

Intramolecular 4π photocyclization of chalconoid-like compounds in solution and antimicrobial activities

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Abstract

Two new (**2** and **8**) and eight known (**1**, **3–7**, **9** and **10**) *o*-, *m*-, *p*-nitro, -methoxy and -methyl substituted chalconoid and alnustone-like compounds with a skeleton of (2*E*,4*E*)-1,5-diarylpenta-2,4-dien-1-one (**1–10**) were synthesized and their $4n(\pi)$ photocyclization reaction gave 10 new *o*-, *m*-, *p*-nitro, -methoxy and -methyl substituted *cis*-3-benzoyl-4-phenylcyclobutenes (**11–20**), stereoselectively, as major products in solution. The antimicrobial activities of all the compounds were also investigated. They showed antibacterial activity against Gram-positive bacteria, moderate antibacterial activity against Gram-negative bacteria, but no antifungal activity was observed against yeast-like fungi.

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1. Introduction

Chalconoids and alnustone are naturally occurring compounds [1–4], which have diarylpropanoid ((2*E*)-1,3-diphenylprop-2-en-1-one) and diarylheptanoid ((4*E*,6*E*)-1,7-diphenylhepta-4,6-dien-3-one) structures having aryl-C₃-aryl and aryl-C₇-aryl skeletons, respectively. They have been shown to exhibit a wide range of biological activities [1–5]. Analogous to chalconoid and alnustone structures, two unknown (**2** and **8**) and eight known (**1** [6,7], **3** [8], **4** [9], **5–6** [10], **7** [6,7,11,12], **9** [13] and **10** [6,11,12]) *o*-, *m*-, *p*-nitro, -methoxy and -methyl substituted chalconoid- and alnustone-like compounds with aryl-C₅-aryl skeleton and (2*E*,4*E*)-1,5-diarylpenta-2,4-dien-1-one structure were synthesized in the current study.

Intramolecular photocyclization of butadiene is a fast and simple method to give a cyclobutene ring [14–19]. The cyclization of the substituted 2*E*,4*E*-diene isomers under

photochemical conditions yields *cis*-products [14–20]. In the literature, various cyclobutene-containing [15–19] and cyclobutane-containing compounds have been reported to be synthesized [13,21–24], and cyclobutane-containing compounds have also been isolated from various plants [25–28]. Although, photocyclization of butadienes has been studied extensively [15–20], photochemical cyclization of (2*E*,4*E*)-1,5-diarylpenta-2,4-dien-1-one and its derivatives in solution has not been reported. Analogous to the cyclobutene and cyclobutane compounds in the literature, 10 new cyclobutene-containing dihydrochalcone-like molecules were synthesized stereoselectively in the current study. Chalcones and dihydrochalcones represent an important family of naturally occurring compounds [1] and exhibit a wide range of biological activities [1,29–35]. Synthesized chiral compounds **11–20** are analogous to dihydrochalcone. One would expect similar biological activities from them.

In the literature, antiviral, antibacterial and antioxidant activities of chalcones were studied [30–35], but antimicrobial activities of the chalconoid-like **1–10** and their electrocyclic products **11–20** were not reported. The antimicrobial

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activity for the compounds **1–20** were tested in vitro using the agar-well diffusion method with nine microorganisms and showed antibacterial activity against Gram-positive and Gram-negative bacteria [36], but no antifungal activity was observed against the two yeast-like fungi.

2. Experimental

2.1. General and instrumentation

NMR spectra were recorded on a Varian Mercury NMR instrument at 200 MHz in CDCl₃. 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 Thermo-var apparatus fitted with a microscope and were uncorrected. UV–vis spectra were obtained on a Unicam UV2-100 spectrophotometer at 25 °C. Thin-layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F₂₅₄ analytical aluminum plates. PTLC was carried out on Merck precoated 60 Kieselgel F₂₅₄ (20 cm × 20 cm, 0.2 mm) silica gel plates.

3. Materials and methods

Cinnamaldehyde, *o*-, *m*-, *p*-nitroacetophenone, *o*-, *m*-, *p*-methoxyacetophenone and *o*-, *m*-, *p*-methylacetophenone were purchased from Aldrich and used without further purification. The solvents (chloroform, *n*-hexane, ethanol and diethyl ether) used were either of analytical grade or bulk solvents distilled before use.

3.1.1. (2*E*,4*E*)-1-(2-Nitro)phenyl-5-phenylpenta-2,4-dien-1-one (**2**)

To a cooled solution (~1–5 °C) of sodium hydroxide (1.2 g, 30 mmol) in 10 ml of 80% EtOH was added *o*-nitroacetophenone (0.76 g, 5 mmol) solution in EtOH (3 ml) dropwise. The resulting mixture was stirred for 15 min, then was added cinnamaldehyde (0.52 g, 5 mmol) solution in EtOH (3 ml) dropwise. After addition was completed, the reaction mixture was stirred at room temperature for 1 h. The mixture was neutralized with 10% HCl. The ethanol was evaporated under vacuum, then the aqueous phase was extracted with CHCl₃ (3 ml × 30 ml). The combined organic phases were dried over Na₂SO₄. Removal of the solvent under the reduced pressure gave compound **2** (2.7 g, 96% yield). *R*_f: 0.65, *n*-hexane-ethyl acetate (0.2:1). mp 115–116 °C; UV λ_{max}^{CHCl₃} (nm): 238, 337 (ε 19508, 27622); ¹H NMR (CDCl₃, 200 MHz) δ (ppm): 7.00, d, *J* = 14.7 Hz (H₂), 7.54, m (H₃), 6.92, m (H₄), 6.56, dd, *J* = 14.6, 2.2 Hz (H₅), 8.14, dd, *J* = 7.6 and 1.6 Hz (H_{3'}), 7.68, m (H_{4'}), 7.44, m (H_{5'}), 7.74, m (H_{6'}), 7.48, m (H_{2''} and H_{6''}), 7.34, m (H_{3''} and H_{5''}), 7.32, m (H_{4''}).

¹³C NMR (CDCl₃, 50 MHz) δ (ppm): 192.81 (C=O), 129.48 (C₂), 146.28 (C₃), 124.44 (C₄), 142.63 (C₅), 135.54 (C_{1'}), 136.23 (C_{2'}), 128.73 (C_{3'}), 133.95 (C_{4'}), 130.44 (C_{5'}), 126.20 (C_{6'}), 135.54 (C_{1''}), 127.35 (C_{2''}), 128.79 (C_{3''}), 129.25 (C_{4''}), 128.79 (C_{5''}), 127.35 (C_{6''}); positive LC–MS/MS *m/z* (%); *m/z* = 279(30) [M]⁺, 249(29) [M – 30]⁺, 248(79) [M – 31]⁺, 247(100) [M – 32]⁺; C₁₇H₁₃NO₃ (279.30): Calcd. C 73.11, H 4.69, N 5.02, found C 72.43, H 4.69, N 5.02; FT-IR (cm⁻¹): 3028, 1655, 1521, 1348, 766, 747 and 686.

The synthesis of compounds **1** and **3–10** was treated in the same way as compound **2**. The spectral data (¹H, ¹³C, FT-IR, UV and MS) of compounds **1** [6,7], **3** [8], **4** [9], **5–6** [10], **7** [6,7,11,12], **9** [13] and **10** [6,11,12] are the same as in the literature.

3.1.2. (2*E*,4*E*)-1-(2-Methyl)phenyl-5-phenylpenta-2,4-dien-1-one (**8**)

Oily; UV λ_{max}^{CHCl₃} (nm): 241, 336 (ε 15960, 22251); 2.61 g, 97% yield. *R*_f: 0.65, *n*-hexane-diethylether (0.5:1). ¹H NMR (CDCl₃, 200 MHz) δ (ppm): 6.98, d, *J* = 14.6 Hz (H₂), 7.28, m (H₃), 6.94, m (H₄), 6.66, d, *J* = 15.2 Hz (H₅), 7.34, m (H_{3'} and H_{5'}), 7.45, m (H_{4''}), 7.48, m (H_{6'}), 7.30, m (H_{2''} and H_{6''}), 7.28, m (H_{3''} and H_{5''}), 7.25 m (H_{4''}), 2.42, s (CH₃). ¹³C NMR (CDCl₃, 50 MHz) δ (ppm): 196.40 (C=O), 126.64 (C₂), 146.04 (C₃), 125.35 (C₄), 141.77 (C₅), 138.96 (C_{1'}), 136.70 (C_{2'}), 129.97 (C_{3'}), 131.17 (C_{4'}), 130.26 (C_{5'}), 129.22 (C_{6'}), 135.80 (C_{1''}), 127.22 (C_{2''}), 128.77 (C_{3''}), 127.22 (C_{4''}), 128.77 (C_{5''}), 127.89 (C_{6''}), 20.11 (CH₃); positive LC–MS/MS *m/z* (%); *m/z* = 248(100) [M]⁺, 247(20) [M – 1]⁺, 230(17) [M – 18]⁺, 144(10) [M – 104]⁺; C₁₈H₁₆O (248.32): Calcd. C 87.06, H 6.49, found C 86.38, H 6.74; FT-IR (cm⁻¹): 3026, 2926, 1658, 1582, 1440, 1269, 1001, 771, 752 and 692.

3.1.3. Synthesis of *cis*-3-benzoyl-4-phenylcyclobutene (**11**)

A solution of compound **1** (250 mg) in 30 ml of diethyl ether, 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 ~6 h. The solution was evaporated and a portion of the residue (40 mg) was purified by PTLC (20 cm × 20 cm, 0.25 mm, 2 plates) to give compound **11** (12 mg, 30% yield, *R*_f = 0.76, *n*-hexane-diethyl ether, 1:1).

The syntheses of compounds **12–20** were performed in the same way as compound **11** with the experimental conditions as stated in Table 1.

3.1.4. *cis*-3-Benzoyl-4-phenylcyclobutene (**11**)

Amorphous solid, mp 68–70 °C; UV λ_{max}^{CHCl₃} (nm): 275 (ε 22916); ¹H NMR (CDCl₃, 200 MHz) δ (ppm): 6.41 bs

Table 1

Experimental conditions for the photocyclization of compounds **1–10** to give compounds **11–20**

Starting compound (mg)	Synthesized compound	Yield (%)	TLC R_f values	Reaction time (h)
1 (250)	11	30	0.76 (1:1) ^a	6
2 (210)	12	22	0.94 (1.5:1) ^a	8
3 (210)	13	24	0.58 (1:0.5) ^a	12
4 (200)	14	45	0.76 (1.5:0.5) ^a	9
5 (146)	15	38	0.64 (0.6:1.4) ^a	4
6 (106)	16	26	0.76 (0.6:1.4) ^a	4
7 (68)	17	21	0.52 (0.6:1.4) ^a	6
8 (86)	18	37	0.88 (1:1) ^a	13
9 (173)	19	23	0.88 (1:1) ^a	13
10 (410)	20	27	0.82 (1:1) ^a	12

^a Solvent system is *n*-hexane-diethyl ether.

(H₁ and H₂), 4.43, ddd, $J=8.6, 5.4, 2$ and 5.6 Hz (H₃), 3.27, bd, $J=5.6$ Hz (H₄), 8.01, dd, $J=7.8$ and 0.8 Hz (H_{2'} and H_{6'}), 7.44, m (H_{3'} and H_{5'}), 7.52, m (H-4'), 7.40, m (H_{2''} and H_{6''}), 7.38, m (H_{3''} and H_{5''}), 7.20, m (H_{4''}). ¹³C NMR (CDCl₃, 50 MHz) δ (ppm) (see Table 2); LC–MS/MS m/z (%); $m/z=235(5)$ [$M+H$]⁺, 234(100) [M]⁺, 113(79) [$M-121$]; C₁₇H₁₄O (234.30): Calcd. C 87.15, H 6.02, found C 86.66, H 6.22; FT-IR (cm⁻¹): 3054, 2953, 1678, 1507, 1448, 966, 748 and 694.

3.1.5. *cis*-3-(2-Nitro)benzoyl-4-phenylcyclobutene (**12**)

Amorphous solid, mp 58–60 °C; UV $\lambda_{\max}^{\text{CHCl}_3}$ (nm): 257 (ϵ 80312); ¹H NMR (CDCl₃, 200 MHz) δ (ppm); 6.32, ddd; $J=11.4, 6.6$ and 1.8 Hz (H₁ and H₂), 4.02, ddd, $J=9.2, 3.4$ and 6.2 Hz (H₃), 3.30, dddd, $J=6.4, 5.0, 2.0$ and 1.8 Hz (H₄), 8.06, dd, $J=8.1$ Hz (H_{3'}), 7.64, m (H_{4'}), 7.53, m (H_{5'}), 7.70, m (H_{6'}), 7.60, m (H_{2''} and H_{6''}), 7.34, m (H_{3''} and H_{5''}), 7.28, m (H_{4''}). ¹³C NMR (CDCl₃, 50 MHz) δ (ppm) (see

Table 2); LC–MS/MS m/z (%); $m/z=279(13)$ [M]⁺, 147(71) [$M-130$]⁺, 141(32) [$M-138$]⁺, 133 (100) [$M-146$]⁺; C₁₇H₁₃NO₃ (279.30): Calcd. C 73.11, H 4.69, N 5.02, found C 72.10, H 4.68, N 4.96; FT-IR (cm⁻¹): 3027, 2951, 1697, 1527, 1346, 750 and 694.

3.1.6. *cis*-3-(3-Nitro)benzoyl-4-phenylcyclobutene (**13**)

Amorphous solid, mp 72–75 °C; UV $\lambda_{\max}^{\text{CHCl}_3}$ (nm): 243 (ϵ 10125); ¹H NMR (CDCl₃, 200 MHz) δ (ppm); 6.42, bs (H₁ and H₂), 4.48, ddd, $J=8.8, 5.4$ and 2.6 Hz (H₃), 3.24, bd, $J=6.4$ Hz (H₄), 8.94, t, $J=1.8$ Hz (H_{2'}), 8.38, dd, $J=8.6$ and 1.2 Hz (H_{4'}), 7.65, dd, $J=8.0$ and 7.8 Hz (H_{5'}), 3.38, dd, $J=7.8$ and 1.2 Hz (H_{6'}), 7.36, m (H_{2''} and H_{6''}), 7.30, m (H_{3''} and H_{5''}), 7.18, m (H_{4''}). ¹³C NMR (CDCl₃, 50 MHz) δ (ppm) (see Table 2); LC–MS/MS m/z (%); $m/z=280(8)$ [$M+H$]⁺, 279(40) [M]⁺, 257(62), 239(78), 213(68), 207(95), 171(68), 157(86), 133(100) [$M-146$]⁺, 123(70) [$M-156$]⁺; C₁₇H₁₃NO₃ (279.30): Calcd. C 73.11, H 4.69, N 5.02, found C 72.70, H 4.98, N 5.17; FT-IR (cm⁻¹): 3083, 2924, 1679, 1531, 1349, 810, 747 and 693.

3.1.7. *cis*-3-(4-Nitro)benzoyl-4-phenylcyclobutene (**14**)

Amorphous solid, mp 56–58 °C; UV $\lambda_{\max}^{\text{CHCl}_3}$ (nm): 264 (ϵ 85161); ¹H NMR (CDCl₃, 200 MHz) δ (ppm); 6.41, dd, $J=5.2$ and 1.8 Hz (H₁ and H₂), 4.47, ddd, $J=8.8, 5.8$ and 2.4 Hz (H₃), 3.28, bm (H₄), 8.25, A₂B₂, $J=10.6$ Hz (H_{2'}, H_{3'}, H_{5'} and H_{6'}), 7.34, m (H_{2''}, H_{3''}, H_{4''}, H_{5''} and H_{6''}). ¹³C NMR (CDCl₃, 50 MHz) δ (ppm) (see Table 2); LC–MS/MS m/z (%); $m/z=279(6)$ [M]⁺, 265(4), 249(8), 148(7), 100(18) [$M-179$]⁺, 79(85) [$M-200$]⁺, 60(100) [$M-219$]⁺; C₁₇H₁₃NO₃ (279.30): Calcd. C 73.11, H 4.69,

Table 2

¹³C NMR data of compounds **11–20**^a in CDCl₃

	11	12	13	14	15	16	17	18	19	20
C=O	198.81	200.63	196.32	197.04	201.29	198.55	197.34	202.58	199.00	198.40
C ₁	129.70	128.75	128.44	128.48	130.26	129.59	128.43	129.70	129.63	129.88
C ₂	133.49	134.06	134.47	133.41	133.64	132.22	130.36	131.86	134.20	131.95
C ₃	46.22	50.36	46.71	46.42	52.33	46.44	45.48	49.03	46.30	46.88
C ₄	45.70	45.98	46.19	46.42	44.08	45.68	43.94	45.89	45.77	45.59
C _{1'}	135.67	136.38	136.05	139.84	127.68	137.01	128.83	136.48	136.64	133.20
C _{2'}	128.94	141.80	130.00	130.04	158.76	112.47	129.73	136.66	129.86	129.26
C _{3'}	128.55	124.47	148.36	123.86	111.20	159.71	112.19	131.67	135.72	129.03
C _{4'}	132.17	132.02	128.06	150.58	130.87	120.59	163.81	125.66	132.13	144.34
C _{5'}	128.55	130.90	129.91	123.86	121.36	129.70	129.73	129.42	128.44	129.03
C _{6'}	128.94	127.81	133.62	130.04	130.49	121.64	112.19	131.82	126.11	129.26
C _{1''}	136.62	136.60	136.72	135.96	137.01	136.56	136.78	138.58	138.30	136.69
C _{2''}	126.28	126.29	126.36	126.33	126.08	126.26	124.72	126.26	126.24	126.26
C _{3''}	128.55	128.52	128.65	128.73	128.42	128.55	126.97	128.53	128.53	128.51
C _{4''}	127.60	127.68	127.84	127.48	127.15	127.63	125.96	127.55	127.57	127.52
C _{5''}	128.55	128.52	128.65	128.73	128.42	128.55	126.97	128.53	128.53	128.51
C _{6''}	126.28	126.29	126.36	126.33	126.08	126.26	127.72	126.26	126.24	126.26
–OCH ₃	–	–	–	–	54.70	55.33	53.90	–	–	–
–CH ₃	–	–	–	–	–	–	–	21.41	21.25	21.63

^a Assignment based on APT, ¹H–¹H COSY, NOESY and HETCOR NMR data and ACD NMR Program.

N 5.02, found C 72.50, H 4.50, N 5.10; FT-IR (cm^{-1}): 3016, 2930, 1680, 1525, 1346, 857, 748 and 694.

3.1.8. *cis*-3-(2-Methoxy)benzoyl-4-phenylcyclobutene (15)

Amorphous solid, mp 44–48 °C; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ (nm): 258 (ϵ 23454); ^1H NMR (CDCl_3 , 200 MHz) δ (ppm); 6.35, bs (H_1 and H_2), 4.24, ddd, $J=8.8$, 5.6 and 2.2 Hz (H_3), 3.36, bd, $J=5.8$ Hz (H_4), 6.76, d, $J=8.2$ Hz ($\text{H}_{3'}$), 7.38, m ($\text{H}_{4'}$), 6.96, dt, $J=7.4$ and 10.0 Hz ($\text{H}_{5'}$), 7.72, dd, $J=7.6$ and 1.8 Hz ($\text{H}_{6'}$), 7.40, m ($\text{H}_{2''}$), 7.38, m ($\text{H}_{3''}$), 7.20, m ($\text{H}_{4''}$), 7.38, m ($\text{H}_{5''}$), 7.40, m ($\text{H}_{6''}$), 3.51, s (OCH_3). ^{13}C NMR (CDCl_3 , 50 MHz) δ (ppm) (see Table 2); LC-MS/MS m/z (%); $m/z=265(38)$ [$M+H$] $^+$, 250(10), 190(32), 134(100); $\text{C}_{18}\text{H}_{16}\text{O}_2$ (264.32): Calcd. C 81.79, H 6.10, found C 81.29, H 6.28; FT-IR (cm^{-1}): 3024, 2938, 1661, 1596, 1485, 1247, 1020, 754 and 693.

3.1.9. *cis*-3-(3-Methoxy)benzoyl-4-phenylcyclobutene (16)

Oily; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ (nm): 258 (ϵ 11217); ^1H NMR (CDCl_3 , 200 MHz) δ (ppm); 6.42, bs (H_1 and H_2), 4.39, ddd, $J=8.8$, 5.4 and 2.0 Hz (H_3), 3.27, bd, $J=5.8$ Hz (H_4), 7.53, dd, $J=2.4$ and 1.6 Hz ($\text{H}_{2'}$), 7.06, m ($\text{H}_{4'}$), 7.31, m ($\text{H}_{5'}$), 7.59, ddd, $J=7.8$, 1.4 and 1.2 Hz ($\text{H}_{6'}$), 7.35, m ($\text{H}_{2''}$ and $\text{H}_{6''}$), 7.28, m ($\text{H}_{3''}$ and $\text{H}_{5''}$), 7.06, m ($\text{H}_{4''}$), 3.76, s (OCH_3). ^{13}C NMR (CDCl_3 , 50 MHz) δ (ppm) (see Table 2); LC-MS/MS m/z (%); $m/z=264(5)$ [M] $^+$, 246(7), 230(6), 220(12), 140(15) [$M-124$] $^+$, 126(100) [$M-138$] $^+$, 112(50) [$M-152$] $^+$; $\text{C}_{18}\text{H}_{16}\text{O}_2$ (264.32): Calcd. C 81.79, H 6.10, found C 81.53, H 6.44; FT-IR (cm^{-1}): 3025, 2934, 1670, 1596, 1487, 1263, 783, 753 and 693.

3.1.10. *cis*-3-(4-Methoxy)benzoyl-4-phenylcyclobutene (17)

Amorphous solid, mp 41–43 °C; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ (nm): 275 (ϵ 17187); ^1H NMR (CDCl_3 , 200 MHz) δ (ppm); 6.41, bd, $J=2.4$ Hz (H_1 and H_2), 4.35, ddd, $J=8.6$, 5.2 and 2.2 Hz (H_3), 3.27, bs, $J=6.0$ Hz (H_4), 7.98, d, $J=8.6$ Hz ($\text{H}_{2'}$ and $\text{H}_{6'}$), 6.88, d, $J=8.6$ Hz ($\text{H}_{3'}$ and $\text{H}_{5'}$), 7.38, m ($\text{H}_{2''}$ and $\text{H}_{6''}$), 7.30, m ($\text{H}_{3''}$ and $\text{H}_{5''}$), 7.24, m ($\text{H}_{4''}$), 3.82, s (OCH_3). ^{13}C NMR (CDCl_3 , 50 MHz) δ (ppm) (see Table 2); LC-MS/MS m/z (%); $m/z=265(19)$ [$M+H$] $^+$, 249(100) [$M-15$] $^+$, 176(38), 148(46), 132(95) [$M-132$] $^+$; $\text{C}_{18}\text{H}_{16}\text{O}_2$ (264.32): Calcd. C 81.79, H 6.10, found C 81.60, H 6.30; FT-IR (cm^{-1}): 3027, 2931, 1660, 1598, 1510, 1257, 1170, 1026, 841, 749 and 694.

3.1.11. *cis*-3-(2-Methyl)benzoyl-4-phenylcyclobutene (18)

Amorphous solid, mp 52–54 °C; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ (nm): 254 (ϵ 13684); ^1H NMR (CDCl_3 , 200 MHz) δ (ppm); 6.35, bs (H_1 and H_2), 4.24, ddd, $J=8.8$, 5.6 and 2.6 Hz (H_3), 3.18, ddd, $J=8.4$, 3.6 and 2.0 Hz (H_4), 7.24, m ($\text{H}_{3'}$), 7.30, m ($\text{H}_{4'}$), 7.18,

m ($\text{H}_{5'}$), 7.66, d, $J=8.0$ Hz ($\text{H}_{6'}$), 7.36, m ($\text{H}_{2''}$ and $\text{H}_{6''}$), 7.24, m ($\text{H}_{3''}$ and $\text{H}_{5''}$), 7.10, m ($\text{H}_{4''}$), 2.74, s (CH_3). ^{13}C NMR (CDCl_3 , 50 MHz) δ (ppm) (see Table 2); LC-MS/MS m/z (%); $m/z=249(100)$ [$M+H$] $^+$, 232(58), 176(22), 154(28), 1118(15), 106(13); FT-IR (cm^{-1}): 3024, 2967, 1671, 1560, 1454, 965, 748 and 694.

3.1.12. *cis*-3-(3-Methyl)benzoyl-4-phenylcyclobutene (19)

Amorphous solid, mp 55–58 °C; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ (nm): 256 (ϵ 38384); ^1H NMR (CDCl_3 , 200 MHz) δ (ppm); 6.41, bd, $J=2.8$ Hz (H_1 and H_2), 4.41, ddd, $J=8.8$, 5.8 and 2.8 Hz (H_3), 3.25, ddd, $J=8.6$, 5.4 and 2.4 Hz (H_4), 7.82, bs ($\text{H}_{2'}$), 7.30, m ($\text{H}_{4'}$), 7.35, m ($\text{H}_{5'}$), 7.82, bs ($\text{H}_{6'}$), 7.36, m ($\text{H}_{2''}$ and $\text{H}_{6''}$), 7.28, m ($\text{H}_{3''}$ and $\text{H}_{5''}$), 7.20, m ($\text{H}_{4''}$), 2.65, s (CH_3). ^{13}C NMR (CDCl_3 , 50 MHz) δ (ppm) (see Table 2); LC-MS/MS m/z (%); $m/z=249(100)$ [$M+H$] $^+$, 237(8), 228(5), 180(4), 154(9), 118(12); FT-IR (cm^{-1}): 3027, 2924, 1668, 1584, 1448, 1257, 966, 781, 748 and 694.

3.1.13. *cis*-3-(4-Methyl)benzoyl-4-phenylcyclobutene (20)

Amorphous solid, mp 65–67 °C; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ (nm): 261 (ϵ 14821); ^1H NMR (CDCl_3 , 200 MHz) δ (ppm); 6.41, bd, $J=2.8$ Hz (H_1 and H_2), 4.39, ddd, $J=8.8$, 5.8 and 2.4 Hz (H_3), 3.28, ddd, $J=8.6$, 5.8 and 2.8 Hz (H_4), 7.90, d, $J=8.2$ Hz ($\text{H}_{2'}$ and $\text{H}_{6'}$), 7.19, d, $J=8.2$ Hz ($\text{H}_{3'}$ and $\text{H}_{5'}$), 7.36, m ($\text{H}_{2''}$ and $\text{H}_{6''}$), 7.24, m ($\text{H}_{3''}$ and $\text{H}_{5''}$), 7.15, m ($\text{H}_{4''}$), 2.38, s (CH_3). ^{13}C NMR (CDCl_3 , 50 MHz) δ (ppm) (see Table 2); LC-MS/MS m/z (%); $m/z=249(100)$ [$M+H$] $^+$, 226(9), 209(6), 154(5), 130(18), 118(22), 100(16); FT-IR (cm^{-1}): 3027, 2924, 1668, 1584, 1448, 1257, 966, 791, 748 and 693.

3.2. Antimicrobial activity assessment

All of the test microorganisms were obtained from Refik Saydam Hizissihha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC, *Serratia marcescens* ATCC, *Pseudomonas aeruginosa* ATCC, *Yersinia pseudotuberculosis* ATCC, *Klebsiella pneumoniae* ATCC, *Enterococcus faecalis* ATCC, *Staphylococcus aureus* ATCC, *Bacillus cereus* 702 Roma and *Candida albicans* ATCC. All the newly synthesized compounds were weighed and dissolved in acetone to prepare stock solutions.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values ($\mu\text{g/ml}$) were determined [36]. 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. MIC is defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. Acetone with a di-

Table 3
Screening results for antimicrobial activity of compounds 1–20

Compounds	MIC ^a (μg/ml)								
	Ec	Sm	Pa	Yp	Kp	Ef	Sa	Bc	Ca
1	800	800	800	800	800	230	230	115	–
2	480	480	480	480	480	60	60	60	–
3	560	560	280	560	280	140	140	140	–
4	1200	1200	2400	2400	1200	300	300	300	–
5	1100	2200	2200	1100	1100	560	280	280	–
6	2900	2900	2900	2900	2900	1450	1450	1450	–
7	440	440	880	880	880	110	110	23	–
8	2265	2265	2265	2265	2265	2265	2265	1132	–
9	1144	1144	1144	1144	1144	286	286	286	–
10	440	440	880	880	880	220	220	23	–
11	150	600	75	150	300	10	10	10	–
12	580	580	1160	1160	1160	290	290	73	–
13	1200	1200	2400	2400	1200	600	600	600	–
14	480	480	960	960	960	55	55	55	–
15	1015	1015	507	1015	1015	126	126	63	–
16	1320	2640	2640	2640	2640	330	330	330	–
17	1150	2300	2300	2300	2300	287	287	287	–
18	245	245	245	490	490	15	15	15	–
19	1800	1800	480	1800	480	240	240	120	–
20	760	760	380	2800	380	190	190	190	–
Amp.	8	32	>128	32	32	2	2	2	–
Flu.									<1

Ec, *Escherichia coli* ATCC 25922; Sm, *Serratia marcescens* ATCC 13880; Pa, *Pseudomonas aeruginosa* ATCC 10145; Yp, *Yersinia pseudotuberculosis* ATCC 911; Kp, *Klebsiella pneumoniae* ATCC 13883; Ef, *Enterococcus faecalis* ATCC 29212; Sa, *Staphylococcus aureus* ATCC 25923; Bc, *Bacillus cereus* 702 Roma; *Candida albicans* ATCC 60193; Amp., Ampicillin; Flu., Fluconazole; (–), no activity (1–100 mg/ml).

^a MIC represents minimum concentration for total inhibition of test microorganism.

lution of 1:10 was used as solvent control. The results are shown in Table 3.

4. Results and discussion

In the current work, Claisen-Schmidt condensation of an appropriate aromatic ketone with cinnamaldehyde according to the route indicated in Scheme 1 yielded two unknown (2 and 8) and eight known (1, 3–7, 9 and 10) [6–13] alnustone and chalconoid-like *o*-, *m*-, *p*-nitro-, -methoxy and -methyl substituted derivatives of (2*E*,4*E*)-1,5-diarylpenta-2,4-dien-1-one (1–10). The most noticeable feature of the structural characterization of compounds 1–10 is the assignment of the proton resonances of their α , β , γ , δ -unsaturated moiety, which was made by a careful analysis of their ¹H, 2D-COSY NMR spectra. From the values of the vicinal coupling constants (³*J*_{H α -H β} = 15 Hz), it was possible to establish the *trans* configuration of these two protons.

These chalcone- and alnustone-like compounds (1–10), when exposed to UV light (400 W high-pressure Hg lamp), are converted to the respective cyclobutenes (11–20) as major products, with the yields (chromatographed products, PTLC) of 30% (11), 22% (12), 24% (13), 45% (14), 38% (15), 26% (16), 21% (17), 37% (18), 23% (19) and 27% (20) in solution.

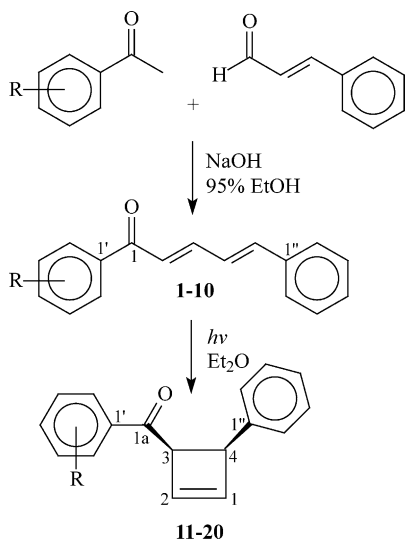
The structures of the cyclobutene rings of the cyclic products (11–20) were elucidated from their ¹H NMR spectra, which show highly shielded CH protons signals at δ -

H_{1,2} ~ 6.38 (bs), δ -H₃ ~ 4.40 (ddd) and δ -H₄ ~ 3.30 (bd or bm), respectively.

Stereochemistry of the compounds 11–20 was determined from NMR spectrometry information. NMR patterns allowed the calculation of the coupling constants of the cyclobutene protons. The obtained values for *J* are in agreement with a *cis* relationship between the A and B part of cyclobutane; the values of *J*_A and *J*_B (~5.6 Hz) are in good agreement with a *cis* relationship between A and B, respectively. The values of these coupling constants suggest that 11–20 were formed by the disrotatory ring closure with *syn* stereochemistry. A more accurate structural determination was attained by NOESY spectra and the important NOESY interactions in compounds 11–20 were seen from H-3 to H-4/H-2 and H-4 to H-3/H-1. Thus, the presence of cyclobutene ring was established.

The structural connectivities of compounds 11–20 were established, in part from ¹H–¹H COSY. The most down field signal for the cyclobutene ring –CH=CH– designated H-1/H-2 at δ ~ 6.38 (bs) was connected to H-3 at δ ~ 4.40, then to H-4 at δ _H ~ 3.30 (bd or bm) for compounds 11–20. Further connectivities for the phenyl parts of the compounds 11–20 were observed between δ ~ 9.0 and 7.0 ppm in the ¹H–¹H COSY NMR.

The positive LC–MS/MS gave [*M*]⁺ or [*M* + 1]⁺ at *m/z* 234(100) for 11, at *m/z* 279(13, 40 and 6) for 12–14, at *m/z* 265(38 and 19) for 15 and 17 and 264(5) for 16 and at *m/z* 249(100, 100 and 100) for 18–20, which were consistent with the molecular formulas to be C₁₇H₁₄O for 11, C₁₇H₁₃NO₃



1: R = H	11: R = H
2: R = <i>o</i> -NO ₂	12: R = <i>o</i> -NO ₂
3: R = <i>m</i> -NO ₂	13: R = <i>m</i> -NO ₂
4: R = <i>p</i> -NO ₂	14: R = <i>p</i> -NO ₂
5: R = <i>o</i> -OCH ₃	15: R = <i>o</i> -OCH ₃
6: R = <i>m</i> -OCH ₃	16: R = <i>m</i> -OCH ₃
7: R = <i>p</i> -OCH ₃	17: R = <i>p</i> -OCH ₃
8: R = <i>o</i> -CH ₃	18: R = <i>o</i> -CH ₃
9: R = <i>m</i> -CH ₃	19: R = <i>m</i> -CH ₃
10: R = <i>p</i> -CH ₃	20: R = <i>p</i> -CH ₃

Scheme 1.

for **12–14**, C₁₈H₁₆O₂ for **15–17** and C₁₈H₁₆O for **18–20**, respectively. The LC–MS/MS also showed typical chalcone fragmentation patterns for all compounds.

Based upon the above observations, the complete chemical shift assignments for **11–20** were deduced and ¹³C NMR data are shown in Table 2. Compounds **11–20** were thus shown to be *o*-, *m*-, *p*-nitro, -methoxy and -methyl substituted *cis*-3-benzoyl-4-phenylcyclobutenes. These 10 new compounds (**11–20**) were synthesized and characterized first time in this work.

As seen in Table 3, all compounds showed antimicrobial activity against Gram-positive and Gram-negative bacteria, but no antifungal activity was observed against yeast-like fungi [36]. The test compounds showed better antibacterial activities against Gram-positive bacteria compared to the activities against Gram-negative bacteria. Compound **11** was the most active substance against *P. aeruginosa*, *E. coli* and *Y. pseudotuberculosis* with the MIC of 75–150 μg/ml. Compounds **2**, **7**, **10**, **11**, **14**, **15** and **18** showed good activity against *B. cereus* with the MIC of 10–63 μg/ml. Compounds **2**, **11**, **14** and **18** were the most active compounds against *E. faecalis* and *S. aureus* with the MIC values of 10–60 μg/ml. Acetone solvent control at 1/10 dilution showed no growth inhibition effect on all tested microorganisms.

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