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Discovery and structure–activity relationships of new steroidal compounds bearing a carboxy-terminal side chain as androgen receptor pure antagonists

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Abstract—Lead optimization of CH4892280 (**4**), an androgen receptor (AR) pure antagonist, was investigated. Compounds **6** and **7**, which have a carboxylic acid at the end of the side chain at the position 7α of dihydrotestosterone (DHT), showed partial agonistic activities in reporter gene assay (RGA). Conversion of the steroidal core structure to 17α-methyltestosterone gave compound **14**, which showed weak pure antagonistic activity. Optimization of the side chain by the insertion of a phenyl ring led to compounds **22** and **28**–**30**, which showed pure antagonistic activities at submicromolar concentrations. The structure–activity relationships were clarified.

Prostate cancer is the most common cancer amongst men in the USA and the second most common malignant cause of male death worldwide after lung cancer.[1] Since the growth of prostate cancer is dependent on androgen, androgen receptor (AR) antagonists such as flutamide (**1**) and bicalutamide (**3**) (Chart 1) are currently used as hormone therapy.[2] These antiandrogens exhibit good efficacy in many cases and comprise an important part of effective therapeutics.[3–6] However, the most considerable problem with these antiandrogens is that recurrence occurs after a short period of response.[7] Since they have partial agonistic activities at high concentration in vitro,[8] this may be attributed to recurrence. Therefore, the search for new antiandrogenic agents that exhibit no agonistic activities, so-called 'AR pure antagonists', has been conducted.[9,10]

We previously reported that CH4892280 (**4**) (Chart 1) exhibited AR pure antagonistic activities using reporter gene assay (RGA).[11] However, it showed no in vivo activities because of metabolic instability. In the lead optimization we decided to convert a scaffold to 17α-methyltestosterone which is known to possess oral activity. Recently our laboratory discovered that estradiol derivatives bearing a carboxy-containing side chain were pure antiestrogens and found that the carboxy moiety is effective for oral absorption.[12,13] These results encouraged us to investigate the effects of a carboxylic group in our AR pure antagonists.

We here report the discovery of new steroidal compounds bearing a carboxy-containing side chain in the position 7α of 17α-methyltestosterone as AR pure antagonists and their structure–activity relationships.

The 7α-substituted dihydrotestosterone (DHT) derivatives **5**–**7** in Table 1 were prepared by the procedures described in the patent[14] and a previous report.[11] Synthesis of the 7α-substituted 17α-methyltestosterone derivatives **14** and **21**–**30**, outlined in Schemes 1 and 2, was performed according to the procedures described in the patent.[15]

The synthesized compounds were evaluated for their in vitro binding affinities and agonist/antagonist activities for AR using the same procedures as described in the previous report.[11] The binding affinity for AR was determined by displacement of [^{3}H]-mibolerone with the test compound utilizing CHO-K1/hAR cells. The

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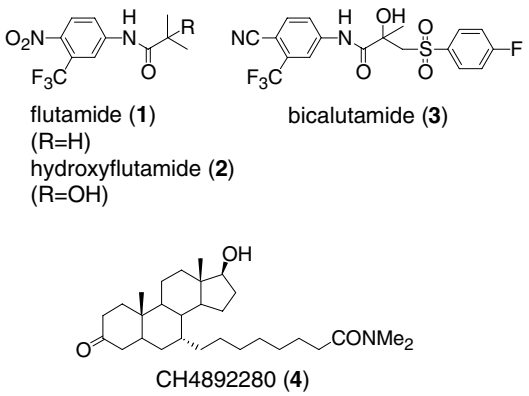


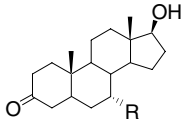
Chart 1. Structures of AR antagonists.

agonistic and antagonistic activities of the compounds for AR were determined by RGA using hAR-transfected Hela cells. Antagonistic activity was described as the IC_{50} value, the concentration of a compound that inhibits the transcriptional activity of 0.1 nM of DHT by 50%. To determine agonistic activity, we calculated the value of 'FI_5', the concentration of a compound-treated group in which the transcriptional activity is five times the transcriptional activity of a group without the addition of a compound. A 'pure antagonist' was defined to have an FI_5 value greater than 10,000 nM.

Initially, we introduced a carboxy-containing side chain in position 7α of DHT to compare the effect on AR agonistic and antagonistic activities with that of the side chain of CH4892280 (Table 1). Compound **5**, which has a side chain similar to the pure antiestrogens of our laboratory,[13] exhibited full agonistic activity (FI_5 = 13 nM) with no antagonistic activity. The unexpected discrepancy of results between AR antagonists and ER antagonists was also found in the compounds with a side chain containing a perfluoroalkyl-sulfoxide moiety in our previous report.[11] Such a long hydrophobic moiety might cause full agonistic activities in AR, although the mode of interaction is unknown. This speculation is supported by the results of compounds **6** and **7**, in which the hydrophobic termini were removed, showed antagonistic activities. However, these compounds still retained partial agonistic activities.

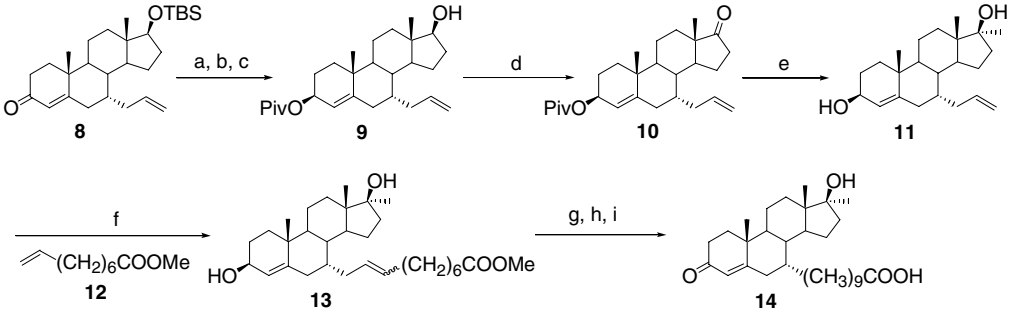
For the next step, the DHT scaffold of **6** was replaced with 17α-methyltestosterone to give compound **14** (Table 2). This compound showed no agonistic activity (FI_5 > 10,000 nM) although compound **6** exhibited agonistic activity with FI_5 of 120 nM. To investigate this remarkable change in agonistic activity by the conversion of the steroidal core structure, a 3D model of these compounds bound to AR was constructed as shown in Figure 1. This model was built based on the X-ray crystal structure of human AR in complex with the ligand

Table 1. Binding and agonistic/antagonistic activities of 7α-substituted DHT derivatives[a]

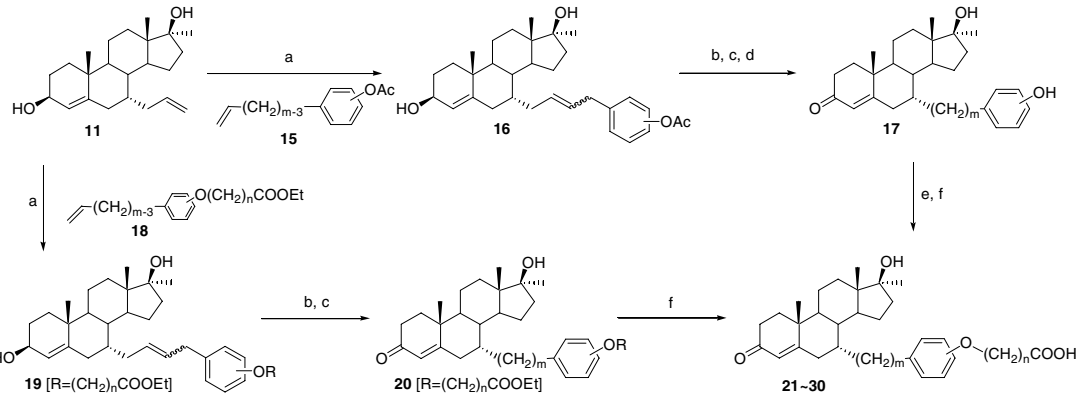


Compound	R	Binding affinity IC_{50} (nM)	RGA	
			FI_5 (nM)	IC_{50} (nM)
5	$(CH_2)_8CH(COOH)(CH_2)_3C_2F_5$	410	13	>10,000
6	$(CH_2)_9$-COOH	350	120	920
7	$(CH_2)_7$-COOH	660	110	730
4 (CH4892280)	$(CH_2)_7$-$CONMe_2$	260	>10,000	190
2 (hydroxyflutamide)	—	200	1000	31
3 (bicalutamide)	—	200	770	140

[a] All data are mean values of duplicate experiments.



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, rt; (b) PivCl, NEt_3, CH_2Cl_2, rt; (c) 2N-HCl, acetone, rt; (d) nPr_4NRuO_4, *N*-methylmorpholine, MS4A, CH_2Cl_2, rt; (e) MeLi, THF, −78 °C; (f) $Cl_2(Cy_3P)_2Ru{=}CHPh$ (cat.), CH_2Cl_2, reflux; (g) H_2, 10% Pd/C (cat.), AcOEt, rt; (h) MnO_2, acetone, rt; (i) 2N-NaOH, H_2O, MeOH, rt.



Scheme 2. Reagents and conditions: (a) $Cl_2(Cy_3P)_2Ru{=}CHPh$ (cat.), CH_2Cl_2, reflux; (b) H_2, 10% Pd/C (cat.), AcOEt, rt; (c) MnO_2, acetone, rt; (d) 2N-NaOH, H_2O, MeOH rt; (e) $Br(CH_2)_nCOOEt$, K_2CO_3, 18-crown-6-ether, *N*,*N*-dimethylacetamide, rt; (f) 2N-NaOH, THF, rt.

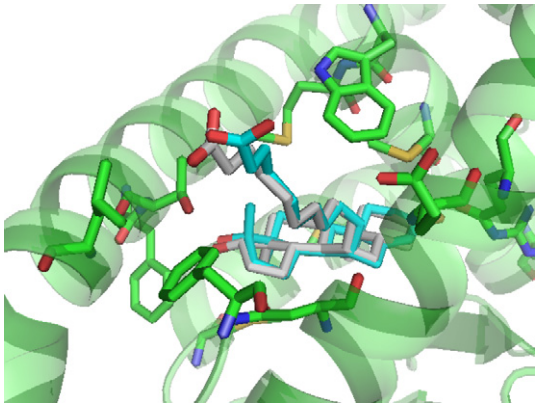
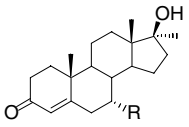


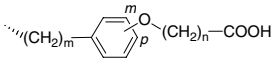
Figure 1. Binding models of compounds **6** (white) and **14** (cyan) to AR. Helix 12 of AR was removed in the model.

R1881 (PDB ID: 1e3g).[16] Compounds **6** and **14** were docked into AR manually. C terminal residues from Glu893 to Thr918 of AR were deleted because these residues collide significantly with the compounds. The model suggests that the 17α-methyl group of compound **14** is located in a position where Helix 12 would be folded to express the agonistic activity. Therefore, it could have the additional effect on a side chain in position 7α of preventing the folding of Helix 12.

Although compound **14** exhibited pure antagonistic activity, its potency was relatively low (IC_{50} = 2000 nM). Therefore, compounds **21**–**30** were synthesized for optimization of the side chain by the insertion of a phenyl ring (Table 2). A phenyl ring could increase antagonistic activities by decreasing the flexibility of the side chain. Furthermore, it is expected to interact with

Table 2. Binding and agonistic/antagonistic activities of 17α-methyltestosterone derivatives[a]



Compound	R			Binding affinity IC_{50} (nM)	RGA	
					FI_5 (nM)	IC_{50} (nM)
14	–$(CH_2)_9$–COOH			600	>10,000	2000
						
	m	Position	*n*			
21	3	*p*	2	300	2500	170
22	3	*p*	3	430	>10,000	660
23	3	*p*	4	660	3200	300
24	3	*m*	3	NT[b]	1500	5800
25	4	*p*	3	NT[b]	170	850
26	4	*p*	4	96	2000	250
27	4	*p*	5	NT[b]	100	420
28	4	*m*	3	670	>10,000	920
29	4	*m*	4	350	>10,000	330
30	4	*m*	5	120	>10,000	530

[a] All data are mean values of duplicate experiments unless otherwise noted.
[b] Not tested.

Trp741, which exists in the proximity of the region where Helix 12 would be folded to express agonistic activity, resulting in potent antagonistic activity (Fig. 2).

For compounds **21**–**23** ($m = 3$, position = para), antagonistic activities increased 3- to 12-fold compared to that of compound **14**. In the case of compound **24** ($m = 3$, position = meta), the antagonistic activity was lower than that of compound **14**.

Although compounds **25**–**27** ($m = 4$, position = para) exhibited antagonistic activities with IC_{50}s of 250–850 nM, they tend to show higher partial agonistic activities compared to other types of compounds. On the contrary, compounds **28**–**30** ($m = 4$, position = meta) exhibited no agonistic activities even at 10,000 nM, retaining the antagonistic activities with IC_{50}s of 330–920 nM. These results may suggest that the substitution position of the terminal carboxyalkyloxy group correlates with the antagonistic activity of compounds **21**–**24** ($m = 3$) and with agonistic activity of compounds **25**–**30** ($m = 4$).

Furthermore, the number of methylene groups (n) has been found to be strongly related to agonistic activities rather than antagonistic activities in compounds **21**–**23** ($m = 3$, position = para) and **25**–**27** ($m = 4$, position = para) since agonistic activity changed considerably by changing n in an identical type (same m, same position).

Compound **22**, one of the pure antagonists discovered in this investigation, exhibited only slight improvement of in vitro metabolic stability against CH4892280 and showed almost no in vivo antiandrogenic activities on seminal vesicle wet weight in castrated mice (Table 3).[17]

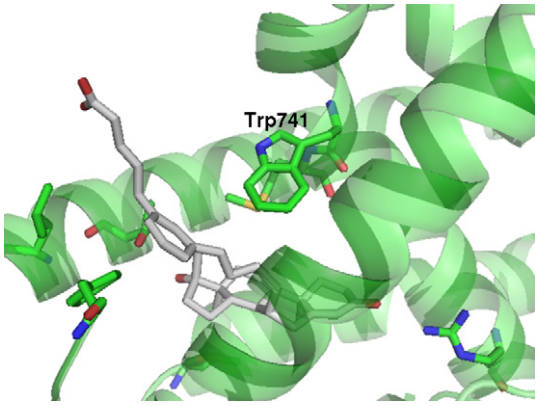


Figure 2. Binding model of compound **29** (white) to AR. This model was built in the same manner as for compounds **6** and **14** (Fig. 1). Helix 12 of AR was removed in the model.

Table 3. Antiandrogenic activities of compound **22**

Dose (mg/body)	Inhibition[a] (%)
1	6.7
3	0.8
10	6.4

[a] Inhibition of TP (testosterone propionate)-stimulated seminal vesicle weight gain by subcutaneous administration of compound **22** ($n = 4$).

Further optimization would be necessary to acquire sufficient metabolic stability for in vivo activity.

In summary, we have discovered new steroidal compounds bearing a carboxy-containing side chain that exhibit AR pure antagonistic activities at submicromolar concentrations. The structure–activity relationships of the compounds were also clarified. It appears that their agonistic/antagonistic activities depend on the structure of the side chain. These findings could be helpful for further investigations in the future.

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