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Synthesis and biological evaluation of polyhydroxycurcuminoids☆

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Abstract—A series of curcumin analogs (**1**–**3**, **5a**–**5t**) was synthesized through the condensation of appropriately protected hydroxybenzaldehydes with acetylacetone, followed by deprotection. The antioxidant activity of these analogs was determined by superoxide free radical nitroblue tetrazolium and DPPH free radical scavenging methods and the polyhydroxycurcuminoids (**5l**–**5s**) displayed excellent antioxidant activity. These analogs showed cytotoxicity to lymphocytes and promising tumor-reducing activity on Dalton's lymphoma ascites tumor cells.

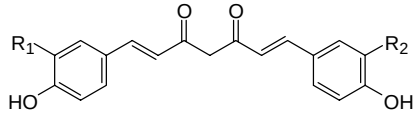
1. Introduction

Free radicals play a major role in the progression of a wide range of pathological disturbances and lead to very serious problems such as cancer, Alzheimer's, Parkinson's, and cardiovascular diseases. In the food industry, free radicals have been found to be responsible in the deterioration of foods during processing and storage.[1,2] In view of this, considerable attention has been given to the addition of antioxidants in foods and supplementation of antioxidants to biological systems to scavenge free radicals. The antioxidative compounds can be classified into two types: phenolics and β-diketones. Phenolic compounds exert their antioxidant activity by acting primarily as hydrogen atom donators, thereby inhibiting the propagation of radical chain reactions. The antioxidant potential of the phenolics depends on the number and arrangement of phenolic hydroxyl groups, as well as the nature of the other substituents on the aromatic rings. A few natural products, such as curcuminoids, have both phenolic and β-diketone groups in the same molecule and thus became potential antioxidants. Curcuminoids, the phenolic diarylheptanoids (**1**–**3**), are characteristic yellow colored constituents of turmeric (*Curcuma longa*) and are widely used in foods and cosmetics. These compounds were reported to posses antioxidant,[3,4] anti-inflammatory,[5] anticancer,[6,7] Alzheimer's[8], and antiviral properties.[9] A few nonphenolic curcumin analogs have been studied for their antioxidant activity.[10,11] Lack of systematic study of antioxidant activity of phenolic curcumin analogs, prompted us to synthesize and evaluate the activity. We report in this paper, the details of synthesis of curcumin analogs (**5a**–**5t**) and their antioxidant, cytotoxicity, and antitumor activities (Fig. 1).

2. Results and discussion

2.1. Synthesis

The curcumin analogs (**1**–**3**, **5a**–**5t**) were synthesized by slight modification of Pabon method.[12] The main steps in this scheme are the protection of active methylene group by reacting with acetyl acetone in the presence of boric oxide in order to get acetyl acetone–boric oxide complex (**4**) and reacting less reactive methyls with the aldehyde group using 1,2,3,4-tetrahydroquinoline as catalyst (Scheme 1). Finally, the boron complex of the



1. Curcumin; R_1=R_2= OCH_3
2. Demethoxycurcumin; R_1= OCH_3, R_2=H
3. Bisdemethoxycurcumin; R_1=R_2=H

Figure 1. Chemical structures of the curcuminoids of *Curcuma longa*.

Keywords: Polyhydroxycurcuminoids; Synthesis; Antioxidative; Tumor reducing.
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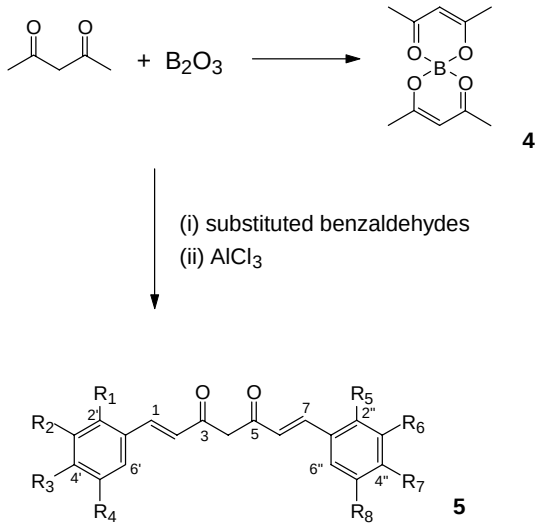

5a R_1=R_5=OH, R_4=R_8=OCH_3, R_2=R_3=R_6=R_7=H
5b R_1=R_5=OH, R_2=R_3=R_4=R_6=R_7=R_8=H
5c R_3=R_7=OCH_3, R_1=R_2=R_4=R_5=R_6=R_8=H
5d R_3=R_7=$N(CH_3)_2$, R_1=R_2=R_4=R_5=R_6=R_8=H
5e R_3=R_7=OH, R_2=R_4=R_6=R_8=OCH_3, R_1=R_5=H
5f R_3=R_7=OH, R_2=R_4=R_6=OCH_3, R_1=R_5=R_8=H
5g R_3=R_7=OH, R_4=R_8=OCH_3, R_2=R_6=Br, R_1=R_5=H
5h R_3=R_7=OH, R_2=R_6=$C(CH_3)_3$, R_1=R_4=R_5=R_8=H
5i R_3=R_7=OH, R_2=R_4=R_6=R_8=$C(CH_3)_3$, R_1=R_5=H
5j R_2=R_3=R_6=R_7=OCH_2Ar, R_4=R_8=OCH_3, R_1=R_5=H
5K R_3=R_7=OH, R_2=R_6=$COOCH_3$, R_1=R_4=R_5=R_8=H
5l R_2=R_3=R_6=R_7=OH, R_1=R_4=R_5=R_8=H
5m R_2=R_3=R_7=OH, R_6=OCH_3, R_1=R_4=R_5=R_8=H
5n R_1=R_4=R_5=R_8=OH, R_2=R_3=R_6=R_7=H
5o R_3=R_6=R_7=OH, R_1=R_2=R_4=R_5=R_8=H
5p R_2=R_3=R_6=R_7=OH, R_4=R_8=OCH_3, R_1=R_5=H
5q R_2=R_3=R_6=R_7=OH, R_4=OCH_3, R_1=R_5=R_8=H
5r R_2=R_3=R_4=R_6=R_7=OH, R_1=R_5=R_8=H
5s R_2=R_3=R_4=R_6=R_7=R_8=OH, R_1=R_5=H
5t R_3=R_7=OH, R_2=R_6=COOH, R_1=R_4=R_5=R_8=H

Scheme 1. General synthetic route for the synthesis of curcumin analogs.

product was decomposed using aqueous acetic acid to get the desired curcumin analogs. But, the polyhydroxy analogs (**5l**–**5s**) could not be prepared by this method, perhaps due to the influence of hydroxyls on the starting benzaldehydes. As such, the hydroxyl groups on the benzaldehydes were protected as benzyl or methyl ethers and carried out the reaction with acetyl acetone–boric oxide complex. Deprotection of benzyl or methyl groups of the protected curcumin analogs (**1**, **2**, **5a**, **5e**, **5f**, and **5j**) was carried out using aluminum chloride[13,14] to obtain the corresponding polyhydroxycurcumins (**5l**–**5s**). The water-soluble analog (**5t**) was obtained by the hydrolysis of the ester group in **5k** using sodium hydroxide. All the curcumin analogs (**1**–**3**, **5a**–**5t**) have been characterized by interpretation of the spectral data (IR, ^{1}H NMR, ^{13}C NMR, and mass). The analog **5f,** a natural product from *Curcuma xanthorrhiza*,[15] and the polyhydroxycurcumins (**5n**–**5t**) were prepared for the first time.

2.2. Antioxidant activity

In the present study, two commonly used antioxidant evaluation methods, superoxide radical scavenging and DPPH free radical scavenging methods, were chosen. Both the methods measure the efficacy of a hydrogen atom transfer from a phenol to a radical.

2.2.1. Superoxide radical scavenging activity. Superoxide radicals were generated in vitro by nonenzymatic system and determined spectrophotometrically (560 nm) by nitro blue tetrazolium (NBT) photo reduction method of McCord and Fridovich.[16,17] The antioxidant activity of the curcumin analogs (**1**–**3**, **5a**–**5t**) was expressed as 50% inhibitory concentration (IC_{50} in μM) and the values are incorporated in Table 1. From the data, all the analogs showed good activity because of the presence of β-diketone moiety. The activity was enhanced by the presence and increasing number of hydroxyl groups on the benzene ring. For example, **5c** (IC_{50}: 810 μM), and **5d** (IC_{50}: 770 μM) having no hydroxyl groups were less potent than the phenolic analogs such as curcumin (**1**, IC_{50}: 41 μM) having two hydroxyl groups, **5o** (IC_{50}: 14.6 μM) with three hydroxyl groups, **5l** (IC_{50}: 11.8 μM) with four hydroxyl groups, pentahydroxycurcumin[18] (**5r**, IC_{50}: 5.6 μM), and hexahydroxycurcumin[18] (**5s**, IC_{50}: 4.8 μM). In addition to the number of phenolic hydroxyls, the substitution pattern of hydroxyls also plays an important role in the antioxidative activity. For example, the tetrahydroxy analog **5p**[18] (IC_{50}: 6.5 μM) having two catechol systems was found to be more active than **5n** (IC_{50}: 11 μM), which has same number of phenolic hydroxyls but does not contain a catechol unit. As expect-

Table 1. Antioxidant activity of curcumin analogs

Compound No.	NBT superoxide scavenging activity (IC_{50} in μM)	DPPH free radical scavenging activity (IC_{50} in μM)
1	41	21
2	40	34
3	38	33
5a	47.6	24.0
5b	260	>500
5c	810	>500
5d	770	52
5e	42.7	26.0
5f	46.5	25.5
5g	68.7	43.0
5h	59.5	48.0
5i	37.6	43.0
5k	117.9	>100
5l	11.8	6.0
5m	12	7.0
5n	11	8.0
5o	14.6	7.6
5p	6.5	5.4
5q	6.8	6.3
5r	5.6	5.2
5s	4.8	4.6
5t	27.8	>100
Vitamin C	852	25.1
Vitamin E	726	>100
BHA	966	34.0
BHT	381	22.5

BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene. The lower the IC_{50} values, the higher is the antioxidant activity.

ed, hexahydroxycurcumin (**5s**) having two pyrogallol units has the most potential and showed 11 times more activity than the parent curcumin (**1**). These polyhydroxycurcumins (**5l–5s**) showed several fold higher activity in comparison with the commercially available antioxidants like vitamin C (IC_{50}: 852 μM), vitamin E (IC_{50}: 726 μM), BHA (IC_{50}: 966 μM), and BHT (IC_{50}: 381 μM). The superior scavenging ability of these analogs (**5l–5s**) lends further support to the fact that the pyrogallol or catechol system enhances the antioxidant activity.[19] Neither the methoxyl (**5e**, **5f**) nor *tert*-butyl groups (**5h**, **5i**) on the benzene ring contributed significantly to the antioxidative activity. Further, the electron-withdrawing groups like carboxylic acid ester (**5k**) or bromine (**5g**) on the benzene ring have reduced the superoxide scavenging ability.

2.2.2. DPPH radical scavenging activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of curcumin analogs (**1–3**, **5a–5t**) was determined by the method of Lamaisen et al.,[20,21] based on the reduction of methanolic solution of the colored DPPH radical. In this model also, phenolic analogs showed enhanced activity (Table 1) in relation to the non-phenolic analogs and the catechol and pyrogallol units have shown further enhanced activity. Hexahydroxycurcumin[18] (**5s**, IC_{50}: 4.6 μM) having two pyrogallol units was the strongest scavenger of DPPH free radical followed by other polyhydroxycurcumins (**5r**, IC_{50}: 5.2 μM, **5p**, IC_{50}: 5.4 μM, and **5q**, IC_{50}: 6.3 μM). The electron-withdrawing groups like carboxylic acid ester (**5k**) or bromine (**5g**) on the benzene ring, as expected, reduced the DPPH free radical scavenging ability.

2.3. Antitumor activity

Cytotoxicity of the curcumin analogs was determined using lymphocytes from normal or patients with leukemia and these analogs were found to be highly cytotoxic at 250 μg (Table 2). These results indicated that the curcumin analogs are potential anticancer agents. The tumor-reducing activity of some selected analogs of curcumin (**1**, **5a**, **5e**, **5h**, **5i**, **5m**, **5n**, and **5p**) were determined on Dalton's lymphoma ascites tumour cells.[6] From the data (Table 3), on 7th day to 30th day, all the tested analogs showed good inhibition and this experiment indicated that these analogs decreased the incidence of tumor formation in experimental animals. However, further studies are needed on different types of tumor models to confirm the efficacy of the compounds.

Table 2. Cytotoxicity of curcumin analogs

Compound No.	Percentage cytotoxicity (μg)		
	25	50	250
1	64	91	100
3	45	74	100
5a	80	86	100
5e	76	89	100
5g	56	85	100
5h	80	84	100
5i	87	89	100
5l	77	94	100
5m	84	90	100
5n	79	86	100
5p	70	93	100
5t	89	92	100

Table 3. Effect of curcumin analogs on solid tumor development

Compound No.	Tumor volumes (cm^3)[a]			
	7th day	15th day	24th day	30th day
Control	0.10 ± .03	0.61 ± .12	3.96 ± .38	5.99 ± .20
1	0.08 ± .02	0.28 ± .01	0.12 ± .03	0.08 ± .01
5a	0.06 ± .01	0.18 ± .01	0.55 ± .03	0.52 ± .02
5e	0.05 ± .01	0.22 ± .01	0.52 ± .05	0.38 ± .01
5h	0.06 ± .02	0.19 ± .01	0.24 ± .02	0.24 ± .02
5i	0.06 ± .01	0.15 ± .01	0.54 ± .02	0.57 ± .03
5m	0.04 ± .01	0.13 ± .01	0.18 ± .01	0.14 ± .01
5n	0.07 ± .01	0.19 ± .01	0.18 ± .01	0.10 ± .01
5p	0.05 ± .01	0.24 ± .01	0.26 ± .01	0.24 ± .02

[a] Values are obtained from the average volume of three animals treated and six animals in control.

3. Conclusion

In conclusion, curcumin analogs (**1–3**, **5a–5t**) were synthesized starting from appropriately substituted benzaldehydes. The polyhydroxycurcumins (**5l–5s**) are potent scavengers of superoxide and DPPH free radicals. The analogs (**5a**, **5e**, **5h**, **5i**, **5m**, **5n**, and **5p**) showed promising tumor-reducing activity on Dalton's lymphoma ascites tumor cells.

4. Experimental

4.1. General

Melting points were recorded on a V Scientific melting point apparatus, in open capillaries and are uncorrected. UV spectra were recorded on a Shimadzu UV-190 Spectrophotometer, IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer and mass spectra on a VG micromass 70-70H mass spectrometer or Agilent 1100 LCMS. Acme silica gel G and silica gel (100–200 meshes) were used for analytical TLC and column chromatography, respectively. Lymphocytes were prepared using the Ficoll–Hypaque method[22] from either normal donors or from patients with leukemia. Daltons lymphoma cells grown in the peritoneal cavity of mice were aspirated and washed in normal saline. The substituted benzaldehydes, 4-hydroxy-3,5-dimethoxybenzaldehyde,[23] 3,4-dibenzyloxy-5-methoxybenzaldehyde,[24] 3-*tert*-butyl-4-hydroxybenzaldehyde,[25] 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde,[26] and methyl 5-formyl-2-hydroxybenzaldehyde[27] were prepared by the literature procedures and the other benzaldehydes were procured from commercial sources.

4.2. General procedure for curcumin analogs

4.2.1. 1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (1). To a solution of boric oxide (350 mg, 5.0 mmol) in DMF (1.0 mL) was added acetylacetone (0.51 mL, 5.0 mmol), followed by tributyl borate (2.7 mL, 10 mmol) at 65 °C and stirred for 15 min. To the above borate complex, 4-hydroxy-3-methoxybenzaldehyde (1.52 g, 10 mmol) was added and stirred for 5 min. A mixture of 1,2,3,4-tetrahydroquinoline (0.1 mL) and acetic acid (0.3 mL) in DMF (1 mL) was added to the reaction mixture and heated to 95 °C for 4 h. After cooling to 15 °C, acetic acid (20%, 50 mL) was added with stirring and again the reaction mixture was stirred at 70 °C for another 1 h. Then it was cooled to 15 °C, the so-formed solid was filtered, washed with water, and dried. The crude curcumin was chromatographed over silica gel column using chloroform/methanol (95:5) as eluent followed by recrystallisation from chloroform/methanol to give curcumin (**1**, 2.39 g, 65%), mp 178–180 °C (lit.[28,29] mp 182–183 °C), IR (KBr) ν_{max} 3428, 1613, 1276, 1154, 1027, 957 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.94 (6H, s, 2× Ar-OCH_3), 5.79 (1H, s, H-4), 6.46 (2H, d, J = 16.0 Hz, H-2,6), 6.92 (2H, d, J = 8.0 Hz, H-5′,5″), 7.04 (2H, d, J = 2.0 Hz, H-2′,2″), 7.11 (2H, dd, J = 8.0, 2.0 Hz, H-6′,6″), 7.58 (2H, d, J = 16.0 Hz, H-1,7); EIMS m/z (%): 368 (M^+, 53), 350 (36), 191 (66), 190 (78), 177 (100), 158 (30), 149 (21), 145 (85), 137 (81), 117 (29), 110 (63), 77 (29).

4.2.2. 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (2). Yield 54%, mp 169–171 °C (lit.[28] mp 172–173 °C); IR (KBr) ν_{max} 3399, 1626, 1374, 1263, 1166, 1136, 1026, 966, 826 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (3H, s, Ar-OCH_3), 6.05 (1H, s, H-4), 6.77 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.71 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.82–6.84 (3H, m, H-5′,3″,5″), 7.15 (1H, dd, J = 8.2, 1.8 Hz, H-6′), 7.33 (1H, d, J = 1.8 Hz, H-2′), 7.52–7.59 (4H, m, H-1,7, 2″,6″); EIMS m/z (%): 338 (M^+, 37), 350 (36), 321 (46), 320 (44), 191 (43), 190 (41), 177 (57), 147 (100), 149 (13), 150 (21), 137 (34), 119 (33), 91 (39).

4.2.3. 1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (3). Yield 55%, mp 222–224 °C (lit.[28] mp 223–224 °C); IR (KBr) ν_{max} 3211, 1620, 1600, 1269, 1168, 1140, 955, 831 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.03 (1H, s, H-4), 6.68 (2H, d, J = 16.0 Hz, H-2,6), 6.80 (4H, d, J = 8.0 Hz, H-3′,5′,3″,5″), 7.50 (2H, d, J = 16.0 Hz, H-1,7), 7.55 (4H, d, J = 8.0 Hz, H-2′,6′,2″,6″); EIMS m/z (%): 308 (M^+, 20), 290 (14), 159 (36), 146 (100), 147 (87), 119 (38), 106 (42), 90 (42), 65 (32).

4.2.4. 1,7-Bis(2-hydroxy-5-methoxyphenyl)-1,6-heptadiene-3,5-dione (5a). Yield 47%, mp 152–154 °C; IR (KBr) ν_{max} 3420, 2920, 1613, 1283, 1210, 1142, 1040, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.74 (6H, s, 2× Ar-OCH_3), 6.11 (1H, s, H-4), 6.85–7.02 (6H, m, H-3′,3″,4′,4″,6′,6″), 7.11 (2H, d, J = 16.0 Hz, H-2,6), 7.88 (2H, d, J = 16.0 Hz, H-1,7), 9.80 (2H, br s, Ar-OH); ^{13}C NMR (DMSO-d_6) δ 183.4, 152.4, 151.5, 135.5, 123.7, 121.7, 118.5, 117.1, 111.7, 101.4, 55.5; LC–MS m/z (%): (ESI-negative mode) 367 [$(M-H)^-$, 100]; Elemental analysis, Calcd for $C_{21}H_{20}O_6$: C, 68.48; H, 5.43. Found: C, 68.42; H, 5.58.

4.2.5. 1,7-Bis(2-hydroxyphenyl)-1,6-heptadiene-3,5-dione (5b). Yield 23%, mp 160–162 °C (lit.[30] mp 163–164 °C); IR (KBr) ν_{max} 3391, 1615, 1255, 1142, 961, 752 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.11 (1H, s, H-4), 6.91 (2H, d, J = 7.5 Hz, H-3′,3″), 6.92 (2H, d, J = 15.8 Hz, H-2,6), 7.21 (4H, m, H-4′,5′,4″,5″), 7.63 (2H, d, J = 7.5 Hz, H-6′,6″), 7.89 (2H, d, J = 15.8 Hz, H-1,7); LC–MS m/z (%): (ESI-negative mode) 307 [$(M-H)^-$, 100].

4.2.6. 1,7-Bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (5c). Yield 54%, mp 156–158 °C (lit.[31] mp 154–158 °C); IR (KBr) ν_{max} 2933, 1625, 1600, 1177, 1028, 977, 826 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (6H, s, 2× Ar-OCH_3), 5.78 (1H, s, H-4), 6.49 (2H, d, J = 15.7 Hz, H-2,6), 6.91 (4H, d, J = 8.7 Hz, H-3′,5′,3″,5″), 7.50 (4H, d, J = 8.7 Hz, H-2′,6′,2″,6″), 7.62 (2H, d, J = 15.7 Hz, H-1,7); LC–MS m/z (%): (ESI-positive mode) 337 [$(M+H)^+$, 100].

4.2.7. 1,7-Bis(4-dimethylaminophenyl)-1,6-heptadiene-3,5-dione (5d). Yield 45%, mp 212–214 °C (lit.[30] mp 210–212 °C); IR (KBr) ν_{max} 2920, 1620, 1362, 962, 814 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.00 (12H, s, 2× Ar-$N(CH_3)_2$), 5.72 (1H, s, H-4), 6.41 (2H, d, J = 15.7 Hz, H-2,6), 6.68 (4H, d, J = 8.7 Hz, H-3′,5′,3″,5″), 7.45 (4H, d, J = 8.7 Hz, H-2′,6′,2″,6″), 7.59 (2H, d, J = 15.7 Hz, H-1,7); LC–MS m/z (%): (ESI-positive mode) 363 [$(M+H)^+$, 100].

4.2.8. 1,7-Bis(4-hydroxy-3,5-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (5e). Yield 53%, mp 200–202 °C; IR (KBr) ν_{max} 3484, 1624, 1606, 1134, 1108, 962 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.93 (12H, s, 4× Ar-OCH_3), 5.81 (1H, s, H-4), 6.46 (2H, d, J = 15.6 Hz, H-2,6), 6.79 (4H, s, H-2′,2″,6′,6″), 6.88 (2H, br s, Ar-OH), 7.57 (2H, d, J = 15.6 Hz, H-1,7); LC–MS m/z (%): (ESI-negative mode) 427 [$(M-H)^-$, 100].

4.2.9. 1-(4-Hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (5f). Yield 16%, mp 146–148 °C (lit.[15] mp 145–146 °C); IR (KBr) ν_{max} 3405, 1627, 1280, 1143, 1118, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.94 (6H, s, 2× Ar-OCH_3), 3.95 (3H, s, Ar-OCH_3), 5.78 (1H, br s, Ar-OH), 5.81 (1H, s, H-4), 5.89 (1H, br s, Ar-OH), 6.47 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.48 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.80 (2H, s, H-2′,6′), 6.93 (1H, d, J = 8.2 Hz, H-5″), 7.05 (1H, d, J = 1.7 Hz, H-2″), 7.12 (1H, dd, J = 8.2, 1.7 Hz, H-6″), 7.57 (1H, d, J = 15.8 Hz, H-1 or H-7), 7.59 (1H, d, J = 15.8 Hz, H-1 or H-7); EIMS m/z (%): 398 (M^+, 75), 380 (55), 368 (14), 327 (13), 302 (17), 232 (16), 220 (41), 207 (47), 191 (31), 190 (29), 180 (32), 177 (100), 161 (25), 150 (30), 145 (41), 137 (41).

4.2.10. 1,7-Bis(3-bromo-4-hydroxy-5-methoxyphenyl)-1,6-heptadiene-3,5-dione (5g). Yield 52%, mp 236–238 °C; IR (KBr) ν_{max} 3400, 1625, 1278, 1139, 1043, 970, 843 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.93 (6H, s, 2× Ar-OCH_3), 5.91 (1H, s, H-4), 6.56 (2H, d, J = 15.6 Hz, H-2,6), 7.10 (2H, s, H-2′, 2″), 7.35 (2H, s, H-6′,6″),

7.49 (2H, d, J = 15.6 Hz, H-1,7); ^{13}C NMR (DMSO-d_6) δ 183.0, 148.5, 146.0, 139.3, 127.6, 125.6, 122.7, 110.5, 109.6, 101.3, 56.4; LC–MS m/z (%): (ESI-positive mode) 525 [$(M+H)^+$, 47], 527 [$(M+2H)^+$, 79], 529 [$(M+5H)^+$, 54]; Elemental analysis, Calcd for $C_{21}H_{18}O_6Br_2$: C, 47.15; H, 3.29. Found: C, 47.06; H, 3.32.

4.2.11. 1,7-Bis(3-*tert*-butyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (5h). Yield 34%, mp 204–206 °C; IR (KBr) ν_{max} 3368, 2953, 1615, 1269, 1138, 968 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.40 (18H, s, 2× Ar–C$(CH_3)_3$), 5.84 (1H, s, H-4), 6.45 (2H, d, J = 15.8 Hz, H-2,6), 6.85 (2H, d, J = 7.9 Hz, H-5′,5″), 7.27 (2H, d, J = 8.0 Hz, H-6′,6″), 7.41 (2H, s, H-2′,2″), 7.56 (2H, d, J = 15.8 Hz, H-1,7), 9.41 (2H, br s, 2× Ar-OH); ^{13}C NMR (DMSO-d_6) δ 184.5, 159.0, 141.7, 137.3, 128.4, 128.1, 127.5, 121.7, 117.8, 101.4, 35.5, 29.7; EIMS m/z (%): 420 (M^+, 78), 402 (73), 324 (51), 216 (48), 217 (11), 203 (88), 176 (18), 163 (20), 161 (61), 147 (54), 57 (100), 42 (31); Elemental analysis, Calcd for $C_{27}H_{32}O_4$: C, 77.14; H, 7.62. Found: C, 77.23; H, 7.58.

4.2.12. 1,7-Bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (5i). Yield 45%, mp 190–192 °C (lit.[30] mp 190–192 °C); IR (KBr) ν_{max} 3627, 2959, 1623, 1207, 969, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (36H, s, 4× Ar–C$(CH_3)_3$), 5.49 (2H, br s, 2× Ar-OH), 5.85 (1H, s, H-4), 6.48 (2H, d, J = 15.8 Hz, H-2,6), 7.39 (4H, s, H-2′,2″,6′,6″), 7.62 (2H, d, J = 15.8 Hz, H-1,7); EIMS m/z (%): 532 (M^+, 45), 436 (100), 259 (38), 219 (11), 57 (46).

4.2.13. 1,7-Bis(3,4-dibenzyloxy-5-methoxyphenyl)-1,6-heptadiene-3,5-dione (5j). Yield 25%, mp 114–116 °C; IR (neat) ν_{max} 1626, 1338, 1125, 964, 735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.81 (6H, s, 2× Ar-OCH_3), 5.01 (4H, s, –OCH_2Ar), 5.06 (4H, s, –OCH_2Ar), 5.75 (1H, s, H-4), 6.41 (2H, d, J = 15.8 Hz, H-2,6), 6.72 (2H, s, H-6′,6″), 6.75 (2H, s, H-2′,2″), 7.2–7.4 (20H, m, Ar–H), 7.47 (2H, d, J = 15.8 Hz, H-1,7).

4.2.14. 1,7-Bis(3-carbomethoxy-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (5k). Yield 9%, mp 210–212 °C; IR (KBr) ν_{max} 3106, 1678, 1630, 1216, 971, 668 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.99 (6H, s, 2× –$COOCH_3$), 5.80 (1H, s, H-4), 6.50 (2H, d, J = 15.6 Hz, H-2,6), 7.01 (2H, d, J = 8.6 Hz, H-5′,5″), 7.60–7.70 (2H, m, H-6′,6″), 7.60 (2H, d, J = 15.6 Hz, H-1,7), 8.03 (2H, d, J = 2.0 Hz, H-2′,2″); EIMS m/z (%): 424 (M^+, 48), 406 (22), 374 (16), 328 (23), 260 (13), 245 (7), 219 (21), 205 (77), 187 (42), 173 (100), 165 (38), 146 (22), 133 (20).

4.3. General procedure for demethylation

4.3.1. 1,7-Bis(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (5l). To an ice cold solution of **1** (1.5 g, 4.0 mmol) in dichloroethane (75 mL) was added aluminum chloride (2.1 g, 15.8 mmol), followed by the dropwise addition of pyridine (4.8 mL, 59.4 mmol) for 15 min and the reaction mixture was heated under reflux for 36 h. After cooling the reaction mixture to 10 °C, cold dil. HCl (20%) was added to decompose aluminum chloride complex and extracted with ethyl acetate (5× 30 mL). The combined ethyl acetate layer was dried over anhydrous sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using chloroform/methanol (95:5) as eluent, followed by recrystallization from methanol to give **5l** (401 mg, 29%), mp 302–304 °C (lit.[9] mp 306–308 °C dec.); IR (KBr) ν_{max} 3488, 3386, 1629, 1617, 1271, 1289, 1142, 1120, 955 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.06 (1H, s, H-4), 6.56 (2H, d, J = 15.6 Hz, H-2,6), 6.77 (2H, d, J = 8.3 Hz, H-5′,5″), 7.00 (2H, d, J = 1.7 Hz, H-2′,2″), 7.06 (2H, dd, J = 8.3, 1.7 Hz, H-6′,6″), 7.44 (2H, d, J = 15.6 Hz, H-1,7); ^{13}C NMR (DMSO-d_6) δ 183.1, 147.8, 145.1, 140.8, 127.7, 126.5, 121.9, 115.9, 114.5, 100.9; LC–MS m/z (%): (ESI-negative mode) 339 [$(M-H)^-$, 100].

4.3.2. 1-(3,4-Dihydroxyphenyl)-7-(3-methoxy-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (5m). Yield 22%, mp 164–166 °C (lit.[9] mp 165–167 °C); IR (KBr) ν_{max} 3484, 1620, 1267, 1132, 1140, 964 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s, Ar-OCH_3), 6.04 (1H, s, H-4), 6.53 (1H, d, J = 16.0 Hz, H-2 or H-6), 6.74 (1H, d, J = 16.0 Hz, H-2 or H-6), 6.76 (1H, d, J = 8.7 Hz, H-5′), 6.80 (1H, d, J = 8.3 Hz, H-5″), 7.07 (1H, dd, J = 8.7, 1.8 Hz, H-6′), 7.00 (1H, d, J = 1.8 Hz, H-2′), 7.12 (1H, d, J = 1.8 Hz, H-2″), 7.29 (1H, dd, J = 8.3, 1.8 Hz, H-6″), 7.44 (1H, d, J = 16.0 Hz, H-1 or H-7), 7.51 (1H, d, J = 16.0 Hz, H-1 or H-7); ^{13}C NMR (DMSO-d_6) δ 183.2, 183.0, 148.6, 147.9, 147.7, 145.1, 140.8, 140.7, 126.5, 122.8, 121.9, 121.0, 120.7, 115.9, 115.6, 114.6, 111.0, 101.0, 55.4; EIMS m/z (%): 354 (M^+, 16), 336 (20), 328 (54), 271 (71), 192 (53), 191 (30), 177 (100), 167 (47), 163 (49), 150 (40), 149 (24), 145 (84), 135 (48), 117 (42), 89 (57), 77 (43).

4.3.3. 1,7-Bis(2,5-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (5n). Yield 9%, mp 202–204 °C; IR (KBr) ν_{max} 3398, 1623, 1354, 1197, 1144, 976 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.12 (1H, s, H-4), 6.69–6.75 (4H, m, H-3′,3″,4′,4″), 6.77 (2H, d, J = 16.0 Hz, H-2,6), 6.96 (2H, d, J = 2.2 Hz, H-6′,6″), 7.81 (2H, d, J = 16.0 Hz, H-1,7), 8.90 (2H, br s, 2× Ar-OH), 9.53 (2H, br s, 2× Ar-OH); LC–MS m/z (%): (ESI-negative mode) 339 [$(M-H)^-$, 100].

4.3.4. 1-(4-Hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (5o). Yield 54%, mp 218–220 °C; IR (KBr) ν_{max} 3338, 1627, 1302, 962 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.06 (1H, s, H-4), 6.59 (1H, d, J = 15.9 Hz, H-2 or H-6), 6.69 (1H, d, J = 15.9 Hz, H-2 or H-6), 6.83 (1H, d, J = 8.2 Hz, H-5″), 6.79 (2H, d, J = 8.0 Hz, H-3′,5′), 7.03 (1H, s, H-2″), 7.09 (1H, d, J = 8.2 Hz, H-6″), 7.45 (1H, d, J = 15.9 Hz, H-1 or H-7), 7.47 (1H, d, J = 15.9 Hz, H-1 or H-7), 7.57 (2H, d, J = 8.0 Hz, H-2′,6′), 9.17 (1H, br s, Ar-OH), 9.63 (1H, br s, Ar-OH), 10.04 (1H, br s, Ar-OH); EIMS m/z (%): 324 (M^+, 18), 306 (8), 299 (34), 298 (90), 242 (30), 241 (100), 163 (49), 161 (26), 162 (38), 147 (87), 110 (43), 119 (39), 91 (21), 44 (34); Elemental analysis, Calcd for $C_{19}H_{16}O_5$: C, 70.36; H, 4.97. Found: C, 69.98; H, 5.02.

4.3.5. 1,7-Bis(3,4-dihydroxy-5-methoxyphenyl)-1,6-heptadiene-3,5-dione (5p). Yield: 24%, mp 230–232 °C; IR (KBr) ν_{max} 3484, 1621, 1384, 1289, 1135, 1091,

962 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.83 (6H, s, 2× Ar-OCH_3), 6.10 (1H, s, H-4), 6.65 (2H, d, J = 15.6 Hz, H-2,6), 6.79 (2H, s, H-2′,2″), 6.98 (2H, s, H-6′,6″), 7.49 (2H, d, J = 15.6 Hz, H-1,7); ^{13}C NMR (DMSO-d_6) δ 182.9, 148.5, 145.8, 141.0, 137.3, 125.2, 121.1, 109.7, 104.2, 100.6; LC–MS m/z (%): (ESI-negative mode) 399 [$(M-H)^-$, 100]; Elemental analysis, Calcd for $C_{21}H_{20}O_8$: C, 62.99; H, 5.04. Found: C, 62.91; H, 5.13.

4.3.6. 1-(3,4-Dihydroxy-5-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (5q). Yield 55%, mp 180–182 °C; IR (KBr) ν_{max} 3379, 1624, 1301, 1279, 1191, 1095, 964 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.82 (3H, s, Ar-OCH_3), 6.07 (1H, s, H-4), 6.57 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.65 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.77 (2H, br s, H-2′,6′), 6.79 (1H, d, J = 8.3 Hz, H-5″), 7.02 (1H, d, J = 8.3 Hz, H-6″), 7.08 (1H, br s, H-2″), 7.45 (1H, d, J = 15.8 Hz, H-1 or H-7), 7.46 (1H, d, J = 15.8 Hz, H-1 or H-7), 8.94 (1H, br s, Ar-OH), 9.13 (1H, br s, Ar-OH), 9.16 (1H, br s, Ar-OH), 9.61 (1H, br s, Ar-OH); ^{13}C NMR (DMSO-d_6) δ 183.2, 182.8, 148.5, 148.4, 145.9, 145.6, 140.7, 140.5, 137.3, 126.3, 125.2, 121.5, 121.2, 120.7, 115.9, 114.7, 109.8, 104.1, 100.8, 56.0; EIMS m/z (%): 370 (M^+, 4), 286 (16), 262 (4), 231 (3), 207 (3), 178 (4), 163 (6), 153 (11), 140 (56), 125 (50), 110 (100), 97 (42); Elemental analysis, Calcd for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90. Found: C, 64.81; H, 4.98.

4.3.7. 1-(3,4,5-Trihydroxyphenyl)-7-(3,4-dihydroxy-phenyl)-1,6-heptadiene-3,5-dione (5r). Yield 9%, mp 202–204 °C; IR (KBr) ν_{max} 3275, 1613, 1361, 1289, 1140, 1117, 1035, 964 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.09 (1H, s, H-4), 6.48 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.56 (1H, d, J = 15.8 Hz, H-2 or H-6), 6.63 (2H, s, H-2′,6′), 6.79 (1H, d, J = 8.0 Hz, H-5″), 7.00 (1H, d, J = 8.0 Hz, H-6″), 7.08 (1H, s, H-2″), 7.37 (1H, d, J = 15.8 Hz, H-1 or H-7), 7.45 (1H, d, J = 15.8 Hz, H-1 or H-7), 8.80–9.40 (5H, br s, Ar-OH); ^{13}C NMR (DMSO-d_6) δ 183.0, 148.4, 146.2, 145.6, 141.1, 140.6, 136.5, 126.3, 125.2, 121.5, 120.7, 115.9, 114.7, 107.8, 100.7; EIMS m/z (%): 356 (M^+, 2), 272 (4), 250 (4), 247 (2), 178 (9), 163 (10), 126 (46), 110 (100), 81 (25); Elemental analysis, Calcd for $C_{19}H_{16}O_7$: C, 64.04; H, 4.53. Found: C, 63.86; H, 4.72.

4.3.8. 1,7-Bis(3,4,5-trihydroxyphenyl)-1,6-heptadiene-3,5-dione (5s). Yield 31%, mp 240–242 °C; IR (KBr) ν_{max} 3356, 1619, 1330, 1195, 1133, 1037, 963 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.11 (1H, s, H-4), 6.48 (2H, d, J = 15.8 Hz, H-2,6), 6.62 (4H, s, H-2′,2″,6′,6″), 7.37 (2H, d, J = 15.8 Hz, H-1,7), 8.85 (2H, br s, 2× Ar-OH), 9.16 (4H, br s, 4 × Ar-OH); ^{13}C NMR (DMSO-d_6) δ 182.9, 146.3, 141.1, 136.5, 125.2, 120.7, 107.8, 101.0; LC–MS m/z (%): (ESI-negative mode) 371 [$(M-H)^-$, 100]; Elemental analysis, Calcd for $C_{19}H_{16}O_8$: C, 61.29; H, 4.33. Found: C, 61.16; H, 4.41.

4.3.9. 1,7-Bis(3-carboxy-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (5t). To a solution of **5k** (200 mg) in ethanol (5 mL) was added aq sodium hydroxide solution (10%, 20 mL) and stirred at room temperature for 15 h. The cooled reaction mixture was acidified with 20% HCl, the solid formed was filtered and dried to give **5t** (140 mg, 75%), mp 278–280 °C; IR (KBr) ν_{max} 2885, 1675, 1622, 1297, 1266, 1139, 962 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.18 (1H, s, H-4), 6.83 (2H, d, J = 15.9 Hz, H-2,6), 7.05 (2H, d, J = 8.7 Hz, H-5′,5″), 7.63 (2H, d, J = 15.9 Hz, H-1,7), 7.94 (2H, dd, J = 8.7, 2.1 Hz, H-6′,6″), 8.11 (2H, d, J = 2.1 Hz, H-2′,2″); EIMS m/z (%): 396 (M^+, 71), 378 (37), 360 (33), 300 (15), 282 (12), 246 (14), 191 (40), 187 (19), 173 (100), 166 (14), 159 (16), 146 (29), 133 (23), 117 (20), 103 (25), 89 (100), 77 (26).

4.4. Antioxidant activity

4.4.1. Superoxide free radical scavenging activity. The superoxide free radical scavenging activity was determined by the NBT method.[16,17] The reaction mixture contained EDTA (6.6 mM), NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), various concentrations of the test drug in ethanol, and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. Optical density was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the optical density was measured again at 560 nm. The percentage inhibition and superoxide radical generation were measured.

4.4.2. DPPH free radical scavenging activity. DPPH radical scavenging activity was measured based on the reduction of methanolic solution of the colored DPPH.[20,21] Free radical scavenging ability of the test drug in ethanol added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption values of DPPH solution at 516 nm. Drug activity is expressed as the 50% inhibitory concentration (IC_{50}). The reaction mixture contained 1×10^{-4} mM methanolic solution of DPPH and various concentrations of test drugs. The percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the plot, drawn for concentration in microgram versus percentage inhibition.

4.5. Antitumor activity

4.5.1. Cytotoxicity. The cytotoxicity of the curcumin analogs was determined by MTT [3-(4′,5′-dimethylthiazol-2′-yl)-2,5- diphenyltetrazolium bromide] method. RPMI-1640 medium containing 10% fetal calf serum and antibiotics were used as culture medium. An amount 0.1 mL of L929 (1×10^4) cells were placed in 96-well flat bottom titer plates and incubated at 37 °C for 24 h. After incubation, a different concentration of curcumin analogs was added and incubated at the same temperature for 48 h. Twenty microliter of MTT solution (5 mg/mL) was added 4 h before termination of incubation period. After incubation, the plate was centrifuged and the remaining solution was removed from each well. One hundred microliters of DMSO (dimethyl sulfoxide) was added to each well to dissolve the formazan crystals. Plates were placed on a plate shaker for 30 min to ensure adequate dissolution. The optical density of each well was measured in ELISA reader at 540 nm for assessing the cell viability.

4.5.2. Tumor reducing activity. Dalton's lymphoma ascites tumor cells (10^6) were injected into the mice peritoneal cavity and two different concentrations of the drug (6 mice/group) were injected from day-1 to day-5 every day. Animals were observed for the development as ascites tumor and death due to tumor burden. Life span % ILS = $T-C/C \times 100$, where T is the number of days the treated animals survived and C is the number of days control animal survived. % ILS more than 25% was considered as significant.[6]

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