Design and Synthesis of Novel Bicalutamide and Enzalutamide Derivatives as Antiproliferative Agents for the Treatment of Prostate Cancer.

Bassetto, M., Ferla, S., Pertusati, F., Kandil, S., Westwall, A. D., Brancale, A., McGuigan, C., Eur. J. Med. Chem., **2016**, 118, 230-243

Wipf group Current Lit, 9-10-16
James Johnson

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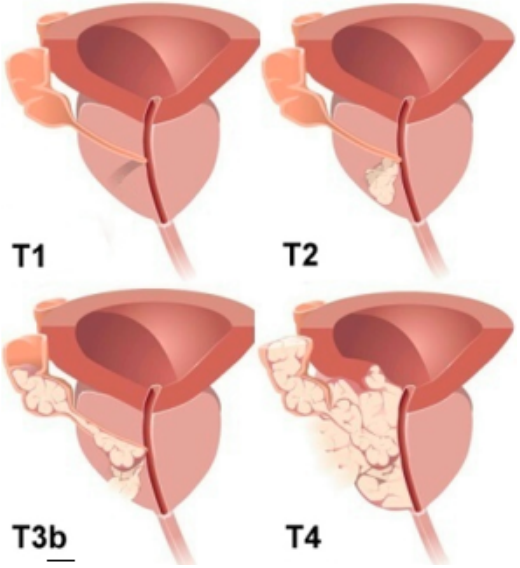
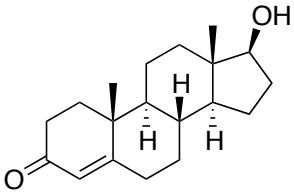
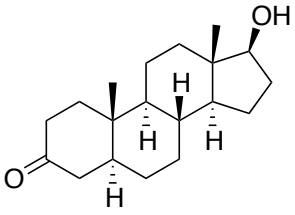
Prostate Cancer and the Androgen Receptor

- In prostate cancer AR has a higher nuclear concentration in the presence of androgens.
- Common treatments are through androgen deprivation therapy:
  - Surgery
  - Radiation
  - Chemical
- Androgen independence/hypersensitivity mutations are the result of a low androgen environment.
- Eventually all patients progress to the lethal castration resistant stage (CRPC).
- CRPC is responsible for all prostate cancer deaths making it the second leading cause of cancer deaths in men in the US
- Current treatments of CRPC only extend patient life by 4-6 months.





testosterone

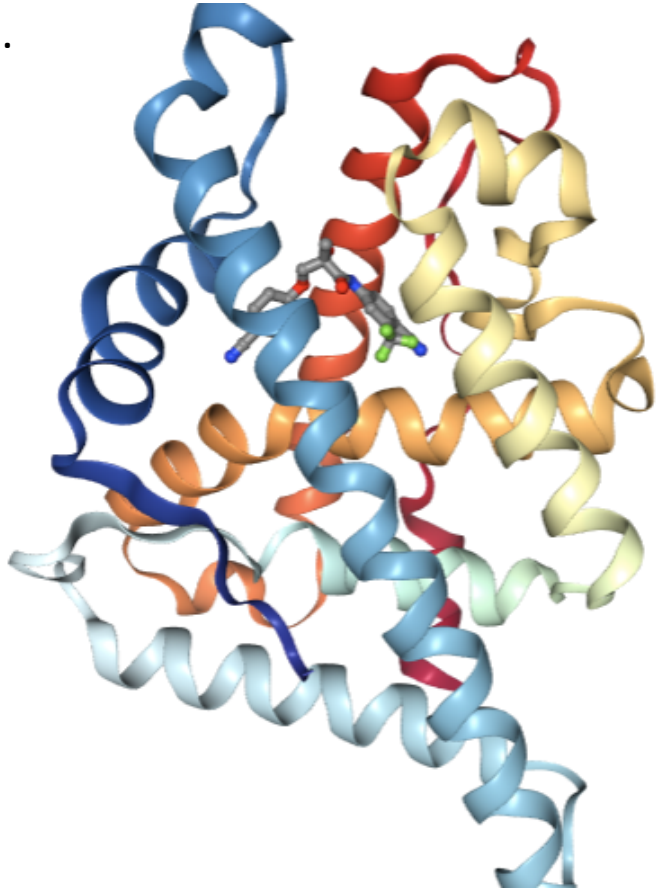


dihydrotestosterone

urologygroupvirginia

The Androgen Receptor

- 110 KDa ligand-inducible nuclear receptor protein.
- Regulates male developmental and physiological characteristics.
- Mutations can increase or decrease binding affinity of androgens and augment transcriptional activity.
- Changes in androgen expression or AR binding affinity directly affect the physiology of the prostate
- Current methods of inhibition involve the use of antiandrogens to block nuclear translocation/AR transcription.



PDB: 3RLJ

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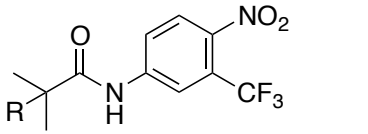
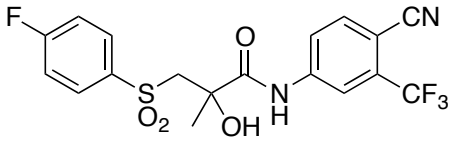
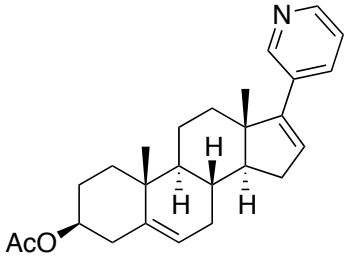
Current FDA approved/preclinical Treatments of Prostate Cancer



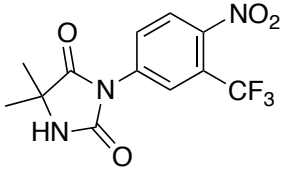
R = H Flutamide
R = OH Hydroxyflutamide



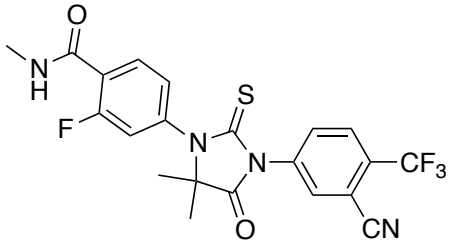
Bicalutamide



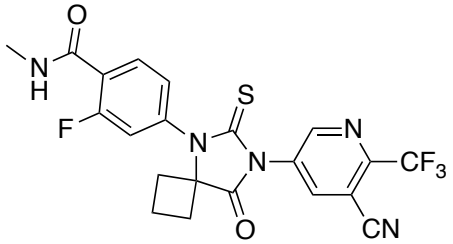
Abiraterone



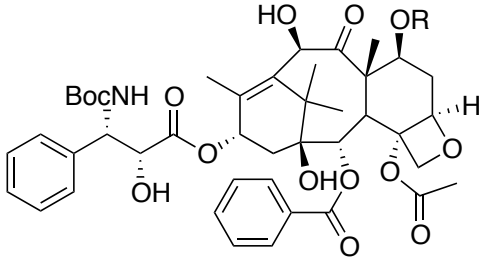
Nilutamide



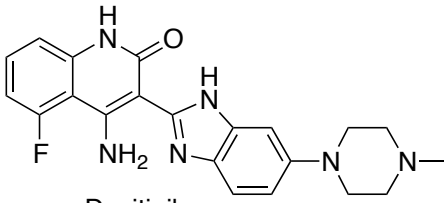
Enzalutamide



Apalutamide
ARN-509
(Phase 3)



R = H, Docetaxel
R = Me, Carbazitaxel



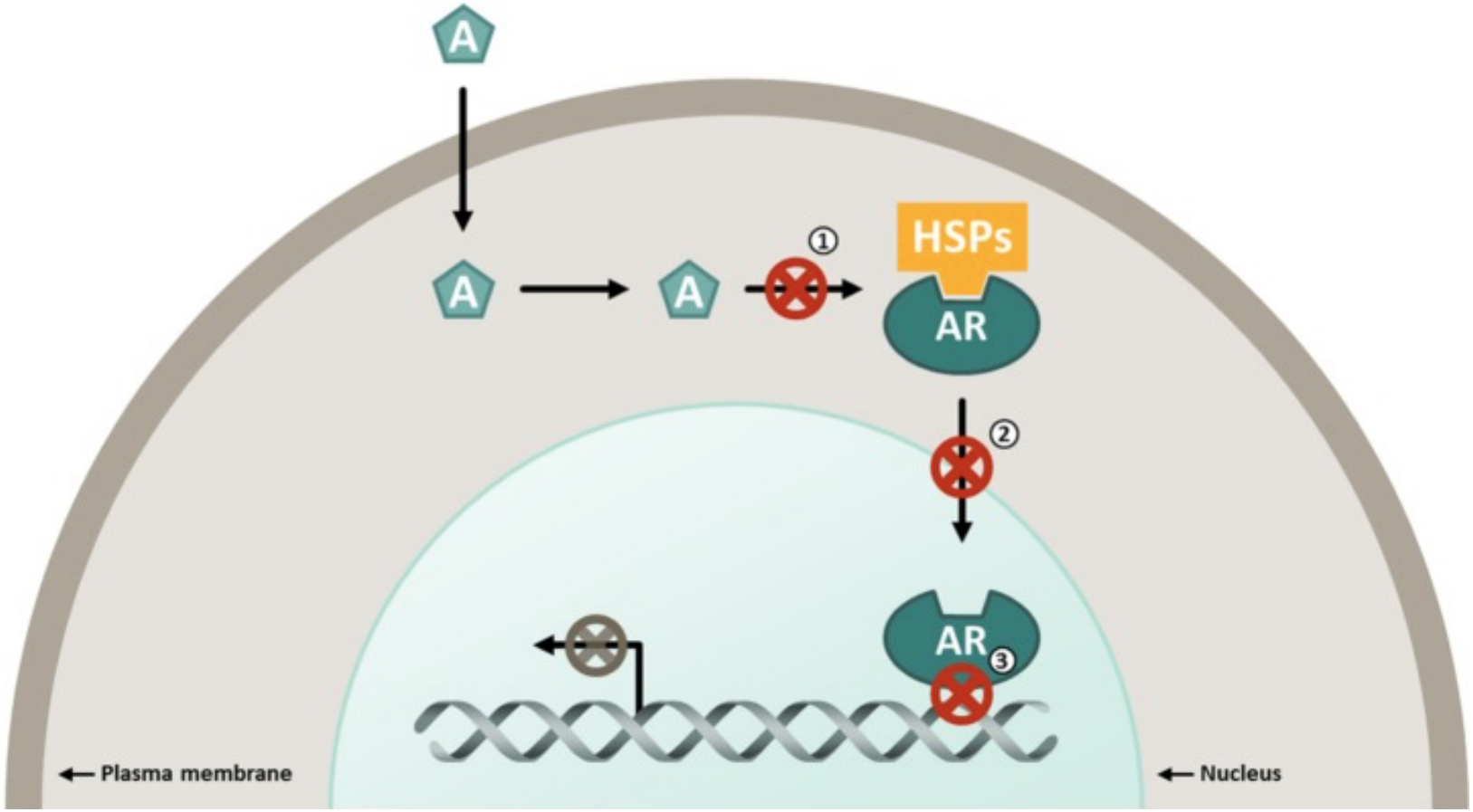
Dovitinib
(phase 2)

TRC105
(Ig)G1 anti-CD105 monoclonal antibody
(phase 2)

Ra^{223}
Alpharadin
(Phase 3)

J. Med. Chem. **2010**, 53, 2779–2796 ; EJMeCh **2016**, 118, 230-243

Enzalutamide/Bicalutamide MOA

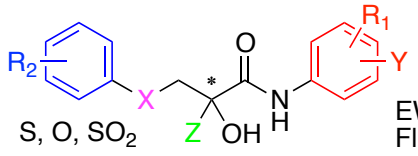


Ther Adv Urol. 2013 Aug; 5(4): 201–210.

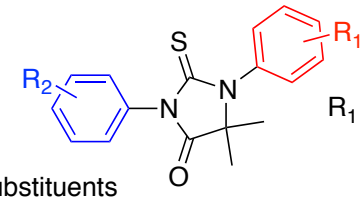
Title Paper

- Improve potency.
- Addition of fluorine atoms to improve pharmacological and physiochemical.
- Decrease cytotoxic/off-target effects.

Fluorinated substituents



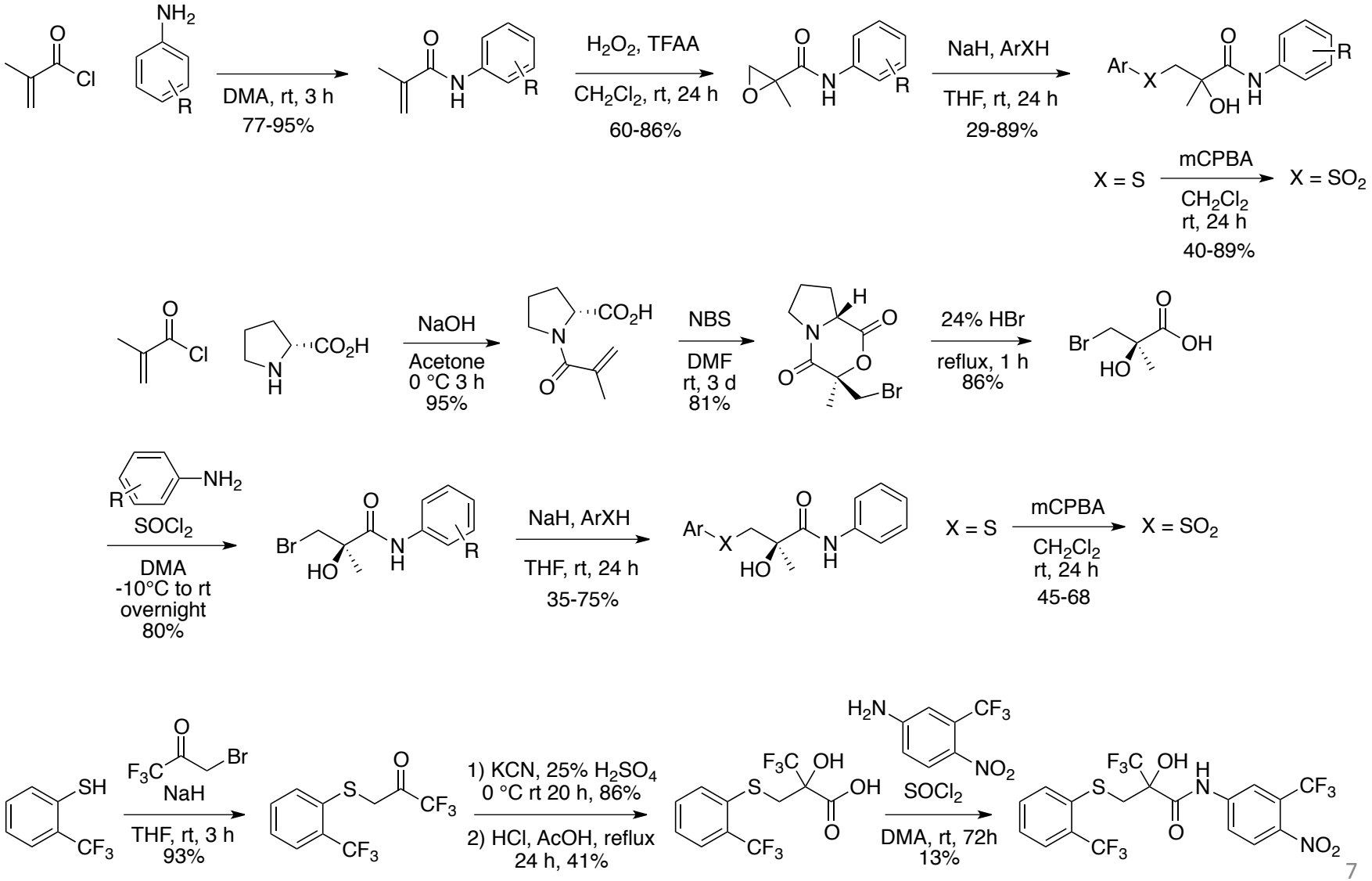
Racemic/R-enantiomer



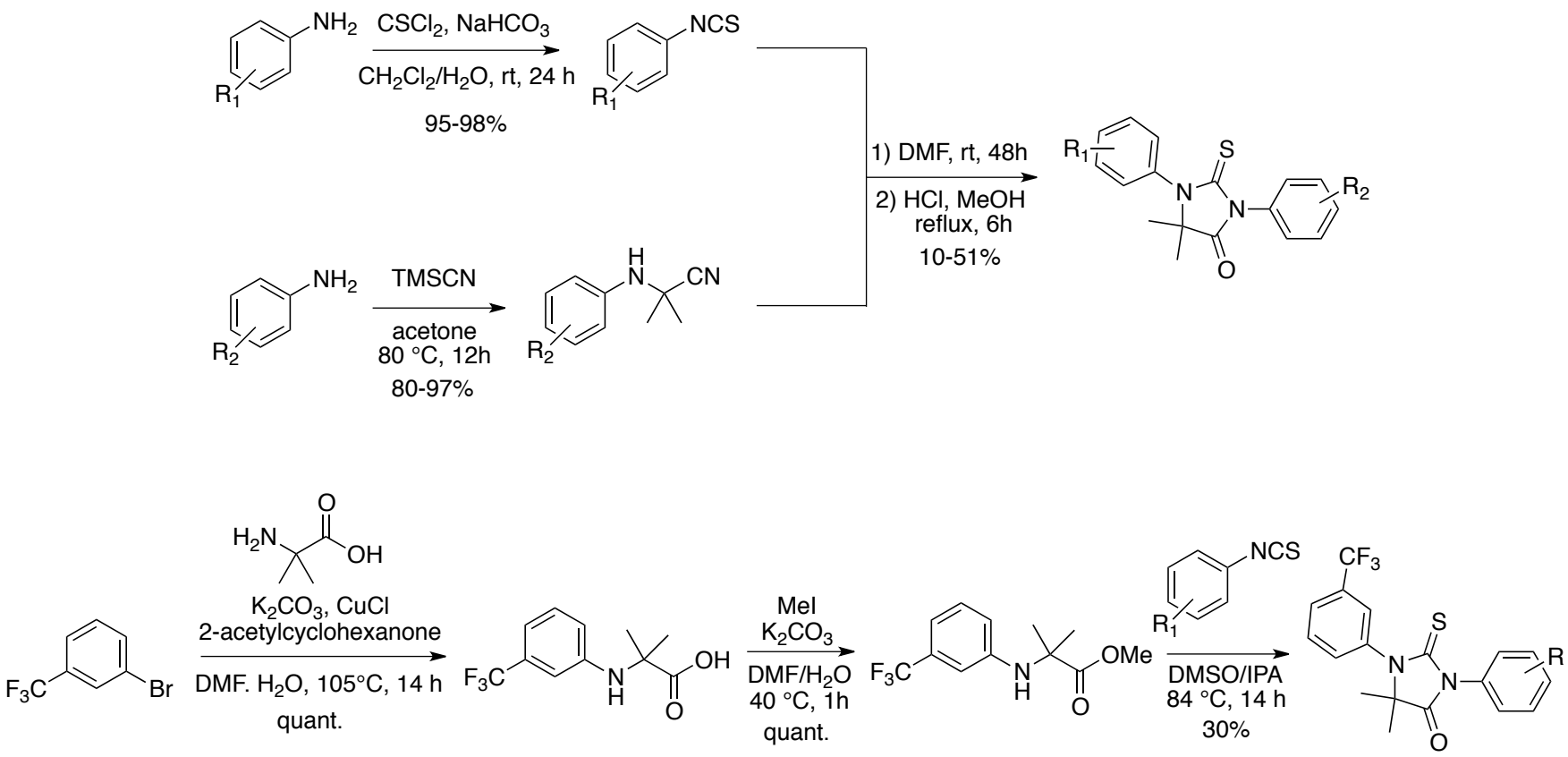
R_1 = *o-*, *m-*, *p*-CF_3

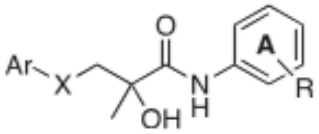
R_2 = EWD substituents
Perfluoronated substituents

Synthesis of bicalutamide analogs

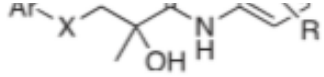


Synthesis of enzalutamide analogs

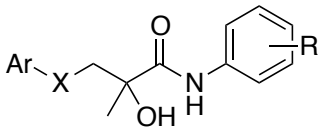




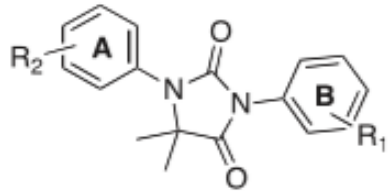
Compound	Ar (B ring)	X	R (A ring)	Absolute IC_{50} (μM)				
				22Rv1	DU-145	LNCaP	VCaP	Geo.mean
3 (Bic.)	4-F-Ph	SO_2	4-CN, 3-CF_3	49.58	49.20	45.27	68.37	52.42
44e (*S*-Eno)	4-CN-Ph	O	4-CN, 3-CF_3	24.77	44.55	20.90	24.47	27.41
45a (*R*-Bic.)	4-F-Ph	SO_2	4-CN, 3-CF_3	46.25	45.41	45.20	51.61	47.05
22b	4-CF_3-Ph	S	4-CN, 3-CF_3	NA				
22c	3-CF_3-Ph	S	4-CN, 3-CF_3	5.56	10.58	5.03	9.23	7.23
22d	2-CF_3-Ph	S	4-CN, 3-CF_3	5.06	7.73	4.91	7.51	6.16
22h	4-OCF_3-Ph	S	4-CN, 3-CF_3	46.62	64.01	42.81	40.33	47.64
22o	4-CF_3-2-Pyridine	S	4-CN, 3-CF_3	53.91	84.10	80.72	89.27	75.60
23b	4-CF_3-Ph	S	4-NO_2, 3-CF_3	16.43	22.52	28.71	21.17	21.78
23c	3-CF_3-Ph	S	4-NO_2, 3-CF_3	5.07	8.73	6.44	8.64	7.04
23d	2-CF_3-Ph	S	4-NO_2, 3-CF_3	**4.6**	**7.57**	**4.78**	**4.45**	**5.17**
24a	4-F-Ph	S	4-CN, 2-CF_3	30.08	43.28	26.45	45.48	35.37
24b	4-CF_3-Ph	S	4-CN, 2-CF_3	NA				
24c	3-CF_3-Ph	S	4-CN, 2-CF_3	15.16	21.05	11.26	23.48	17.04
24d	2-CF_3-Ph	S	4-CN, 2-CF_3	11.64	18.42	10.72	18.27	14.32
25a	4-F-Ph	S	4-NO_2, 2-CF_3	17.63	26.40	11.93	21.10	18.50
25b	4-CF_3-Ph	S	4-NO_2, 2-CF_3	14.63	19.20	8.66	17.07	14.28
25c	3-CF_3-Ph	S	4-NO_2, 2-CF_3	12.58	14.35	10.07	16.51	13.16
25d	2-CF_3-Ph	S	4-NO_2, 2-CF_3	11.97	17.53	11.66	12.79	13.30
25f	3,4-F-Ph	S	4-NO_2, 2-CF_3	83.59	100	83.04	100	91.28
25g	2,4-F-Ph	S	4-NO_2, 2-CF_3	14.93	21.43	11.00	16.64	15.56
25h	4-OCF_3-Ph	S	4-NO_2, 2-CF_3	61.92	69.59	100	100	81.02
25i	3-OCF_3-Ph	S	4-NO_2, 2-CF_3	15.80	21.63	9.15	15.88	14.93
25l	2-OCF_3-Ph	S	4-NO_2, 2-CF_3	**4.64**	**6.57**	**3.31**	**5.27**	**4.80**
25o	4-CF_3-2-Pyridine	S	4-NO_2, 2-CF_3	13.58	19.74	11.06	17.18	15.02
25p	5-CF_3-2-Pyridine	S	4-NO_2, 2-CF_3	11.52	14.03	5.52	17.36	11.15
26c	3-CF_3-Ph	S	4-CF_3	18.57	30.71	22.56	19.50	22.44
26d	2-CF_3-Ph	S	4-CF_3	5.20	8.76	7.22	5.86	6.63
26i	3-OCF_3-Ph	S	4-CF_3	15.90	27.84	13.88	18.82	18.44



Compound	Ar (B ring)	X	R (A ring)	Absolute IC_{50} (μM)				
				22Rv1	DU-145	LNCaP	VCaP	Geo.mean
27b	4-CF_3-Ph	O	4-CN, 3-CF_3	6.91	16.28	6.45	9.65	9.15
27c	3-CF_3-Ph	O	4-CN, 3-CF_3	5.89	10.84	8.31	9.04	8.32
27d	2-CF_3-Ph	O	4-CN, 3-CF_3	11.55	17.32	9.68	16.60	13.39
27f	3,4-F-Ph	O	4-CN, 3-CF_3	35.54	45.19	18.98	25.64	29.73
27g	2,4-F-Ph	O	4-CN, 3-CF_3	34.00	44.26	37.30	38.69	38.39
27h	4-OCF_3-Ph	O	4-CN, 3-CF_3	17.20	18.36	13.56	17.59	16.57
27i	3-OCF_3-Ph	O	4-CN, 3-CF_3	9.79	16.36	9.83	9.91	11.17
27o	4-CF_3-2-Pyridine	O	4-CN, 3-CF_3	100	100	100	100	100
28b	4-CF_3-Ph	O	4-NO_2, 3-CF_3	16.19	25.93	17.24	10.91	16.76
28c	3-CF_3-Ph	O	4-NO_2, 3-CF_3	8.58	10.02	9.91	10.28	9.68
28d	2-CF_3-Ph	O	4-NO_2, 3-CF_3	20.16	35.20	22.60	27.16	25.69
28e	4-CN-Ph	O	4-NO_2, 3-CF_3	30.92	32.68	18.69	26.88	26.69
28f	3,4-F-Ph	O	4-NO_2, 3-CF_3	18.54	21.61	14.50	18.92	18.21
28g	2,4-F-Ph	O	4-NO_2, 3-CF_3	20.16	22.39	10.06	20.92	17.56
28h	4-OCF_3-Ph	O	4-NO_2, 3-CF_3	7.53	10.43	17.66	19.55	12.83
28i	3-OCF_3-Ph	O	4-NO_2, 3-CF_3	9.31	13.14	9.95	9.60	10.40
28l	2-OCF_3-Ph	O	4-NO_2, 3-CF_3	6.60	9.88	7.04	9.61	8.15
28m	4-CN,2-CF_3-Ph	O	4-NO_2, 3-CF_3	5.55	7.65	5.69	8.38	6.71
28n	4-CN,3-F-Ph	O	4-NO_2, 3-CF_3	11.77	16.39	7.71	12.39	11.65
28o	4-CF_3-2-Pyridine	O	4-NO_2, 3-CF_3	35.22	100	36.51	100	59.88
29b	4-CF_3-Ph	O	4-CN, 2-CF_3	29.91	32.39	28.61	23.02	28.26
29c	3-CF_3-Ph	O	4-CN, 2-CF_3	18.70	32.12	15.23	21.59	21.08
29d	2-CF_3-Ph	O	4-CN, 2-CF_3	19.60	33.17	14.03	21.93	21.15
29e	4-CN-Ph	O	4-CN, 2-CF_3	42.935	100	34.722	61.370	55.00
29f	3,4-F-Ph	O	4-CN, 2-CF_3	37.03	41.93	34.80	47.18	39.95
29g	2,4-F-Ph	O	4-CN, 2-CF_3	60.92	99.48	30.64	50.62	55.37
29h	4-OCF_3-Ph	O	4-CN, 2-CF_3	27.99	34.64	27.65	22.37	27.83
29i	3-OCF_3-Ph	O	4-CN, 2-CF_3	28.65	35.86	63.39	56.34	43.76
29l	2-OCF_3-Ph	O	4-CN, 2-CF_3	16.68	27.09	33.83	43.85	28.61
29m	4-CN,2-CF_3-Ph	O	4-CN, 2-CF_3	18.45	27.52	9.39	17.70	17.04
29n	4-CN,3-F-Ph	O	4-CN, 2-CF_3	45.34	68.78	78.27	77.20	65.89
29o	4-CF_3-2-Pyridine	O	4-CN, 2-CF_3	100	100	100	100	100
30b	4-CF_3-Ph	O	4-NO_2, 2-CF_3	18.93	22.08	13.57	18.56	18.01
30c	3-CF_3-Ph	O	4-NO_2, 2-CF_3	15.17	18.14	12.93	19.34	16.20
31c	3-CF_3-Ph	O	4-CF_3	25.68	27.45	18.16	18.64	22.100
31d	2-CF_3-Ph	O	4-CF_3	6.42	26.41	6.98	11.49	10.80
31i	3-OCF_3-Ph	O	4-CF_3	15.43	29.40	2.16	4.28	8.05

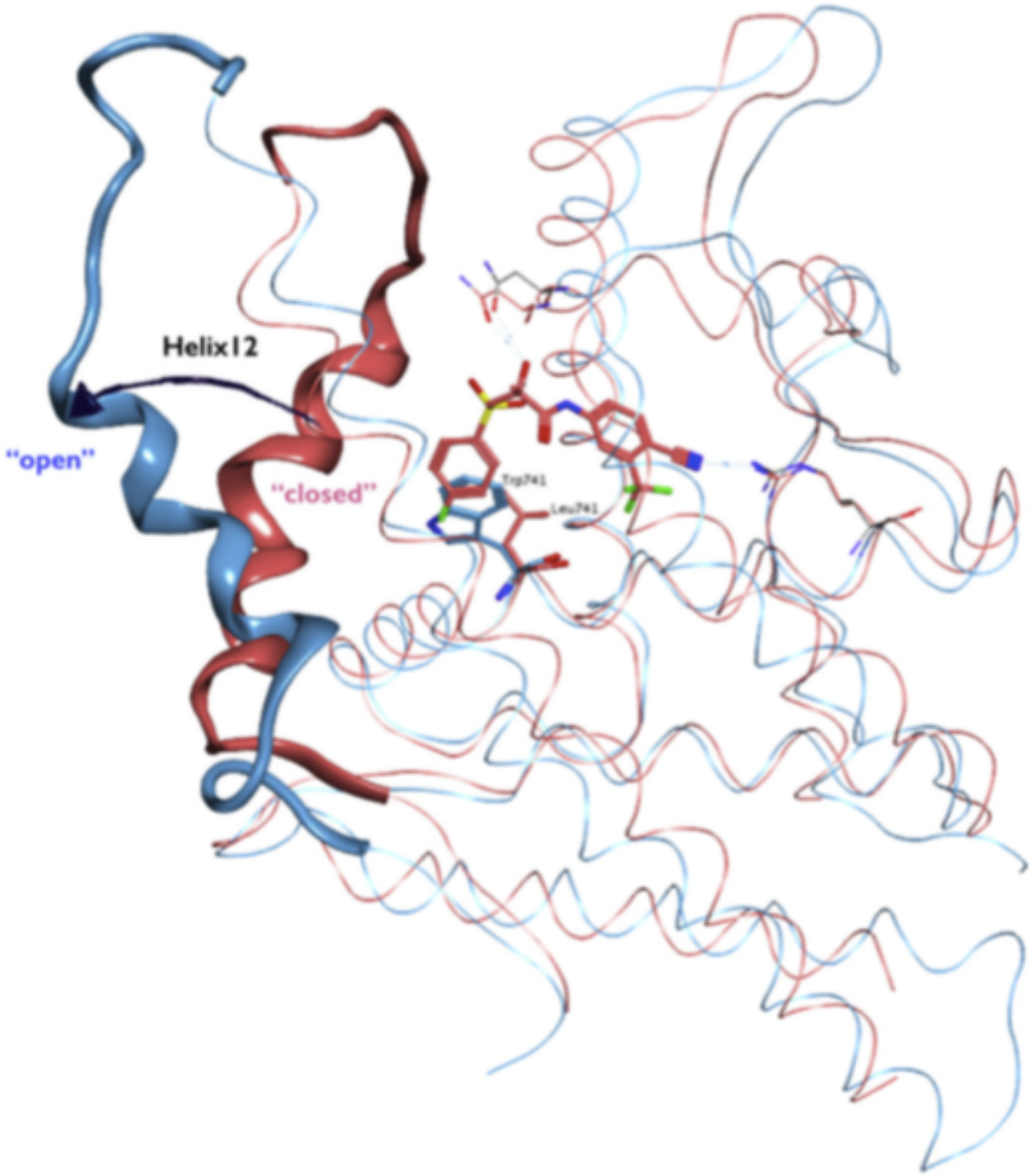


Compound	Ar (B ring)	X	R (A ring)	Absolute IC_{50} (μM)				
				22Rv1	DU-145	LNCaP	VCaP	Geo.mean
32b	4-CF_3-Ph	SO_2	4-CN, 3-CF_3	21.54	32.84	20.05	29.11	25.345
32c	3-CF_3-Ph	SO_2	4-CN, 3-CF_3	20.954	39.112	14.94	39.66	26.40
32d	2-CF_3-Ph	SO_2	4-CN, 3-CF_3	46.55	55.03	42.74	58.55	50.32
32h	4-OCF_3-Ph	SO_2	4-CN, 3-CF_3	17.02	27.28	31.64	32.81	26.35
32o	4-CF_3-2-Pyridine	SO_2	4-CN, 3-CF_3	100	100	100	100	100
33b	4-CF_3-Ph	SO_2	4-NO_2, 3-CF_3	18.66	24.68	17.94	31.53	22.59
33c	3-CF_3-Ph	SO_2	4-NO_2, 3-CF_3	16.14	31.62	16.20	30.83	22.47
33d	2-CF_3-Ph	SO_2	4-NO_2, 3-CF_3	18.56	37.51	25.70	27.55	26.50
34a	4-F-Ph	SO_2	4-CN, 2-CF_3	77.00	100	91.59	65.22	82.36
34b	4-CF_3-Ph	SO_2	4-CN, 2-CF_3	27.87	44.56	32.93	43.29	36.48
34c	3-CF_3-Ph	SO_2	4-CN, 2-CF_3	34.35	45.94	32.08	44.92	38.83
34d	2-CF_3-Ph	SO_2	4-CN, 2-CF_3	98.82	100	74.02	100	92.48
35a	4-F-Ph	SO_2	4-NO_2, 2-CF_3	46.67	60.73	38.65	58.21	50.25
35b	4-CF_3-Ph	SO_2	4-NO_2, 2-CF_3	25.39	40.91	29.30	46.89	34.56
35c	3-CF_3-Ph	SO_2	4-NO_2, 2-CF_3	18.14	32.01	16.77	38.66	24.77
35d	2-CF_3-Ph	SO_2	4-NO_2, 2-CF_3	28.33	46.09	20.46	38.58	31.86
35f	3,4-F-Ph	SO_2	4-NO_2, 2-CF_3	33.58	48.09	19.47	37.26	32.90
35g	2,4-F-Ph	SO_2	4-NO_2, 2-CF_3	30.24	46.58	17.92	57.96	34.78
35h	4-OCF_3-Ph	SO_2	4-NO_2, 2-CF_3	100	100	100	100	100
35i	3-OCF_3-Ph	SO_2	4-NO_2, 2-CF_3	17.96	27.72	12.23	20.27	18.74
35l	2-OCF_3-Ph	SO_2	4-NO_2, 2-CF_3	28.79	38.97	17.77	51.74	31.87
35o	4-CF_3-2-Pyridine	SO_2	4-NO_2, 2-CF_3	100	100	100	100	100
35p	5-CF_3-2-Pyridine	SO_2	4-NO_2, 2-CF_3	46.46	57.66	45.03	58.47	51.54
42b (*R*)	4-CF_3-Ph	S	4-CN, 3-CF_3	9.75	17.18	14.45	9.28	12.24
42c (*R*)	3-CF_3-Ph	S	4-CN, 3-CF_3	13.14	19.45	14.31	8.91	13.43
42d (*R*)	2-CF_3-Ph	S	4-CN, 3-CF_3	**5.30**	**7.69**	**7.90**	**2.95**	**5.55**
42g (*R*)	2,4-F-Ph	S	4-CN, 3-CF_3	17.15	28.46	25.19	18.29	21.78
42h (*R*)	4-OCF_3-Ph	S	4-CN, 3-CF_3	28.81	29.78	19.83	17.39	23.32
43a (*R*)	4-F-Ph	S	4-CN, 2-CF_3	NA				
45b (*R*)	4-CF_3-Ph	SO_2	4-CN, 3-CF_3	20.19	26.76	17.47	31.42	23.34
45c (*R*)	3-CF_3-Ph	SO_2	4-CN, 3-CF_3	25.66	45.19	13.17	39.92	27.95
45d (*R*)	2-CF_3-Ph	SO_2	4-CN, 3-CF_3	34.75	44.31	32.23	37.30	36.89
45g (*R*)	2,4-F-Ph	SO_2	4-CN, 3-CF_3	42.25	60.20	42.26	60.90	50.58
45h (*R*)	4-OCF_3-Ph	SO_2	4-CN, 3-CF_3	18.82	31.56	18.91	31.195	24.33
46a (*R*)	4-F-Ph	SO_2	4-CN, 2-CF_3	100	100	85.55	100	96.17
52	2-CF_3-Ph	S	4-NO_2, 3-CF_3	**1.28**	**2.40**	**0.72**	**2.78**	**1.57**



Compound	R_1	R_2	IC_{50} (μM)				
			22Rv1	DU-145	LNCaP	VCaP	Geo.mean
61b	4-CN, 3-CF_3	4-CF_3	63.58	100	9.37	30.06	36.58
62b	4-NO_2, 3-CF_3	4-CF_3	100	100	**2.12**	73.13	35.28
62c	4-NO_2, 3-CF_3	3-CF_3	100	100	15.16	100	62.40
63b	4-CN, 2-CF_3	4-CF_3	100	100	8.36	100	53.78
63c	4-CN, 2-CF_3	3-CF_3	59.31	100	18.3	100	57.40
64b	4-NO_2, 2-CF_3	4-CF_3	16.39	100	**3.73**	100	27.96
64c	4-NO_2, 2-CF_3	3-CF_3	5.01	7.88	4.07	13.26	6.80
65b	3-CF_3	4-CF_3	100	100	5.95	72.40	45.56
65c	3-CF_3	3-CF_3	60.68	100	5.61	75.66	40.05
71b	4-CF_3	3-CF_3	92.47	100	16.59	100	57.34
71d	2-CF_3	3-CF_3	100	100	28.28	100	77.79
5 (Enzal.)	–	–	31.76	32.27	11.47	53.04	28.10

Compound	Antagonistic effect (%)*	Agonistic effect (%)**	IC_{50} (μM)***
45a (***R*-Bic.**)	83	5	0.490
44e (***S*-Enob.**)	59	26	0.0364
5 (**Enzal.**)	95	N.E.	0.361
22d	93	11	0.425
23c	93	N.E.	0.625
23d	91	4	0.280
25d	94	2	0.332
28d	94	7	0.183
28h	96	–	0.625
28i	106	–	0.407
28l	103	N.E.	0.198
28m	108	N.E.	0.0722
28n	65****	N.E.	0.0492
29m	99	–	0.736
33d	85	1	0.811
33c	104	–	1.07
42b	92	9	2.170
45b	93	–	0.775
45h	61****	N.E.	0.777
52	110	–	0.245
62b	91	–	0.0803
63b	96	–	0.402
64b	102	–	0.265
64c	87	–	0.111
65c	100	–	0.123



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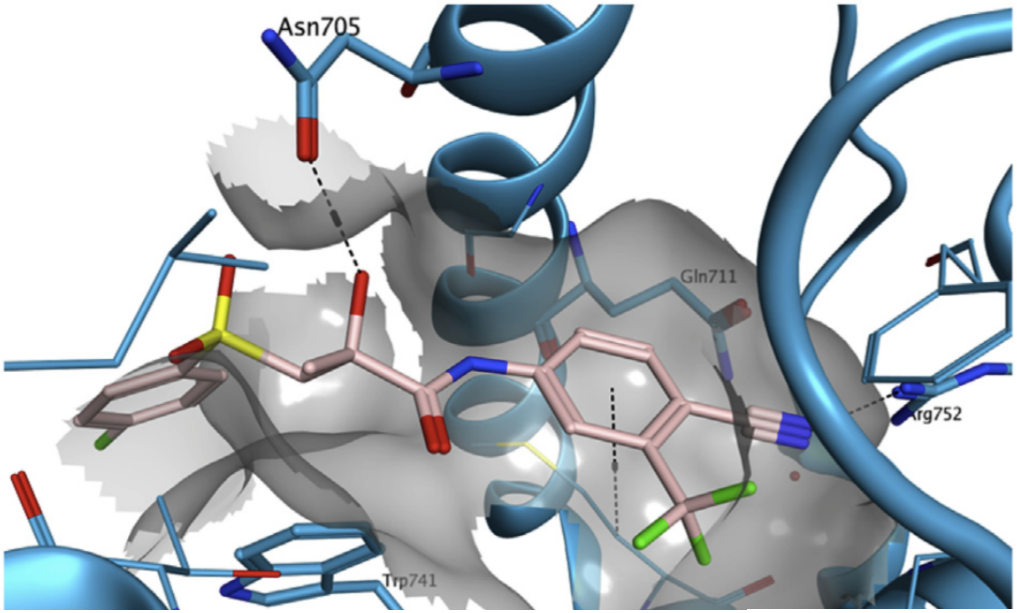
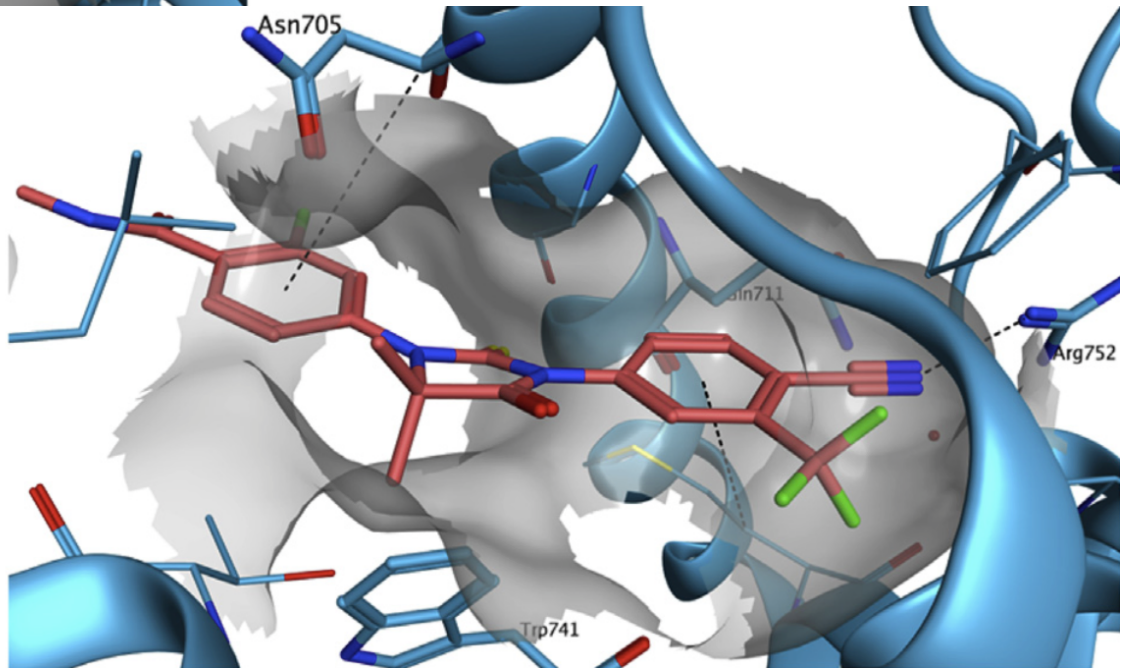
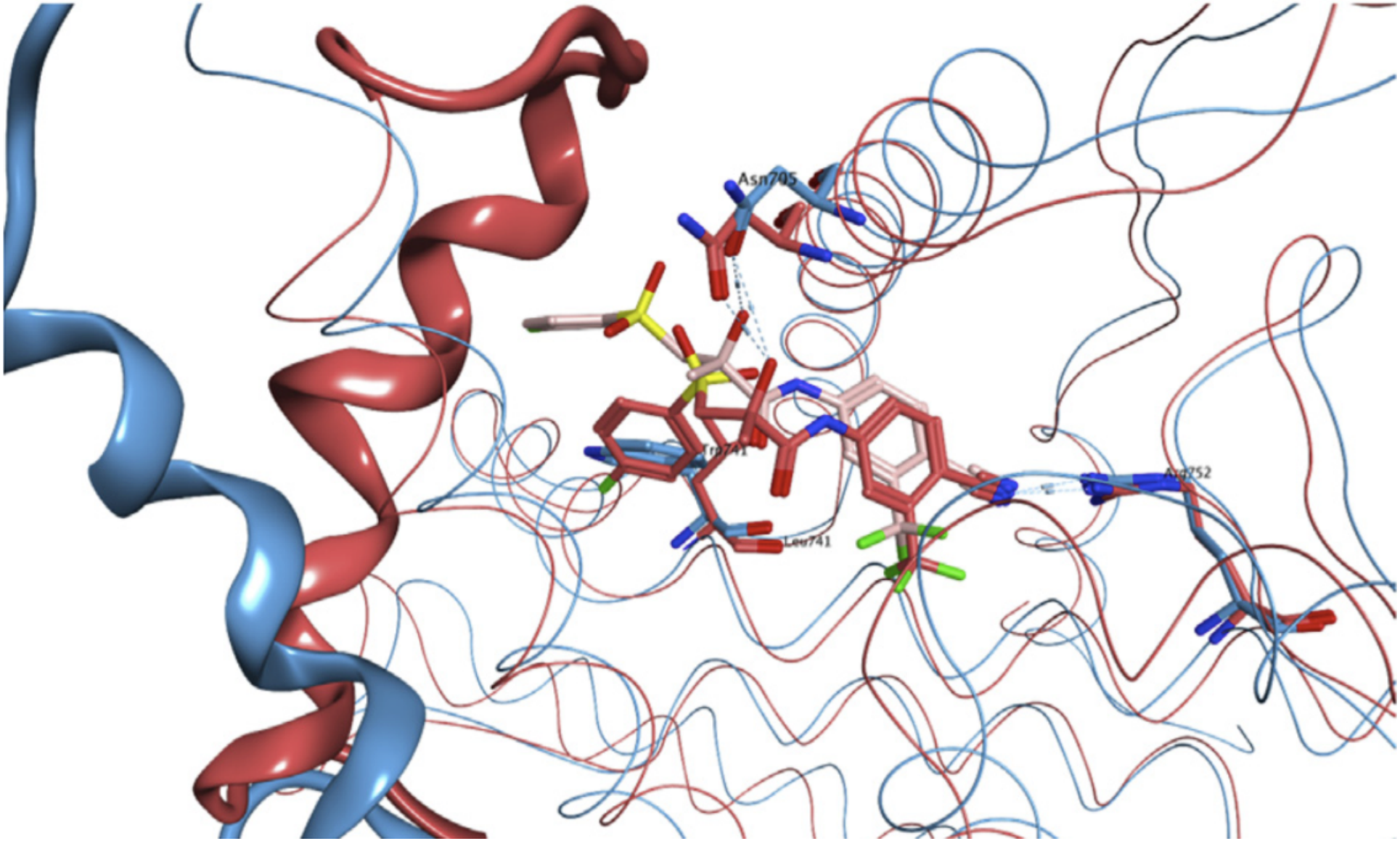
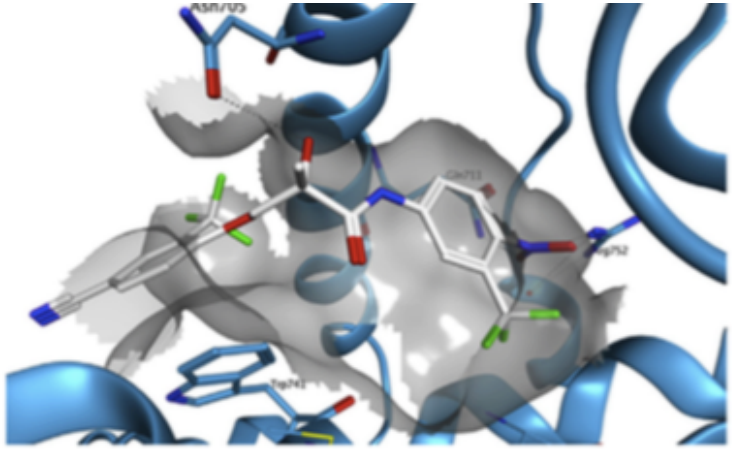
Superposition between the crystal structure of the AR in the closed form (PDB ID: 1Z95) (pink) and the new open AR model (light blue).

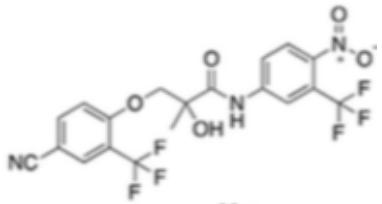




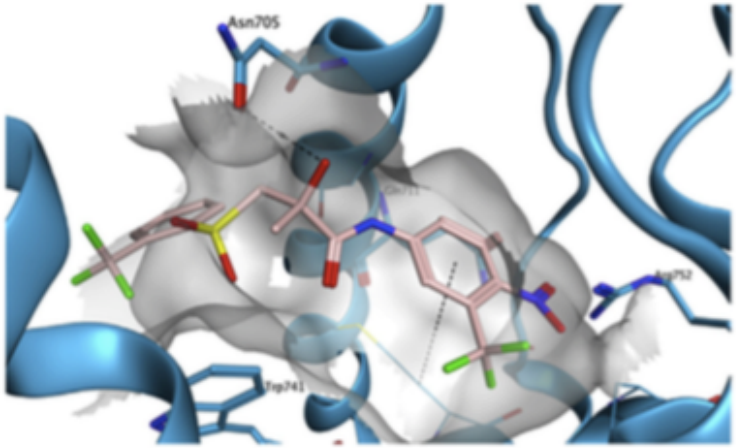
Docking of (R)-bicalutamide (carbon atoms in pink) in the AR homology model (light blue) 15

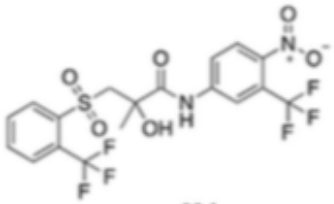

Asn705
Trp741
Leu741



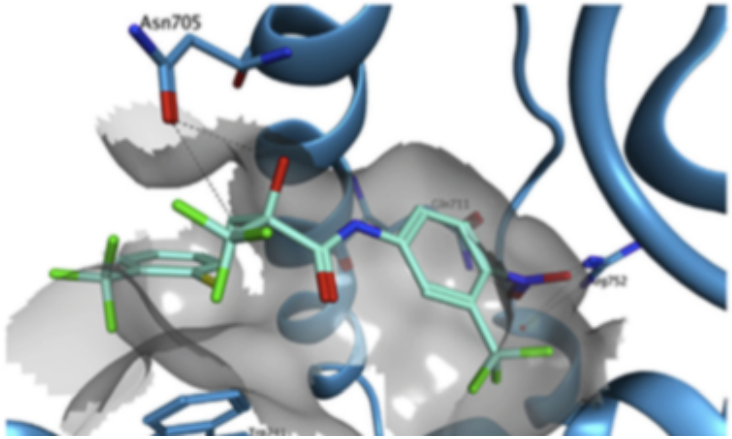


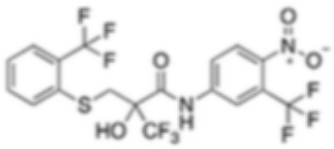
28m

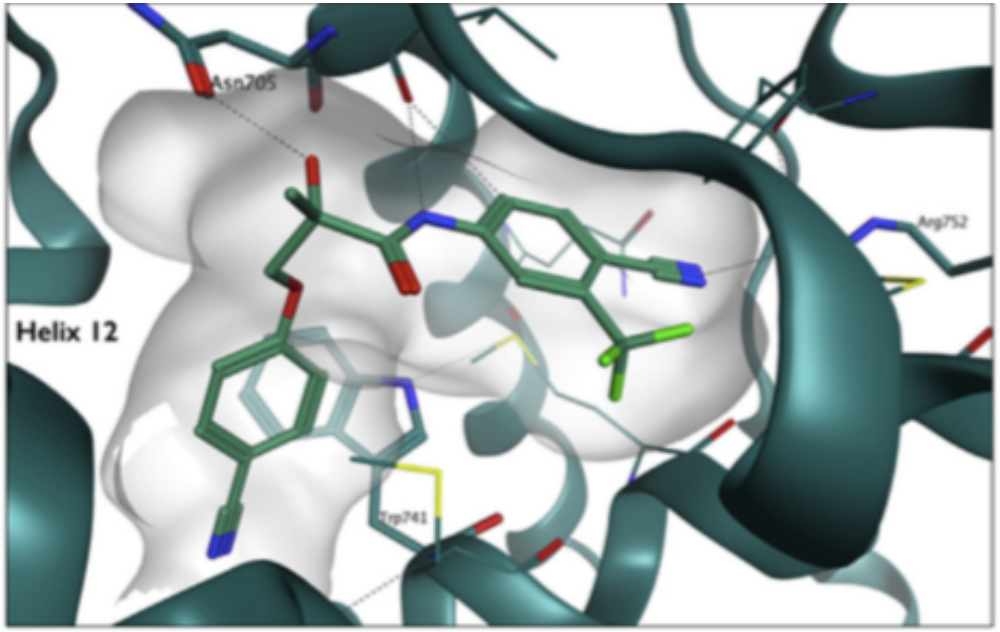


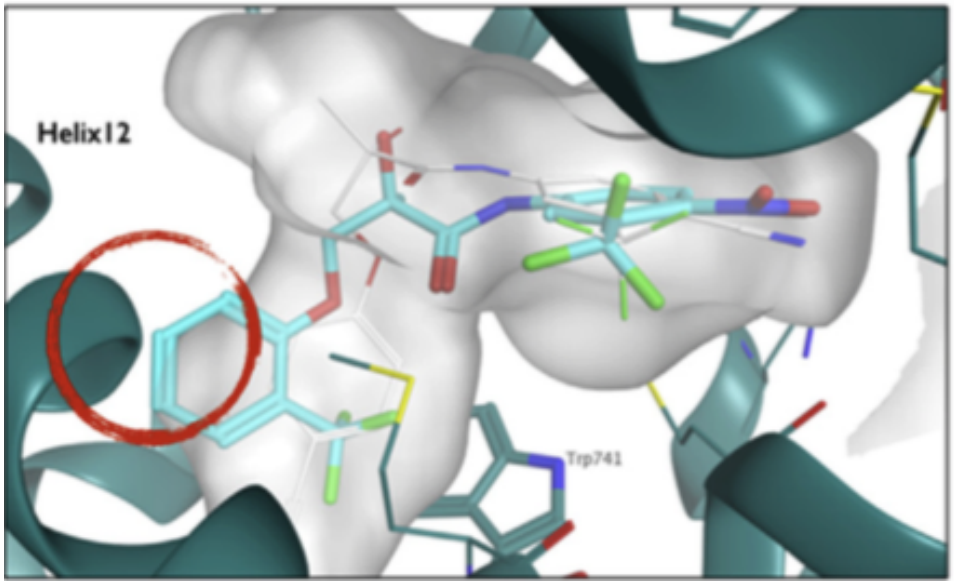


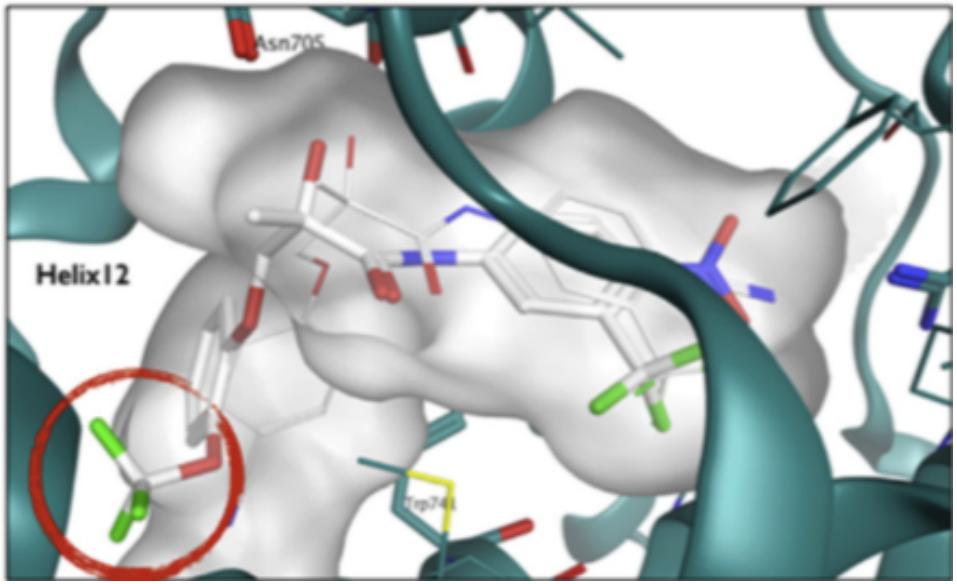
33d






Asn705
Arg752
Helix 12
Trp741


Helix12
Trp741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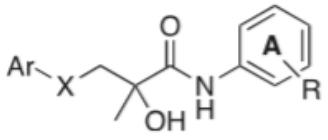
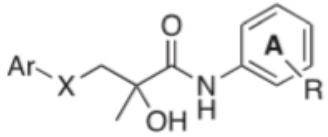


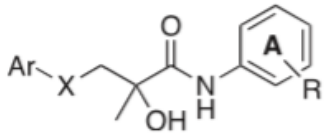
Compound	Ar (B ring)	X	R (A ring)	Metabolic stability		
				CL_{int} (μL/min/mg protein)*	SE CL_{int}**	$t_{1/2}$ (min)
45a (***R*-Bic.**)	4-F-Ph	SO_2	4-CN, 3-CF_3	6.48	2.52	214
44e (***S*-Eno**)	4-CN-Ph	O	4-CN, 3-CF_3	Metabolically Stable***		
22c	3-CF_3-Ph	S	4-CN, 3-CF_3	232	5.89	
					5.97	
22d	2-CF_3-Ph	S	4-CN, 3-CF_3	379	12.8	
					3.66	
23c	3-CF_3-Ph	S	4-NO_2, 3-CF_3	202	2.33	
					6.87	
23d	2-CF_3-Ph	S	4-NO_2, 3-CF_3	409	46.8	
					3.39	
27b	4-CF_3-Ph	O	4-CN, 3-CF_3	Metabolically Stable***		
28m	4-CN,2-CF_3-Ph	O	4-NO_2, 3-CF_3	2.59	2.45	
					534	
33d	2-CF_3-Ph	SO_2	4-NO_2, 3-CF_3	Metabolically Stable***		
42b	4-CF_3-Ph	S	4-CN, 3-CF_3	248	11.8	
					5.60	
42c	3-CF_3-Ph	S	4-CN, 3-CF_3	302	5.66	
					4.59	

Caco-2 cell permeability test



Compound	Ar (B ring)	X	R (A ring)	Permeability data		
				Mean P_{app} (A → B) (x 10^{-6} cm s^{1})*	Mean P_{app} (B → A) (x 10^{-6} cm s^{-1})**	Efflux ratio BA/AB***
45a (***R*-Bic.**)†	4-F-Ph	SO_2	4-CN, 3-CF_3	32.0	38.1	1.19
44e (***S*-Eno.**)†	4-CN-Ph	O	4-CN, 3-CF_3	31.5	26.7	0.848
22c	3-CF_3-Ph	S	4-CN, 3-CF_3	3.92	4.73	1.21
22d	2-CF_3-Ph	S	4-CN, 3-CF_3	3.08	7.12	2.31
23c	3-CF_3-Ph	S	4-NO_2, 3-CF_3	5.00	4.27	0.853
23d	2-CF_3-Ph	S	4-NO_2, 3-CF_3	3.44	2.90	0.843
27b	4-CF_3-Ph	O	4-CN, 3-CF_3	5.09	4.80	0.943
28m	4-CN,2-CF_3-Ph	O	4-NO_2, 3-CF_3	4.35	3.49	0.803
33d†	2-CF_3-Ph	SO_2	4-NO_2, 3-CF_3	13.3	17.2	1.29
42b	4-CF_3-Ph	S	4-CN, 3-CF_3	5.80	4.74	0.817
42c	3-CF_3-Ph	S	4-CN, 3-CF_3	6.84	12.2	1.78

MTT assay



Compound	Ar (B ring)	X	R (A ring)	MTT test		Antiproliferative data
				MEC (μM)*	AC_{50} (μM)**	Abs. IC_{50} (μM)***
45a (**R-Bic.**)	4-F-Ph	SO_2	4-CN, 3-CF_3	19.2	54.3	47.05
44e (***S*-Eno.**)	4-CN-Ph	O	4-CN, 3-CF_3	21.8	32.8	27.41
22c	3-CF_3-Ph	S	4-CN, 3-CF_3	18.4	36.6	7.23
22d	2-CF_3-Ph	S	4-CN, 3-CF_3	14.7	25.8	6.16
23c	3-CF_3-Ph	S	4-NO_2, 3-CF_3	13.2	26.1	7.04
23d	2-CF_3-Ph	S	4-NO_2, 3-CF_3	**1.71**	**2.73**	**5.17**
27b	4-CF_3-Ph	O	4-CN, 3-CF_3	11.3	23.9	9.15
28m	4-CN,2-CF_3-Ph	O	4-NO_2, 3-CF_3	10.6	20.2	6.71
33d	2-CF_3-Ph	SO_2	4-NO_2, 3-CF_3	22.7	32.6	26.50
42b	4-CF_3-Ph	S	4-CN, 3-CF_3	49.6	61.2	12.24
42c	3-CF_3-Ph	S	4-CN, 3-CF_3	26.6	34.6	13.43

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Conclusions

- Synthesized several new antagonists of AR
- New analogs show increased potency to enzalutamide resistant cell lines.
- Compounds still show cytotoxicity.
- Prepared a homology model of AR to predict future structural modifications.
- Possible new mechanism of antagonism of AR