

Antitumor Agents. 250.[†] Design and Synthesis of New Curcumin Analogues as Potential Anti-Prostate Cancer Agents

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In a continuing study of curcumin analogues as potential drug candidates to treat prostate cancer at both androgen-dependent and androgen-refractory stages, we designed and synthesized over 40 new analogues classified into four series: monophenyl analogues (series A), heterocycle-containing analogues (series B), analogues bearing various substituents on the phenyl rings (series C), and analogues with various linkers (series D). These new compounds were tested for cytotoxicity against two human prostate cancer cell lines, androgen-dependent LNCaP and androgen-independent PC-3. Antiandrogenic activity was also evaluated in LNCaP cells and PC-3 cells transfected with wild-type androgen receptor. Ten compounds possessed potent cytotoxicity against both LNCaP and PC-3 cells, seven only against LNCaP, and one solely against PC-3. This study established an advanced structure–activity relationship (SAR), and these correlations will guide the further design of new curcumin analogues with better anti-prostate cancer activity.

Introduction

Prostate cancer is the most common cancer among males of Western countries² and is a complex heterogeneous disease that acts differently in different men. The real cause of prostate cancer is still unknown. However, androgen and the androgen receptor (AR) are postulated to play crucial roles in the development of prostate cancer.³ The current treatment for prostate cancer is a combination of surgery, radiation, and chemotherapy. The therapeutic agents used clinically include steroidal antiandrogens, such as cyproterone acetate, and nonsteroidal antiandrogens, such as flutamide and bicalutamide. The steroidal antiandrogens possess partial agonistic activity and overlapping effects with other hormonal systems, leading to many complications including severe cardiovascular problems, gynecomastia, loss of libido, and erectile dysfunction.^{4–6} The nonsteroidal antiandrogens show fewer side effects and have improved oral bioavailability; therefore, they are favored over the steroidal antiandrogens. However, antiandrogen withdrawal syndrome has been discovered in patients receiving nonsteroidal antiandrogens for several months.^{7,8} Long-term drug usage probably leads to mutation of the AR, and the nonsteroidal antiandrogens now exhibit agonistic activity to the mutant AR.⁹ In addition, the clinically available antiandrogens are unable to kill prostate cancer cells, and within one to three years of drug administration, the cancer usually develops into an androgen-refractory stage, which is not curable. Therefore, it is urgent to develop new classes of anti-prostate cancer drugs.

Prostate cancer incidence is much lower in Asian than in Western countries,¹⁰ possibly due to differences in diet. Turmeric is a spice and medicine much more highly consumed in Asian countries such as India, Thailand, China, and Japan. Curcumin (**1**) is the major constituent in the rhizome of *Curcuma longa* (Zingiberaceae), commonly named turmeric. Over the last few

decades, **1** has been studied fairly extensively and has been found to have various biological properties including anti-inflammatory,¹¹ anti-oxidant,¹² anti-HIV,¹³ chemopreventive,¹⁴ and anticancer¹⁵ effects in several cell types. In our laboratory, we used **1** as a lead compound to design and synthesize **1**-analogues as a new class of potential antiandrogenic agents for the treatment of prostate cancer.^{16–18} Certain **1**-analogues showed antiandrogenic activity in human prostate cancer cells, and two potent antiandrogens previously developed in our laboratory, dimethylated curcumin (DMC, **2**) and 4-ethoxycarbonylethyl curcumin (ECECur, **3**), are currently under in vivo investigation (Figure 1). Most recently, we found that the curcumin analogue **4** possesses potent antiandrogenic activity. From our prior studies, a preliminary antiandrogenic structure–activity relationship (SAR) was established. In a continuing study, we have now prepared four series of new **1**-analogues including monophenyl curcumin analogues (series A), heterocycle-containing curcumin analogues (series B), curcumin analogues bearing various substituents on the phenyl rings (series C), and curcumin analogues with various linkers (series D). These new **1**-analogues were evaluated in cytotoxicity and antiandrogenic assays in two human prostate cancer cell lines, LNCaP and PC-3. Based on the structures and anti-prostate cancer activities of the new **1**-analogues, a more detailed SAR has been formulated. This advanced SAR better reveals the structural features of **1**-analogues responsible for the cytotoxic as well as antiandrogenic activities in human prostate cancer cells. This information will guide our optimization of **1**-analogues with better pharmacological profiles as potential drug candidates for the treatment of prostate cancer.

Chemistry

The general synthetic scheme for series A is shown in Scheme 1. To avoid the aldol condensation taking place at both terminals of 2,4-pentanedione, excess pentanedione was used in this reaction.¹⁹ Boric anhydride was first added to form a complex with 2,4-pentanedione. The aim of this complexation is to protect C-3 from Knoevenagel condensation so that the aldol condensation takes place at the terminal carbon.

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[†] Antitumor agents 250. For the prior paper in the series, see ref 1.

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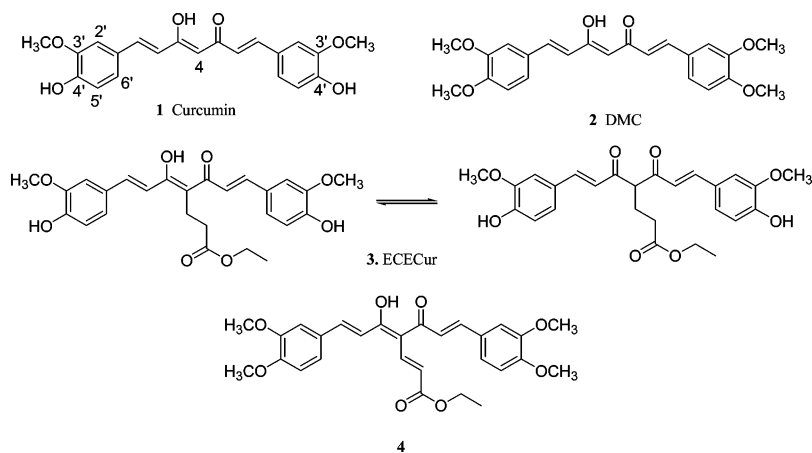
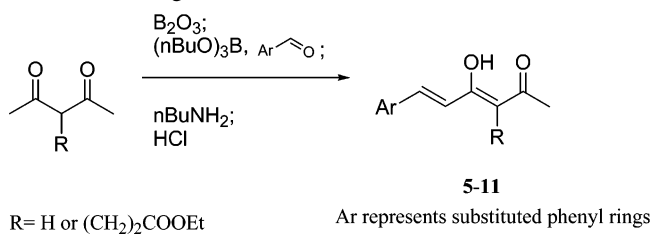
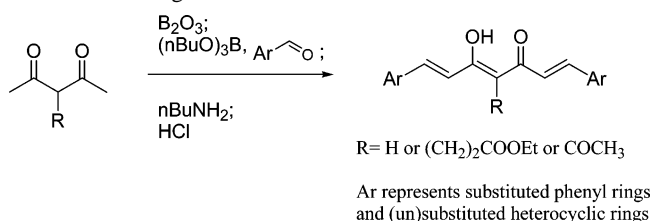


Figure 1. Structures of curcumin (**1**), DMC (**2**), ECECur (**3**), and **4**

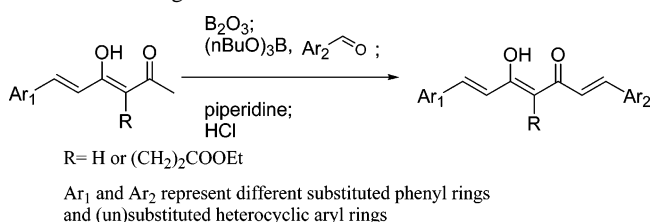
Scheme 1. The General Synthetic Method for Monophenyl Curcumin Analogues



Scheme 2 The General Synthetic Strategies for Symmetric Curcumin Analogues



Scheme 3. The General Synthetic Scheme for Asymmetric Curcumin Analogues



The synthetic strategies for series B (heterocycle-containing curcumin analogues) and series C (symmetric and asymmetric curcumin analogues with various substituents on the phenyl rings) are shown in Scheme 2 and Scheme 3, respectively. The symmetric compounds were synthesized by using Pederson's method.²⁰ At least two equivalents of the aldehyde are needed to ensure aldol condensation at both terminals of the dione. To prepare asymmetric compounds having different aryl rings, a monoaryl intermediate was first prepared by using the method shown in Scheme 1 and subsequently condensed with an appropriate second aldehyde to give the target compounds.

The syntheses of series D with various linkers between the two phenyl rings are shown in Schemes 4–9.

In the general synthesis of **1**, 5-diphenyl-1, 4-pentadien-3-ones, one equivalent of acetone and two equivalents of substituted benzaldehyde were treated with 0.25 M NaOH

solution and 0.25 equivalent of 25% aqueous solution of cetyltrimethylammonium bromide to afford the product (Scheme 4).

Curcumin analogues with an elongated linker were prepared by employing Pederson's synthetic method.²⁰ 2,4-Pentanedione or ethyl 4-acetyl-5-oxohexanoate was reacted with 4-hydroxy-3-methoxy-cinnamaldehyde to give **35** or **36** as the target analogue (Scheme 5).

Hydrogenation of DMC in the presence of 10% Pd/C gave the saturated analogue **37** as the product (Scheme 6). The partially saturated analogue **38** has a hydroxy group in the linker instead of an enol, and was prepared as shown in Scheme 7. 3,4-Dimethoxycinnamone and 3, 4-dimethoxycinnamaldehyde were both prepared from 3,4-dimethoxybenzaldehyde by condensation with acetone and acetaldehyde, respectively. After treating 3,4-dimethoxycinnamone with LDA at –78 °C, 3,4-dimethoxycinnamaldehyde was added to provide **38**.²¹

To synthesize the imide curcumin analogue (**39**), 3,4-dimethoxycinnamic acid was first converted to the acid chloride by treatment with thionyl chloride. Then, two equivalents of the acid chloride were reacted with one equivalent of (Me₃-Si)₂NH in the presence of triethylamine to give the target product **39**.²² (Scheme 8).

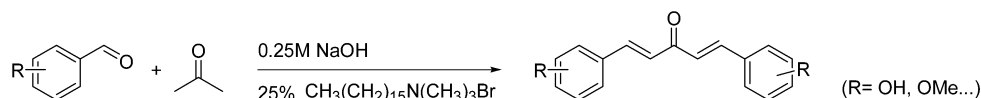
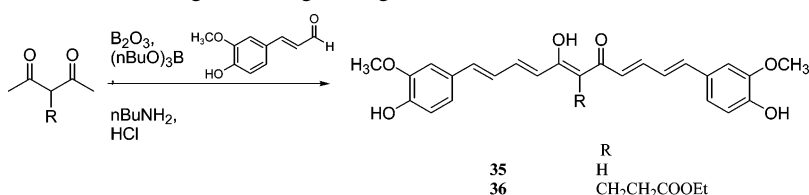
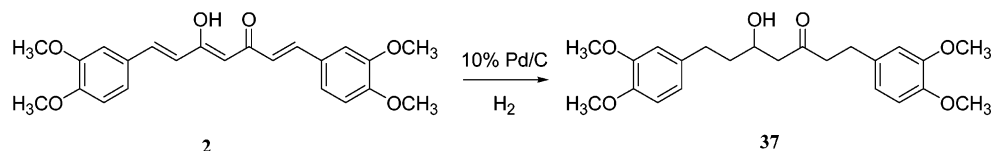
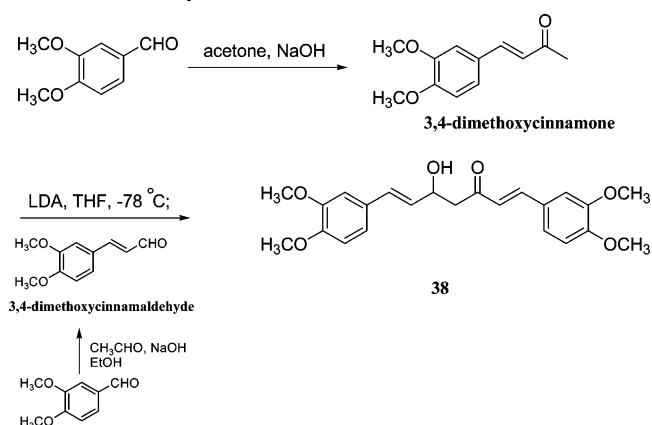
The syntheses of curcumin analogues **4**, **40–45**, **47**, and **48**, which have mono- or disubstitution at C-4, have been described and discussed elsewhere.¹⁸ The preparation of **49** and **50** is shown in Scheme 9. 4-Methyl DMC (**49**) was prepared by using the methodology shown in Scheme 2 and then fluorinated by SelectFluor under basic conditions to give 4-fluoro-4-methyl DMC (**50**).²³

Results and Discussion

The target compounds were tested for cytotoxicity against two human prostate cancer cell lines, LNCaP and PC-3. The LNCaP cell line is an androgen-dependent human prostate cancer cell line that expresses mutant AR, and the PC-3 cell line is an androgen-independent human prostate cancer cell line that does not express functional AR. The antiandrogenic activity of these **1**-analogues was examined in LNCaP cells and PC-3 cells transfected with wild-type AR.

The seven monophenyl analogues (**5–11**) did not exhibit significant cytotoxicity in either LNCaP or PC-3 cells (Table 1A). The absence of one phenyl ring in the curcumin skeleton apparently results in decreased cytotoxicity, indicating that both phenyl rings must be present to retain cytotoxicity against human prostate cancer cells.

In addition, none of the four heterocycle-containing series B analogues (**12–15**) showed significant cytotoxicity in either

Scheme 4. The General Synthetic Method of 1,5-Diphenyl-1,4-pentadien-3-ones**Scheme 5.** The Synthesis of Curcumin Analogues Having a Long Linker**Scheme 6.** The Preparation of **37** from DMC**Scheme 7.** The Synthesis of **38**

LNCaP or PC-3 cells (Table 1.B). These results suggest that replacing the phenyl ring(s) with five-membered-heterocycle(s) will not improve the desired activity. This information will guide us to design new **1**-analogues with the phenyl rings intact.

Series C contains symmetric and asymmetric curcumin analogues with various substituents on the phenyl rings. None of these newly synthesized analogues showed better cytotoxicity than DMC (**2**), which has IC₅₀ values of 1.1 μ M (PC-3) and

1.3 μ M (LNCaP) (Table 1.C). Compound **19**, which has 3',4',6'-dimethoxy substitution on both phenyl rings, was active against both human prostate cancer cell lines. Compounds **24** and **25**, which have 3',5'-dimethoxy-4'-hydroxy and 3'-methyl-4'-hydroxyl substituted phenyl rings, respectively, were active against androgen-dependent LNCaP cells. Among the five asymmetric analogues, **27** and **31** also exhibited activity against LNCaP cells. Both compounds have 3',4'-dimethoxy substitution on one phenyl ring and 3'-methoxy-4'-hydroxy substitution on the other (Table 1.D). In addition, **31** has an ethoxycarbonyl ethyl side chain at position C-4 of the linker. Thus, symmetry is not a vital requirement for selective cytotoxic activity. The common features of the phenyl rings of these active compounds are as follows. (1) The C-2' positions should be unsubstituted. (2) The C-3' positions should be substituted. However, while methoxy or methyl substituents are favorable, hydroxy groups are not favorable at these positions. (3) The C-4' positions should also be substituted. The substituents can either be two methoxy groups, two hydroxy groups, or one methoxy and one hydroxy. Ethoxy and isoprenyl substituents are not favorable at these positions. (4) Except for **24**, most of these synthesized compounds are unsubstituted at the C-5' position. However, **24** has C-5' methoxy substituents, and it was active in LNCaP cells. (5) Most of the new analogues have no substitution on the C-6'

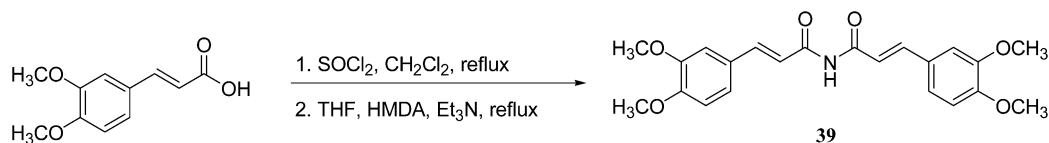
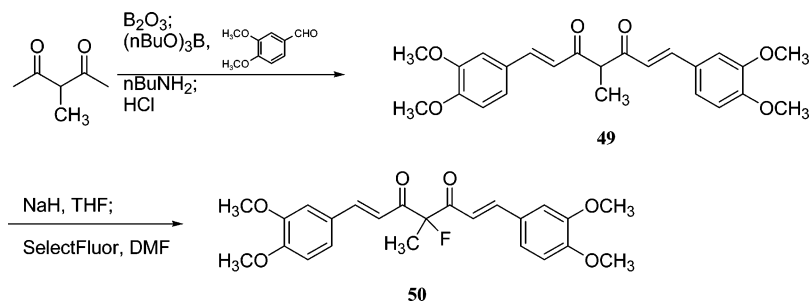
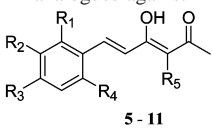
Scheme 8. The Synthesis of Imide Analogue **39****Scheme 9.** The Synthesis of **49** and **50**

Table 1. Cytotoxicity of **1**-analogues against LNCaP and PC-3 Human Prostate Cancer Cells^a

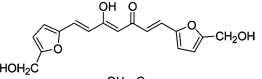
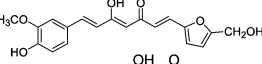
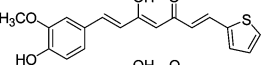
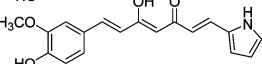
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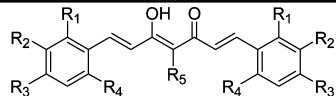
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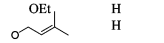
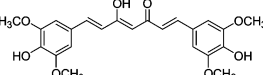
Cmpd	R1	R2	R3	R4	R5	Cytotoxicity IC ₅₀ (μ M) [†]	
						PC-3	LNCaP
5	H	OMe	OH	H	H	8.0	6.2
6	H	OMe	OMe	H	H	7	6.6
7	H	OH	OMe	H	H	6.1	5.3
8	OMe	H	OMe	H	H	12.3	9.9
9	OMe	OMe	OMe	H	H	7.8	5.8
10	H	OMe	OMe	OMe	H	10.5	12.5
11	H	OMe	OH	H	(CH ₂) ₂ COOEt	43.7	51.5

B.

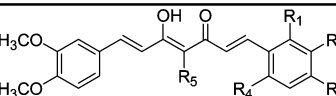
Compound	Chemical Structure	Cytotoxicity IC ₅₀ (μ M) [†]	
		LNCaP	PC3
12		[37]**	[37]
13		7.3	9.0
14		6.3	7.7
15		13.6	15.4

C.



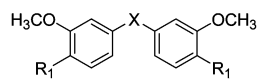
Compound	R1	R2	R3	R4	R5	PC-3	LNCaP
						IC ₅₀ (μ M) [*]	IC ₅₀ (μ M)
1 (Curcumin)	H	OMe	OH	H	H	7.7	3.8
2 (DMC)	H	OMe	OMe	H	H	1.1	1.3
3 (ECECur)	H	OMe	OH	H	CH ₂ CH ₂ COOEt	5.1	1.5
16	H	OH	OMe	H	H	8.1	10.9
17	OMe	H	OMe	H	H	[45]**	11.8
18	OMe	OMe	OMe	H	H	8.1	4.8
19	H	OMe	OMe	OMe	H	2.4	2.9
20	H	OMe	OTHP	H	CH ₂ CH ₂ COOEt	12.3	4.2
21	H	OMe	OTHP	H	H	ND***	ND
22	H	OMe	OEt	H	H	20.3	6.5
23	H	OMe		H	H	>19.8 [41]	6.1
24						14.0	2.6
25	H	Me	OH	H	H	4.8	1.8
26	H	Me	OMe	H	H	>27.4 [44]	7.7

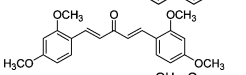
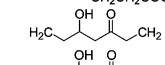
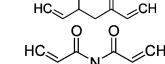
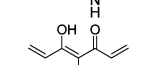
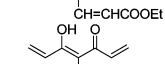
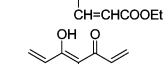
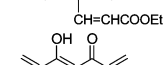
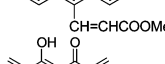
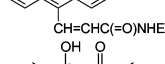
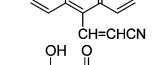
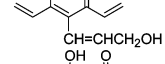
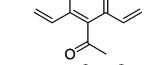
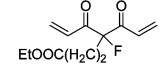
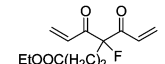
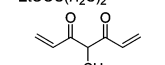
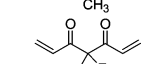
D.



Cmpd	R1	R2	R3	R4	R5	PC-3	LNCaP
						IC ₅₀ (μ M) [*]	IC ₅₀ (μ M)
27	H	OMe	OH	H	H	6	3.3
28	H	OH	OMe	H	H	5.5	4.8
29	OMe	OMe	OMe	H	H	9.1	7.7
30	H	OMe	OMe	OMe	H	8.2	8.6
31	H	OMe	OH	H	CH ₂ CH ₂ COOEt	6.3	2.1

E.



Compound	R1	X	PC-3	LNCaP
			IC ₅₀ (μ M) [*]	IC ₅₀ (μ M)
32	OH	HC=CH=CH=CH	2.4	1.4
33	OMe	HC=CH=CH=CH	3.8	3.4
34		HC=CH=CH=CH	18.7	21.8
35	OH	HC=CH=CH=CH	10.6	4.4
36	OH	HC=CH=CH=CH	6.4	3.1
37	OMe		[33]**	10.8
38	OMe		18.4	17.7
39	OMe		>10 [36]	2.2
4	OMe		1.0	0.2
40	OTHP		3.1	2.6
41	OH		11.7	7.0
42	OMe		0.2	0.4
43	OMe		1.2	0.6
44	OMe		0.7	0.2
45	OMe		1.3	0.2
46	OH		23.4	8.8
47	OTHP		6.3	7.3
48	OH		9.8	6.3
49	OMe		3.5	5.8
50	OMe		4.6	6.4

^a *: IC₅₀ values are mean concentrations that inhibit growth by 50% and variation between replicates was less than 5%. The cutoff line is 4 μ M. Compound with IC₅₀ less than 4 μ M is considered to be active. **: []: % inhibition of cell growth at 20 μ g/mL. ***: ND, not determined.

positions. An exception is **19**, which has methoxy substituents on C-6', and retained activity in both cell lines. Additional investigation will be needed to probe what functional groups can be accommodated at the C-5' and C-6' positions to keep or even enhance the activity of curcumin analogues.

With 3',4'-dimethoxy or 3'-methoxy-4'-hydroxy substituents on the phenyl rings, compounds having a 1,4-pentadien-3-one linker (e.g. **32** and **33**) showed significant cytotoxicity against both human prostate cancer cell lines (Table 1.E). However, **34**, an analogue that also possesses a 1,4-pentadien-3-one linker but has 2,4-dimethoxy substituted phenyl rings, was inactive. These results once again confirm the importance of 3',4'-disubstitution on the phenyl rings. Curcumin analogues **35** and **36** have elongated linkers (11 carbons) connecting the 3'-

methoxy-4'-hydroxyphenyl rings. Compound **35** was not cytotoxic in either prostate cancer cell line, while **36** showed cytotoxicity against LNCaP cells (IC₅₀ 3.1 μ M), but activity against PC-3 cells was not significant. Overall, compared with **1** and **3**, the cytotoxicity of **35** and **36** decreased. Thus, elongation of the linker did not improve the cytotoxic activity of **1**-analogues against prostate cancer cells. Compounds **37** (saturated DMC: 3-hydroxy-heptan-5-one linker) and **38** (partially saturated DMC: 3-hydroxy-1,6-heptadien-5-one linker) were not cytotoxic toward prostate cancer cells, suggesting that an unsaturated and conjugated linker, such as the 3-hydroxy-1,4,6-heptatrien-5-one found in **1** and **2**, is required for the bioactivity of **1**-analogues. When NH replaced the CH₂ between the two carbonyl groups in a similar linking group, the resulting

Table 2. The Antiandrogenic Activity of Compounds **3**, **4**, **40**, and **44**^a

DHT (1 nM) + tested compounds	LNCaP transactivation (%)	PC3+wt. AR transactivation (%)
DHT (control)	100	100
DHT+ HF (5 μ M)	65	31
DHT+ 3 (5 μ M)	4	13
DHT+ 4 (5 μ M)	6	40
DHT+ 40 (3 μ M)	54	46
DHT+ 44 (5 μ M)	42	68

^a LNCaP and PC-3 human prostate cell lines were seeded and cotransfected with reporter MMTV-luciferase (both cell lines), wild-type AR expression plasmid (PC-3) using SuperFect. Subsequently, the transfected cells were harvested and replated in 10% charcoal-stripped fetal bovine serum DMEM medium. The cells were then treated with dehydrotestosterone (DHT, 1 nM) and tested compounds (3 μ M or 5 μ M) and harvested for detection of the luciferase activity. (cf. Experimental Section).

imide analogue (**39**) lost cytotoxicity against PC-3 cells but remained cytotoxic against LNCaP cells with an IC₅₀ of 2.2 μ M. Compounds with both fluorine and either a propionic ethyl ester or a methyl group at the middle (C-4) carbon (e.g. **47**, **48**, **50**) were not cytotoxic against either tested cell line. However, various unsaturated (acrylic ester, acrylamide, acrylonitrile, allyl alcohol) monosubstitution at C-4 of the linker (e.g. **40**, **42–45**) resulted in high cytotoxicity against both human prostate cancer cell lines. These analogues were generally more potent than **1** and as potent as **2–4**. From the bioassay results of Series D, we can see that it is essential to design a proper linker connecting the substituted phenyl rings in order to obtain potent anti-prostate cancer analogues. The best linkers discovered so far are 3-hydroxy-1,4,6-heptatrien-5-one (the original curcumin linker), 1,4-pentadien-3-one (a shorter linker), and several 4-monosubstituted 3-hydroxy-1,4,6-heptatrien-5-ones that are shown in the structures of ECECur (**3**), **4**, **40**, and **42–45**.

New **1**-analogues also were evaluated in an anti-AR assay in LNCaP cells and PC-3 cells transfected with wild-type AR. Table 2 shows the antiandrogenic activity of the most potent compounds (**3**, **4**, **40**, and **44**); the remaining analogues exhibited no or weak activity in the antiandrogenic bioassay. All four potent compounds have similar phenyl substitutions, linking groups, and side chains. Because the most cytotoxic compounds (**42**, **43**, and **45**) were inactive in the anti-AR bioassay, they likely have mechanisms of action other than interrupting the AR signaling pathway in human prostate cancer cells. Either with or without antiandrogenic activity, these cytotoxic **1**-analogues have potential to treat both androgen-dependent prostate cancer and androgen-refractory prostate cancer.

An advanced SAR has been established on the basis of the structure information and bioactivities of the newly synthesized **1**-analogues discussed above. The following SAR conclusions were made. (1) Biphenyl rings are required for the cytotoxic and antiandrogenic activities. (2) The C-2' positions of the phenyl rings should be unsubstituted. (3) The C-3' and C-4' positions should be substituted, with 3',4'-dimethoxy and 3'-methoxy-4'-hydroxy substituents on the phenyl rings found to be most favorable. Substitution at the C-5' and C-6' positions probably will not affect the cytotoxicity. (4) The length of the linker impacts the cytotoxicity as well as antiandrogenic activity in human prostate cancer cells. Elongation of the linker results in the loss of cytotoxicity and antiandrogenic activity. (5) An unsaturated and conjugated linker is required for the cytotoxic and antiandrogenic activities. (6) Substitution at the C-4 position of the linker is very crucial for improved anti-AR activity in this compound class. Monosubstitution with proper side chains improved the cytotoxicity significantly.

Conclusions

In conclusion, we synthesized over 40 new **1**-analogues and first examined their cytotoxicity against androgen-dependent LNCaP and androgen-independent PC-3 human prostate cancer cell lines. Ten compounds (**2**, **4**, **19**, **32**, **33**, **40**, and **42–45**) showed significant cytotoxicity against both androgen-dependent LNCaP cells and androgen-independent PC-3 cells. Seven compounds [**1** (curcumin), **3** (ECECur), **24**, **25**, **27**, **31**, and **39**] were cytotoxic only against androgen-dependent LNCaP cells. Only one compound (**49**) was active against PC-3 cells but inactive against LNCaP cells. The most potent antiandrogenic compounds were **3** (ECECur), **4**, **40**, and **44**. Those analogues that were active in the cytotoxicity assay but inactive in the antiandrogenic assay are presumed to act by a different mechanism other than interrupting the AR signaling pathway. All active compounds are potential drug candidates for the treatment of prostate cancer at the androgen-dependent or androgen-refractory stage. The study of their mechanism(s) of action will be useful in exploring the causes of prostate cancer. In addition, an advanced SAR has been established from this work. This extensive SAR information will guide us to design optimized **1**-analogues having better, selective anti-prostate cancer activity.

Experimental Section

Melting points were determined on a Fisher-Johns melting apparatus and are uncorrected. ¹H 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. MS spectra were recorded on Agilent 1100 series LC/MS trap and API-3000 LC/MS/MS spectrometer. Column chromatography was carried out on CombiFlash Companion (Isco, Inc.), and thin-layer chromatography was performed on precoated silica gel or aluminum plates (Aldrich, Inc.). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and agreed with theoretical values to within $\pm 0.4\%$.

Preparation of Monophenyl Analogues 5–11. To a solution of 2,4-pentanedione or 4-acetyl-5-oxo-hexanoate in EtOAc (3 equiv) was added boric anhydride (0.7 equiv). The solution was stirred at 70 °C for 0.5 h. To the solution were added the aldehyde (1 equiv) and tributyl borate (1 equiv). The mixture was stirred for 30 min. At 85 °C, butylamine (1 equiv) dissolved in EtOAc was added dropwise over 15 min. The stirring continued for 1 h at 100 °C. The mixture was then hydrolyzed by adding 1 N HCl at 50 °C and stirring for 0.5 h at 50 °C. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed until neutral and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the crude products were purified by flash column chromatography eluting with a hexanes–EtOAc gradient.

4-Hydroxy-6-(4-hydroxy-3-methoxyphenyl)-hexa-3,5-dien-2-one (5): (from vanillin and 2,4-pentanedione) Yellow powder, 50% yield. mp 146–147 °C (lit.²⁴ 146–147 °C); ESI MS *m/z* 235.0 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.16 (3H, s), 3.94 (3H, s), 5.63 (H, s), 6.33 (H, d, *J* = 15.9 Hz), 6.92 (H, d, *J* = 8.1 Hz), 7.01 (H, d, *J* = 1.8 Hz), 7.10 (H, dd, *J* = 8.1 Hz, *J* = 1.8 Hz), 7.53 (H, d, *J* = 15.9 Hz).

6-(3,4-Dimethoxyphenyl)-4-hydroxyhexa-3,5-dien-2-one (6): (from 3,4-dimethoxybenzaldehyde and 2,4-pentanedione) 48% yield. mp 78–79 °C; ESI MS *m/z* 249.0 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.10 (3H, s), 3.92 (6H, s), 5.64 (H, s), 6.35 (H, d, *J* = 16.2 Hz), 6.87 (H, d, *J* = 8.4 Hz), 7.04 (H, d, *J* = 1.8 Hz), 7.11 (H, dd, *J* = 8.4 Hz, *J* = 1.8 Hz), 7.55 (H, d, *J* = 16.2 Hz).

4-Hydroxy-6-(3-hydroxy-4-methoxyphenyl)-hexa-3,5-dien-2-one (7): (from 3-hydroxy-4-methoxybenzaldehyde and 2,4-pentanedione) 45% yield. mp 163–164 °C (lit.²⁴ 160–162 °C); ESI MS *m/z* 233.2 (M – 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.16 (3H, s), 3.93 (3H, s), 5.63 (H, s), 6.32 (H, d, *J* = 15.6 Hz), 6.85

(H, d, $J = 8.7$ Hz), 7.02 (H, dd, $J = 8.7$ Hz, $J = 2.1$ Hz), 7.14 (H, d, $J = 2.1$ Hz), 7.51 (H, d, $J = 15.6$ Hz).

6-(2,4-Dimethoxyphenyl)-4-hydroxyhexa-3,5-dien-2-one (8): (from 2,4-dimethoxybenzaldehyde and 2,4-pentanedione) 44% yield. mp 92–93 °C; ESI MS m/z 249.0 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.15 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 5.63 (H, s), 6.48 (H, d, $J = 15.9$ Hz), 6.50 (H, d, $J = 2.1$ Hz), 6.51 (H, dd, $J = 8.1$ Hz, $J = 2.1$ Hz), 7.46 (H, d, $J = 8.4$ Hz), 7.84 (H, d, $J = 15.9$ Hz). Anal. (C₁₄H₁₆O₄·1/8 H₂O) C, H.

4-Hydroxy-6-(2,3,4-trimethoxyphenyl)-hexa-3,5-dien-2-one (9): (from 2,3,4-trimethoxybenzaldehyde and 2,4-pentanedione) 45% yield. mp 66–67 °C; ESI MS m/z 279.2 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.16 (3H, s), 3.88 (3H, s), 3.90 (3H, s), 3.92 (3H, s), 5.64 (H, s), 6.48 (H, d, $J = 15.9$ Hz), 6.70 (H, d, $J = 8.7$ Hz), 7.27 (H, d, $J = 8.7$ Hz), 7.79 (H, d, $J = 15.9$ Hz). Anal. (C₁₅H₁₈O₅) C, H.

4-Hydroxy-6-(2,4,5-trimethoxyphenyl)-hexa-3,5-dien-2-one (10): (from 2,4,5-trimethoxybenzaldehyde and 2,4-pentanedione) 48% yield. mp 107–108 °C; ESI MS m/z 279.2 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.15 (3H, s), 3.88 (3H, s), 3.89 (3H, s), 3.94 (3H, s), 5.66 (H, s), 6.42 (H, d, $J = 15.9$ Hz), 6.50 (H, s), 7.02 (H, s), 7.89 (H, d, $J = 15.9$ Hz). Anal. (C₁₅H₁₈O₅) C, H.

4-Acetyl-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-hepta-4,6-dienoic acid ethyl ester (11): (from vanillin and ethyl 4-acetyl-5-oxohexanoate) 40% yield. Yellow oil; ESI MS m/z 335.0 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (3H, t, $J = 7.2$ Hz), 2.18 (3H, s), 3.96 (3H, s), 4.13 (2H, quart, $J = 7.2$ Hz), 5.97 (0.5H, s), 6.67 (H, d, $J = 15.9$ Hz), 6.94 (H, d, $J = 8.1$ Hz), 7.06 (H, d, $J = 1.8$ Hz), 7.14 (H, dd, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.63 (H, d, $J = 15.9$ Hz). Anal. (C₁₈H₂₂O₆·7/4 H₂O) C, H.

5-Hydroxy-1,7-bis-(5-hydroxymethyl-furan-2-yl)-hepta-1,4,6-trien-3-one (12). 2,4-Pentanedione (0.2 mL, 2 mmol) and boric anhydride (100 mg, 1.4 mmol) were dissolved in 15 mL of EtOAc. The solution was stirred at 70 °C for 0.5 h. 5-Hydroxymethyl-2-furaldehyde (506 mg, 4 mmol) and tributyl borate (1.08 mL, 4 mmol) were added. After stirring for 30 min, butylamine (0.3 mL, 3 mmol) dissolved in 4 mL of EtOAc was added dropwise over 15 min. The stirring continued for 5 h at 85 °C. The mixture was then hydrolyzed by adding 8 mL of 1 N HCl and stirring for 0.5 h at 60 °C. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed until neutral and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the crude product was purified by CombiFlash column chromatography eluting with hexanes–EtOAc to give 68 mg of red powder, obtained in 12% yield. mp 129–130 °C; ESI MS m/z 339.2 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 4.67 (4H, s), 5.74 (H, s), 6.40 (2H, d, $J = 3.3$ Hz), 6.53 (2H, d, $J = 15.3$ Hz), 6.58 (2H, d, $J = 3.3$ Hz), 7.43 (2H, d, $J = 15.6$ Hz). Anal. (C₁₇H₁₆O₆) C, H.

Preparation of Heterocycle-Containing Curcumin Analogues 13–15. Compound **5** and boric anhydride (0.7 equiv.) dissolved in EtOAc were stirred at 70 °C for 0.5 h. The appropriate benzaldehyde (1 equiv) and tributyl borate (2 equiv) were added, and the mixture was stirred for 0.5 h. Piperidine dissolved in EtOAc was added dropwise. After increasing the temperature to 100 °C, stirring was continued for 1 h. The mixture was then hydrolyzed by adding 1 N HCl and stirring at 60 °C for 0.5 h. The organic layer was separated, and the aqueous layer was extracted with EtOAc three times. The combined organic layers were washed with water until neutral. The solvent was removed in vacuo. The crude products were purified by flash column chromatography eluting with hexanes–EtOAc.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(5-hydroxymethyl-furan-2-yl)-hepta-1,4,6-trien-3-one (13): (from 5-hydroxymethyl-2-furaldehyde) 32% yield. mp 140–142 °C; ESI MS m/z 343.3 (M + H)⁺, 366.2 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.90 (3H, s), 4.64 (2H, s), 5.71 (H, s), 6.36 (H, d, $J = 2.7$ Hz), 6.43 (H, d, $J = 7.5$ Hz), 6.47 (H, d, $J = 7.2$ Hz), 6.53 (2H, d, $J = 3.0$ Hz), 6.90 (H, d, $J = 8.1$ Hz), 7.01 (H, s), 7.08 (H, d, $J = 7.5$ Hz), 7.33 (H, d, $J = 15.3$ Hz), 7.55 (H, d, $J = 15.6$ Hz). Anal. (C₁₉H₁₈O₆) C, H.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-thiophen-2-yl-hepta-1,4,6-trien-3-one (14): (from thiophene-2-carbaldehyde) 38% yield. mp 130–132 °C; ESI MS m/z 327.1 (M – 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.89 (3H, s), 5.28 (H, s), 5.74 (H, s), 6.37 (H, d, $J = 15.6$ Hz), 6.45 (H, d, $J = 15.9$ Hz), 6.91 (H, d, $J = 7.8$ Hz), 7.01 (H, d, $J = 2.4$ Hz), 7.02 (H, d, $J = 1.2$ Hz), 7.04 (H, s), 7.08 (H, dd, $J = 1.2$ Hz, $J = 7.8$ Hz), 7.21 (H, d, $J = 3.6$ Hz), 7.34 (H, d, $J = 4.8$ Hz), 7.57 (H, d, $J = 15.6$ Hz), 7.73 (H, d, $J = 15.3$ Hz). Anal. (C₁₈H₁₆O₄S) C, H.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(1H-pyrrol-2-yl)-hepta-1,4,6-trien-3-one (15): (from 1H-pyrrole-2-carbaldehyde) 24% yield. mp 138–139 °C; ESI MS m/z 334.2 (M + Na)⁺; ¹H NMR (300 MHz, CD₃COCD₃): δ 3.92 (3H, s), 5.86 (H, s), 6.25 (H, s), 6.47 (H, d, $J = 15.9$ Hz), 6.64 (H, s), 6.68 (H, d, $J = 15.9$ Hz), 6.88 (H, d, $J = 7.8$ Hz), 7.06 (H, s), 7.16 (H, d, $J = 7.8$ Hz), 7.32 (H, s), 7.57 (H, d, $J = 15.9$ Hz), 7.59 (H, d, $J = 15.9$ Hz). Anal. (C₁₈H₁₇NO₄) C, H, N.

Preparation of Symmetric Curcumin Analogues 16–26. Compounds **16–19** and **22–26** were prepared from corresponding benzaldehydes by using the same procedure described above for **12** from 5-hydroxymethyl-2-furaldehyde. The preparation of **20** and **21** was reported elsewhere by us.

5-Hydroxy-1,7-bis-(3-hydroxy-4-methoxyphenyl)-hepta-1,4,6-trien-3-one (16): (from 3-hydroxy-4-methoxybenzaldehyde and 2,4-pentanedione) 45% yield. mp 181–183 °C (lit.²⁵ 190–192 °C); ESI-MS m/z 367.1 (M – 1)⁺; ¹H NMR (300 MHz, CD₃COCD₃): δ 4.01 (6H, s), 6.01 (H, s), 6.66 (2H, d, $J = 15.6$ Hz), 7.03 (2H, d, $J = 8.1$ Hz), 7.20 (2H, dd, $J = 2.1$ Hz, $J = 8.1$ Hz), 7.26 (2H, d, $J = 2.1$ Hz), 7.65 (2H, d, $J = 15.3$ Hz).

1,7-Bis-(2,4-dimethoxyphenyl)-5-hydroxy-hepta-1,4,6-trien-3-one (17): (from 2,4-dimethoxybenzaldehyde and 2,4-pentanedione) 48% yield. mp 135–137 °C; ESI-MS m/z 367.1 (M + 1)⁺; ¹H NMR (300 MHz, CD₃COCD₃): δ 3.87 (12H, s), 5.81 (H, s), 6.46 (2H, d, $J = 2.4$ Hz), 6.52 (2H, dd, $J = 2.4$ Hz, $J = 8.7$ Hz), 6.63 (2H, d, $J = 15.6$ Hz), 7.49 (2H, d, $J = 8.7$ Hz), 7.90 (2H, d, $J = 15.9$ Hz). Anal. (C₂₃H₂₄O₆·1/4H₂O) C, H.

5-Hydroxy-1,7-bis-(2,3,4-trimethoxyphenyl)-hepta-1,4,6-trien-3-one (18): (from 2,3,4-trimethoxybenzaldehyde and 2,4-pentanedione) 30% yield. mp 108–109 °C; ESI MS m/z 457.2 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.89 (6H, s), 3.90 (6H, s), 3.94 (6H, s), 5.83 (H, s), 6.64 (2H, d, $J = 15.9$ Hz), 6.71 (2H, d, $J = 9$ Hz), 7.31 (2H, d, $J = 8.7$ Hz), 7.85 (2H, d, $J = 16.2$ Hz). Anal. (C₂₅H₂₈O₈) C, H.

5-Hydroxy-1,7-bis-(2,4,5-trimethoxyphenyl)-hepta-1,4,6-trien-3-one (19): (from 2,4,5-trimethoxybenzaldehyde and 2,4-pentanedione) 28% yield, mp 140–142 °C; ESI MS m/z 457.2 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.88 (6H, s), 3.89 (6H, s), 3.94 (6H, s), 5.86 (H, s), 5.57 (2H, d, $J = 15.9$ Hz), 6.51 (2H, s), 7.06 (2H, s), 7.95 (2H, d, $J = 15.9$ Hz). Anal. (C₂₅H₂₈O₈) C, H.

5-Hydroxy-7-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-4-[3-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-acryloyl]-hepta-4,6-dienoic acid ethyl ester (20): Yellow powder, 59% yield, mp 60–61 °C; ESI MS m/z 635.2 (M – 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (3H, t), 1.57–2.17 (12H, m), 2.96 (0.57H, t), 3.62 (4H, t), 3.91 (6H, s), 4.13 (2H, q), 5.47 (2H, t), 6.72 (2H, d, $J = 15.6$ Hz), 6.90–7.18 (6H, m), 7.44 (2H, d, $J = 15.6$ Hz); Anal. (C₃₀H₃₂O₈) C, H.

5-Hydroxy-1,7-bis-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-hepta-1,4,6-trien-3-one (21): Yellow powder; 67% yield, mp 67–69 °C; ESI MS m/z 535.0 (M – 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.57–2.17 (12H, m), 3.62 (4H, t), 3.91 (6H, s), 5.47 (2H, t), 5.83 (1H, s), 6.50 (2H, d, $J = 15.9$ Hz), 7.09–7.16 (6H, m), 7.60 (2H, d, $J = 15.9$ Hz); Anal. (C₃₁H₃₆O₈·H₂O) C, H.

1,7-Bis-(4-ethoxy-3-methoxyphenyl)-5-hydroxy-hepta-1,4,6-trien-3-one (22): (from 4-ethoxy-3-methoxybenzaldehyde and 2,4-pentanedione) 31% yield, mp 139–140 °C (lit.²⁶ 102 °C); ESI-MS m/z 425.1 (M + 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.49 (6H, t, $J = 7.2$ Hz), 3.93 (6H, s), 4.15 (4H, quart, $J = 8.4$ Hz), 5.82 (H, s), 6.49 (2H, d, $J = 15.6$ Hz), 6.88 (2H, d, $J = 8.1$ Hz), 7.08 (2H, d, $J = 2.1$ Hz), 7.14 (2H, dd, $J = 2.1$ Hz, $J = 8.1$ Hz), 7.61 (2H, d, $J = 15.6$ Hz).

3-Methoxy-4-(3-methyl-but-2-enyloxy)-benzaldehyde. Vanillin (1.52 g, 10 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) were dissolved in dry acetone (20 mL). 1-Bromo-3-methyl-but-2-ene (3.0 mL) was then added to the solution, and stirring continued for 12 h. The solvent was evaporated in a vacuum, and the resulting solid was extracted with CH₂Cl₂ three times. The CH₂Cl₂ solution was washed with water and dried over anhydrous sodium sulfate. The organic solvent was removed in a vacuum. A colorless liquid (418 mg) was obtained by flash column chromatography eluting with a gradient hexanes–EtOAc solvent. ¹H NMR (300 MHz, CDCl₃): δ 1.76 (3H, s), 1.80 (3H, s), 3.94 (3H, s), 4.68 (2H, d, *J* = 6.6 Hz), 5.52 (H, t, *J* = 6.6 Hz), 6.98 (H, d, *J* = 8.4 Hz), 7.41 (H, d, *J* = 2.1 Hz), 7.44 (H, dd, *J* = 2.1 Hz, *J* = 8.4 Hz), 9.85 (H, s).

5-Hydroxy-1,7-bis-[3-methoxy-4-(3-methyl-but-2-enyloxy)-phenyl]-hepta-1,4,6-trien-3-one (23): (from 3-methoxy-4-(3-methyl-but-2-enyloxy)-benzaldehyde and 2,4-pentanedione) 25% yield, mp 124–125 °C; ESI MS *m/z* 527.2 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.75 (6H, s), 1.79 (6H, s), 3.92 (6H, s), 4.63 (4H, d, *J* = 6.6 Hz), 5.52 (2H, t, *J* = 6.6 Hz), 5.82 (H, s), 6.50 (2H, d, *J* = 15.6 Hz), 6.88 (2H, d, *J* = 8.4 Hz), 7.08 (2H, d, *J* = 1.8 Hz), 7.12 (2H, dd, *J* = 1.8 Hz, *J* = 8.4 Hz), 7.61 (2H, d, *J* = 15.9 Hz). Anal. (C₃₁H₃₆O₆) C, H.

5-Hydroxy-1,7-bis-(4-hydroxy-3,5-dimethoxyphenyl)-hepta-1,4,6-trien-3-one (24): (from 4-hydroxy-3,5-dimethoxybenzaldehyde and 2,4-pentanedione) 17% yield, mp 198–199 °C (lit.²⁷ 188–190 °C); ESI MS *m/z* 451.2 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.94 (6H, s), 5.80 (H, s), 6.49 (2H, d, *J* = 15.3 Hz), 6.80 (4H, s), 7.58 (2H, d, *J* = 15.3 Hz).

5-Hydroxy-1,7-bis-(4-hydroxy-3-methylphenyl)-hepta-1,4,6-trien-3-one (25): (from 4-hydroxy-3-methylbenzaldehyde and 2,4-pentanedione) 52% yield, mp 217–218 °C; ESI MS *m/z* 337.2 (M + 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.26 (6H, s), 5.81 (H, s), 6.50 (2H, d, *J* = 15.9 Hz), 6.86 (2H, d, *J* = 8.4 Hz), 7.27 (2H, d, *J* = 8.4 Hz), 7.36 (2H, s), 7.58 (2H, d, *J* = 15.9 Hz). Anal. (C₂₁H₂₀O₄·1/4H₂O) C, H.

5-Hydroxy-1,7-bis-(4-methoxy-3-methylphenyl)-hepta-1,4,6-trien-3-one (26): (from 4-methoxy-3-methylbenzaldehyde and 2,4-pentanedione) 55% yield, mp 131–132 °C; ESI MS *m/z* 365.1 (M + 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.24 (6H, s), 3.87 (6H, s), 5.78 (H, s), 6.49 (2H, d, *J* = 15.6 Hz), 6.83 (2H, d, *J* = 8.7 Hz), 7.37 (2H, d, *J* = 8.7 Hz), 7.38 (2H, s), 7.60 (2H, d, *J* = 15.6 Hz). Anal. (C₂₃H₂₄O₄·1/8H₂O) C, H.

Preparation of Asymmetric Curcumin Analogues 27–31. By using the same procedure described above for **13–15** from **5**, compounds **27–30** were prepared from **6**, and **31** from **11**.

1-(3,4-Dimethoxyphenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,4,6-trien-3-one (27): (from **6** and vanillin) 55% yield, mp 83–84 °C (lit.¹⁷ 89–91 °C); ESI-MS *m/z* 381.1 (M – 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.94 (9H, s), 5.81 (H, s), 6.48 (2H, d, *J* = 15.6 Hz), 6.87–7.14 (6H, m), 7.60 (2H, d, *J* = 15.6 Hz).

7-(3,4-Dimethoxyphenyl)-5-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-hepta-1,4,6-trien-3-one (28): (from **6** and 3-hydroxy-4-methoxybenzaldehyde) 45% yield, mp 157–158 °C; ESI-MS *m/z* 405.3 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.93 (3H, s), 3.94 (6H, s), 5.80 (H, s), 6.48 (H, d, *J* = 16.2 Hz), 6.51 (H, d, *J* = 15.9 Hz), 6.86 (H, d, *J* = 8.4 Hz), 6.89 (H, d, *J* = 8.4 Hz), 7.06 (H, dd, *J* = 8.4 Hz, *J* = 1.8 Hz), 7.09 (H, d, *J* = 1.8 Hz), 7.15 (H, dd, *J* = 8.4 Hz, *J* = 1.8 Hz), 7.18 (H, d, *J* = 1.8 Hz), 7.58 (H, d, *J* = 15.9 Hz), 7.61 (2H, d, *J* = 15.6 Hz).

1-(3,4-Dimethoxyphenyl)-5-hydroxy-7-(2,3,4-trimethoxyphenyl)-hepta-1,4,6-trien-3-one (29): (from **6** and 2,3,4-trimethoxybenzaldehyde) 55% yield, mp 138–139 °C; ESI-MS *m/z* 427.2 (M + H)⁺, 449.3 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.89 (3H, s), 3.91 (3H, s), 3.93 (3H, s), 3.94 (6H, s), 5.83 (H, s), 6.51 (H, d, *J* = 15.9 Hz), 6.63 (H, d, *J* = 15.9 Hz), 6.71 (H, d, *J* = 8.7 Hz), 6.89 (H, d, *J* = 8.4 Hz), 7.09 (H, d, *J* = 2.1 Hz), 7.15 (H, dd, *J* = 1.8 Hz, *J* = 8.7 Hz), 7.31 (H, d, *J* = 8.7 Hz), 7.61 (H, d, *J* = 15.9 Hz), 7.85 (H, d, *J* = 16.2 Hz). Anal. (C₂₄H₂₆O₇·1/2H₂O) C, H.

1-(3,4-Dimethoxyphenyl)-5-hydroxy-7-(2,4,5-trimethoxyphenyl)-hepta-1,4,6-trien-3-one (30): (from **6** and 2,4,5-trimethoxybenzaldehyde) 48% yield, mp 128–129 °C; ESI-MS *m/z* 427.2 (M + H)⁺, 449.3 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.89 (3H, s), 3.90 (3H, s), 3.93 (3H, s), 3.94 (3H, s), 3.95 (3H, s), 5.84 (H, s), 6.50 (H, d, *J* = 15.6 Hz), 6.51 (H, s), 6.57 (H, d, *J* = 16.2 Hz), 6.88 (H, d, *J* = 8.1 Hz), 7.06 (H, s), 7.08 (H, d, *J* = 2.1 Hz), 7.14 (H, dd, *J* = 2.1 Hz, *J* = 8.1 Hz), 7.60 (H, d, *J* = 15.9 Hz), 7.96 (H, d, *J* = 15.9 Hz). Anal. (C₂₄H₂₆O₇·1/4H₂O) C, H.

4-[3-(3,4-Dimethoxyphenyl)acryloyl]-7-(4-hydroxy-3-methoxyphenyl)-5-oxo-hept-6-enoic acid ethyl ester (31): (from **11** and 3,4-dimethoxybenzaldehyde) 38% yield, mp 67–68 °C; ESI-MS *m/z* 483.4 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (3H, t, *J* = 7.2 Hz), 2.32–2.37 (2H, m), 2.55 (2H, t, *J* = 7.8 Hz), 2.95 (0.5H, t, *J* = 7.8 Hz), 3.91–3.97 (9H, m), 4.14 (2H, q, *J* = 7.2 Hz), 6.70 (H, dd, *J* = 4.2 Hz, *J* = 15.6 Hz), 6.84–7.19 (7H, m), 7.62–7.76 (2H, m); Anal. (C₂₇H₃₀O₈·1/4H₂O) C, H.

Preparation of 32, 33, and 34. To a solution of acetone (0.36 mL, 5 mmol) and the appropriate benzaldehyde (10 mmol) in 50 mL of a 0.25 M solution of aqueous NaOH was added 1.5 mL of a 25% w/w aqueous solution of cetyltrimethylammonium bromide. The mixture was allowed to stir vigorously at room temperature for 20 h, diluted with brine, and extracted with EtOAc. The EtOAc solution was concentrated and then subjected to column chromatography to obtain the target product.

1,5-Bis-(4-hydroxy-3-methoxyphenyl)-penta-1,4-dien-3-one (32): 50% yield, mp 83–84 °C (lit.²⁵ 84–86 °C); ESI MS *m/z* 327.3 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.94 (6H, s), 6.90 (2H, d, *J* = 8.4 Hz), 6.95 (2H, d, *J* = 15.6 Hz), 7.11 (2H, d, *J* = 1.8 Hz), 7.20 (2H, dd, *J* = 1.8 Hz, *J* = 8.4 Hz), 7.68 (2H, d, *J* = 15.6 Hz).

1,5-Bis-(3,4-dimethoxyphenyl)-penta-1,4-dien-3-one (33): Bright yellow powder. 76% yield; mp 74–75 °C (lit.²⁵ 72–75 °C); ESI-MS *m/z* 355.2 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.94 (6H, s), 3.96 (6H, s), 6.90 (2H, d, *J* = 8.4 Hz), 6.95 (2H, d, *J* = 15.9 Hz), 7.15 (2H, d, *J* = 1.8 Hz), 7.20 (2H, dd, *J* = 1.8 Hz, *J* = 8.4 Hz), 7.69 (2H, d, *J* = 15.9 Hz).

1,5-Bis-(2,4-dimethoxyphenyl)-penta-1,4-dien-3-one (34): Yellow powder. 85% yield; mp 130–132 °C (lit.²⁵ 138–140 °C); ESI-MS *m/z* 355.2 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.86 (3H, s), 3.90 (3H, s), 6.47 (2H, d, *J* = 2.4 Hz), 6.53 (2H, dd, *J* = 2.4 Hz, *J* = 8.4 Hz), 7.09 (2H, d, *J* = 15.9 Hz), 7.57 (2H, d, *J* = 8.4 Hz), 7.69 (2H, d, *J* = 15.9 Hz).

Preparation of 35 and 36. Compounds **35** and **36** were prepared from 4-hydroxy-3-methoxy-cinnamaldehyde by using the same procedure described above for **12** from 2,4-pentanedione and 5-hydroxymethyl-2-furaldehyde.

7-Hydroxy-1,11-bis-(4-hydroxy-3-methoxyphenyl)-undeca-1,3,6,8,10-pentaen-5-one (35): (from 4-hydroxy-3-methoxy-cinnamaldehyde and 2,4-pentanedione) 15% yield; mp 162–164 °C; ESI-MS *m/z* 421.4 (M + 1)⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.95 (6H, s), 5.78 (H, s), 6.13 (2H, d, *J* = 15.6 Hz), 6.78–7.04 (10H, m), 7.41 (H, d, *J* = 15.0 Hz), 7.44 (H, d, *J* = 15.3 Hz). Anal. (C₂₅H₂₄O₆) C, H.

5-Hydroxy-9-(4-hydroxy-3-methoxyphenyl)-4-[5-(4-hydroxy-3-methoxyphenyl)-penta-2,4-dienoyl]-nona-4,6,8-trienoic acid ethyl ester (36): (from 4-hydroxy-3-methoxy-cinnamaldehyde and ethyl 4-acetyl-5-oxohexanoate) 22% yield; mp 143–144 °C; ESI-MS 519.3 (M – 1)[–]; ¹H NMR (300 MHz, CD₃COCD₃): δ 1.24 (3H, t, *J* = 7.2 Hz), 2.80–2.86 (4H, m), 3.89 (6H, s), 4.11 (2H, quart, *J* = 7.2 Hz), 6.78 (2H, d, *J* = 14.4 Hz), 6.85 (2H, d, *J* = 8.1 Hz), 7.02 (2H, d, *J* = 15.0 Hz), 7.07 (2H, dd, *J* = 1.8 Hz, *J* = 8.1 Hz), 7.26 (2H, d, *J* = 1.8 Hz), 7.52 (H, d, *J* = 15.0 Hz), 7.55 (H, d, *J* = 14.4 Hz). Anal. (C₃₀H₁₆O₄·3/8H₂O) C, H.

1,7-Bis-(3,4-dimethoxyphenyl)-5-hydroxy-heptan-3-one (37). Dimethylcurcumin (**2**) (80 mg, 0.2 mmol) was dissolved in 3 mL of EtOAc. 10% Pd/C (80 mg) was added to the solution. After hydrogenation for 24 h at 45 psi, the solution was filtered, and the filtrate was concentrated. Flash column chromatography (hexane/EtOAc = 2:1) afforded white product in 25% yield, mp 93–94 °C (lit.¹⁷ 94–95 °C). ESI-MS *m/z* 425.1 (M + Na)⁺; ¹H NMR (300 MHz,

CDCl_3): δ 1.61–1.85 (2H, m), 2.56–2.87 (8H, m), 3.85 (6H, s), 3.86 (6H, s), 4.07 (H, m), 6.69–6.90 (6H, m).

3,4-Dimethoxycinnamone. 3,4-Dimethoxybenzaldehyde (1.5 g, 9 mmol) was dissolved in 10 mL of acetone. After 10 mL of sodium hydroxide solution (0.5 g NaOH in 10 mL of H_2O) was added, the mixture was stirred for 24 h. Then the excess acetone was removed in vacuo. Upon acidification with 1 N HCl, a green precipitate was obtained. The precipitate was extracted with EtOAc. The organic layer was dried over sodium sulfate and solvent removed in vacuo. Flash column chromatography gave the desired product (1.5 g in 81% yield). $^1\text{H NMR}$ (300 MHz, CD_3COCD_3): δ 2.27 (3H, s), 3.87 (6H, s), 6.67 (H, d, $J = 16.2$ Hz), 7.00 (H, d, $J = 8.1$ Hz), 7.04 (H, d, $J = 0.9$ Hz), 7.22 (H, dd, $J = 2.1$ Hz, $J = 8.1$ Hz), 7.32 (H, d, $J = 2.1$ Hz), 7.55 (H, d, $J = 16.2$ Hz).

3, 4-Dimethoxycinnamaldehyde. To a solution of acetaldehyde (0.84 mL, 15 mmol) in EtOH (10 mL) was added 3 M NaOH (5 mL, 15 mmol) at 0 °C. The solution was stirred for an additional 20 min. After 3, 4-dimethoxybenzaldehyde (2.5 g, 15 mmol) in EtOH (5 mL) was added to the stirring solution dropwise, the reaction was brought to room temperature and stirred for 2 h. Then the mixture was poured into water and adjusted to pH 7 by adding 1 N HCl. After extraction with EtOAc, the organic layer was washed with water three times and dried over anhydrous sodium sulfate. After removal of the solvent under vacuum, the crude product was purified with flash column chromatography. Pale yellow product (1.1 g) was obtained in 38% yield. $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 3.93 (3H, s), 3.94 (3H, s), 6.62 (H, dd, $J = 7.8$ Hz, $J = 16.2$ Hz), 6.91 (H, d, $J = 8.1$ Hz), 7.08 (H, d, $J = 2.1$ Hz), 7.17 (H, dd, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.42 (H, d, $J = 15.9$ Hz), 9.67 (H, d, $J = 7.8$ Hz).

1,7-Bis-(3,4-dimethoxyphenyl)-5-hydroxy-hepta-1,6-dien-3-one (38). To a stirring solution of lithium diisopropylamine (0.29 mL, 0.58 mmol) in THF (3 mL) was added a THF (3 mL) solution of 3, 4-dimethoxycinnamone (100 mg, 0.48 mmol) at -78 °C. After 15 min, 3,4-dimethoxycinnamaldehyde (85 mg, 0.44 mmol) in THF (3 mL) was added. After stirring for an additional 20 min at -78 °C, the mixture was quenched with saturated NH_4Cl solution. The solution was allowed to warm to ambient temperature and extracted with EtOAc. The organic layer was washed with water and saturated NaCl solution and dried over anhydrous sodium sulfate. The crude product was purified by flash column chromatography to give 22 mg of pure product in 13% yield. mp 88–89 °C; ESI MS m/z 399.3 ($\text{M} + \text{H}$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 2.93 (2H, d), 3.80 (6H, s), 3.85 (6H, s), 4.13 (H, d), 6.25 (H, dd, $J = 6$ Hz, $J = 15.9$ Hz), 6.58 (H, d, $J = 15.9$ Hz), 6.80 (H, d, $J = 16.2$ Hz), 6.88 (H, d, $J = 8.7$ Hz), 6.90 (H, dd, $J = 0.9$ Hz, $J = 8.7$ Hz), 7.00 (H, d, $J = 8.4$ Hz), 7.04 (H, d, $J = 0.9$ Hz), 7.25 (H, dd, $J = 0.9$ Hz, $J = 8.7$ Hz), 7.34 (H, d, $J = 0.9$ Hz), 7.61 (1H, d, $J = 16.2$ Hz). Anal. ($\text{C}_{23}\text{H}_{26}\text{O}_6$) C, H.

3-(3,4-Dimethoxyphenyl)-N-[3-(3,4-dimethoxyphenyl)-acryloyl]-acrylamide (39). 3,4-Dimethoxycinnamic acid (624 mg, 3 mmol) was dissolved in 15 mL of dry methylene chloride. Thionyl chloride (0.3 mL, 3.6 mmol) was added at 0 °C. The solution was stirred under reflux for 5 h. The solvent was removed under vacuum to give a yellow solid. In the same flask, 10 mL of anhydrous THF was added, and the mixture was heated to reflux. HMDA (0.3 mL) was added very slowly to the refluxing solution, followed by the addition of triethylamine (0.4 mL). The solution was stirred under reflux overnight. The solvent was then removed in vacuo. The solid was extracted with CH_2Cl_2 three times. The combined CH_2Cl_2 solution was washed with water three times and brine once and then dried over anhydrous sodium sulfate. The crude product was obtained after flash column chromatography. 78 mg (13% yield), pale yellow powder. mp 220–221 °C; ESI MS m/z 420.2 ($\text{M} + \text{Na}$) $^+$; $^1\text{H NMR}$ (300 MHz, DMSO): δ 3.82 (12H, s), 7.04 (2H, d, $J = 8.7$ Hz), 7.10 (2H, d, $J = 15.9$ Hz), 7.23 (2H, s), 7.24 (2H, d, $J = 8.7$ Hz), 7.66 (2H, d, $J = 15.9$ Hz), 10.51 (0.5 H, s). Anal. ($\text{C}_{22}\text{H}_{23}\text{NO}_6 \cdot 3/2\text{H}_2\text{O}$) C, H; N: 2.61.

The preparation of compounds **4**, **40–45**, **47**, and **48** was reported by us in a recent publication.¹⁸

7-(3,4-Dimethoxyphenyl)-4-[3-(3,4-dimethoxyphenyl)-acryloyl]-5-hydroxy-hepta-2,4,6-trienoic acid ethyl ester (4): Red powder, 54% yield; mp 170–171 °C; ESI MS m/z 494.6 ($\text{M} + \text{H}$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 1.34 (3H, t), 3.95 (12H, s), 4.29 (2H, quart), 5.98 (2H, d, $J = 15.6$ Hz), 6.95 (2H, d, $J = 8.4$ Hz), 7.00 (1H, d, $J = 15.6$ Hz), 7.08 (2H, d, $J = 1.8$ Hz), 7.22 (2H, dd, $J = 8.4$ Hz, $J = 1.8$ Hz), 7.77 (2H, d, $J = 15.3$ Hz), 7.91 (1H, d, $J = 15.6$ Hz). Anal. ($\text{C}_{28}\text{H}_{30}\text{O}_8 \cdot 1/4\text{H}_2\text{O}$) C, H.

5-Hydroxy-7-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-4-[3-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-acryloyl]-hepta-2,4,6-trienoic acid ethyl ester (40): Orange powder; 62% yield; mp 72–73 °C; ESI MS m/z 634.7 M^+ ; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 1.34 (3H, t), 1.5–2.2 (12H, m), 3.62 (4H, t), 3.92 (6H, s), 4.28 (2H, q), 5.49 (2H, t) 5.96 (H, d, $J = 15.6$ Hz), 7.00 (2H, d, $J = 15.6$ Hz), 7.08–7.16 (6H, m), 7.76 (2H, d, $J = 15.3$ Hz), 7.83 (H, d, $J = 15.9$ Hz); Anal. ($\text{C}_{36}\text{H}_{42}\text{O}_{10}$) C, H.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-4-[3-(4-hydroxy-3-methoxyphenyl)-acryloyl]-hepta-2,4,6-trienoic acid ethyl ester (41): Orange powder, 93% yield; mp 106–106.5 °C; ESI MS m/z 465.2 ($\text{M} - 1$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 1.34 (3H, t), 3.95 (6H, s), 4.29 (2H, quart), 5.96 (2H, d, $J = 15.6$ Hz), 6.95 (2H, d, $J = 8.2$ Hz), 6.96 (1H, d, $J = 15.6$ Hz), 7.05 (2H, d, $J = 2.1$ Hz), 7.17 (2H, dd, $J = 8.2$ Hz, $J = 2.1$ Hz), 7.75 (2H, d, $J = 15.3$ Hz), 7.90 (1H, d, $J = 15.9$ Hz). Anal. ($\text{C}_{26}\text{H}_{26}\text{O}_8 \cdot 11/8\text{H}_2\text{O}$) C, H.

7-(3,4-Dimethoxyphenyl)-4-[3-(3,4-dimethoxyphenyl)-acryloyl]-5-hydroxy-hepta-2,4,6-trienoic acid methyl ester (42): Orange powder; 50% yield; mp 167–168 °C; ESI MS m/z 481.6 ($\text{M} + \text{H}$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 3.82 (3H, s), 3.93 (12H, s), 5.98 (H, d, $J = 15.6$ Hz), 6.90 (2H, d, $J = 8.4$ Hz), 6.98 (2H, d, $J = 15.6$ Hz), 7.07 (2H, d, $J = 1.8$ Hz), 7.20 (2H, dd, $J = 8.4$ Hz, $J = 1.8$ Hz), 7.76 (2H, d, $J = 15.6$ Hz), 7.90 (H, d, $J = 15.9$ Hz); Anal. ($\text{C}_{27}\text{H}_{28}\text{O}_8 \cdot 1/4\text{H}_2\text{O}$) C, H.

7-(3,4-Dimethoxyphenyl)-4-[3-(3,4-dimethoxyphenyl)-acryloyl]-5-hydroxy-hepta-2,4,6-trienoic acid ethylamide (43): Yellow powder, 16% yield; mp 219–221 °C; ESI MS m/z 516.2 ($\text{M} + \text{Na}$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 1.32 (3H, t), 3.92 (6H, s), 3.92 (6H, s), 4.00 (2H, quart), 5.85 (H, d, $J = 15.3$ Hz), 6.88 (2H, d, $J = 8.4$ Hz), 6.95 (2H, d, $J = 15.6$ Hz), 7.06 (2H, d, $J = 1.5$ Hz), 7.18 (2H, dd, $J = 8.4$ Hz, $J = 1.5$ Hz), 7.73 (2H, d, $J = 15.6$ Hz), 7.82 (H, d, $J = 15.3$ Hz); Anal. ($\text{C}_{28}\text{H}_{31}\text{NO}_7 \cdot 3/2\text{H}_2\text{O}$) C, H.

7-(3,4-Dimethoxyphenyl)-4-[3-(3,4-dimethoxyphenyl)-acryloyl]-5-hydroxy-hepta-2,4,6-trienenitrile (44): 19% yield; mp 204–205 °C; ESI MS m/z 448.3 ($\text{M} + \text{H}$) $^+$; $^1\text{H NMR}$ (300 MHz, DMSO): δ 3.70 (3H, s), 3.75 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 6.32 (H, d, $J = 9.6$ Hz), 6.99 (2H, dd, $J = 8.4$ Hz, $J = 1.5$ Hz), (2H, d, $J = 15.6$ Hz), (2H, d, $J = 1.8$ Hz), (2H, dd, $J = 8.4$ Hz, $J = 1.8$ Hz), (2H, d, $J = 15.6$ Hz), (H, d, $J = 15.9$ Hz); Anal. ($\text{C}_{26}\text{H}_{25}\text{NO}_6 \cdot 9/4\text{H}_2\text{O}$) C, H.

1,7-Bis-(3,4-dimethoxyphenyl)-5-hydroxy-4-(3-hydroxypropenyl)-hepta-1,4,6-trien-3-one (45): Red powder. 19% yield; mp 178–179 °C; ESI MS m/z 453.2 ($\text{M} + \text{Na}$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 3.92 (6H, s), 3.93 (6H, s), 4.40 (2H, d, $J = 4.5$ Hz), 5.30 (0.4 H, s), 5.88 (H, triplet of doublet, $J = 15.6$ Hz, $J = 4.5$ Hz), 6.59 (H, d, $J = 15.6$ Hz), 6.88 (2H, d, $J = 8.4$ Hz), 6.97 (2H, d, $J = 15.6$ Hz), 7.06 (2H, d, $J = 1.8$ Hz), 7.17 (2H, dd, $J = 8.4$ Hz, $J = 1.8$ Hz), 7.68 (2H, d, $J = 15.6$ Hz); Anal. ($\text{C}_{26}\text{H}_{28}\text{O}_7 \cdot 3/4\text{H}_2\text{O}$) C, H.

3-[1-Hydroxy-3-(4-hydroxy-3-methoxyphenyl)-allylidene]-6-(4-hydroxy-3-methoxy-phenyl)-hex-5-ene-2,4-dione (46): Compound **46** was obtained from vanillin and triacetyl methane by using the same procedure described above for **12** from 5-hydroxymethyl-2-furaldehyde and 2,4-pentanedione. 8% yield; mp 162–164 °C; ESI MS m/z 433.2 ($\text{M} + \text{Na}$) $^+$; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 2.17 (3H, s), 3.94 (6H, s), 5.80 (H, s), 6.48 (2H, d, $J = 15.6$ Hz), 6.94 (2H, d, $J = 8.1$ Hz), 7.05 (2H, d, $J = 1.8$ Hz), 7.13 (2H, dd, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.59 (2H, d, $J = 15.6$ Hz). Anal. ($\text{C}_{23}\text{H}_{22}\text{O}_7$) C, H.

4-Fluoro-7-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-4-[3-[3-methoxy-4-(tetrahydropyran-2-yloxy)-phenyl]-acryloyl]-5-oxo-hept-6-enoic acid ethyl ester (47): 13% yield; mp 59–60 °C; EIMS m/z 677.3 (M + Na)⁺, 655.3 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (3H, t), 1.5–2.1 (12H, m), 2.45 (2H, m), 2.65 (2H, m), 3.62 (4H, t), 3.90 (6H, s), 4.13 (2H, q), 5.49 (2H, t), 7.06 (2H, dd, $J = 3$ Hz, $J = 15.6$ Hz), 7.12–7.15 (6H, m), 7.75 (2H, d, $J = 15.6$ Hz); Anal. (C₃₆H₄₄FO₁₀) C, H.

4-Fluoro-7-(4-hydroxy-3-methoxyphenyl)-4-[3-(4-hydroxy-3-methoxyphenyl)-acryloyl]-5-oxo-hept-6-enoic acid ethyl ester (48): 97% yield; mp 63–63.5 °C; EIMS m/z 509.3 (M + Na)⁺, 487.3 (M + H)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (3H, t), 2.45 (2H, m), 2.65 (2H, m), 3.95 (6H, s), 6.93 (2H, d, $J = 7.8$ Hz), 7.06 (2H, dd, $J = 3$ Hz, $J = 15.6$ Hz), 7.09 (2H, d, $J = 1.8$ Hz), 7.16 (2H, dd, $J = 1.8$ Hz, $J = 7.8$ Hz), 7.75 (2H, d, $J = 15.6$ Hz); Anal. (C₂₆H₂₇FO₈·3/4H₂O) C, H.

1,7-Bis-(3,4-dimethoxyphenyl)-4-methyl-hepta-1,6-diene-3,5-dione (49). Compound **49** was prepared from 3-methyl-2,4-pentanedione and 3,4-dimethoxybenzaldehyde by using the same procedure described above for **12** from 2,4-pentanedione and 5-hydroxymethyl-2-furaldehyde. 51% yield; mp 129–130 °C (lit.²⁸ 142–145 °C); ESI MS m/z 433.2 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 2.19 (3H, s), 3.91 (3H, s), 3.93 (3H, s), 3.95 (3H, s), 6.89 (2H, d, $J = 8.1$ Hz), 6.99 (2H, d, $J = 15.3$ Hz), 7.10 (2H, d, $J = 1.8$ Hz), 7.19 (2H, dd, $J = 8.1$ Hz, $J = 1.8$ Hz), 7.70 (2H, d, $J = 15.6$ Hz).

1,7-Bis-(3,4-dimethoxyphenyl)-4-fluoro-4-methyl-hepta-1,6-diene-3,5-dione (50): Yellow powder. 48% yield; mp 44–45 °C; ESI MS m/z 451.2 (M + Na)⁺; ¹H NMR (300 MHz, CDCl₃): δ 1.97 (3H, s), 3.90 (6H, s), 3.95 (6H, s), 6.83 (2H, d, $J = 7.8$ Hz), 6.87 (2H, dd, $J = 3$ Hz, $J = 15.6$ Hz), 6.90 (2H, d, $J = 1.8$ Hz), 6.95 (2H, dd, $J = 1.8$ Hz, $J = 7.8$ Hz), 7.75 (2H, d, $J = 15.6$ Hz); Anal. (C₂₄H₂₅FO₆·5/2H₂O) C, H.

Cytotoxicity Bioassay in Human Prostate Cancer Cell Lines LNCaP and PC-3. The in vitro cytotoxicity bioassay was performed according to the procedures described in Rubinstein et al.²⁹ Drug stock solutions were prepared in DMSO, and the final solvent concentration was not greater than 1% DMSO (v/v), a concentration without effect on cell replication. The human prostate cancer cells were exposed to doses of the compounds for 2 days and variation between replicate experiments was ≤5%. The IC₅₀ values were determined from dose–response graphs.

Antiandrogenic Bioassay in Human Prostate Cancer Cells: Human prostate cancer LNCaP and PC-3 cells were maintained in RPMI medium and Dulbecco's minimum essential medium (DMEM), respectively. Both media were supplemented with penicillin (25 units/mL), streptomycin (25 μg/mL), and 10% fetal calf serum. For the androgen receptor transactivation assay, an androgen-dependent reporter gene transcription test was employed as the primary screening for potential antiandrogen identification. This assay was first performed in LNCaP cells, which express a clinically relevant mutant AR. Once antiandrogenic activity was detected in the LNCaP AR transactivation assay, compounds were reexamined for their potential activity against wild type AR. Wild type AR transactivation assay was performed in PC-3 host cells, which lack an endogenous, functional AR. The method and conditions of cell and gene transfection have been described previously. In brief, cells were plated in 24-well tissue culture dishes for 24 (PC-3 cells) or 48 (LNCaP cells) h prior to transfection. Subsequently, LNCaP cells were transfected with a reporter gene, MMTV-luciferase, which contains MMTV-LTR promoter and androgen receptor binding element, and PRL-SV40, which served as an internal control for transfection efficiency. PC-3 cells were transfected with a wild type AR expression plasmid, pSG5AR, in addition to the above-mentioned MMTV-luciferase reporter gene and PRL-SV40 internal control. SuperFect (Qiagen, Chatsworth, CA) was employed as the transfection reagent following manufacturer's recommendations. At the end of a 5-h transfection, the medium was changed to DMEM or RPMI supplemented with 10% charcoal dextran-stripped, i.e., androgen-depleted, serum. After 24 h, the cells were treated with 1 nM of DHT and/or test compounds at the designated concentration

for another 24 h. The cells were harvested for luciferase activity assay using Dual Luciferase Assay System (Promega, Madison, WI). The derived data were expressed as relative luciferase activity normalized to the internal luciferase control. Cells cultured in medium containing DHT (androgen), as a positive control, induced a marked reporter gene expression. Test compounds capable of significantly suppressing this DHT-induced reporter gene expression were identified as potential antiandrogens.

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Supporting Information Available: Results of the elemental analysis of **4**, **8–15**, **17**, **19–21**, **23**, **25**, **26**, **29–31**, **35**, **36**, **38–48**, and **50** are reported. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Tatsuzaki, J.; Bastow, K. F.; Nakagawa-Goto, K.; Nakamura, S.; Itokawa, H.; Lee, K. H. Antitumor Agents 249. Synthesis and evaluation of dehydrozingerone analogues as cytotoxic agents. *Cancer Lett.*, submitted.
- (2) Jemal, A.; Samuels, A. A., G.; Ward, E.; Thun, M. Cancer statistics, 2003. *CA Cancer J. Clin.* **2003**, *53*, 5–26.
- (3) Ross, R. K.; Pike, M. C.; Coetzee, G. A.; Reichards, M. C.; Yu, M. C.; Feigelson, H.; Stanczyk, F. Z.; Kolonel, L. N.; Henderson, B. E. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. *Cancer Res.* **1998**, *58*, 4497–4504.
- (4) Goldenberg, S. L.; Bruchofsky, N. Use of cyproterone acetate in prostate cancer. *Urol. Clin. North Am.* **1991**, *18*, 111–112.
- (5) de Voogt, H. J. The position of cyproterone acetate (CPA), a steroid anti-androgen, in the treatment of prostate cancer. *Prostate* **1992** (Suppl. 4), 91–95.
- (6) de Voogt, H. J.; Smith, P. H.; Pavone-Macaluso, M.; de Pauw, M.; Suci, S. Cardiovascular side effects of diethylstilbestrol, cyproterone acetate, medroxyprogesterone acetate and estraumustine phosphate used for the treatment of advanced prostate cancer: Results from European Organization for Research on Treatment of Cancer Trials 30761 and 30762. *J. Urol.* **1986**, *135*, 303–307.
- (7) Kelly, W. K.; Scher, H. I. Prostate specific antigen decline after antiandrogen withdrawal: The flutamide withdrawal syndrome. *J. Urol.* **1993**, *149*, 607–609.
- (8) Suzuki, H.; Akakura, K.; Komiya, A.; Aida, S.; Akimoto, S.; Shimazaki, J. Codon 877 mutation in the androgen receptor gene in advanced prostate cancer: relation to antiandrogen withdrawal syndrome. *Prostate* **1996**, *29*, 153–158.
- (9) Bohl, C. E.; Gao, W.; Miller, D. D.; Bell, C. E.; Dalton, J. T. Structural basis for antagonism and resistance of bicalutamide in prostate cancer. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6201–6206.
- (10) Hsing, A. W.; Tsao, L.; Devesa, S. S. International trends and patterns of prostate cancer incidence and mortality. *Int. J. Cancer* **2000**, *85*, 60–67.
- (11) Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekharan, K. N.; Kuttan, R. Antitumor and antioxidant activity of natural curcuminoids. *Cancer Lett.* **1995**, *94*, 79–83.
- (12) Huang, M.-T. Antioxidant and antitumor properties of curcumin. *Food Factors for Cancer Prevention, [International Conference on Food Factors: Chemistry and Cancer Prevention], Hamamatsu, Japan, Dec., 1995*; Springer: Tokyo, 1997, pp 249–252.
- (13) Jordan, W. C.; Drew, C. R. Curcumin—a natural herb with anti-HIV activity. *J. Natl. Med. Assoc.* **1996**, *88*, 333.
- (14) Kawamori, T.; Lubet, R.; Steele, V. E.; Kelloff, G. J.; Kasey, R. B. Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res.* **1999**, *59*, 597–601.
- (15) Aggarwal, B. B.; Kumar, A. P.; Bharti, A. C. Anticancer potential of curcumin preclinical and clinical studies. *Anticancer Res.* **2003**, *23*, 363–398.
- (16) Ohtsu, H.; Itokawa, H.; Xiao, Z.; Su, C.-Y.; Shih, C. C. Y.; Chiang, T.; Chang, E.; Lee, Y.; Chiu, S.-Y.; Chang, C.; Lee, K.-H. Antitumor agents 222. Synthesis and anti-androgen activity of new diarylheptanoids. *Bioorg. Med. Chem.* **2003**, *11*, 5083–5090.
- (17) Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H.-K.; Itokawa, H.; Su, C.-Y.; Shih, C.; Chiang, T.; Chang, E.; Lee, Y.; Tsai, M.-Y.; Chang, C.; Lee, K.-H. Antitumor agents. 217. Curcumin analogues as novel androgen receptor antagonists with potential as anti-prostate cancer agents. *J. Med. Chem.* **2002**, *45*, 5037–5042.
- (18) Lin, L.; Shi, Q.; Su, C.-Y.; Shih, C. C. Y.; Lee, K.-H. Antitumor agents 247. New 4-ethoxycarbonyl ethyl curcumin analogs as potential antiandrogenic agents. *Bioorg. Med. Chem.* **2005**, in press.

- (19) Masuda, T.; Matsumura, H.; Oyama, Y.; Takeda, Y.; Jitoe, A.; Kida, A.; Hidaka, K. Synthesis of (\pm)-cassumunins A and B, new curcuminoid antioxidants having protective activity of the living Cell against oxidative damage. *J. Nat. Prod.* **1998**, *61*, 609–613.
- (20) Pedersen, U.; Rasmussen, P. B.; Lawesson, S.-O. Synthesis of naturally occurring curcuminoids and related compounds. *Liebigs Ann. Chem.* **1985**, 1557–1569.
- (21) Baranovsky, A.; Schmitt, B.; Fowler, D. J.; Schneider, B. Synthesis of aew biosynthetically important diarylheptanoids and their oxo- and fluoro- analogues by three different strategies. *Synth. Commun.* **2003**, *33*, 1019–1045.
- (22) Bowser, J. R.; Williams, P. J.; Kurz, K. Cleavage of silicon–nitrogen bonds by acid chlorides: an unusual synthetic route to amides. *J. Org. Chem.* **1983**, *48*, 4111–4113.
- (23) Lal, G. S. Site-selective fluorination of organic compounds using 1-alkyl-4-fluoro-1,4-diazabicyclo[2,2,2]octane salts (Selectfluor Reagents). *J. Org. Chem.* **1993**, *58*, 2791–2796.
- (24) Belliotti, T. R.; Connor, D. T.; Flynn, D. L.; Kostlan, C. R.; Nies, D. E. Preparation of novel styrylpyrazoles, styrylisoxazoles, and analogs as 5-lipoxygenase inhibitors. Eur. Pat. Appl. (Warner-Lambert Co., USA), 1987; p 58.
- (25) Weber, W. M.; Hunsaker, L. A.; Abcouwer, S. F.; Deck, L. M.; Vander Jagt, D. L. Anti-oxidant activities of curcumin and related enones. *Bioorg. Med. Chem.* **2005**, *13*, 3811–3820.
- (26) Chowdhury, H.; Walia, S.; Saxena, V. S. Isolation, characterization and insect growth inhibitory activity of major turmeric constituents and their derivatives against *Schistocerca gregaria* (Forsk) and *Dysdercus koenigii* (Walk). *Pest Manage. Sci.* **2000**, *56*, 1086–1092.
- (27) Youssef, K. M.; El-Sherbeny, M. A.; El-Shafie, F. S.; Farag, H. A.; Al-Deeb, O. A.; Awadalla, S. A. A. Synthesis of curcumin analogues as potential antioxidant, cancer chemopreventive agents. *Arch. Pharm.* **2004**, *337*, 42–54.
- (28) Ishida, J.; Ohtsu, H.; Tachibana, Y.; Nakanishi, Y.; Bastow, K. F.; Nagai, M.; Wang, H.-K.; Itokawa, H.; Lee, K.-H. Antitumor agents. Part 214: Synthesis and evaluation of curcumin analogues as cytotoxic agents. *Bioorg. Med. Chem.* **2002**, *10*, 3481–3487.
- (29) Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simo, R. M.; Tosini, S.; Skehan, P.; Scudiero, P. A.; Monks, A.; Boyd, M. R. Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J. Natl. Cancer Inst.* **1990**, *82*, 1113.

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