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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of curcumin and ethylcurcumin bioconjugates as potential antitumor agents

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Abstract Some new curcumin and ethylcurcumin bioconjugates with various functionalities supported on the curcumin skeleton were synthesized and evaluated for antitumor activity. Most of the newly synthesized compounds are more active than curcumin and ethyl curcumin but are less cytotoxic than the reference compound doxorubicin. Surprisingly, many of these compounds are not cytotoxic to noncancer cells. Compounds **5c**, **5e**, **5g**, **5j**, **6b**, and **6g** having 5-methylthiadiazole, 6-methoxy-benzothiazole, diethylaminoethyl and the usual alkylating bis(2-chloroethyl)amino moieties showed the highest cytotoxic activity against SK-MEL cancer cells. Compounds **5k**, **6c**, and **6g** are less cytotoxic to KB cancer cells. Moreover, compounds **5c**, **5e**, **5j**, **5k**, **6d**, **6e**, **6f**, and **6g** showed cytotoxicity against BT-549 cancer cells with **5j** being the most active compound. Curcumin and the new intermediate di-*O*-chloroacetylcurcumin (**3a**) were also cytotoxic against the same cell line but are less active than the target compounds. Compound **6b** is the only one exhibiting cytotoxicity against SK-OV-3 cancer cells.

Keywords Curcumin derivatives · Curcumin and ethylcurcumin bioconjugates · Antitumor activity

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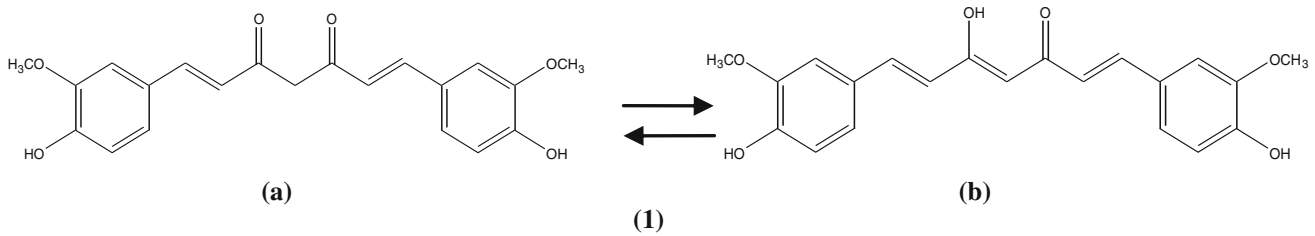
Introduction

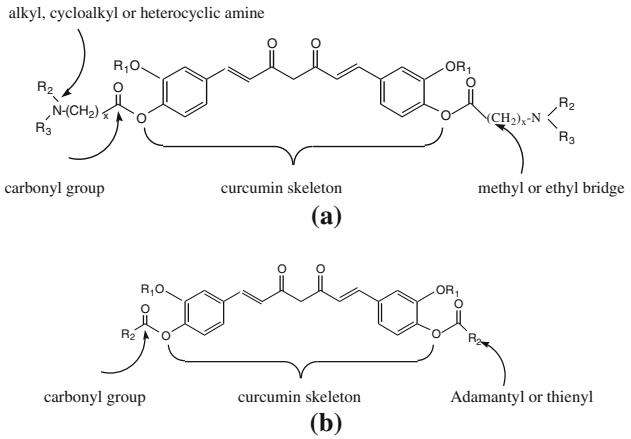
Curcumin (**1**), [diferuloylmethane, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadi-ene-3,5-dione], is a well-known acyclic diarylheptanoid which has been identified as the major constituent of turmeric powder extracted from the rhizome of the plant *curcuma longa* (Anderson *et al*., 2000). Recently, numerous studies have demonstrated the remarkable antioxidant and free radical scavenging activities of curcumin (Mukhopadhyay *et al*., 1982; Sharmam, 1976; Srimal and Dhawan, 1973; Rao *et al*., 1982; Kunchandy and Rao, 1990; Soudamini and Kuttan, 1989; Youssef *et al*., 2004; Al-Omar *et al*., 2005). Also, it has long been used as a natural occurring medicine for the treatment of inflammatory diseases (Toda *et al*., 1985; Jovanovic *et al*., 2001). In addition, several studies have shown that curcumin and curcumin derivatives possess antiproliferative activities against tumor cells in vitro (Mehta *et al*., 1997; Al-Omar *et al*., 2005). Curcumin is a potent inhibitor of tumor initiation in vivo (Huang *et al*., 1988, 1994, 1997) and is also a potent chemopreventive agent inhibiting tumor promotion in skin, oral, intestinal, and colon carcinogenesis (Huang *et al*., 1994; Kelloff *et al*., 1994). Besides, it possesses several other biological activities including antibacterial (Kumar *et al*., 2001), antiviral (Mazumder *et al*., 1995), antihepatotoxic (Kiso *et al*., 1983; Lin *et al*., 1998), hypotensive (Rajakrishnan *et al*., 1999), and anticholesterolemic (Masuda *et al*., 2001) activities.

Curcumin, having an unique conjugated structure including two methoxylated phenols (**1a**) and an enol form of β-diketone (**1b**), shows a typical radical trapping ability as a chain breaking antioxidant (see Scheme 1). The antioxidant mechanism of curcumin and curcumin-related phenols, also found in edible or medicinal plants, has attracted much attention (Roughley and Whiting, 1973; Rao *et al*., 1982;



Scheme 1

Ruby *et al*., 1995; Sreejayan and Rao, 1994; Khopde *et al*., 1999; Jovanovic *et al*., 1999; Snyder and Arnone, 2002);. Accordingly, it became of interest to synthesize some new bioconjugates with various functionalities supported on the curcumin skeleton to be evaluated for anticancer activity. Curcumin bioconjugates can serve a dual purpose of systemic delivery as well as therapeutic agents against cancer diseases. This design would allow enzyme-mediated transformation of the bioconjugate within the target organ. The selected moieties involved in such structural modification featured sulfonamide, alkyl, cycloalkyl, and heterocyclic amino functionalities attached to curcumin or ethyl curcumin through an acetyl or propionyl bridge to investigate the effect of molecular modification on the biological activity of compounds (**A**) (see Structure 1). Furthermore, we were motivated to select adamantoyl chloride, heptanoyl chloride, and 2-thienoyl chloride and directly attach them through an ester function to the curcumin and ethyl curcumin core to furnish compounds (**B**). The conjugate bonds reported herein are ester or amino ester linkages which are enzyme sensitive to produce the expected systemic delivery. Recently, curcumin, having a planar topology, has been shown to inhibit topoisomerase II in a similar fashion to the antineoplastic agent etoposide (Snyder *et al*., 2002). Results pointed to DNA damage induced by topoisomerase II poisoning as a possible mechanism by which curcumin initiated apoptosis. With the hope to go a step forward in the field of anticancer agents, the synthesized compounds were screened for their cytotoxic activity as well as topoisomerase inhibitory activity. The synthesis of the target compounds is outlined in Schemes 2, 3, and 4.



Structure 1

Experimental part

Synthesis

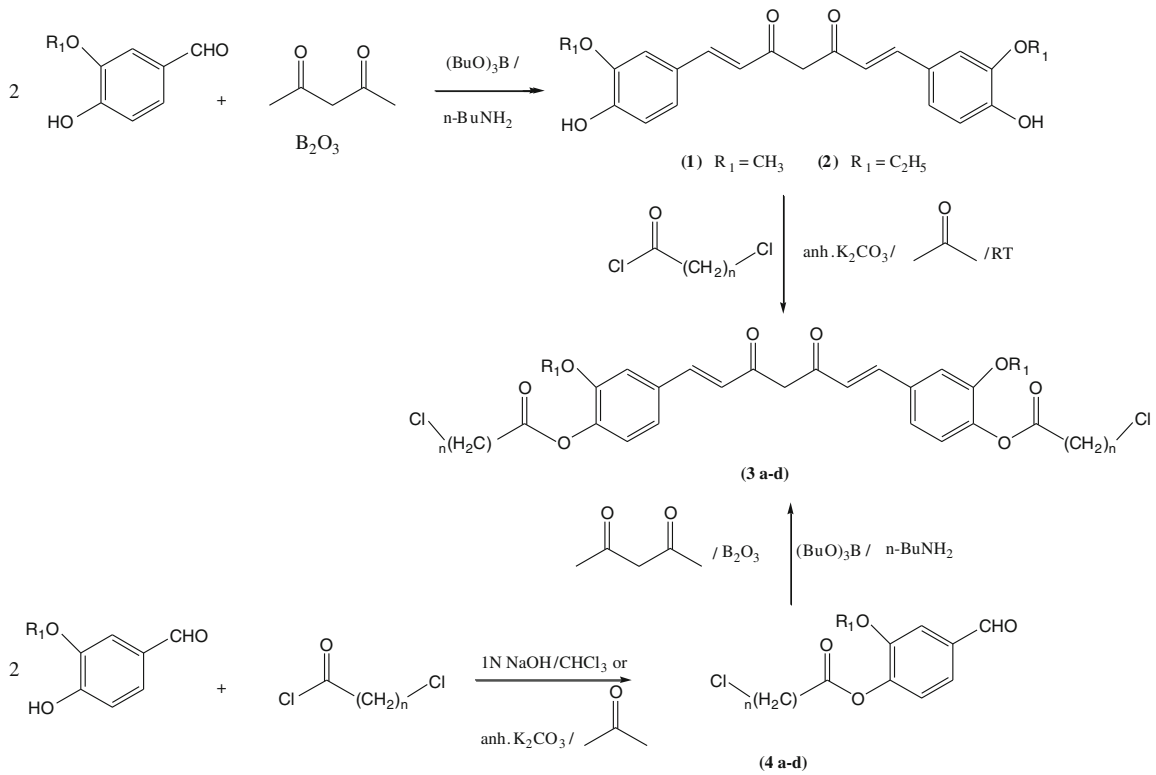
Melting points were determined in open glass tubes on a Branstead/Electrothermal IA9100 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded, for potassium bromide disks, ν (cm^{-1}) on Perkin Elmer 1430 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined on Jeol (300 MHz, 500 MHz) and Ultrashield Bruker Biospin (500 MHz) spectrometers. Chemical shifts are expressed as δ values (ppm) using tetramethylsilane (TMS) as internal reference. Signals are indicated by the following letters: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra (MS) were obtained on GC/MS QP 5000 (Ver. 2), Class-5000 (Ver. 1.2) Shimadzu apparatus. Follow up of the reaction and checking the homogeneity of the compounds were made by ascending thin layer chromatography (TLC) run on precoated (0.25 mm) (GF 254) silica gel plates. The ratio of the solvent systems used as eluents were volume to volume. Visualization of the spots was performed by exposure to UV lamp at 254 nm. Silica gel (60-230 mesh E. Merck) was employed for routine column chromatography separations.

According to Scheme 2, the key starting materials, commercially available, curcumin (**1**) and ethyl curcumin (**2**) were either purchased from Sigma-Aldrich (St. Louis, MO) or prepared according to previously reported methods (Mohri *et al*., 2003).

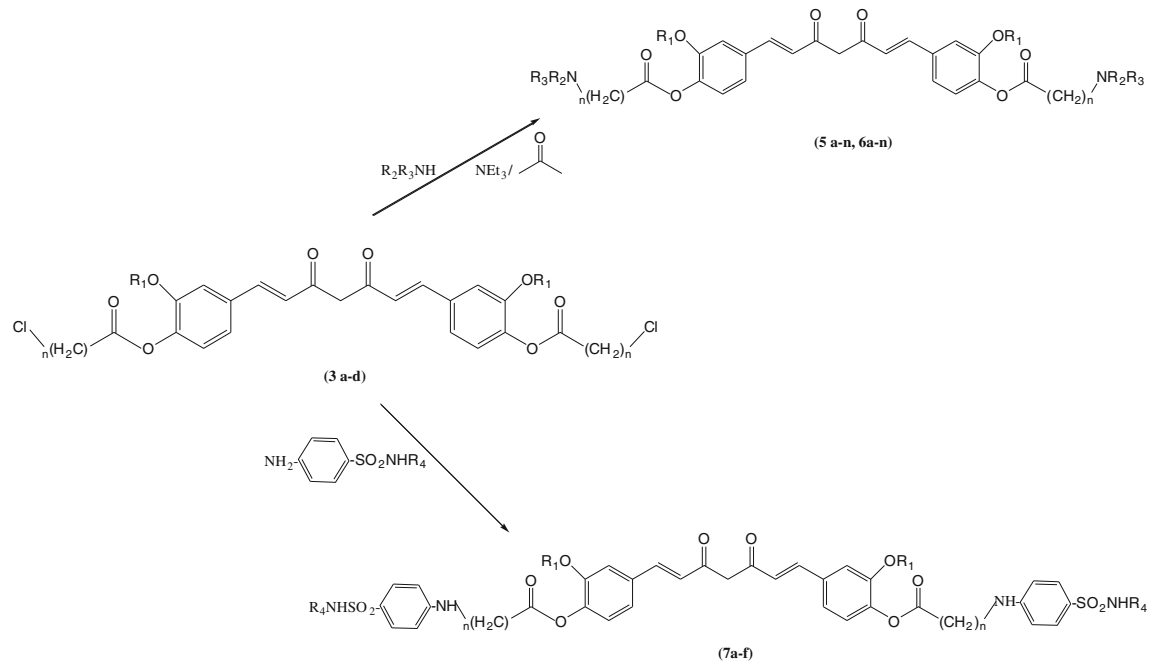
Di-*O*-chloroacetylcurcumin (**3a**) and di-*O*-chloropropionylcurcumin (**3b**)

Method A

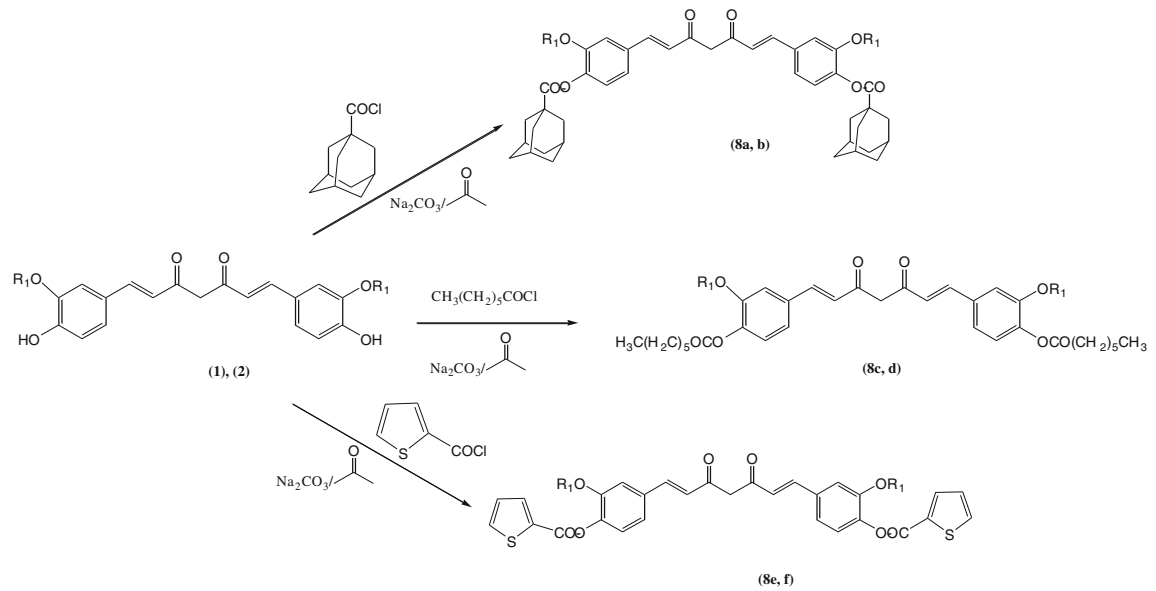
Sodium carbonate (2 g) was added to a stirred ice-cold solution of curcumin (**1**) (3.68 g, 0.01 mol) in dry acetone



Scheme 2 Preparation of Di-*O*-chloroacylcurcumin and di-*O*-chloroacylethylcurcumin (**3a**–**d**)

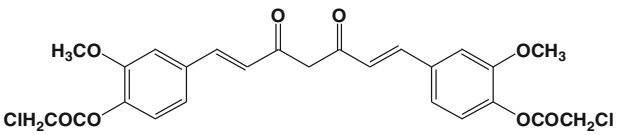
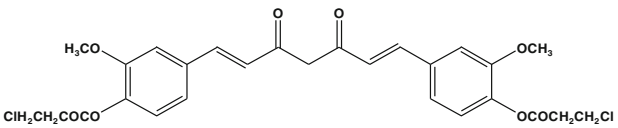


Scheme 3 Preparation of 1,7-Bis(4-alkyl(cycloalkyl or heteroaryl)-aminoacyloxy)-3-(methoxyphenyl)-1,6-hepta-diene-3,5-dione (**5a**–**n**) and 1,7-bis(4-alkyl (cycloalkyl or heteroaryl)aminoacyloxy)-3-(ethoxyphenyl)-1,6-hepta- diene-3,5-dione (**6a**–**n**) and preparation of 1, 7-Bis(4-(4-substituted sulfanilamido)acyloxy)-3-(methoxyphenyl)-1, 6-heptadiene-3,5-dione (**7a**, **b**) and 1,7-bis(4-(4-substituted sulfanilamido)acyloxy)-3-(ethoxy-phenyl)-1,6-heptadiene-3,5-dione (**7c**–**f**)



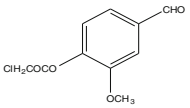
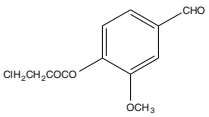
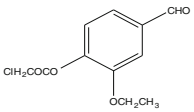
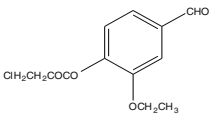
Scheme 4 Preparation of Di-*O*-adamantoylcurcumin (**8a**), di-*O*-adamantoylethyl curcumin (**8b**, Di-*O*-heptanoylcurcumin (**8c**) and di-*O*-heptanoylethyl curcumin (**8d**) and Di-*O*-(2-thienoyl)curcumin (**8e**) and di-*O*-(2-thienoyl)ethyl curcumin (**8f**)

Table 1 Physicochemical data of di-*O*-chloroacetylcurcumin (**3a**) and di-*O*-chloropropionylcurcumin (**3b**)

Compds no.	Structures	Yield%	M.p. °C	Molecular formula	M. wt.
3a		43	136	$C_{25}H_{22}Cl_2O_8$	521.5
3b		38	143	$C_{27}H_{26}Cl_2O_8$	549.5

(50 ml) for 15 min. Chloroacetyl chloride or chloropropionyl chloride (0.025 mol) was dropwise added and the mixture was stirred for 3 days at RT. The reaction mixture was filtered, evaporated, and extracted with EtOAc (3 × 30 ml). The organic layer was dried over anhydrous $MgSO_4$ (5 g) and evaporated under reduced pressure to give an orange oil. Addition of EtOH (20 ml) gave a yellow precipitate which was filtered off. The product was purified by column chromatography using toluene/MeOH (97.5:2.5 v/v) as eluent (Table 1). IR of di-*O*-chloroacetylcurcumin (**3a**) v (cm^{-1}): 3414.8 (OH, intramolecularly H-bonded), 1768.7 (C=O, ester), 1635.7 (C=O, ketone), 1599.9, 1507.6 (C=C Ar), 1254.8, 1122.4 (v_{as} and v_s C–O–C). ^{1}H-NMR of **3a** ($CDCl_3$) δ ppm (300 MHz): 3.88 (s, 6H, 2 OCH_3), 4.10 (s, 4H, 2 CH_2), 5.86 (s, 2H, H_a), 6.57 (d, dist, 2H, $2H_b$, $J = 15.9$ Hz), 7.08–7.26 (m, 6H, 2 (3 Ar–H)), 7.61 (d, dist, 2H, $2H_c$, $J = 15.7$ Hz). IR of di-*O*-chloropropionylcurcumin (**3b**) v (cm^{-1}): 3414.2 (OH, intramolecularly H-bonded), 1746.6, (C=O, ester), 1635.1 (C=O, ketone), 1607.4, 1506.4 (C=C Ar), 1253, 1129.3 (v_{as} and v_s C–O–C). ^{1}H-NMR of **3b** (DMSO-d_6) δ ppm (300 MHz): 3.12 (t, 4H, 2 CH_2–Cl, $J = 6.1$ Hz), 3.84 (s, 6H, 2 OCH_3), 3.89 (t, 4H, 2 $COCH_2$, $J = 6.33$ Hz), 6.2156 (s, 2H, H_a), 7.0095 (d, dist, 2H, 2 × H_b, $J = 15.9$ Hz), 7.15–7.53 (m, 6H, 2 × 3 Ar–H), 7.65 (d, dist, 2H, 2 × H_c, $J = 15.9$ Hz); MS of **3b**: *m/z* (% relative abundance): M^+ 549.5 (absent), 73 (100).

Table 2 Physicochemical data of the synthesized compounds **4a–d**

Compds. no.	Structures	Method A reaction time (h)	Method B reaction time (h)	Yield% A	Yield% B	M.p. °C	Molecular formula	M. wt.
4a		3	24	43	78	68–71	$C_{10}H_9ClO_4$	228.5
4b		24	3	41	72	65–69	$C_{11}H_{11}ClO_4$	242.5
4c		24	3	42	67	61–64	$C_{11}H_{11}ClO_4$	242.5
4d		24	3	39	61	55–59	$C_{12}H_{13}ClO_4$	256.5

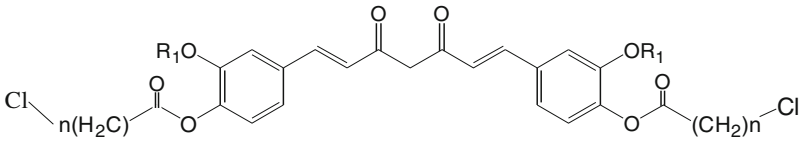
Method B

*3-Alkoxy-4-chloroacyloxybenzaldehyde (**4a–d**)* The appropriate acid chloride (0.01 mol) in $CHCl_3$ (20 ml) was added dropwise over a period of 5 min to an ice-cold stirred solution of the aldehyde (0.01 mol) and 1 N NaOH (2 ml) in chloroform (5 ml). The mixture was stirred at RT for 1 day and then evaporated to dryness. The residue was extracted (3 × 20 ml) with EtOAc and 0.1 N NaOH. The combined organic layers were washed with water (3 × 20 ml), dried over anhydrous $MgSO_4$ (5 g) and removed under reduced pressure to give a colorless oil. Trituration of the crude product with EtOH (20 ml) gave colorless crystals of **4a–d** which were crystallized from EtOH (Table 2).

The selected acid chloride (0.01 mol) was dropwise added to an ice-cold stirred solution of the appropriate aldehyde (0.01 mol) in dry acetone and anhydrous K_2CO_3. The mixture was further stirred in an ice bath for 3 h, filtered, and concentrated. Dilution with EtOH (20 ml) precipitated colorless crystals of **4a–d** which were filtered and crystallized from EtOH (Table 2). IR of **4a** ν (cm^{-1}): 2933.4, 2847 (CHO), 1765 (C=O ester), 1698 (C=O aldehyde), 1600, 1505 (C=C aromatic), 1180, 1150, 1100, 1020 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **4a** (DMSO-d_6) δ ppm (500 MHz): 3.84 (s, 3H, OCH_3), 4.72 (s, 2H, CH_2–Cl), 7.39 (d, 1H, H_A, $J_{AB} = 8.40$, *ortho* coupling), 7.56, 7.58 (dd, 1 H, H_B, $J = 1.55$ and 7.65 Hz, *meta* and *ortho* coupling), 7.61 (d, 1H, H_X, $J_{AX} = 1.55$ Hz, *para* coupling), 9.95 (s, 1H, CHO). IR of **4b** ν (cm^{-1}): 3073, 2940 (CHO), 1760 (C=O ester), 1698 (C=O aldehyde), 1601, 1495 (C=C aromatic), 1154, 1120, 1068, 1025 (ν_{as} and ν_s C–O–C). ^{1}H-NMR of **4c** (DMSO-d_6) δ ppm (300 MHz): 1.31 (t, 3H, OCH_2CH_3, $J = 6.87$ Hz), 4.16 (q, 2H, OCH_2CH_3, $J = 6.87$ Hz), 4.74 (s, 2H, CH_2Cl), 7.41 (d, 1H, H_A, $J_{AB} = 7.98$ Hz, *ortho* coupling), 7.58, 7.6051 (dd, 1H, H_B, $J = 1.65$ and 8.07 Hz, *meta* and *ortho* coupling), 7.63 (d, 1H, H_X, $J_{AX} = 1.63$ Hz, *para* coupling), 9.97 (s, 1H, CHO).

*Di-O-chloroacylcurcumin (**3a**, **b**) and di-O-chloroacylethylcurcumin (**3c**, **d**)* To a stirred solution of the appropriate 3-alkoxy-4-chloroacyloxybenzaldehyde (**4a–d**) (0.04 mol) in dry EtOAc (20 ml) was added tri-(*n*-butyl) borate (21 ml, 0.08 mol) and the mixture was stirred at RT for 10 min. The previously prepared complex formed by stirring for 1 h acetylacetone (2 g, 0.02 mol) with boric anhydride (1 g, 0.014 mol) was then added to this solution. After stirring for 5 min at RT, *n*-butylamine (0.1 ml) was dropwise added every 10 min (total amount 0.4 ml) and the reaction mixture was stirred at RT for an overnight. 0.4 N HCl (30 ml, 60°C) was then added and the mixture was further stirred for 2 h followed by extraction with EtOAc (3 × 25 ml). The combined organic layers were washed with H_2O, dried over anhydrous $MgSO_4$ (5 g) and concentrated to $\simeq$15 ml. MeOH (15 ml) was added and the mixture was allowed to stand in the refrigerator for 3 h to give a yellow precipitate of **3a–d** which was filtered off, washed with cold MeOH, and dried (Table 3). IR and ^{1}H-NMR spectra of compounds **3a**, **b** were coinciding with those obtained from method A. IR of **3c** ν (cm^{-1}): 3435.6 (OH, intramolecularly H-bonded), 1746 (C=O, ester), 1630.9 (C=O, ketone), 1558.1, 1496.3 (C=C aromatic), 1270, 1157 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **3c** ($CDCl_3$) δ ppm (300 MHz): 1.42 (t, 6H, 2 OCH_2CH_3, $J = 6.87$ Hz),

Table 3 Physicochemical data of the synthesized compounds **3a–d**



(3a-d)

(**3a–d**)

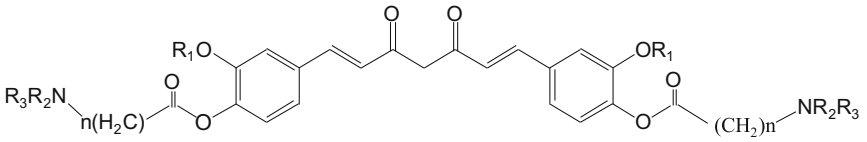
Compds. no.	R_1	*n*	Yield%	M. p. °C	Molecular formula	M. wt.
3a	CH_3	1	61	136	$C_{25}H_{22}Cl_2O_8$	521.5
3b	CH_3	2	56	143	$C_{27}H_{26}Cl_2O_8$	549.5
3c	C_2H_5	1	59	130	$C_{27}H_{26}Cl_2O_8$	549.5
3d	C_2H_5	2	54	145	$C_{29}H_{30}Cl_2O_8$	577.5

4.11 (q, 4H, $2OCH_2CH_3$, $J = 6.87$ Hz), 4.33 (s, 4H, 2 CH_2–Cl), 5.85(s, 2H, H_a), 6.56 (d, 2H, $2H_b$, $J = 15.93$ Hz), 7.08–7.18 (m, 6H, 2 (3-Ar–H)), 7.61 (d, 2H, $2H_c$, $J = 15.93$ Hz); MS of **3c** *m/z* (% relative abundance): M^+ 548 (0.24), M^+ +2 550 (0.21), M^+ +4 552, 83 (100). ^{1}H-NMR of **3d** ($CDCl_3$) δ ppm (500 MHz): 1.44 (t, 6H, 2 × OCH_2CH_3, $J = 7$ Hz), 3.10 (t, 4H, 2 $COCH_2$, $J = 7$ Hz), 3.90 (t, 4H, 2 CH_2–Cl, $J = 7$ Hz), 4.13 (q, 4H, 2 OCH_2CH_3, $J = 7$ Hz), 5.87 (s, 1H, H_a), 6.57 (d, 2H, 2 H_b, $J = 16$ Hz), 7.09 (d, 2H, 2 H_A, $J_{AB} = 8$ Hz, *ortho* coupling), 7.17 (d, 2H, 2 H_B, $J_{AB} = 8.5$ Hz, *ortho* coupling), 7.28 (s, 2H, 2 H_X), 7.55 (d, 2H, 2 H_c, $J = 16$ Hz), 9.96 (s, 1H, enol OH).

*1,7-Bis(4-alkyl(cycloalkyl or heteroaryl)aminoacyloxy)-3-(methoxyphenyl)-1,6-hepta-diene-3,5-dione (**5a–n**) and 1,7-bis(4-alkyl (cycloalkyl or heteroaryl)aminoacyloxy)-3-(ethoxyphenyl)-1,6-hepta-diene-3,5-dione (**6a–n**)*

NEt_3 (five drops) were added to a solution of di-*O*-chloroacylcurcumin **3a**, **b** or di-*O*-chloroacylethyl curcumin **3c**, **d** (0.005 mol) in EtOH (30 ml). The appropriate amine (0.01 mol) was then added and the mixture was heated under reflux for the specified time (Table 4). EtOH was evaporated under reduced pressure and the residue was crystallized from $CHCl_3$ (15 ml) giving compounds **5a–n** and **6a–n** (Table 4). ^{1}H-NMR of **5a** (DMSO-d_6) δ ppm (300 MHz): 1.16–1.99 (hump, br, 30H, 2 Ad-H), 2.09 (s, 4H, 2 CO–CH_2), 3.83 (s, 6H, 2 OCH_3), 6.03 (s, br, 2H, H_a), 6.74 (d, 2H, 2 H_b, $J = 15.66$ Hz), 6.80 (d, 2H, 2 H_A, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.13, 7.16 (dd, dist, 2H, 2 × H_B, $J = 1.5$ and 8.22 Hz, *meta* and *ortho* coupling), 7.31 (s, dist, 2H, 2 NH), 7.17 (d, 2H, 2 H_X, $J_{AX} = 0.24$ Hz, *para* coupling), 7.53 (d, 2H, 2 × H_c, $J = 15.66$ Hz); MS of **5a** *m/z* (% abundance): M^+ 750 (absent), 135 (100). IR of **5b** ν (cm^{-1}): 3550, 3414.4 (NH + OH intramolecular H-bonded), 1733.7 (C=O, ester), 1637.4 (C=O, ketone), 1618 (C=N mixed with C=C aromatic), 1510.2 (C=C, aromatic), 1272.1 1122.9 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **5b** (DMSO-d_6) δ ppm (300 MHz): 2.09 (s, 4H, 2 CH_2), 3.84 (s, 12H, 4 OCH_3), 6.06 (s, 2H, H_a), 6.77 (d, 2H, 2 H_b, $J = 15.66$ Hz), 6.82 (d, 2H, 2 H_A, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.14, 7.17 (dd, 2H, 2 H_B, $J = 1.65$ and 8.25 Hz, *meta* and *ortho* coupling), 7.33 (d, 2H, H_X, $J_{AX} = 1.65$ Hz, *para* coupling), 7.55 (d, 2H, 2 H_c, $J = 15.66$ Hz, overlapping with benzothiazole multiplet), 7.53–7.61 (m, 6H, 2 (3-benzothiazole-H)), 9.69 (s, 2H, 2 NH, D_2O-exchangeable); MS of **5b** *m/z* (% abundance): M^+ 808 (absent), 125 (100). IR of **5c** ν (cm^{-1}): 3425 (NH + OH intramolecularly H-bonded), 1727.3 (C=O, ester), 1630 (C=O, ketone mixed with C=N), 1575, 1506 (C=C aromatic), 1272.5, 1120.7 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **5c** (DMSO-d_6) δ ppm (300 MHz): 2.50 (s, 4H, 2 × CH_2), 3.35 (s, 6H, 2 CH_3), 3.84 (s, 6H, 2 OCH_3), 6.06 (s, 2H, H_a), 6.76 (d, 2H, 2 H_b, $J = 15.93$ Hz), 6.82 (d, 2H, 2 H_A, $J_{AB} = 8.22$ Hz, *ortho* coupling), 7.14, 7.17 (dd, 2H, 2 H_B, $J = 1.65$ and 8.01 Hz, *meta* and *ortho* coupling), 7.33 (d, 2H, 2 H_X, $J_{AX} = 1.65$ Hz), 7.55 (d, 2H, 2 H_c, $J = 15.66$ Hz), 9.69 (s, 2H, 2 × NH, D_2O-exchangeable). ^{1}H-NMR of **5d** (CD_3OD) δ ppm (500 MHz): 2.29 (s, 2H, 2 NH), 2.31 (t, dist, 4H, 2 × CH_2–N), 3.23 (t, 4H, 2 CH_2–Cl, $J = 3.5$ Hz), 3.33 (s, 4H, 2 × $COCH_2$–N), 3.92 (s, 6H, 2 × OCH_3), 4.90 (s, 2H, 2 × H_a), 6.64 (d, 2H, 2 H_b, $J = 15.5$ Hz), 6.84 (d, 2H, 2 H_A, $J_{AB} = 8$ Hz, *ortho* coupling), 7.12 (d, 2H, 2 H_B, 7.7 Hz, *ortho* coupling), 7.23 (s, 2H, 2 H_X), 7.58 (d, 2H, 2 × H_c, $J = 15.5$ Hz). IR of **5e** ν (cm^{-1}): 3414.8 (OH intramolecularly H-bonded), 1728.4 (C=O, ester), 1637.6 (C=O, ketone), 1618.1, 1511 (C=C aromatic), 1272.6, 1122.9 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **5e** (DMSO-d_6) δ ppm (300 MHz): 2.09 (s, 4H, 2 $COCH_2$–N), 2.50 (t, 8H, 4N-CH_2CH_2–Cl, $J = 3.57$ Hz), 3.48 (t, 8H, 4 NCH_2CH_2–

Table 4 Physicochemical data of the synthesized compounds **5a–n** and **6a–n**



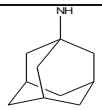
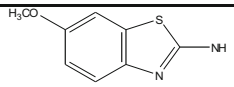
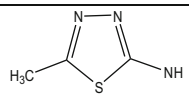
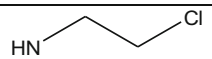
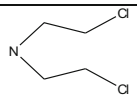
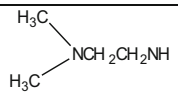
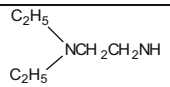
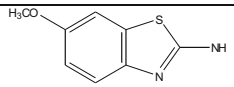
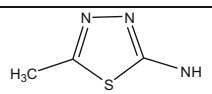
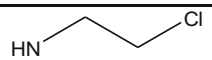
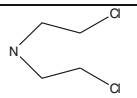
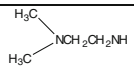
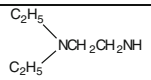
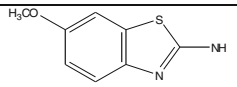
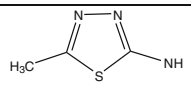
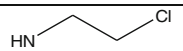
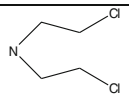
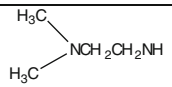
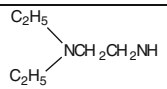
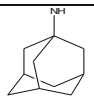
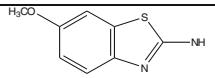
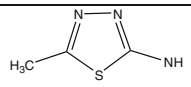
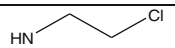
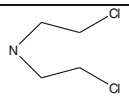
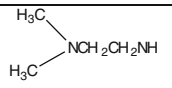
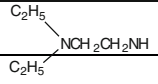
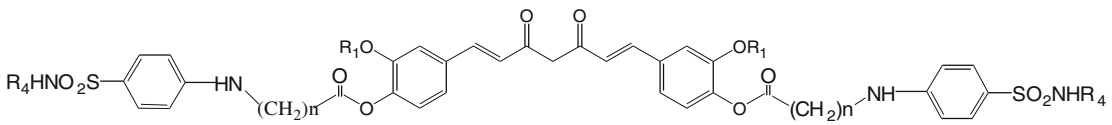
Compd. no.	R_1	*n*	R_2 R_3	Reaction time (h)	Yield%	M. p. (°C)	Molecular formula	M. wt.
5a	$-CH_3$	1		12	86	110–115	$C_{45}H_{54}N_2O_8$	750
5b	$-CH_3$	1		12	59	160–163	$C_{41}H_{36}N_4O_{10}S_2$	808
5c	$-CH_3$	1		24	63	170–173	$C_{31}H_{30}N_6O_8S_2$	678
5d	$-CH_3$	1		6	83	87–93	$C_{29}H_{32}Cl_2N_2O_8$	607
5e	$-CH_3$	1		4–5	95	85–90	$C_{33}H_{38}Cl_4N_2O_8$	732
5f	$-CH_3$	1		1	69	160–165	$C_{33}H_{44}N_4O_8$	624
5g	$-CH_3$	1		1	62	150–155	$C_{37}H_{52}N_4O_8$	680
5h	$-CH_3$	2		12	69	140	$C_{47}H_{58}N_2O_8$	778
5i	$-CH_3$	2		36	47	170–174	$C_{43}H_{40}N_4O_{10}S_2$	836
5j	$-CH_3$	2		36	43	120–123	$C_{33}H_{34}N_6O_8S_2$	706
5k	$-CH_3$	2		36	71	90–95	$C_{31}H_{36}Cl_2N_2O_8$	635
5l	$-CH_3$	2		36	74	87–93	$C_{35}H_{42}Cl_4N_2O_8$	760

Table 4 continued

5m	$-CH_3$	2		3–4	66	160–165	$C_{35}H_{48}N_4O_8$	652
5n	$-CH_3$	2		3–4	69	155–160	$C_{39}H_{56}N_4O_8$	708
6a	CH_2CH_3	1		4–5	71.5	115–120	$C_{47}H_{58}N_2O_8$	778
6b	CH_2CH_3	1		12	66	137–140	$C_{43}H_{40}N_4O_{10}S_2$	836
6c	CH_2CH_3	1		12	72	105–110	$C_{33}H_{34}N_6O_8S_2$	706
6d	CH_2CH_3	1		9	56	107–110	$C_{31}H_{36}Cl_2N_2O_8$	635
6e	CH_2CH_3	1		9	65	95–100	$C_{35}H_{42}Cl_4N_2O_8$	760
6f	CH_2CH_3	1		6	67	115–120	$C_{35}H_{48}N_4O_8$	652
6g	CH_2CH_3	1		6	69	105–110	$C_{39}H_{56}N_4O_8$	708
6h	CH_2CH_3	2		6	67	85–90	$C_{49}H_{62}N_2O_8$	806
6i	CH_2CH_3	2		16	58	165–170	$C_{45}H_{44}N_4O_{10}S_2$	864
6j	CH_2CH_3	2		36	79	105–110	$C_{35}H_{38}N_6O_8S_2$	734
6k	CH_2CH_3	2		12	91	115–120	$C_{33}H_{40}Cl_2N_2O_8$	663
6l	CH_2CH_3	2		12	64	125–130	$C_{37}H_{46}Cl_4N_2O_8$	788
6m	CH_2CH_3	2		3–4	89	155–160	$C_{37}H_{52}N_4O_8$	680
6n	CH_2CH_3	2		3–4	81	155–160	$C_{41}H_{60}N_4O_8$	736

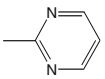
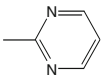
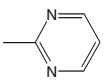
Cl, overlapping with solvent signal), 3.84 (s, 6H, 2 OCH_3), 6.06 (s, 1H, H_a), 6.77 (d, 2H, 2 H_b, $J = 15.9$ Hz), 6.83 (d, 2H, 2 H_A, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.14, 7.16 (dd, 2H, 2 H_B, $J = 1.65$ and 8.37 Hz, *meta* and *ortho* coupling), 7.33 (d, 2H, 2 H_X, $J_{AX} = 1.38$ Hz, *para* coupling), 7.57 (d, 2H, 2 H_c, $J = 15.9$ Hz), 9.69 (s, br, 1H, enol OH). ^{1}H-NMR of **5f** (DMSO-d_6) δ ppm (300 MHz): 3.17 (s, 12H, 2N(CH_3)$_2$), 3.54 (t, dist, 4H, 2 CH_2–N), 3.67 (t, 4H, 2 CH_2–NH, $J = 5.49$ Hz), 3.80 (s, dist, 2H, 2 NH, overlapping with OCH_2), 3.83 (s, 4H, 2 $COCH_2$–N), 4.06 (s, 6H, 2 OCH_3), 6.06 (s, 1H, H_a), 6.76 (d, 2H, 2 H_b, $J = 15.75$ Hz), 6.83 (d, 2H, 2 H_A, $J_{AB} = 8.07$ Hz, *ortho* coupling), 7.15 (d, 2H, 2 H_B, $J = 8.07$ Hz, *ortho* coupling), 7.32 (s, 2H, 2 H_X), 7.54 (d, 2H, 2 H_c, $J = 15.75$ Hz), 9.74 (s, 1H, enol H). ^{1}H-NMR of **5h** (CD_3OD) δ ppm (500 MHz): 1.18–2.17 (hump, br, 30H, 2 Ad-H), 2.631 (t, dist, 4H, 2 × CH_2–N, $J = 6.2$ Hz), 3.064 (t, 4H, 2 CO–CH_2, $J = 6.5$ Hz), 3.88 (s, 2H, 2 NH overlapping with OCH_3 signal), 3.92 (s, 6H, 2 OCH_3), 4.94 (s, 2H, H_a), 6.63 (d, 2H, 2 H_b, $J = 15.7$ Hz), 6.84 (d, 2H, 2 H_A, $J_{AB} = 8$ Hz, *ortho* coupling), 7.11 (d, 2H, 2 H_B, $J = 7.8$ Hz, *ortho* coupling), 7.22 (d, 2H, 2 H_X), 7.58 (d, 2H, 2 H_c, $J = 15.8$ Hz); ^{13}C-NMR of **5h** (CD_3OD) δ ppm (500 MHz): 39.51 (2 CH_2N), 40.48 (2 CH_2CO), 47.10, 47.27, 47.44, 47.61, 47.78, 47.90, 47.95, 48.07, 48.12, 48.24 (2 (10 Ad-C)), 55.07 (2 OCH_3), 101.37 (C-4), 110.27 (2 C-2′), 115.04 (2 C-5′), 120.79 (2 C-6′), 122.77 (2 C-1′), 127.07 (C-2 + C-6), 129.77 (C-1 + C-7), 140.94 (2 C-3′), 148.06 (2 C-4′), 149.25 (C-3 + C-5), 183.23 (2 CO); MS of **5h** *m/z* (% relative abundance): M^+ at 778 (absent), 45 (100). IR of **5j** ν (cm^{-1}): 3435.7 (NH + OH intramolecularly H-bonded), 1790.6 (C=O, ester), 1658.9 (C=O, ketone), 1558 (C=N mixed with C=C aromatic), 1498.8 (C=C aromatic), 1273.2, 1169.9 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **5j** (CD_3COCD_3) δ ppm (500 MHz): 3.61 (s, 6H, 2 × CH_3), 3.91 (s, 6H, 2 OCH_3), 4.08 (t, dist, 4H, 2 CH_2–N), 4.61 (t, dist, 4H, 2 CH_3–CO), 5.79 (s, br, 2H, H_a), 6.70 (d, 2H, 2 H_b, $J = 15.5$ Hz), 6.89–7.68 (m, 6H, 2 Ar-H), 7.74 (d, 2H, 2 H_c, $J = 15.5$ Hz), 8.32 (s, 2H, 2 NH); ^{13}C-NMR of **5j** (CD_3COCD_3) δ ppm (500 MHz): 45.35 (2 CH_2N), 47.25 (2 CH_2CO), 60.85 (2 OCH_3), 105.75 (C-4), 111.80 (2 C-2′), 116.85 (2 C-5′), 120.0 (2 × C-6′), 123.75 (2 × C-1′), 128.82 (C-2 + C-6), 134.93 (C-1 + C-7), 142.00 (2 C-3′), 147.85 (2 C-4′), 148.95 (C-3 + C-5), 170.40 (2 × thiazole C-2), 180.85 (2 × thiazole C-5), 192.75 (2 CO); MS of **5j** *m/z* (% relative abundance): M^+ +1 707 (0.7), 43 (100). ^{1}H-NMR of **6a** ($CDCl_3$) δ ppm (300 MHz): 1.25–2.36 ((hump, br, 30H, 2 × Ad-H), 1.48 (t, 6H, 2 × OCH_2CH_3, $J = 6.87$ Hz), 2.35 (s, 4H, 2 × CH_2CO), 2.63 (s, 2H, 2 × NH), 4.15 (q, 4H, 2 × OCH_2CH_3, $J = 6.87$ Hz), 5.78 (s, 1H, H_a), 6.45 (d, 2H, 2 × H_b, $J = 15.66$ Hz), 6.93 (d, 2H, 2 × H_A, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.03 (s, 2H, 2 × H_X),

Table 5 Physicochemical data of the synthesized compounds (**7a–f**)



(7a-f)

(**7a–f**)

Cpds. No	R_1	*n*	R_4	Reaction time (h)	Yield%	m.p. °C	Molecular formula	M. wt.
7a	CH_3	1	H	16	69	155–60	$C_{37}H_{36}N_4O_{12}S_2$	792
7b	CH_3	1		12	50	175–78	$C_{45}H_{40}N_8O_{12}S_2$	948
7c	C_2H_5	1	H	18	63	143–48	$C_{39}H_{40}N_4O_{12}S_2$	820
7d	C_2H_5	1		24	60	185–90	$C_{47}H_{44}N_8O_{12}S_2$	976
7e	C_2H_5	2	H	18	78	115–20	$C_{41}H_{44}N_4O_{12}S_2$	848
7f	C_2H_5	2		24	57	115–20	$C_{49}H_{48}N_8O_{12}S_2$	1004

7.11 (d, 2H, 2 × H_B, $J = 8.25$ Hz, *ortho* coupling), 7.57 (d, 2H, 2 × H_c, $J = 15.66$ Hz), 9.81 (s, 1H, enol OH).

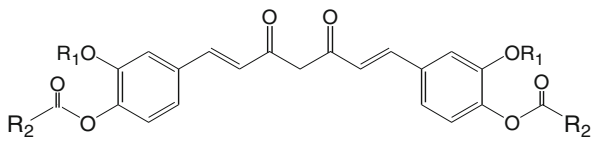
*1,7-Bis(4-(4-substituted sulfanilamido)acyloxy)-3-(methoxyphenyl)-1,6-heptadiene-3,5-dione (**7a, b**) and 1,7-bis(4-(4-substituted sulfanilamido)acyloxy)-3-(ethoxyphenyl)-1,6-heptadiene-3,5-dione (**7c–f**)*

Following the same procedure as for the preparation of compounds **5a–n** and **6a–n**, the appropriate sulfonamide derivative (0.01 mol) was added to a solution of di-*O*-chloroacylcurcumin **3a**, **b** or di-*O*-chloroacylethyl curcumin **3c**, **d** (0.005 mol) in EtOH (30 ml) containing triethylamine (five drops). The mixture was heated under reflux for the specified time (Table 5), EtOH was evaporated under reduced pressure and the residue was purified by elution on a column of silica gel using a mixture of toluene–EtOH (97:3 v/v) to give compounds **7a–f** (Table 5).

*Di-O-adamantoylcurcumin (**8a**) and di-O-adamantoylethyl curcumin (**8b**)*

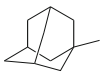
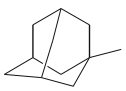
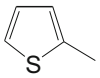
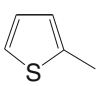
Adamantoyl chloride (0.03 mol) dissolved in the least amount of EtOH (10 ml) was added to a mixture of curcumin (**1**) or ethyl curcumin (**2**) (0.01 mol) and Na_2CO_3 (2 g) in acetone (50 ml). The mixture was heated under reflux for 9–19 h, filtered, and evaporated to dryness. The residue was extracted with EtOAc (3 × 25 ml). The combined organic layer was dried over anhydrous $MgSO_4$ (5 g), filtered, evaporated, and crystallized from EtOH (15 ml) to give a yellow precipitate of **8a**, **b** (Table 6). IR of **8a** ν (cm^{-1}): 3435.8 (OH intramolecularly H-bonded), 1750.4 (C=O, ester), 1630.9 (C=O, ketone), 1599, 1507.4 (C=C aromatic), 1255.2, 1122.8 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **8a** ($CDCl_3$) δ ppm (300 MHz): 1.23–2.19 (hump, br, 30H, 2 Ad-H), 3.85 (s, 6H, 2 OCH_3), 5.85 (s, 2H, H_a), 6.55 (d, 2H, 2 H_b, $J = 15.9$ Hz), 7.01 (d, 2H, 2 × H_A, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.10 (s, 2H, 2 H_X), 7.13, 7.16 (dd, 2H, 2 × H_B, $J = 1.65$ and 8.04 Hz, *meta* and *ortho* coupling), 7.61 (d, 2H, 2 H_c, $J = 15.9$ Hz); ^{13}C-NMR of **8a** ($CDCl_3$) δ ppm (500 MHz): 56.01 (2 OCH_3), 27.88, 27.94, 36.48, 38.73, 38.81, 41.14, 76.79, 77.04, 77.29 (2 Ad-C), 101.75 (C-4), 111.48 (2 C-2′), 121.16 (2 C-5′), 123.32 (2 C-6′), 124.03 (2 C-1′), 129.18 (C-2 + C-6), 140.13 (C-1 + C-7), 141.95 (2 × C-3′), 151.52 (2 × C-4′), 175.60 (C-3 + C-5), 183.16 (2 × CO); MS of **8a** *m/z* (% relative abundance): M^+ 692 (2.1), 135 (100. ^{1}H-NMR of **8b** ($CDCl_3$) δ ppm (300 MHz): 1.25–2.36 (hump, br, 30H, 2 × Ad-H overlapping with t of OCH_2CH_3), 1.48 (t, 6H, 2 × OCH_2CH_3, $J = 6.87$ Hz), 4.16 (q, 4H, 2 × OCH_2CH_3, $J = 6.97$ Hz), 5.78 (s, 1H, H_a), 6.45 (d, 2H, 2 × H_b, $J = 15.66$ Hz), 6.93 (d, 2H, 2 × H_A, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.03 (s, 2H, 2 × H_X), 7.11 (d, 2H, 2 × H_B, $J_{AB} = 8.25$ Hz, *ortho* coupling), 7.57 (d, 2H, 2 × H_c, $J = 15.66$ Hz); 9.81 (s, 1H, enol OH).

Table 6 Physicochemical data of the synthesized compounds **8a–f**



(8a-f)

(**8a–f**)

Cpds. no.	R_1	R_2	Reaction time (h)	Yield%	m.p.	Molecular formula	M. wt.
8a	CH_3		9	47	215	$C_{43}H_{48}O_8$	692
8b	C_2H_5		16	36	220	$C_{45}H_{52}O_8$	720
8c	CH_3	C_6H_{13}	4–5	63	99.5	$C_{35}H_{44}O_8$	606
8d	C_2H_5	C_6H_{13}	4–5	77	128–30	$C_{37}H_{48}O_8$	620
8e	CH_3		4	81.5	199–203	$C_{31}H_{24}O_8S_2$	588
8f	C_2H_5		6	51	217–19	$C_{33}H_{28}O_8S_2$	616

*Di-O-heptanoylcurcumin (**8c**) and di-O-heptanoylethyl curcumin (**8d**)*

Heptanoyl chloride (0.03 mol) was added dropwise to an ice-cold mixture of curcumin (**1**) or ethyl curcumin (**2**) (0.015 mol) and Na_2CO_3 (2 g) in acetone (50 ml). The mixture was stirred at room temperature for 4–6 h, evaporated to dryness and the residue was extracted with EtOAc (2 × 20 ml). The combined organic extracts were dried over anhydrous $MgSO_4$, evaporated, and crystallized from EtOH to give a yellow precipitate of **8c**, **d** (Table 6). IR of **8c** ν (cm^{-1}): 1768 (C=O, ester), 1629 (C=O, ketone), 1598, 1512 (C=C aromatic), 1120, 1010 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **8c** ($CDCl_3$) δ ppm (300 MHz): 0.91 (t, 6H, 2 × $(CH_2)_5$-CH_3, J = 6.96 Hz), 1.30–1.79 (m, 16H, 2 × $(CH_2)_4$), 2.58 (t, 4H, 2 × CH_2CO, J = 7.32 Hz), 3.87 (s, 6H, 2 × OCH_3), 5.85 (s, 2H, H_a), 6.55 (d, 2H, 2 × H_b, J = 15.75 Hz), 7.05 (d, 2H, 2 × H_A, J_{AB} = 8.04 Hz, *ortho* coupling), 7.11, 7.15 (dd, 2H, 2 × H_B, J = 1.8 and 9 Hz, *meta* and *ortho* coupling), 7.27 (s, 2H, 2 × H_X), 7.62 (d, 2H, 2 × H_c, J = 15.75 Hz). IR of **8d** ν (cm^{-1}): 1769 (C=O, ester), 1629 (C=O, ketone), 1590, 1500 (C=C aromatic), 1115, 1020 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **8d** ($CDCl_3$) δ ppm (300 MHz): 0.90 (t, 6H, 2 × $(CH_2)_5$-CH_3, J = 6.96 Hz), 1.33–1.57 (m, 16H, 2 × $(CH_2)_4$), 1.77 (t, 6H, 2 × OCH_2CH_3, J = 7.3 Hz), 2.57 (t, 4H, 2 × CH_2–CO, J = 7.3 Hz), 4.09 (q, 4H, 2 × OCH_2CH_3, J = 6.96 Hz), 5.84 (s, 2H, H_a), 6.54 (d, 2H, 2 × H_b, J = 15.75 Hz), 7.04 (d, 2H, 2 × H_A, J_{AB} = 8.4 Hz, *ortho* coupling), 7.11 (d, 2H, 2 × H_B, J_{AB} = 7.5 Hz, *ortho* coupling), 7.26 (s, 2H, 2 × H_X), 7.60 (d, 2H, 2 × H_c, J = 15.75 Hz); ^{13}C-NMR of **8d** ($CDCl_3$) δ ppm (300 MHz): 14.164, 14.813, 22.592, 25.157, 28.874 (5 peaks for 5Cs of side chain), 31.569 (2 × OCH_2CH_3), 34.157 (2 × –$OCOCH_2C_5H_{11}$), 64.454 (2 × OCH_2CH_3), 101.874 (C-4), 112.477 (2 × C-2′), 121.034 (2 × C-5′), 123.316 (2 × C-6′), 124.133 (2 × C-1′), 133.812 (C-2 + C-6), 140.171 (C-1 + C-7), 141.728 (2 × C-3′), 150.911 (2 × C-4′), 171.743 (C-3 + C-5), 183.216 (2 × CO); Examination of 2D ^{1}H-NMR (COSY) ($CDCl_3$) and ^{1}H, ^{13}C-NMR (C, H correlation) ($CDCl_3$) provided further confirmation for the structure of the compound **8d**.

*Di-O-(2-thienoyl)curcumin (**8e**) and di-O-(2-thienoyl)ethyl curcumin (**8f**)*

Compounds **8e**, **f** (Table 6) were prepared by adding thiophene-2-carbonyl chloride (0.03 mol) adopting the previously mentioned procedure. IR of **8e** ν (cm^{-1}): 3339.6 (OH intramolecularly H-bonded), 1746.7 (C=O, ester), 1672.9 (C=O, ketone), 1606.8, 1494.5 (C=C aromatic), 1278.3, 1122 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **7c** (DMSO) δ ppm (500 MHz): 3.86 (s, 6H, 2 × OCH_3), 6.23 (s, 2H, H_a), 7.051 (d, 2H, 2 × H_b, J = 16 Hz), 7.32, 7.34 (dd, 2H, 2 × H_B, J = 3.5 and 7.8 Hz, *meta* and *ortho* coupling), 7.39 (d, 2H, 2 × H_A, J_{AB} = 7.5 Hz, *ortho* coupling), 7.59 (s, 2H, 2 × H_X), 7.69 (d, 2H, 2 × H_c, J = 16 Hz), 7.86 (d, 2H, 2 × thienyl-5-H, J = 8 Hz), 8.04, 8.06 (dd, 2H, 2 × thienyl-4-H), J = 1 and 4 Hz *ortho* and *meta* coupling), 8.11 (d, 2H, 2 × thienyl-3-H, J = 4 Hz). IR of **8f** ν (cm^{-1}): 3431.1 (OH intramolecularly H-bonded), 1727 (C=O, ester), 1626.5 (C=O, ketone), 1596.7, 1508.7 (C=C aromatic), 1250.7, 1117.2 (ν_{as} and ν_s C–O–C); ^{1}H-NMR of **8d** (DMSO-d_6) δ ppm (300 MHz): 1.23 (t, 6H, 2 × 3 OCH_2CH_3, J = 7.00 Hz), 4.14 (q, 4H, 2 × OCH_2CH_3, J = 7.04 Hz), 6.21 (s, 2H, H_a), 7.02 (d, 2H, 2 × H_b, J = 15.93 Hz), 7.32 (d, 2H, 2 × H_A, J_{AB} = 8.52 Hz, *ortho* coupling), 7.33 (s, 2H, 2 × H_X), 7.67 (d, 2H, 2 × H_c, J = 15.93 Hz), 7.38 (d, dist, 2H, 2 × H_B, J_{AB} = 8.52 Hz, *ortho* coupling), 7.56 (d, dist, 2H, 2 × thienyl-H-4), 8.02 (br, 2H, 2 × thienyl-H-5, J = 3.57 Hz), 8.10 (d, 2H, 2 × thienyl-H-3, J = 3.57 Hz); ^{13}C-NMR of **8f** (DMSO) δ ppm (500 MHz): 40.13 (2 × CH_3), 64.95 (2 × OCH_2), 102.31 (C-4), 113.79 (2 × C-2′), 121.94 (2 × C-5′), 123.94 (2 × C-6′), 125.22 (2 × C-1′), 129.26 (C-2 + C-6), 131.84 (thienyl-C-4), 134.46 (thienyl-C-3), 135.72 (thienyl-C-5), 135.83 (thienyl-C-2), 140.30 (C-1 + C-7), 141.37 (2 × C-3′), 151.02 (2 × C-4′), 159.88 (C-3 + C-5), 183.69 (2 × CO); MS of **8f** *m/z* (% relative abundance): M^+ 616 (0.7), 43 (100).

Anticancer screening

Materials and methods

Cytotoxicity to mammalian cells

The cytotoxic activity of the tested compounds **1**, **2**, **3a**, **3c**, **3d**, **5a–k**, **6a–h**, **6m**, **n**, **7a**, **8a**, and **8c–f** was determined against human cancer cell line SK-MEL (malignant, melanoma), KB (epidermal carcinoma, oral), BT-459 (ductal carcinoma, breast), and SK-OV-3 (ovary carcinoma). Vero cells, derived from monkey kidney fibroblasts, and LLC-PK1, from pig kidney epithelial tissue, were used representing noncancerous cells. The assay was performed in 96-well tissue culture treated microplates according to the neutral red staining procedure as modified by Lin *et al.*, 1998. The results are reported in Table 7 and represented in Figs. 1–4.

Interaction with topoisomerases

Measurement of the catalytic activity of topoisomerase I (topo I) was based on the conversion of supercoiled DNA to relaxed DNA. Cleavage complex stabilization was

Table 7 Cytotoxicity of the synthesized compounds to mammalian cells

Compds. no.	IC 50 value (μM)[a]					
	Cancer cells				Noncancer cells	
	SK-MEL	KB	BT-549	SK-OV-3	VERO	LLC-PK1
1	13.75	>25	10.5	NA	NC	NC
2	16	>25	18.0	NA	NC	NC
3a	12.5	>25	10.0	NA	NC	NC
3c	NA	NA	NA	NA	NC	NC
3d	NA	NA	NA	NA	NC	NC
5a	15.0	>25	19.4	NA	NC	NC
5b	15.0	23	18.1	NA	NC	22.5
5c	7.5	22.5	8.75	NA	23.0	22.5
5d	NA	NA	NA	NA	NC	NC
5e	7.0	19.5	6.75	NA	>25	20.0
5f	15.0	NA	20.0	22.5	NA	NC
5g	8.5	24.0	16.3	15.5	19.5	NC
5h	18.5	>25	20.50	NA	NC	NC
5i	23.8	>25	23.75	NA	NC	NC
5j	7.5	16.3	4.25	NA	15.0	15.5
5k	15.0	11.5	11.00	23.8	12.5	16.8
6a	NA	NA	NA	NA	NC	NC
6b	4.75	NA	NA	2.8	NC	NC
6c	12.5	11.5	12.00	15.8	11.5	13.0
6d	13.3	12.5	9.00	NA	11.0	6.8
6e	14.5	21.5	11.0	13.5	15.0	13.8
6f	NA	NA	11.50	NA	NC	22.0
6g	11.5	11.5	10.00	14.8	5.0	5.8
6h	NA	15	NA	NA	NC	NC
6m	NA	NA	NA	NA	NC	NC
6n	NA	NA	NA	NA	NC	NC
7a	18.8	14	10.5	22.5	11	15.75
8a	NA	NA	NA	NA	NC	NC
8c	NA	NA	NA	NA	NC	NC
8d	NA	NA	NA	NA	NC	NC
8e	NA	NA	NA	NA	NC	NC
8f	NA	NA	NA	NA	NC	NC
Doxorubicin	0.55	<0.55	<0.55	0.8	>5.0	0.75

NA not active up to 25 μM. *NC* not cytotoxic up to 25 μM. *SK-MEL* human malignant, melanoma. *KB* human epidermal carcinoma, oral. *BT-549* ductal carcinoma, breast. *SK-OV-3* human ovary carcinoma. *Vero* monkey kidney fibroblasts. *LLC-PK1* pig kidney epithelial

[a] Stock solution = 5 mM in DMSO; test concentrations = 25, 8.33, and 2.78 μM

assayed by measuring the nicked DNA produced in the presence of the tested compounds. Human topo I and the topo I drug kit were purchased from Topogen Inc. (Columbus, Ohio). A supercoiled plasmid DNA (pHOT1) with a high affinity topo I recognition element was used as substrate. Enzyme activity was assayed in a total volume of 20 μl containing 250 ng of DNA, test compound, 2–4 U of purified enzyme, 10 mM EDTA, 0.15 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine, and 5% glycerol. The reaction mixture was incubated at 37°C for 30 min. Reactions were then terminated by the addition of 1% sodium dodecyl sulfate (SDS) followed by treatment with proteinase K (50 μg/ml) at 37°C for 30 min. DNA was extracted with chloroform–isoamyl alcohol (24:1 v/v) and analyzed by electrophoresis on a 1% agarose gel in TAE buffer (40 mM Tris acetate, 2 mM EDTA, pH 8.5). The gel was stained with ethidium bromide, destained in water, and photographed on a UV transilluminator followed by a densitometric analysis using NIH image software 1.52. Enzyme activity was measured as a percentage of substrate DNA converted to product. The concentration of test compound that prevented 50% of the substrate from being converted into product (IC_{50}) was calculated.

For the determination of cleavage complex formation activity with topo I, the assay was performed with a minimum of 4 U of purified enzyme as described above, except that ethidium bromide was included in both the agarose gel and running buffer to resolve nicked DNA from supercoiled or relaxed species. Drug-induced stabilization of the cleaved complex was determined in terms of the percent nicked DNA produced. The relaxation activity of Topo II was analyzed in the same manner, except that the reaction mixture contained 10 mM Tris–HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/ml BSA, 1 mM ATP, and 4 units Topo IIa. Decatenation of kDNA (TopoGEN Inc.) was performed in the same condition except that supercoiled plasmid DNA was substituted by 400 ng kDNA. All compounds tested **1**, **2**, **3a**, **3c**, **3d**, **5a–k**, **6a–e**, **6m**, **6n**, **7a**, **8a**, and **8c–f** were dissolved in DMSO provided that the DMSO concentration did not exceed 2.5% in all the assays and a DMSO control was always included. The results are reported in Table 8.

Results and discussion

Chemistry background

In this investigation, the synthetic method of preparation of curcumin (**1**) and ethyl curcumin (**2**) involved an one step condensation of the appropriate aromatic aldehyde with acetylacetone–boric oxide complex. This method was originally reported (Crabbe *et al*., 1971) and modified by Roughley and Whiting (1973). The method founds large application because of its versatility, wide application for the preparation of symmetrical and unsymmetrical curcuminoids and other diketones, high yield and purity (Nurfina *et al*., 1997; Mohri *et al*., 2003). Curcumin (**1**) and

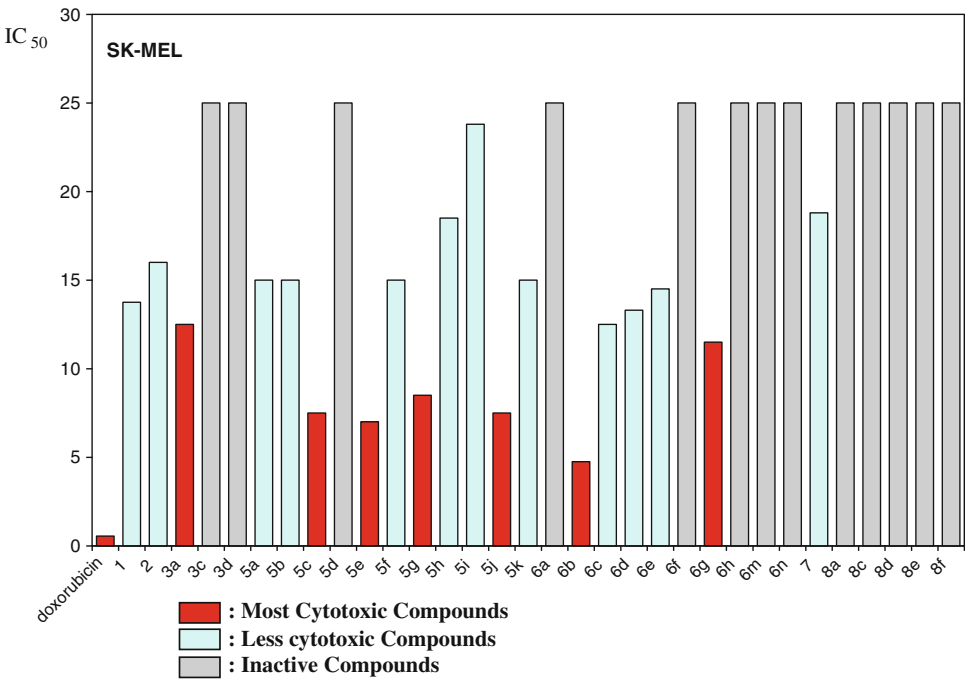


Fig. 1 Cytotoxic effect of the synthesized compounds to SK-Mel cells

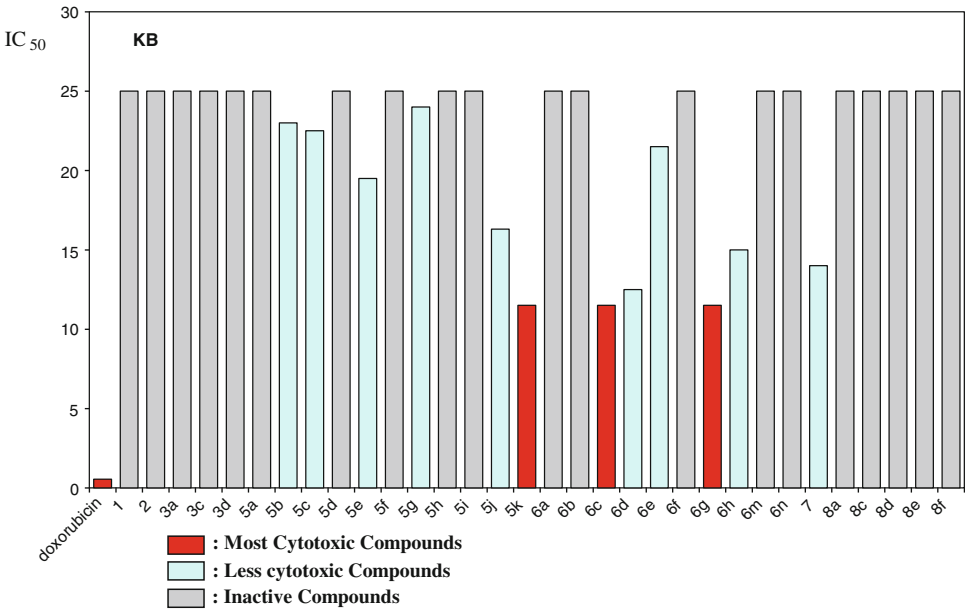


Fig. 2 Cytotoxic effect of the synthesized compounds to KB Cells

ethyl curcumin (**2**) were prepared as shown in Scheme 2. Di-*O*-chloroacetylcurcumin (**3a**), di-*O*-chloropropionylcurcumin (**3b**), di-*O*-chloroacetylethyl curcumin (**3c**), and di-*O*-chloropropionylethyl curcumin (**3d**) were prepared by two methods: (1) treating the appropriate curcumin and ethylcurcumin with acid chlorides in the presence of Na_2CO_3 (Crabbe *et al*., 1971; Nurfina *et al*., 1997; Ishida *et al*., 2002, (2) preparation of the target intermediates **3a**–**d**, through a two-step reaction involving the treatment of 3-alkoxy-4-chloroacyloxybenzaldehyde (**4a**–**d**) with acetylacetone (Scheme 2) (Roughley and Whiting, 1973). 1,7-Bis(4-alkyl-(cycloalkyl or heteroaryl)-aminoacyloxy)-3-(methoxy-phenyl)-1,6-heptadiene-3,5-dione (**5a**–**n**) and 1,7-bis(4-alkyl (cycloalkyl or heteroaryl)aminoacyloxy)-3-(ethoxyphenyl)-1,6-heptadiene-3,5-dione (**6a**–**n**) were synthesized by heating under reflux an ethanolic solution of di-*O*-chloroacylcurcumin (**3a**,**b**) or di-*O*-chloroacylethylcurcumin (**3c**, **d**) and the appropriate alkyl, cycloalkyl, or heteroarylamine in the presence of triethylamine (Scheme 3). 1,7-Bis(4-(4-substituted sulfanilamido)acyloxy)-3-(methoxyphenyl)-1,

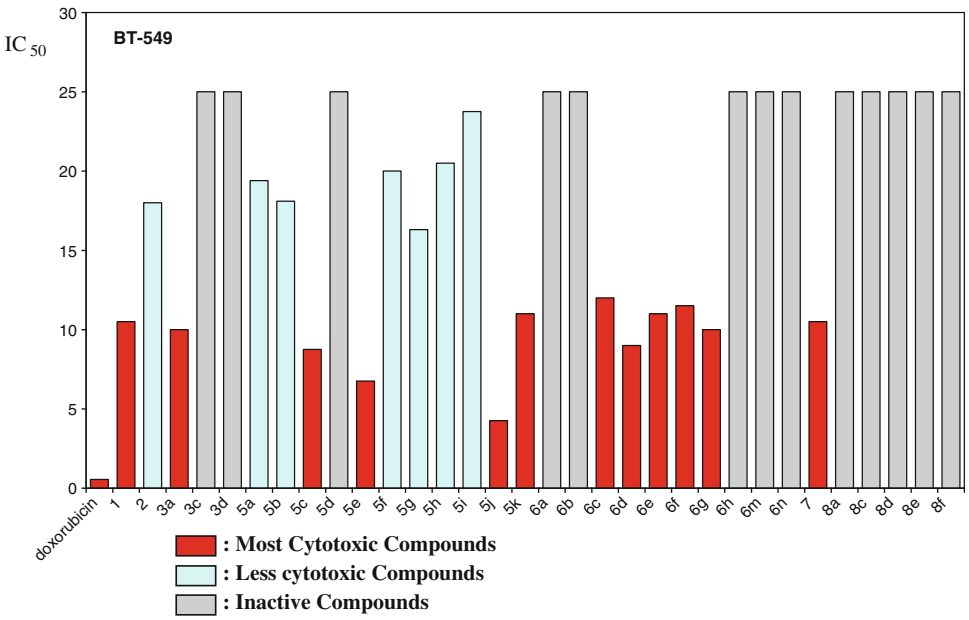


Fig. 3 Cytotoxic effect of the synthesized compounds to BT-549 Cells

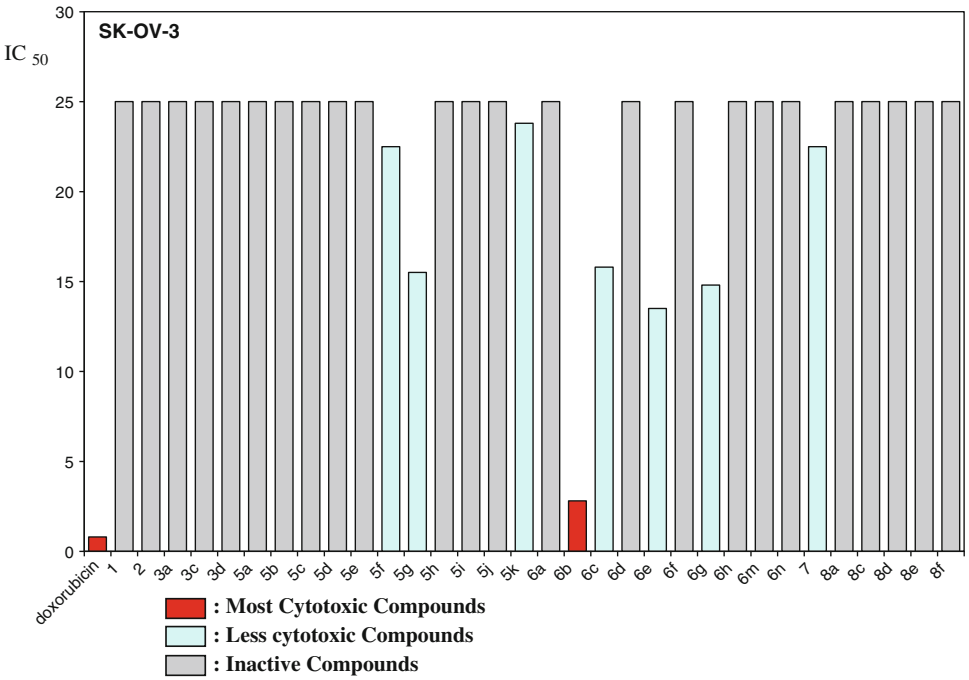


Fig. 4 Cytotoxic effect of the synthesized compounds to SK-OV-3 cells

6-heptadiene-3,5-dione (**7a**, **b**) and 1,7-bis(4-(4-substituted sulfanilamido)acyloxy)-3-(ethoxyphenyl)-1,6-hepta-diene-3,5-dione (**7c**–**f**) (Scheme 3) were synthesized using the same general amination procedure as for the preparation of the compounds **5a**–**n** and **6a**–**n**. Di-*O*-adamantoylcurcumin (**8a**) and di-*O*-adamantoylethylcurcumin (**8b**) were prepared by esterification of curcumin (**1**) or ethyl curcumin (**2**) as previously mentioned (Luo *et al.*, 2003; Bischoll and Hoffmann, 2002. A solution of either **1** or **2** in acetone containing anhydrous Na_2CO_3 was treated with 2 molar equivalents of adamantoyl chloride (Scheme 4). Di-*O*-heptanoylcurcumin (**8c**) and di-*O*-heptanoylethylcurcumin (**8d**) were prepared by esterification of curcumin **1** or ethyl curcumin **2** in acetone containing anhydrous Na_2CO_3 (2 g) with 2 molar equivalents of 2-heptanoyl chloride at rt (Scheme 4). Di-*O*-(2-thienoyl)curcumin (**8e**) and di-*O*-(2-thienoyl)-ethylcurcumin (**8f**) were prepared following the previous procedure.

Table 8 Anticancer activity of the synthesized compound (inhibition of topoisomerase activity)

Samples	IC 50 value (μM)			
	Topoisomerase I		Topoisomerase II	
	Cleavage[a]	Catalytic[b]	Cleavage[a]	Catalytic[b]
1	NA	NA	ND	15
2	NA	NA	ND	7.5
3a	NA	NA	ND	20
3c	NA	NA	ND	12
3d	NA	NA	ND	20
5a	NA	NA	ND	4
5b	NA	NA	ND	15
5c	NA	NA	ND	15
5d	NA	NA	ND	0.35
5e	NA	NA	ND	5
5f	NA	NA	ND	16
5g	NA	NA	ND	3
5h	NA	NA	ND	18
5i	NA	NA	ND	19
5j	NA	NA	ND	3
5k	NA	NA	ND	2.9
6a	NA	NA	ND	3.4
6b	NA	NA	ND	12
6c	NA	NA	ND	2.7
6d	NA	NA	ND	4.1
6e	NA	NA	ND	3.4
6f	NA	NA	ND	4
6g	NA	NA	ND	10
6h	NA	NA	ND	2.1
6m	NA	NA	ND	NA
6n	NA	NA	ND	7.1
7a	NA	NA	ND	3
8a	NA	NA	ND	4.5
8c	NA	NA	ND	2.9
8d	NA	NA	ND	3
8e	NA	NA	ND	8
8f	NA	NA	ND	23

ND not determined, *NA* not active up to 25 μM

[a] Cleavage complex stabilization activity (similar to camptothecin for topo I and etoposide for topo II)

[b] Inhibition of catalytic activity of topoisomerase i.e., relaxation of supercoiled DNA by topoisomerase I and decatenation of KDNA by topoisomerase II

Cytotoxic activity

The cytotoxic activity of the tested compounds **1**, **2**, **3a**, **3c**, **3d**, **5a–k**, **6a–h**, **6m**, **6n**, **7a**, **8a**, and **8c–f** was determined against human cancer cell line SK-MEL (malignant, melanoma), KB (epidermal carcinoma, oral), BT-459 (ductal carcinoma, breast), and SK-OV-3 (ovary carcinoma). Vero cells, derived from monkey kidney fibroblasts, and LLC-PK1, from pig kidney epithelial tissue, were used representing noncancerous cells. The overall results (Figs. 1–4, Tables 7, 8) demonstrated that, in general, some of the synthesized compounds showed cytotoxic activity against the tested cancerous cell lines less then doxorubicin, which was used as a reference. While the cytotoxic mechanism of doxorubicin remains somewhat controversial, there is substantial evidence to suggest that the following events play a role (Burke and Koch 2004): (1) intercalation and alkylation of DNA, (2) induction of topo II mediated strand breaks, (3) interference with DNA unwinding and helicase activity, (4) lipid peroxidation, and (5) direct membrane effects at low concentrations resulting in modification of membrane function and related cytotoxicity (Foye and Sengupta, 1995).

Although curcumin was documented (Snyder *et al*., 2002) to damage DNA by catalytic topo II poisoning, many of the tested compounds were more active than curcumin **1** and ethyl curcumin **2** as cytotoxic agents. The most active compound **6b** was tenfold less potent than the reference doxorubicin in various cancer cell lines. Curcumin showed only moderate activity against BT-549 while being completely nontoxic to the noncancerous Vero and LLC-PK1 cells. Some of the synthesized compounds, namely 1,7-bis(4-(5-methylthiadiazol-2-yl)aminoacetyloxy)-3-(methoxy-phenyl)-1,6-heptadiene-3,5-dione (**5c**), 1,7-bis(4-(bis(2-chloroethyl)-amino-acetyloxy)-3-(methoxyphenyl)-1,6-heptadiene-3,5-dione (**5e**), 1,7-bis(4-(2-diethylaminoethyl)amino-acetyloxy)-3-(methoxyphenyl)-1,6-heptadiene-3,5-dione (**5g**), 1,7-bis(4-(5-methylthia-diazol-2-yl)aminopropionyloxy)-3-(methoxyphenyl)-1,6-heptadiene-3,5-dione (**5j**), 1,7-bis(4-(6-methoxybenzothiazol-2-yl)aminoacetyloxy)-3-(ethoxy-phenyl)-1,6-heptadiene-3,5-dione (**6b**) and 1,7-bis(4-(2-diethylaminoethyl)aminoacetyloxy)-3-(ethoxyphenyl)-1,6-heptadiene-3,5-dione (**6g**) showed cytotoxic activity against SK-MEL cancerous cell lines (Fig.1) with no or little effect on the noncancerous cells. Compound **6b** was the most active against SK-MEL cells with $IC_{50} = 4.75$ μM followed by compound **5e** with an IC50 = 7 μM. Compounds **5c** and **5j** were also cytotoxic to the same cell line with $IC_{50} = 7.5$ μM while compounds **5g** had $IC_{50} = 8.5$ μM and **6g** had IC50 = 11.5 μM. Compounds **5j**, **5e**, and **5c** and 1,7-bis(4-(2-chloroethyl)aminoacetyloxy)-3-(ethoxyphenyl)-1,6-heptadiene-3,5-dione (**6d**) exhibited cytotoxic activity against BT-549 cells with $IC_{50} = 4.25$, 6.75, 8.75, and 9 μM, respectively (Fig. 3). Di-*O*-chloroacetylcurcumin (**3a**), **6g** and 1,7-bis(4-(4-sulfanilamido)acetyloxy)-3-(methoxy-phenyl)-1,6-heptadiene-3,5-dione (**7a**) were almost as active as curcumin against BT-549 exhibiting $IC_{50} = 10$ μM. 1,7-Bis(4-(bis(2-chloroethyl)aminoacetyloxy)-3-(ethoxy-phenyl)-1,6-heptadiene-3,5-dione (**6e**) and 1,7-bis(4-(2-dimethylaminoethyl)amino-acetyloxy)-3-(ethoxyphenyl)-1,6-heptadiene-3,5 dione (**6f**)

were slightly less active than curcumin exhibiting IC_{50} of 11 and 11.5 μM, respectively (Fig. 3). Moreover, 1,7-bis(4-(2-chloroethyl)amino-propionyloxy)-3-(methoxyphenyl)-1,6-heptadiene-3,5-dione (**5k**), 1,7-bis(4-(5-methyl-thiadiazol-2-yl)aminoacetyloxy)-3-(ethoxyphenyl)-1,6-hepta-diene-3,5-dione (**6c**) and **6g** were the most cytotoxic against KB cells with $IC_{50} = 11.5$ μM (Fig. 2). Regarding SK-OV-3 cell line (Fig. 4), compound **6b** was the most cytotoxic among these series with $IC_{50} = 2.8$ μM. It was also not cytotoxic to any of the noncancer cells tested up to 25 μM. All other compounds were inactive against this type of cancer cells. It was also noted that none of the tested compounds having an adamantoyl, heptanoyl, and thienoyl functions directly esterified to the phenolic hydroxyl groups (compounds **8a–f**), showed any activity against all cell lines used indicating that this linkage is not appropriate for activity.

Inhibition of topoisomerases

Topo I and II have been established as targets of many chemotherapeutic drugs currently in clinical usage (Ronison *et al*., 2001). Inhibition of either enzyme can result in aberrant mitosis in cancer cells which has been characterized as the primary form of cell death caused by inhibitors of Topo I and II (Lock and Stribinskiene, 1996). Therefore, creating DNA-binding compounds which recognize specific sequences is a central goal in the development of DNA-targeted drugs. The in vitro cytotoxic activity and the inhibition of the topoisomerases I and II, as possible molecular targets, of some of the newly synthesized compounds were carried out to explain the biological mechanism of action of these potential curcumin bioconjugates. It is also worth-mentioning that none of the tested compounds showed any inhibition of the topoisomerase I. Some compounds exhibited very low inhibition of the catalytic activity of topoisomerase II which does not correlate with the cytotoxic effect indicating that the cytotoxicity of these compounds should be explained through a mechanism of action different from inhibition of topoisomerases. Similar to doxorubicin, the cytotoxicity of these compounds, especially for compound **6b**, may be due to their ability to bind to DNA.

Conclusion

Most of the newly synthesized compounds are more active than curcumin and ethyl curcumin but are less cytotoxic than the reference compound doxorubicin. Many of these compounds are not cytotoxic to noncancer cells. Within our series, compounds **5c**, **5e**, **5g**, **5j**, **6b**, and **6g** having 5-methylthiadiazole, 6-methoxy-benzothiazole, diethylaminoethyl and the usual alkylating bis(2-chloroethyl)amino moieties showed the highest cytotoxic activity against SK-MEL cancer cells (Fig. 1). Compounds **5k**, **6c**, and **6g** are less cytotoxic to KB cancer cells (Fig. 2). Moreover, compounds **5c**, **5e**, **5j**, **5k**, **6d**, **6e**, **6f**, and **6g** showed cytotoxicity against BT-549 cancer cells (Fig. 3) with **5j** being the most active compound. Curcumin and our intermediate di-*O*-chloroacetylcurcumin (**3a**) are also cytotoxic against the same cell line but are less active than the synthesized compounds. Finally, compound **6b** is the only one exhibiting cytotoxicity against SK-OV-3 cancer cells (Fig. 4). Therefore, these compounds and especially compound **6b** should be very promising candidates for further studies.

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References

Al-Omar MA, Youssef KM, El-Sherbeny MA, Awadalla AA, El-Subbagh HI (2005) Synthesis and in vitro antioxidant activity of some new fused pyridine analogs. Arch Pharm Pharm Chem Life Sci 338:181–189

Anderson AM, Mitchell MS, Mohan RS (2000) Isolation of curcumin from turmeric. J Chem Educ 77:359–360

Burke PJ, Koch TH (2004) Design, synthesis, and biological evaluation of doxorubicin–formaldehyde conjugates targeted to breast cancer cells. J Med Chem 47:1193–1206

Crabbe P, Maldonado LA, Sanchez I (1971) Synthesis of new heterocyclic derivatives of estradiol. Tetrahedron 27:711–725

Foye WO, Sengupta SK (1995) Cancer chemotherapy. In: Foye WO, Lemke TL, Williams DA (eds) Principles of medicinal chemistry, chap 37. Lippincott Williams and Wilkins, Philadelphia, p 839

Huang MT, Smart RC, Wong CQ, Conney AH (1988) Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on 13-acetate. Cancer Res 48:5941–5946

Huang MT, Lou YR, Ma W, Newmark HL, Reuhl KR, Conney AH (1994) Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res 54:5841–5847

Huang MT, Newmark HL, Frenkel K (1997) Inhibitory effects of curcumin on tumorigenesis in mice. Cancer Res 27:26–34

Ishida J, Ohtsu H, Tachibana Y, Nakanishi Y, Bastow KF, Nagai M, Wang HK, Itokawa H, Lee KH (2002) Antitumor agents. Part 214: synthesis and evaluation of curcumin analogues as cytotoxic agents. Biorg Med Chem 10:3481–3487

Jovanovic SV, Steenken S, Boone CW, Simic G (1999) How curcumin works preferentially with water soluble antioxidants. J Am Chem Soc 121:9677–9681

Jovanovic SV, Boone CW, Steenken S, Trinoga M, Kaskey RB (2001) How curcumin works preferentially with water soluble antioxidants. J Am Chem Soc 123:3064–3068

Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC (1994) Chemopreventive drug development: perspectives and progress. Cancer Epidemiol Biomarkers Prev 3:85–98

Khopde SM, Priyadarsini KI, Venkatesan P, Rao MNA (1999) Free radical scavenging ability and antioxidant efficiency of curcumin and its substituted analogue. Biophys Chem 80:85–91

Kiso Y, Suzuki Y, Suzuki N, Watanabe N (1983) Antihepatotoxic principles of curcuma longa rhizomes. Planta Med 49:185–187

Kumar S, Narian U, Tripathi S, Misra K (2001) Syntheses of curcumin bioconjugates and study of their antibacterial activities against β-lactamase-producing microorganisms. Bioconjugate Chem 12:464–469

Kunchandy E, Rao MNA (1990) Oxygen radical scavenging activity of curcumin. Int J Pharm 58:237–240

Lin LI, Ke YF, Ko YC, Lin JK (1998) Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion in vitro and suppress matrix metalloproteinase-9 secretion. Oncology 55:349–353

Lock RB, Stribinskiene L (1996) Dual modes of death induced by etoposide in human epithelial tumor cells allow Bcl-2 to inhibit apoptosis without affecting clonogenic survival. Cancer Res 56(17):4006–4012

Luo QL, Li JY, Liu ZY, Chen LL, Li J, Qian Z, Shen Q, Li Y, Lushington GH, Ye QZ, Nan FJ (2003) Discovery and structural modification of inhibitors of methionine aminopeptidases from *Escherichia coli* and *Saccharomyces cerevisiae*. J Med Chem 46:2631

Masuda T, Maekawa T, Hidaka K, Bando H, Takeda Y, Yamaguchi H (2001) Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling products from curcumin and linoleate. J Agric Food Chem 49(5):2539–2547

Mazumder A, Raghavan K, Weinstein J, Kohn KW, Pomier Y (1995) Inhibition of human immunodeficiency virus type-1 integrase by curcumin. Biochem Pharmacol 49:1165–1170

Mehta K, Pantazis P, McQueen T, Aggarwal BB (1997) Antiproliferative effect of curcumin (diferuloylmethane) against human breast tumor cell lines. Anticancer Drugs 8:470–481

Mohri K, Watanabe Y, Yoshida Y, Sato M, Isobe K, Sugimoto N, Tsuda Y (2003) Synthesis of glycosylcurcuminoids. Chem Pharm Bull 51:1268–1272

Mukhopadhyay A, Basu N, Ghatak N, Gujral PK (1982) Anti-inflammatory and irritant activities of curcumin analogues in rats. Agents Actions 12:508–515

Nurfina AN, Reksohadiprodjo MS, Timmerman H, Jenie UA, Sugiyanto D, Van Der Goot H (1997) Synthesis of some symmetrical curcumin derivatives and their antiinflammatory activity. Eur J Med Chem 32:321–328

Rajakrishnan V, Viswanathan P, Rajasekharan KN, Menon VP (1999) Neuroprotective role of curcumin from curcuma longa on ethanol-induced brain damage. Phytother Res 13:571–574

Rao TS, Basu N, Siddiqui HH (1982) Antiinflammatory activity of curcumin analogues. Ind J Med Res 75:574–578

Roninson IB, Broude EV, Chang BD (2001) Treatment-induced senescence and mitotic catastrophe in tumor cells. Drug Resist Update 4:303–313

Roughley PJ, Whiting DA (1973) Experiments in the biosynthesis of curcumin. J Chem Soc Perkin Trans I 20:2379–2388

Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R (1995) Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett 94:79–83

Sharma OP (1976) Antioxidant activity of curcumin and related compounds. Biochem Pharmacol 25:1811–1812

Snyder RD, Arnone MR (2002) Putative identification of functional interactions between DNA intercalating agents and topoisomerase II using the V79 in vitro micronucleus assay. Mutat Res 503:21–35

Soudamini KK, Kuttan R (1989) Inhibition of chemical carcinogenesis by curcumin. J Ethnopharm 27:227–233

Sreejayan RMN, Rao MNA (1994) Curcuminoids as potent inhibitors of lipid peroxidation. J Pharm Pharmacol 46:1013–1016

Srimal RC, Dhawan BN (1973) Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. J Pharm Pharmacol 25(6):447–452

Toda S, Miyase T, Arichi H, Tanizawa H, Takino Y (1985) Natural antioxidants. III. Antioxidative components isolated from rhizome of Curcuma longa L. Chem Pharmaceut Bull 33(4):1725–1728

Youssef KM, El-Sherbeny MA, El-Shafie FS, Farag HA, Al-Deeb OA, Awadalla SAA (2004) Synthesis of curcumin analogues as potential antioxidant, cancer chemopreventive agents. Arch Pharm Pharm Med Chem 337:42–54