Current and emerging approaches to noncompetitive AR inhibition

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Abstract
The androgen receptor (AR) has been shown to be a key determinant in the pathogenesis of castration-resistant prostate cancer (CRPC). The current standard of care therapies targets the ligand-binding domain of the receptor and can afford improvements to life expectancy often only in the order of months before resistance occurs. Emerging preclinical and clinical compounds that inhibit receptor activity via differentiated mechanisms of action which are orthogonal to current antiandrogens show promise for overcoming treatment resistance. In this review, we present an authoritative summary of molecules that noncompetitively target the AR. Emerging small molecule strategies for targeting alternative domains of the AR represent a promising area of research that shows significant potential for future therapies. The overall quality of lead candidates in the area of noncompetitive AR inhibition is discussed, and it identifies the key chemotypes and associated properties which are likely to be, or are currently, positioned to be first in human applications.

KEYWORDS
androgen receptor, developability properties, noncompetitive inhibition, prostate cancer

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Despite decades of research, prostate cancer remains the second most common cancer in men with estimates exceeding 1.4 million new diagnoses and 370,000 deaths worldwide in 2020.\(^1\) With the advent of modern screening techniques enabling earlier detection and intervention, primary localized therapy can successfully treat 65%–80% of prostate cancer cases. However, for the proportion of patients that relapse, disease progression to castration-resistant prostate cancer (CRPC) invariably occurs.\(^2\)–\(^4\) Upon relapse, systemic treatment is pursued in the form of androgen deprivation therapy (ADT), which is achieved by surgical and/or chemical castration and reduces serum androgen levels by up to 95%.\(^5\) These approaches have been developed over the last several decades since the pioneering discoveries by Charles Huggins in 1941, where he described how surgical and chemical castration reduces androgen levels, leading to tumor regression and symptom alleviation.\(^6\) Although ADT affords an initial improvement, progression to CRPC usually occurs within 12–48 months, at which point, further treatment is limited to radiotherapy or chemotherapy, with the median survival ranging from 9 to 30 months.\(^7,8\) Recent efforts to develop new treatments have been relatively successful. Indeed, with the introduction of new antiandrogenic drugs such as enzalutamide (nonsteroidal) and abiraterone (steroidal), the patient outlook has improved, with a 15% increase in 5-year overall survival.\(^9\) Nevertheless, the prognosis for CRPC remains poor and the need for continued improvement of its treatment is clear.

At the core of the pathogenesis of prostate cancer is the androgen receptor (AR), a 110 kDa nuclear receptor (NR) primarily responsible for the androgen-mediated regulation of gene expression. Its function is vital for the growth and maintenance of both normal and carcinogenic prostate tissue.\(^10\) The AR protein consists of four domains: the C-terminal ligand-binding domain (LBD) where the ligand-binding pocket (LBP) resides, the DNA-binding domain (DBD), the amino-terminal domain (NTD), and a hinge region that connects the LBD and the DBD (Figure 1).

The AR gene is commonly mutated in prostate cancer and the receptor itself has been extensively validated as a drug target.\(^11\) When disease progression to CRPC occurs, the AR remains a potent driver for cancer growth and metastasis.\(^12,13\) There is considerable evidence implicating the aberrant activation of the AR during ADT as a potential cause for the development of CRPC.\(^11\)–\(^13\) Antiandrogens are antagonists that compete with androgens to bind the AR-LBP and have been one of the most significant targets for drug development for CRPC therapies. Steroidal and more recently nonsteroidal compounds have been developed for the treatment of advanced and metastatic prostate cancer in recent decades.\(^14\)–\(^16\) Although these drugs are initially successful, a complex variety

![Figure 1](https://wileyonlinelibrary.com/doi/吸收)
of resistance mechanisms have emerged that drive tumor progression. The development of treatment resistance and potential methods of overcoming it has garnered significant interest in recent years.\textsuperscript{12,17–19} Emerging approaches have shifted attention away from the LBP, focusing on the development of compounds that target the AR protein via alternative domains.\textsuperscript{20–22}

In the current review, our aim is to provide a detailed account of contemporary treatments that target the AR beyond the LBP and to critically evaluate the developability of emerging lead compounds. The direct comparison of compounds reported herein is intrinsically difficult due to the heterogeneous nature of the reported data, which spans multiple laboratories, and the variety of biological assays utilized. Hence, a compound-by-compound approach will be taken, outlining activity against full-length AR (AR-FL) and mutant variants, lacking the LBD (AR-V) commonly associated with CRPC, such as AR-V7.\textsuperscript{23} Our analysis aims to highlight structural properties from a medicinal chemistry perspective and employs the use of in silico physicochemical property prediction data. Due to the multifaceted nature of lead optimization impacting on the potency, selectivity, drug metabolism, and pharmacokinetics (DMPK) of a specific compound, attention will be paid to several developability metrics and molecular properties that can be used to characterize candidate drugs. Of particular importance throughout this review are molecular weight (MW), topological polar surface area (TPSA), calculated lipophilicity coefficient (cLogP) calculated using the open source software DataWarrior, number of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) defined by Lipinski’s rules,\textsuperscript{24,25} and intrinsic property forecast index (iPFI), a metric proposed by Young et al. that is the sum of cLogP and aromatic ring count which is important with considercations such as solubility and off-target effects.\textsuperscript{26}

2 | TARGETING THE LBD

Currently, there are several approved nonsteroidal antiandrogen drugs such as \textit{flutamide}, \textit{bicalutamide}, and \textit{enzalutamide} which share a structural motif—an anilide-bearing electron-withdrawing cyano, nitro, and/or trifluoromethyl groups. Contemporary antiandrogens \textit{apalutamide} and \textit{darolutamide} deviate slightly from this, incorporating a pyridine ring and omitting the anilide moiety, respectively; however, they maintain a degree of structural similarity with their predecessors (Figure 2).

Several molecular properties can give discovery teams an indication of how a given molecule might behave in vivo, particularly pertaining to MW and LogP. These properties allow insight into the dynamics of ADME behavior; that is, how a molecule is absorbed, distributed, metabolized, and excreted from a site of action.\textsuperscript{27} The compounds shown in Figure 2 will be nonionized under physiological conditions, and despite their low aqueous solubility, are well absorbed in the gastrointestinal tract, likely due to good permeability as demonstrated experimentally using Caco-2 cells.\textsuperscript{28} Oral absorption of these drugs is good, except for \textit{darolutamide} which is considered to be moderate.\textsuperscript{29} The high bioavailability observed in the remaining examples can be attributed to cLogP values in the range of 2.0–3.6, MWs below 500 Da, and a minimal rotatable bond count.\textsuperscript{30} \textit{Enzalutamide} can penetrate the blood–brain barrier (BBB), possibly due to a low TPSA (<110 Å), giving rise to off-target effects at the \(\gamma\)-aminobutyric acid receptor, presenting a potential risk of seizure.\textsuperscript{15} Next-generation analogs, \textit{apalutamide} and \textit{darolutamide} mitigated these risks via reduced penetration of the BBB and have also displayed efficacy in \textit{enzalutamide}-resistant models of CRPC.\textsuperscript{15,31} Despite these advancements resistance eventually occurs, warranting further investigation of novel treatments.\textsuperscript{32}

The current understanding of AR-dependant resistance mechanisms include the following: (1) constitutively or conditionally active AR-Vs which lack the LBD entirely, (2) AR point mutations that can confer ligand promiscuity or increased stability to degradation, (3) gene amplification and receptor overexpression, (4) intra-tumoural or adrenal androgen synthesis, (5) interference with AR coregulation, and (6) ligand-independent receptor activation.\textsuperscript{17,19,33–35} Some of these mechanisms do not rely on the availability of androgens or antiandrogens; thus, the exclusive
development of LBP antagonists is likely to encounter resistance similar to current treatments. This can be observed in reports of receptor point mutations and splice variants giving rise to enzalutamide resistance.36,37

3 ACTIVATION FUNCTION 2-TARGETING COMPOUNDS

When agonists bind to the LBP, a conformational change occurs, forming activation function 2 (AF-2)—a surface-accessible hydrophobic pocket available for protein–protein interactions (PPIs).38–40 The AF-2 region interacts with cofactors, the NTD, and the LBD, making it an attractive target to regulate AR activity via orthosteric inhibition of key PPIs.41,42 The AF-2 domain has been validated as a route for inhibition using peptide antagonists with multiple supporting studies.43–45 Based on this, the following small molecules have been identified or designed to directly, orthosterically disrupt the interaction between the AR AF-2 and coregulatory proteins in the AR signaling pathway. The development of small molecule coactivator binding inhibitors was described by Gunther et al. in 2009.46 A pyrimidine-core estrogen receptor (ER) AF-2 inhibitor was modified, increasing the steric bulk of side chains, thus affording selectivity for the AR AF-2 over other hormone receptors, exemplified by Compound 7 (Table 1). The series displayed efficacy against both wild-type AR (AR-WT) and the flutamide-resistant T877A mutant with IC50 values of 1.6 and 9.4 µM, respectively, in a luciferase assay. Noncompetitive receptor inhibition with respect to dihydrotestosterone (DHT), as well as a radiometric binding assay using tritium-labeled synthetic androgen R1881, both provide evidence for orthosteric inhibition of the AR-steroid receptor coactivator (AR-SRC) interaction.

Initially developed as structure-based peptidomimetics, the introduction of flexible aliphatic chains (compound 1 and 3) and several aromatic rings (compound 2 and 7) afforded specificity toward the desired target. However,
these structural features also contribute to increased lipophilicity, which could lead to off-target effects, albeit toxicity was not seen at even high concentrations. The main drawback of these compounds remains poor solubility; however, this could be circumvented via the incorporation of heterocycles and polar substituents into the peripheral aromatic rings.

Computer-aided drug discovery approaches have been used to screen the ZINC lead-like database—a curated collection of approximately 4 million biologically relevant compounds. Utilizing a docking study, combined with experimental approaches, Axerio-Cilies et al. identified two hits, compounds 4 and 6 that bound directly and specifically to AF-2 to inhibit recruitment of AR coactivator SRC2 (Figure 3).

The more potent analog, compound 4, displaced SRC2 with an IC₅₀ of 8.2 µM and inhibited AR transactivation with an IC₅₀ of 34.4 µM determined by a nondestructive cell-based enhanced green fluorescent protein (eGFP) assay (Table 2); compound 6 performed similarly in the eGFP assay and was threefold less potent in the SRC displacement assay. Direct and reversible interaction between the compounds and the AR was established via biolayer interferometry (BLI). Any interaction with the LBP was excluded via an androgen displacement assay. Moreover, a crystal structure was determined with a closely related analog (compound 5) shown to be directly situated at AF-2 (Figure 4).

Compounds 4 and 6 both comply with Lipinski’s rules, and the cLogP and iPFI imply good solubility; however, the presence of polar groups combined with relatively low lipophilicity could potentially have a negative impact on membrane permeability. Compound 4 did not show toxicity liabilities; however, compound 6 displayed cytotoxicity in an MTS assay (50% inhibition at 50 µM) which could be ascribed to the hydrazide moiety, a known toxicophore.

A further virtual screening campaign for AF-2 antagonists yielded a diarylhydrazide series typified by MDG506, which inhibited AR-WT and T877A in a TR-FRET assay with IC₅₀ values of 26 and 33 µM, respectively (Figure 5A). Lloyd et al. highlighted the potentially problematic electrophilic hydrazide linker necessitating...

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**Table 1**: Peptidomimetic ER/AR AF-2 inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Properties</th>
<th>IC₅₀ (µM)¹⁶</th>
<th>ER</th>
<th>AR-WT</th>
<th>AR-T877A</th>
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<td>1</td>
<td><img src="image1" alt="Structure" /></td>
<td>MW: 250.4, cLogP: 3.1, HBA: 2, HBD: 4, TPSA: 49.8</td>
<td>7.9</td>
<td>No binding</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
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<td><img src="image2" alt="Structure" /></td>
<td>MW: 360.5, cLogP: 4.9, HBA: 4, HBD: 2, TPSA: 49.8, iPFI: 7.9</td>
<td>4.1</td>
<td>2.6</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Structure" /></td>
<td>MW: 278.4, cLogP: 3.8, HBA: 4, HBD: 2, TPSA: 49.8, iPFI: 4.8</td>
<td>3.6</td>
<td>5.6</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><img src="image4" alt="Structure" /></td>
<td>MW: 410.6, cLogP: 6.1, HBA: 3, HBD: 2, TPSA: 49.8, iPFI: 10.1</td>
<td>&gt;30</td>
<td>1.6</td>
<td>9.4</td>
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</tr>
</tbody>
</table>

Note: All values obtained by luciferase reporter gene assay.

Abbreviations: AF-2, activation function 2; AR-WT, wild-type androgen receptor; cLogP, calculated lipophilicity coefficient; ER, estrogen receptor; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area.
Figure 3  Activation function 2 inhibitors developed using computer-aided drug discovery. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area. [Color figure can be viewed at wileyonlinelibrary.com]

Table 2  Inhibitory activity and assay description of AF-2 targeting compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reported activity (IC₅₀)</th>
<th>Assay description</th>
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<tr>
<td>7⁴⁶</td>
<td>1.6, 9.4</td>
<td>MMTV-luciferase assay in HEC-1 cells</td>
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<tr>
<td>4⁴⁸</td>
<td>34.4, eGFP assay in LNCaP cells</td>
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<td></td>
<td>8.2, fluorescence polarization assay for SRC2-3 displacement</td>
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</tr>
<tr>
<td>6⁴⁸</td>
<td>33.4, eGFP assay in LNCaP cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26, fluorescence polarization assay for SRC2-3 displacement</td>
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<tr>
<td>MDG506⁵⁰</td>
<td>26.3, 33.2</td>
<td>TR-FRET assay</td>
</tr>
<tr>
<td>SPC002⁵¹,⁵²</td>
<td>24</td>
<td>TR-FRET assay</td>
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<tr>
<td>7b⁵³</td>
<td>≤40</td>
<td>MMTV-luciferase assay in CV-1 cells</td>
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<td>D2⁵⁴</td>
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<td>14d⁵⁵</td>
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<td>MTT cell viability assay in LNCaP cells</td>
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<td>TPCK⁵⁶</td>
<td>0.764/2.4*, AR-RFP TIF2-GFP biosensor assay in U-2OS/*AR-transfected PC-3 cells</td>
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<tr>
<td>Parthenolide⁵⁶</td>
<td>1.17/0.925*</td>
<td>AR-RFP TIF2-GFP biosensor assay in U-2OS/* AR-transfected PC-3 cells</td>
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<td>IMB-A6⁵⁸</td>
<td>10*, PSA-Luciferase in LNCaP cells/*confirmed in AR-transfected PC-3 cells</td>
<td></td>
</tr>
<tr>
<td>T1-12⁵⁹</td>
<td>0.47/1.42</td>
<td>eGFP/*PSA assay in LNCaP cells</td>
</tr>
</tbody>
</table>

Abbreviations: AF-2, activation function 2; AR-WT, wild-type androgen receptor; eGPF, enhanced green fluorescent protein.
additional optimization to replace the undesired moiety, and instead utilize the series as a mechanistic tool.61 Of the
diarylhydrazide series, MDG506 displayed the lowest cytotoxicity, with cell viability of 80% at 50 µM. Subsequent
use of their designed hydrazide library, coupled with published data relating to non–LBP AR inhibitors, enabled the
authors to identify the structurally novel non-LBP AR antagonist SPC002 from the SPECS database via the use of
molecular topology-derived quantitative structure–activity relationship (SAR). SPC002 displayed a comparable IC₅₀ of 24 µM and molecular dynamics and docking studies predicted SPC002 to have a higher affinity for AF-2 than for binding function 3 (BF-3) of the AR, vide infra. 

MDG506 combines several aromatic rings which despite the presence of a number of polar groups, gives the molecule an overall high iPFI. Incorporation of further heteroatoms into the tricyclic aromatic motif could reduce the high iPFI, thereby improving the pharmacodynamic profile of MDG506. The presence of a hydrazide, a known toxicophore, may also present future toxicity concerns. SPC002 consists of a comparatively more flexible scaffold, along with three minimally functionalised phenyl rings. This gives rise to a high cLogP with a low TPSA, and potential for promiscuous off-target effects. Future optimization could focus on the incorporation of heterocycles to tune logP, increased rigidity, and higher degrees of saturation to increase sp³ character.

Biaryl PPI inhibitors have been identified and evaluated against both the ERα and AR. These compounds were designed to antagonize the binding of coactivator proteins to the receptor coactivator binding domain. Compound 7b exhibited significant AR inhibition at concentrations of ≤40 µM and was the only analog reported to possess selectivity for the AR with reliable cell viability (Figure 5B). Various substitution patterns on the biaryl linker resulted in cytotoxicity at concentrations above 50 µM. Further exploration of the SAR has solely focused on activity against ERα.

Compound 7b has a high cLogP value, 12 rotatable bonds, and an iPFI >7. Considering the presence of a basic amine and its comparatively high lipophilicity, the molecule is likely to exhibit off-target liabilities, demonstrated by the cytotoxicity of structurally related analogs. In addition to this, the presence of a basic tertiary alkyl amine and an aromatic core indicates a potential alert for hERG channel inhibition and associated cardiotoxicity.

Ravindranathan et al. designed compound D2 to mimic the leucine-rich motifs, LXXLL of proteins that bind to AF-2 (Figure 6A). Compound D2 was shown to disrupt the interaction between the AR and coregulator PEPL1 and inhibit AR-mediated prostate cancer cell proliferation, both with an IC₅₀ of 40 nM. Furthermore, compound D2 demonstrated cytostatic effects in xenograft prostate tumors in vivo and cultured human tumors ex vivo. Subsequent SAR exploration afforded compound 14d, which demonstrated higher antiproliferative activity with an IC₅₀ of 16 nM against LNCaP cells and similar efficacy in the PEPL1 displacement assay.

The cLogP and iPFI values of compound D2, combined with the presence of polar functional groups suggest an acceptable profile in relation to absorption considering solubility and permeability. This profile is improved upon in the case of compound 14d because replacing the methyl ester with a carboxamide affords an improved cLogP and likely imparts a degree of solubility. Unfortunately, the presence of a nitro group is a potential liability as the moiety is known to undergo metabolism to nitroso compounds and hydroxylamines which are toxicophores and anilines which are often genotoxic.

Johnston et al. recently described the development of a fluorescence-based bioassay for a high-throughput screening (HTS) campaign of novel inhibitors of AR-coactivator PPIs, specifically AR-TIF2. Using the Library of Pharmacologically Active Compounds (LOPAC) to test this assay, five nonsteroidal compounds were identified to inhibit DHT-induced AR-TIF2 PPIs with IC₅₀ values ranging from 0.56 to 1.17 µM including TPCK and Parthenolide (Figure 6B).

Despite relatively suitable physicochemical properties, TPCK is likely to be highly promiscuous due to the presence of an activated α-chloroketone functionality and hence, prone to covalent bonding to a broad range of proteins. Parthenolide possesses no HBD and has a very low TPSA which will likely confer poor solubility and makes absorption a potential issue. The compound also possesses a Michael acceptor and an epoxide moiety, making promiscuous binding to off-target proteins through covalent modification, a potential concern. While the identified hits are far from lead-like, the authors noted the small and outdated nature of the LOPAC library and subsequently screened a larger, more diverse compound deck. Of the 286 confirmed active compounds, approximately 60% were able to inhibit or disrupt AR-TIF2 PPIs with an IC₅₀ <40 µM. Johnston et al. have since reported the development of five assays that target the AF-2 domain, although the structures of the hits have not been disclosed to date.
More recent work has utilized computational modeling to screen the ZINC database for chemical entities that interact with the AR AF-2. This led to the synthesis of an oxadiazole series of which LHJ-647 was the most potent (Figure 7A).57 LHJ-647 disrupted the interaction between AF-2 and the coactivator RIPK1 and, it also suppressed AR transcription in AR-transfected PC-3 cells with an IC50 of ~1 µM.

LHJ-647 is a small compound with modest lipophilicity furnished with a balance of polar groups that observes Lipinski’s rules and possesses a favorable iPFI value. This indicates an acceptable developability profile and potential for further optimization. One particular feature of note is the embedded aniline, which as previously acknowledged, has the potential to be associated with genotoxicity.

Recently, Liu et al. have developed a pharmacophore model based on structures of the AR with AF-2 bound small molecules available from the Protein Data Bank database.58 This model was used to conduct a virtual screen
of the ZINC database and identified IMB-A6, which inhibited AR-WT activity in LNCaP and AR-transfected PC-3 cells with an IC\textsubscript{50} of 10 µM (Figure 7B). IMB-A6 also inhibited clinically relevant AR mutants; T877A, W741L, and F876L in PC-3 cells. A library of 248 chemically similar analogs was screened and 14 compounds were selected to be tested against the same AR mutants. Eight of the compounds retained modest activity at 10 µM concentration, validating the efficacy of the chemotype represented by IMB-A6. The low MW and cLogP, accompanied by a good iPFI score and balance of functionality make IMB-A6 an attractive compound with likely potential for further development, although the embedded ketone represents a potential metabolic liability.

Recently, Chai et al. employed a structure-based virtual screen followed by SAR exploration of the sulfonamide moiety to identify a series exemplified by T1-12 (Figure 7C).\textsuperscript{59} The most potent analog, T1-12 effectively displaced coactivator peptides from the AF-2 pocket and displayed AR transcriptional inhibition with an IC\textsubscript{50} of 0.47 µM in LNCaP cells. T1-12 also inhibited tumor growth by 65% in xenograft models at a dose of 2.5 mg/kg/week when administered intratumorally.

T1-12 has a desirable in silico developability profile, displaying a low MW, cLogP, and TPSA. The favorable iPFI score combined with the balance of polar and nonpolar functionality makes IMB-A6 an attractive compound with likely potential for further development, although the embedded ketone represents a potential metabolic liability.

The developability profile of each of the compounds discussed in this section can be summarized by applying three main parameters (MW, cLogP, and iPFI) relating to solubility and permeability (absorption), along with important considerations (TPSA, rotatable bonds, HBD, and HBA). In addition, to avoid toxicity and promiscuous off-target binding, a low MW and cLogP are desirable.\textsuperscript{69} In summary, Parthenolide, TPCK, compound 4, T1-12, LHJ-647, compound 6, and IMB-A6 all maintain low MW, and acceptable cLogP and iPFI values (Figure 8). In terms
of structural alerts, Parthenolide has a Michael acceptor and an epoxide which are not considered to be optimal for further drug development. Having stated this, Michael acceptors and other covalent inhibitors are increasingly viewed as being acceptable in, for example, oncology applications. \textsuperscript{70} TPCK and SPC002 are likely to be involved in Nonspecific covalent binding. Compound 7 has a high cLogP value >5 which could lead to off-target effects. Compound 6, MDG506, and compounds D2 and 14d displayed general cytotoxicity which can be ascribed to the hydrazide and nitro moieties, respectively. Compound 7b has a potential hERG issue through the basic tertiary amine. From consideration of physicochemical properties, reported potency for AR inhibition, and the presence of structural alerts, our analysis indicates that LHJ-647, IMB-A6, and T1-12 are optimal starting points for the future development of AF-2-directed AR inhibitors.

4 | BF-3-TARGETING COMPOUNDS

In pursuit of new AR AF-2 binders, Estebanez-Perpiña et al. identified a novel surface binding site termed BF-3, which displayed allosteric activity for AF-2.\textsuperscript{71} In addition, Nonspecific AF-2/BF-3 binders were reported, including TRIAC and Tolfenamic acid (Figure 9A). These compounds disrupted coactivator SRC2 binding with IC\textsubscript{50} values of \sim 50 \mu M (Table 3) and inhibited AR activity in a dose-dependent manner in the 10–30 \mu M range in vitro, serving as proof of principle for receptor inhibition via BF-3.

Both compounds present a common pharmacophore and are similar in terms of the developability profiles. The three iodine atoms present in TRIAC dramatically increase the MW; however, there is potential for exploring
alternative substitution patterns to alter the properties of the compound. **Tolfenamic acid** is a marketed pharmaceutical that inhibits the COX pathway.\textsuperscript{79}

Joseph et al. exploited the interaction between the AR and cofactor gelsolin to develop an assay for identifying small molecules that induce inactive conformations of the AR.\textsuperscript{72} This conformation-based assay identified two compounds \textbf{D36} and \textbf{D80} (Figure 9B). \textbf{D36} was the most potent, allosterically competing with the synthetic androgen, R1881 with an IC\textsubscript{50} of 10 µM. These compounds were reported to bind an AR surface site, which was proposed to be BF\textsubscript{3}, and essentially induce a conformation similar to that of the unoccupied receptor. \textbf{D36} inhibited PSA transcription and proliferation in LAPC4 cells, as well as reducing proliferation in two CRPC cell models, \textit{SR\alpha}AR LNCaP and VCaP.

Both compounds possess multiple aromatic rings which impact the iPFI, making solubility a potential concern. \textbf{D80} has a particularly high cLogP which consequently impacts iPFI, also the thiophene moiety can be prone to oxidation and metabolic instability.\textsuperscript{80} The considerations make \textbf{D36} the more developable compound in terms of the available metrics, the core indole scaffold is also a well-known pharmacophore that offers potential for SAR exploration and further optimization.

The Cherkasov group has reported improvements to the modest potency and selectivity of BF-3-targeting antiandrogens.\textsuperscript{49,73} Virtual screening efforts followed by cellular assays afforded 55 hits (>50% inhibition at 50 µM

**FIGURE 9**  (A) Nonspecific binders of activation function 2 and binding function 3; (B) hits identified via a conformation-based assay. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area. [Color figure can be viewed at wileyonlinelibrary.com]
by eGFP assay) including compound 3, compound 4, and ZINC13574823 (Figure 10). The most potent of these hits demonstrated AR transcriptional IC50 values ranging from 0.9 to 50 μM. Mechanistic action via AF-2 or the LBP was disproved via SRC2 and DHT displacement assays, respectively.

Compound 3 has both a high cLogP and iPFI; it is likely that these characteristics will lead to low solubility and possible off-target effects. In addition, the ether chains have the propensity to be rapidly metabolized. Compound 4 possesses a number of rotatable bonds and consists of two catechol moieties, which are pan-assay interference (PAINS) structural alerts that are known to yield false positives.81,82 ZINC13574823 has a high cLogP and a low TPSA, which combined with a lack of polar groups could again lead to concerns over solubility and off-target

### TABLE 3  Inhibitory activity and assay description of BF-3 targeting compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reported activity AR–IC50 (μM)</th>
<th>Assay description</th>
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<tr>
<td>TRIAC71</td>
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<td>Fluorescence polarization assay for SRC2-3 displacement</td>
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Abbreviations: BF-3, binding function 3; eGFP, enhanced green fluorescent protein.
effects. From a biostructural perspective, compounds 3 and 4 have been confirmed through X-ray crystallography to bind the AR BF-3 (Figure 11). A thorough SAR exploration of compound 3 led to the identification of compound 54, compound 18, compound 23, and VPC-13566, accompanied by sequential increases in potency in the variety of assays employed, see Table 3 vide infra (Figure 12). Ultimately, the studies found VPC-13566 to be a selective BF-3 inhibitor with nanomolar potency in multiple assays against both AR-WT and a range of clinically relevant mutants. In addition, VPC-13566 also reduced PSA expression and cell viability of enzalutamide-resistant MR49F cells and reduced PSA expression and tumor volume at levels comparable with enzalutamide in a castration-resistant xenograft model.

Compounds 54 and 18 broadly occupy drug-like space in terms of Lipinski’s rules. Further optimization led to compounds 23 and VPC-13566, which possess a higher cLogP and iPFI and lower MW, however, are accompanied by significant increases in potency, Table 3 (vide infra).

Most recently, Leblanc et al. conducted a pharmacokinetic optimization of VPC-13566, which despite its potent activity in vivo, suffers from metabolic instability. Efforts to circumvent this led to the development of VPC-13789, which demonstrated a significant improvement in microsomal half-life from 21 to 206 min, whilst maintaining an IC50 of <200 nM (Figure 13). In an androgen displacement assay, VPC-13789 had no effect at 10 µM concentration, discounting a mechanism that functions via the LBP; furthermore, VPC-13789 had no effect on the growth of AR-negative PC-3 cells, indicating mechanistic targeting of the AR. Due to high peak plasma concentration and rapid clearance, the authors subsequently developed VPC-13822, a methylene phosphate prodrug (Figure 13). VPC-13822 inhibited PSA expression in LNCaP cells with a similar IC50 to the parent compound 0.66 and 0.52 µM, respectively. It underwent steady conversion to the parent drug with a half-life of 2 h, and significantly reduced tumor growth in a xenograft model.

The pharmacokinetic parameters for VPC-13789 remain largely similar to the other members of the series, and while the metabolism issue was addressed, it still exhibited a cLogP close to the recommended upper limit of Lipinski’s rules and accordingly a high iPFI value. This was successfully addressed utilizing a prodrug strategy, with a significant drop in cLogP and iPFI being reflected by a twofold increase in solubility. Notably, VPC-13822 demonstrated no significant signs of toxicity over the 4-week xenograft study following a per oral (po) dose of 115 mg/kg twice daily. The relatively high MW of VPC-13822 violates Lipinski’s rule; however, Protti et al. recently...
recommended an expansion of widely used parameters that determine drug-like space, based on the prevalence of orally bioavailable prodrugs and natural products that exceed the existing guidelines.\textsuperscript{83}

All of the BF\textsubscript{3} targeting compounds comply with Lipinski’s rules, except for compound 3, TRIAC, and the prodrug, VPC\textsubscript{13822} (Figure 14). Compounds 54 and 18 have the most favorable developability profile; their substantial optimization to VPC\textsubscript{13789} has resulted in the lead VPC\textsubscript{13822} which has a good cLogP and iPFI, a reasonable HBD/A count, and TPSA, with the methylene phosphophate group attributing to the high MW. Compound D80 contains thiophene which is potentially metabolically unstable. Compound D36, however, displays a moderate cLogP and MW and has been underexplored in terms of SAR, presenting an opportunity for future development. Compound 4, unfortunately, contains a number of rotatable bonds and also known structural alerts for PAINS. ZINC13574823 has a novel structural scaffold with desirable sp\textsuperscript{3} character and a high degree of potency for an initial hit; however, the comparatively high cLogP and low TPSA pose potential issues that would require addressing via the introduction of polar functionality.\textsuperscript{84,85}

Targeting AF-2 and BF-3 are evidently valid approaches toward novel CRPC treatments; however, this is only possible in cases where the LBD is retained (i.e., no splice variants which lack the LBD). Unfortunately, this approach fails to provide viable treatments to patients suffering from AR mutations that circumvent the inhibition of the different LBD regions. Consequently, a multi-targeted approach considering other regions of the AR is crucial to achieving improved outcomes for CRPC patients.

\textbf{FIGURE 11} Compounds 3 and 4 situated at binding function 3 (BF\textsubscript{3}). (A) Full protein, testosterone (red), BF\textsubscript{3} and compound 3 (blue); (B) BF\textsubscript{3} binding site, ?-? interaction (cyan) (PDB:2YLO); (C) full protein, testosterone (red), BF\textsubscript{3}, and compound 4 (blue); (D) BF\textsubscript{3} binding site, hydrogen-bonding (green) (PDB:3ZQT). [Color figure can be viewed at wileyonlinelibrary.com]
Deletion studies involving the NTD have demonstrated that it is essential for receptor function, making it as appealing as it is challenging as a drug target. A raft of data indicates that the NTD is required for transcription, and is present in all forms of the AR, including the range of known mutants and splice variants.86

5 | TARGETING THE NTD

Deletion studies involving the NTD have demonstrated that it is essential for receptor function, making it as appealing as it is challenging as a drug target. A raft of data indicates that the NTD is required for transcription, and is present in all forms of the AR, including the range of known mutants and splice variants.86
Based on this, it is anticipated that treatments targeting the NTD would provide efficacy against AR variants resistant to current treatments formed from alternative splicing, for example, enzalutamide-resistant F876L and AR-Vs lacking the LBD. The main challenge of this approach is developing drugs that would target the intrinsically disordered domain, which is not amenable to Structure-Based Drug Design. It has been proposed that the disorder of the NTD might allow it to act as a center for signaling pathways via PPIs with coactivators of varying sizes, adopting different conformations for each. Recent cryoelectron microscopy studies have elucidated the structure of the AR dimer complexed with DNA and coactivators p300 and SRC3. In this study, antibody labeling occurred with differing efficiency for the two NTDs of the dimer, substantiating the proposal that distinct NTD conformations result in differences in coactivator recruitment. Computational methods are beginning to emerge for the rational design of drugs that target intrinsically disordered proteins and have been recently reviewed. However, it is a contemporary research area, and more experience is required before its utility can be fully established.

Mutations occur proportionately across the whole AR and the NTD is no exception; 10% of its residues have the potential for mutation in prostate cancer. Results from our laboratories demonstrated that NTD-mutations generally produce a loss of function or no significant difference compared to AR-WT, with only a minority leading to a constitutive gain of function. Nevertheless, mutations with no apparent change in WT activity have been proposed to drive cancer progression via other routes such as altered binding to coregulators, increased protein stability, and gene amplification, which suggests that various NTD mutations can influence the progression of CRPC.
The development of drugs that target the NTD has garnered much attention in recent years, largely due to the discovery of AR-Vs.\textsuperscript{20,22,94–97} Although this topic has been reviewed previously, we intend to address both attempts to target the NTD pharmacologically and appraise the capacity of natural products identified as pharmaceutical leads.

5.1 NTD-targeting natural products

Natural products are an abundant source of bioactive compounds and potential innovative drug leads, evidenced by the fact that they account for more than 40% of all pharmaceuticals on the current market.\textsuperscript{98–100} In addition to this, approximately half of the available cancer therapies also originate from natural products.\textsuperscript{101,102}

In 2008, Sadar et al., isolated \textit{sintokamides A–E} and \textit{dysamide A} (Figure 15A) from the marine sponge \textit{Dysidea} sp. collected near Palau Sintok, Indonesia.\textsuperscript{103} The \textit{sintokamide} family is a group of polychlorinated marine peptides derived from chlorinated leucine and were the first small molecules shown to inhibit the AR via the NTD.

\textbf{Sintokamide A} was shown to inhibit forskolin-induced transactivation of the AR-NTD when pretreated at 5 \textmu M for 1 h via a luciferase reporter assay.\textsuperscript{103} Furthermore, \textit{sintokamide A} exhibited an AR transcriptional IC\textsubscript{50} of 10 \textmu M and antiproliferative IC\textsubscript{50} of 35 \textmu M in LNCaP cells. \textit{Dysamide A} and \textit{sintokamides B, C, and E}, also displayed antiandrogenic activity at 5 \textmu g/mL in LNCaP cells determined by PSA-luciferase reporter gene assay.\textsuperscript{104} Further analysis identified the AF-1 region in the NTD as the binding site for \textit{sintokamide A} and selectivity over other hormone receptors was established. In addition, efficacy against both AR-FL and AR-Vs was demonstrated by utilizing luciferase and proliferation assays, as well as xenograft models.\textsuperscript{105}

Recently, Sadar and Anderson explored the SAR surrounding the \textit{sintokamide} pharmacophore, using a PSA-luciferase assay to tune the potency.\textsuperscript{106} It was established that transcriptional inhibition was proportional to the number of chlorine atoms present within the scaffold. Another modification was the replacement of the propionamide moiety at the N-terminus with a bulkier, more lipophilic N-pivaloyl group to afford the more potent synthetic analog \textit{LPY36} (Figure 15A). During these studies, the synthesis of the scaffold was simplified via the removal of two of the four stereogenic centers, which had no effect on the antagonist profile. This was accompanied by a threefold increase in potency via luciferase assay and increased suppression of LNCaP cell proliferation.

Further investigation of the \textit{sintokamide} family provided a beachhead for the development of NTD-targeting inhibitors. However, the core scaffold possesses a large degree of molecular flexibility, and the compounds have a high MW and cLogP which are likely indicative of poor developability properties such as solubility and permeability. From a DMPK perspective, this profile is problematic for the same reasons, with a number of metabolic hotspots evident in the \textit{sintokamide} family. Furthermore, the activity requirement of multiple alkyl halides raises concerns about off-target effects, although these are anomerically stabilized. Having stated this, a comparatively lower (fourfold) selectivity of \textit{LYP36} for AR-positive LNCaP over AR-negative PC-3 cell viability was observed, indicating the potential for off-target effects.

Meimitis \textit{et al.} isolated \textit{niphatehones A} and \textit{B} from the marine sponge \textit{Niphates digitalis} collected in Dominica, assessed their ability to inhibit AR transcription, and conducted an SAR study (Figure 15B).\textsuperscript{107} The natural products were found to be the most potent compounds of the SAR study, with IC\textsubscript{50} values of approximately 5 \textmu M via two luciferase assays.\textsuperscript{108} Interestingly, both enantiomers of \textit{Niphathene B} demonstrated comparable IC\textsubscript{50} values of 5.2 and 6.3 \textmu M.\textsuperscript{108} However, the Michael acceptor displayed reactivity with glutathione, suggesting that in turn, this would lead to off-target binding and thus limiting its potential drug scaffold for further development.

The \textit{niphathene} compounds consist of an extensive aliphatic carbon chain which results in increased molecular flexibility and a higher than desirable cLogP. For these reasons, aqueous solubility is predicted to be poor with a likely high degree of plasma protein binding, allied with the potential for extensive metabolic oxidation. Most importantly, as a general thiol alkylating agent, off-target toxicity for these molecules is likely.
Mahanine is a carbazole alkaloid, isolated from *Murraya koenigii*, a curry leaf plant cultivated in Southeast Asia (Figure 16A). Mahanine was also found to be present in the edible Thai vegetable *Micromelum minutum*, rendering it readily available. In terms of pharmacology, mahanine has been shown to inhibit DHT-induced transactivation of the AR in a dose-dependent manner in the 2.5–10 µM range, assessed by ARR3-TK-luciferase and PSA-luciferase assays. Inhibition of ligand-independent transactivation was also demonstrated using a Gal4-DBD-AR-NTD fusion protein via luciferase assay; activity towards a protein construct with the DBD of the Gal4 transcription factor precludes a mechanism of action via the AR-DBD, providing evidence that inhibition functions through the AR-NTD. In addition to direct inhibition of the AR, mahanine was reported to display other mechanisms of AR disruption, including enhanced ubiquitination which causes proteasomal degradation, inhibition of nuclear translocation, and reduction of phosphorylation at serine-81 due to suppression of CDK1 activity.
Mahanine exhibits a high cLogP and associated iPFI, indicative of poor solubility and risk of off-target effects. In addition, the phenol moiety is a likely site of phase II metabolism. Having stated this, the low MW of the compound potentially provides latitude for further development of the scaffold; the introduction of polar functional groups and replacement of the prenyl moiety could address the high cLogP and low TPSA.

Cinobufagin-3-acetate is a steroidal natural product secreted by the asian toad, *Bufo gargarizans* (Figure 16B). Studies have shown that cinobufagin-3-acetate binds directly to the NTD thus inhibiting AR-STAT3 signaling—a pathway that is a key driver in the progression to CRPC. The compound demonstrated static levels of AR inhibition in the presence of varying concentrations of R1881, discounting a mechanism that functions via the LBP. Cinobufagin-3-acetate displayed significant inhibition against the clinical variants AR-V7 and ARv567es in LNCaP cells at concentrations of 1 and 10 µM, respectively, and was found to reduce AR protein expression without altering AR mRNA levels or reducing AR nuclear translocation.

Cianobufagin-3-acetate exhibits a comparatively good cLogP for a steroidal lead, this is likely due to the presence of hydrophilic pyranone and acetate groups, which perhaps warrants further optimization of its pharmacokinetic profile, particularly against the background of the rich history of steroids as pharmaceutical agents. However, the epoxide moiety should be considered for possible nonspecific covalent interactions and consequently, potential off-target toxic effects.

5.2 | The EPI family

A library of compounds was isolated from the marine sponge, *Geodia lindgreni* by Sadar and Andersen in 2010 and are structurally similar to bisphenol A diglycidyl ether (BADGE). The collected library was then screened for its ability to block transactivation of the AR NTD, with EPI-001 emerging as the most potent compound (Figure 17).
FIGURE 17  Early EPI analogs of BADGE, prodrug EPI-506, and next-generation analog EPI-7170. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area. [Color figure can be viewed at wileyonlinelibrary.com]
The compounds identified termed the EPI series, are derived from the racemic mixture EPI-001, and represent the most extensively investigated class of NTD inhibitor. EPI-001 was initially shown to selectively block transactivation of the NTD via AF-1 and induce cytoreduction of CRPC xenografts without overt toxicity at a dose of 50 mg/kg.\textsuperscript{114} EPI compounds display activity against truncated AR-Vs and require a chlorohydrin moiety to covalently bind to the AR, but do not possess nonspecific thiol alkylating abilities, as demonstrated with mercaptoethanol and glutathione.\textsuperscript{108,115} However, the chlorohydrin moiety is known to convert to the epoxide under neutral and basic pH, and BADGE is reactive to nucleophiles. EPI-001 under acidic conditions did not form thiol adducts, but at pH 7.4 trace amounts of thiol adduct were observed, and at basic pH nearly complete conversion to the thiol adduct was noted.\textsuperscript{116} Placement of one of the hydroxy groups next to a basic site in the AF-1 pocket was proposed to facilitate the selective irreversible covalent binding. EPI-002 (later termed ralaniten), a stereoisomer of EPI-001 was evaluated via a luciferase assay to inhibit AR transcription with an IC\textsubscript{50} of 7.4 μM and oral administration at 200 mg/kg reduced the growth of VCaP xenografts.\textsuperscript{115}

In more recent mechanistic studies, the interaction of EPI-002 was localized to TAU-5 within the AF-1 region of the NTD, and it was shown to disrupt interactions between TAU-5 and essential coactivators CBP/p300 and RAP74.\textsuperscript{117,118} EPI-002 has demonstrated efficacy against AR-V7 in LNCaP95 xenograft models, as well as AR mutant variants possessing gain-of-function mutations in either the LBD or the NTD.\textsuperscript{118} A clinical trial [NCT02606123] assessing EPI-506, an acetylated EPI prodrug, was initiated in 2015 but terminated two years later due to an excessive dose regimen (18 capsules per day).\textsuperscript{119}

More recently, a next-generation analog, EPI-7170 was reported to inhibit androgen-induced PSA-luciferase activity with an IC\textsubscript{50} of 1.1 μM (Figure 17).\textsuperscript{120} EPI-7170 has demonstrated inhibition of both AR-FL and truncated AR-Vs, which have been implicated in regulating DNA damage repair in prostate cancer.\textsuperscript{121} Accordingly, EPI-7170 has been shown to sensitize AR-V-driven LNCaP95 cells to infrared radiation, increasing DNA damage and reducing cell survival and proliferation.

The effectiveness of the EPI series in combination with other therapeutics has also been demonstrated preclinically.\textsuperscript{122} Enhanced anticancer activity has been observed when combined with docetaxel, autophagy inhibitors, and the co-targeting of the AR and PI3K/Akt/mTOr pathways with mTOR inhibitor BEZ235.\textsuperscript{122–124} Most recently, EPI-7170 has been combined with the CDK inhibitor palbociclib and the Pin1 inhibitor all-trans retinoic acid, with increased combination efficacy compared to monotherapy treatment observed in both cases in cell-based and xenograft models.\textsuperscript{125,126} A possible combination of EPI with sintokamide A has also been proposed, as the two compounds reportedly target separate sites in AF-1 and exhibit additive transcriptional inhibition.\textsuperscript{105}

A phase-1 clinical trial [NCT04421222] is currently underway exploiting the next iteration of the EPI scaffold, EPI-7386 (structure undisclosed). EPI-7386 displays improved metabolic stability and a 20-fold increase in potency compared to EPI-002.\textsuperscript{127}

The EPI family remains the most promising set of compounds for AR inhibition via the NTD and represents a potential new approach in the treatment of CRPC. Although the MW of some analogs exceeds 500 Da, the remaining parameters of the series are within a reasonable range. Potential concerns surrounding the alkyl halide moiety are minimal, as although it is essential for activity, the potential for nonspecific alkylation has been considered. Off-target effects of the series have been explored, as EPI-002 has been shown to increase the transcription of metallothionein genes, driven by the transcription factor MTF-1. However, this effect was not observed for contemporary analogs in the EPI series and had no impact on AR inhibition.\textsuperscript{128} Furthermore work by Brand et al. invokes nonselective action of EPI-001 at much higher concentrations than previously employed to inhibit AR which suggests PPAR\textsubscript{y} as a target.\textsuperscript{116} Concerning toxicity, EPI-002 has been examined in xenograft models up to a dose of 200 mg/kg, with only minor body weight loss observed indicating the minimal potential for toxicity.\textsuperscript{115} EPI-7170 has also been assessed in murine models and induced no alteration in body weight at an efficacious dose of 30 mg/kg.\textsuperscript{129} First-generation EPI analogs have a reported half-life of 3.3 h in mice and PK optimization led to the development of EPI-7386 with a half-life of 24 h in humans.\textsuperscript{115,127}
Very recently, Asim and Spring reported the combination of two covalently linked AR inhibitors, **EPI-001** and **enzalutamide** (Figure 18). The strategy was aimed at appending enzalutamide via the amide portion to the 1,2-diol of EPI-001 through an epoxide opening maintaining the active chlorohydrin pharmacophore. Utilizing a biocompatible triazole with a PEG linker allowed different linker lengths to be investigated. This new class of hybrid compounds would simultaneously target the NTD and the LBD of the AR overcoming resistance to the standalone FDA-approved standard of care enzalutamide. Compounds 9a–e were screened in a C4-2b prostate cancer cell line, known to express several AR-Vs in which enzalutamide possessed an LC50 value of 63.5 µM and EPI-001 was slightly less potent at 84.8 µM. In comparison, compounds 9a-9e exhibited an 18-53 fold improvement with LC50 values of 1.7–4.6 µM. After further investigative biological studies, 9b emerged as the lead compound. 9b was shown to inhibit AR-mediated gene transcription in a luciferase assay and was able to significantly inhibit transcription of the PSA gene proving its direct engagement with the AR; furthermore, it produced negligible toxicity in PC-3 cells. The authors hypothesized that the improvement in cell toxicity was due to an entropic effect mediated by the linker which increases the local concentration of the second inhibitor that binds to the AR. The hypothesis is supported by studies whereby the dual inhibitors outperformed an equimolar concentration of EPI-001 and enzalutamide by a factor of 80.

Despite the advantages of targeting multiple domains of the AR, compound 9b will potentially suffer from poor pharmacokinetic properties due to high MW, HBA, TPSA, and rotatable bond count inferring poor solubility and membrane permeability. Asim and Spring stated that the compounds could have limited membrane permeability as they were less potent than enzalutamide in the luciferase assay, although cLogP and HBD for the compound are within Lipinski's rules. Nevertheless, compound 9b, is an excellent example of the incorporation of two known AR inhibitors, and its discovery may trigger interest in generating further compounds of this type.

### 5.3 NTD targeting analogs identified via high-throughput and virtual screening campaigns

Recent advances in the field of genomics have shortened the time it takes to develop new drugs. However, HTS campaigns remain a powerful means for hit identification, often providing novel structures and future lead candidates. **Tethered EPI-enzalutamide** via a linker for targeting the N-terminal domain and ligand-binding domain. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area. [Color figure can be viewed at wileyonlinelibrary.com]
molecules for a wide variety of targets.\textsuperscript{132} HTS is hampered by the need for extensive physical infrastructure and economic resources, but this drawback can be partially addressed through the use of virtual methodologies. Through outsourcing and engaging in collaborations between different bodies, screening campaigns are still a significant part of the armamentarium of pharmaceutical companies and academic groups.

An HTS campaign to identify AR nuclear localization inhibitors identified three hits with IC\textsubscript{50} values within 1–5 µM, one being IMTPPE, which was selected for further investigation (Figure 19).\textsuperscript{133} The compound exhibited an IC\textsubscript{50} of 1 µM in a luciferase assay and inhibited the growth of LNCaP and C4-2 cells (AR-positive) and 22Rv1 cells (AR-V-driven), but not DU145 or PC-3 cells (AR-negative). IMTPPE also demonstrated efficacy against enzalutamide-resistant 22Rv1 xenografts.\textsuperscript{134} An SAR study of IMTPPE, resulted in the discovery of the JJ-450 scaffold, with the (−)-enantiomer exhibiting an IC\textsubscript{50} value of 1.7 µM via PSA-luciferase assay.\textsuperscript{135,136} (−)-JJ-450 has been shown to inhibit the enzalutamide-resistant AR F876L mutant by both luciferase and cell proliferation assays, and slow androgen-mediated nuclear import of the AR. Further efficacy was also demonstrated against both AR-FL and AR-V-driven xenografts. Recently, Cole et al. reported the next-generation analog (+)-JJ-74-138, which possessed increased potency in the enzalutamide-resistant LN95 cell line as measured by PSA expression and cell proliferation.\textsuperscript{137}

The compounds above were shown to bind directly to the AR and inhibit AR-Vs that lack the LBD, suggesting a mechanism of action independent from this region, however, the specific binding site has not yet been determined. (±)-JJ-74-138 was shown to inhibit PC-3 cell proliferation, suggesting potential for off-target effects. However, no change in body weight was observed at doses of 10 and 75 mg/kg for (+)-JJ-74-138 and (−)-JJ-450, respectively, reducing concerns over possible toxicity.

In the optimization of IMTPPE, the substitution of the thioether for a chiral cyclopropane will likely increase metabolic stability and target specificity; however, the replacement of the isoxazole for a phenyl ring also increased the lipophilicity of (−)-JJ-450. The introduction of a pentafluorosulfanyl group, associated with high lipophilicity, exacerbated this increase, resulting in unfavorable cLogP and iPFI values which make (+)-JJ-74-138 likely to have poor solubility and permeability.\textsuperscript{138}

In a previous review of molecules targeting the AR beyond the LBD, Elshan et al. disclosed their own series of analogs that target the NTD, termed JN compounds.\textsuperscript{22} JN018 was the initial hit exemplifying the series and elicited a dose-dependent reduction in cell viability in a range of AR-positive prostate cancer cell lines in the 0.1–4 µM range after 5 days of exposure (Figure 20A).\textsuperscript{139} JN018 was also shown to inhibit tumor growth in xenograft models.

![Figure 19](https://wileyonlinelibrary.com/doi/10.1002/med.21961)
with similar efficacy to enzalutamide, although signs of toxicity in mice were reported with increased treatment duration.

**JN018** exhibits a high cLogP and iPFI, indicative of poor developability. The TPSA of the compound is also particularly low, suggesting it may be able to cross the BBB, leading to the potential for further off-target effects in the CNS. In addition, two Michael acceptors are present in **JN018**; although they could potentially be required for the reported covalent mechanism of action, concerns with off-target toxicity via nonspecific reaction with endogenous nucleophiles should be addressed. Moreover, the basic tertiary amine in the molecule, allied with a cLogP of greater than 5, raises concerns regarding hERG inhibition, leading to cardiac side effects.

In 2017, Ponnusamy et al. reported the selective androgen receptor degrader **UT-155**, which binds to both the NTD and the LBD of the AR (Figure 20B). Degradation of AR-Vs was demonstrated by **UT-155** in vitro, inhibiting transactivation with an IC50 of 78 nM. Substitution of the indole with a fluorinated pyrazole motif afforded...
second-generation compound UT-34, which demonstrated improved in vivo efficacy, despite a lower in vitro IC$_{50}$ of 200 nM.\textsuperscript{143} This was followed by a further SAR study to improve the pharmacodynamic profile of the series, which yielded compound 26f.\textsuperscript{144}

Given that the UT series shares structural features with traditional LBP-antagonists, it is not surprising that UT-155 displayed an affinity for the LBP; however, mutation of key LBP residues did not decrease anti-AR activity, suggesting that inhibition can be attributed to an alternative mechanism of action which may be consistent with degradation via interaction with the NTD.\textsuperscript{142} In addition to this, steady-state fluorescence emission spectroscopy and Biacore surface plasmon resonance studies were utilized to demonstrate the binding of UT-155 and UT-34 to the AR-NTD. Considering their developability properties, the UT compounds are an attractive scaffold, with low MW and cLogP values likely contributing to favorable physicochemical properties. The most recent iteration, compound 26f demonstrates in vivo efficacy against enzalutamide-resistant xenograft models and vastly improved DMPK properties in mouse liver microsomes ($T_{1/2} = 265$ min) compared to UT-155 ($T_{1/2} = 12$ min) and UT-34 ($T_{1/2} = 78$ min).\textsuperscript{144} The safety profile of UT-34 has been evaluated, with no off-target effects observed for GPCRs, kinases, other NRs, or inhibition of the hERG ion channel. The favorable DMPK properties and the balanced developability profile of compound 26f make it a promising lead for addressing treatment resistance in CRPC.

QW07 was identified from a screening campaign via AR-NTD-Gal4DBD-luciferase assay, designed to identify NTD-targeting AR inhibitors (Figure 20C).\textsuperscript{145} The compound displayed an IC$_{50}$ value of 5 µM through a luciferase assay in LNCaP cells and was also validated against multiple AR splice variants. QW07 represents a novel small molecule that demonstrated greater in vitro efficacy than EPI-001 and inhibited the expression of four genes that are typically stimulated by the AR (PSA, FKBP5, SLC45A3, and TMPRSS2).

Surface plasmon resonance and biotin antibody assay studies confirmed that the interaction of QW07 with the AR resides within the NTD.\textsuperscript{145} At a dose of 40 mg/kg/day, a significant reduction in tumor size was observed in enzalutamide-resistant xenograft models bearing AR amplification and splice variants; doubling the dose caused no organ damage (judged by histopathological analysis) or detectable changes in body weight. QW07 was shown not to reduce AR expression or inhibit nuclear translocation and it was proposed that the activity could be attributed to disruption of the interaction between the AR-NTD and CREB-binding protein—a bridging factor that potentially stabilizes the binding of the AR to androgen response elements (AREs).\textsuperscript{145} The comparatively high cLogP and iPFI of QW07 suggest that properties such as solubility or permeability may pose future problems. Having stated this, the encouraging in vivo efficacy data suggest that ADME properties are at least acceptable, although the exact method of administration was not disclosed.

In 2013, Cherkasov et al. discovered VPC-2055 in a screening campaign for structurally novel AR-LBP antagonists and did not exhibit the same protein degradation mechanism as the other hits identified (Figure 21).\textsuperscript{146} Interestingly, VPC-2055 possesses two chlorohydrin moieties, the same motif responsible for the activity of known NTD inhibitor EPI-001. This prompted an SAR exploration that ultimately provided VPC-220010, which inhibited AR-V7 with an IC$_{50}$ of 2.7 µM, fivefold more potent than EPI-001.\textsuperscript{147} VPC-220010 also reduced cell viability of LNCaP and AR-V7-dependent 22Rv1 cells more potently than EPI-001, whilst having no effect on AR-negative PC-3 cells.

A mechanism of action via the AR-NTD was evidenced by sustained efficacy against an AR-NTD-Gal4-DBD fusion protein in a luciferase assay. Selectivity for the AR over other steroid hormone receptors was also demonstrated using luciferase reporter gene assays. The small and polar nature of VPC-220010 makes it an attractive compound from a development perspective, with favorable values for MW, cLogP, and iPFI. The presence of multiple reactive groups raises possible concerns regarding off-target toxicity, although the chlorohydrin moiety is present in the well-established EPI scaffold. The microsomal half-life of VPC-220010 is 30 min, which would likely require optimization in successor compounds. Further development of this molecule may also yield improvements to potency, as in the case of second-generation EPI analogs such as EPI-7170.

Work in the Lilly group has taken a different approach to AR inhibition in the form of bispecific antibodies (biAbs).\textsuperscript{148} A ligation of two single-chain variable fragments, 3E10—an anti-DNA antibody, and AR441—an anti-AR
antibody displayed blockage of genomic signaling of both AR-FL and AR-V7 in LNCaP cells. The biAbs successfully met the design rationale of effective cell penetration and target engagement; moreover, the successful inhibition of AR-V7 provided evidence for action via the NTD.

5.4 | Novel liquid–liquid phase separation approach to target the NTD

Very recently, Xie et al. disclosed that the AR forms hormone-dependent nuclear puncta that have properties of liquid-like condensates and are associated with drug resistance. They demonstrated that antiandrogens, bicalutamide, and hydroxyflutamide blocked puncta formation and transcriptional activity of AR-WT but promoted condensate formation in W742C and T878A. The correlation of liquid–liquid phase separation (LLPS) with AR transcriptional activity postulates that the formation of liquid condensates by antiandrogens with receptor mutants may give rise to drug resistance. Independent isolated regions of the AR were unable to form condensates, but NTD-DBD sequences were able to form puncta similar to the AR-FL. ET516 was identified through a chemical library screen and inhibited condensate formation and transcriptional activity both in AR-WT and mutant AR bound to the NTD with an IC50 value of 0.2 µM (Figure 22). ET516 inhibited the growth of cultured prostate cells and xenographs in vivo. Overall, the work by Xie et al. highlights LLPS and AR-NTD as viable drug targets in CRPC.

ET516 possesses a somewhat similar scaffold to the EPI family and resembles EPI-7170. ET516 like EPI-7170 also exhibits a high MW, above the acceptable limit according to Lipinski’s rules and it also possesses a higher cLogP value and inferred iPFI > 7. It is likely that these characteristics will lead to low solubility with permeability issues leading to promiscuous off-target effects. ET516 lacks the hydroxy moiety present in EPI-7170 which avoids epoxide generation under physiological or basic conditions leading to fewer off-target effects. The introduction of the alkyne as a bioisostere for the ether linker could aid in modulating the pharmacokinetic profile of ET516. Overall, targeting the novel vulnerability of liquid condensates could help aid the clinical challenge that is CRPC and will be of great interest to see what further compounds will be disclosed (Table 4).

The molecules presented for targeting the NTD span a broad range of physicochemical properties and a number of low MW, and low cLogP hits are amenable to optimization (Figure 23). EPI-002 is one example that has already undergone extensive investigation to afford the lead compound EPI-7170, which possesses good potency,
cLogP, and iPFI, albeit a somewhat high MW. VPC-220010 took the related chlorohydrin pharmacophore of the EPI series, and applied it to a more compact naphthalene core, concomitantly reducing the MW and cLogP of the scaffold. Further investigation, for example through the replacement of the lipophilic core with heterocyclic derivatives, could modulate the potency and DMPK properties of the series, and a direct comparison with the EPI compounds may clarify the mechanism of action.

UT-34 and IMTPPE also emerged as developable hits that have undergone optimization, resulting in compound 26f which possessed favorable physicochemical properties, and (+)-JJ-74-138, for which the cLogP and MW are inflated by the pentafluorosulfanyl group, respectively. QW07 is a novel scaffold with reasonable potency and MW that presents an opportunity for further examination of its structure–activity and structure–property relationships; a hit-to-lead optimization could tune the efficacy and reduce cLogP, improving this novel chemotype for NTD-directed AR inhibition. Finally, cinobufagin-3-acetate presents a new steroidal structure that acts via the NTD and maintains a low cLogP and iPFI. Optimization of this scaffold could center around the requirement of the epoxide moiety to ensure this moiety is not required for the compound’s mechanism of action, and seek to reduce the MW.

### 6 | TARGETING THE DBD

The binding of the DBD to AREs within DNA of androgen-regulated genes is essential for transcriptional activity. The structure of the DBD is highly conserved amongst other NRs, therefore achieving specificity for the AR is a likely challenge for DBD-targeted therapeutics.20,97 Recently, advances in computational approaches combined with the disclosure of structural data for the AR-DBD, have enabled the identification of hypothetical sites that are potentially selective for the AR.152,153 Approximately 11% of residues in the DBD are subject to a mutation in prostate cancer, highlighting the significance of the DBD in advanced disease.92 One possible disadvantage of targeting the DBD rests with the transcription-independent roles of the AR implicated in CRPC and treatment resistance.154,155 As the DBD is related solely to the genomic function of AR, molecules that target the region may fail to disrupt any mechanisms whereby CRPC persists unrelated to gene transcription. Nevertheless, the...
<table>
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<th>Compound</th>
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<td>MTS cell proliferation assay in LNCaP cells</td>
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<td>Alamar blue proliferation assay in LNCaP cells</td>
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<td>Dysamide A</td>
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<tr>
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<td>mEGFP assay in LNCaP cells expressing AR (F877L/T878A)/*AR-V7</td>
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Abbreviations: AR, androgen receptor; NTD, N-terminal domain.
exploration of multiple approaches is likely to be vital for the successful treatment of CRPC. Although reviewed previously, we believe a critical evaluation of the reported structures will afford insight and aid in the future development of drugs for CRPC.22,156,157

6.1 | DBD targeting compounds

The first reported example of AR inhibition through interaction with DNA takes the form of a sequence-specific, hairpin polyamide minor groove binder, ARE-1 (Figure 24).158 The polyamide was reported to bind to AREs within the cognate DNA system resulting in an observed decrease in PSA expression in LNCaP cells with a potency comparable to bicalutamide. Polyamide 1 was shown to exhibit cytotoxicity against LNCaP and VCaP cell lines with IC₅₀ values of 6.5 and 7.0 µM, respectively, and demonstrated efficacy in xenograft models.159,160 Subsequently, an analogous cyclic polyamide was reported to significantly inhibit PSA expression at 3 µM, whilst demonstrating low hepatic and cardiac toxicity with high microsomal stability, with a half-life greater than 3 h.161 Recently, the
acetylated analog, ARE-1 was disclosed and displayed a reduction in murine toxicity and improved efficacy in enzalutamide-resistant models, both in vitro and in vivo.\textsuperscript{162-164}

Although these compounds do not directly target the AR protein, the initial reports set an important precedent for direct inhibition of the AR-DNA interface. Since AREs are not identical across the genome, if genes specific to CRPC were to be identified they could potentially be inhibited selectively with tuneable polyamides to minimize off-target effects.\textsuperscript{158}

The first examples of small molecule inhibition of the AR directly via the DBD were reported in 2009.\textsuperscript{165,166} Pyrvinium pamoate and Harmol hydrochloride were two hits obtained from a FRET-based conformational assay (Figure 25). Chromatin immunoprecipitation studies identified Harmol as blocking DNA occupancy, whereas Pyrvinium inhibited the recruitment of RNA pol II to the DNA-bound AR dimer. Luciferase and proliferation assays identified Pyrvinium as the lead compound in terms of potency with an IC\textsubscript{50} value of 13-24 nM in vitro.

Harmol was excluded from further characterization in vivo due to rapid clearance most likely due to metabolic liabilities, for example, the benzylic position in the pyridine and conjugation of the phenolic alcohol during phase II metabolism. However, Harmol possesses a low MW and cLogP, potentially contributing to good solubility and permeability, making the compound a promising structure for future developments which address the metabolic

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**FIGURE 24** Pyrazole-imidazole scaffolds targeting the DNA-binding domain. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area.
instability. Furthermore, the iPFI value of 5.1 remains in a good range for aqueous solubility, enabling future developments.

The nonspecific toxicity of Pyrvinium has been considered, with separate studies demonstrating apoptosis at concentrations of 0.3 and 50 µM in an MTS assay and Western blot analysis for PARP, respectively.\textsuperscript{167,168} In xenograft models, Pyrvinium alone did not display a significant reduction of prostate weight; however, when combined with bicalutamide, castration levels of tumor growth suppression were observed.\textsuperscript{166} Drug affinity responsive target stability assays and deletion studies have been utilized, which confirm that Pyrvinium binds directly to the DBD/hinge of the AR, and has demonstrated activity against homologous hormone receptors such as GR. Pan-receptor activity has been argued both for and against in the area of prostate cancer, possibly avoiding functional replacement of the AR as a driver of CRPC in the case of the former, and being viewed as an undesirable source of off-target effects in the latter.\textsuperscript{167,169} Pyrvinium was also shown to be tissue selective and active against a range of AR-Vs in both LNCaP cells and against 22Rv1 in xenograft models.\textsuperscript{170} A more recent mechanistic study determined that the activity of Pyrvinium is attributed to interactions with residues 609 and 612 in the AR dimer-DNA complex, and proposed that the observed efficacy was due to a conformational change that disrupts PPIs with important cofactors such as RNA pol II, and splicing factors DDX17 and DDX5.\textsuperscript{169} Despite these mechanistic studies being specific to the AR, Pyrvinium has been implicated in numerous additional signaling pathways, which suggests that the mechanism of any functional activity is likely multidimensional.\textsuperscript{171} In addition, structural optimization of Pyrvinium for enhanced aqueous solubility has been undertaken, resulting in the analog P24.\textsuperscript{170} The structure of this was not disclosed, therefore it cannot be examined in terms of its optimization campaign and if it addressed issues associated with structural alerts embedded in the compound (e.g., pyridinium species, anilino motif).

Cherkasov et al. undertook the rational design of DBD-targeted therapeutics utilizing the available crystal structure of the AR DBD dimer-DNA complex.\textsuperscript{153,167} Aided by computational modeling, a binding site was proposed to exist in the DBD adjacent to the P-Box region—the region of the DBD that interacts with the DNA major groove.\textsuperscript{172} Virtual screening of the ZINC database, followed by an eGFP assay yielded five hit compounds, the most potent of which underwent extensive SAR development, resulting in \textit{VPC-14449} which had an IC\textsubscript{50} value of 100 nM determined by eGFP assay (Figure 26A).\textsuperscript{167}

Binding of \textit{VPC-14449} to the intended DBD region was evidenced using mutagenesis studies, defining the interaction to exist between residues 592 and 594 in the DBD. Moreover, bilayer interferometry was used to

![FIGURE 25 Seminal small molecule inhibitors of the AR-DBD. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area. [Color figure can be viewed at wileyonlinelibrary.com]]
Further substantiate the DBD as the site of action by excluding the possibility of a binding event at the LBP, AF-2, or BF-3 within the LBD, or AREs within target DNA. Despite the conserved nature of nuclear hormone DBD regions, VPC-14449 displayed negligible inhibition of the ER, GP, and PR. VPC-14449 was shown to disrupt DBD-chromatin interactions in vitro and inhibit growth of LNCaP cells, enzalutamide-resistant MR49C cells, and AR-V-driven 22Rv1 cells. VPC-14449 also reduced PSA expression and tumor volume in xenograft models at a dose of 100 mg/kg twice daily, at which no toxicity was observed. Notably, optimization of previous hits from the initial screen was also carried out, affording potent DBD-inhibitors VPC-14332 and VPC-14452; however, these assets have only been reported in the patent literature, and therefore are not as extensively characterized as VPC-14449. Subsequently, Xu et al. further explored the SAR of VPC-14449, but despite extensive analog generation, the potency was not improved.

In recent reports from the Cherkasov group, their focus has turned to targeting the o-Box, the region of the DBD involved in homodimerization, which is typically a requirement for DNA binding. Using the same virtual screening methodology that they applied to P-Box, four hit compounds were identified and validated. VPC-17005 was the most potent with an IC₅₀ of 0.7 µM, and demonstrated activity against enzalutamide-resistant MR49F cells and AR-V-driven 22Rv1 cells, but not AR-negative PC-3 cells, which suggests selectivity for the AR pathway (Figure 26B).
Treatment of LNCaP and 22Rv1 cells with VPC-17005 did not influence the expression of AR protein, which excludes receptor degradation as a possible mechanism of action. In addition, selectivity over other the NRs (ER, GR, and PR) was attributed to the nonconserved residues S597, F606, and S613 by sequence alignment. Mechanistic action via the v-box dimerization interface was demonstrated through the use of a mammalian-two hybrid assay with fused proteins, FRET imaging, BLI experiments, and a lack of inhibition for the dimerization-deficient AR mutant A596T/S597T.

Low MW and moderate cLogP suggest that VPC-17005 will possess adequate solubility and permeability, although the benzothiophene moiety may present a potential site of metabolic liability. In addition to this, the aminothiazole portion constitutes a known toxicophore which will likely preclude further development. The fragment-like nature of the hit compound, allied with availability of structural data for the AR-DBD enables structure-based optimization of the molecule, for the pursuit of additional receptor-ligand interactions with increased potency.

In subsequent attempts to address the metabolic instability of VPC-17005, the authors conducted a further virtual screen of the ZINC database, and utilized a pharmacophore model of VPC-17005 to identify novel chemotypes with a similar ability to inhibit AR DBD-dimerization. VPC-17160 and VPC-17281 were identified and displayed improved antidimerization capacity against AR-V7 compared to VPC-17005 (Figure 26C). VPC-17281, demonstrated marked improvements to microsomal stability and antiproliferative capacity against AR-V7-driven 22Rv1 cells.

From an optimization perspective, VPC-17281 is likely to be the superior lead compound, due to the comparably favorable physicochemical properties. Furthermore, the microsomal stability is significantly higher, potentially due to the propensity for CYP450-mediated metabolism at 2- and 5-position of the thiophene moiety present. The acrylamide moiety of VPC-17281 should be considered in future optimization as a possible source of off-target covalent modification, especially given that it is not required for the proposed reversible mechanism of action.

The dihydrochalcone MF-15 is a synthetic analog of a series of recently reported chalcone natural products, isolated from Melodorum fruticosum (Figure 27A). MF-15 acts as a dual inhibitor of the AR and AKR1C3, an enzyme involved in intra-tumoral androgen biosynthesis, which is implicated in enzalutamide-resistant CRPC. At a concentration of 10 μM, MF-15 inhibited AKR1C3 activity by 87% and significantly inhibited androgen-induced PSA expression. In addition, MF-15 inhibited AR-FL and AR-V7 in a dose-dependent manner between concentrations of 2.5-10 μM, and reduced cell viability of enzalutamide resistant 22Rv1 cells in the 0.2-100 μM range. Kafka et al. proposed that MF-15 interacts with the P-box in the DBD based on molecular docking studies and nonspecific inhibition for both the AR and GR, which share DBD sequence homology. MF-15 has acceptable properties in terms of MW and cLogP, leaving opportunities for structural growth and exploration. Replacement of the phenyl rings with heterocycles, or alternative incorporation of polar functionality could modulate activity and favorably influence cLogP. However, it should be noted that the polyphenol functionality is a PAINS structural alert.

Recently, Lee et al. reported a DBD-targeting proteolysis-targeting chimera (PROTAC). MTX-23 was found to degrade AR-FL, and the clinically relevant splice variants AR-V7 and ARv567, while leaving other steroid hormone receptor levels unaffected (Figure 27B). MTX-23 also reduced cellular proliferation in AR-positive cell lines, but not AR-negative cell lines. The degradation-based mechanism of MTX-23 was substantiated through the identification of polyubiquitinated AR-FL and AR-V7 by immunoblot assay. Notably, MTX-23 also displayed efficacy against LNCaP, VCaP, and 22Rv1 cells that had been cultured to display resistance to the current standard of care CRPC treatments: abiraterone, apalutamide, enzalutamide, and darolutamide. In enzalutamide-resistant xenograft models, MTX-23 significantly reduced tumor size over 5 weeks in combination with enzalutamide, compared to enzalutamide treatment alone.

Despite their advantages of higher potency and potential for catalytic protein deterioration, PROTACs often suffer from poor physicochemical properties due to the high MW and a large number of rotatable bonds.
MTX-23 exhibits similar characteristics, with large values for MW, cLogP, HBA, and TPSA which increases the likelihood of poor solubility and membrane permeability. Nevertheless, MTX-23 displayed efficacy in xenograft models when administered both intraperitoneally and orally, suggesting at least moderate bioavailability.

In general, the molecules that target the DBD all comply with Lipinski's rules, with the exception of MTX-23 (Figure 28). Despite rapid clearance, Harmol possesses good potency and fragment-like properties, making it an attractive starting point to optimize inhibition of DNA occupancy. Pyrvinium also resides in druglike chemical space and displays potent AR inhibition in a luciferase assay, optimization has already been carried out and we await the disclosure of next-generation analog, P24. The P-box-targeting VPC compounds all have druglike properties in terms of MW, cLogP, HBD/A, TPSA, and iPFI, making oral absorption likely. They have also undergone extensive SAR exploration, particularly VPC-14449, and represent the most thoroughly validated class of AR-DBD inhibitors to date. The lead D-box-targeting compound, VPC-17281 also presents with good physicochemical properties for solubility and permeability, and a balance of polar and lipophilic groups (Table 5).

7 | TARGETING THE HINGE REGION

The HR is a short flexible linker between the DBD and the LBD that contains one half of a bipartite nuclear localization signal. Mutation and deletion studies have demonstrated that the HR, particularly residues 628 to 646, exerts inhibitory regulation over ligand-dependant AF-2 function, modulates transcriptional activity and is implicated in constitutively active double mutants. It also plays a role in DNA binding, nuclear translocation, transactivation and C–N interaction of the AR. While the role of the HR is more than a passive linker, it has received scant attention and is widely overlooked in the context of targeting the AR in the treatment of CRPC.
Despite the implication of the HR in numerous functional processes, no small molecule inhibitors of AR that directly target the HR have been reported to date. In terms of chemologics, EZN-4176 is an antisense oligonucleotide that targets the HR of AR mRNA with some efficacy, although technically lies outside the principal focus of this review as it does not target the AR protein.²⁹⁵,²⁹⁶ There are also examples of AR inhibition via the interruption of posttranslational modifications that occur at the HR, but are known to target other proteins such as histone acetyltransferases, rather than engaging in direct interaction with the AR itself.²⁹⁷ Further mechanistic investigation of the HR and its roles could afford valuable insight and lead to possible therapeutic targets for CRPC.

### 8 | MISCELLANEOUS COMPOUNDS

In addition to the above, ASC-J9 (dimethylcurcumin) is a small molecule enhancer of AR degradation (Figure 29A).²⁹⁸,²⁹⁹ Mechanistically, it is reported to function via selectively interrupting interactions between the AR and its coregulators, ultimately resulting in reduced transcriptional activity and enhancing proteasomal degradation. ASC-J9 has been shown to inhibit growth of androgen-sensitive and castration resistant cell lines and suppresses tumor growth in xenograft models. Addressing the lipophilic nature of ASC-J9 and removing the central Michael acceptor moieties, despite their presence in the natural curcuminoid scaffold, may serve as potential focus points during future optimization.

Finally, AZD3514 is a small molecule inhibitor of the AR, which has a bimodal mechanism of action, acting both via disruption of androgen-promoted nuclear translocation, and downregulation of receptor levels (Figure 29B).³⁰⁰ Two phase 1 clinical trials (NCT01351688, NCT01162395) assessing AZD3514 have taken place, wherein moderate efficacy was demonstrated, albeit accompanied by an unacceptable side effect profile. Although the MW of AZD3514 is above 500, the remaining metrics such as cLogP, iPFI, and TPSA are acceptable; combined with demonstrable efficacy, this encourages further investigation of this chemotype.
9 \quad **CONCLUSIONS**

Many of the compounds presented in this review combine good developability profiles with promising efficacy against the AR in a range of assays that demonstrate novel modes of action (Figure 30). Those assets with reasonable lipophilicity and low MWs are more likely to be developed into advanced leads, or ultimately clinical candidates; not only because there are further opportunities for structural growth and exploration but also because they avoid the majority of the potential liabilities present in suboptimal molecules, that is, off-target effects, low
solubility, poor permeability, and presence of metabolic hotspots. Through careful control of the developability properties of candidate molecules in early-stage drug development, researchers can ensure that time and resources are focused on higher-quality compounds to minimize drug attrition rates throughout the discovery pipeline. Many of the current approaches are well-balanced in this regard and are likely to be explored in future publications.

**Figure 29** (A) AR degradation enhancer ASC-J9; (B) dual mechanism AR inhibitor AZD3514. cLogP, calculated lipophilicity coefficient; HBA, hydrogen bond acceptor; HBD, hydrogen bond donors; iPFI, intrinsic property forecast index; MW, molecular weight; TPSA, topological polar surface area. [Color figure can be viewed at wileyonlinelibrary.com]

**Figure 30** Developability overview of all compounds considered within this review. MTX-23, (+)-JJ-74-138, and compound 9b were omitted for clarity. [Color figure can be viewed at wileyonlinelibrary.com]
It remains to be seen whether the development of more efficient and mechanistically diverse inhibitors of the AR will overcome treatment resistance in the clinical setting. The success seen with second-generation antiandrogens, coupled with the fact that the AR gene is the most commonly upregulated gene in CRPC patients, warrants additional future development of AR-based CRPC therapies that do not target the canonical LBD.202,203

DATA AVAILABILITY STATEMENT
Data relating to calculated molecular properties of molecules reported in the review is available from the corresponding authors on request.

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AUTHOR BIOGRAPHIES

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