ABSTRACT: The androgen receptor (AR) plays a key role in the maintenance of muscle and bone and the support of male sexual-related functions, as well as in the progression of prostate cancer. Accordingly, AR-targeted therapies have been developed for the treatment of related human diseases and conditions. AR agonists are an important class of drugs in the treatment of bone loss and muscle atrophy. AR antagonists have also been developed for the treatment of prostate cancer, including metastatic castration-resistant prostate cancer (mCRPC). Additionally, selective AR degraders (SARDs) have been reported. More recently, heterobifunctional degrader molecules of AR have been developed, and four such compounds are now in clinical development for the treatment of human prostate cancer. This review attempts to summarize the different types of compounds designed to target AR and the current frontiers of research on this important therapeutic target.

1. INTRODUCTION

The androgen receptor (AR) is a transcriptional factor that is essential for the growth, survival, and proliferation of cells. Testosterone and its more active metabolite, 5α-androstan-17β-ol-3-one, or dihydrotestosterone (DHT), are endogenous androgens. Androgens and AR signaling are essential in the male sexual system, for secondary sexual characteristics, and for muscle and skeleton development and maintenance in both males and females. Androgen deficiency leads to decreasing libido and dysfunction in males, which causes loss of muscle mass and strength and a depressed mood. AR agonists are therefore useful in treating age- and disease-related androgen deficiency, muscle atrophy, and bone loss.

AR signaling is important in prostate development and homeostasis, and AR proteins are necessary for the function, survival, and differentiation of prostatic tissue in the normal prostate. However, the functions of ARs and their signaling can switch during prostate carcinogenesis from tumor suppressive to tumor promoting. Androgens and ARs also play a critical role in the initiation and progression of prostate cancer and a subset of breast cancer. Androgen deprivation therapy (ADT), achieved either by surgical castration or with drugs that block androgen synthesis, has proved to be effective in the treatment of androgen-dependent advanced and metastatic prostate cancer, which is also termed androgen-dependent prostate cancer. Unfortunately, the therapeutic efficacy of castration is not long-lasting, and after a few years of treatment, prostate cancer progresses into what is termed castration-resistant prostate cancer (CRPC). However, AR signaling continues to play an important role in CRPC, and blocking AR signaling is therefore a useful therapeutic strategy in the treatment of CRPC.

The current clinical paradigm for the treatment of prostate cancer, even in the castration-resistant state, is focused on blocking AR signaling. Potent AR-directed therapies, such as the second generation, pure AR-antagonist enzalutamide, can effectively block AR signaling. However, resistances to AR-directed therapies rapidly develop in the clinic. AR gene amplification, activating mutations of the AR receptor on the AR ligand-binding domain (LBD), and splice variants (ARVs) are some of the important resistance mechanisms to AR-directed therapies.

AR exerts its function in both androgen-dependent and androgen-independent mechanisms. In the androgen-dependent pathway, androgen binding to AR causes conformational changes in the AR to make AR dissociate from heat shock proteins (HSP), followed by the recruitment of coregulators and subsequent dimerization and translocation into the nucleus to bind to targeted DNAs for gene transcription. In the androgen-independent AR pathway, AR and AR variants (ARV) can dimerize without initiation by an androgen. In the latter scenario, AR can be activated in the absence of androgens.
by epidermal growth factor (EGF), insulin-like growth factor (IGF), and interleukin-6 (IL-6) signaling pathways via phosphorylation, which leads to increased nuclear translocation and gene transcription.27

AR exists in different forms: full-length AR (FLAR), AR variants (ARV), and mutant forms (Figure 2).28−31 Full-length AR is a 110 kDa 919-amino acid protein that consists of an N-terminal transcriptional domain (NTD), a C-terminal ligand-binding domain (LBD), and a DNA-binding domain (DBD) that links the N- and C-terminal domains through a hinge region. The N-terminal domain is capped by polyglutamines and polyglycines. The average number of polyglutamines is 21, and they comprise a region that is responsible for the interaction with AR coregulators. The NTD contains a transcriptional activation function 1 (AF1). This FXXLF motif interacts with the LBD upon androgen binding to AR, known as an N/C interaction. DBD binds to an androgen response element (ARE) during gene transcription and has two zinc fingers that are attached to the hinge region. The LBD includes a ligand-binding pocket (LBP), a transcriptional activation function 2 (AF2) domain, and a binding function 3 (BF3) domain. ARVs, lacking a LBD at the C-terminal, are alternative spliced ARs.32 More than 20 ARVs have been identified in human prostate cancers, including in cell lines and clinical specimens. Indeed, overexpression of ARV7 has been found in enzalutamide-resistant mCRPC patients. Among numerous mutations of AR (http://androgendb.mcgill.ca), F877L, T878A, H875Y, and W742L/C point mutants have been associated with resistance to the prostate cancer drugs abiraterone, bicalutamide, enzalutamide, and apalutamide.33−36

2. AR DRUGGABLE POCKETS

AR contains a number of potential druggable pockets within different domains. The ligand-binding domain (LBD) contains the well-known internal ligand-binding pocket (LBP).37,38 The AF1 (activation function 1), AF2 (activation function 2),39,40 DBD (DNA-binding domain),41,42 and BF3 (the binding function 3)43 each contain a potentially druggable pocket. A number of cocrystal structures revealing these pockets, with the exception of AF1, have been determined.

The LBP pocket is formed by 5 helices (H3, H5, H10, H11, and H12). Agonists and antagonists compete with testosterone or DHT for binding to the LBP pocket (Figure 3A,B). Agonists and antagonists induce different conformational changes in AR, which leads to different poses of the H12 helix. Molecular dynamics simulations of AR with agonists and antagonists have been performed on the basis of the estrogen receptor,44 but precise experimental details of these conformational changes for the H12 helix remain to be determined.

The LBP pocket remains to be solved. The AF2 domain contains an androgen-dependent activation site, located near the LBP on AR, the H12 helix remains to be determined. All agonists and most antagonists have been established. All agonists and most antagonists discovered to date bind to the LBP, initiating or disrupting gene transcription process. Compounds binding to the AF1 site can be either inhibitors or selective AR degraders (SARD), but their precise mechanisms of action remain to be established.45−47 Compounds, which bind to the AF2, BF3, and DBD pockets, can inhibit AR gene transcription, thus functioning as antagonists.48 Compounds, which bind to the AF2, BF3, and DBD pockets, can inhibit AR gene transcription, thus functioning as antagonists.49 Several mechanisms of action of small molecules targeting AR have been established. All agonists and most antagonists discovered to date bind to the LBP, initiating or disrupting gene transcription process. Compounds binding to the AF1 site can be either inhibitors or selective AR degraders (SARD), but their precise mechanisms of action remain to be established.50,51 Compounds, which bind to the AF2, BF3, and DBD pockets, can inhibit AR gene transcription, thus functioning as antagonists.52 More recently, heterobifunctional small-molecules based upon the proteolysis-targeting chimera (PROTAC) technology have been designed to induce the degradation of AR.53 This review summarizes and analyzes different therapeutic approaches targeting AR, including AR agonists, antagonists, SARDs, and PROTACs.

Figure 1. Androgen-dependent and -independent gene expression pathways. A, androgen; AR, androgen receptor; HSP, heat shock protein; ARV, androgen receptor variant.

Figure 2. Illustration of structures of FLAR and ARVs. NTD, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain; PolyQ, poly glutamines; AF1, activation function 1; LBP, ligand-binding pocket; AF2, activation function 2; BF3, binding function 3.

AR is a 110 kDa 919-amino acid protein that consists of an N-terminal transcriptional domain (NTD), a C-terminal ligand-binding domain (LBD), and a DNA-binding domain (DBD) that links the N- and C-terminal domains through a hinge region. The N-terminal domain is capped by polyglutamines and polyglycines. The average number of polyglutamines is 21, and they comprise a region that is responsible for the interaction with AR coregulators. The NTD contains a transcriptional activation function 1 (AF1). This FXXLF motif interacts with the LBD upon androgen binding to AR, known as an N/C interaction. DBD binds to an androgen response element (ARE) during gene transcription and has two zinc fingers that are attached to the hinge region. The LBD includes a ligand-binding pocket (LBP), a transcriptional activation function 2 (AF2) domain, and a binding function 3 (BF3) domain. ARVs, lacking a LBD at the C-terminal, are alternative spliced ARs. More than 20 ARVs have been identified in human prostate cancers, including in cell lines and clinical specimens. Indeed, overexpression of ARV7 has been found in enzalutamide-resistant mCRPC patients. Among numerous mutations of AR (http://androgendb.mcgill.ca), F877L, T878A, H875Y, and W742L/C point mutants have been associated with resistance to the prostate cancer drugs abiraterone, bicalutamide, enzalutamide, and apalutamide.

2. AR DRUGGABLE POCKETS

AR contains a number of potential druggable pockets within different domains. The ligand-binding domain (LBD) contains the well-known internal ligand-binding pocket (LBP). The AF1 (activation function 1), AF2 (activation function 2), DBD (DNA-binding domain), and BF3 (the binding function 3) each contain a potentially druggable pocket. A number of cocrystal structures revealing these pockets, with the exception of AF1, have been determined.

The LBP pocket is formed by 5 helices (H3, H5, H10, H11, and H12). Agonists and antagonists compete with testosterone or DHT for binding to the LBP pocket (Figure 3A,B). Agonists and antagonists induce different conformational changes in AR, which leads to different poses of the H12 helix. Molecular dynamics simulations of AR with agonists and antagonists have been performed on the basis of the estrogen receptor, but precise experimental details of these conformational changes for the H12 helix remain to be determined.

The AF1 domain consists of residues 142−485 of the N-terminal transactivation domain (NTD). and its crystal structure remains to be solved. The AF2 domain contains an androgen-dependent activation site, located near the LBP pocket on the LBD (Figure 3A). After androgen binds to LBP on AR, the H12 folds back to enclose the LBP and create a surface pocket formed by helices H3, H4, and H12, which together comprise the AF2 pocket. BF3 (Figure 3B) contains an allosteric site located on LBD in the vicinity of, but distinct from, the LBP and AF2 pockets. Small molecule binding to the BF3 site leads to conformational change in AR, which renders it inaccessible to AR coactivators. The interface between DBD and DNA generates another pocket (Figure 3C). Compared with estrogen receptor (ER), progesterone receptor (PR), and other nuclear receptors, AR DBD has two unique amino acids Q592 and Y594 that may provide an opportunity to design AR DBD-selective inhibitors.

Several mechanisms of action of small molecules targeting AR have been established. All agonists and most antagonists discovered to date bind to the LBP, initiating or disrupting gene transcription process. Compounds binding to the AF1 site can be either inhibitors or selective AR degraders (SARD), but their precise mechanisms of action remain to be established. Compounds, which bind to the AF2, BF3, and DBD pockets, can inhibit AR gene transcription, thus functioning as antagonists. More recently, heterobifunctional small-molecules based upon the proteolysis-targeting chimera (PROTAC) technology have been designed to induce the degradation of AR. This review summarizes and analyzes different therapeutic approaches targeting AR, including AR agonists, antagonists, SARDs, and PROTACs.
3. AR AGONISTS

3.1. Steroid-Based AR Agonists. Testosterone was isolated and synthesized in 1935. It has a $K_d$ value of 0.4–1.0 nM to human AR (hAR), and DHT has a higher affinity with a $K_d$ value of 0.25–0.5 nM (Figure 4). With its EC$_{50}$ of 0.1–0.2 nM, dihydrotestosterone (DHT) is approximately 10 times more potent than testosterone in the activation of AR. Testosterone and DHT have short half-lives of 50 and 30 min, respectively. Testosterone is metabolized by aromatases into estrogens, and DHT is metabolized by 3$\alpha$-hydroxysteroid dehydrogenase reduction within the cells of androgen sensitive tissues. Both testosterone and DHT have short half-lives of 50 and 30 min, respectively. Testosterone is metabolized by aromatases into estrogens, and DHT is metabolized by 3$\alpha$-hydroxysteroid dehydrogenase reduction within the cells of androgen sensitive tissues. Metabolism of testosterone and DHT, as well as their half-lives, will be discussed in more detail below.

Introduction of a 17$\alpha$-methyl into a steroid prevents the oxidation of the hydroxyl group at C17 and greatly increases the metabolic stability and bioavailability of the compound (Figure 4). Methyl testosterone (3) has a half-life of approximately 3 h, significantly longer than that of testosterone, but its affinity is 4 times lower than that of testosterone. Metribolone (R1881, 4), also with a 17$\alpha$-methyl substituent, has a 1.5–2 times greater affinity for AR than DHT. However, metribolone leads to severe liver toxicity in animals and has not been used clinically. Medroxyprogesterone acetate (MPA, 5) is a full AR agonist with a $K_d$ value of 1 nM, but it is a pan-nuclear hormone agonist for PR and GR, among others. Although it is a potent AR agonist, MPA is used as a birth control agent, and its activity is primarily related to its PR agonist effect. Oxandrolone (6) is an anabolic-selective AR agonist that has approximately 10 times greater selectivity for anabolic AR than androgenic AR. Oxandrolone has a $K_i$ value of 62 nM against AR. Stanozolol (7), with a pyrazoline moiety has greater solubility and a binding affinity similar to that of testosterone but no selectivity for anabolic AR over androgenic AR. YK-11 (8) is an anabolic-selective AR agonist that activates AR without an N/C interaction and induces C2C12 myoblast cell division more significantly than DHT.

All these steroid AR agonists have common side effects, including acne, abnormal hair growth, increased sexual desire, and liver damage. Most of them were used as performance-enhancing drugs and are therefore controlled substances.
Steroid-based AR agonists are being gradually phased out and replaced by nonsteroidal agonists.

3.2. Nonsteroidal AR Agonists. Early nonsteroid AR agonists were testosterone mimetics obtained from ligand-based drug design efforts. These compounds usually contain a hydrogen bond acceptor moiety that mimics the carbonyl group at C3 in DHT, a group that mimics the 17-hydroxy group in DHT, and a polycyclic ring (bicyclic, tricyclic, or tetracyclic) as epitomized by DHT and LGD2226 in Figure 5a.68,69 The determination of AR agonist cocrystal structures led to the discovery of cyano- or nitrophenyl-based compounds. The cyano motif mimics the carbonyl group at C3 in DHT, as illustrated in Figure 5b.70

3.2.1. Polycyclic AR agonists. On the basis of a systematic SAR study, Ligand Pharmaceuticals reported a series of trifluoromethyl quinolinone compounds (9−13) (Figure 6).71−80 These compounds have Ki values for binding against human AR (hAR) protein from 0.9 to 17 nM, a low-digit EC50 agonist potency against hAR in cell, and 82−132% efficacy compared with that of DHT (Table 1). In a mouse model, these compounds selectively target anabolic AR and are capable of stimulating muscle and bone marrow growth. Among them, 13 (LGD2941) had been in phase I clinical trials by Ligand Pharmaceuticals for the treatment of hypogonadism, female sexual dysfunction, and menopausal syndrome.81 Compounds 14 and 15 were developed by Merck as orally available full AR agonists.82,83 Compound 14 has an AR protein binding IC50 of 59 nM, an agonist EC50 in the TAMAR cells of 17 nM, and efficacy of 141% compared with that of DHT.82

Table 1. Activities of Compounds from Ligand Pharmaceuticals Incorporated

<table>
<thead>
<tr>
<th>compound no.</th>
<th>hAR agonist EC50 (nM)</th>
<th>hAR agonist efficacy (%)</th>
<th>hAR Ki (nM) (K_DHT=0.2)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>4</td>
<td>100</td>
<td>17</td>
<td>71, 72</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>95</td>
<td>4.6</td>
<td>73−75</td>
</tr>
<tr>
<td>11</td>
<td>1.1</td>
<td>132</td>
<td>8.7</td>
<td>76</td>
</tr>
<tr>
<td>12</td>
<td>1.1</td>
<td>82</td>
<td>0.9</td>
<td>77−79</td>
</tr>
<tr>
<td>13</td>
<td>7.1</td>
<td>109</td>
<td>6.5</td>
<td>80</td>
</tr>
</tbody>
</table>

Compound 15 (MK0773) is an analogue of 14 and has an AR binding IC50 of 6.6 nM.84 The heteroaromatic ring in 14 or 15 improved the pharmokinetic (PK) profile and oral bioavailability. In a rat study, compound 14 was reported to exhibit osteoblastic and muscle tissue selectivity as an oral agent. Compound 15 was selected for clinical study and, in a phase II clinical trials for sarcopenia in women, it was found to increase lean body mass but failed to improve muscle strength and function.85 Compound 16 is a tetracyclic indole disclosed by Johnson & Johnson with a pyridine nitrogen mimicking the carbonyl at C3 in DHT.85 Compound 16 has an IC50 of 29 nM in the rat AR COS-7 whole-cell binding assay, whereas R1881 (compound 4 in Figure 5) has an IC50 of 1.5 nM in the same assay. The carbazole derivative (17) (RAD35010) is an anabolic-selective agonist with an AR binding IC50 of 27 nM.86 In a castrated male rat model, 17 as an oral agent was able to restore levator ani weight to the sham level without increasing the weight of the prostate.

Figure 5. Alignment of DHT with nonsteroid AR agonists in AR. (a) Alignment of DHT (PDB ID: 1I37)68 with a nonsteroid LGD2226 (PDB ID: 2HVC)69 in a cocrystal structure. (b) Alignment of DHT (PDB ID: 1I37) with a cyano aromatic agonist (PDB ID: 2IHQ)70 in a cocrystal structure.

Figure 6. Structures of orally available polycyclic AR agonists.
3.2.2. Cyano/Nitroanilines. RAD140 (18) is a tissue-selective anabolic AR agonist with an AR binding $K_i$ of 7 nM (Figure 7). Interestingly, it binds to AR in breast cancer cells but not to AR in prostate cancer cells. RAD140 significantly increases fat and lean body mass in rats and monkeys at 0.1 mg/kg or higher with oral administration. In an AR+/ER+ breast cancer PDX model, RAD140 activates AR and suppresses ER.

RAD140 has been evaluated as a single agent in a phase I clinical trial in patients with AR+/ER+ breast cancer. Compound 19, a compound developed by Eli Lilly, has a $K_i$ value of 2 nM against AR and an EC$_{50}$ of 0.5 nM in AR-expressing C2C12 cells. It demonstrates an ED$_{50}$ of 0.14 mg/kg in muscle but has little effect on the prostate. Compound 19 has high skin permeability, which makes it suitable as a transdermal AR agonist.

Compounds 20–21 were developed by the Takeda Corp. as tissue-selective agonists (Figure 7). Compound 20 has an IC$_{50}$ value of 2.4 nM to hAR and an EC$_{50}$ of 0.32 nM in an hAR luciferase reporter assay in the Cos-7 cell line, and compound 21 has the corresponding values of 1.0 and 0.29 nM, respectively. In the male rat, compounds 20 [3 mg/kg, subcutaneous (SQ), 4 days] or 21 (1.5 mg/kg, SQ, 4 days) double the weight of the levator ani muscle but have no influence in prostate or seminal vesicles. Both compounds, however, demonstrate high brain concentrations in a rat tissue distribution study and are therefore unsuitable for further clinical development. Compound 22 was developed by Acadia Pharma. It has an AR binding IC$_{50}$ of 1 nM and efficacy equal to 81% of DHT. It also showed good oral bioavailability in rats and dogs. It was found to be tissue-selective in a male rat efficacy study, in which the levator ani weight increased by 68% compared with a 21% increase of the prostate after oral administration of 0.75 mg/kg for 14 days. LGD4033/VKS211 (23), an agonist developed by Ligand Pharmaceuticals, has a $K_i$ value of 1 nM against hAR. In rats, it shows anabolic selectivity in muscle and bone over prostate. Compound 23 is currently being developed by Viking Pharmaceuticals for the treatment of acute hip fractures in male patients over 65 years old.

Compound 24 is a full agonist with an imidazolopyrazole scaffold that was developed by Johnson & Johnson (Figure 7). During lead compound optimization, compounds were evaluated in a male rat model following PO administration. Compound 24 increases levator ani weight by 91% and prostate weight by 36% after 2 mg/kg dosing for 5 days. Compound 25 from BMS is a highly affinity AR agonist with a $K_i$ of 0.3 nM and an EC$_{50}$ of 0.2 nM, but no tissue selectivity in a male
castrated rat model. After 0.1 mg/kg PO (by mouth) of 25 for 14 days, the weight of both the levator ani muscle and the prostate increased by 100%. In addition to the absence of tissue selectivity, cyano phenylimino compounds (24, 25) are thought to have stability concerns, and this has hindered their further development.101,102

Compounds 26 and 27 from Karen Pharmaceuticals are designed to match the pharmacophore of DHT (Figure 7).98,99 The distances between the nitro group and the dimethyl in 26 and 27 are similar to that between the 3-carbonyl and the 18-methyl in DHT. Both compounds are pure agonists with rat AR binding IC50 values of 13 and 26 nM, and agonist EC50 values of 9.2 and 13 nM respectively. Although 26 and 27 bind selectively to AR better than other nuclear receptors, they lack tissue selectivity. Andarine (GTx-007) (Figure 8), an orally available tissue-selective agonist from GlaxoSmithKline (GSK) that restores the levator ani muscle to the sham level in a castrated rat model after 0.3 mg/kg PO for 28 days, but has little impact on prostate tissue. In a phase I clinical trial for the muscle atrophy of COPD patients, GSK2881078 was well tolerated and capable of increasing lean body mass in a dose-dependent manner. It has also completed the phase II clinical trial, but the results are yet to be released.103

Compound 30, a pyrazoline amide, shows pure agonist effects in both anabolic and androgenic systems (Figure 8).105 In rats, at 0.11 mg/day PO over 14 days, compound 30 achieves 90% of levator ani muscle stimulation compared with testosterone. At 0.76 mg/day, it demonstrates 51% prostate stimulation compared with testosterone, thereby indicating low tissue selectivity. Andarine (GTx-007) (31) and ostarine (GTx-024/MK-2866) (32) are AR agonists with AR binding Ki values of 4.0 and 0.55 nM, respectively.104,105 Ostarine modulates skeletal muscle through the selective potentiation of anabolic AR. In several phase II double-blind clinical trials, ostarine significantly improved total lean body mass and physical function.106

Compound 33 is a potent cyanophenyl cyclourea AR agonist developed by Bristol Meyers Squibb (BMS). It binds to AR with a Ki of 0.9 nM and an EC50 of 1.8 nM (Figure 8).107 In rats, it achieves a levator ani muscle ED50 of 0.09 mg/kg PO and exhibits a >50-fold difference of muscle over prostate selectivity. Furthermore, this compound demonstrated a 5.5 h plasma half-life and 65% oral bioavailability in a rat PK study. Compound 34 is a cyano phenylactam agonist developed by the Takeda Corp.108 It demonstrates an AR binding IC50 of 3.6 nM and EC50 of 4.7 nM. It also shows selectivity for muscle over prostate. Although both these compounds present overall promising profiles as drug candidates, to date, neither compound has been evaluated in clinical trials.

Compounds 35, an acyl and sulfonyl imide, and BMS564929 (36), a diaicyl imide, were designed by BMS using a structure-based drug design approach (Figure 8).109 Both compounds bind tightly to AR with a Ki of 0.45 and 3.2 nM, and an EC50 of 11.9 and 2.3 nM, respectively. Both compounds demonstrate tissue selectivity in rat with a simulation of levator ani muscle to prostate of >250- and >50-fold, respectively. However, 35 achieves good rat PK with iv administration while 36 is orally available. Compound 36 has been evaluated in a clinical trial, in which the side effect of an enlarged prostate was observed. Compound 37 is a hydantoin derivative developed by Johnson & Johnson.111 During the SAR study, the compounds were directly evaluated in a castrated rat model without in vitro assessment. Compound 37 stimulates levator ani muscle growth of 75% but only 11% growth of prostate after 2 mg/day for 5 days PO, which indicates it is a muscle-selective and orally available agonist.

The hydantoin compounds (38, 39) were obtained by modification of the AR antagonist nilutamide (40) (Figure 8).112,113 Compound 38 is a partial agonist with an AR binding IC50 of 0.9 nM. In a rat castrated model, it shows strong levator ani muscle stimulation and a weak antiandrogenic effect on ventral prostate with 30 mg/kg PO. Compound 39 (GLPG0492) is also a partial agonist with an AR IC50 of 13 nM. Compound 39 achieves >50% bioavailability compared with 9% of compound 38. Compound 39 has been evaluated in a phase I clinical trial, but its further clinical development was not reported.114

Compound 41, developed by Novartis, is a full agonist with an hAR binding IC50 of 0.7 nM and an EC50 of 0.5 nM in an AR functional assay (Figure 9).115 It demonstrates excellent human skin permeation in the absence of a penetration enhancer. In the Hershberger rat model, it completely restores skeletal muscle without any effect on the prostate. Compound 42 is an AR agonist developed by Pfizer to treat osteoporosis and frailty.116 It has an EC50 of 0.34 nM in ARE-Luciferase transfected CV-1 cells, but a low N/C (N-terminal/C-terminal) interaction with an EC50 of 1206 nM. In a rat efficacy study, 42 selectively stimulated the levator ani muscle over the prostate but achieved only modest efficacy at 10 mg/kg IP (intraperitoneal). The benzimidazole (43) is an agonist lacking cyano substitution on the phenyl ring, developed by Johnson & Johnson.117 Compound 43 exerts an agonist effect in muscle but an antagonist effect in the prostate. It has an AR binding IC50 of 2 nM and an ED50 of 0.01 mg/kg in rat. NEP28 (44) has a thiopeine ring in place of the phenyl ring and has an IC50 of ~10 nM in 22Rv1 pARE-LUC.118 It is selective for levator ani muscle over prostate in a rat castration model. However, it is capable of penetrating the CNS and modulating the amyloid-β degradation enzyme neprilysin, which indicates that it is not purely an AR agonist. Compound 45 was developed by GSK with optimized potency and physicochemical properties and has a high oral availability of 83%.119 The isoquinoline derivative (46) was developed by Pfizer to treat muscle weakness and has been evaluated in a phase 1 clinical trial.119

Stimulation of the levator ani over prostate in male rat is the standard method to evaluate the AR agonists’ in vivo anabolic-to-androgenic-selectivity. High in vivo efficacy and high selectivity.
of the levator ani over prostate are necessary for further evaluation. Some of the aforementioned AR agonists have been studied for their in vivo effects on the levator ani and prostate, and their results are summarized in Table 2.

4. ANDROGEN-COMPETITIVE ANTAGONISTS

Unlike agonists, which bind to AR and initiate gene transcription, antagonists bind to AR and recruit coregulators that inactivate AR gene transcription. Antagonists that bind to LBP are androgen-competitive AR inhibitors. AR antagonists have been developed for the treatment of human prostate cancer and other human conditions such as acne.

4.1. AR Antagonists for Prostate Cancer

Flutamide (53) and hydroxyflutamide (54) are two of the early AR antagonists reported (Figure 10).120,121 Flutamide is a prodrug and metabolizes to an active form, hydroxyflutamide. Hydroxyflutamide has an IC50 of 84 nM in androgen-sensitive SEM-107 Shionogi cells and an IC50 of 45 nM in T-47D breast cells. Both drugs are not tissue-selective and are used to treat prostate cancer as well as acne, excessive hair growth, and high androgens in women. However, because of their metabolically susceptible nitro group, both drugs have a very short half-life in vivo and must be taken three times a day. Both drugs have been replaced by new generation antagonists. Bicalutamide (55) is an antagonist drug used along with other androgen-deprived therapies.122 The IC50 of bicalutamide in LNCaP cells is 160 nM. The activity of bicalutamide is from the R-isomer, which binds to AR with a Ki value of 11 nM. The S-isomer is less active with an AR binding Ki of 365 nM. However, development of resistance has plagued bicalutamide. One resistance mechanism for bicalutamide is a switch from antagonist to agonist after AR mutation. Hence, modifications of bicalutamide to develop newer antagonists to overcome mutation-related resistance have been undertaken by different groups. Guerrini et al.123 introduced a benzyl ring to the hydroxyl carbon of R-bicalutamide to generate 56, which has an AR binding affinity and LNCaP-inhibitory activity similar to that of R-bicalutamide. It demonstrates inhibitory activity in bicalutamide-resistant CRPC- and AIPC-mutated cells and in androgen-independent PC-3 and DU145 cells. However, 56 is less effective than R-bicalutamide in the suppression of tumor growth in mouse xenograft models. GTx corp. found that replacement of the nitro group, both drugs have a very short half-life in vivo and must be taken three times a day. Both drugs have been replaced by new generation antagonists. Bicalutamide (55) is an antagonist drug used along with other androgen-deprived therapies.122 The IC50 of bicalutamide in LNCaP cells is 160 nM. The activity of bicalutamide is from the R-isomer, which binds to AR with a Ki value of 11 nM. The S-isomer is less active with an AR binding Ki of 365 nM. However, development of resistance has plagued bicalutamide. One resistance mechanism for bicalutamide is a switch from antagonist to agonist after AR mutation. Hence, modifications of bicalutamide to develop newer antagonists to overcome mutation-related resistance have been undertaken by different groups. Guerrini et al.123 introduced a benzyl ring to the hydroxyl carbon of R-bicalutamide to generate 56, which has an AR binding affinity and LNCaP-inhibitory activity similar to that of R-bicalutamide. It demonstrates inhibitory activity in bicalutamide-resistant CRPC- and AIPC-mutated cells and in androgen-independent PC-3 and DU145 cells. However, 56 is less effective than R-bicalutamide in the suppression of tumor growth in mouse xenograft models. GTx corp. found that replacement of the
phenyl ring by a bulkier aromatic naphthyl ring, as in 57, increased the potency against wild-type and W742L- and T878A-mutated AR.\textsuperscript{124−126}

Nilutamide (58) was discovered in 1977 and is used together with other androgen-deprived therapies (Figure 11).\textsuperscript{132} In the Shionogi cell proliferation assay, nilutamide has an IC\textsubscript{50} of 412 nM while the IC\textsubscript{50} of bicalutamide is 243 nM. Nilutamide is not tissue-selective and has been replaced by a new generation of antagonists, which include bicalutamide and enzalutamide. Substituents on the N of nilutamide appear to be tolerated and increase the AR binding affinity. RU-58441 (59) is 4 times more potent than nilutamide, but it is not orally available and has never been evaluated in clinical trials.\textsuperscript{133} BMS developed compounds 60 and 61 by extending the five-membered ring in nilutamide.\textsuperscript{127} Compounds 60 and 61 have K\textsubscript{i} values of 73 and 18 nM, respectively, in displacement of \textsuperscript{3}H-DHT in the AR+ MDA-453 breast cancer cell line (Table 3). These two compounds show IC\textsubscript{50} values of 6 and 35 nM, respectively, in MDA-MB-453 cells, and 4.75 and 3.92 \mu M, respectively, in LNCaP cells. Further modifications of 60 and 61 by altering the bicyclic ring and introducing heteroatoms and optimizing the tail substitution led to compounds 62−65. In MDA-453 cells, compounds 62−65 have K\textsubscript{i} values ranging from 1.7 to 14 nM, and IC\textsubscript{50} values from 16 to 91 nM.\textsuperscript{128−131,134} Compounds 62, 64, and 65 achieve micromolar activity in CWR22R cells. Compound 65 reduces seminal vesicle weight by 42% with 1 mg/kg PO dosing and by 74% at 10 mg/kg. As a result, 65 was evaluated in phase I dose escalation trials in CRPC but failed to reach phase II.\textsuperscript{135}

RU56187 (66), obtained by modification of nilutamide, is a prodrug that undergoes N-demethylation to produce an active compound (Figure 12).\textsuperscript{136,137} RU56187 is slightly less potent than DHT in binding to AR and is 3-fold more potent than bicalutamide in rat efficacy studies. However, RU56187 was used simply as a proof of concept of the thioxoimidazolidine scaffold and was not evaluated in clinical trials. In a follow-up study, Chugai Pharmaceuticals extended the substitution to obtain 67, which is stable in microsomes and does not undergo dealkylation.\textsuperscript{138} Compound 67 has an IC\textsubscript{50} of 440 nM in AR binding and 410 nM in LNCaP. In the LNCaP xenograft mouse model, 67 demonstrates efficacy comparable with that of bicalutamide.

Enzalutamide (68) and apalutamide (69) were both discovered by the Michael E. Jung group and have a similar

<table>
<thead>
<tr>
<th>compound no.</th>
<th>\textsuperscript{3}H-DHT displacement (MDA-453) K\textsubscript{i} (nM)</th>
<th>MDA-453 IC\textsubscript{50} (nM)</th>
<th>LNCaP IC\textsubscript{50} (nM)</th>
<th>seminal vesicle and prostate weight decrease</th>
<th>PO CWR22R mice dose (24 h exposure)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>73</td>
<td>60</td>
<td>4750</td>
<td></td>
<td>30 mg/kg (6 \mu M) 90 mg/kg (24 \mu M)</td>
<td>127</td>
</tr>
<tr>
<td>61</td>
<td>18</td>
<td>350</td>
<td>3920</td>
<td></td>
<td></td>
<td>127</td>
</tr>
<tr>
<td>62</td>
<td>2.4</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td>128</td>
</tr>
<tr>
<td>63</td>
<td>14</td>
<td>32</td>
<td>125</td>
<td></td>
<td></td>
<td>129</td>
</tr>
<tr>
<td>64</td>
<td>8</td>
<td>91</td>
<td></td>
<td></td>
<td>15 mg/kg (2.8 \mu M)</td>
<td>130</td>
</tr>
<tr>
<td>65</td>
<td>1.7</td>
<td>16</td>
<td>153</td>
<td>42% (15 mg/kg) 74% (10 mg/kg)</td>
<td>1 mg/kg (0.79 \mu M) 10 mg/kg (4 \mu M)</td>
<td>131</td>
</tr>
</tbody>
</table>

Figure 12. Structures of cyano phenyl thioxoimidazolidine AR antagonists.

Figure 13. Structures of cyano phenyl cycloalkyl ether AR antagonists.
Enzalutamide has an IC$_{50}$ of 36 nM in LNCaP cells. In mechanistic studies, it inhibits AR nuclear translocation, DNA binding, and gene transcription. In a mouse LNCaP xenograft model with overexpressed AR, enzalutamide achieves tumor regression at 10 mg/kg. Apalutamide has a similar xenograft model with overexpressed AR, enzalutamide achieves approximately 2-fold lower IC$_{50}$ in LNCaP than enzalutamide.

Several groups have pursued further modifications of enzalutamide. Cyclization of the five-membered enzalutamide ring with the second phenyl ring leads to a rigid compound (70), which is 2-fold less potent in LNCaP cells than enzalutamide.

Pfizer reported a series of cyanophenyl cycloalkyl ether compounds (72–77) as AR antagonists. Compounds 72 and 73 inhibit cell growth in AR-overexpressed LNCaP with IC$_{50}$ values of 59 and 144 nM, respectively. Both compounds possess good druglike properties including solubilities of 12 and 11.4 μM and calculated cLogP values of 3.8 and 4.5, respectively. In an LNCaP xenograft mouse model, with 100 mg/kg SQ administration for 72 and 25 mg/kg SQ administration for 73, both compounds strongly inhibited tumor growth after 5 weeks, at which time point both groups showed a prostate-specific antigen (PSA) decrease of >90%. However, 73 is rapidly metabolized by aldehyde oxidases, and consequently, two optimized compounds (74 and 75) were identified and proved to be unsusceptible to aldehyde oxidase. The lactam phenyl ether scaffold (76) has an AR antagonist IC$_{50}$ of 131 nM but it also demonstrates 89% of the agonist effect at 1 μM. In a rat PK study, 76 shows 64% oral bioavailability. Replacing the trifluoromethyl group in 76 with a chlorine afforded 77, which has an AR antagonist IC$_{50}$ of 68 nM and 89% of agonist effect at 1 μM.

The Takeda Corp. discovered a series of phenyl pyrrole and pyrazole AR antagonists (78–80). Unlike 79 and 80, compound 78 has hydroxymethyl and chlorine substituents on the phenyl ring. Compounds 78–80 have wild-type AR binding IC$_{50}$ values of 0.037, 0.034, and 0.43 μM and bicalutamide-resistant T877A AR binding IC$_{50}$ values of 0.1, 0.16, and 0.34 μM, respectively. In a COS-7 gene transcriptional assay, compounds 78–80 have antagonist IC$_{50}$ values of 0.025, 0.59, and 1.0 μM against wild-type AR and 0.11, 1.1, and 0.19 μM against T877A AR mutant, respectively. In an LNCaP-xenograft mouse model, 78 at 25 mg/kg after 28 days PO administration achieves 15% T/C (treatment group/control group), 79 at 40 mg/kg achieves 23% T/C, and 80 at 50 mg/kg achieves 17% T/C.

Darolutamide (81), which contains a central pyrazole, was recently approved by the U.S. Food and Drug Administration (FDA) for the treatment of CRPC. Darolutamide is a mixture of two diastereomers, which are interconvertible through a ketone form. Both diastereomers and the ketone form are active AR antagonists. In vitro, darolutamide has 8–10 times stronger affinity in AR binding than enzalutamide and apalutamide. It inhibits N/C interactions and AR homodimerization in both wild-type and W742C/L-mutated AR. In vivo, darolutamide inhibits tumor growth in VCaP, MR49F, and other AR mutant xenograft animal models.

Poutiainen et al. reported a cyclohexyl isoxazole compound (82) as an AR antagonist that inhibits 99.2% of DHT binding to AR. It has a PIC$_{50}$ of 7.56 in COS-1 cells, measured as AR-selective antagonists (Figure 16). In a rat PK study, 86 binds to hAR with a K$_{i}$ of 4.6 nM and to rat AR with a K$_{i}$ of 6.2 nM.
an AR-mediated transcriptional activation assay, 85 has an IC_{50} of 0.2 μM, and 86 has an IC_{50} of 0.11 μM. In a male Wistar rat efficacy study of 10 mg/kg for 15 days PO, 85 failed to decrease ventral prostate weight, but 86 reduced it by 67%. A further rat efficacy study determined the ED_{50} of 86 to be 2.2 mg/kg/day. Compound 85 is an antagonist designed by the Takeda Corp. on the basis of its in-house agonist (21). Compound 87 binds to wild-type AR with an IC_{50} of 0.83 μM and to T878A-mutated AR with an IC_{50} of 1.7 μM. In an LNCaP cell growth study, 87 was found to have an IC_{50} of 0.29 μM in wild-type and 1.7 μM in T878A mutation. At 1 μM concentration, it inhibits PSA expression by >60% in LNCaP cells. In an LNCaP xenograft mouse model, 87 at 30 mg/kg PO twice daily for 4 weeks has a minimal effect on tumor growth, consistent with no PSA reduction.

A series of benzimidazoles (88–89) and indoles (90–92) have been developed by Johnson & Johnson (Figure 17). Compounds 88–90 were directly evaluated in vivo without any in vitro assessment. In testosterone-treated castrated rats with dosing of 2 mg/day PO for 5 days, 88–90 reduced prostate weight by 96, 84, and 80%, respectively, which resulted in calculated ID_{50} values of 0.32, 0.13, and 0.13 mg/day. Compounds 91 and 92 were evaluated in vitro and have AR binding IC_{50} values of 140 and 100 nM compared with the bicalutamide value of 1300 nM.

AstraZeneca identified a trifluoromethyl triazolopyridazine (93) as a novel antagonist, with a PIC_{50} in AR binding of <4.1 and a PIC_{50} in AR downregulation of <5.8 (Figure 18). Compound 93 demonstrates 100% rat oral availability and reduces rat seminal vesicle weight by about 80% after dosing of 258 μM/kg twice daily PO for 7 days. Further optimization of 93 yielded AZD3514 (94). AZD3514 shows a PIC_{50} in AR downregulation of <5.7. In a PK study, AZD3514 demonstrates 74% oral availability in rats and 75% in dogs. In addition to inhibiting AR, AZD3514 induces AR degradation at 1 μM, which indicates that it has more than one mechanism of action. AZD3514 has been evaluated in a phase I clinical trial for CRPC. It provides modest efficacy and reduces PSA in patients but has significant side effects.

Compounds 95 and 96 are examples from two different series of antagonists reported by the Tanatani research group (Figure 19). Compounds 95 and 96 have IC_{50} values of 0.11 μM in wild-type AR, 0.006 μM in T878A-mutated AR, and an IC_{50} in SC-3 cells of 0.44 μM. Compound 96 has IC_{50} values of 0.18 μM in...
SC-3 cells and 0.56 μM in LNCaP and is capable of reducing ~80% of PSA expression in LNCaP. Compound 97, developed by Astellas, has IC_{50} values of 0.75 μM in SC-3, 0.043 μM in T887A-mutated LNCaP, 0.22 μM in H874Y-mutated 22RV1, and >10 μM in AR-independent PC-3. The Wipf group identified JJ-450 after systemic optimization of a high-throughput screening lead compound. The dextrorotary enantiomer (98) has an EC_{50} of 1.7 μM in C4-2-PSA-rl cell PSA luciferase assay, in which enzalutamide had an EC_{50} of 1.1 μM. The levorotary enantiomer 99 is 10 times less active. The binding site of this compound has not been identified and it may be involved in multiple mechanisms of action. It inhibits AR transcriptional activity by retarding AR translocation and also inhibits ARV7 transcriptional activity and ARV7 gene expression.

4.2. AR Antagonists for Topical Use. Pfizer reported a series of AR antagonists (100–106) (Figure 20). These compounds have hAR binding IC_{50} values of 20 to 100 nM and AR MDA-kb2 cell function IC_{50} values from 0.2 to 90 nM (Table 4). Most of these compounds reduce wax ester in an animal topical sebum model, but none of them has been evaluated in the clinic.

<table>
<thead>
<tr>
<th>compound no.</th>
<th>hAR binding IC_{50} (nM)</th>
<th>AR function IC_{50} (MDA-kb2) (nM)</th>
<th>cLogP</th>
<th>in vivo wax ester inhibition (3% formulation, 2 weeks)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>39.9</td>
<td>0.2</td>
<td>3.55</td>
<td>86%</td>
<td>167</td>
</tr>
<tr>
<td>101</td>
<td>20</td>
<td>45</td>
<td>3.38</td>
<td>83%</td>
<td>168</td>
</tr>
<tr>
<td>102</td>
<td>60</td>
<td>12</td>
<td>4.15</td>
<td>64%</td>
<td>169</td>
</tr>
<tr>
<td>103</td>
<td>26</td>
<td>90</td>
<td>3.75</td>
<td>85%</td>
<td>170</td>
</tr>
<tr>
<td>104</td>
<td>60</td>
<td>57</td>
<td>3.75</td>
<td>&gt;80%</td>
<td>171</td>
</tr>
<tr>
<td>105</td>
<td>43</td>
<td>78</td>
<td>3.65</td>
<td>&gt;80%</td>
<td>172</td>
</tr>
<tr>
<td>106</td>
<td>100</td>
<td>38</td>
<td>4.70</td>
<td>&gt;80%</td>
<td>173, 174</td>
</tr>
</tbody>
</table>

5. RELATION BETWEEN AGONIST AND ANTAGONIST

5.1. Methyl on the Phenyl Ring. As summarized above, cyano phenyl-based AR agonists may or may not possess a methyl at the meta position relative to the cyanophenyl group in the phenyl ring. However, none of the AR antagonists has a methyl substituent. The methyl moiety on the cyanophenyl ring may have certain functions contributing to the AR agonist effect. Sunden et al. proposed that the methyl groups could lead to a conformational difference in the whole molecule illustrated by compounds 108 and 109 (Figure 21). The methyl compound (109) is an AR agonist, while the desmethyl compound (108) is an AR antagonist. Sunden et al. suggests that the methyl dictates the conformation of the molecule (109), which allows H12 to flip back to the agonist position. However, the desmethyl compound (108) remains in a stable conformation that prevents H12 from switching back to the agonist position.

The methyl moiety may also have other functions that contribute to the agonist effect. Antagonists for prostate cancer are designed to target androgenic AR, and agonists are designed to target anabolic AR. It is possible that the difference between androgenic AR and anabolic AR may the methyl-containing compound better suited for anabolic AR. An example is RAD140 (18), which comprises a methyl moiety and is selective for AR in breast tissue over AR prostate tissue.

5.2. Size of the Molecule. Theoretically, agonists bind to AR and induce H12 to flip back to enclose the LBP, while antagonists bind to AR, and H12 remains open. Therefore, it may be thought that antagonists should be bulkier than agonists. However, this size theory is not necessarily correct. Hydroxyflutamide is an antagonist of androgenic AR (Figure 22). Andarine, which is significantly larger than hydroxyflutamide, is an agonist in anabolic AR and a partial agonist in androgenic AR.

5.3. Antagonist–Agonist switch. One of the resistance mechanisms to AR antagonists involves the antagonist–agonist switch. Upon AR mutation, an antagonist can turn into an agonist for the mutated AR (Figure 23). Bicalutamide is an agonist for wild-type AR, but it becomes an agonist for T878A- and T878S-mutated AR. Similarly, enzalutamide and apalutamide are weak agonists for F877L and F876L AR, but strong agonists in F877L/T878A-mutated AR.

6. CLINICAL DEVELOPMENT OF SECOND-GENERATION AR ANTAGONISTS

Among the many AR antagonists discussed above, enzalutamide, apalutamide, and darolutamide have been recently approved by the FDA for the treatment of human prostate cancer and are regarded as second-generation AR antagonists. These antagonists are AR-selective and have also eliminated the agonist effects associated with those first-generation AR antagonists such as flutamide and bicalutamide. The second-generation antagonists achieve improvements in potency and efficacy and have diminished side effects.

Enzalutamide (68) was the first second-generation antagonist approved by the FDA (Figure 24). In a preclinical study,
enzalutamide demonstrated high potency, efficacy, favorable oral PK, and few adverse effects (Section 4.1).\textsuperscript{180} In phase I/II studies, it is well tolerated for up to 4 years, with fatigue as the major side effect.\textsuperscript{181} It also demonstrates durable antitumor efficacy in naive and post chemotherapeutic CRPC, as measured by a reduction of PSA levels and tumor radiography. In phase II trials, >90\% of patients who are eligible for ADT on a treatment cohort achieved more than 80\% reduction of PSA at week 25.\textsuperscript{182} In a phase III study, enzalutamide afforded a significant survival benefit and improved quality of life over a placebo in prostate cancer patients. This was observed in androgen-sensitive prostate cancer and pre- and postdocetaxel mCRPC.\textsuperscript{183} Currently, combinations of enzalutamide with different classes of drugs are being extensively evaluated in clinical trials.

An uncommon but lethal side effect with enzalutamide is seizure resulting from its ability to cross the blood brain barrier (BBB) and bind to the GABA-gated chloride channel.\textsuperscript{184} Apalutamide and enzalutamide have a similar GABA binding affinity and IC\textsubscript{50} values of ∼3 \(\mu\)M. Apalutamide, with a polar nitrogen on the phenyl ring, is more hydrophilic than enzalutamide and, thus, has less BBB penetration. The concentration of apalutamide in brain tissue is four times lower than that of enzalutamide.\textsuperscript{185} A phase I study in mCRPC showed that apalutamide is capable of reducing the median PSA level >85\% at week 12.\textsuperscript{186} A phase II study with apalutamide demonstrated durable activity in non-mCRPC, pre- and post-abiraterone acetate, and prednisone mCRPC arms with a median PSA level at week 12 decreasing to 85\%, 88\%, and 22\%, respectively.\textsuperscript{187} Apalutamide in phase III trials of CRPC achieved median metastasis-free survival of 40 months compared with 16 months for the placebo.\textsuperscript{188} A common side effect of apalutamide is skin rash, which can be attributed to the cyano pyrimidine moiety, which is capable of attaching to cysteine residues in proteins through a reversible covalent bond.\textsuperscript{189}

JNJ-63576253 (110) is a clinical stage second-generation AR antagonist capable of overcoming a subset of enzalutamide and apalutamide resistance.\textsuperscript{190} It has an IC\textsubscript{50} of 54 nM in LNCaP cells in a transcriptional reporter assay. It is also capable of inhibiting enzalutamide- and apalutamide-resistant F877L mutant LNCaP with an IC\textsubscript{50} of 37 nM in the transcriptional reporter assay. In addition, it inhibits VCaP proliferation with an IC\textsubscript{50} of 290 nM. JNJ-63576253 achieves 45\% oral availability in mouse. In an in vivo efficacy study in an LNCaP F877L xenograft mouse model, JNJ-63576253 achieved 87\% tumor growth inhibition with daily dosing of 30 mg/kg PO. This compound is currently being evaluated in phase I/II clinical trials in CRPC patients with F877L mutation.\textsuperscript{191}

A preclinical study of darolutamide revealed multiple advantages over enzalutamide, including higher potency, the
achieve its end point. An optimized compound, EPI-7386, phase I/II clinical trial for EPI-506 in CRPC patients failed to do so. Furthermore, darolutamide exhibits very poor BBB permeation with the ratio of brain-to-blood concentration in mice >10-fold lower than for enzalutamide. Phase I/II studies demonstrated that darolutamide is well tolerated and effective in mCRPC and reduces PSA by >50% in 70% of patients at week 12. In a phase III study, darolutamide provided, on average, ~7 months improvement in overall survival, progression-free time, and quality of life.

Despite its clinical success, resistance to enzalutamide is observed in the clinic within 3 to 6 months that is cross-resistant with abiraterone. The mechanisms of resistance to enzalutamide have been reviewed elsewhere. Because of their similar mechanisms, it is expected that resistance to apalutamide and darolutamide will be reported in the future.

7. NONANDROGEN COMPETITIVE AGENTS

7.1. AF1 Site. EPI-001 (111) is a biphenyl derivative and the first compound known to inhibit the AF1 of NTD (Figure 25). It blocks the N/C interaction required for the transcriptional activity of AR and its splice variants by covalently inhibiting transcriptional activation units 1 and 5 on NTD. EPI-001 has an IC50 of ~6 μM for the inhibition of AR NTD transactivation. However, as a covalent inhibitor, EPI-001 also modulates peroxisome proliferator-activated receptor gamma, which indicates multiple mechanisms of action (MOAs) may contribute to its activity. EPI-002 is an enantiomerically pure version of EPI-001. EPI-506 is the acetate prodrug of EPI-002 and it inhibits AR NTD transactivation with an IC50 of ~9.6 μM. A phase I/II clinical trial for EPI-506 in CRPC patients failed to achieve its end point. An optimized compound, EPI-7386, with an undisclosed structure, is a member of the latest generation of bisphenol NTD inhibitors. It has an IC50 of 535 nM for AR NTD transactivation.

The dihydrosoquinolinicotinamide compound (114) is a novel scaffold that binds to the AF1 site. At a concentration of 10 μM, it demonstrates a >95% AR antagonist effect and a <5% DHT agonist effect and inhibits LNCaP cell growth by >90%.

Compound 114 has an IC50 of 0.78 μM in NTD-overexpressed HEK293T cells. Western blotting assays showed this compound reduced AR and ARV levels in LNCaP, CWR22rv, DU145, PPC1, and HEK293T cell lines at a concentration of 10 μM. In a cellular proliferative assay, this compound has IC50 values around 1 μM in AR-independent C4-2 and CWR22rv cell lines. Compound 114 inhibits tumors with 10 mg/kg of 21 days IP dosing in CWR22rv xenograft mouse with T/C of 65%.

The Miller group cyclized the linker and the B-ring in bicalutamide and obtained UT-155 (115) and UT-034 (116). Both UT-155 and UT-034 bind to the AF1 site and function as SARDs. Although they are derivatives of bicalutamide, UT-155 and UT-034 do not bind to the LBP pocket with LBP K values greater than 10 μM; both reduce full-length AR and ARV at a 1 μM concentration in LNCaP and 22Rv1 cell lines and inhibit cellular growth >70% in an enzalutamide-resistant MR49F cell line. In a mouse efficacy study with 100 mg/kg dosing, both compounds suppressed tumor growth in both an LNCaP castrated model and an MR49F model.

7.2. AF2 Site. Compounds 117 to 120 were identified by virtual screening and have micromolar IC50 values in the inhibition of AR and co-regulatory SRC2 and SRC3 interactions (Figure 26). Compound 120 has IC50 values of 26.3 μM against wild-type AR and 33.2 μM in T878A-mutated AR. At a 10 μM concentration, it inhibits LNCaP cell growth by ~30% and also reduces PSA expression by ~30%. However, 120 consists of an acylhydrazone moiety that is known to be labile in the acidic environment found in cancer cells.

7.3. BF3 Site. Similar to AF2 binders, BF3 binders (121–123) are tool compounds (Figure 27). In an enhanced green fluorescent protein transcriptional (eGFPT) assay, the IC50 values are 0.43 μM for 121 and 1.5 μM for 122. Compound 122 decreases PSA in LNCaP with an IC50 of 0.53 μM. At a 6 μM concentration, 122 reduces PSA >80% in LNCaP cells and >60% in enzalutamide-resistant LNCaP. Compound 123 has an IC50 of 6.26 μM in an AR luciferase activity binding assay, whereas that of bicalutamide is 0.27 μM. Compound 123 has an IC50 of 11.57 μM in LNCaP, compared with bicalutamide whose IC50 is 23.79 μM. Compound 124 was identified from virtual screening, and 125 is an orally bioavailable prodrug of 124. Compound 125 inhibits the PSA level in LNCaP cell line with an IC50 of 0.66 μM.
125 inhibits tumor growth in LNCaP xenograft mice, but is less efficacious than enzalutamide.

7.4. DBD. Pyrvinium (126) was identified as the first DBD inhibitor with an IC\textsubscript{50} of 0.19 \(\mu\)M in AR transcriptional activity (Figure 28).\textsuperscript{212} Compound 127 is a lead compound obtained from virtual screening and has an IC\textsubscript{50} of 0.33 \(\mu\)M in an eGFPT assay and an IC\textsubscript{50} of 0.28 \(\mu\)M in reducing PSA in LNCaP. An SAR study and optimization of 127 led to 128, which has IC\textsubscript{50} values of 0.10 \(\mu\)M in an enhanced green fluorescence cellular AR transcription assay and 0.17 \(\mu\)M in an LNCaP PSA assay. In a cell proliferation assay, the IC\textsubscript{50} values for 128 were <0.5 \(\mu\)M in LNCaP and <0.5 \(\mu\)M in MR47F.\textsuperscript{213}

8. SARD

The rapid emergence of resistance to AR antagonists in the clinic has necessitated new approaches of targeting AR. Taking a page from selective estrogen receptor degraders (SERD) in the estrogen receptor arena, the selective androgen receptor degrader (SARD) is thought to be a potentially promising strategy with which to treat prostate cancer.\textsuperscript{214} SARD is proposed to disrupt the AR coregulatory protein–protein interaction and lead to a proteasome-dependent degradation of the AR protein, potentially through an enhanced association of AR with MDM2, which is an E3 ligase.\textsuperscript{215} However, the exact mechanism of SARDs is still not clear.

Some compounds, such as 114, 119, and 120 described above, when binding to the AF1 site, are found to be able to degrade full-length AR and ARVs. Further clinical evaluation of these compounds, which may help to understand the role of ARV in CRPC, is awaited.

Compounds 129 and 94 are also identified as SARDs, but their binding site is unknown. At 7.5 \(\mu\)M, ASC-J9 degrades >80% of AR and ARV3, completely inhibits DHT (1 nM and 10 nM), and inhibits the growth of C4-2, C81, CWR22RV1, and LNCaP cells at a concentration of 5 \(\mu\)M (Figure 29).\textsuperscript{216} A mechanism of action study showed that ASC-J9 interrupts the AR-AR70 and AR-SRC interactions in LNCaP cells and it interrupts the AR-AR5 interaction in WPMY-1 cells. ASC-J9 inhibits C81, C4-2, and LNCaP xenograft mouse tumor growth with 75 mg/kg dosing. It is currently being evaluated in a phase II clinical trial as an acne cream. As discussed above, AZD3514 can be classified as a SARD, but its mechanism is unknown.

Compound 130, a derivative of RU56187 can also degrade AR. It achieves a maximum of >50% of AR degradation in LNCaP cells. It also blocks cell growth in LNCaP and MR49F at a 3 \(\mu\)M concentration.\textsuperscript{217} Although 130 is a weak degrader, it validates a concept that AR can be degraded by AR inhibitor conjugates.

9. PROTACs

In 2001, the laboratories of Deshaies at the California Institute of Technology and Crews at Yale University published their groundbreaking paper, which formally documented the concept of proteolysis-targeting chimeras (PROTACs).\textsuperscript{218} A PROTAC is a bifunctional small molecule consisting of a ligand for the protein of interest (POI) and a ligand to bind to and recruit an E3 ligase or an E3 ligase complex, tethered together through a linker. A PROTAC molecule brings the POI to close proximity of the E3 ligase/E3 ligase complex for ubiquitination, followed by proteasome-dependent degradation. In the past few years, major progresses have been made in the discovery and development of PROTAC degraders for a large number of proteins, including AR.

For the design of PROTAC degraders against AR, the AR ligand can be agonists, antagonists, or AR allosteric site binders, as described above. E3 ligase systems, such as inhibitors of
MDM2, apoptosis proteins (IAP), the von Hippel–Lindau (VHL)/cullin 2, and the cereblon/cullin 4A, have been used in the design of PROTAC degrader molecules. The E3 ligase systems and their utilities in PROTACs have been recently reviewed.\(^{219}\)

In 2008, the Crews laboratory reported the first PROTAC AR degrader (131), which was designed using an MDM2 inhibitor as the E3 ligase ligand and bicalutamide as the AR antagonist.\(^{220}\) While compound 131 only degraded AR protein in cells at micromolar concentrations, it provided an important proof-of-concept (Figure 30).

Scientists from the Takeda Corp. employed IAP ligands for the design of bifunctional degraders, which they named specific and nongenetic inhibitors of apoptosis protein-dependent protein erasers (SNIPERs). Compound 132 was one such SNIPER molecule for AR.\(^{221}\) This compound (132) reduces AR at 1 μM in 22RV1 and VCaP cells. In the VCaP cell line, it also inhibits AR-mediated gene expression and suppresses cell growth. In addition to AR degradation, 132 induces the degradation of cIAP1 protein by binding to cIAP1, which leads to the activation of caspase and the apoptosis of VCaP cell line.
PROTAC 133 is obtained by linking the cereblon ligand thalidomide to a relatively weak AR antagonist RUS6187. PROTAC 133 demonstrates AR degradation in LNCaP and VCaP in a dose- and time-dependent manner. It also inhibits LNCaP cell proliferation, migration, and invasion, but it is a weak AR degrader with potency or efficacy not superior to that of enzalutamide. Kim et al. designed the PROTAC 134 using a bicalutamide derivative as the AR ligand and thalidomide as a cereblon ligand. Compound 134, however, is a weak AR PROTAC with a DC₅₀ value of 5.2 μM in LNCaP cells.

Highly potent AR PROTACs were designed using potent AR antagonists and VHL ligands, represented by compounds 135−137. Crews et al. reported an enzalutamide VHL-based PROTAC ARCC-4 (135), which achieves low nanomolar degradation potencies in VCaP, LNCaP, 22RV1, and other AR+ prostate cancer cell lines, as well as in the AR+/ER+ T47D breast cancer cell line. It also inhibits the growth of prostate cancer cells. Our group discovered ARD69 after a systematic optimization of the VHL ligand portion by testing different AR antagonists and optimizations of the linker. ARD69 reduces AR and PSA effectively. In a follow-up study, a weak VHL ligand was used for the successful design of a potent and efficacious AR PROTAC ARD266 (137). Despite their exceptional degradation potency, compounds 136−137 have low oral bioavailability in animals, which limits their further development. ARD69 and ARD266 showed that PROTACs with highly rigid linkers not only are feasible but also may provide better potency.

AR PROTACs designed from a potent AR ligand and cereblon ligand with rigid linkers have demonstrated excellent AR degradation potency, potent cell growth inhibitory activity, good PK profiles, and efficacious antitumor efficacy. ARV-110 from Arvinas is the first AR PROTAC evaluated in clinical trials. ARV110 utilized a potent AR antagonist, which is derivative of 72 (Section 4) and a thalidomide analogue. In a preclinical study, ARV-110 degrades AR at low nM concentrations in LNCaP and VCaP cells. It also reduces AR-related gene expression in low nanomolar concentrations and inhibits LNCaP and VCaP cell growth at nM concentrations. In a mouse VCaP xenograft model, a single dose of ARD69 reduces AR and PSA effectively. In a follow-up study, a weak VHL ligand was used for the successful design of a potent and efficacious AR PROTAC ARD266 (137). ARD2585 demonstrates excellent PK in mice and has 51% oral bioavailability. It is more efficacious than enzalutamide in suppressing tumor growth in mice VCaP xenograft without any sign of toxicity.

The data on ARV-110, ARD-2128, and ARD-2585 showed that highly potent and orally active AR PROTAC molecules can be successfully designed using a potent AR antagonist and a cereblon ligand.

PROTACs based on inhibitors binding to NTD pockets should be able to degrade ARV and full-length AR simultaneously. AF1 and DBD are the two known binding sites in NTD. Some of the AF1 site inhibitors are SARDs (Section 8). Hence, an AR PROTAC based on a DBD site inhibitor would be a good choice for an ARV degrader. MTX-23 is the first PROTAC based on a derivative of 128 (section 7.4) and a VHL ligand. MTX-23 degrades ARV7 and full-length AR with DC₅₀ values of 0.37 and 2 μM, respectively (Figure 31).

MTX-23 inhibits cellular growth of apalutamide- and darolutamide-resistant 22RV1 and VCaP cell lines at a concentration of 1 μM and suppresses tumor growth in enzalutamide-resistant 22RV1 mouse xenografts. Although a modestly potent degrader of ARV7 and full-length AR, MTX-23 is a useful tool compound with which to validate the concept of AR PROTACs.

10. FUTURE PERSPECTIVES

In the past half century, significant efforts from pharmaceutical companies and academic research laboratories have been invested in the development of AR agonists. Besides the early achievements in steroid-based AR agonists, no nonsteroid AR agonist has been approved by the FDA to date. However, exploration at the molecular level of nonsteroid AR agonists with LBP has led to great progress in AR antagonists since the year 2000. Second-generation AR antagonists enzalutamide, apalutamide, and darolutamide are selective, potent, and efficacious and have fewer side effects in the clinic. However, the rapid emergence of resistance to these drugs necessitates new strategies to target AR.
The recently established surface druggable pockets, AF2, BF3, and DBD, may allow circumvention of some of the LBP-related drug resistance mechanisms. Some tool compounds have been identified for each binding site. More potent and selective compounds for these sites may be needed to further understand their MOAs. With the cocrystal structures of the AF2, BF3, and DBD binding pockets, structure-based drug design is feasible.

Although the cocrystal structure of the AF1 site is still unavailable, compounds 115 and 116 from the Miller group shed some light in this direction.203–205 These compounds are minor modifications of bicalutamide but bind to the AF1 site and act as SARD molecules. Given the success in selective estrogen receptor degraders (SERD), AF1-binding SARDs are an important direction in AR research.

In the past few years, AR PROTACs have become a hot topic in AR research and AR-targeted therapy. Interestingly, despite a large number of agonists and antagonists, reported AR PROTACs have employed only a few AR inhibitors and their large number of agonists and antagonists, reported AR inhibitors in AR research and AR-targeted therapy. Interestingly, despite a large number of agonists and antagonists, reported AR PROTACs have employed only a few AR inhibitors and their large number of agonists and antagonists, reported AR inhibitors in AR research and AR-targeted therapy. Interestingly, despite a large number of agonists and antagonists, reported AR inhibitors in AR research and AR-targeted therapy.

Inhibitor, can degrade ARV7. Further optimization of MTX-23 in inhibiting ARV7. PROTACs like MTX-23, based on a DBD and BF3, may allow circumvention of some of the LBP-related drug resistance mechanisms. Some tool compounds have been identified for each binding site. More potent and selective compounds for these sites may be needed to further understand their MOAs. With the cocrystal structures of the AF2, BF3, and DBD binding pockets, structure-based drug design is feasible.

In the past few years, AR PROTACs have become a hot topic in AR research and AR-targeted therapy. Interestingly, despite a large number of agonists and antagonists, reported AR PROTACs have employed only a few AR inhibitors and their large number of agonists and antagonists, reported AR inhibitors in AR research and AR-targeted therapy. Interestingly, despite a large number of agonists and antagonists, reported AR inhibitors in AR research and AR-targeted therapy.

Inhibitor, can degrade ARV7. Further optimization of MTX-23 in inhibiting ARV7. PROTACs like MTX-23, based on a DBD and BF3, may allow circumvention of some of the LBP-related drug resistance mechanisms. Some tool compounds have been identified for each binding site. More potent and selective compounds for these sites may be needed to further understand their MOAs. With the cocrystal structures of the AF2, BF3, and DBD binding pockets, structure-based drug design is feasible.

Inhibitor, can degrade ARV7. Further optimization of MTX-23 in inhibiting ARV7. PROTACs like MTX-23, based on a DBD and BF3, may allow circumvention of some of the LBP-related drug resistance mechanisms. Some tool compounds have been identified for each binding site. More potent and selective compounds for these sites may be needed to further understand their MOAs. With the cocrystal structures of the AF2, BF3, and DBD binding pockets, structure-based drug design is feasible.

Wild-Type Androgen Receptor in Muscle Recapitulates Polyglutamine 2013

Resistance in Castration-Resistant Prostate Cancer Cell Lines. 2013, 3 (9), 1020–1029.


Controlled Clinical Trial to Study the Efficacy and Safety of the Selective Androgen Receptor Modulator (Sarm) Mk-0773 in Female Participants with Sarcopenia. J. Nutr Health Aging 2013, 17 (6), 533–543.


(102) Neil, D.; Clark, R. V.; Magee, M.; Billiard, J.; Chan, A.; Xue, Z.; Russell, A. Gsk2881078, a Sarm, Produces Dose-Dependent Increases in Lean Mass in Healthy Older Men and Women. J. Clin Endocrinol Metab 2018, 103 (9), 3215–3224.


Androgen Receptor Antagonists.


(184) Crona, D. J.; Whang, Y. E. Posterior Reversible Encephalopathy Syndrome Induced by Enzalutamide in a Patient with Castration-Resistant Prostate Cancer. Invest New Drugs 2011, 29 (3), 751–754.


Benzimidazole Derivatives.

The Binding Function 3 (Bf3) Site of the Androgen Receptor through Hydrophobic Tagging.


---

**Recommended by ACS**

**Designing Selective Drug-like Molecular Glues for the Glucocorticoid Receptor/14-3-3 Protein–Protein Interaction**

Jakob S. Pallesen, Gavin O’Mahony, et al.

DECEMBER 09, 2022
JOURNAL OF MEDICINAL CHEMISTRY

**Discovery of Spiro[pyrrolidine-3,3′-oxindole] LXRβ Agonists for the Treatment of Osteoporosis**

Hao Chen, Qiong Gu, et al.

DECEMBER 20, 2022
JOURNAL OF MEDICINAL CHEMISTRY

**Structure-Based Design of Novel Alkynyl Thio-Benzoxazepinone Receptor-Interacting Protein Kinase-1 Inhibitors: Extending the Chemical Space from the Alloster...**

Danni Quan, Chunlin Zhuang, et al.

FEBRUARY 01, 2023
JOURNAL OF MEDICINAL CHEMISTRY

**Discovery of Nanomolar DCAF1 Small Molecule Ligands**

Alice Shi Ming Li, Masoud Vedadi, et al.

MARCH 22, 2023
JOURNAL OF MEDICINAL CHEMISTRY

Get More Suggestions >