Antitumor Agents. 217.† Curcumin Analogues as Novel Androgen Receptor Antagonists with Potential as Anti-Prostate Cancer Agents

Hironori Ohtsu,‡ Zhiyan Xiao,‡ Junko Ishida,§ Masahiro Nagai,§ Hui-Kang Wang,‡ Hideji Itokawa,‡ Ching-Yuan Su,|| Charles Shih,|| Tzuying Chiang,⊥ Eugene Chang,⊥ YiFen Lee,⊥ Meng-Yin Tsai,⊥ Chawnshang Chang,⊥ and Kuo-Hsiung Lee‡,*

Natural Products Laboratory, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7360, Hoshi University, 2-4-41 Ebara, Shinagawa, Tokyo 158-0098, Japan, AndroScience Corporation, 11175 Flintkote Avenue, Suite F, San Diego, California 92121, and George Whipple Laboratory for Cancer Research, Departments of Pathology, Urology and Radiation Oncology, University of Rochester Medical Center, 601 Elmwood Avenue, Box 626, Rochester, New York 14642

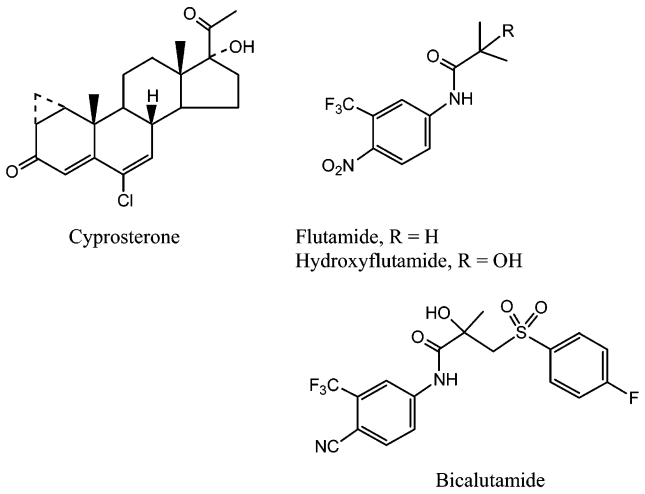
Received May 10, 2002

A number of curcumin analogues were prepared and evaluated as potential androgen receptor antagonists against two human prostate cancer cell lines, PC-3 and DU-145, in the presence of androgen receptor (AR) and androgen receptor coactivator, ARA70. Compounds **4** [5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one], **20** [5-hydroxy-1,7-bis[3-methoxy-4-(methoxycarbonylmethoxy)phenyl]-1,4,6-heptatrien-3-one], **22** [7-(4-hydroxy-3-methoxyphenyl)-4-[3-(4-hydroxy-3-methoxyphenyl)acryloyl]-5-oxohepta-4,6-dienoic acid ethyl ester], **23** [7-(4-hydroxy-3-methoxyphenyl)-4-[3-(4-hydroxy-3-methoxyphenyl)acryloyl]5-oxohepta-4,6-dienoic acid], and **39** [bis(3,4-dimethoxyphenyl)-1,3-propanedione] showed potent antiandrogenic activities and were superior to hydroxyflutamide, which is the currently available antiandrogen for the treatment of prostate cancer. Structure−activity relationship (SAR) studies indicated that the bis(3,4-dimethoxyphenyl) moieties, the conjugated β-diketone moiety, and the intramolecular symmetry of the molecules seem to be important factors related to antiandrogenic activity. The data further suggest that the coplanarity of the β-diketone moiety and the presence of a strong hydrogen bond donor group were also crucial for the antiandrogenic activity, which is consistent with previous SAR results for hydroxyflutamide analogues. When the pharmacophoric elements of dihydrotestosterone (DHT) and compound **4** are superposed, the resulting construct implies that the curcumin analogues may function as a 17α-substituted DHT. Compounds **4**, **20**, **22**, **23**, and **39** have been identified as a new class of antiandrogen agents, and these compounds or their new synthetic analogues could be developed into clinical trial candidates to control androgen receptor-mediated prostate cancer growth.

Introduction

The androgen receptor (AR) is a member of a large family of ligand-dependent transcriptional factors known as the steroid receptor superfamily.[2,3] Androgens and the AR play an important role in the growth of prostate cancer and normal prostate. Prostate cancer represents the most common male malignancy in the United States.[4] Recently, antiandrogens such as hydroxyflutamide (HF) in combination with surgical or medical castration have been widely used for the treatment of prostate cancer.[5] Both steroidal and nonsteroidal derivatives are presently available and have shown clinical benefit as chemotherapeutic agents for prostate cancer (Chart 1). The synthetic steroidal antiandrogen cyprosterone is one of the first antiandrogens used clinically in Europe;[6] however, this steroidal antiandrogen showed agonistic activity and overlapping effects with other hormonal systems, in addition to a range of unpleasant side effects.[7] HF and bicalutamide are nonsteroidal antiandrogens, both of which are thought to be pure antiandrogens without agonistic activity. Bicalutamide has a longer half-life (6 days) and a higher binding affinity to the AR than HF.[8,9]

Chart 1. Structures of Cyprosterone, Hydroxyflutamide, and Bicalutamide



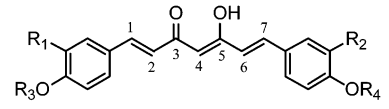
Cyprosterone

Flutamide, R = H
Hydroxyflutamide, R = OH

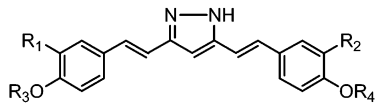
Bicalutamide

* To whom correspondence should be addressed. Phone: (919)-962−0066, Fax: (919)-966−3893. E-mail: khlee@unc.edu.
† Antitumor Agents. 217. For paper 216, see ref 1.
‡ University of North Carolina at Chapel Hill.
§ Hoshi University.
|| AndroScience Corporation.
⊥ University of Rochester Medical Center.

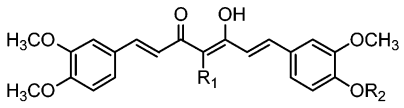
10.1021/jm020200g CCC: $22.00
Published on Web 10/12/2002



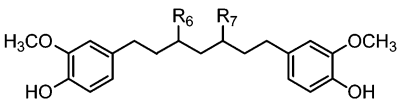
	R_1	R_2	R_3	R_4
1	OCH_3	OCH_3	H	H
2	OCH_3	H	H	H
3	H	H	H	H
4	OCH_3	OCH_3	CH_3	CH_3



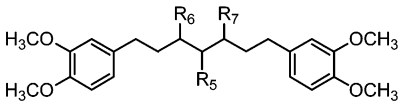
	R_1	R_2	R_3	R_4
5	OCH_3	OCH_3	H	H
6	OCH_3	H	H	H
7	H	H	H	H
8	OCH_3	OCH_3	CH_3	CH_3



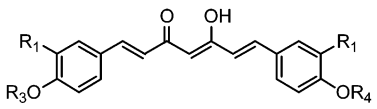
	R_1	R_2
9	H	H
10	CH_3	CH_3



	R_6	R_7
11	=O	=O
12	=O	-OH
13	-OH	-OH



	R_5	R_6	R_7
14	H	=O	=O
15	H	=O	-OH
16	H	-OH	-OH
17	CH_3	=O	-OH
18	CH_3	-OH	-OH



	R_1	R_3	R_4
19	OCH_3	CH_2COOCH_3	H
20	OCH_3	CH_2COOCH_3	CH_2COOCH_3

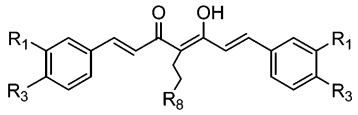
Figure 1. Structures of Curcumin Analogues **1**−**20**.

The agonistic activity of the antiandrogen may result in "antiandrogen withdrawal syndrome".[10] A currently accepted hypothesis postulates that mutations in ARs may account for why HF, the active metabolite of flutamide, can activate AR target genes and stimulate prostate cancer growth.[10] The same mechanism is used to explain the "flutamide withdrawal syndrome", in which patients who experience an increase in prostate-specific antigen (PSA) while taking flutamide, have a decrease in PSA after withdrawal of treatment. Indeed, HF can activate AR target genes, such as PSA and MMTV-LTR (a reporter gene which expresses luciferase), in the presence of ARA70, the first identified AR coactivator.[11] Because this syndrome often leads to the failure of androgen-ablative therapy, it is desirable to develop better antiandrogens without agonist activity.

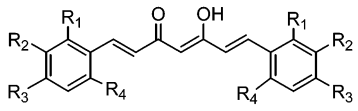
The phenolic diarylheptanoid curcumin (**1**) is the major pigment in turmeric. Curcumin and its analogues show potent antioxidant activity, antiinflammatory activity,[12] cytotoxicity against tumor cells,[13] and antitumor-promoting activities.[14,15] In a previous paper, we reported that two cyclic diarylheptanoids, 13-oxomyricanol and myricanone, exhibited potent antitumor-promoting effects on DMBA-initiated and TPA-induced mouse skin carcinogenesis.[16] Very recently, we have also evaluated the cytotoxic effects of curcumin analogues and 1,3-diaryl-1,3-diketopropane derivatives against a panel of human tumor cell lines.[17] In the present study, we have prepared a number of curcumin analogues and evaluated their antagonistic activity against the AR in the presence of ARA70, using two human prostate cancer cell lines, PC-3 and DU-145. 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 ARA70.

Chemistry

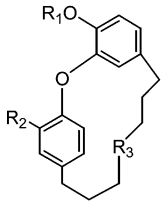
Figures **1** and **2** show the structures of curcumin analogues and 1,3-diaryl-1,3-diketopropane derivatives. Curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**) were obtained by column chromatography (silica gel, $CHCl_3$−MeOH) of commercially available curcumin (Aldrich), which contained **2** and **3** as minor components. Treatment of **1** with diazomethane gave dimethylated curcumin (**4**) and monomethylated curcumin (**9**). Methylation of **1** with methyl iodide and K_2CO_3 furnished the trimethylated derivative **10**, in which a methyl group was also introduced at the C-4 position. Compounds **5**−**8** were synthesized by heating **1**−**4** with histidine hydrazide, AcOH, and *p*-TsOH overnight. Hydrogenation of **1** with 10% Pd−C gave a mixture of **11**−**13**. Similarly, compounds **14**−**16** and **17**, **18** were obtained by hydrogenation of **4** and **10**, respectively. Heating **1** with methyl chloroacetate, NaI, and K_2CO_3 in acetone furnished a mixture of mono(methoxycarbo-



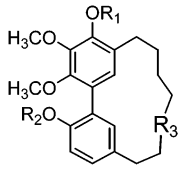
	R_1	R_3	R_8
21	H	H	COOH
22	OCH_3	OH	$COOC_2H_5$
23	OCH_3	OH	COOH



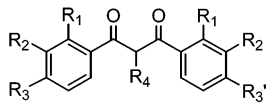
	R_1	R_2	R_3	R_4
24	H	H	F	H
25	H	F	H	H
26	F	H	H	H
27	H	F	OMe	H
28	H	CF_3	F	H
29	H	H	OCF_3	H



	R_1	R_2	R_3
30	H	H	C=O
31	H	H	C=N-OH
32	CH_3	OH	C=O



	R_1	R_2	R_3
33	H	H	C=O
34	CH_3	H	C=O
35	CH_3	CH_3	C=O
36	Ac	Ac	C=O
37	H	H	
38	H	H	



	R_1	R_2	R_3	R_3'	R_4
39	H	OCH_3	OCH_3	OCH_3	H
40	OCH_3	H	OCH_3	OCH_3	H
41	H	H	OCH_3	NO_2	Br
42	H	H	NO_2	NO_2	Br
43	H	H	NO_2	NO_2	H
44	H	H	H	H	CH_2COPh

Figure 2. Structures of Curcumin Analogues **21**−**44**.

nylmethyl) ether **19** and bis(methoxycarbonylmethyl) ether **20**, which were separated by preparative TLC (PLC). Compounds **21**−**23** were prepared from benzene or vanillin and ethyl 4-acetyl-5-oxohexanoate as described previously in the literature.[22] Compounds **21**−**23** constitute an unseparable mixture of keto−enol tautomeric isomers. The syntheses of **24**−**38** were described in our previous paper.[16,17] Compounds **39**−**44** were purchased from Aldrich, Inc. (Milwaukee, WI).

Results and Discussion

Forty-four curcumin derivatives (**1**−**44**) were tested for antagonistic activity against the AR using two different human prostate cancer cells, PC-3 and DU-145 (Figures 3A−C). The parental compound, curcumin (**1**), was inactive in all cases. However, dimethylated curcumin (**4**) showed significant antagonistic activity (reducing 70% of DHT-induced AR activity) when assayed in PC-3 cells transfected with wild-type AR (wt.AR) and was more potent than HF (which reduced 16% of DHT-induced AR activity, Figure 3A). Compound **4** also showed the highest antagonist activity when assayed in DU-145 cells transfected with a mutant LNCaP AR and ARA70 (showing a 45% reduction in DHT-induced AR activity, Figure 3B), indicating that compound **4** is an effective antagonist for both wt.AR and mutant AR.

To determine the structural requirements for AR antagonist activity in this series of compounds, an SAR study was conducted in PC-3 and DU-145 cell assay systems. Compared with **4**, monomethylated curcumin (**9**) lacks one *O*-methyl group at the *para* position on one benzene ring and was significantly less active than **4** (Figure 3A). Thus, the bis(3,4-dimethoxyphenyl) groups of **4** are important to the activity. Compounds **14** and **15**, which were obtained by hydrogenation of **4**, were as potent as HF with a 18% reduction in DHT-induced AR activity but were considerably less active compared to **4** (Figure 3A). Introducing a methyl group at C-4 of **4** (**10**) resulted in decreased activity (Figure 3B). Converting the β-diketone moiety of **4** to the corresponding pyrazole derivative **8** greatly reduced the activity (data not shown). Furthermore, 1,3-bis(3,4-dimethoxyphenyl)-1,3-propandione (**39**), which contains the bis-aryl groups found in **4**, but lacks the conjugated double bonds, was less active than **4** (Figure 3A and 3B), indicating that the conjugated double bonds also contribute to the activity of **4**. These observations suggested that the bis-(3,4-dimethoxyphenyl) groups and the conjugated β-diketone moiety are crucial for the activity.

Data in Figure 3C shows a somewhat different cell assay system where antiandrogen activity was assayed in DU-145 cells transfected with wt.AR and ARA70. Compounds **4**, **20**, **22**, **23**, and **39** showed comparable or more potent antiandrogen activity than HF in this assay system. Compounds **20** and **22** were almost equipotent (54% and 53.8% reduction, respectively) and were slightly more active than **4** (49.9%). Because curcumin (**1**) itself was not active, introducing either methoxycarbonylmethyl groups at the phenolic hydroxyls (**20**) or an ethoxycarbonylethyl group at C-4 (**22**) greatly contributed to the anti-AR activity in DU-145 cells in the presence of wt.AR and ARA70.

In this study, we also examined the antiandrogen activity of fluorodiarylheptanoids **24**−**29** and cyclic

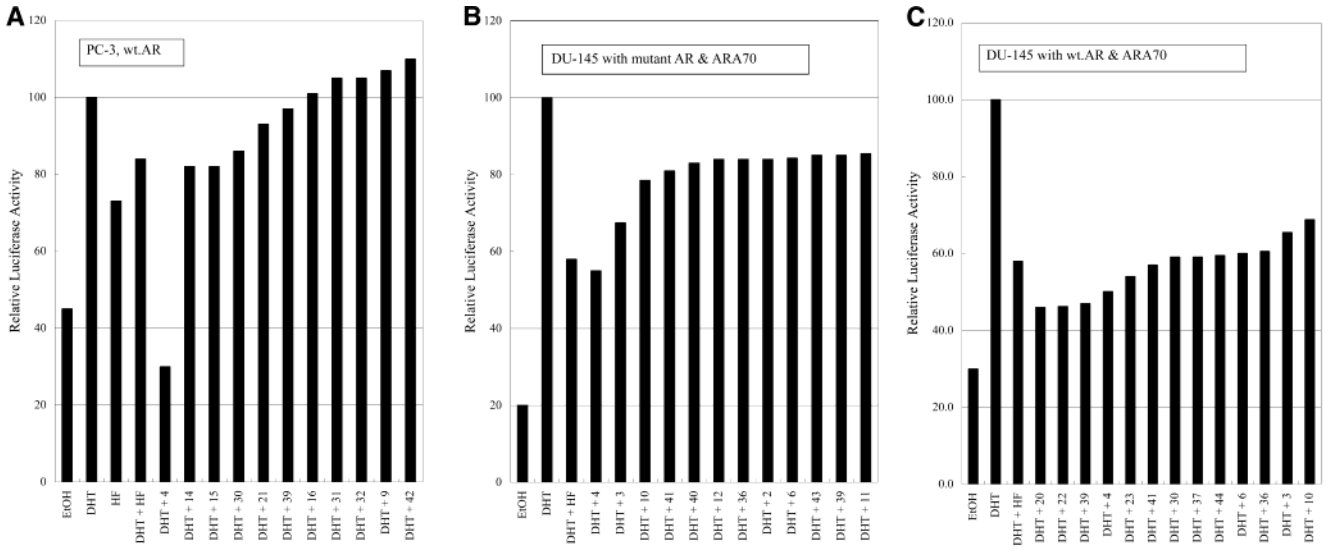


Figure 3. Suppression of DHT-mediated MMTV (mouse memory tumor virus) transcription AR activity by hydroxyflutamide (HF) and selected compounds. PC-3 and DU-145 cell lines were seeded and cotransfected with reporter MMTV-luciferase (**A** and **C**) or mutant AR expression plasmid (**B**) and ARA70 (**B** and **C**) using SuperFect. Subsequently the transfected cells were harvested and replated in 10% charcoal-stripped fetal bovine serum DMEM (Dulbecco's Modified Eagle Media) medium. The cells were then treated with dehydrotestosterone (DHT, 1 nM) and antiandrogens (1 μM) and harvested for detection of the luciferase activity. The results were averaged from two independent experiments.

diarylheptanoids **30**−**38**. Compounds **24**−**29** have fluorine or trifluoromethyl substituents on both benzene rings but showed weak activity or were inactive (data not shown). Among the cyclic diarylheptanoids **30**−**38**, compound **30** was the most active and was almost as active as HF (Figure 3A and 3C). The remaining cyclic diarylheptanoids showed weak antagonistic activity.

By closely examining the structures and activities of the curcumin analogues, additional structural features, besides the bis(3,4-dimethoxyphenyl) groups and the conjugated β-diketone moiety, seem to be related to the antiandrogenic activity. The conjugated β-diketone moiety in the most active compounds (**4**, **20**, **22**, **23**, and **39**) can be effectively fixed into a conformation that ensures coplanarity. However, in compounds **11**−**18**, the coplanarity of the β-diketone moiety is lost and the antiandrogenic activity decreases dramatically. This observation is consistent with the correlation between the antiandrogenic activity and the coplanarity of the −NH−CO−OH− moiety in HF analogues.[18] The hydrogen bond donor ability of the hydroxyl group in the most active compounds is enhanced by the conjugated system (this ability will be decreased by the formation of intramolecular hydrogen bond with the carbonyl group; however, the conjugated system makes the carbonyl group itself a very weak hydrogen bond acceptor), but certain compounds (e.g., **30**−**38**) lacking such a hydroxyl group are significantly less active. The presence of a strong hydrogen bond donor group is also consistent with the previous SAR for HF analogues.[18] In addition, intramolecular symmetry seems to be another important factor related to antiandrogenic activity.

It was previously suggested that the steroid A-ring is a pharmacophoric component necessary for molecular recognition between steroid receptors and the ligands, while the remaining steroid structure confers receptor selectivity/specificity.[19,20] Additionally, the "17β-hydroxyl-3-one" substructures have been considered essential for effective binding of steroids to AR.[21] Using these predefined pharmacophoric elements, we have superposed

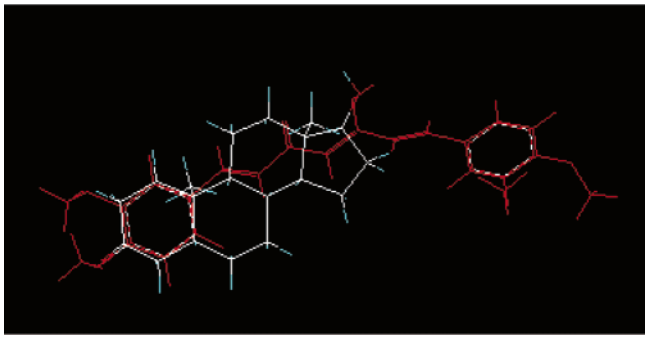


Figure 4. Superposition of DHT and compound **4.** DHT and compound **4** are represented in white and red, respectively. Both molecules were constructed and optimized with SYBYL 6.0. Compound **4** was subjected to a genetic algorithm conformational search. The lowest-energy conformer of **4** was superposed to DHT by the six-membered rings (A-ring of DHT and phenyl of **4**) and the hydroxyl groups and was then field-fit minimized using DHT as template.

DHT, the naturally occurring ligand of AR, with compound **4** (Figure 4) by aligning the six-membered rings (A-ring of DHT and a phenyl ring of **4**) and the hydroxyl groups. The resulting superposition suggests that the curcumin analogues may assume a conformation similar to that of 17α-substituted DHT in order to exert their antiandrogenic activity. The remaining structural elements of compound **4** extend into the D-ring C17 vicinity, which coincides with a sterically favorable area defined by a previous three-dimensional quantitative structure−activity relationship (3D-QSAR) study using the comparative molecular field analysis (CoMFA) technique.[20] The consistency between the SAR of curcumin analogues and known antiandrogens implies that this new class of compounds may have a ligand−receptor recognition pattern and interaction mode similar to that of known antiandrogens. This knowledge would facilitate future mechanic investigations of curcumin analogues.

Conclusion

In conclusion, we have prepared a number of curcumin analogues and evaluated their potential antiandrogen activity in three different assay conditions using human prostate cancer cell lines. Compounds **4** showed promising antiandrogen activities in all assays. Compounds **4**, **20**, **22**, **23**, and **39** have been identified as a new class of antiandrogen agents. SAR studies revealed that bis(3,4-dimethoxyphenyl) moieties, a conjugated β-diketone, and an ethoxycarbonylethyl group at the C-4 position play important roles in the antagonistic activity. Further mechanistic studies and the development of new antiandrogens are actively underway in our laboratory.

Experimental Section

Melting points were determined on a Fisher-Johns melting apparatus and are uncorrected. Optical rotations were determined with a DIP-1000 polarimeter. ^{1}H NMR spectra were recorded on a Bruker AC-300 and JMN GX-500 spectrometer. The chemical shifts are presented in terms of ppm with TMS as the internal reference. MS spectra were recorded on HP5989A and JMS D-300 instruments. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and agreed with theoretical values to within ±0.4%. New compounds **9**, **16**, and **18** were homogeneous by HPLC analyses in three different solvent systems. Compounds **1**−**3** were obtained by column chromatography (silica gel, $CHCl_3$−MeOH) of commercially available curcumin (Aldrich), which contained **2** and **3** as minor components. Compounds **39**−**44** were purchased from Aldrich, Inc (Milwaukee, WI).

Dimethylcurcumin (4). Curcumin (**1**) in Et_2O and MeOH was treated with excess of diazomethane in ether for 24 h. The solvents were removed in vacuo, and the residue was purified by silica gel column chromatography and PLC to yield yellow needles of **4** (yield 19.8%); mp 129−130 °C (MeOH) (lit.[23] 128−130 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (12H, s, $OCH_3 \times 4$), 5.82 (1H, s, 1-H), 6.48 (2H, d, 16 Hz), 6.88 (2H, d, $J = 8$ Hz), 7.08 (2H, bs), 7.15 (2H, bd), 7.61 (2H, $J = 16$ Hz); ^{13}CNMR (300 MHz, $CDCl_3$): δ 55.9, 56.0, 101.3, 109.8, 111.1, 122.0, 122.6, 128.1, 140.4, 149.2, 151.0, 183.2.

Preparation of Pyrazole Derivative 8. To a solution of **1**−**4** in butanol and ethanol were added histidine hydrazide (1 equiv), acetic acid, and *p*-TsOH. The solution was refluxed for 24 h, and then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography and PLC.

Compound 8. Yellow powder (yield 17.5%), mp 166−168 °C (MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.92 (6H, s, $OCH_3 \times 2$), 3.94 (6H, s, $OCH_3 \times 2$), 6.62 (1H, s, 1-H), 6.86 (2H, d, $J = 8$ Hz), 6.93 (2H, d, $J = 16$ Hz), 7.04 (2H, dd, $J = 8$, 2 Hz), 7.06 (2H, bs), 7.05 (2H, d, $J = 16$ Hz); ^{13}CNMR (300 MHz, $CDCl_3$): δ 55.8, 55.9, 99.6, 108.6, 111.2, 115.8, 120.1, 129.7, 130.6, 149.1, 149.3; Anal. Calcd (theoretical) for $C_{23}H_{24}N_2O_4 \cdot {}^5/_4H_2O$: C, 66.57; H, 6.44; N, 6.75. Found: C, 66.44; H, 6.19; N, 6.27.

Monomethylcurcumin (9). Curcumin (**1**) in MeOH was treated with excess diazomethane in Et_2O for 24 h. After removal of solvents, the residue was purified by silica gel column chromatography and PLC to yield a yellow amorphous solid (yield 20%); mp 89−91°C, $[\alpha]_D$ −3.6° ($c = 1.14$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (9H, s, $OCH_3 \times 3$) , 5.81 (1H, s, 1-H), 5.94 (1H, bs, OH), 6.49 (2H, bd, $J = 15$ Hz), 6.93 (1H, d, $J = 8$ Hz), 6.97 (1H, d, $J = 8$ Hz), 7.10 (4H, m), 7.60 (2H, bd, $J = 15$ Hz); EIMS *m*/*z* 382 (M^+), HRFABMS 382.1396 ($M + H^+$) (calcd for $C_{22}H_{22}O_6$: 382.1416).

Hydrogenation of 1, 4, and 10 (11−18). A solution of starting material in EtOAc was shaken with 10% Pd−C under H_2 (45 psi) overnight using a Parr's apparatus. The solution was filtered and concentrated in vacuo to give a residue, which was purified by silica gel column chromatography and PLC.

Tetrahydrocurcumin (11). White powder, mp 92−93 °C (lit.[23] 95−96 °C), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.53−2.58 (3H, m), 2.78−2.88 (5H, m), 3.87 (6H, s, $OCH_3 \times 2$), 5.43 (1H, s, 1-H), 5.50 (2H, s, ArOH), 6.65 (2H, d, $J = 8$ Hz), 6.69 (2H, s), 6.83 (2H, d, $J = 8$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 31.3, 40.4, 55.8, 99.8, 111.0, 114.3, 120.8, 132.6, 144.0, 146.4, 193.2.

Hexahydrocurcumin (12). White powder, mp 87−88 °C (lit.[23] 78−80 °C), ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60−1.81 (2H, m), 2.53−2.97 (8H, m), 3.85 (6H, s, $OCH_3 \times 2$), 4.06 (1H, m, 2-H), 6.70 (4H, m), 6.80 (2H, d, $J = 8$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 29.7, 31.7, 38.8, 45.8, 49.8, 56.3, 67.4, 111.5, 111.6, 114.8, 114.9, 121.2, 121.4, 133.0, 134.2, 144.2, 144.5, 146.9, 147.9, 211.9.

Octahydrocurcumin (13). Colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (2H, m), 1.75 (4H, m), 2.53−2.70 (4H, m), 3.80 (6H, s, $OCH_3 \times 2$), 3.91 (2H, brs), 6.13 (2H, s, ArOH), 6.65 (2H, d, $J = 8$ Hz), 6.69 (2H, bs) 6.82 (2H, bd, $J = 8$ Hz), ^{13}C NMR (300 MHz, acetone-d_6): δ 31.1, 39.8, 42.6, 35.6, 72.0, 111.0, 114.3, 120.6, 133.6, 143.6, 146.4.

Compound 14. White powder (yield 26.0%), mp 60−61 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.56 (3H, m), 2.86 (5H, m), 3.85 (12H, s, $OCH_3 \times 4$), 5.44 (1H, s, 1-H), 6.71 (4H, m), 6.78 (2H, bd); Anal. Calcd (theoretical) for $C_{23}H_{28}O_6 \cdot {}^1/_4H_2O$: C, 68.21; H, 7.09. Found: C, 68.25; H, 7.06.

Compound 15. White powder (yield 20.0%), mp 94−95 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65−1.80 (2H, m), 2.53−2.84 (8H, m), 3.85 (12H, s, $OCH_3 \times 4$), 4.05(1H, bs, 2′-H), 6.68−7.23 (4H, m), 6.79 (2H, bd), Anal. Calcd (theoretical) for $C_{23}H_{30}O_6 \cdot {}^1/_4H_2O$: C, 67.88; H, 7.55. Found: C, 67.73; H, 7.49.

Compound 16. Colorless oil (yield 4.2%), mp 60−61 °C, δ ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55−1.65 (4H, m), 1.73−1.82 (3H, m), 2.60−2.72 (3H, m), 3.86 (6H, s, $OCH_3 \times 2$), 3.87 (8H, bs, $OCH_3 \times 2$, 2,2′-H), 6.72−6.78 (4H, m), 6.79 (2H, bd), 7.27-(2H, s, OH × 2), EIMS *m*/*z*: 404 (M^+), HRFAB-MS *m*/*z* 404.219070 $(M + H)^+$ (calcd for $C_{23}H_{32}O_6$: 404.2198891).

Compound 17. Colorless oil (yield 5.9%),^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (3H, d), 1.80 (1H, m), 2.43−2.82 (8H, m), 3.86 (6H, s, $OCH_3 \times 2$), 3.87 (6H, s, $OCH_3 \times 2$), 3.94 (1H, bs, 2′-H),6.70−6.78 (6H, m), EIMS *m*/*z* 416 (M^+).

Compound 18. Colorless oil (yield 6.95%), ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (3H, d, 1-CH_3), 1.52 (1H, m), 1.84 (2H, m), 2.67 (6H, m), 3.83 (14H, bs, $OCH_3 \times 4$, 2, 2′-H), 6.78 (6H, m); EIMS *m*/*z*: 418 (M^+), HRFAB-MS *m*/*z* 418.236618 $(M + H)^+$ (calcd for $C_{24}H_{34}O_6$: 418.2355392).

Preparation of 19 and 20. A mixture of curcumin (**1**, 100 mg, 0.81 mmol) in acetone (20 mL) with methyl chloroacetate (2 mL) and NaI (20 mg) was refluxed with anhydrous potassium carbonate (176 mg) for 24 h with stirring. After filtration and removal of solvent, the residue was purified by silica gel column chromatography to yield the corresponding methyl acetates **19** and **20**.

Compound 19: Yellow powder (yield 20.0%), mp 60−61 °C, mp 66−67 °C, $[\alpha]_D$ −2.4° ($c = 2.08$, $CHCl_3$); ^{1}H NMR (300 MHz, acetone-d_6): δ 3.73 (3H, s, $COOCH_3$), 3.86 (6H, s, $OCH_3 \times 2$), 4.79 (2H, s, OCH_2COO), 5.99 (1H, s, 1-H), 6.70 and 6.73 (both 1H, d, $J = 15.3$ Hz), 6.88 (1H, d, $J = 8$ Hz), 6.94 (1H, d, $J = 8$ Hz), 7.17 (2H, m), 7.33 (2H, m), 7.59 and 7.61 (both 1H, d, $J = 15.3$ Hz), ^{13}C NMR (300 MHz, $CDCl_3$): d 51.8, 55.9, 55.9, 65.9, 101.4, 111.2, 111.6, 114.3, 115.9, 121.8, 122.6, 123.0, 123.5, 127.6, 128.7, 129.8, 140.3, 141.3, 148.4, 149.8, 150.0, 150.4, 169.4, 183.4, 184.6; Anal. Calcd (theoretical) for $C_{24}H_{24}O_8 \cdot {}^3/_4H_2O$: C, 63.50; H, 5.66. Found: C, 63.53; H, 5.65.

Compound 20: Yellow powder (yield 20.0%), mp 141−142 °C (MeOH), $[\alpha]_D$ −0.29° ($c = 5.86$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80 (6H, s), 3.93 (6H, s), 4.73 (4H, s, $OCH_2COO \times 2$), 5.82 (1H, s, 1-H), 6.50 (2H, d, $J = 16$ Hz), 6.79 (2H, d, $J = 8$ Hz), 7.09 (4H, bs), 7.58 (2H, d, $J = 16$ Hz), ^{13}C NMR (300 MHz, $CDCl_3$): δ 52.3, 56.0, 66.0, 101.4, 110.7, 113.6, 122.0, 122.7, 129.5, 140.1, 149.0, 149.7, 169.0, 183.1; Anal. Calcd (theoretical) for $C_{27}H_{28}O_{10} \cdot {}^1/_2H_2O$: C, 62.18; H, 5.60. Found: C, 62.31; H, 5.57.

Compound 21: Yellow amorphous solid (yield 3.0%), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.58 (2H, m), 2.95 (2H, m), 7.12 (2H, d, $J = 15$ Hz), 7.40 (6H, m), 7.60 (4H, m), 7.81(2H, d, J

= 15 Hz), 12.65(1H, bs); Anal. Calcd (theoretical) for $C_{22}H_{20}O_4$: C, 75.84, H, 5.79. Found: C, 75.56, H, 5.74.

Compound 22: Yellow amorphous solid (yield 25.0%), Anal. Calcd (theoretical) for $C_{26}H_{28}O_8$: C, 66.66, H, 6.02. Found: C, 66.38, H, 6.16.

Compound 23: Yellow powder (yield 45.0%), mp 144−146 °C (MeOH) [lit.[22] 71−73 °C (CH_2Cl_2)]; Anal. Calcd (theoretical) for $C_{24}H_{26}O_8 \cdot {}^5/_2H_2O$: C, 59.87; H, 5.23. Found C, 59.94; H, 5.11.

The structures of **1**−**4**, **10**−**13**, **22**, and **23** were confirmed by comparison of their physical spectral data with those reported in the literature.[22,23]

Suppression of DHT-Mediated Transcription Activity. Cell Culture and Transfections. Human prostate cancer DU145 and PC-3 cells were maintained in Dulbecco's minimum essential medium (DMEM) containing penicillin (25 units/mL), streptomycin (25 μg/mL), and 10% fetal calf serum (FCS). For AR transactivation assay, PC-3 cells were transfected with an AR expression plasmid and reporter gene. Because of a low content of endogenous AR coactivators, DU-145 cells were transfected with expression plasmids for AR and ARA70, and reporter gene. The previously described conditions were followed with minor modifications.[10] Transfections were performed using the SuperFect kit according to manufacturer's procedures (Qiagen, Chatsworth, CA). Briefly, 1×10^5 cells were plated on 35-mm dishes 24 h before transfection, and then a reporter plasmid, MMTV−Luciferase, which contains MMTV-LTR promoter and AR-binding element, was cotransfected with AR expression plasmid (wild type or mutant), or pSG5ARA70. PRL-TK was used as an internal control for transfection efficiency. The total amount of DNA was adjusted to 3.0 μg with pSG5 in all transcriptional activation assays. After a 2 h transfection, the medium was changed to DMEM−10% charcoal stripped serum medium, and 14−16 h later, the cells were treated with DHT, antiandrogen, or test compounds. After another 14−16 h, the cells were harvested and tested for luciferase activity in luciferase assays (Promega, Dual Luciferase Assay System, Madison, WI). Data were expressed as relative luciferase activity as compared to an internal luciferase positive control.

Acknowledgment. This work was supported by National Cancer Institute Grant CA-17625 awarded to K. H. Lee and DK-60905 to C. Chang.

References

(1) For the previous paper in this series, see Ohtsu, H.; Nakanishi, Y.; Bastow, K. F.; Lee, K. H. Antitumor Agents 216. Synthesis and Evaluation of Paclitaxel−Camptothecin Conjugates as Novel Cytotoxic Agents. *Bioorg. Med. Chem*., in press.

(2) Chang, C.; Kokontis, J.; Liao, S. Structural Analysis of Complementary DNA and Amino acid Sequences of Human and Rat Androgen Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7211−7215.

(3) Beato, M. Gene Regulation by Steroid Hormones. *Cell* **1989**, *56*, 335−344.

(4) Landis, S. H.; Murray, T.; Bolden, S.; Wingo, P. A. Cancer Statistics 1998. *Cancer J. Clin.* **1998**, *48*, 6−29.

(5) Crawford, E. D.; Eisenberger, M. A.; McLeod, D. G.; Spaulding, J. T.; Benson, R.; Dorr, F. A.; Blumenstein, B. A.; Davis, M. A.; Goodman, P. J. A Controlled Trial of Leuprolide with and without Flutamide in Prostate Carcinoma. *New Engl. J. Med.* **1989**, *321*, 419−424.

(6) McLeod, D. G. Antiandrogenic Drugs. *Cancer* **1993**, *71*, 1046−1049.

(7) Neumann, F.; Jacobi, G. H. Antiandrogens in Tumor Therapy. *J. Clin. Oncol.* **1982**, *1*, 41−65.

(8) Verhelst, J.; Denis, L.; Vliet, V.; Poppel, H. V.; Braeckman, J.; Cangh, P. V.; Mattelaer, J.; D'Hulster, D.; Mahler, C. Endocrine Profile during Administration of the New Nonsteroidal Antiandrogen Casodex in Prostate Cancer. *Clin. Endocrinol.* **1994**, *41*, 525−530.

(9) Kelly, W. K.; Scher, H. I. Prostate Specific Antigen Decline after Antiandrogen Withdrawal: the Flutamide Withdrawal Syndrome. *J. Urol.* **1993**, *149*, 607−609. (b) Scher, H. I.; Kelly, W. K. Flutamide Withdrawal Syndrome: Its Impact on Clinical Trials in Hormone-refractory Prostate Cancer. *J. Clin. Oncol.* **1993**, *11*, 1566−1572.

(10) Miyamoto, H.; Yeh, S.; Wilding, G.; Chang, C. Promotion of Agonist Activity of Antiandrogens by the Androgen Receptor Coactivator, ARA70, in Human Prostate Cancer DU145 Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7379−7384.

(11) Yeh, S.; Miyamoto, H.; Chang, C. Hydroxyflutamide May Not Always Be a Pure Antiandrogen. *The Lancet* **1997**, *349*, 852−853.

(12) Nurfina, A. N.; Reksohadiprodjo, M. S.; Timmerman, H.; Jenie, U. A.; Sugiyanto, D.; van der Goot, H. Synthesis of Some Symmetrical Curcumin Derivarives and their Antiinflammatory Activity. *Eur. J. Med. Chem.* **1997**, *32*, 321−328.

(13) Syu, W., Jr.; Shen, C. C.; Don, M. J.; Ou, J. C.; Lee, G. H.; Sun, C. M. Cytotoxicity of Curcuminoids and Some Novel Compounds from *Curcuma zedoaria*. *J. Nat. Prod.* **1998**, *61*, 1531−1534.

(14) Sugiyama, Y.; Kawakishi, S.; Osawa, T. Involvement of the β-Diketone Moiety in the Antioxidative Mechanism of Tetrahydrocurcumin. *Biochem. Pharmacol.* **1996**, *52*, 519−525.

(15) Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekharan, K. N.; Kettan, R. Anti-tumor and Antioxidant activity of Natural Curcuminoids. *Cancer Lett.* **1995**, *94*, 79−83.

(16) Ishida, J.; Kozuka, M.; Wang, H.-K.; Konoshima, T.; Tokuda, H.; Okuda. M.; Mou, X.-Y.; Nishino, H.; Sakurai, N.; Lee, K.-H.; Nagai, M. Antitumor-promoting Effects of Cyclic Diarylheptanoids on Epstein−Barr Virus Activation and Two-Stage Mouse Skin Carcinogenesis. *Cancer Lett.*, **2000**, *159*. 135−140.

(17) Ishida, J.; Ohtsu, H.; Tachibana, Y.; Nakanishi, Y.; Bastow, K. F.; Nagai, M.; Wang, H. K.; Itokawa, H.; Lee, K. H. Synthesis and Evaluation of Curcumin Analogues as Cytotoxic Agents. Unpublished data.

(18) Morris, J. J.; Hughes, L. R.; Glen, A. T. and Taylor, P. J. Non-Steroidal Antiandrogens. Design of Novel Compounds Based on an Infrared Study of the Dominant Conformation and Hydrogen-Bonding Properties of a Series of Anilide Antiandrogens. *J. Med. Chem.* **1991**, *34*, 447−455.

(19) McKinney, J. D. and Waller, C. L. Polychlorinated Biphenyls as Hormonally Active Structural Analogues. *Environ. Health. Perspect.* **1994**, *102*, 290−297.

(20) Waller, C. L.; Juma, B. W.; Gray. L. E., Jr.; Kelce, W. R. Three-Dimensional Quantitative Structure−Activity Relationships for Androgen Receptor Ligands. *Toxicol. Appl. Pharmacol.* **1996**, *137*, 219−227.

(21) Singh, S. M.; Gauthier S.; Fernand L. Androgen Receptor Antagonists (Antiandrogens): Structure−Activity Relationships. *Curr. Med. Chem.* **2000**, *7*, 211−247.

(22) Pedersen, U.; Rasmussen, P. B.; Lawesson, S. O. Synthesis of Naturally Occurring Curcuminoids and Related Compounds. *Liebigs Ann. Chem.* **1985**, 1557−1569.

(23) Roughley, P. J.; Whiting, D. A. Experiments in the Biosynthesis of Curcumin. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2379−2388.

JM020200G