at the University of Tennessee Health Science Center in Memphis, TN, with Dr. Duane D. Miller. In 2007, he was hired at GTx, Inc. as a Research Scientist. He has also worked in the fields of antimicrotubule agents, *N*-acyliminium ion cyclization, HIV integrase inhibitors, antibacterial agents, and liquid crystalline compounds.

James T. Dalton is Vice-President of Preclinical Research and Development at GTx, Inc., in Memphis, TN. He received his B.S. in Pharmacy from University of Cincinnati (1986) and Ph.D. (1990) in Pharmaceutics and Pharmaceutical Chemistry from The Ohio State University. He was a faculty member in the College of Pharmacy at The University of Tennessee, Memphis, from 1992 to 2000. He then returned to The Ohio State University as an Associate Professor in the Division of Pharmaceutics where he served as Division Chair (2002−2005) and was promoted to Professor (2004). He joined GTx, Inc., in 2005, where he now leads all preclinical drug discovery and development efforts in SARMs, other selective nuclear hormone receptor modulators, and novel anticancer agents.

Duane D. Miller is the Director of Medicinal Chemistry at GTx, Inc. He obtained his B.S. (1966) at Kansas University. He was an NIH Fellow while at the University of Washington and obtained his Ph.D. in 1969. He joined The Ohio State University Faculty in 1969, where he became Chairman of the Division of Medicinal Chemistry and Pharmacognosy in 1982. He moved to the University of Tennessee, Memphis, TN, in 1992 as the Van Vleet Professor and became Chair and Associate Dean in 2001. He has over 250 publications and 300 presentations nationally and internationally. He is the recipient of many awards including the 2008 Medicinal Chemistry Division Award for his work in SARMs.

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