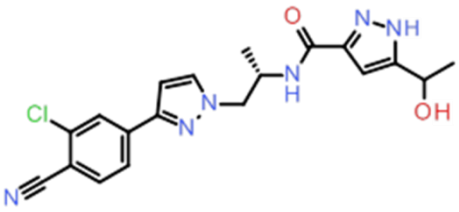
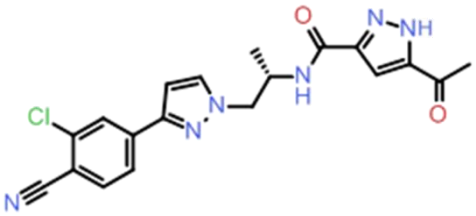
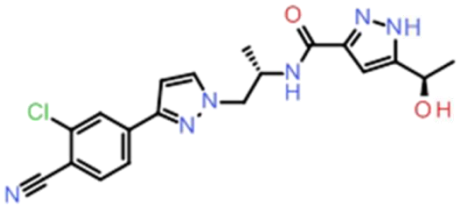


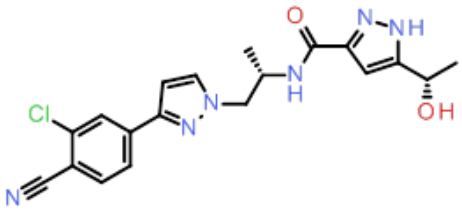
darolutamide
(399)



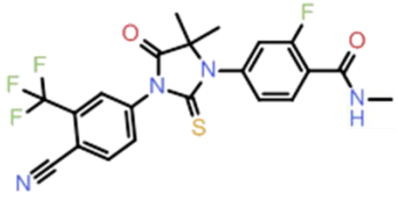
keto-darolutamide
(397)



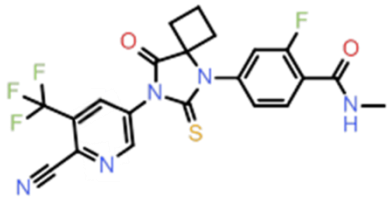
(S,R)-darolutamide
(399)



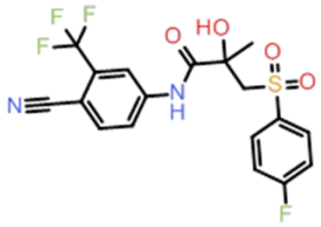
(S,S)-darolutamide
(399)



enzalutamide
(464)



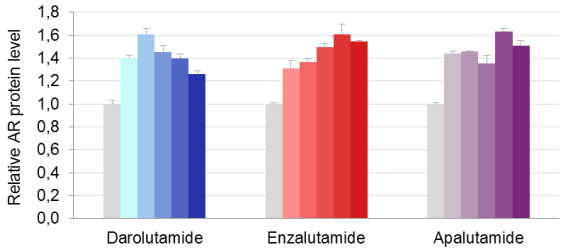
apalutamide
(477)



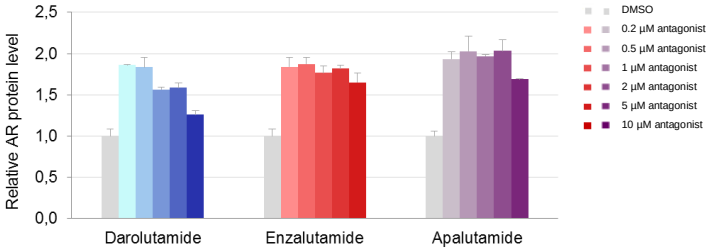
bicalutamide
(430)

Supplementary Figure 1. Chemical structure of darolutamide and derivatives, and of the other AR antagonists analyzed. The molecular weight of each compound is indicated in parentheses.

VCaP

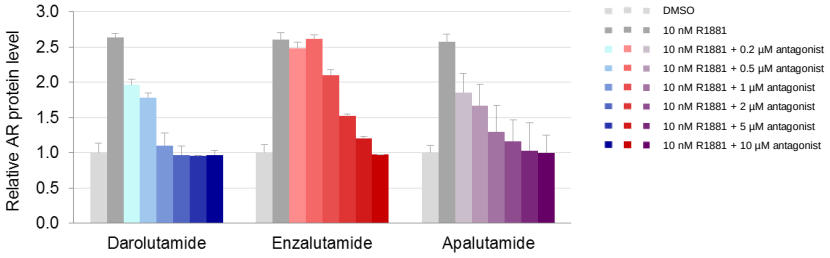


AR full-length



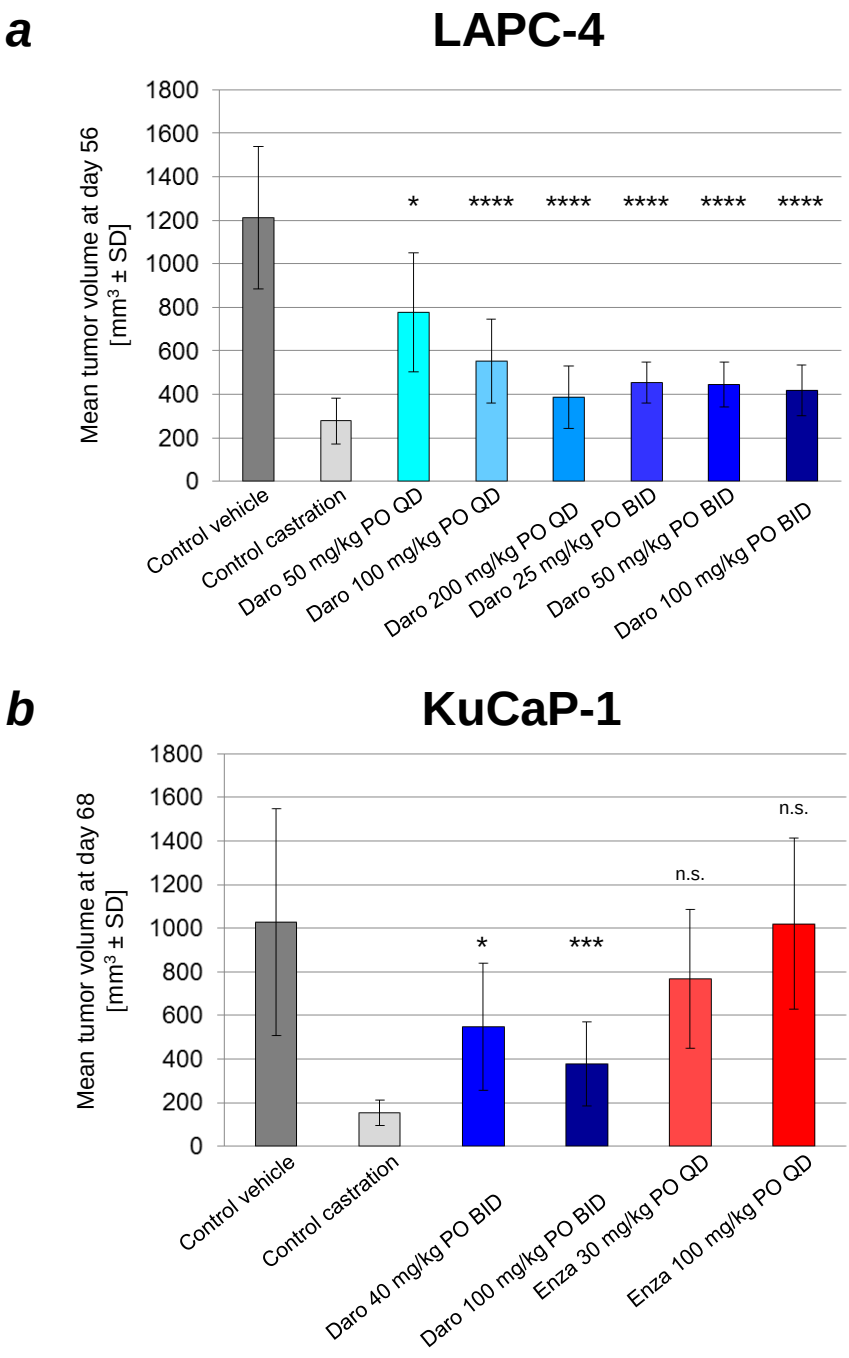
AR V7

LAPC-4



AR full-length

Supplementary Figure 2. Impact of AR antagonists on AR protein levels. Lysates of cells treated with different AR antagonist concentrations were analyzed using a sandwich AR ELISA kit.



Supplementary Figure 3. *(a) In vivo* efficacy in LAPC-4 model. Mean tumor volume ± SD at day 56 is shown. Significance vs. control vehicle group was determined at day 56 using one-way ANOVA with Dunnett's post hoc test on Log-transformed tumor volumes: *, $p = 0.04$; ****, $p = 0.0001$. *(b) In vivo* efficacy in KuCaP-1 model. Mean tumor volume ± SD at day 68 is shown. Significance vs. control vehicle group was determined at day 68 using one-way ANOVA with Dunnett´s post hoc test on Log-transformed tumor volumes: *, $p = 0.0169$; ***, $p = 0.0001$, n.s.: not significant. Daro: darolutamide, Enza: enzalutamide.

Primer	Sequence (5´-3´)
KLK3 forward	CAAACCTGCTCAGCCTTTGTC
KLK3 reverse	TGTTCCTCCAGAGTAGGTCTGTT
FKBP5 forward	GAGGCTGCCTGGGCTTC
FKBP5 reverse	AGAACCTCGTGTTCCAGCAA
FEZ1 forward	GAGGCTCCTCGTTGATACCG
FEZ1 reverse	CCACCTCCTCTCCAGATCCA

Supplementary Table 1. Primers used for ChIP-qPCR analysis.

Compound	R1881 (nM)	V731M	H875Y	F877L	T878A
Darolutamide	0.1	90 ± 30	200 ± 90	140 ± 30	1540 ± 90
	1	570 ± 180	1740 ± 530	850 ± 190	9830 ± 300
	10	7160 ± 2640	>10000	7200 ± 2150	>10000
Enzalutamide	0.1	120 ± 20	320 ± 120	**Agonism**	130 ± 10[2]
	1	740 ± 160	3050 ± 1360	**20 ± 10%**	720 ± 420[2]
	10	8510 ± 1830	>10000		>10000
Apalutamide	0.1	120 ± 10	140 ± 70	**Agonism**	310 ± 40[2]
	1	740 ± 280	1430 ± 350	**40 ± 10%**	1120 ± 760
	10	7360 ± 1790	>10000		>10000
Bicalutamide	0.1	210 ± 140	960 ± 280[1]	430 ± 120[1]	860 ± 260
	1	1350 ± 690	7610 ± 2930[1]	2140 ± 320[1]	3410 ± 290[2]
	10	9470 ± 580	>10000	>10000[1]	>10000

Compound	R1881 (nM)	I882L	M896T	M896V
Darolutamide	0.1	30 ± 10	n.d.	50 ± 10
	1	110 ± 20	50 ± 20	240 ± 60
	10	1270 ± 400	330 ± 230	2840 ± 1740
Enzalutamide	0.1	40 ± 10	n.d.	480 ± 270[1]
	1	170 ± 40	470 ± 140[1]	1270 ± 590[1]
	10	1760 ± 490	2590 ± 1280[1]	>10000[1]
Apalutamide	0.1	40 ± 10	n.d.	50 ± 5
	1	170 ± 30	200 ± 50[1]	260 ± 40
	10	1710 ± 170	1210 ± 370[1]	2930 ± 1030
Bicalutamide	0.1	90 ± 10	**Agonism**	**Agonism**
	1	330 ± 10	**50 ± 10%**	**60 ± 10%**
	10	3140 ± 620		

Supplementary Table 2. Transactivation assays for AR mutants. Mean IC_{50} ± SD values from 3 to 6 biological replicates are given in nM. Cells were treated with the indicated R1881 concentrations (in nM) and the mentioned AR antagonist. In some cases agonism was found and the % activity measured in the presence of 1 µM compound, in comparison to 1 nM R1881 treatment which was set to 100%, is given in bold. Statistical analysis was performed with t-test on average pIC_{50} values. Superscripts indicate antagonism significantly lower[1] or higher[2] compared to darolutamide. Abbreviation: n.d., not determined.

Compound	AR wild-type	AR W742C	AR W742L
Darolutamide	600 ± 80	550 ± 90	910 ± 170
(*S,R*)-darolutamide	550 ± 20	500 ± 30	780 ± 180
(*S,S*)-darolutamide	740 ± 40	600 ± 70	1000 ± 250
Keto-darolutamide	630 ± 60	480 ± 40	1130 ± 380
Enzalutamide	930 ± 170[1]	2340 ± 310[1]	6190 ± 1020[1]
Apalutamide	800 ± 210	1410 ± 340[1]	3910 ± 1950[1]
Bicalutamide	1430 ± 130[1]	**Ago: 90 ± 20%**	**Ago: 50 ± 10%**

Supplementary Table 3. Effects of AR antagonists on AR wild-type and mutant N/C interaction. Mean IC_{50} ±SD values from at least 2 biological replicates are given in nM. Cells were treated with 10 nM R1881. In case agonistic activity was observed, the value is shown in bold and gives the % activity measured with 10 µM compound only, in comparison to treatment with 10 nM R1881 which was set to 100 %. Statistical analysis was performed with t-test on average pIC_{50} values. A superscript indicates inhibitory activity that is significantly lower than that of darolutamide. Ago: agonism.