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Antitumor Agents 222.† Synthesis and Anti-androgen Activity of New Diarylheptanoids

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Abstract—Fifteen new diarylheptanoids were synthesized and evaluated for antagonistic activity against androgen receptor (AR)-mediated reporter gene transcription using DU145, PC-3, and LNCaP prostate cancer cell lines. Most compounds showed activity in a 5α-dihydrotestosterone (DHT)-induced reporter gene expression assay in DU145 cells transfected with wild-type AR. Ten compounds (**5**, **8**, **10**, **14**–**15**, and **18**–**22**) were equipotent with hydroxyflutamide (HF), the anti-androgen currently available for the treatment of prostate cancer. However, except for compounds **5** and **10**, none of the tested compounds was significantly effective in attenuating DHT-induced reporter gene expression in LNCaP cells, which contain endogenous mutant AR.

Introduction

Prostate cancer is the second most common cancer diagnosed in US males and the androgen receptor (AR) has been well documented to play an essential role in its progression.[2–4] The AR functions as a ligand-dependent transcription factor and belongs to the super family of nuclear receptors.[5,6] High levels of androgens may constitutively activate the AR signaling pathway so to be responsible for the progression of prostate cancer. Logical approaches to treat prostate cancer include ablation of androgens by either anti-androgens (AR antagonists) neutralization or bilateral orchiectomy. Currently, anti-androgens, in combination with surgical or medical castration, are widely used for the treatment of prostate cancer.[7]

Both steroidal and non-steroidal anti-androgens are presently available and have shown clinical benefit as chemotherapeutic agents for prostate cancer. However, their agonistic activity, as well as their overlapping effects with other hormonal systems, causes a range of unpleasant side effects including thrombosis, fluid retention, and loss of libido, which hinder their use as anti-prostate cancer drugs.[8] The agonistic activity of the anti-androgen may result in 'anti-androgen withdrawal syndrome' and failure of androgen-ablative therapy after several months' treatment. The predominant mechanism of action of the available anti-androgens is to compete with the natural androgens at their binding site on AR. Due to the high sensitivity of AR to androgens, it is desirable to develop anti-androgens with high specificity and affinity to AR as potential anti-prostate cancer drugs.

The phenolic diarylheptanoid, curcumin (**1**), is the major pigment in turmeric (the rhizome of *Curcuma longa*). Several curcumin analogues showed cytotoxicity against multiple tumor cell lines. Notably, some compounds were moderately active (with ED_{50} ranging from 2.8 to 8.0 μg/mL) against two prostate cancer cell lines (PC-3 and LNCaP clone FGC), which are usually not responsive to chemotherapeutic agents.[9] In a previous paper, we reported that several diarylheptanoids showed potent antagonistic activity against the androgen receptor (AR) (compounds **2**–**7**, Fig. 1).[10] Among

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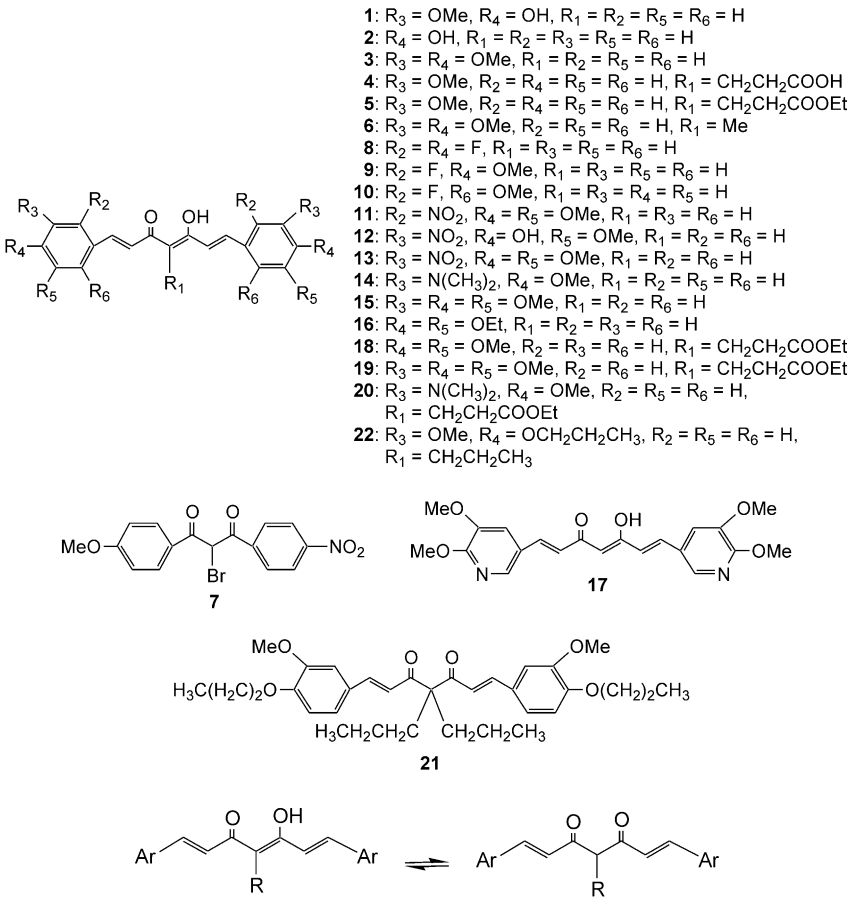
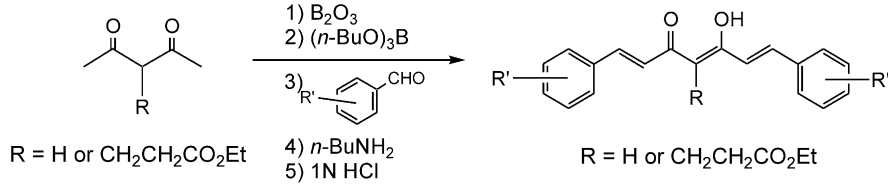


Figure 1. Structures of diarylheptanoids.

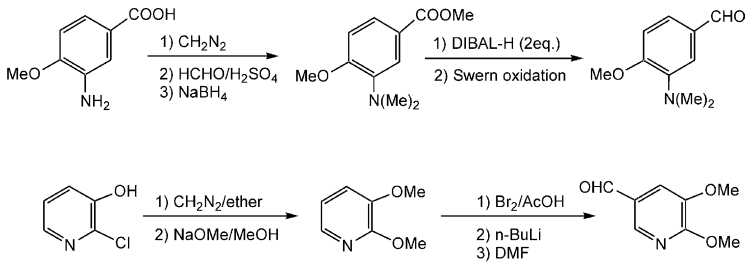
these prior compounds, **3** [5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one] and **5** [7-(4-hydroxy-3-methoxyphenyl)-4-[3-(4-hydroxy-3-methoxyphenyl)-acryloyl]-5-oxo-hept-6-enoic acid ethyl ester] showed the most promising anti-AR activity and were as potent as hydroxyflutamide (HF), which is the currently available anti-androgen for the treatment of prostate cancer. The previous structure–activity relationship (SAR) study indicated that the bis(3,4-dimethoxyphenyl) and conjugated β-diketone moieties are important to the activity but modification of the β-diketone moiety led to decreased potency. However, chemical modification on the phenyl rings was well tolerated and, thus, could probably result in significantly optimized activity profiles. In an effort to obtain compounds with more potent antagonistic activity against the AR, we next focused on chemical modification of the bis-aromatic ring system. Phenyl rings bearing fluorine, nitro, and dimethylamino, as well as methoxy groups were incorporated into new diarylheptanoid derivatives as described herein.

Chemistry

The general procedure for synthesizing diarylheptanoids **8–20** is summarized in Scheme 1. 2,4-Pentanedione or ethyl 4-acetyl-5-oxo-hexanoate was condensed with the appropriate benzaldehyde in EtOAc at 40 °C using the method of Pedersen et al.[11] Protection with a boron complex was necessary in order to avoid Knoevenagel condensation at C-3 of 2,4-pentanedione. Although such protection should not be needed with ethyl 4-acetyl-5-oxo-hexanoate, reactions without the complexation did not succeed.[11] Two commercially unavailable benzaldehydes were synthesized as shown in Scheme 2. To prepare 4-dimethylamino-3-methoxybenzaldehyde, 4-amino-3-methoxybenzoic acid was first esterified with diazomethane then *N*-dimethylated in good yield with formaldehyde and sodium borohydride in aqueous THF.[12] Attempted direct reduction of the carbomethoxy group to the aldehyde with 1.1 equiv of DIBAL-H resulted in a mixture of alcohol and aldehyde. Reduction



Scheme 1. Synthesis of diarylheptanoids **7–19**.



Scheme 2. Synthesis of aldehydes.

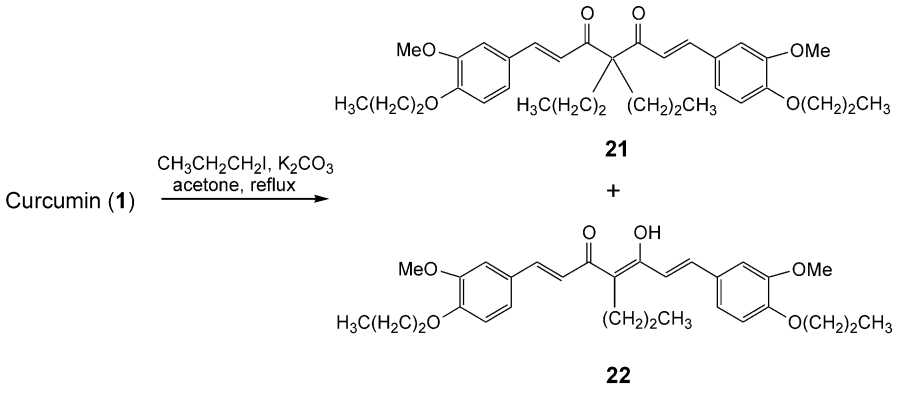
of the methyl benzoate with excess DIBAL-H (2 equiv) followed by Swern oxidation gave 4-dimethylamino-3-methoxybenzaldehyde in an acceptable yield (60% in two steps). 5,6-Dimethoxynicotinaldehyde was prepared from 2-chloro-3-pyridinol.[13] Compound **13** was synthesized by treating compound 12 with trimethylsilyldiazomethane and *N*,*N*-diisopropylethylamine in methanol (41% yield). Alkylation of curcumin with iodopropane and K_2CO_3 in refluxing acetone for 48 h gave a mixture of tetrapropyl (compound **21**, *m*/*z* 559 $[M+Na]^+$) and tripropyl (compound **22**, *m*/*z* 517 $[M+Na]^+$) derivatives (Scheme 3). The structures of all new compounds were confirmed by ^1H NMR and MS data.

Results and Discussion

The curcumin analogues **2**–**22** were first evaluated for their antagonistic activity against the AR in an AR-mediated transactivation assay following the prior conditions.[14] Three different human prostate cancer cell lines, PC-3, DU-145, and LNCaP, were chosen. PC-3 cells are androgen-independent tumor cells that do not express functional AR; DU-145 cells are androgen-independent tumor cells that express neither functional AR nor endogenous ARA (AR co-activator); and LNCaP are prostate cancer cells expressing mutated AR (threonine 877 to alanine). The DU145 cells allow a more sensitive detection of AR agonist or antagonist activity than PC3 or LNCaP cells.

Using DU145 cells transfected with wild-type AR and ARA70, compounds **2** and **4**–**22** were tested for their ability to block AR function in the presence of DHT. At a dose of 1 μM, most compounds inhibited DHT-induced reporter gene expression to various degrees as shown in Table 1A (column 1). Ten compounds (**5**, **8**, **10**, **14**–**15**, and **18**–**22**) were equipotent with hydroxyflutamide (HF), the anti-androgen currently available for the treatment of prostate cancer. Surprisingly, compounds **16** and **17** did not show any suppression. These results were confirmed using PC-3 cells transfected with wild-type AR (Table 1A, column 2). In the latter assay system, compounds **5**–**13** displayed detectable activity and were able to block DHT-induced wild-type AR transactivation; however, they were less active than HF in this assay system. The greater potency of these compounds in the DU145 cells, in which AR/ARA70-mediated reporter gene expression is involved, suggests that ARA70 plays a role in enhancing the antagonist activity of these diarylheptanoid compounds.

We further tested if these compounds could block mutated AR-mediated transactivation and cell growth in LNCaP cells (Table 1A and B, respectively). At a test dose of 1 μM (Table 1A, column 3), only compounds **5** and **10** were significantly effective (equipotent with HF) in attenuating DHT-induced reporter gene expression in LNCaP cells. When the test dose was raised to 2.5 μM (Table 1A, column 4), compounds **19** and **20** showed detectable activity in blocking AR-mediated transactivation. However, at 2.5 μM, compounds **19** and **20** also negatively affected the growth of fetal rat heart-derived H9c2 myocytes (a 10–20% reduction, compared to vehicle control). Therefore, the above-observed AR transactivation blockage may reflect a nonspecific cytotoxicity of compounds **19** and **20** at an elevated dose. These data suggest that most of these compounds may have no or only marginal activity in blocking mutant AR activation by DHT.



Scheme 3. Synthesis of diarylheptanoids **21** and **22**.

Table 1. Suppression of DHT-induced AR activation and cell growth by compounds **2–22**

Compd	A: Suppression of DHT-induced AR activation[a]					B: Suppression of cell growth[b]	
	DU145/wtAR[c]	PC3/wtAR[c]	LNCaP[c]	LNCaP[d]	PC3/mtAR[c]	LNCaP + DHT[c]	LNCaP-DHT[c]
HF	+ + +	+ + + +	+ +		+ +	0	0
2	+ +	+ +	+		+ +	+	+
3					+ +	+ +	+
4	+ +					0	0
5	+ + +	+ + +	+ +		+ +	+ +	+
6	+ +	+ +	+		+	0	+
7	+ +	+ +	+		+	+	+
8	+ + +	+	+				
9	+	+	0				
10	+ + +	+	+ +		0		
11	+ +	+ +	+			+	0
12	+ +	+ +	+			+	0
13	+ +	+ +	+			0	0
14	+ + +		0	0			
15	+ + +		0	0			
16	0	0	0				
17	0	0	0				
18	+ + +		0	0			
19	+ + +		0	+ +			
20	+ + +		0	+ +			
21	+ + +		0	0			
22	+ + +		0	0			

0: not effective (−5 to +5%) of DHT value; +: 10–20% reduction; + +: 20–30% reduction; + + +: 30–40% reduction; + + + +: 40–50% reduction.

[a]DU-145, PC-3, and LNCaP cell lines were seeded and cotransfected with reporter MMTV-luciferase (all cell lines), wild-type (wt) or mutant (mt) AR expression plasmid (DU-145, PC-3) and ARA70 (DU-145) using SuperFect. Subsequently, the transfected cells were harvested and re-plated in 10% charcoal-stripped fetal bovine serum DMEM medium. The cells were then treated with dehydrotestosterone (DHT, 1 nM), and antiandrogens (1 μM) and harvested for detection of the luciferase activity. (cf. Experimental).

[b]Suppression of cell growth was accessed with the MTT assay (cf. Experimental).

[c]1.0 μM of test compound.

[d]2.5 μM of test compound.

It should be noted that, although compound **10** was able to block DHT-induced mutant AR transactivation in LNCaP cells, it did not effectively reduce DHT-induced reporter gene expression in PC-3 cells transfected with the same mutant AR (compare the third and fifth columns, Table 1A). Cellular factors in different cell lines may affect the antagonistic activity of compound **10** toward mutant AR; thus, compound **10** would not have consistent antagonistic activity among various prostate cancer cell types. It is also noteworthy that when compounds **11–13** were further studied using a LNCaP cell growth assay (Table 1B), they caused only minimal suppression of prostate cancer cell growth in the presence of DHT.

Dorai et al.[15] reported an inhibitory effect of curcumin (~10 μM) on the growth of LNCaP (AR-positive) and PC-3 (AR-negative) prostate cancer cell lines, which implied that general non-specific cytotoxicity might be responsible for the above observed effects. To eliminate this possibility, compound **3** was tested in parallel with HF against PC-3 and DU145 cells, which are AR negative and, hence, androgen-insensitive. The results indicated that, at a dose up to 5 μM, compound **3** displayed no adverse effect on the growth of either PC-3 or DU145 cells. The reference compound, HF, had no inhibitory effect on the growth of PC-3 cells, but slightly increased the growth of DU145 cells. Compound **3** was further tested for growth inhibition of two AR-negative normal non-prostate cells, human coronary artery endothelial cells (Clonetics) and fetal rat heart-derived H9c2 muscle cells (American Type Cell Collection). Absence of the AR was demonstrated by Western blotting in primary cultures of both cell lines. Their growth after a 5-day incubation with compound **3** at 1–5 μM was comparable to that with vehicle control (0.2% DMSO). Therefore, we concluded that compound **3**, and probably other diarylheptanoid compounds, might selectively block wild-type AR function and, thus, their inhibitory activity against prostate cancer cell growth might be mediated by the AR.

Furthermore, the new compounds were evaluated for AR agonist activity in several different systems (Table 2A). In this study, HF, an anti-androgen with residual agonist activity, was included as a positive control to validate the conditions for detection of weak or moderate AR agonist activity. In PC-3 cells transfected with wild-type or mutant AR, compounds **8–10** at a dose of 1 μM, did not stimulate reporter gene expression, although HF at the same test dose caused an ~200% increase in wild-type AR-mediated reporter gene expression. Using DU145 cells transfected with wild-type AR and ARA70 (which enhances AR's response to agonists), compounds **11–22** at a concentration of 1 μM also did not increase reporter gene expression, although HF at the same dose evoked AR/ARA70-mediated transactivation by ~50%. Compounds **11–13** were also evaluated in an LNCaP cell growth assay (Table 2B) and, at a dose of 1 μM, did not stimulate cell growth. Therefore, we concluded that these compounds have little AR agonist activity and do not stimulate the biological

Table 2. AR agonist activity of compounds **2**–**22**

Compd	A: AR activation				B: LNCaP cell growth inhibition
	DU145/ wtAR	PC3/ wtAR	PC3/ mtAR	LNCaP	
HF	+	+ +		+ +	+
2					0
3	0	0	0	0	0
4					0
5	0	0	0	0	0
6		0			0
7		0			0
8		0	0		
9		0	0		
10		0	0		
11	0			0	0
12	0			0	0
13	0			0	0
14	0				
15	0				
16	0				
17	0				
18	0				
19	0				
20	0				
21	0				
22	0				

0: not effective (−5 to +5%); +: 50–60% increase above vehicle control; + +: 175–200% increase. The same protocols described in Table 1's legend were employed.

function of wild-type or mutant AR, which represents a significant therapeutic advantage of this compound class over the currently available anti-androgens.

At the present time, accurate SAR conclusions are difficult to formulate from semi-quantitative biological data. Compounds with various substitution patterns and both electron-attracting and -releasing substituents showed anti-androgen activity. The sole compound (**16**) with a nitrogen heteroatom incorporated into the phenyl ring was inactive.

In conclusion, driven by previous SAR, we synthesized a number of new curcumin analogues by diversely substituting the phenyl rings. Biological evaluation of these compounds confirmed our previous observations that curcumin analogues are a novel class of anti-androgens. Further chemical modification is ongoing to optimize their anti-androgen activity.

Experimental

Melting points were determined on a Fisher-Johns melting apparatus and are uncorrected. All reagents and solvents were reagent grade or were purified by standard methods before use. ^{1}H NMR spectra were recorded on a Varian Gemini-300 spectrometer. The chemical shifts are presented in terms of ppm with TMS as the internal reference. IR spectra were recorded on a MIDAC M series spectrometer. MS spectra were recorded on HP5989A and JMS D-300 instruments. Column chromatography was carried out on silica gel 60 (Merck 70–230 and 230–400 mesh), and thin-layer chromatography (TLC) was performed using pre-coated silica gel on aluminum plates (Aldrich, Inc.). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, USA.

Preparation of 3-dimethylamino-4-methoxybenzaldehyde

3-Amino-4-methoxybenzoic acid (5 g, 30 mmol) was treated with excess diazomethane-etherate to give methyl 3-amino-4-methoxybenzoate (5.16 g, 95% yield). A slurry of methyl 3-amino-4-methoxybenzoate (3.7 g, 20.4 mmol) and finely crushed sodium borohydride (4.42 g, 117 mmol) in dry THF (40 mL) was added to a stirred solution of 3 M H_2SO_4 (6 mL) and 37% aqueous formaldehyde (6.1 mL, 81.6 mmol) in THF (40 mL) at 0∼20 °C (ice bath to room temperature). After the first half of the addition, the mixture was acidified with 3 M H_2SO_4 (6 mL) and the addition was continued. To the resultant mixture, water (50 mL) was added with stirring, and then the solution was made strongly basic by addition of solid KOH. The organic phase was separated and the aqueous phase was extracted with diethyl ether (50 mL×2). The combined organic layers were washed with brine and dried over Na_2SO_4. The solution was concentrated in vacuo to give methyl 3-dimethylamino-4-methoxybenzoate as a brown oil (4.18 g, 98% yield). IR ν_{max} ($CHCl_3$) 2800, 1710, 1598, 1509; ^{1}H NMR ($CDCl_3$) δ 2.19 (6H, s), 3.89 (3H, s), 3.95 (3H, s), 6.87 (1H, d, J = 8.4 Hz), 7.63 (1H, d, J = 1.8 Hz), 7.72 (1 h, dd, J = 8.4, 1.8 Hz); positive ESI-MS m/z 210 $[M+1]^+$.

To a stirred solution of 3-dimethylamino-4-methoxybenzoate (3.06 g, 14.6 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C, DIBAL-H (1.5 M toluene solution, 20 mL, 30 mmol) was added in one portion. The reaction was stirred for 30 min at 0 °C, and then quenched by adding MeOH (5 mL). After stirring at room temperature for an additional 30 min, the resulting slurry was filtered and washed thoroughly with CH_2Cl_2. The solvent was removed in vacuo to give 3-dimethylamino-4-methoxybenzylalcohol (2.16 g, 82% yield) as a pale-yellow oil. IR ν_{max} ($CHCl_3$) 3432 (OH), 2789, 1602, 1509; ^{1}H NMR ($CDCl_3$) δ 2.77 (6H, s), 3.87 (3H, s), 4.58 (2H, s), 6.81 (1H, brd, J = 8.2 Hz), 6.94 (1H, dd, J = 8.2, 2.4 Hz), 6.95 (1H, brs); positive ESI-MS m/z 182 $[M+1]^+$.

Dry DMSO (3.35 mL, 47.2 mmol) in CH_2Cl_2 (5 mL) was added to oxalyl chloride (2.06 mL, 23.6 mmol) in CH_2Cl_2 (5 mL) at −78 °C, and the mixture was stirred for 10 min at −78 °C. 3-Dimethylamino-4-methoxybenzylalcohol (2.14 g, 11.8 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to the mixture over 10 min and stirring was continued at −78 °C for 15 min then at −40 to −45 °C for 2 h. Triethylamine (8.2 mL, 59.0 mmol) was added to the mixture, which was stirred for 20 min at 0 °C. The reaction was quenched with sat. NH_4Cl solution, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2, and the combined CH_2Cl_2 solution was washed successively with water and brine, dried (Na_2SO_4), and concentrated to give 3-dimethylamino-4-methoxybenzaldehyde (1.55 g, 74% yield) as a brown oil. IR ν_{max} ($CHCl_3$) 2800, 1686 (Ar–CHO), 1591, 1506; ^{1}H NMR ($CDCl_3$) δ 2.83

(6H, s), 3.98 (3H, s), 6.97 (1H, d, $J=8.1$ Hz), 7.48 (1H, dd, $J=8.1$, 1.8 Hz), 7.52 (1H, d, $J=1.8$ Hz), 9.85 (1H, s); positive ESI-MS m/z 180 $[M+1]^+$.

General procedure for synthesis of diarylheptanoids (8–20)

The procedure reported by Pedersen et al.[11] was used to prepare compounds **8**–**20**. In general, 2,4-pentanedione (1 equiv, for compounds **4**–**17**) or ethyl 4-acetyl-5-oxohexanoate (1 equiv, for compounds **18**–**20**) and boric anhydride (0.7 equiv) dissolved in 10 mL of EtOAc were stirred for 30 min at 40 °C. The appropriate benzaldehyde (2 equiv) and tributylborate (4 equiv) were added, and the mixture was stirred for 30 min. *n*-Butylamine (1.5 equiv) dissolved in 10 mL of EtOAc was added dropwise over 20 min. Stirring was continued for 18 h at 40 °C. The mixture was then hydrolyzed by adding 10 mL of 1 N HCl and heating at 60 °C for 1 h. The organic layer was separated, and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with H_2O until neutral, then dried over Na_2SO_4, and the solvent was removed in vacuo. The crude products were purified by silica gel column chromatography eluting with *n*-hexane–EtOAc.

Compound 8. 3% yield (started with 5 mmol of 2,4-difluorobenzaldehyde), yellow powder, mp 155.5–156 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 5.86 (1H, s), 6.68 (2H, d, $J=16.0$ Hz), 6.84–6.96 (4H, m), 7.56 (2H, m), 7.71 (2H, d, $J=16.0$ Hz). ESI-MS m/z 347 $[M-1]^+$. Anal. calcd for $C_{19}H_{12}F_4O_2$: C, 65.52; H, 3.47. Found: C, 65.66; H, 3.57.

Compound 9. 10% yield (started with 5 mmol of 2-fluoro-4-methoxybenzaldehyde), yellow powder, mp 163–165 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 3.84 (6H, s), 5.82 (1H, s), 6.62 (2H, d, $J=16.0$ Hz), 6.70 (4H, m), 7.48 (2H, t, $J=8.7$ Hz), 7.71 (2H, d, $J=16.0$ Hz). ESI-MS m/z 371 $[M-1]^+$. Anal. calcd for $C_{21}H_{18}F_2O_4$: C, 67.74; H, 4.87. Found: C, 67.58; H, 4.92.

Compound 10. 9% yield (started with 5 mmol of 2-fluoro-6-methoxybenzaldehyde), yellow needles, mp 144–145 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 3.82 (6H, s), 5.90 (1H, s), 6.72 (2H, d, $J=16.0$ Hz), 6.88 (2H, m), 7.56 (2H, m), 7.00-7.07 (4H, m), 7.74 (2H, d, $J=16.0$ Hz). ESI-MS m/z 371 $[M-1]^+$. Anal. calcd for $C_{21}H_{18}F_2O_4$: C, 67.74; H, 4.87. Found: C, 67.60; H, 4.95.

Compound 11. 29% yield (started with 5 mmol of 6-nitroveratraldehyde), yellow needles, mp 240–241 °C (*n*-hexane-EtOAc). ^{1}H NMR ($CDCl_3$) δ 3.99 (6H, s), 4.04 (6H, s), 6.05 (1H, brs), 6.50 (2H, brd, $J=15.3$ Hz), 7.03 (2H, brs), 7.64 (2H, brs), 8.18 (2H, d, $J=15.3$ Hz). EI–MS m/z 440 $[M-NO_2]^+$. Anal. calcd for $C_{23}H_{22}N_2O_{10}$: C, 56.79; H, 4.56. Found: C, 56.58; H, 4.65.

Compound 12. 64% yield (started with 5 mmol of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde), yellow powder, mp 280 °C (decomp, *n*-hexane–EtOAc). ^{1}H NMR (DMSO-d_6) δ 3.94 (6H, s), 6.12 (1H, s), 6.97 (2H, d, $J=15.3$ Hz), 7.61 (2H, d, $J=15.3$ Hz), 7.63 (2H, s), 7.82 (2H, s). ESI-MS m/z 457 $[M-1]^+$. Anal. calcd for $C_{21}H_{18}N_2O_{10}$: C, 55.03; H, 3.96. Found: C, 55.07; H, 3.98.

Compound 13. To a solution of **12** (116 mg, 0.253 mmol) in dioxane–MeOH (10 mL, 1:1), *N*,*N*-diisopropylethylamine (122 μL, 0.70 mmol) and 2 M hexane solution of trimethylsiliyldiazomethane (350 μL, 0.70 mmol) were added and the mixture was stirred at room temperature overnight under N_2. The solvent was removed in vacuo and the resulting residue was recrystallized from hexane–EtOAc to give 40.8 mg of pure **13**. The mother liquor was subjected to silica gel column chromatography ($CHCl_3$) to give additional **13** (10 mg). Forty-one percent yield, yellow powder, mp 209–210 °C (*n*-hexane–EtOAc). ^{1}H NMR (acetone-d_6) δ 3.99 (6H, s), 4.06 (6H, s), 6.12 (1H, s), 7.01 (2H, d, $J=16.0$ Hz), 7.68 (2H, d, $J=16.0$ Hz), 7.70 (4H, s). ESI-MS m/z 485 $[M-1]^+$. Anal. calcd for $C_{23}H_{22}N_2O_{10}$: C, 56.79; H, 4.56. Found: C, 56.50; H, 4.55.

Compound 14. 20% yield (started with 5 mmol of 3-dimethylamino-4-methoxybenzaldehyde), orange needles, mp 126–127 °C (*n*-hexane-EtOAc). ^{1}H NMR ($CDCl_3$) δ 2.83 (12H, s), 3.93 (6H, s), 5.83 (1H, s), 6.50 (2H, d, $J=16.0$ Hz), 6.86 (2H, d, $J=16.0$ Hz), 7.14 (2H, d, $J=1.8$ Hz), 7.20 (2H, d, $J=8.4$, 1.8 Hz), 7.61 (2H, d, $J=16.0$ Hz). ESI-MS m/z 421 $[M-1]^+$. Anal. calcd for $C_{25}H_{30}N_2O_4$: C, 71.07; H, 7.16. Found: C, 71.17; H, 7.35.

Compound 15. 28% yield (started with 5 mmol of 3,4,5-trimethoxybenzaldehyde), yellow needles, mp 189–190 °C (*n*-hexane–EtOAc, lit:[16] mp 108–109 °C). ^{1}H NMR ($CDCl_3$) δ 3.90 (6H, s), 3.91 (12H, s), 5.87 (1H, s), 6.53 (2H, d, $J=15.6$ Hz), 6.79 (4H, s), 7.59 (2H, d, $J=15.6$ Hz). ESI-MS m/z 455 $[M-1]^+$. Anal. calcd for $C_{25}H_{28}O_8$: C, 65.78; H, 6.18. Found: C, 65.66; H, 6.16.

Compound 16. 27% yield (started with 5 mmol of 3,4-diethoxybenzaldehyde), yellow powder, mp 138–139 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 1.47 (6H, t, $J=6.9$ Hz), 1.48 (6H, t, $J=6.9$ Hz), 4.14 (8H, q, $J=6.9$ Hz), 5.80 (1H, s), 6.47 (2H, d, $J=15.6$ Hz), 6.87 (2H, d, $J=8.4$ Hz), 7.09 (2H, brs), 7.11 (2H, brd, $J=15.6$ Hz), 7.56 (2H, d, $J=15.6$ Hz). ESI-MS m/z 451 $[M-1]^+$. Anal. calcd for $C_{27}H_{32}O_6$: C, 71.66; H, 7.13. Found: C, 71.46; H, 7.06.

Compound 17. 17% yield (started with 5 mmol of 5,6-dimethoxynicotinaldehyde), yellow needles, mp 168–170 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 3.94 (6H, s), 4.06 (6H, s), 5.84 (1H, s), 6.50 (2H, d, $J=16.0$ Hz), 7.21 (2H, brs), 7.61 (2H, dd, $J=16.0$ Hz), 7.89 (2H, brs). ESI-MS m/z 397 $[M-1]^+$. Anal. calcd for $C_{21}H_{22}N_2O_6$: C, 63.31; H, 5.57; N, 7.03. Found: C, 63.84; H, 5.40; N, 6.99.

Compound 18. *N*,*N*-Diisopropylethylamine (185 μL, 1.06 mmol) and trimethylsilyldiazomethane (0.53 mL, 2 M hexane solution, 1.06 mmol) were added to a solution

of compound 5 (116 mg, 0.354 mmol) in MeOH–benzene (10 mL, 1:1), and the mixture was stirred at room temperature overnight under N_2. The solvent was removed in vacuo and the resulting residue was subjected to silica gel column chromatography using *n*-hexane–EtOAc to give 78 mg of pure **18** as a yellow oil. 44% yield, yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, $J=7.2$ Hz), 2.33–2.58 (2H, m), 2.95 (1H, m), 3.91 (6H, s), 3.93 (3H, s), 3.96 (3H, s), 4.14 (2H, q, $J=7.2$ Hz), 6.72 (2H, d, $J=16.0$ Hz), 6.85–7.22 (6H, m), 7.73 (2H, d, $J=16.0$ Hz). ESI-MS m/z 495 $[M-1]^+$.

Compound 19. 25% yield (started with 5 mmol of 3,4,5-trimethoxybenzaldehyde), yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, $J=7.2$ Hz), 2.38 (1H, m), 2.56 (1H, t, $J=7.2$ Hz), 2.97 (1H, t, $J=7.2$ Hz), 3.90 (6H, s), 3.93 (12H, s), 4.13 (2H, q, $J=7.2$ Hz), 6.84 (4H, s), 7.04 (2H, d, $J=15.3$ Hz), 7.70 (2H, d, $J=15.3$ Hz); ESI-MS m/z 555 $[M-1]^+$. Anal. calcd for $C_{30}H_{36}O_{10}$: C, 64.70; H, 6.52. Found: C, 64.86; H, 6.48.

Compound 20. 28% percent yield (started with 5 mmol of 3-dimethylamino-4-methoxybenzaldehyde), yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, $J=6.9$ Hz), 2.37 (1H, m), 2.55 (1H, s), 2.80 (6H, s), 2.84 (6H, s), 2.96 (1H, m), 3.92 (3H, s), 3.94 (3H, s), 4.09–4.19 (2H, m), 6.69–7.25 (8H, m), 7.73 (2H, d, $J=15.3$ Hz). ESI-MS m/z 521 $[M-1]^+$.

7-(3-Methoxy-4-propyloxyphenyl)-4-propyl-4-[3-(3-methoxy-4-propyloxyphenyl)-acryloyl]-hept-6-en-5-one (21) and 7-(3-methoxy-4-propyloxyphenyl)-4-[3-(3-methoxy-4-propyloxyphenyl)-acryloyl]-hept-6-en-5-one (22). Iodopropane (3.34 mL, 34.2 mmol) was added to a stirred solution of curcumin (628 mg, 1.71 mmol) and K_2CO_3 (473 mg, 34.2 mmol) in dry acetone (35 mL), and the mixture was refluxed for 48 h. After cooling the mixture to room temperature and filtering, the solvent was removed in vacuo. The resulting yellow solid was subjected to silica gel column chromatography (*n*-hexane–EtOAc) to give compounds **21** (283 mg, 31% yield) and **22** (112 mg, 13% yield).

Compound 21. Pale-yellow needles, mp 118–119 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 0.93 (6H, t, $J=6.9$ Hz), 1.03 (6H, t, $J=7.5$ Hz), 1.26 (3H, m), 1.80–1.91 (4H, m), 1.98–2.05 (4H, m), 3.88 (6H, m), 3.99 (4H, t, $J=6.9$ Hz), 6.64 (2H, d, $J=15.3$ Hz), 6.82 (2H, d, $J=8.1$ Hz), 6.99 (2H, d, $J=1.8$ Hz), 7.11 (2H, dd, $J=8.1$, 1.8 Hz), 7.68 (2H, d, $J=15.3$ Hz); ESI-MS m/z 559 $[M+Na]^+$. Anal. calcd for $C_{33}H_{44}O_6$: C, 73.85; H, 8.26. Found: C, 73.97; H, 8.53.

Compound 22. Pale-yellow needles, mp 132–133 °C (*n*-hexane–EtOAc). ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, $J=6.9$ Hz), 1.05 (3H, t, $J=7.2$ Hz), 1.06 (3H, t, $J=7.2$ Hz), 1.05 (2H, m), 1.80-1.95 (4H, m), 2.44 (2H, t, $J=8.4$ Hz), 2.69 (1H, dd, $J=16.5$, 3.3 Hz), 2.94 (1H, dd, $J=16.5$, 13.8 Hz), 3.90 (3H, s), 3.91 (3H, s), 4.01 (2H, t, $J=6.9$ Hz), 4.02 (2H, t, $J=6.9$ Hz), 5.31 (1H, dd, $J=14.0$, 3.0 Hz), 6.84 (2H, d, $J=8.1$ Hz), 6.93 (1H, d, $J=9.0$ Hz), 7.01 (2H, dd, $J=6.0$, 1.8 Hz), 7.09 (1H, dd, $J=8.1$, 1.8 Hz), 7.32 (2H, d, $J=16.0$ Hz). ESI-MS m/z 517 $[M+Na]^+$. Anal. calcd for $C_{30}H_{38}O_6$: C, 72.85; H, 7.74. Found: C, 72.89; H, 7.77.

Biological assays

Cell culture and gene transfection.[14] Human prostate cancer DU145, PC-3, and LNCaP cells were maintained in Dulbecco's minimum essential medium (DMEM) or RPMI medium containing penicillin (25 units/mL), streptomycin (25 g/mL), and 10% fetal calf serum. For gene transfection, the previously described conditions were followed with minor modifications. LNCaP cells, containing an endogenous mutant AR, were transfected with reporter gene. PC-3, lacking functional AR, were transfected with an AR expression plasmid and reporter gene. DU145 cells with a low content of endogenous AR coactivators, were transfected with expression plasmids for AR and ARA70 coactivator and a reporter gene. Transfections were performed using the SuperFect kit (Qiagen, Chatsworth, CA, USA). Briefly, $1–2\times10^5$ cells were plated on each 35-mm dish 24 h before transfection, and then a reporter gene, MMTV-luciferase, which contains MMTV-LTR promoter and AR-binding element, was co-transfected with AR expression plasmid (wild-type or mutant), or pSG5ARA70. pRL-TK was used as an internal control for transfection efficiency. After a 3-h transfection, the medium was changed to DMEM or RPMI supplemented with 10% charcoal stripped serum, and 20 h later, the cells were treated with DHT (1 nM) and/or test compounds for another 20 h. The cells were harvested and assayed for luciferase activity using Dual Luciferase Assay System (Promega, Madison, WI, USA). Data were expressed as relative luciferase activity normalized to the internal luciferase positive control.

LNCaP and H9c2 cell growth assay.[17] MTT assay, a colorimetric assay that measures the conversion of a colorless substrate to reduce into a colored tetrazolium by mitochondrial dehydrogenase, was employed to assess cell proliferation. Briefly, LNCaP and H9c2 cells were plated at a density of 1×10^3/well and 3×10^3/well, respectively, in 96-well tissue culture plates. Subsequently, the cells were incubated with 10% charcoal stripped serum-containing RPMI supplemented with DHT (1 nM) and/or test compounds for 5 consecutive days. At the end of incubation, the cells were added with an MTT-containing solution (5 mg/mL in PBS) in 1/10 of volume for 3 h at 37 °C. The resultant precipitate was dissolved in lysis buffer containing 50% dimethyl formamide, 5% sodium dodecyl sulphate, 0.35 M acetic acid and 50 mM HCl. The lysate was read at a wavelength of 595 nm using a microplate reader. To ensure the accuracy of the data derived from the MTT assay, cell counts from duplicate wells was also performed in some growth assays.

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