Available online at www.sciencedirect.com
ScienceDirect
Journal of Photochemistry and Photobiology A: Chemistry 188 (2007) 161–168
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Synthesis of *N*-alkyl derivatives and photochemistry of nitro (*E*)-3-azachalcones with theoretical calculations and biological activities

Nurettin Yaylı [a,*], Murat Küçük [a], Osman Üçüncü [a], Ahmet Yaşar [a], Nuran Yaylı [a], Şengül Alpay Karaoğlu [b]

[a] *Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey*
[b] *Department of Biology, Faculty of Arts and Sciences at Rize, Karadeniz Technical University, 53100 Rize, Turkey*

Received 14 February 2006; received in revised form 23 November 2006; accepted 1 December 2006
Available online 3 December 2006

Abstract

Three new *N*-alkyl substituted nitro (*E*)-3-azachalconium bromides (**4–6**) and two new stereoselective dimerization products (**7** and **8**) of *o*-, *m*-, and *p*-nitro substituted (*E*)-3-azachalcones were synthesized and tested for antimicrobial and antioxidant activities. Compounds **4–6** showed very good antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida tropicalis* and no activity against *Klebsiella pneumoniae* and *Yersinia pseudotuberculosis*. The monomers of *o*-, *m*-, and *p*-nitro substituted (*E*)-3-azachalcones (**1–3**) and their *N*-alkyl derivatives (**4–6**) showed good DPPH radical scavenging activity with IC_{50} values in the range of 0.25–0.90 mg/mL. The dimerization product **8** showed the highest activity among the eight compounds synthesized, while compound **7** was inactive in DPPH test. Compounds **4** and **8** showed similar antioxidant activity as the standard antioxidants Trolox® and Vitamin C.

The possible photochemical dimerization products of compounds **1–3** were calculated theoretically based on transition state structures. The experimentally obtained β-truxinic type dimers were also the expected products from transition state energy calculations.

Keywords: Nitro-(*E*)-3-azachalcones; Nitro-(*E*)-*N*-decyl-3-azachalconium bromide; Photodimerization; Antimicrobial and antioxidant activities

1. Introduction

Chalcones are among the most widely occurring natural compounds with various biological activities [1,2]. Azachalcones are homologs of chalcones with an annular nitrogen atom in the phenyl ring. They have been shown to posses a wide variety of biological activities, such as antituberculostatic, antimicrobial, anti-inflammatory, and antibacterial potential [3]. The azachalcones and their *N*-alkyl derivatives have been reported [4–6] to be the most potent of the chalcones series as inhibitors of myeloperoxidase release from rat polymorphonuclear leukocytes and microtubule polymerization inhibitors which bind to the colchicine-binding site of microtubules [7].

In the last few years, the isolation of some cyclobutane derivatives of natural compounds from *Agelas spectrum*, *Agelas conifera* [8], *Combretum albopunctatum* [9], and *Goniothalamus thwaitesii* [10] have been reported. The synthesis of cyclobutane ring is one of the most studied reactions due to dimerization of α,β-unsaturated carbonyl compounds in particular of 1,3-diaryl-2-propene-1-one (chalcones) [11–18] in organic photochemistry. Analogous to these dimers of chalcones, two new dimers (**7** and **8**) of (*E*)-3-azachalcones were synthesized stereoselectively in the current study.

The present work deals with the synthesis, spectral characterization, and biological activities of unreported three new *o*-, *m*-, and *p*-nitro substituted (*E*)-*N*-decyl-3-azachalconium bromide (**4–6**) and two new dimers of *m*- and *p*-nitro substituted 3-azachalcones (**7** and **8**) (Scheme 1).

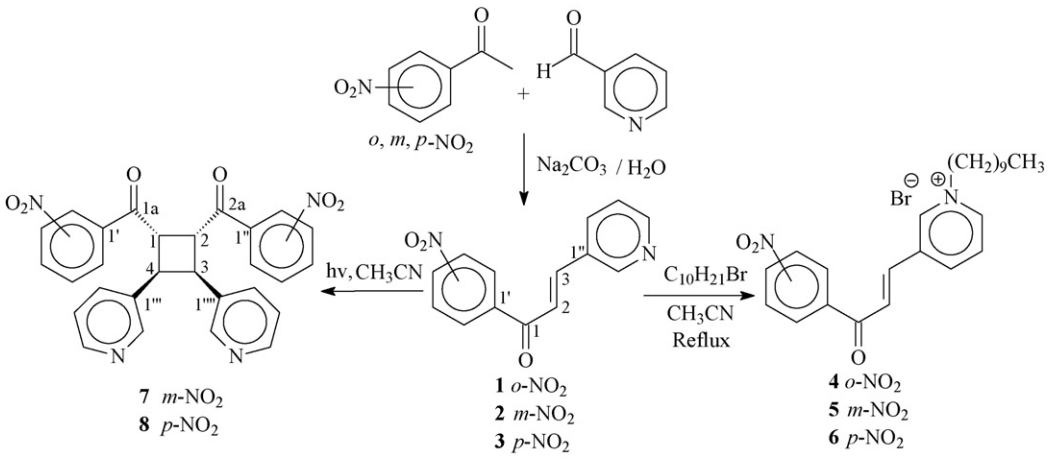
2. Experimental

2.1. General and instrumentation

NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$. The mass spectral analyses were carried out on a Micromass Quattro LC–MS/MS spectrophotometer.

* Corresponding author. Tel.: +90 462 3772486; fax: +90 462 3253196.
E-mail address: yayli@ktu.edu.tr (N. Yaylı).

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2006.12.004



Scheme 1. Reactions for the synthesis of compounds **1–8**.

The elemental analyses were performed on a Leco CHNS 932 instrument. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000–400 cm^{-1}) spectrometer. Melting points were determined by using a Thermo-var apparatus fitted with a microscope and are uncorrected. UV–vis spectral analyses were carried out on a Unicam UV2-100 spectrophotometer at 25 °C. Thin-layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminum plates. PTLC was carried out on Merck precoated 60 Kieselgel F_{254} (20 mm × 20 mm × 0.25 mm) silica gel plates.

3. Materials and methods

o-, *m*- and *p*-Nitroacetophenone and 3-pyridinecarboxaldehyde were purchased from Aldrich/Merck and used without further purification. The solvents (chloroform, *n*-hexane, ethanol, methanol, acetonitrile, and diethyl ether) used were either of analytical grade or bulk solvents distilled before use. The compounds **1–3** were prepared according to the literature [19–23].

*3.1. General procedure for synthesis of compounds **4–6***

A mixture of *o*-, *m*-, and *p*-nitro substituted (*E*)-3-azachalcone (0.02 mol) and 1-bromodecane (0.05 mol) in acetonitrile (30 mL) was refluxed for 24–36 h. Then acetonitrile was removed using a rotary evaporator. The residue was purified by column chromatography (column, 30 cm × 2 cm) on a silica gel (25 g, Merck, 230–400 mesh). The column was eluted successively with the following solvent and solvent mixture: *n*-hexane (30 mL) chloroform–methanol (70:3, 73 mL and 70:5, 75 mL). Fractions (10–15 mL each) were collected and monitored by analytical TLC. The desired products **4–6** were obtained from fractions 5–9 (47, 44, and 46% yield, $R_f = 0.31$, 0.18, and 0.23, respectively, chloroform–methanol, 10:1).

*3.1.1. (2E)-1-(2-nitrophenyl)-3-(N-decyl-3-pyridinium bromide)-2-propen-1-one (**4**)*

Light brown amorphous solid, mp 122–124 °C; UV λ_{max} ($CHCl_3$) (nm) 271 (ε/$dm^3\,mol^{-1}\,cm^{-1}$ 21,800); ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table 1; positive LC–MS/MS *m/z* (%); *m/z* = 476(20) $[M+2(^{81}Br)]^+$, 474(52) $[M(^{79}Br)]^+$, 427(10), 396(30) $[M-{}^{79}Br+H]^+$, 395(100) $[M-{}^{79}Br]^+$ or $[M+2-{}^{81}Br]^+$, 255(8), 248(78), 108(28). Calcd. for $C_{24}H_{31}N_2O_3Br$ (475.43): C 60.63, H 6.57, N 5.89; found (475.28): C 60.83, H 6.69, N 5.95; FT-IR cm^{-1}: 3435, 3076, 2923, 2853, 1653, 1625, 1530, 1466, 1343, 1283, 1105, 1062, 991, 855, 789.

*3.1.2. (2E)-1-(3-nitrophenyl)-3-(N-decyl-3-pyridinium bromide)-2-propen-1-one (**5**)*

Oily; UV λ_{max} ($CHCl_3$) (nm) 281 (ε/$dm^3\,mol^{-1}\,cm^{-1}$ 31,800); ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table 1; positive LC–MS/MS *m/z* (%); *m/z* = 476(78) $[M+2(^{81}Br)]^+$, 474(23) $[M(^{79}Br)]^+$, 427(8), 396(27) $[M-{}^{79}Br+H]^+$, 395(100) $[M-{}^{79}Br]^+$ or $[M+2-{}^{81}Br]^+$. Calcd. for $C_{24}H_{31}N_2O_3Br$ (475.43): C 60.63, H 6.57, N 5.89; found (475.35): C 60.89, H 6.81, N 6.05; FT-IR cm^{-1}: 3435, 3015, 2925, 2854, 1670, 1614, 1533, 1454, 1347, 1221, 1088, 976, 808, 705, 670.

*3.1.3. (2E)-1-(4-nitrophenyl)-3-(N-decyl-3-pyridinium bromide)-2-propen-1-one (**6**)*

Light yellowish amorphous solid, mp 94–96 °C; UV λ_{max} ($CHCl_3$) (nm) 291 (ε/$dm^3\,mol^{-1}\,cm^{-1}$ 37,400); ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table 1; positive LC–MS/MS *m/z* (%); *m/z* = 476(18) $[M+2(^{81}Br)]^+$, 474(44) $[M(^{79}Br)]^+$, 427(32), 396(30) $[M-{}^{79}Br+H]^+$, 395(100) $[M-{}^{79}Br]^+$ or $[M+2-{}^{81}Br]^+$, 254(39), 208(3), 159(3). Calcd. for $C_{24}H_{31}N_2O_3Br$ (475.43): C 60.63, H 6.57, N 5.89; found (475.68): C 60.49, H 6.52, N 5.85; FT-IR cm^{-1}: 3435, 3105, 3005, 2923, 2853, 1670, 1614, 1601, 1522, 1462, 1347, 1222, 1108, 1023, 976, 838, 760.

*3.1.4. Photodimerization of **2** and **3** in solution*

Solutions of compounds **2** and **3** (0.217 g, 0.214 g, respectively) in 30 mL of acetonitrile, kept in beakers, were exposed to UV light (400 W high-pressure Hg lamp). The progress of the reactions was followed by silica gel TLC (ethyl

Table 1
NMR data of compounds **4–6** in $CDCl_3$

Position	**4**[a]		**5**[a]		**6**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	–	190.82	–	186.35	–	186.88
2	7.46, AB, 16.0	136.48	7.74, AB, 15.4	136.21	7.72, AB, 15.6	135.99
3	7.80, AB, 16.0	129.09	8.60, AB, 15.4	128.23	8.60, AB, 15.6	128.60
1′	–	134.11	–	137.30	–	140.53
2′	–	146.46	8.74, bs	123.22	8.08, d, 8.6	130.40
3′	7.93, d, 8.2	124.26	–	147.79	8.43, d, 8.6	123.42
4′	7.60, m	134.91	8.72, d, 7.8	127.18	–	149.80
5′	7.60, m	131.69	7.55, dd, 7.8, 8.0	129.93	8.43, d, 8.6	123.42
6′	7.60, m	131.46	8.18, d, 8.0	135.29	8.08, d, 8.6	130.40
1″	–	134.22	–	135.21	–	135.39
2″	10.21, bs	144.37	10.61, bs	144.37	10.58, bs	144.46
4″	9.21, d, 5.8	143.74	9.21, d, 6.0	143.83	9.18, d, 6.0	143.77
5”	8.15, dd, 5.8, 8.0	128.43	8.17, dd, 6.0, 8.2	128.23	8.16, dd, 6.0, 8.2	128.22
6″	8.72, d, 8.0	144.37	8.81, d, 8.2	144.19	8.82, d, 8.2	144.16
1‴	4.90, t, 7.4	61.79	4.96, t, 6.6	61.49	4.97, t, 7.0	61.63
2‴	1.97, m	31.68	1.98, m	31.62	1.99, m	31.68
3‴–8‴	1.09, m	25.77	0.99, m	25.58	1.01, m	25.64
		28.78		28.59		28.64
		28.91		28.68		28.73
		29.04		28.84		28.90
		29.14		28.93		28.98
		31.43		31.24		31.29
9‴	1.09, m	22.32	0.99, m	22.08	1.01, m	22.13
10‴	0.74, t, 6.6	13.82	0.63, t, 6.8	13.58	0.65, t, 6.8	13.63

[a] Assignment based on ^1H, ^{13}C, ^1H–^1H COSY, and comparison with ACD NMR program.

acetate–methanol, 10:1). The reactions were stopped after ∼12 and 24 h, respectively. The solutions were evaporated, and the residues were purified by column chromatography (column, length 30 cm, diameter 2 cm) on a silica gel (25 g, Merck, 230–400 mesh). The columns were eluted successively with the following solvent and solvent mixtures: chloroform (30 mL), chloroform–ethyl acetate (1:1, 30 mL); chloroform–methanol (25:2, 30 mL; 20:5, 30 mL; 20:7, 30 mL). Fractions (10–15 mL each) were collected and monitored by analytical TLC. The desired products for **7** were obtained from fractions 6–9 (146 mg, 42% yield, $R_f = 0.42$, ethyl acetate–methanol, 10:1) and for **8** from fractions 8–11 (106 mg, 49% yield, $R_f = 0.34$, ethyl acetate–methanol, 10:1).

*3.1.5. (1α,2α)-di-(3-nitrobenzoyl)-(3β,4β)-di-(3-pyridyl)cyclobutane (**7**)*

Oily; UV λ_{max} ($CHCl_3$) (nm) 241 (ε/dm^3 mol^{-1} cm^{-1} 53,800); ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table 2; positive LC–MS/MS *m/z* (%); *m/z* = 509(100) $[M+H]^+$, 475(3), 413(5), 304(3), 255(5), 182(2), 156(2), 122(2). Calcd. for $C_{28}H_{20}N_4O_6$ (508.49): C 66.14, H 3.96, N 11.02; found (508.27): C 65.93, H 4.07, N 11.00; FT-IR cm^{-1}: 3082, 3027, 2928, 2857, 1693, 1614, 1575, 1531, 1479, 1428, 1349, 1222, 1096, 1026, 996, 812, 757, 711.

*3.1.6. (1α,2α)-di-(4-nitrobenzoyl)-(3β,4β)-di-(3-pyridyl)cyclobutane (**8**)*

Yellowish amorphous solid, mp 101–103 °C; UV λ_{max} ($CHCl_3$) (nm) 266 (ε/dm^3 mol^{-1} cm^{-1} 15,300); ^1H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) see Table 2; positive LC–MS/MS *m/z* (%); *m/z* = 509(100) $[M+H]^+$, 479(6), 395(2), 340(4), 254(6), 224(3), 182(2), 154(3), 107(4). Calcd. for $C_{28}H_{20}N_4O_6$ (508.49): C 66.14, H 3.96, N 11.02; found (508.20): C 65.91, H 3.94, N 11.12; FT-IR cm^{-1}: 3082, 3025, 2925, 2857, 1692, 1603, 1525, 1423, 1345, 1314, 1219, 1113,1026, 850, 749, 707.

3.2. Theoretical calculations

Theoretical investigations were performed with HYPERCHEM 7.5 program on an IBM PC Pentium IV computer. The HOMO and LUMO energies in the ground state and the HSOMO and LSOMO energies in the exited state were calculated by using the PM3 and PM3-RHF-CI semi-empirical methods [24–26].

3.3. Antimicrobial activity assessment

All test microorganisms were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows:

Table 2
NMR data of compounds **7–8** in $CDCl_3$

Position	**7**[a]		**8**[a]	
	δ_H	δ_C	δ_H	δ_C
1,2	4.96, AA′BB′, 6.4, 4.0, 1.6	48.19	4.88, AA′BB′, 6.2, 4.2, 1.8	48.45
3,4	4.56, AA′BB′, 6.4, 4.0, 1.6	42.23	3.95, AA′BB′, 6.2, 4.2, 1.8	42.35
1a, 2a	–	195.12	–	196.51
1′/1″	–	136.34	–	139.40
2′/2″	8.41, bs	122.77	7.96, AB, 9.0	129.00
3′/3″	–	149.17	8.26, AB, 9.0	124.19
4′/4″	8.43, dd, 1.8, 8.2	127.80	–	150.46
5′/5″	7.64, dd, 8.0, 8.2	133.54	8.26, AB, 9.0	124.19
6′/6″	8.15, dt, 1.4, 8.0	130.31	7.96, AB, 9.0	129.00
1‴/1⁗	–	135.38	–	135.46
2‴/2⁗	8.59, t, 2.0	148.75	8.40, t, 1.6	148.97
3‴/4⁗	8.40, d, 4.6	148.31	8.44, dd, 1.6, 6.4	148.80
5‴/5⁗	7.16, dd, 4.6, 8.2	123.48	7.16, dd, 6.4, 8.0	123.56
6‴/6⁗	7.37, dt, 2.0, 8.2	132.82	7.33, dt, 2.0, 8.0	132.84

[a] Assignment based on ^1H, ^{13}C, ^1H–^1H COSY, and comparison with ACD NMR program.

Escherichia coli ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 911, *Candida tropicalis* ATCC 13803. All the newly synthesized compounds were weighed and dissolved in dimethylsulfoxide (DMSO) to prepare the stock solutions of 1 mg/mL.

The antimicrobial activities of the substances were tested quantitatively in respective broth media by using double dilution, and the minimal inhibitory concentration (MIC) values (μg/mL) were determined [27]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulfoxide (DMSO) with dilution of 1:10 was used as solvent control.

3.4. Antioxidant activity

The antioxidant activity of the compounds was tested by utilizing DPPH scavenging [28]. Briefly, 750 μL samples of various concentrations (0.015–1.00 mg/mL in DMSO) were added to 750 μL of 50 mM ethanolic DPPH solution. After a 50 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 are expressed as IC_{50} (mg/mL), the compound concentration providing 50% scavenging of the DPPH radical present in the solution. The results were compared with those of Trolox® and Vitamin C.

4. Results and discussion

Scheme 1 illustrates the synthetic approach chosen for the preparation of *N*-alkyl azachalconium bromides (**4–6**) and dimerization products (**7**, **8**) of known *o*-, *m*-, and *p*-nitro substituted (*E*)-3-azachalcones (**1–3**) [19–23].

The most noticeable feature of the structural characterization of *o*-, *m*-, and *p*-nitro substituted (*E*)-3-azachalcones (**1–3**) is the assignment of the proton resonances of their α,β-unsaturated moiety, which was made by a careful analysis of their ^1H and 2D-COSY NMR. From the values of the vicinal coupling constants ($^3J_{H\alpha-H\beta} \sim 16.4/15.8/15.8$ Hz, respectively), it was possible to establish the *trans* configuration of these two protons [4–6].

Over the last 25 years, the synthesis of *N*-alkyl derivatives of (*E*)-3-azachalcones attract widespread interest because many of them have exhibited antimicrobial activities [4–6,10]. The length of the *N*-alkyl chain influences the antimicrobial activity and the highest activity has been observed with the presence of 10 carbon atoms in the bromoalkyl chain of the aza-compounds [3]. Thus, *n*-decyl bromide was chosen for the *N*-alkylation of compounds **1–3**. A series of three new *N*-alkyl substituted derivatives of *o*-, *m*-, and *p*-nitro substituted (*E*)-3-azachalcones (**4–6**) were synthesized by the reaction of compounds **1–3** with 1-bromodecane in boiling acetonitrile (Scheme 1). The geometry at the ethenylene bridge of (*E*)-*N*-alkyl-4-azachalconium bromides (**4–6**) was also assigned as *E* based on the olefin ^1H NMR coupling constants ($^3J_{H\alpha-H\beta} = 16.0/15.4/15.6$ Hz, respectively) [4–6].

m- and *p*-Nitro-(*E*)-3-azachalcone (**2** and **3**), when exposed to UV light (400 W high-pressure Hg lamp) in acetonitrile, were converted to the respective cyclobutanes (**7** and **8**) with the yields (chromatographed product, PTLC) of 42 and 49%, respectively. The minor products of these reactions were less than 5% and not characterized.

The structures of the cyclobutyl rings of products **7** and **8** were elucidated from their ^1H NMR spectra, which show highly shielded CH proton signals at δ_H 4.96(H_{1-2})/4.56(H_{3-4}) for **7** and δ_H 4.88(H_{1-2})/4.49(H_{3-4}) for **8**, respectively [4–7]. Stereochemistries of the dimers (**7** and **8**) were established on the basis of the ^1H NMR spectra and comparison with the literature data [11–14]. The obtained values for *J* are in agreement with a *cis*

relationship between the A and B part of system, while the values of J_A and J_B are in agreement with *cis* relationship between A and A′ and B and B′, when the values are 6.4 and 6.2 Hz for **7** and **8**, respectively [11–14]. Two symmetrical multiplets (AA'BB' system) at δ_H 4.96 (δ_C 48.19)/δ_H 4.56 (δ_C 42.23) for compound **7** and at δ_H 4.88 (δ_C 48.45)/δ_H 4.49 (δ_C 42.35) for compound **8** were observed for the cyclobutyl protons in ^{1}H NMR spectra. Simulation of these NMR patterns has allowed the calculation of the coupling constants of the cyclobutyl protons ($J_{AA'}$ = 6.4/6.2, J_{AB} = 4.0/4.2, $J_{AB'}$ = 1.6/1.8, $J_{BB'}$ = 6.4/6.2, respectively). The values of these coupling constants and ^{1}H and ^{13}C NMR patterns of the cyclobutyl moieties of compounds **7** and **8** suggest that the formation of cyclobutane ring occurs by *cis* head-to-head junction to give β-truxinic structure [12,13], different from the findings of earlier studies [10–18] and an on-going investigation.

The positive LC–MS/MS gave $[M+H]^+$ at *m/z* 255(100%) for **1–3**, $[M+2(^{81}Br)]^+$ at *m/z* 476(20, 78, and 18%) and $[M(^{79}Br)]^+$ at *m/z* 474(52, 23, and 44%) for **4–6**, and $[M+H]^+$ at *m/z* 509(100) for **7–8**, which were consistent with the molecular formulas to be $C_{14}H_{10}N_2O_3$ for **1–3**, $C_{24}H_{31}N_2O_3Br$ for **4–6**, and $C_{28}H_{20}N_4O_6$ for **7** and **8**. The rest of the fragmentation patterns of compounds **1–8** are shown in Scheme 2.

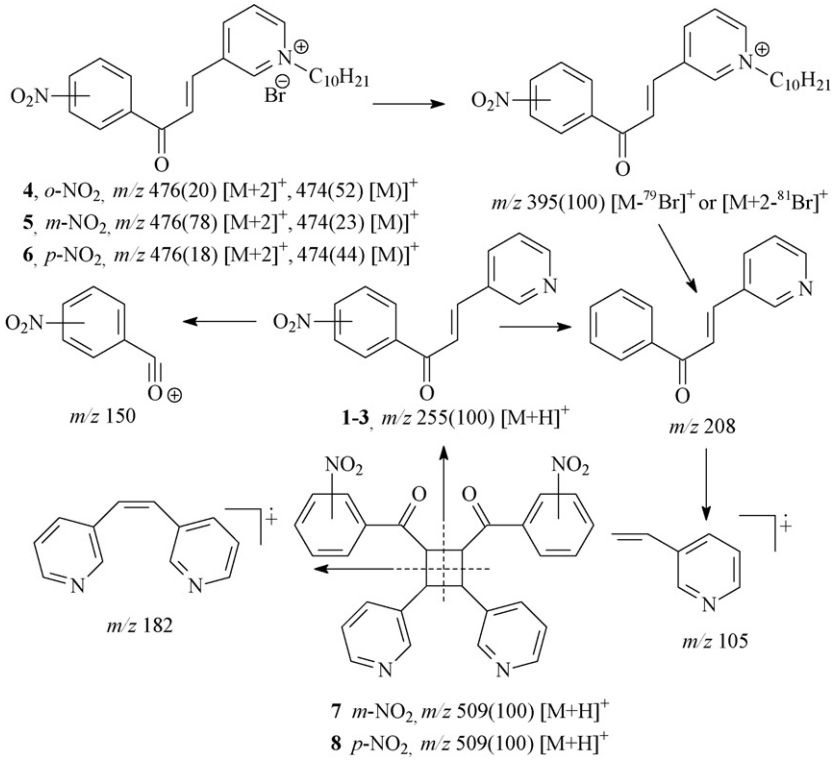
The compounds **4–8** were characterized on the basis of spectral data evaluations (^{1}H, ^{13}C, ^{1}H–^{1}H COSY NMR, FT-IR, UV–vis, and LC–MS/MS), whose results were in agreement with the proposed structures (Tables 1 and 2).

The photochemical irradiation of *o*-nitro-(*E*)-3-azachalcone (**1**) in acetonitrile or diethyl ether with or without benzophenone or benzoylperoxide as sensitizer in solution and solid state did not give any photodimer. The photochemical behavior of this substrate is similar to that reported in the case of ethyl cinnamate and cinnamonitrile [11], and α-methyl furyl acrylates [12]. In fact, the substrate was also recovered unchanged after prolonged reaction time in this case. The reason for this behavior could be explained after theoretical calculations (Table 3).

Table 3
HOMO/LUMO and HSOMO/LSOMO energies and electron coefficients of compounds **1–3**

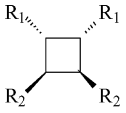
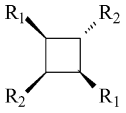
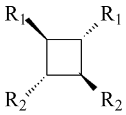
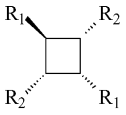
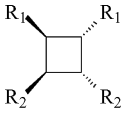
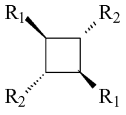
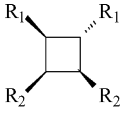
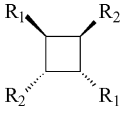
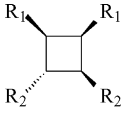
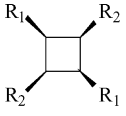
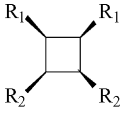
	1		**2**		**3**	
Electronic state	S_0	S_1	S_0	S_1	S_0	S_1
HOMO (eV)	−9.89		−9.84		−9.94	
C_α	0.50		−0.49		−0.49	
C_β	0.30		−0.29		−0.30	
LUMO (eV)	−1.49		−1.45		−1.71	
C_α	−0.26		0.23		−0.17	
C_β	0.36		−0.32		0.26	
HSOMO (eV)		−4.41		−4.33		−4.99
C_α		0.44		−0.46		−0.43
C_β		0.39		0.39		0.43
LSOMO (eV)		−6.84		−6.84		−6.95
C_α		0.61		0.60		0.60
C_β		−0.23		0.23		0.24

The irradiation of the compounds **2** and **3** led to the formation of **7a** and **8a** isomers, but compound **1** did not give any dimerization reaction. On the basis of this failure in the formation of any photoaddition product of compound **1**, we have examined the possibility of frontier orbital control in the stereochemical behavior, and some theoretical calculations were done to see



Scheme 2. Molecular and fragment ions observed in the positive LC–MS/MS spectra of compounds **1–8**.

Table 4
The total electronic energy of dimers and the transition state energy for the ring closure reaction for isomers of **7** and **8** (kcal/mol)

Isomers (head-to-head)	$-E$	Biradicals $\Delta H^{\neq}$	Isomers (head-to-tail)	$-E$	Biradicals $\Delta H^{\neq}$
 **a**	152185.8 **(7)** 152193.1 **(8)**	126.4 **(7)** 120.0 **(8)**	 **g**	152184.4 **(7)** 152173.6 **(8)**	128.2 **(7)** 130.0 **(8)**
 **b**	152183.8 **(7)** 152192.0 **(8)**	128.8 **(7)** 120.5 **(8)**	 **h**	152183.9 **(7)** 152176.4 **(8)**	128.8 **(7)** 136.2 **(8)**
 **c**	152182.2 **(7)** 152186.7 **(8)**	129.4 **(7)** 125.9 **(8)**	 **i**	152183.4 **(7)** 152183.6 **(8)**	129.2 **(7)** 129.1 **(8)**
 **d**	152180.8 **(7)** 152185.3 **(8)**	131.8 **(7)** 127.3 **(8)**	 **j**	152180.9 **(7)** 152186.4 **(8)**	131.8 **(7)** 126.3 **(8)**
 **e**	152178.9 **(7)** 152179.1 **(8)**	134.2 **(7)** 133.5 **(8)**	 **k**	152174.2 **(7)** 152169.2 **(8)**	138.5 **(7)** 143.5 **(8)**
 **f**	152175.7 **(7)** 152174.5 **(8)**	136.9 **(7)** 138.2 **(8)**			

R_1 = *m*- and *p*-$O_2NC_6H_4CO$-, R_2 = 3-pyridyl.

the optimized structure of compounds **1**–**3**. We estimated the HOMO and LUMO energies on the ground state and HSOMO and LSOMO energies in the exited state by using the PM3 [26] and PM3-RHF-CI semi-empirical methods (Table 3). The calculated electron density coefficients of compounds **1**–**3** are shown in Table 3. On the basis of the data for compound **1**, the superposition of HOMO/LSOMO and LUMO/HSOMO is not allowed (Table 3). The dimerization reaction of **2** takes place between HSOMO and LUMO orbitals because of energy barrier (Table 3). The energy gap between HSOMO and LUMO is smaller than that between HOMO and LSOMO. These data can explain the experimental formation of only head-to-head dimers. Similarly, the major product of the dimerization reaction of **3** is head-to-head dimer as expected from the total electronic energy of dimers and the transition state energy for the ring closure reaction for all possible dimers (Table 4).

Experimental results showed that single isomers were obtained by the dimerization of compounds **2** and **3**. Theoretical calculations were done in order to see compounds **7** and **8** to be kinetically the most stable isomers. In the photochemical reactions of compounds **2** and **3,** possible eleven different isomers are obtain according to kinetic theory [11–14]. As a result of experimental irradiation of compound **2** and **3**, isomers **7a** and **8a** were obtained, respectively. We calculate all possible isomers to show how to closure dimerization of cyclobutane and the energy of the transition state of the ring-closure reactions from the biradical *syn* and *anti* forms, [11–14] and results are reported in Table 4. According to the results obtained with semi-empirical method, the most stable of the dimers possible to form, having the lowest strain energy and heat of formation in transition state, is head-to-head isomer that has R_1 and R_2 groups in cyclobutane ring at *trans–cis–trans–cis* configuration. The results showed that the obtained isomers **7a** and **8a** are kinetically favored and have the most stable transition state energy for the two compounds (Table 4). As a result, the kinetically favorable photoadducts **7a** and **8a** were formed.

Based upon the above observations, the complete chemical shift assignments for **7,** and **8** were deduced to be (1α,2α)-di-(3-nitrobenzoyl)-(3β,4β)-di-(3-pyridyl)cyclobutane (**7**) and (1α,2α)-di-(4-nitrobenzoyl)-(3β,4β)-di-(3-pyridyl)cyclobutane (**8**).

The antimicrobial activity of all the compounds (**1**–**8**) was determined (Table 5). The activities of the synthesized compounds were investigated by broth microdilution method [27].

Table 5
Screening for antimicrobial activity of the compounds **1–8** (<0.35–1000 μg/mL)

Compound no.	Microorganisms and minimal inhibitory concentration (μg/mL)							
	Ec	*Kp*	*Yp*	*Pa*	*Ef*	*Sa*	*Bc*	*Ct*
1	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–
3	–	–	–	–	–	12	–	–
4	6	–	–	25	<0.35	<0.35	<0.35	3
5	6	–	–	25	<0.35	<0.35	<0.35	3
6	3	–	–	25	<0.35	0.75	0.75	3
7	–	50	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–
Amp.	8	32	32	>128	2	2	<1	
Flu.								8

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Yersinia pseudotuberculosis* ATCC 911, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 911, *Candida tropicalis* ATCC 13803. Amp.: Ampicillin, Flu.: Fluconazole, (–) no activity (1000 μg/mL).

The compounds **1–6** showed antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and a yeast-like fungus, but the other compounds showed no antimicrobial activity. The compounds showed better antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria. Compounds **4–6** exhibited broad-spectrum antimicrobial activity. These three compounds were active against all test organisms except for *K. pneumoniae* and *Y. pseudotuberculosis*. The MIC values for test microorganisms were between <0.35 and 25 μg/mL. Compound **3** and **7** were specifically effective against *S. aureus* and *K. pneumoniae* with the MIC values of 12 and 50 μg/mL, respectively. Compounds **1**, **2**, and **8** did not show any activity against the test microorganisms. The solvent control dimethylsulfoxide showed no inhibition effect on all test microorganisms.

The antioxidant activity of the synthesized monomeric, alkyl derivative and dimeric compounds of nitro substituted (*E*)-3-azachalcones (**1–3**) were also tested based on their ability to scavenge the stable radical DPPH (2,2-diphenyl-1-picrylhydrazine) [28]. The antioxidant activities are expressed as the compound concentration providing 50% scavenging of the available radicals (IC_{50}, mg/mL) (Fig. 1). All the compounds except **7** showed antioxidant activity with IC_{50} values in the range of 0.19–0.90 mg/mL. The seven active azachalcones showed about 10-fold better radical scavenging activity as compared to the heteroaryl chalcones containing thiophenyl ring tested in our earlier study, with IC_{50} values in 1.92–7.6 mg/mL range [17]. The monomeric compounds exhibited lower radical scavenging potential when compared to their alkyl derivatives in general (Fig. 1), with higher IC_{50} values. The dimerization product **8** showed the highest activity among the eight compounds tested, while compound **7** was inactive in DPPH test. Compounds **4** and **8** were found to be highly effective antioxidants as similar to the standard antioxidants Trolox® and Vitamin C. An increase was observed in the antioxidant activity in two of the monomers (**1** and **2**) when alkylated. The dimeric compound **8** showed higher activity as compared to its monomer and alkyl derivative of the monomer. The higher antimicrobial activities of the alkylated azachalcones **4** and **5** were paralleled with their high antioxidant activities. The both antimicrobial and antioxidant activities of the alkyl derivatives of azachalcones make them potential agents for the cure of bacterial infections accompanied by oxidative stress.

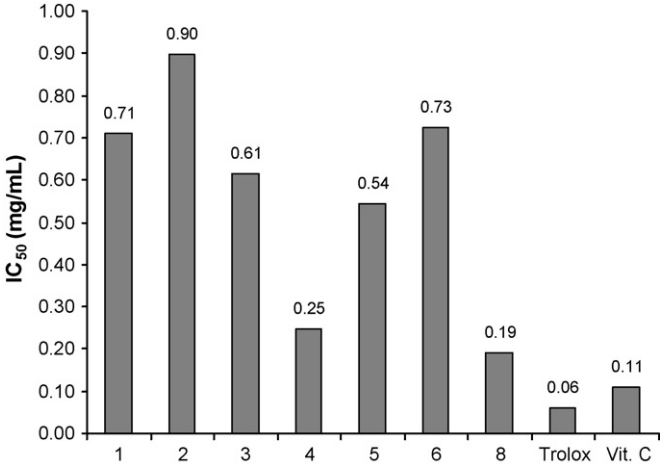


Fig. 1. The antioxidant capacities of the synthesized compounds based on DPPH radical scavenging activities. The results are given as IC_{50} (mg/mL), the concentration of the test compound that provides 50% scavenging of the DPPH radicals already available in the solution. Trolox® and Vitamin C are used as the standard reference antioxidants. Compound **7** was inactive.

Acknowledgements

This study was supported by grants from Karadeniz Technical University and the scientific and technological research council (TUBITAK) of Turkey.

References

[1] J.B. Harborne, The Flavonoids. Advances in Research, Chapman & Hall, London, 1988, pp. 329–388.
[2] J.P.J. Marais, D. Ferreira, D. Slade, Phytochemistry 66 (2005) 2145–2176.
[3] Z. Nowakowska, E. Wyrzykiewicz, B. Kedzia, Il Farmaco 56 (2001) 325–329.
[4] Z. Nowakowska, E. Wyrzykiewicz, B. Kedzia, Il Farmaco 57 (2002) 657–661.
[5] Z. Nowakowska, Magn. Reson. Chem. 38 (2000) 382–383.

[6] B.Z. Jovanović, M.M. Vuković, A.D. Marinković, J. Csanádi, J. Mol. Struct. 482/483 (1999) 371–374.
[7] M.L. Edwards, D.M. Stemarick, J.S. Sabol, K.A. Diekema, R.J. Dinerstein, J. Med. Chem. 37 (1994) 4357–4362.
[8] V. Seidel, F. Bailleul, P.G. Waterman, Phytochemistry 55 (2000) 439–446.
[9] D.R. Katerere, A.I. Gray, A.R. Kennedy, R.J. Nash, R.D. Waigh, Phytochemistry 65 (2004) 433–438.
[10] M. D'Auria, R. Racioppi, J. Photochem. Photobiol. A: Chem. 112 (1998) 145–148.
[11] M. D'Auira, R. Racioppi, Tetrahedron 53 (1997) 17307–17316.
[12] M. D'Auria, L. Emanuele, V. Esposito, R. Racioppi, Arkivoc xi (2002) 65–78.
[13] M. D'Auria, Heterocycles 54 (2000) 475–496.
[14] M. D'Auria, L. Emanuele, G. Mauriello, R. Racioppi, J. Photochem. Photobiol. A: Chem. 134 (2000) 147–154.
[15] N. Yaylı, A. Yaşar, O. Üçüncü, S.Ö. Sivrikaya, C. Güleç, M. Küçük, R. Abbasov, J Photochem. Photobiol. A: Chem. 171 (2005) 291–298.
[16] N. Yaylı, O. Üçüncü, A. Yaşar, Y. Gök, M. Küçük, S. Kolaylı, Turk. J. Chem. 28 (2004) 515–521.
[17] N. Yaylı, O. Üçüncü, E. Aydın, Y. Gök, A. Yaşar, C. Baltacı, N. Yıldırım, M. Küçük, J. Photochem. Photobiol. A: Chem. 169 (2005) 229–234.
[18] N. Yaylı, Y. Gök, O. Üçüncü, A. Yaşar, Ç. Atasoy, E. Şahinbaş, M. Küçük, J. Chem. Res. (2005) 155–159.
[19] J. Durinda, L. Szucs, J. Kolena, A. Nagy, E. Misikova, J. Heger, J. Farm. Fak. Univ. Komenskeho, Bratislava, Czech. Cesko-Slovenska Farmacie, 26 (1977) 140–149.
[20] T. Stefano, C. Sergio, R. Fabio, C. Lucia, J. Chem. Soc. Chem. Commun. 15 (1994) 1741–1742.
[21] M.J. Mphahlele, J. Mol. Struct. 688 (2004) 129–136.
[22] S.S. Chaphekar, S.D. Samant, J. Chem. Tech. Biotech. 79 (2004) 769–773.
[23] X. Huang, J. Org. Chem. 53 (1988) 4862–4864.
[24] R.B. Woodward, R. Hoffmann, The Conservation of Orbital Symmetry, Verlag Chemie Academic Press, 1970.
[25] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.P.S. Stewart, J. Am. Chem. Soc. 105 (1985) 3902–3909.
[26] J.J.P. Stewart, J. Comput. Chem. 101 (1989) 209–220.
[27] National Committee for Clinical Laboratory Standard, NCCLS Document M7-A3, 13 (25), Willanova, PA, USA, 1993.
[28] M. Cuendet, K. Hostettmann, O. Potterat, Helv. Chim. Acta 80 (1997) 1144–1152.