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Synthesis and Biological Activities of *N*-Alkyl Derivatives of *o*-, *m*-, and *p*-Nitro (*E*)-4-Azachalcones and Stereoselective Photochemistry in Solution, with Theoretical Calculations

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The *N*-alkyl derivatisation and photochemical dimerisation of 3 *o*-, *m*-, and *p*-nitro substituted 4-azachalcones (**1-3**) yielded 3 new *o*-, *m*-, and *p*-nitro substituted (*E*)-*N*-decyl-4-azachalconium bromides, (2*E*)-1-(2-nitrophenyl)-3-(*N*−decyl-4-pyridinium bromide)-2-propen-1-one (**4**), (2*E*)-1-(3-nitrophenyl)-3-(*N*-decyl-4-pyridinium bromide)-2-propen-1-one (**5**), and (2*E*)-1-(4-nitrophenyl)-3-(*N*-decyl-4-pyridinium bromide)-2-propen-1-one (**6**), and 3 new dimers in solution, (1β,2α)-di-(3-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (**7**), (1β,2α)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (**8a**), and (1β,2β)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (**8b**), stereoselectively. The monomeric compounds showed good antimicrobial activity against test micro-organisms. The most sensitive micro-organisms were Gram-positive bacteria. The monomers also showed high antioxidant activity, while the dimerisation products **7-8a,b** were less active. Compound **6** was found to have similar or even higher activity when compared to the standard antioxidants Trolox® and vitamin C, respectively.

The possible dimerisation products of compounds **1-3** were calculated theoretically. Experimental and theoretical calculations showed that δ-truxinic type dimer is the most stable isomer.

Key Words: Nitro-(*E*)-4-azachalcones, nitro-(*E*)-*N*-decyl-4-azachalconium bromide, photodimerisation, antimicrobial and antioxidant activities.

Introduction

Chalcones are a class of naturally occurring compounds with various biological activities[1]. They are known as the precursors of all flavonoid-type natural products in biosynthesis[2]. Among the various biological activities of chalcones are their insecticidal, antimicrobial, antichinoviral and antipicorniviral, and bacteriostatic

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properties[3]. Azachalcones, the derivatives of chalcones with an annular nitrogen atom in the phenyl ring, have also been reported to have a wide variety of biological activities, such as antibacterial, antituberculostatic, and anti-inflammatory potential[3]. The 4-azachalcones and their *N*-alkyl derivatives have been reported to be the most potent of the chalcones series as inhibitors of myeloperoxidase release from rat polymorphonuclear leukocytes and microtubule polymerisation inhibitors which bind to the colchicine-binding site of microtubules[4].

The cycloaddition reaction of alkenes to give cyclobutane dimers is one of the most studied reactions in organic photochemistry. Various cyclobutane-containing substituted compounds were synthesised by the photochemical dimerisation of α,β-unsaturated carbonyl compounds, in particular of 1,3-diaryl-2-propene-1-one (chalcones)[5−11], and isolated from various plants[12−14]. Analogous to these dimers of chalcones, 3 new dimers of 4-azachalcones (**7**, **8a,** and **8b**) were synthesised stereoselectively in the current study.

The present work deals with the synthesis, spectral characterisation and results of biological activity assays of 3 new *o*-, *m*-, and *p*-nitro substituted (*E*)-*N*-decyl-4-azachalconium bromides (**4**, **5**, and **6**), and 3 new dimers of *m*- and *p*-nitro substituted 4-azachalcones (**7**, **8a,** and **8b**).

Experimental

General and instrumentation

NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$,$CDCl_3$-CD_3COOD (10 drops), and $CDCl_3$-CD_3OD (10 drops). NMR data assignment was based on ^{1}H, ^{13}C, APT, ^{1}H-^{1}H COSY, and ACD NMR program. The mass spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrophotometer. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000-400 cm^{-1}) spectrometer. Melting points were determined using a Thermo-var apparatus fitted with a microscope and are uncorrected. UV-vis spectral analyses were carried out on a Unicam UV2-100 at 25 °C. Thin-layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminium plates. PTLC was carried out on Merck precoated 60 Kieselgel F_{254}(20 x 20 0.25 mm) silica gel plates.

Materials and methods. *o*-, *m*-, and *p*-nitroacetophenone and 4-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. Compounds **1-3** were prepared according to the literature[14−20].

General procedure for synthesis of compounds 4-6. *o*-, *m*- and *p*-nitro substituted (*E*)-4-azachalcone (**1-3**) (0.02 mol) and 1-bromodecane (0.05 mol) in acetonitrile (30 mL) were refluxed separately for 24-48 h. Then the acetonitrile was removed using a rotary evaporator. The residue was purified by column chromatography (column, length 30 cm, diameter 2 cm) on silica gel (25 g, Merck, 230-400 mesh). The column was eluted successively with the following solvent and solvent mixture: *n*-hexane (20 mL), and 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 7-9 (75%, 82%, and 66% yield, R_f = 0.60, 0.87, and 0.73, chloroform-methanol, 3:1), respectively.

(2*E*)-1-(2-nitrophenyl)-3-(*N*-decyl-4-pyridinium bromide)-2-propen-1-one (4): Oily; UV λ_{max}^{CHCl3} nm: 283 (ε, 5819); ^{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(28) $[M+H]^+$, 475(17) $[M]^+$, 396(30) $[M-Br+H]^+$,

395(100) [M-Br]$^+$, 309(14), 278(12), 248(22), 179(35), 151(94), 126(13); molecular weight (m/z) calcd. for $C_{24}H_{31}N_2O_3Br$, 475.43; found, 475.19; FT-IR cm^{-1}: 3411, 3010, 2926, 2855, 1671, 1638, 1527, 1466, 1346, 1226, 1018, 975, 856, 755.

Table 1. NMR data of compounds **4-6**.

	4^{a,b}		**5**^{a,b}		**6**^{a,c}	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	-	190.9	-	186.7	-	187.6
2	7.25, AB, 16.4	136.7	7.77, AB, 15.4	137.7	7.83, AB, 15.6	137.7
3	7.48, AB, 16.4	131.4	8.58, AB, 15.4	132.1	8.46, AB, 15.6	132.1
1′	-	134.2	-	137.5	-	140.7
2′	-	145.9	8.86, d, 2.6	123.7	8.37, d, 6.6	130.4
3′	7.97, d, 7.4	124.3	-	148.2	8.46, d, 6.6	123.9
4′	7.68, t, 7.4	135.0^d	8.68, d, 8.0	127.6	-	150.6
5′	7.56, t, 7.4	134.4^d	7.68, t, 8.0	130.3	8.46, d, 6.6	123.9
6′	7.45, d, 7.4	128.6	8.32, d, 8.0	135.3	8.37, d, 6.6	130.4
1″	-	149.6	-	150.4	-	150.5
2″-6″	8.29, d, 6.4	126.6	8.73, d, 6.0	127.3	8.56, d, 6.6	127.1
3″-5″	9.30, d, 6,4	144.9	9.34, d, 6.0	144.9	9.13, d, 6.6	144.9
1‴	4.73, t, 6.8	61.3	4.88, t, 6.4	61.6	4.80, t, 7.2	61.9
2‴	1.86, m	31.3	1.99, m	31.7	2.07, m	31.7
3‴-9‴	1.04, m	22.2	1.19, m	22.4	1.28, m	22.5
		25.6		25.9		26.0
		28.6		28.9		28.9
		28.7		29.0		29.1
		28.9		29.1		29.2
		29.0		29.2		29.3
		31.3		31.6		31.4
10‴	0.68, t, 6.6	13.7	0.75, t, 6.0	13.9	0.87, t, 6.8	13.8

[a] Assignment based on ^{1}H, ^{13}C, APT, ^{1}H-^{1}H COSY, and ACD NMR program.
[b] In $CDCl_3$.
[c] In $CDCl_3$-CD_3COOD (10 drops).
[d] May exchange.

(2*E*)-1-(3-nitrophenyl)-3-(*N*-decyl-4-pyridinium bromide)-2-propen-1-one (5): Oily; UV λ_{max}^{CHCl3} nm: 294 (ε, 37336); ^{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(30) [M+H]$^+$, 475(20) [M]$^+$, 396(32) [M-Br+H]$^+$, 395(100) [M-Br]$^+$, 254(72), 208(8), 108(11); molecular weight (m/z) calcd. for $C_{24}H_{31}N_2O_3Br$, 475.43; found, 475.88; FT-IR cm^{-1}: 3413, 3082, 3010, 2925, 2854, 1672, 1637, 1615, 1531, 1465, 1349, 1222, 1087, 983, 811, 727, 697.

(2*E*)-1-(4-nitrophenyl)-3-(*N*-decyl-4-pyridinium bromide)-2-propen-1-one (6): Yellowish amorphous solid, mp 179-180 °C; UV λ_{max}^{CHCl3} nm: 241, 289 (ε, 7123, 9917); ^{1}H NMR ($CDCl_3$-CD_3COOD (10 drops), 200 MHz) and ^{13}C NMR ($CDCl_3$-CD_3COOD (10 drops), 50 MHz) δ (ppm) see Table 1; positive LC-MS/MS m/z (%); m/z = 476(18) [M+H]$^+$, 475(26) [M]$^+$, 396(20) [M-Br+H]$^+$, 395(100) [M-Br]$^+$, 255(3), 209(3), 154(5), 135(4); molecular weight (m/z) calcd. for $C_{24}H_{31}N_2O_3Br$, 475.43; found, 475.44; FT-IR cm^{-1}: 3435, 3108, 3003, 2918, 2852, 1670, 1637, 1601, 1522, 1470, 1338, 1220, 1009, 976, 856, 837, 732.

Photodimerisation of 2 in solution. A solution of compound **2** (2 g) in 50 mL of acetonitrile, kept in a beaker, 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 ∼12 h. The solution was evaporated and a portion of the residue was purified by PTLC (0.5 mm, 20 x 20 cm, 2 plates) to give compound **7** (27 mg, 45% yield, R_f = 0.15, ethyl acetate-methanol, 10:1).

Photodimerisation of 3 in solution. A solution of compound **3** (1 g) in 35 mL of acetonitrile, kept in a beaker, 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 ∼24 h. The solution was evaporated and a portion of the residue purified by PTLC (220 mg, 0.5 mm, 20 x 20 cm, 3 plates) to give compounds **8a** and **8b** (112 mg and 93 mg, 51% and 42% yield, R_f = 0.62 and 0.82, ethyl acetate-methanol (3:1), respectively).

(1β,2α)-di-(3-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (7): Yellowish amorphous solid, mp 104-106 °C; UV λ_{max}^{CHCl3} nm: 245 (ε, 30088); ^{1}H 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(8), 417(5), 329(5), 290(3), 236(3), 186(4), 154(6), 114(48); molecular weight (m/z) calcd. for $C_{28}H_{20}N_4O_6$, 508.49; found, 508.34; FT-IR cm^{-1}: 3082, 3021, 2926, 2857, 1682, 1597, 1531, 1473, 1416, 1350, 1223, 1102, 993, 818, 752, 713.

Table 2. NMR data of compounds **7**-**8a,b**.

	7[a,b]		**8a**[a,b]		**8b**[a,c]	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1,2	4.69, AA′BB′	46.6	4.66, AA′BB′	46.7	5.55, dd, 9.8, 9.2 4.90, dd, 9.4, 9.2	48.2 46.4
3,4	3.98, AA′BB′	46.1	3.95, AA′BB′	46.1	4.99, dd, 9.8, 9.4 4.71, dd, 9.8, 9.4	43.3 40.3
1a, 2a	-	195.5	-	196.2	-	197.5 199.8
1′/ 1″	-	135.8	-	138.9	-	139.6 138.9
2′/ 2″	8.65, bs	123.7	7.99, AB, 8.8	129.8	8.40, d, 9.2 7.89, d, 8.8	130.0 128.9
3′/ 3″	-	148.1	8.22, AB, 8.8	124.0	8.55, d, 9.2 8.20, d, 8.8	124.4 123.8
4′/ 4″	8.39, dd, 8.0, 2.0	128.4	-	148.3	-	150.6 150.2
5′/5″	7.61, dd, 8.0, 7.6	130.2	8.22, AB, 8.8	124.0	8.55, d, 9.2 8.20, d, 8.8	124.4 123.8
6′/ 6″	8.17, d, 7.6	134.1	7.99, AB, 8.8	129.8	8.40, d, 9.2 7.89, d, 8.8	130.0 128.9
1‴/ 1⁗	-	148.4	-	150.8	-	147.0 144.8
2‴/ 2⁗ 6‴/ 6⁗	7.22, d, 6.0	122.1	7.20, d, 6.2	122.1	6.88, d, 5.4 6.84, d, 6.4	122.1 123.7
3‴/ 3⁗ 5‴/ 5⁗	8.63, d, 6.0	150.7	8.62, d, 6.2	150.6	8.32, d, 5.4 8.16, d, 6.4	148.8

[a] Assignment based on ^{1}H, ^{13}C, APT, ^{1}H-^{1}H COSY, and ACD NMR program.
[b] In $CDCl_3$.
[c] In $CDCl_3$-CD_3OD (10 drops).

(1β,2α)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (8a): Yellowish amorphous solid, mp 217-219 ^{o}C; UV λ_{max}^{CHCl3} nm: 266 (ε, 31034); ^{1}H 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) $[M+H]^+$, 494(32), 466(15), 457(135), 429(13), 385(5), 313(8), 254(146), 225(16), 183(4), 154(7), 123(22); molecular weight (m/z) calcd. for $C_{28}H_{20}N_4O_6$, 508.49; found, 508.28; FT-IR cm^{-1}: 3080, 3025, 2925, 2846, 1681, 1598, 1525, 1410, 1347, 1319, 1224, 1108, 982, 853, 757, 713.

(1β,2β)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (8b): Oily; UV λ_{max}^{CHCl3} nm: 266 (ε, 80285); ^{1}H NMR ($CDCl_3$-CD_3OD (10 drops), 200 MHz) and ^{13}C NMR ($CDCl_3$-CD_3OD (10 drops), 50 MHz) δ (ppm) see Table 2; positive LC-MS/MS m/z (%); m/z = 509(100) $[M+H]^+$, 510(30) $[M+2]^+$, 511(7) $[M+3]^+$, 479(6), 273(63), 254(7), 182(3), 154(4), 126(3); molecular weight (m/z) calcd. for $C_{28}H_{20}N_4O_6$, 508.49; found, 508.57; FT-IR cm^{-1}: 3104, 3016, 2927, 2851, 1682, 1600, 1525, 1415, 1346, 1325, 1216, 1108, 980, 845, 753, 710.

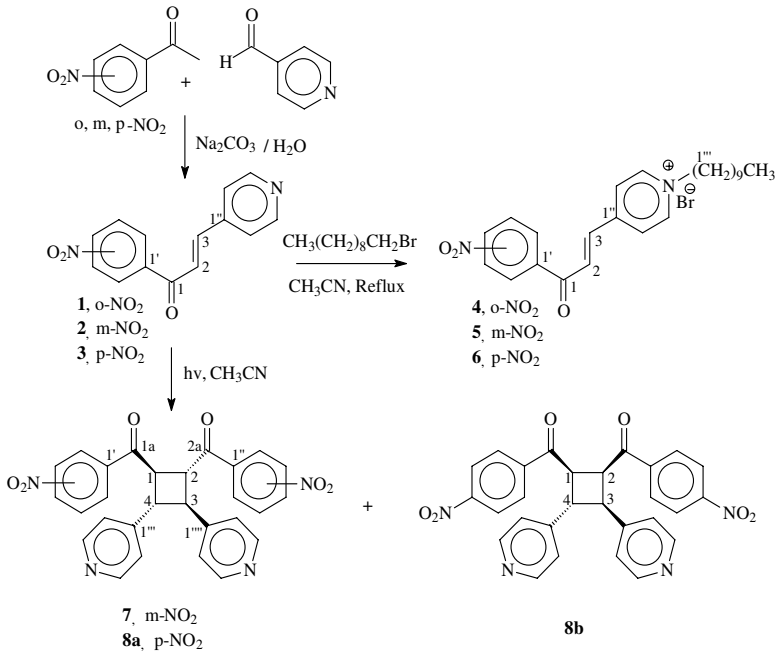
Antimicrobial activity assessment. All test micro-organisms were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 911, and *Candida tropicalis* ATCC 13803. All the newly synthesised 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 inhibition concentration (MIC) values (μg/mL) were determined[21]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI, USA) at pH 7.3 and buffered Yeast Nitrogen Base (Difco) 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. DMSO with a dilution of 1:10 was used as solvent control.

Antioxidant activity. The antioxidant activity of the compounds was tested by utilising DPPH scavenging[22]. Briefly, 50 μL samples of various concentrations were added to 5 mL of 0.004% ethanolic DPPH solution. 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 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.

Results and Discussion

In our work, *o*-, *m*-, and *p*-nitro substituted (*E*)-4-azachalcones (**1-3**)[15,16] were prepared with the Claisen-Schmidt condensation of an appropriate aromatic ketone with 4-pyridinecarboxaldehyde[17] according to the route indicated in the Scheme. The most noticeable feature of the structural characterisation of compounds **1-3** is the assignment of the proton resonances of their α,β-unsaturated moiety, which was done by a careful analysis of their ^{1}H and 2D-COSY NMR. From the values of the vicinal coupling constants ($^{3}J_{H\alpha-H\beta}$ ~15.8/16.4 Hz) it was possible to establish the *trans* configuration of these 2 protons.



Scheme

N-Alkyl derivatives of (*E*)-4-azachalcones attract widespread interest because many of them have exhibited antimicrobial activities[3,18,19]. Literature data revealed that the length of the *N*-alkyl chain influences the antimicrobial activity[3,18,19]. The highest activity is connected with the presence of 10 carbon atoms in the bromoalkyl chain of the aza-compounds[3,18]. Thus, *n*-decyl bromide was chosen for the *N*-alkylation of compounds **1-3**. A series of 3 new bromides of (*E*)-*N*-alkyl substituted derivatives of *o*-, *m*-, and *p*-nitro substituted (*E*)-4-azachalcones (**4-6**) were synthesised by the reaction of compounds **1-3** with 1-bromodecane in boiling acetonitrile. All the compounds were characterised on the basis of spectral data studies (^{1}H, ^{13}C, APT, ^{1}H-^{1}H COSY NMR, FT-IR, UV-Vis, and LC-MS/MS)[3,18–20], whose results were in agreement with the proposed structure. The geometry at the ethylene bridge of (*E*)-*N*-alkyl-4-azachalconium bromides (**4-6**) was also assigned as *E* based on the olefin ^{1}H NMR coupling constants ($^3J_{H\alpha-H\beta}$ = 16.4/15.4/15.6 Hz, respectively).

m-Nitro-(*E*)-4-azachalcone (**2**), when exposed to UV light (400 W high-pressure Hg lamp) in acetonitrile, was converted to the respective cyclobutane (**7**) with the yield (chromatographed product, PTLC) of 42%. The irradiation of *p*-nitro-(*E*)-4-azachalcone (**3**) under the same conditions gave a mixture of 2 dimers, **8a** and **8b**, as major products, with the yields (chromatographed products, PTLC) of 53% and 42%, respectively. From a synthetic point of view it is noteworthy that the compounds obtained in the highest yield (**7** and **8a**) showed the same stereochemistry as the naturally occurring cyclobutanes[11–13] and the stereochemistry of compound **8b** was rel. 1β,2β,3β,4α[4–7]. The minor products of these reactions were less than 5% and were not characterised.

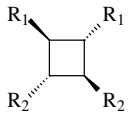
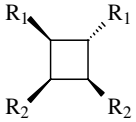
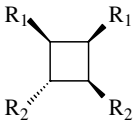
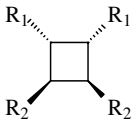
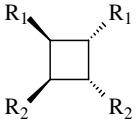
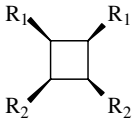
The photochemical irradiation of *o*-nitro-(*E*)-4-azachalcone (**1**) in acetonitrile or diethyl ether with or without benzophenone or benzoylperoxide as sensitiser in solution and solid state did not yield the anticipated products. The photochemical behaviour of this substrate is similar to that reported in the case of cinnamonitrile and ethyl cinnamate[4]. In fact, the substrate was also recovered unchanged after a

prolonged reaction time in this case. The photochemical dimerisation of compounds **2** and **3** was successfully performed experimentally in acetonitrile solution. Experimental results showed that 1 and 2 stable isomers were obtained by the dimerisation of compounds **2** and **3**, respectively.

In the photochemical reactions of compounds **2** and **3,** theoretically 11 different isomers can be obtained according to kinetic theory[4−7]. We calculated the heat of formation of 6 isomers obtained by head-to-head addition using the AM1 semi-empirical method. The results showed that isomers **7** and **8a** were the most stable isomers in this method.

As a result of experimental irradiation of compound **3**, 2 isomers were obtained in 53% and 42% yields. According to spectroscopic results, these are isomers **8a** and **8b**. The most stable isomer obtained experimentally was in parallel with computational data, but the second was not. To solve this apparent problem, we calculated the energy of the transition state of the ring-closure reactions from the biradical *syn* and *anti* forms[4−7]. The biradical *syn* can convert into 3 dimers, and biradical *anti* can convert into 3 other dimers[5,6]. The results are listed in Table 3. The calculations showed that isomer **8a** has the most stable transition state energy and isomer **8b** is second. The results showed that the second obtained isomer is kinetically favoured. As a result, the ring closure is a kinetic process and it cannot be explained by the thermodynamic stability of the products.

Table 3. The total electronic energy of head-to-head dimers and the transition state for the ring closure reaction for isomers of **8**.

Isomers	-E	Biradicals $\Delta H^{\neq}$ [kcal/mol^{-1}]	Isomers	-E	Biradicals $\Delta H^{\neq}$ [kcal/mol^{-1}]
 **8a**	152191.95	120.70	 **8d**	152184.45	127.83
 **8b**	152188.62	124.02	 **8e**	152173.64	139.02
 **8c**	152186.31	126.34	 **8f**	152162.58	150.07

R_1= *p*-NO_2PhCO-, R_2= 4-Pyridinyl

The structures of the cyclobutyl rings of products **7** and **8** were elucidated from their ^{1}H NMR spectra, which show highly shielded CH protons signals at δ_H 4.69(H_{1-2})/3.98(H_{3-4}) for **7**, δ_H 4.66(H-1,2)/3.95(H-3,4) for **8a**, and δ_H 5.55(H-1)/4.90(H-2)/4.99(H-3)/4.71(H-4)/ for **8b**[4−7]. Stereochemistries of the compounds **7** and **8a** were determined from NMR spectrometry information. Two symmetrical multiplets (AA′BB′) at δ_H 4.69 (δ_C 46.57) / δ_H 3.98 (δ_C 46.14) for compound **7** and at δ_H 4.66 (δ_C 46.69) / δ_H 3.95 (δ_C 46.09) for compound **8a** were observed for the cyclobutyl protons in ^{1}H NMR spectra. Simulation of these NMR patterns allowed the calculation of the coupling constants of the cyclobutyl protons ($J_{AA'} = 9.2$,

J_{AB} = 5.8, $J_{AB'}$ = not detected, $J_{BB'}$ = 9.2). The values of these coupling constants suggest that **7** and **8a** were formed by head-to-head coupling, but they do not allow a certain assignment with respect to *syn* / *anti* stereochemistry. 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 in compounds **7** and **8a**[4−10].

The structural connectivities of compounds **7-8a,b** were established, in part from ^{1}H-^{1}H COSY. It was found that for the most downfield for the cyclobutyl ring, methine designated H-1/H-2 at δ_H 4.69 was connected to H-3/H-4 at δ_H 3.98 for **7,** H-1/H-2 at δ_H 4.66 was connected to H-3/H-4 at δ_H 3.95 for **8a,** and H-1at δ_H 5.55 (dd, 9.8, 9.2 Hz) was connected to H-2 at δ_H 4.90 (dd, 9.4, 9.2 Hz) and then H-3 at δ_H 4.99 (dd, 9.8, 9.4 Hz) to H-4 at δ_H 4.71 (dd, 9.8, 9.4 Hz) for **8b**. The chemical shifts of compounds **7** and **8a** are in total agreement with those of similar structures in the literature with the δ-truxinic structure[4−10], and the stereochemistry of cyclobutane ring in compound **8b** was rel.1β,2β,3β,4α as in the literature.[4] Further connectivities for the *o*-, *m*-, and *p*-nitro substituted phenyl and pyridinyl parts of the compounds **7-8a,b** were observed from the ^{1}H-^{1}H COSY NMR spectra.

The positive LC-MS/MS gave M+H at m/z 255 (100%) for **1-3,** at m/z 476 (28%, 30%, and 18%) for **4-6,** and at m/z 509 (100%) for **7-8a** and **8b**, which were consistent with the molecular formulae being $C_{14}H_{10}N_2O_3$ for **1-3,** $C_{24}H_{31}N_2O_3Br$ for **4-6,** and $C_{28}H_{20}N_4O_6$ for **7-8a** and **8b**.

In our previous[9−11] and present work and literature reports[4−7,11,12], dimerisation of chalconoid compounds showed half of the total carbon resonance peak in their ^{13}C NMR spectra as expected due to the symmetry of structures as we found in compounds **7** and **8a**. However, in this study, we saw some of the carbon resonances for compound **8b** to be 2 peaks for each symmetrical carbon such as C_{1a}/C_{2a} at δ ∼199.75/197.52 ppm in their ^{13}C NMR spectra. This could be the unsymmetrical substitution of the cyclobutyl ring of compound **8b**, which causes the loss of symmetry of this molecule.

Based upon the above observations, the complete chemical shift assignments for **7, 8a,** and **8b** were deduced and are shown in the experimental section. Compounds **7**, **8a**, and **8b** were thus shown to be (1β,2α)-di-(3-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (**7**), (1β,2α)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (**8a**), and (1β,2β)-di-(4-nitrobenzoyl)-(3β,4α)-di-(4-pyridinyl)cyclobutane (**8b**).

The antimicrobial activity of all the compounds (**1-8a,b**) was determined (Table 4). The activities of the synthesised compounds were investigated by broth microdilution[21]. Compounds **1-6** showed antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and yeast-like fungi, but the other compounds showed no antimicrobial activity. The compounds showed better antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria. Compounds **5** and **6** exhibited broad-spectrum antimicrobial activity. These 6 compounds were active against all test organisms with the exception of *K. pneumoniae* and *Y. pseudotuberculosis*. The MIC values (MBC) for the test micro-organisms were between <0.35 and 25 μg/mL. Compound **3** was specifically effective against *S. aureus*, with a MIC value of 25 μg/mL. Compound **1** showed activity against only Gram-positive bacteria, *B. cereus* and *S. aureus*, with MIC values <0.35 and 3 μg/mL, respectively. The solvent control DMSO showed no inhibition effect on the test micro-organisms.

The synthesised compounds were also tested for their antioxidant activity based on their ability to scavenge the stable radical DPPH (2,2-diphenyl-1-picrylhydrazine)[22]. The results are expressed as IC_{50} (mg/mL), the compound concentration providing 50% scavenging of the available radicals. All the

compounds showed antioxidant activity. The monomeric compounds exhibited higher radical scavenging potential in general (Figure), with low IC_{50} values. Dimeric compounds **7**, **8a**, and **8b** showed lower antioxidant activity when compared to both monomers and standard antioxidants Trolox® and vitamin C. Compounds **1** and **6** were highly active in DPPH scavenging, and the radical scavenging capacity of compound **6** was either equal to or higher than the reference antioxidants Trolox® and vitamin C, respectively. The higher antimicrobial activities of the alkylated azachalcones **4-6** were parallel with their high antioxidant activities. The combined biological activities of these monomeric azachalcones make them potential agents for intervention in bacterial infections causing oxidative stress.

Table 4. Screening for antimicrobial activity of the compounds **1-8a,b** (<0.35-1000 μg/mL).

Comp. No.	Micro-organisms and Minimal Inhibition Concentration Value							
	Ec	***Kp***	Yp	***Pa***	***Ef***	***Sa***	***Bc***	***Ct***
1	-	-	-	-	-	3	<0.35	-
2	-	25	-	12	3	6	3	25
3	-	-	-	-	-	25	-	-
4	-	-	-	12	3	1.5	-	-
5	6	-	-	25	0.75	0.75	0.75	<0.35
6	3	-	-	25	0.75	0.35	0.75	<0.35
7	-	-	-	-	-	-	-	-
8a	-	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-	-
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).

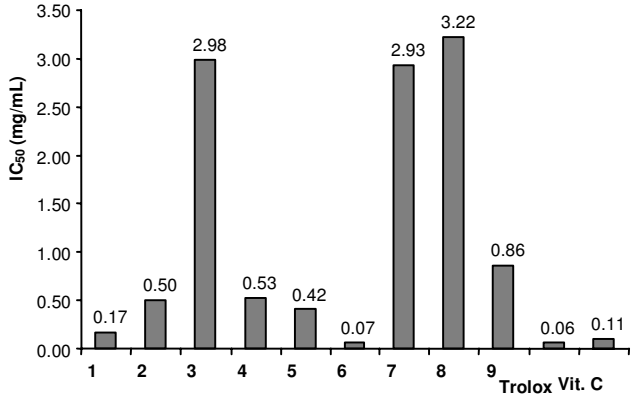


Figure. The antioxidant capacities of the synthesised compounds based on DPPH radical scavenging activities. The results are given as IC_{50} (mg/mL), the concentration of the test compound that provides 50% scavenging of the DPPH radicals already available in the solution. Trolox® and vitamin C are used as the standard reference antioxidants.

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