Stereoselective photochemistry of substituted chalcones in solution and their antioxidant activities

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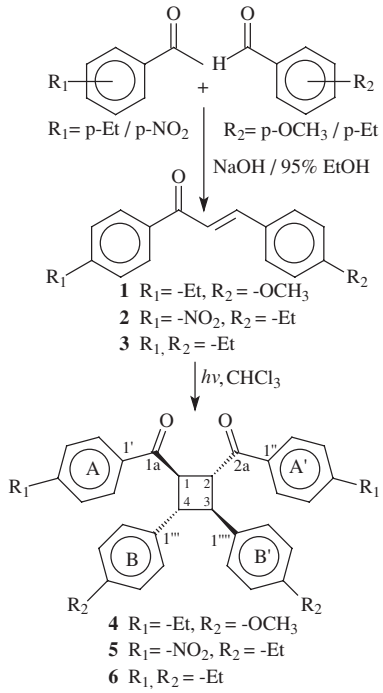
Three new δ-truxinic type cyclobutanes [(1β,2α)-di-(4-ethylbenzoyl)-(3β,4α)-di-(4-methoxyphenyl)cyclobutane (**4**), (1β,2α)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-ethylphenyl) cyclobutane (**5**), and (1β,2α)-di-(4-ethylbenzoyl)-(3β,4α)-di-(4-ethylphenyl) cyclobutane (**6**)] have been prepared by stereoselective photodimerisation of the corresponding chalcone monomers (**1**-**3**) in solution. NMR and MS of the dimers are discussed. The precursor chalcones and the dimeric products showed antioxidant activities to different extents with respect to the individual compounds as well as to the antioxidant methods used.

Keywords: chalcones, photodimerisation, cyclobutanes, antioxidants

Cycloaddition reaction of alkenes to give cyclobutane dimers is one of the most studied reactions in organic photochemistry,[1-9] although there is still need for investigation in so-far unstudied areas of the chalcones. The cycloadditions of *trans*-chalcones are known to give four possible stereoisomers as *anti*, *syn*, head-to-tail, and head-to-head. These reactions are stereospecific and have been explained by means of the Woodward-Hoffmann selection rules.[10] The formation of different stereoisomers from the dimerisation of chalcones and related compounds may be dependent on the physical state of the substrate (solid, solution, or molten state) and the reaction conditions.[1-4] Various cyclobutane containing dimers of chalcones have been reported to be synthesised[1,4,7,9] and isolated from various plants,[11-16] and some have shown antimicrobial activity.[16]

In the present study, two new disubstituted chalcones (**1** and **2**) and one known example (**3**)[17,18] (Scheme 1) were prepared by the standard procedure.[19] Their spectral data are given in Table 1. These disubstituted chalcones, when exposed to UV light (400 W high-pressure Hg lamp), were converted into the respective cyclobutanes (**4**–**6**) as major products, in yields (chromatographed products, PTLC) of 48, 38, and 44 %, respectively. The yields in this type of reaction have usually been low, as in our case, or even lower.[1-9] The minor



Scheme 1

Table 1 NMR data[a] of compounds **1–3** in $CDCl_3$

	1		**2**		**3**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	–	189.84	–	189.01	–	189.72
2	7.48, d, *J* = 15.6	119.65	7.80, d, *J* = 15.8	120.30	7.80, d, *J* = 15.5	120.95
3	7.42, d, *J* = 15.6	144.20	7.44, d, *J* = 15.8	146.94	7.49, d, *J* = 15.5	144.35
1'	–	149.56	–	150.02	–	149.56
2',6'	7.95, d, *J* = 8.2	128.71	8.28, d, *J* = 8.8	123.91	7.95, d, *J* = 8.2	128.72
3',5'	7.54, d, *J* = 8.2	128.13	8.10, d, *J* = 8.8	129.51	7.51, d, *J* = 8.2	128.45
4'	–	136.14	–	143.23	–	135.95
Et	2.63, q, *J* = 7.3	28.99	–	–	2.62, q, *J* = 7.3	28.82
	1.23, t, *J* = 7.3	15.32	–	–	1.21, t, *J* = 7.3	15.25
1"	–	127.69	–	148.34	–	147.10
2",6"	7.27, d, *J* = 7.9	130.25	7.56, d, *J* = 8.0	129.05	7.16, d, *J* = 7.9	128.56
3",5"	6.87, d, *J* = 7.9	114.43	7.24, d, *J* = 8.0	128.79	7.25, d, *J* = 7.9	128.08
4"	–	161.63	–	131.91	–	132.45
OCH_3	3.76, s	55.36	–	–	–	–
Et	–	–	2.63, q, *J* = 7.3	29.03	2.58, q, *J* = 7.3	28.93
		–	1.24, t, *J* = 7.3	15.41	1.18, t, *J* = 7.3	15.34

[a]*J* in Hz. Assignments based on ^1H, APT, ^1H–^1H COSY and ACD NMR program

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products of the reactions were in small quantities and were not characterised.

Chalcones belong to the largest class of plant secondary metabolites, which, in many cases, serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores.[20] They are known to possess antioxidant character to various extents.[21,22] The antioxidant activity of natural compounds like chalconoids is related to a number of different mechanisms such as free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl.[23] Reactive oxygen species produced in normal or diseased tissues or organisms can change the structures and functions of most biological molecules. Elevated concentrations of these species, examples for which are free radicals like super oxide and hydroxyl radicals and molecules like hydrogen peroxide and peroxynitrite, cause oxidative stress in various organs, tissues, or organisms, which in turn cause many diseases or malfunctions. Oxidative stress can be overcome in organisms by the use of agents either synthesised or obtained from outside sources. Organisms cannot produce these antioxidant components, proteins or small organic compounds, in sufficient quantities under certain high need conditions. External antioxidant sources have become more important in recent years with heightened awareness of the importance of these chemicals. Scientists have become more interested in natural sources to fight oxidative stress, looking for active components of plants in this respect in recent years. Antioxidants which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction, therefore, appear to be very important in the prevention of many diseases.

Several methods have been developed in recent years to evaluate the total antioxidant capacity of potential antioxidant compounds. The basis of most of these methods relies on a substrate that is oxidised in the procedures, and oxygen consumption, oxidation product generation, or substrate loss, is monitored in various ways.[24]

In the literature, the antiviral and antimicrobial activities of chalcone dimers have been studied,[25-29] but the antioxidant activities of **1–6** have not been reported. Thus, the antioxidant activity of the chalcones **1–3** and their dimerisation products **4–6** were measured according to a basic method that utilises linoleic acid as the substrate of oxidation and measures the fluorescence persistence time of each sample spot placed on a fluorescent-silica coated TLC plate.[30,31] The longer the fluorescence persisted, the higher the was antioxidant activity. The second antioxidant assay method used is based on scavenging of the stable free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) by the potential antioxidant compound. Any compound that can donate an electron or hydrogen to DPPH reacts with it and decreases its light absorbance at 517 nm, allowing the spectrophotometric determination of the antiradical antioxidant activity of any potential compound.[32] The lower the amount of the test compound providing 50% scavenging of the radicals present meant higher activity.

In this study, we found that [2+2] photodimerisations of disubstituted (ethyl-, methoxy-, and nitro-) chalcone derivatives **1–3** proceeded efficiently and stereoselectively in solution and gave the corresponding *anti*-head-to-head dimers **4–6** in relatively good yields. This cyclisation allows the formation of the most stable δ-truxinic type isomers.

The structures of the cyclobutyl rings of products **4–6** were elucidated from their ^{1}H NMR spectra, which show highly shielded CH proton signals at δ_H 4.54/3.83, δ_H 4.62/3.82, and δ_H 4.53/3.93, respectively.[1-9]

The stereochemistry of compounds **4–6** was also revealed by NMR spectroscopy. Two symmetrical multiplets (AA'BB') at δ_H 4.54 (δ_C 47.63)/δ_H 3.83 (δ_C 47.79) for compound **4**, at δ_H 4.62 (δ_C 47.77)/δ_H 3.82 (δ_C 48.84) for compound **5,** and at δ_H 4.53 (δ_C 47.76)/δ_H 3.93 (δ_C 47.48) for compound **6** were observed for the cyclobutyl protons in the ^{1}H NMR. Simulation of these NMR patterns allowed the calculation of the coupling constants of the cyclobutyl protons ($J_{AA'}$ = 8.8/9.0, J_{AB}=5.8/5.6 , $J_{AB'}$ = not detected, $J_{BB'}$ = 8.8/9.0). The values of these coupling constants suggest that the dimerisation of **1–3** occurred by head-to-head coupling, but they do not allow

Table 2 NMR data of compounds **4–6** in $CDCl_3$

	4[a]		5[a]		6[a]	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1, 2	4.54, AA'BB', *J* = 8.8, 5.8, nd, 8.8	47.63	4.62, AA'BB', *J* = 9.0, 5.8, nd, 9.0	47.77	4.53, AA'BB', *J* = 8.8, 5.6, nd, 8.8	47.76
3, 4	3.83, AA'BB', *J* = 8.6, 5.2, nd, 8.6	47.79	3.82, AA'BB', *J* = 9.2, 5.6, nd, 9.2	48.84	3.93, AA'BB', *J* = 8.8, 5.6, nd, 8.8	47.48
1a, 2a	–	198.73	–	197.35	–	198.83
2'/ 1"	–	150.27	–	150.44	–	150.28
2'/ 2"	7.76, AX, *J* = 8.0	128.98	8.16, AB, *J* = 8.4	123.70	7.24, AX, *J* = 8.4	129.03
3'/ 3"	7.13, AX, *J* = 8.0	127.94	7.94, AB, *J* = 8.4	129.92	7.12, AX, *J* = 8.4	127.95
4'/ 4"	–	133.30	–	143.99	–	133.39
5'/ 5"	7.13, AX, *J* = 8.0	127.94	7.94, AB, *J* = 8.4	129.92	7.12, AX, *J* = 8.4	127.95
6'/ 6"	7.76, AX, *J* = 8.0	128.98	8.16, AB, *J* = 8.4	123.70	7.24, AX, *J* = 8.4	129.03
Et	2.60, q, *J* = 7.6	28.80	–	–	2.62, q, *J* = 7.6	28.85
	1.17, t, *J* = 7.6	15.01	–	–	1.18, t, *J* = 7.6	15.06
1'"/ 1""	–	133.69	–	139.64	–	142.96
2'"/ 2""	7.24, AX, *J* = 8.6	128.46	7.18, bs	128.48	7.23, AB, *J* = 8.2	128.01
3'"/ 3""	6.83, AX, *J* = 8.6	113.86	7.18, bs	127.33	7.12, AB, *J* = 8.2	127.38
4'"/ 4""	–	158.51	–	137.39	–	138.84
5'"/ 5""	6.83, AX, *J* = 8.6	113.86	7.18, bs	127.33	7.12, AB, *J* = 8.2	127.38
6'"/ 6""	7.24, AX, *J* = 8.6	128.46	7.18, bs	128.45	7.23, AB, *J* = 8.2	128.01
OCH_3	3.76, s	55.14	–	–	–	–
Et	–	–	2.64, q, *J* = 7.6	28.47	2.62, q, *J* = 7.6	28.46
			1.26, t, *J* = 7.6	15.56	1.21, t, *J* = 7.6	15.60

[a]Assignment based on ^{1}H, APT, ^{1}H–^{1}H COSY, NOESY, HETCOR and ACD NMR programme.; nd: AB' coupling constant was not detected.

a certain assignment with respect to *syn*/*anti* stereochemistry. A more accurate structural determination was attained by ^{1}H-^{1}H COSY, ^{1}H–^{13}C-COSY, and NOESY spectra and literature data.[1-9] The close similarity of the ^{1}H and ^{13}C NMR patterns of the cyclobutyl moieties with δ-truxinic structure strongly suggests that the formation of the cyclobutane ring occurs by *anti* head-to-head junction with compounds **1–3**.[1-6]

The structural connectivities of compounds **4–6** were established in part from ^{1}H-^{1}H COSY. It was found that the most downfield for the cyclobutyl ring methines, designated H-1/H-2 at δ_H 4.54 (δ_C 47.63), was connected to H-3/H-4 at δ_H 3.83 (δ_C 47.79) in **4**, H-1/H-2 at δ_H 4.62 (δ_C 47.77) to H-3/H-4 at δ_H 3.82 (δ_C 48.84) in **5**, and H-1/H-2 at δ_H 4.53 (δ_C 47.76) to H-3/H-4 at δ_H 3.93 (δ_C 47.48) in **6**. The important NOESY interactions in compounds **4–6** were seen between H-1 and H-3 and between H-2 and H-4. Thus the presence of the cyclobutane ring was established. The chemical shifts of compounds **4–6** are in total agreement with those of similar compounds with δ-truxinic structure in the literature.[1-8]

(±)-LC-MS/MS gave M+Na at *m/z* 555 (100) for **4**, M-H at *m/z* 561 (19) for **5,** and M+Na at *m/z* 551 (36) for **6**, which were consistent with the molecular formulas to be $C_{36}H_{36}O_4$ for **4**, $C_{34}H_{30}N_2O_6$ for **5,** and $C_{38}H_{40}O_2$ for **6,** requiring dimeric structures for **1**–**3**. LC-MS/MS also showed other diagnostic fragments for compounds **1–6**, which are shown in Scheme 2.

Based on the above observations, the complete chemical shift assignments for **4–6** were deduced and are shown in Table 2. Compounds **4–6** were thereby shown to be (1β,2α)-di-(4-ethylbenzoyl)-(3β,4α)-di-(4-methoxyphenyl) cyclobutane, (1β,2α)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-ethylphenyl)cyclobutane, and (1β,2α)-di-(4-ethylbenzoyl)-(3β,4α)-di-(4-ethylphenyl)cyclobutane, respectively. These three chiral compounds (**4–6**) were synthesised and characterised for the first time in this work.

The antioxidant activities of the compounds synthesised were evaluated according to two different methods. The fluorescence disappearance times of spots on TLC plates irradiated continuously with a UV source at 254 nm were measured. The monomer **1** and its dimeric form **4** showed similar antioxidant activities (Table 3). The other four compounds appeared inactive with this method.

Table 3 Antioxidant activities of the monomeric (**1–3**) and dimeric chalcones (**4–6**).

Sample	Fluorescence disappearance time[a] /%activity	DPPH scavenging activity IC_{50}[b] /mg/ml
Trolox	34	0,01
BHT	100	0,06
1	26	1,29
2	–	1,95
3	–	2,33
4	23	1,61
5	–	0,28
6	–	0,71

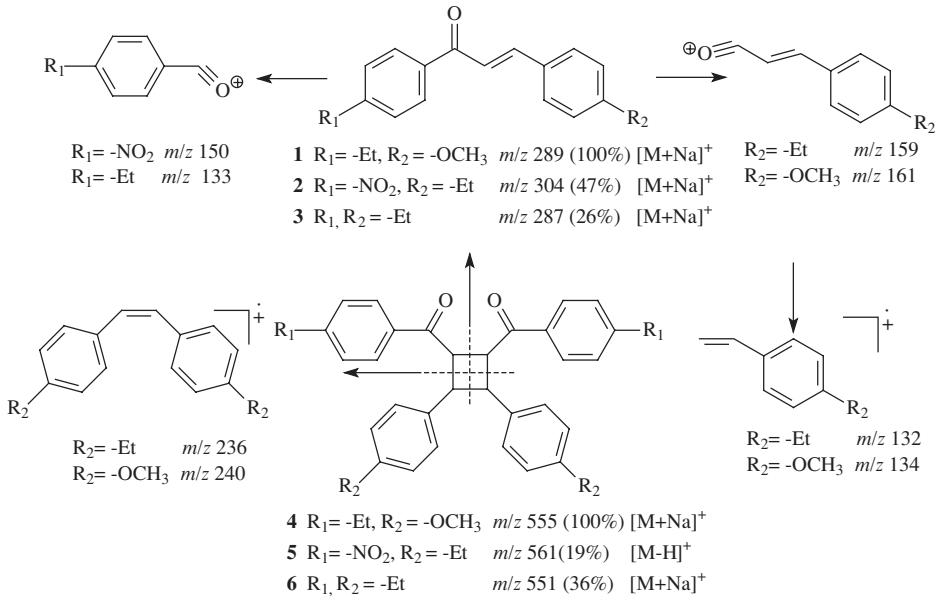
[a]:Relative activity is calculated by taking the activity of BHT as 100%
–:No activity observed
[b]:IC_{50} represents the concentration providing 50 percent scavenging of DPPH radical.

The DPPH radical scavenging assay revealed that the dimers **5** and **6** were more active in scavenging DPPH radicals in comparison to their monomers **2** and **3**. On the other hand, the monomer **1** was more active than its dimer **4**. All six compounds exhibited radical scavenging and, thus, antioxidant activity, though lower than the reference antioxidants butylated hydroxytoluene (BHT) and Trolox® (Table 3).

Experimental

General and instrumentation: NMR spectra were recorded on a Varian Mercury NMR instrument at 200 MHz in $CDCl_3$. The mass spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrometer. Elemental analyses were performed on a Carlo Erba 1106 apparatus. Infrared spectra were measured on a Perkin-Elmer 1600 FT-IR (4000-400 cm^{-1}) spectrometer. Melting points were obtained using a Gallenkamp apparatus. UV-vis spectra and absorbance values were obtained with a Unicam UV2-100 spectrophotometer at 25 °C. Thin-layer chromatography (TLC) and fluorescence persistence time measurements were carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminum plates. PTLC was carried out on Merck precoated 60 Kieselgel F_{254} (20 cm × 20 cm, 0.2 mm) silica gel plates. A Camag UV source at 254 nm was used for antioxidant activity measurements.

Materials and methods: 4-methoxybenzaldehyde, 4-ethylbenzaldehyde, 4-ethylacetophenone, and 4-nitroacetophenone were purchased from Aldrich and used without further purification. 60% α-linoleic acid in hexane and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma. Butylated hydroxytoluene (BHT) was



Scheme 2 Fragment ions observed in the (±)-LC-MS/MS spectra of 1–6

purchased from Applichem. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) was obtained from Aldrich. The solvents chloroform, *n*-hexane, ethanol, and diethyl ether were either of analytical grade or bulk solvents distilled before use.

(2E)-1-(4-Ethylphenyl)-3-(4-methoxyphenyl)propen-1-one (**1**): To a cooled solution (~1–5 °C) of sodium hydroxide (1.0 g, 25 mmol) in 10 ml of 80% EtOH *p*-ethylacetophenone (1.48 g, 10 mmol) in EtOH was added dropwise. The resulting mixture was stirred for 10 minutes, then *p*-methoxybenzaldehyde (1.36 g, 10 mmol) in EtOH was added dropwise. After addition was complete the reaction mixture was stirred at room temperature for 1 h. The mixture was neutralised with 10% HCl. The ethanol was evaporated under vacuum, and the aqueous phase was extracted by $CHCl_3$ (3 × 30 ml). The combined organic phases were dried over Na_2SO_4. Removal of the solvent under reduced pressure gave compound **1** as an oil (2.5 g, 93%). R_f 0.5, *n*-hexane-diethyl ether (1 : 0.5). IR: ν_{max} (cm^{-1}) 3050, 2962, 2932, 1663, 1599, 1511, 1458, 1420, 1252, 1174, 1031, 975, 824. ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz): see Table 1. UV: λ_{max} ($CHCl_3$): 260, 338 nm (ε 17 700, 6170); positive LC-MS/MS: *m/z* (%) 289 (100) $[M+Na]^+$, 290 (20) $[M+1+Na]^+$, 266 (9) $[M]^+$, 160 (6), 132 (10). Calcd. for $C_{18}H_{18}O_2$ (266.34): C 81.17, H 6.81; found C 80.95, H 7.10 %.

(2E)-1-(4-Nitrophenyl)-3-(4-ethylphenyl)propen-1-one (**2**): To a cooled solution (~1–5 °C) of sodium hydroxide (1.00 g, 25 mmol) in 10 ml of 80% EtOH *p*-nitroacetophenone (1.51g, 9.15 mmol) in EtOH was added dropwise. The resulting mixture was stirred for 20 minutes, then *p*-ethylbenzaldehyde (1.34g, 10 mmol) in EtOH was added dropwise. The rest of the procedure was followed as for compound **1** to give compound **2** (2.7 g, 96% yield). m.p. 111.7 °C; R_f 0.63, *n*-hexane-diethyl ether (1 : 0.5). IR: ν_{max} (cm^{-1}) 3047, 2969, 1660, 1591, 1523, 1418, 1329, 1212, 984, 824. ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz): see Table 1. UV: λ_{max} ($CHCl_3$) 272, 341 (nm) (ε 27100, 22100). Positive LC-MS/MS *m/z* (%) 304 (47) $[M+Na]^+$, 305 (13) $[M+1+Na]^+$, 198 (24), 177 (10), 118 (14). Calcd. for $C_{17}H_{15}NO_3$ (281.31): C 72.58, H 5.37, N 4.98; found C 72.24, H 5.49, N 5.11 %.

The synthesis of compound **3** was treated in the same way as compounds **1** and **2.** The spectral data of compound **3** are reported here for comparison.[17,18]

(2E)-1,3-Di-(4-ethylphenyl)propen-1-one (**3**): oil (2.5 g. 95%). R_f: 0.75, *n*-hexane-diethyl ether (1 : 0.5). IR: ν_{max} (cm^{-1}) 3050, 2966, 2930, 1664, 1603, 1560, 1416, 1325, 1224, 1016, 975, 824. ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz): see Table 1. UV: ν_{max} (cm^{-1}) 259, 322 (ε 14700, 5800). Positive LC-MS/MS: *m/z* (%) 287 (26) $[M+Na]^+$, 264 (13) $[M]^+$, 265 (3) $[M+1]^+$, 158 (8), 132 (100). Calcd. for $C_{17}H_{20}O$ (264.37): C 86.32, H 7.63 found C 85.96, H 7.76 %.

Photodimerisation of **1** *in solution*: A solution of compound **1** (250 mg, 0.94 mmol) in 30 ml of chloroform-diethyl ether (1 : 1), kept in a Pyrex flask, was exposed to UV light (400 W high-pressure Hg lamp). The progress of the reaction was followed by silica gel TLC (*n*-hexane-diethyl ether, 1:1). The reaction was stopped after ~5 h. The solution was evaporated and the residue purified by PTLC (0.5 mm, 20 cm × 20 cm, 2 plates) to give compound **4** (120 mg, 48%), R_f 0.6, *n*-hexane-diethyl ether, 1 : 1).

Photodimerisation of **2** *in solution*: A solution of compound **2** (120 mg, 0.42 mmol) was treated as for compound **1** to give compound **5** (45 mg, 38%), R_f 0.4, reaction time ~6 h).

Photodimerisation of **3** *in solution*: A solution of compound **3** (160 mg, 0.60 mmol) was treated as in compound **1** to give compound **6** (70 mg, 44%), R_f 0.7, reaction time ~9 h).

(1β,2α)-Di-(4-ethylbenzoyl)-(3β,4α)-di-(4-methoxyphenyl)cyclobutane (**4**): Yellowish oily compound. IR: ν_{max} (cm^{-1}) 3021, 2965, 2835, 1666, 1606, 1513, 1248, 1178, 1035, 828. UV: λ_{max} ($CHCl_3$): 262 nm (ε 9050). ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz): see Table 1. Positive LC-MS/MS *m/z* (%): 555 (100) $[M+Na]^+$, 556 (13) $[M+1+Na]^+$, 437 (75), 438 (23), 248 (50), 249 (8). Calcd. for $C_{36}H_{36}O_4$ (532.68): C 81.17, H 6.81; found C 80.74, H 6.96 %.

(1β,2α)-Di-(4-nitrobenzoyl)-(3β,4α)-di-(4-ethylphenyl) cyclobutane (**5**): Yellowish oily compound. IR: ν_{max} (cm^{-1}) 3109, 2925, 2857, 1681, 1602, 1526, 1459, 1345, 1219, 1036, 852, 770. UV: UV: λ_{max} ($CHCl_3$) 262 nm (ε 7880). ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table 1. Negative LC-MS/MS: *m/z* (%) 561 (19) $[M-1]^+$, 451 (6), 281 (16), 204 (12). Calcd. for $C_{34}H_{30}N_2O_6$ (562.62): C 72.58, H 5.37, N 4.98; found C 72.24, H 5.32, N 4.86 %.

(1β,2α)-Di-(4-ethylbenzoyl)-(3β,4α)-di-(4-ethylphenyl) cyclobutane (**6**): Yellowish oily compound. IR: ν_{max} (cm^{-1}) 3080, 2965, 2930, 2873, 1668, 1606, 1455, 1234, 1058, 845, 828. UV: λ_{max} ($CHCl_3$) 261 nm (ε 3566); ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz): see Table 1. Positive LC-MS/MS: *m/z* (%) 551 (36) $[M+Na]^+$, 529 (3) $[M+1]^+$, 364 (22), 365 (8), 120 (20). Calcd. for $C_{38}H_{40}O_2$ (528.73): C 86.32, H 7.63; found C 86.06, H 7.55 %.

Antioxidant activity by fluorescence persistence time

The TLC plate method[28,29] was used for the determination of antioxidant activity. A fluorescent-labelled silica TLC plate (silica gel 60 F254) was dried at 105 °C for 30 min, and a 5 µl aliquot from each sample solution and from the solutions of reference standards BHT and Trolox® (1.0 mg/ml) was spotted onto the plate twice with a semiautomatic pipette, drying in between. The plate was then plunged into 3% α-linoleic acid solution in hexane twice, drying in between and at the end. The dried plate was then placed 2.5 cm below a UV (254 nm) light source and the background of the spots appeared within the first 10–15 min under continuous irradiation. The TLC plate was observed every 15 min under continuous irradiation, and the time each fluorescent spot disappeared was considered to be the induction period for lipid peroxidation. The antioxidant activities of the samples and of Trolox® were evaluated by comparing their fluorescence disappearance times with those of the reference standard BHT and given as per cent of the activity of BHT.

Free radical scavenging activity

The free radical scavenging activity of the compounds was tested by utilising DPPH scavenging.[30] Briefly, 50 µl samples of various concentrations were added to 5 ml 0.004% ethanolic DPPH solutions. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity. The results were compared with those of BHT and Trolox®.

This study was supported by grants from Karadeniz Technical University and DPT of Turkey.

Received 20 June 2004; accepted 16 December 2004
Paper 04/2588

References

1 F. Toda, K. Tanaka and M. Kato, *J. Chem. Soc., Perkin Trans. 1,* 1998, 1315.
2 M. D'Auria, *Heterocycles*, 2001, **54**, 475.
3 M. D'Auria, L. Emanuele, G. Mauriello and R. Racioppi, *J. Photochem. Photobiol. A: Chemistry*, 2000, **134**, 147.
4 D. Cesarin-Sobrinho and J. Netto-Ferreira, *Quim. Nova*, 2002, 62.
5 T. Ishikawa, N. Koseki, T. Furukawa, E. Sakurada, C. Koseki, Y. Saito, K. Ogata and T. Harayama and H. Ishii, *Tetrahedron*, 1994, **50**, 9287.
6 I. Turowska-Tryk, K. Grzesniak, E. Trzop and T. Zych, *J. Solid State Chem.*, 2003, **174**, 459.
7 B. Montaudo and S. Caccamese, *J. Org. Chem*., 1973, **38**, 710.
8 D.M.S. Atkinson, J.M. Almond, P. Hollins and S. Jenkins, *Spectrochim. Acta,* 2003, **59A**, 629.
9 N. Yaylı, O. Üçüncü, A. Yaşar, Y. Gök, M. Küçük and S. Kolaylı, *Turk. J. Chem.*, 2004, **28**, 515.
10 R.B. Woodward and R. Hoffmann, *The Conservation of Orbital Symmetry*, Verlag Chemie, Academic Press, 1970.
11 V. Seidel, F. Bailleul and G.P. Waterman, *Phytochemistry*, 2000, **55**, 439.
12 R.D. Katerere, I.A. Gray, R.A. Kennedy, J.R. Nash and D.R. Waigh, *Phytochemistry*, 2004, **65**, 433.
13 M. D'Auria and R. Racioppi, *J. Photochem. Photobiol. A: Chemistry*, 1998, **112**, 145.
14 V.S. Parmar, S.C. Jain, K.S. Bisht, R. Jajni, P. Yaneja, A. Jha, O.D. Tyagi, A.K. Prasad, J. Wengel, C.R. Olsen and P.M. Boll, *Phytochemistry*, 1997, **46**, 597.
15 P.A. Keifer, R.E. Schwartz, M.E.S. Koker, R.G. Hughes, J.D. Rittschof and K.L. Rinehart, *J. Org. Chem*., 1991, **56**, 2965.
16 M. D'Auria and R. Racioppi, *Tetrahedron*, 1997, **53**, 17307.
17 N. Yaylı, *Ind. J. Chem*. 1994, **33B**, 556.
18 Ç. Atasoy, *Synthesis of Chalconoid type Compounds*, MSc thesis, Karadeniz Technical University, Turkey 2000.
19 L. Claisen, *Chem. Ber*.**,** 1912, **45**, 3157.
20 J. Vaya, P.A. Belinky and M. Aviram, *Free Rad. Biol. Med*., 1997, **23**, 302.

21 G. Cioffi, L.M. Escobar, A. Braca and N.D. Tommasi, *J. Nat. Prod.,* 2003, **66**, 1061.
22 Y. Nakamura, S. Watanabe, N. Miyake, H. Kohno and T. Osawa, *J. Agric. Food. Chem.*, 2003, **51**, 3309.
23 K. Robards, P.D. Prenzlere, G. Tucker, P. Swatsitang and W. Glover, *Food Chem*., 1999, **66**, 401.
24 R. Fukumoto and G. Mazza, *J. Agric. Food Chem*., 2000, **48**, 3597.
25 J. Konkane, J. Lukkari, P. Posenen, R. Sillonpaa, H. Laine, K. Hormaa and C. Visy, *Macromolecules*, 1994, **27**, 4327.
26 A. Arcoria, F.D. Ballistreri, G. Musumarra and S. Occhipinti, *Org. Mass Spectr.*, 1981, **16**, 54.
27 D. Binder, C.R. Neo, W. Holzer and W.B. Resen, *Arch. Pharm.* (Weinheim, Germany), 1985, **318**, 48.
28 R. Kumer, M. Singh and K.N. Singh, *Indian J. Chem. Technol*., 1998, **5**, 227.
29 K.M. Ghoneim, M.M. Bardan, M.A. Shaaban and S. El-Meligie, *Egypt. J. Pharm. Sci.*, 1988, **29**, 555.
30 W.H. Chang, H.X. Luu and A.C. Cheng, *J. Food Sci.*, 1983, **48**, 658.
31 S. Kolayli, M. Küçük, C. Duran, F. Candan and B. Dinçer, *J. Agric. Food. Chem*., 2003, **51**, 748.
32 M. Cuendet, K. Hostettmann and O. Potterat, *Helv. Chim. Acta*, 1997, **80**, 1144.