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Design of oxobenzimidazoles and oxindoles as novel androgen receptor antagonists

Chuangxing Guo *, Mason Pairish *, Angelica Linton, Susan Kephart, Martha Ornelas, Asako Nagata, Benjamin Burke, Liming Dong, Jon Engebretsen, Andrea N. Fanjul

Oncology Medicinal Chemistry, Pfizer PharmaTherapeutics R&D, San Diego, CA 92121, USA

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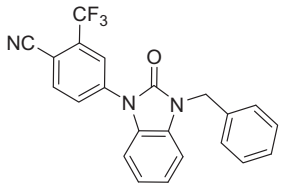
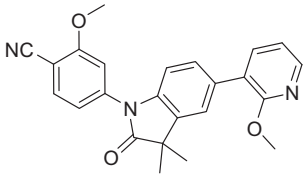
ABSTRACT

Oxobenzimidazoles (e.g., **1**), a novel series of androgen receptor (AR) antagonists, were discovered through de novo design guided by structure-based drug design. The compounds in this series were reasonably permeable and metabolically stable, but suffered from poor solubility. The incorporation of three dimensional structural features led to improved solubility. In addition, the observation of a 'flipped' binding mode of an oxobenzimidazole analog in an AR ligand binding domain (LBD) model, led to the design and discovery of the novel oxindole series (e.g., **2**) that is a potent full antagonist of AR.



1
oxobenzimidazole

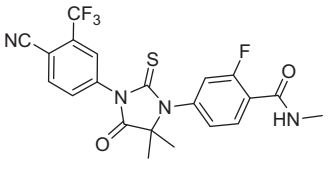


2
oxindole

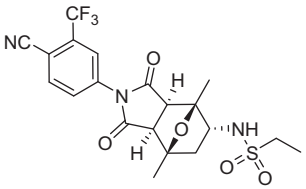
Most prostate cancer patients receiving hormonal therapies progress to more aggressive castration-resistant prostate cancer (CRPC).[1] Recently, full AR antagonists such as **MDV3100** and **BMS-641988** (Fig. 1) have demonstrated efficacy against CRPC in preclinical models[2] and more importantly in patients.[3] These encouraging results instigated our interest[4] in searching for new, non-steroidal full AR antagonists.

There are no reported crystal structures of a full antagonist bound to AR, but the crystal structure of a full antagonist bound to the closely related estrogen receptor (ER) may be illustrative of the conformational changes responsible for full antagonism to a nuclear hormone receptor (NHR). As shown in Figure 2A[5] when an agonist (diethylstilbestrol) (shown in orange) binds to ER, helix 12 (H12) (shown in yellow) adopts a 'closed' conformation, which facilitates binding of a coactivator (shown in blue) to ER and triggers downstream gene transcription. In Figure 2B, an ER antagonist (4-hydroxytamoxifen) (shown in orange) 'pushes' H12 open and prevents the binding of the coactivator. As a result, gene transcription is not initiated.

It was rationalized that since the LBD domain of NHRs have a highly conserved structure,[6] a full antagonist of AR may require



MDV-3100
(Phase II/III)



BMS-641988
(Phase I)

Figure 1. Androgen receptor antagonists.

* Corresponding authors.
E-mail addresses: alexguo01@gmail.com (C. Guo), mason.pairish@pfizer.com (M. Pairish).

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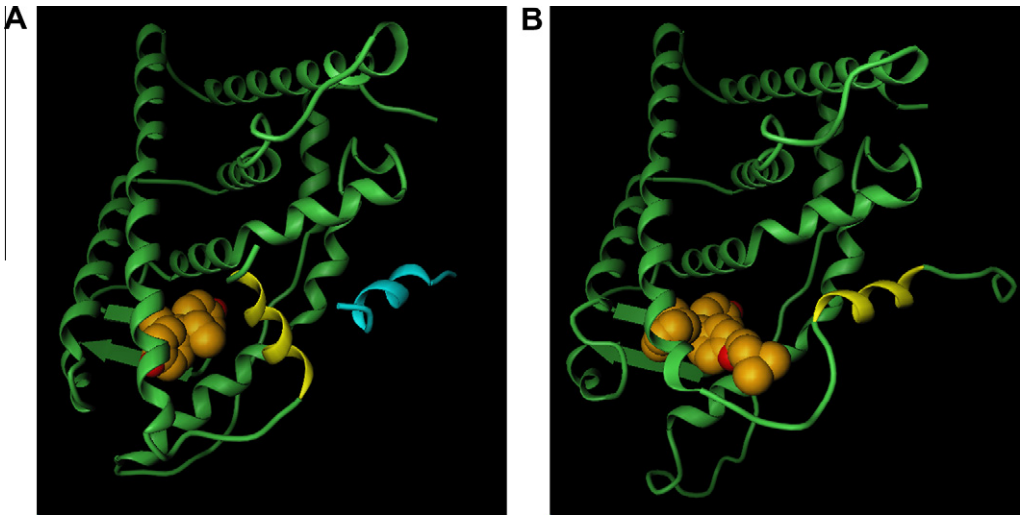


Figure 2. Crystal structures of estrogen receptor. Helix 12 in yellow. Coactivator in blue. Ligand in orange. (A) ER with agonist (diethylstilbestrol) (PDB: 3ERD). (B) ER with antagonist (4-hydroxytamoxifen) (PDB: 3ERT).

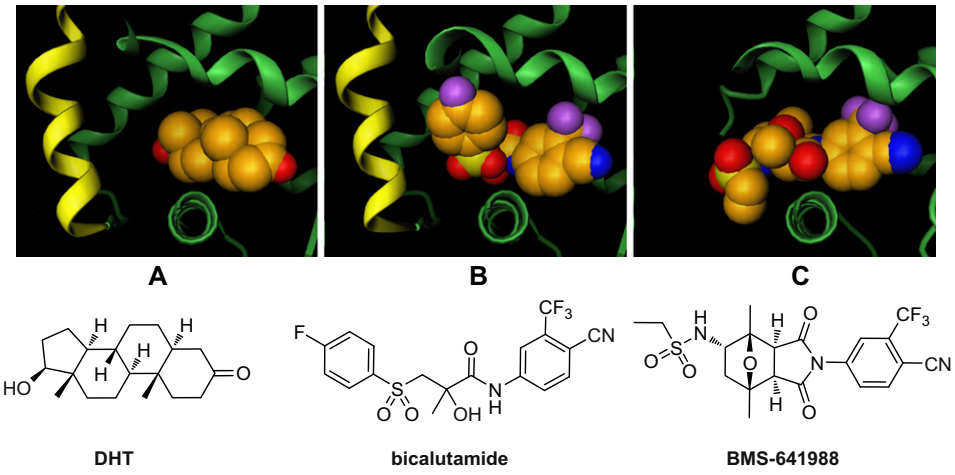


Figure 3. Crystal structures and truncated model of AR. Helix 12 in yellow. Ligand in orange. (A) AR with agonist (DHT) (PDB: 3L3X). (B) AR with partial-antagonist (bicalutamide) (PDB: 1Z95). (C) Model of truncated AR with full antagonist BMS-641988).

H12 movement[7] similar to that seen in ER. In co-crystal structures[8] of AR with its natural agonist 5-alpha-dihydrotestosterone (DHT) (Fig. 3 A) and the co-crystal structures[9] of a mutant AR with a partial antagonist[10] (bicalutamide) (Fig. 3B), H12 adopts a closed conformation. Our docking[11] of the reported full antagonist BMS-641988 (Fig. 3C) into the truncated[12] ligand binding domain (LBD) suggests that it would keep H12 from closing and prevent the binding of coactivator, thereby inhibiting gene transcription.

Comparison of the possible binding modes of BMS-641988 and compound **3** (an AR antagonist previously reported by Pfizer[13]), suggested that an oxobenzimidazole (e.g., **1**) could be used as a novel core which would provide rigid vectors for attaching the left hand side group and the right hand side group (Fig. 4). In particular, the right hand side benzyl group may be able to push H12 into an open form for achieving full antagonism. To test this design, analogs **1, 7–9** were synthesized by the methods shown in Scheme 1.[14] Nucleophilic displacement of commercially available aryl fluoride **4** with urea **5** yielded product **6**, which was then alkylated or coupled with boronic acids to provide compounds **1, 7–9** in good yields. Compounds **1, 7–9** were tested in a cell-based (CRPC model) AR antagonism assay.[15] Compound **1** was found to be a full AR antagonist (Table 1) with reasonable potency. Interestingly, more rigid analogs **7** and **8** were inactive in the same assay, implying the vector coming off the right hand side nitrogen may be different from that of **MDV3100**.

Despite its high lipophilicity, the metabolic stability (HLM ER = 0.46)[16] of **1** is good. The major drawback of this molecule is its poor aqueous solubility. In order to improve solubility, we looked into two initial strategies: truncation and substitution. The effect on potency with the change in polarity was monitored with the measure of lipophilicity ligand efficiency (LipE).[17] LipE accounts for compound lipophilicity and antagonism potency (in one term). First, truncation of the right-hand region was investigated. Although this was an effective way to improve solubility, either increased agonism[18] and/or decreased potency was observed with the changes (Table 2). A second strategy to improve solubility was the introduction of nitrogen atoms into the benzene rings of our lead compounds to give pyridyl analogs (Table 3). Unfortunately, potency and solubility did not track together with these substitutions.

A third approach to improve aqueous solubility was to disrupt molecular planarity and symmetry.[19] Branching in the right-hand region (Table 4) was effective in increasing solubility but the resulting compounds were either less potent (**20**/**21**) or prone to

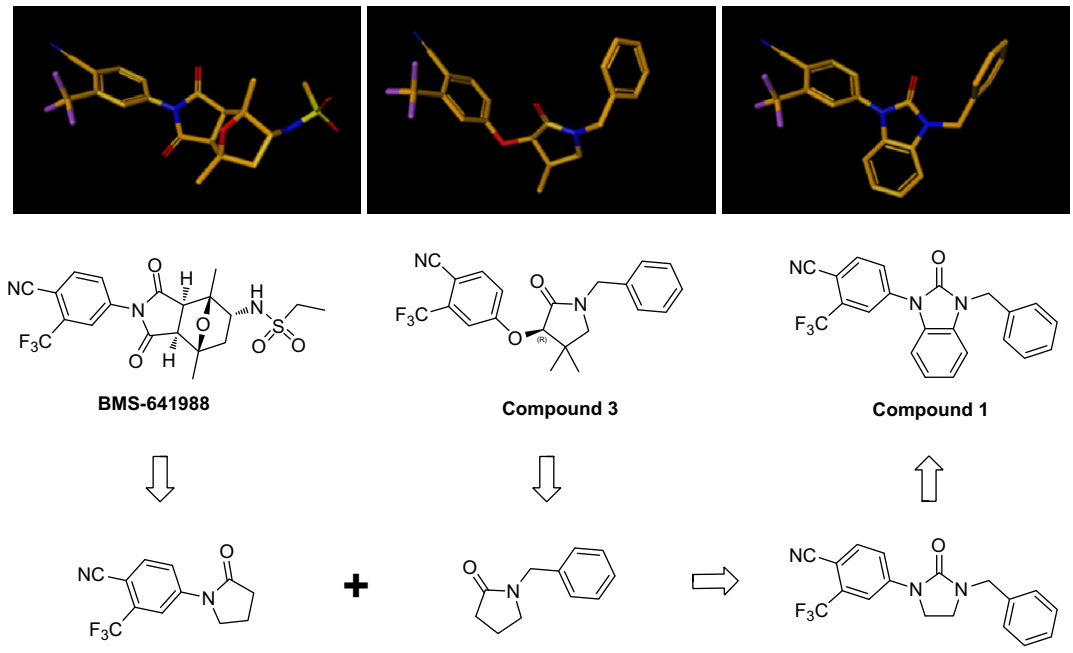
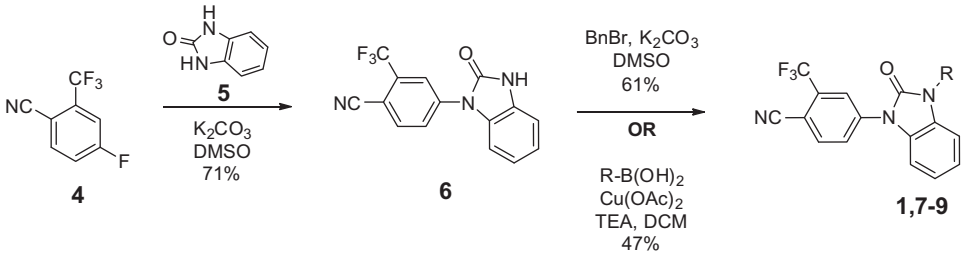
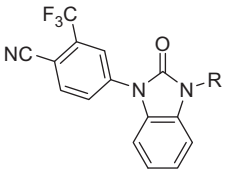


Figure 4. Design of oxobenzimidazole core.



Scheme 1. Synthesis of compounds **1**, **7**–**9**.

Table 1
Initial SAR of oxobenzimidazole series



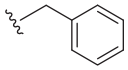
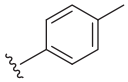
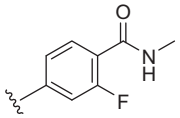
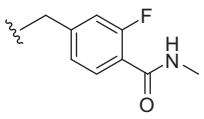
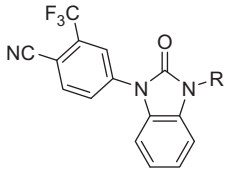
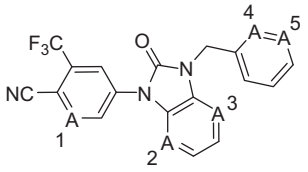
Compd	R	*c*Log*P*	Antagonism IC_{50}, nM (or% @1.0 μM)	Agonism fold induction @1.0 μM	Sol (μM)	LipE
1		5.43	170	1.04×	<0.16	1.34
7		6.38	(12.6%)	0.88×	0.79	
8		4.66	(28.7%)	1.44×	8.81	
9		4.08	(72.5%)	1.08×	9.9	

Table 2
SAR of analogs with truncated right-hand substituents



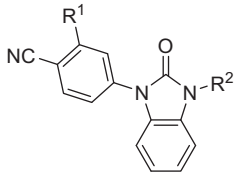
Compd	R	*c*Log*P*	Antagonism IC_{50}, nM (or% @1.0 μM)	Agonism fold induction @ 1.0 μM	Sol (μM)
10	CH_2CN	3.39	(94.8%)	1.42×	18.9
11	*i*Pr	4.86	373	0.965×	9.3
12	H	4.18	(60.1%)	6.2×	7.9
13	Et	4.55	293	1.08×	3.47

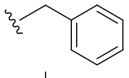
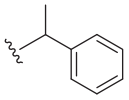
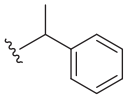
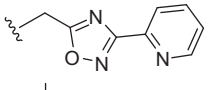
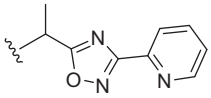
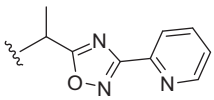
Table 3
SAR of pyridyl isomers

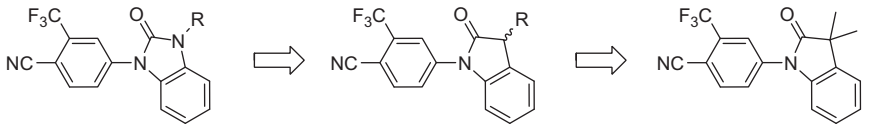


Compd	N position	*c*Log*P*	Antagonism IC_{50}, nM (or% @1.0 μM)	Agonism fold induction @ 1.0 μM	LipE	Sol (μM)
14	A1	4.4	(91%)	1.04×		5.3
15	A2	4.27	60.6	1.13×	2.95	<0.500
16	A3	4.27	588	0.951×	1.96	
17	A4	3.93	301	1.03×	2.59	12
18	A5	3.93	389	0.984×	2.48	2.5
19	A1, A2	3.23	(96.3%)	1.1×		1.7

Table 4
SAR of analogs with branched right-hand groups



Compd	R^1	R^2	Optical rotation	*c*Log*P*	Antag. IC_{50} (nM)	Agonism fold induction @1.0 μM	LipE	Sol (μM)
1	CF_3		NA	5.43	170	1.04×	1.34	<0.16
20	CF_3		(−)	5.74	363	0.833×	0.70	5.58
21	CF_3		(+)	5.74	292	0.904×	0.79	4.41
22	Cl		NA	3.38	126	0.941×	3.52	5.9
23	Cl		(−)	3.68	189	0.945×	3.04	
24	Cl		(+)	3.68	178	0.874×	3.07	9.45



25

Antagonism % @ 1.0 uM = (75.70%)
Agonism fold induction @ 1.0 uM = 1.96x
cLogP = 3.3

Figure 5. Design of dimethyloxindole core.

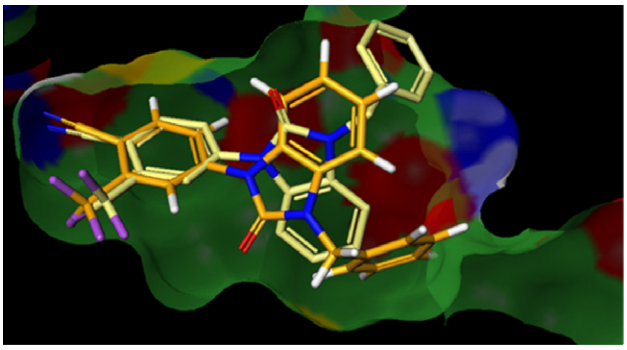


Figure 6. Two different binding modes observed when docking compound **1**.

epimerization (**23**/**24**).[20] Lipophilicity ligand efficiency (LipE).[17] was improved in the case of compounds **23/24**. Therefore, we turned our attention to adding a three dimensional structural feature to the oxobenzimidazole core.

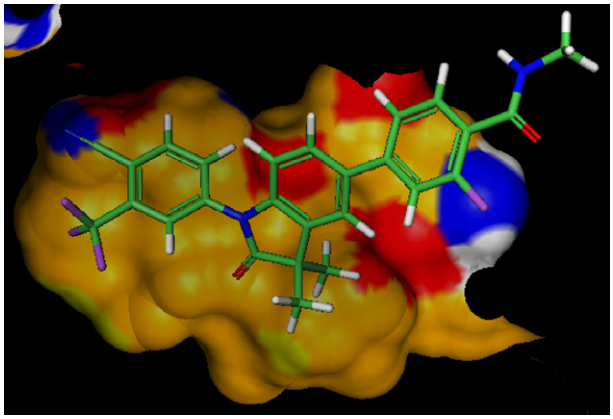
Figure 5 illustrates how replacement of one of the nitrogens of the oxobenzimidazole core with a carbon would introduce a non-planar sp^3 center. The dimethyl-substituted oxindole analog **25** had submicromolar antagonism, but unfortunately proved to possess significant AR agonism.[18]

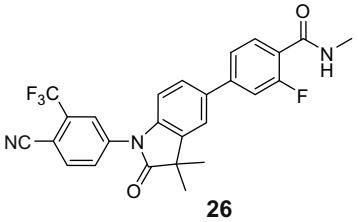
In order to suppress the agonism, computer modeling was employed to design oxindole molecules which would have the potential to push AR H12 open. As a surrogate for the oxindole core, the modeled bound conformations of the closely related oxobenzimidazole **1** were examined for hints of a suitable vector to extend out for interaction with the H12. Docking[11] of **1** into a truncated AR model (Fig. 6).[12] suggested that the oxobenzimidazole ring could adopt two conformations. In one conformation (shown in yellow), the benzyl group extends towards H12 as originally hypothesized. In the second, 'flipped' conformation (shown in orange), the benzyl group binds deeper in the LBD and the 5-position of the oxobenzimidazole is oriented toward H12. This suggested a new vector for the introduction of H12 disrupting extensions.

We reasoned that the extended oxindole structure such as **26** would dock well in this conformation (Fig. 7). Therefore it was synthesized by the method shown in Scheme 2.[14] Nucleophilic displacement of commercially available aryl fluoride **4** with oxindole **27** yielded product **28**. Suzuki cross-coupling with boronic acid **29** provided compound **26** which was found to be a potent AR full antagonist.

To improve ADME properties of **26**, we wanted to reduce lipophilicity by replacing the trifluoromethyl group with a methoxy group. The aryl methoxy group was introduced via a copper catalyzed cross coupling (Scheme 3)[14] of arylbromide **30** and oxindole **31** to give compound **32**. Bromination of **32** proceeded regiospecifically to afford **33** as the only product. Subsequent Suzuki couplings provided **35** and **2**. Both compounds have improved solubility over the original lead (**1**) and good pharmacology and ADME profiles (Table 5). Lipophilicity ligand efficiency (LipE).[17] was also significantly improved (3.58/3.79 for **35** and **2** vs 1.34 for **1**). The oxindole has been established as a novel core for AR full antagonists.

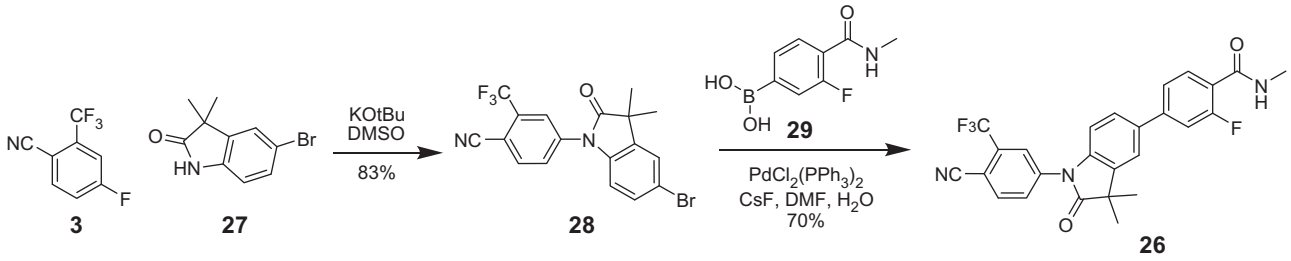
In summary, a series of novel, non-steroidal full AR antagonists have been discovered through design of AR LBD binders possessing sufficient length and rigidity to force H12 movement. The initial oxobenzimidazole leads suffered from low solubility that is likely due to high lipophilicity and the planarity of the oxobenzimidazole core. The crystallinity of the oxobenzimidazole core was reduced by introduction of three dimensional structural features (sp^3 centers), most notably, by replacing oxobenzimidazole with the less planar dimethyloxindole. The design idea was inspired by detailed analysis of ligand binding conformations docked into a truncated AR LBD model. The discovery of the dimethyloxindole series not



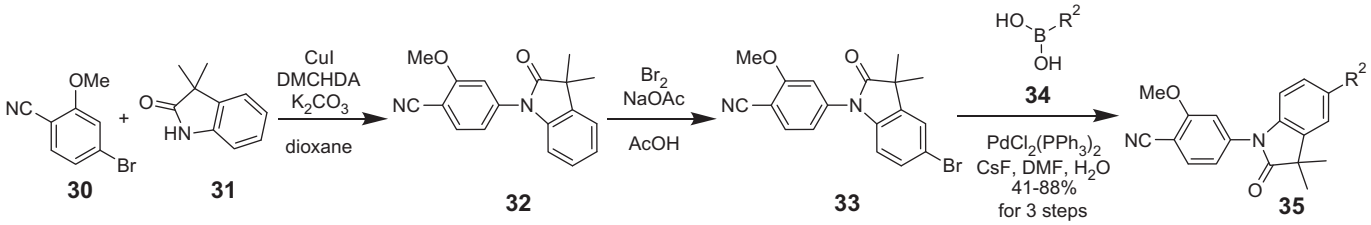


26

Figure 7. Docking experiment of extended dimethyloxindole structure **26** suggested that the 'flipped conformation' is preferred.

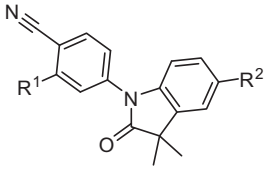


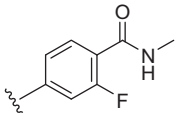
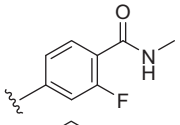
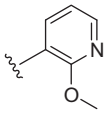
Scheme 2. Synthesis of oxindole analogs bearing trifluoromethyl groups.



Scheme 3. Synthesis of oxindole analogs bearing methoxy groups.

Table 5
SAR of extended dimethyloxindole series



Compd	R^1	R^2	*c*Log*P*	Antag. IC_{50} (nM)	Agonism fold induction @1.0 μM	Sol (μM)	HLM[a]	LipE
26	CF_3		3.84	197	0.941×	6.25	0.4	2.87
35	OMe		3.2	165	0.884×	9.7	<0.25	3.58
2	OMe		3.31	79.6	1.04×	9.8	[b]	3.79

[a] see Ref. 16.
[b] Compound **2** ionizes poorly under the MS conditions used in the HLM assays, so reliable data for this compound could not be obtained from this assay.

only benefits the search for novel second generation AR antagonists, but also sheds light on the AR protein conformation changes responsible for full antagonism.

References and notes

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11. Docking simulation was performed with Glide docking program, and compounds were docked into a LBD binding pocket of the truncated AR model (see Ref. 12).
12. Although many agonist-bound NHR crystal structures have been published in the last decade, only a few antagonist-bound structures have been reported in the PDB. The AR modeling was based on three ER co-crystal structures that have antagonistic actions in some cells (PDB code: 1ERR, 3ERT, 1NDE). These co-crystal structures bound to these modulators displayed a different ER conformation from the ''closed'' agonist-bound form. Instead, these structures adopted a conformation where the C-terminal alpha helix (helix12; H12) was changed dramatically and H12 blocked its co-activator binding site. When developing a model for our antagonist designs, we made two main assumptions. We assumed that the agonist-bound crystal structures were not proper for modeling since antagonists would bump H12 in the agonist-bound 'closed' conformation. We also assumed that AR would have a similar inactive conformation to ER when AR antagonists were bound. However, since the C-terminal residues were not well conserved among NHRs and uncertainty remained regarding its exact conformation in AR, we built an AR model based on AR crystal structure (PDB code: 3B5R) with a truncated C-terminus (Leu881∼) where H12 was absent. We applied this truncated, antagonist-ligand-accepting model for evaluating our designed molecules by docking simulations.
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14. See Supplementary data for synthetic details.
15. CRPC cell-based HTS assay: In this assay, prostate cancer refractory cells (LNAR), stably expressing an AR Response Element DNA sequence (ARE)-luciferase reporter gene construct (PSA-LUC), were treated with the testing compounds in the absence (agonistic mode) or the presence (antagonistic mode) of a potent agonist (R1881). Upon activation and binding of the AR to the ARE, the luciferase gene transcribed and translated into active luciferase enzyme and luminescence was read as signal by the plate reader. Testing compounds were dissolved in 100% DMSO as 10 mM stock solution. Serial dilutions were prepared from 0.17 nM to 10 μM and final DMSO concentration never exceeded 0.1%. For agonism, values obtained from the compounds under study were compared to those of untreated cells, which were assigned an arbitrary number of 1.0 to indicate no agonism. For antagonism, cells were treated with 0.1 nM R1881 alone (corresponding to max receptor activation = 100%) or in combination with the various compounds.
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18. The cut-off for significant residual AR agonism was set to >1.11 fold induction in CRPC cell-based assay (agonism mode, see Ref. 7) based on statistic analysis of multiple test results ($n = 176$) of RD-162 in the same assay. RD-162, a full AR antagonist reported in literature (Refs. 4a,b), was used as a reference standard. In the agonism assay, the averaged agonism fold induction of RD-162 ($n = 176$) is 0.876 with a standard deviation of 0.0779. An AR ligand with an agonism fold induction >1.11 ($0.876 + 3 \times 0.0779$) is likely (>99.7% confidence) to have more residual agonism than RD-162.
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