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# Synthesis of New C-C Linked Bichalcones and Their Antimicrobial and Antioxidant Activities

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**Abstract:** Bichalcones are obtained naturally from the genus *Rhus*. Bichalcones have attracted attention not only from a synthetic perspective but also because of their broad-spectrum biological activities. A series of methoxy-substituted chalcones (**1**–**9**) yielded C-C linked bichalcones (**10**–**18**) by the Ullmann coupling method, and their antimicrobial and antioxidant activities were assessed in this study. The confirmation of the synthesized compounds' structures was performed by NMR ($^1$H, $^{13}$C, APT, and COSY), FT-IR, UV, and HRESI-TOF-MS data. According to the preliminary results, a number of the said compounds exhibited an interesting activity against *Candida tropicalis*. Moreover, the antioxidant activities of bichalcones (**10**–**18**) were evaluated according to DPPH and FRAP methods. Concerning antioxidant activities, compounds **15** and **16** were the most active compared to Trolox used as a standard. Of all the synthesized new bichalcone derivatives, compounds **10, 11, 12, 14**, and **17** displayed higher activity against to fungal microorganism *Candida tropicalis*. Compounds **15** and **16** were found to have the highest antioxidant activity according to both DPPH and FRAP methods.

**Keywords**: bichalcone, Ullmann coupling, antimicrobial activity, antioxidant activity.

## INTRODUCTION

CHALCONES (1, 3-diphenyl-2-propen-1-one) and their bis-form, called bichalcones, are pharmacologically and naturally occurring bioactive compounds that belong to the flavonoid family and have an $\alpha,\beta$-unsaturated carbonyl group.[1] Chalcones are conventionally synthesized *via* the Claisen-Schmidt condensation reaction of aldehydes and ketones in an alkaline medium. It is possible to synthesize bichalcones by the condensation of chalcones by Suzuki homo-coupling,[2] Suzuki-Miyaura cross-coupling,[3–6] or Ullmann coupling.[7–9] Rhuschalcones II-VI are bichalcones that are isolated from the root bark of Rhus pyroides and exhibit a strong antiplasmodial activity and a moderate antiproliferative activity against 2 colorectal cancer cell lines.[10] In recent years, it has been revealed that some bischalcones related to rhuschalcone VI display moderate anti-trypanosomal and anti-protozoal activities.[3]

In the literature, it has been stated that some synthetic bichalcone analogs cause apoptosis in 4 human cancer cell lines[11] and ionone-based terpenoid-like bichalcones exhibit antibacterial activities against human pathogenic microbes.[12] The synthesis of a number of novel bichalcones has been performed, and their *in vitro* antiplasmodial activities against the erythrocytic stages of *Plasmodium falciparum* have been assessed. Some bichalcones displayed significant antiplasmodial activity (Chloroquine-sensitive $Pf_3D_7$ $IC_{50}$ (mM): 2.0, 1.5, and 2.5, respectively).[13] In addition to these, there are many studies in the literature showing that bischalcone compounds have various biological activities such as antibacterial and antioxidant activity.[14–18]

The biological evaluation of the synthetic and isolated bichalcones reported that the inhibitory investigation of bichalcones against enzymes caused medicinal products, such as anti-browning substances, to be discovered and harmful bacteria and insects to be controlled.[11,13,19,20] The *in*

*vitro* antiplasmodial activities of a series of bichalcones were reported against the erythrocytic stages of *Plasmodium falciparum*, and some of them displayed remarkable antiplasmodial activity sensitive to $Pf_3D_7$ with $IC_{50}$ values of 1.5-2.5 mM.[12] The isolation of Rhuschalcones II-VI from the root bark of *Rhus pyroides* was mentioned, and their cytotoxic activity against the HT29 and HCT-116 colon tumor cell lines was reported.[10]

Therefore, concerning the pharmacological significance of bichalcones, we wanted to synthesize a novel series of methoxy-substituted bichalcones with identical substitutions at various positions on the phenyl ring (A) of chalcones. First, we investigated the *in vitro* antimicrobial potencies of the synthesized bichalcones (**10**–**18**) against Gram-positive and Gram-negative bacteria and yeast and then determined the antioxidant activity of all the synthesized compounds (**10**–**18**) with DPPH and FRAP methods.

# MATERIALS AND METHODS

## Materials

We purchased 2/3/4/ methoxy acetophenone, 2/3/4/ bromo benzaldehyde, NaOH, $PPh_3$, $[CH_3(CH_2)_3]_4NBr$, $NiCl_2.6H_2O$, Zn powder from Aldrich, Fluka, or Sigma. The solvents (n-hexane, diethyl ether, ethyl acetate, ethanol, and THF) were analytical grade such as Sigma-Aldrich or Sigma or bulk solvents distilled prior to usage.

The crude reaction mixtures formed as a result of organic reactions were controlled by using normal phase silica gel 60 F254 coated aluminum plates in thin layer chromatography, and then purified by extraction and normal phase column chromatography. A cabinet UV lamp with a wavelength of 254 and 366 nm was used to control the silica gel 60 $F_{254}$ separation. NMR spectra were taken with Varian Mercury 200 MHz NMR instrument in $CDCl_3$ and NMR solvent. The IR spectra were taken in the form of KBr tablets or with the help of $CHCl_3$ and $CH_3OH$ solvents on NaCl in Perkin-Elmer 1600 Series FT-IR (4000–400 $cm^{-1}$) spectrophotometer. UV spectra were taken at 25 °C in Unicam UV2-100 Spectrophotometer. Mass spectra were performed using an Agilent 6230-LC-TOF/MS instrument in EtOAc.

$^1H$ and COSY NMR spectra were adjusted according to TMS peak, $^{13}C$ and APT spectra were adjusted according to $CDCl_3$ solvent peak ($\delta$ 77.0 ppm). Mass spectra were taken using the electron spray (ES) method.

Normal phase 230–400 mesh acidic silica gel was used in column chromatography and a 254 nm UV lamp in the cabinet was used to control the separation in thin layers. Chloroform was used as a solvent for UV spectra. The samples were placed in 10 mm quartz cells and measurements were made in the 200–700 nm region and at 25 °C. $CDCl_3$ was used as solvent for NMR spectra. Measurements were made by placing the samples in quartz NMR tubes. While FT-IR spectra were taken, chloroform was used as the solvent. After the samples were applied on NaCl plates and the solvents were evaporated, measurements were made in the region of 400–4000 $cm^{-1}$.

## Methods

In this study, the reaction of methoxy acetophenone (0.01 mmol) in ethanol (5 mL) and bromo benzaldehyde (0.01 mmol) with NaOH (2 eqiv.) at 0–5 °C in ethanol solution yielded the known methoxy-substituted bromochalcones (**1**–**9**) by the Claisen-Schmidt condensation.[21–25] The combined organic solvent was dried over $MgSO_4$ and concentrated in a vacuum. The residue purification was carried out by crystallization. The structure of the chalcones **(1**–**9)** was revealed by $^1H$-NMR, $^{13}C$-NMR, LC-MS/MS, and FT-IR spectroscopy.

Then, bichalcones (**10**-**18**) were prepared by the Ullmann coupling reactionin the presence of 2-/,3-/,4-methoxy-substitute 2-/,3-/,4-bromo chalcones (~0.6214 g or 1.96 mmol) (**1**-**9**) as starting materials, respectively, with $PPh_3$ (~0.6214 g or 1.96 mmol), $[CH_3(CH_2)_3]_4NBr$ (TBAB) (0.74 g, 4.9 mmol), $NiCl_2.6H_2O$ (155 mg, 0.65 mmol), and Zn powder (198 mg, 3.0 mmol) in an aqueous THF solvent (30 mL).[4,7,9]

2-/3-/4-methoxy-substitute 2-/3-/4-bromo chalcones and $PPh_3$, $NiCl_2.6H_2O$, and $[CH_3(CH_2)_3]_4NBr$ were dissolved in anhydrous THF and then stirred until the boiling temperature under $N_2$ for 5 min. Zinc dust was added to the reaction vessel, which was stirred for a period of 1 h under $N_2$. The chalcone solution was injected into the reaction flask and stirred at room temperature for 24 h. The resultant mixture was neutralized, the excess solvent was evaporated, and the residue was poured over ice/water. The extraction of an aqueous mixture was carried out with ethyl acetate. The combined organic layers were washed using water and brine and dried. The solvent removal was performed under vacuum, and the residue purification was carried out by column chromatography (Silica gel) (*n*-hexane-diethyl ether (5 : 1, 3 : 1, and 2 : 1). Bischalcone compounds were taken with the hexane mobile phase from the column with yields in the range from 12 % to 20 %. The structures of the obtained oily bichalcone compounds were clarified by their spectral properties ($^1H$, $^{13}C$/APT, NMR, FT-IR, UV, and HRESI-TOF-MS), which were consistent with the proposed structure, and by comparing them with information in the literature.[26–29]

One of the most remarkable features of chalcones is observed from the $^1H$ NMR spectrum. The signal corresponding to 2-propen-1-one double bond proton, called Hα and Hβ and observed at $\delta$H 7.3–7.5 ppm and $\delta$H 7.7–8.0 ppm, gave the coupling constant as 3J = 15.6–16.0 Hz,

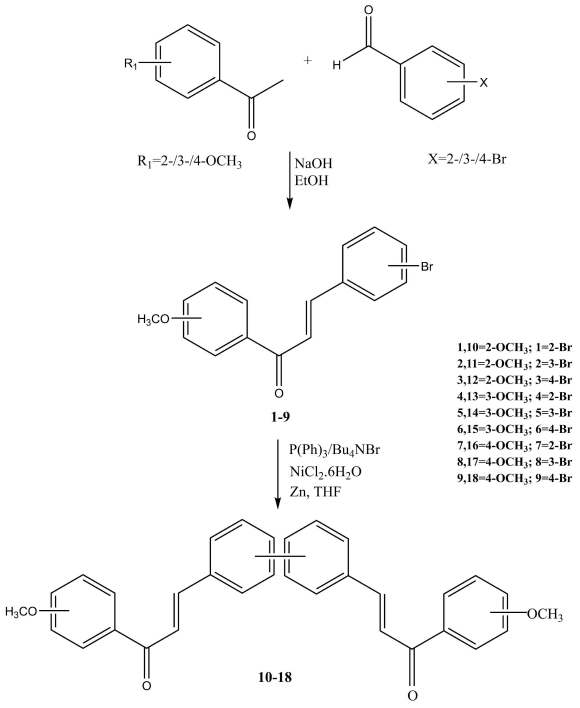


**Figure 1.** Synthetic pathway of bichalcones (**10**–**18**).

suggesting the E geometry of the double bond, as previously indicated for the other chalcones. The above-mentioned results were supported by the $^{13}C$ NMR data for Cα at *δ*C 122-125 ppm and Cβ at *δ*C 140–143 ppm for the E geometry of the chalcone's double bond, as reported in the literature.[30–34]

Figure 1 shows the reaction sequences utilized for synthesizing target compounds **10**–**18**.

Bichalcones, which are among the flavonoid subclasses, are dimers with C-C or C-O-C linkages between monomeric chalcones.[35–37] It is possible to synthesize C-C linked bichalcones by dimerizing chalcone units where the direct C-C coupling of two monomeric chalcone units takes part via the Stille coupling,[38] Suzuki-Miyaru,[3,4,39] and Ullmann-type synthesis.[7] In this study, symmetrical C-C bichalcones were synthesized by dimerizing 2'-, 3'-, 4'-bromo chalcone units via the Ullmann-type synthesis.[7]

## Antimicrobial Activity

Bichalcones **(10–18)** were tested individually against 10 bacterial species and one yeast, which were obtained from the American Type Culture Collection (ATCC) (Table 1).

### DISK DIFFUSION METHOD

The disk diffusion method was employed with the objective of determining the antimicrobial activity of bichalcones.[40–43] The inoculation of bacterial cultures into the Mueller-Hinton broth was carried out, and they were incubated at a temperature of 37 °C for a period of 16 hours and then adjusted to $OD_{625}$ = 0.08–0.1 (about $1\times10^7$ – $1\times10^8$ CFU/mL). One hundred microliters of every bacterial suspension were put onto the Mueller-Hinton agar's surface. Disks (6.0 mm in diameter) were put on the agar's surface, which contained every bacterium, and were impregnated with 10 µL of the synthetic compounds' methanol solution (10 µg/disk), and the incubation of Petri dishes was performed at a temperature of 37 °C for a period of 24 h. Kanamycin was utilized as a positive reference at 10 µg/disk (Sigma). After incubation, the diameter of the inhibition zones was measured and recorded. Likewise, every plate had a blank disk with 10 µL of methanol and an antibiotic disk. Every experiment was conducted with three repetitions. The bacteria, inhibition zone in a diameter ≥ 6 mm around the disks that were impregnated with methanol extract, were utilized for minimal inhibitory concentration (MIC).

**Table 1.** The names and ATCC numbers of microorganisms.

| Name of a microorganism | ATCC | Name of a microorganism | ATCC |
|---|---|---|---|
| **Gram-positive** | | **Gram-negative** | |
| *Bacillus subtilis* | ATCC 6633 | *Escherichia coli* | ATCC 25922 |
| *Enterococcus faecalis* | ATCC 29212 | *Klebsiella pneumonia* | ATCC 13883 |
| *Staphylococcus aureus* | ATCC 25923 | *Proteus vulgaris* | ATCC 13315 |
| *Staphylococcus epidermidis* | ATCC 12228 | *Salmonella typhimirium* | ATCC 14028 |
| **Yeast** | | *Yersinia pseudotuberculosis* | ATCC 911 |
| *Candida tropicalis* | ATCC 13803 | *Enterobacter cloaceae* | ATCC 13047 |

### MICROPLATE DILUTION METHOD

MIC values were found for the bichalcone (**10–18**)-sensitive bacterial strains in the disk diffusion assay. The bacterial strains' inoculum was prepared from 12 h agar cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. First, the extracts dissolved in methanol were diluted to the maximum concentration (500 µg $mL^{-1}$) for testing. Afterward, serial 2-fold dilutions were prepared with the objective of acquiring a concentration range from 500 µg $mL^{-1}$ to 0.49 in 1 mL sterile test tubes with the Mueller-Hinton broth. The microplate dilution method was employed to determine the MIC values of bichalcones against bacterial strains.[40–43] Kanamycin was utilized as a standard drug for positive control and with the inoculum on every strip was utilized as a negative control. The incubation of the 96-well plates was performed at a temperature of 37 °C for a period of 24 h. Microbial growth in every medium was identified by reading the respective absorbance (Abs) at 600 nm with a spectrophotometer (Molecular Devices, SpectraMax M2) and confirmed by plating 10 µL

samples from every well on the Mueller-Hinton agar medium. The bichalcones examined in the present research were screened two times against every organism. The lowest concentration at which the growth of the test microorganism was completely inhibited is indicated as the MIC (μg $mL^{-1}$) value.

## Antioxidant Activity

### DPPH• ACTIVITY AND FRAP

In the study, the most preferred method for testing antioxidant activity in the literature was used. The DPPH• radical scavenging activity is based on scavenging (conversion to a non-radical form) the DPPH radical in the test solution by the substance under test. The DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) is a stable radical, and a 100 μM methanolic solution of this radical was used in the present study.[44] The synthesized compounds and standards (BHT, Trolox, vitamin C) were prepared at five concentrations.

In this method, the three-dimensional structure and size of the test item are important. Some compounds may not reach the radical site of DPPH due to steric hindrance and are reported as inactive as a result of the test.

The second method used in the study is based on reducing iron(III) ion in the test medium to iron (II) ion and measuring the absorbance of the complex given by TPTZ in solution with iron (II).[45] The method is among the most common antioxidant determination methods in different studies. The results were interpreted by comparing them with ascorbi c acid, which has a high reducing potential and is also a standard antioxidant substance. A graph of absorbance versus concentration was plotted.

# RESULTS

## Spectral Data of Synthetic Compounds 10–18

Among the synthesized compounds **1**–**18**, nine compounds **1**–**9** have already been reported, and their spectral data match each other.[46–48] However, compounds **10**–**18** are new, and their spectral data are presented below.

Some of the H$\alpha$ and H$\beta$ protons of bichalcones emerge as two doublets in the ranges of 7.4–7.6 ppm (H$\alpha$) and 7.6–7.8 ppm (H$\beta$) with the coupling constant (*J*) value of 15–16 Hz. The high *J* value of protons obviously indicated *E* conformation. The APT NMR data for $C_\alpha$ at $\delta_C$ 120.4–121.8 ppm and $C_\beta$ at $\delta_C$ 143.2–144.83 ppm gave the *E* geometries for the bichalcone's double bonds, as indicated in the literature.[21–25] In general, the $^1$H NMR spectrum of bichalcones showed overlapped resonance at $\delta_H$ 7.0–8.0 ppm. The $^{13}$C-NMR spectra of bichalcones gave the carbonyl carbon

**Table 2.** $^{13}$C NMR (50 MHz, $CDCl_3$) data of bichalcones (**10**–**18**).

| Bichal. No | Bichalcones ($\delta_C$, ppm)[a][b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** |
| C=O | 192.5 | 193.0 | 192.8 | 190.1 | 190.2 | 190.2 | 188.4 | 188.6 | 188.4 |
| α | 120.6 | 120.4 | 120.7 | 121.9 | 121.0 | 121.9 | 121.7 | 121.7 | 121.8 |
| β | 143.2 | 143.2 | 143.2 | 144.8 | 144.8 | 144.8 | 143.9 | 143.9 | 143.9 |
| 1 | 135.0 | 158.1 | 135.3 | 139.5 | 159.8 | 134.7 | 135.0 | 163.6 | 135.0 |
| 2 | 135.0 | 128.8 | 128.8 | 136.9 | 128.9 | 128.9 | 135.0 | 130.3 | 128.9 |
| 3 | 128.3 | 134.9 | 128.4 | 129.5 | 134.8 | 128.4 | 128.9 | 134.9 | 128.3 |
| 4 | 128.8 | 130.4 | 135.3 | 133.5 | 128.1 | 134.7 | 130.8 | 128.3 | 135.0 |
| 5 | 128.8 | 132.8 | 128.4 | 128.3 | 129.5 | 128.4 | 128.3 | 130.7 | 128.3 |
| 6 | 128.3 | 128.3 | 128.8 | 131.9 | 128.4 | 128.9 | 130.3 | 128.8 | 128.9 |
| 1' | 129.1 | 129.1 | 129.2 | 137.2 | 139.5 | 139.4 | 130.2 | 130.7 | 131.0 |
| 2' | 158.0 | 158.0 | 158.1 | 111.1 | 112.7 | 112.7 | 130.1 | 130.9 | 130.3 |
| 3' | 111.5 | 111.5 | 111.5 | 159.8 | 159.8 | 159.8 | 113.8 | 113.8 | 113.8 |
| 4' | 132.8 | 132.8 | 132.9 | 119.3 | 119.3 | 119.2 | 163.3 | 163.4 | 163.4 |
| 5' | 126.9 | 126.9 | 127.0 | 130.4 | 130.5 | 129.5 | 113.8 | 113.8 | 113.8 |
| 6' | 130.2 | 130.2 | 130.2 | 120.7 | 122.0 | 120.9 | 130.1 | 130.9 | 130.3 |
| -OCH3 | 55.6 | 55.6 | 55.7 | 55.5 | 55.5 | 55.4 | 55.2 | 55.4 | 55.5 |

[a] Chemical shift values are relative to $CDCl_3$ ($\delta_C$, 77.0 ppm).

[b] The ACD NMR program was utilized to interpret $^{13}$C NMR data for bichalcones.

(C=O) at $\delta_C$ 188.4–193.0 ppm. The $^{13}$C and APT spectra of bichalcones clearly gave especially the C–C linked carbon peaks at $\delta_C$ 134.0-136.9 ppm,[4,13,44,45] whereas the bromo-substituted aromatic carbon peaks (=C–Br) of chalcone were observed at $\delta_C$ 122.0–125.7 ppm. These $^{13}$C NMR data shifts clearly indicated the C–C linkage of chalcone (Table 2).

**(2*E*,2'*E*)-3,3'-(BIPHENYL-2,2'-DIYL)-BIS-[1-(2-METHOXYPHENYL)PROP-2-EN-1-ONE], (10)**

Yield: 13 %, oily, $R_f$: 0.52 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3065 (=CH), 1596 (C=C, aromatic ring), 1243 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 239 (3.3), 304 (3.6); $^1$H-NMR (400 MHz, $CDCl_3$ ($\delta$, ppm): Ar-H and CH=CHCO: 7.0–7.9 (m, 20H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 192.5 (2C=O), 120.6 ($2CH_2$), 143.2 ($2CH_2$), ar-C [129.1 (2CH), 158.0 (2CH), 111.5 (2CH), 132.8 (2CH), 126.9 (2CH), 130.2 (2CH), 135.0 (4-CH), 135.0 (2C-C), 128.3 (2CH), 128.8 (2CH), 55.6 (2-$OCH_3$)].HRESI-TOF-MS: (*m* / *z*, %) $[M+H]^+$: 475.1905 (70), calc. 475.1917: $[M-CH_3+H_2O]^+$: 477.2046 (100), calc. 477.2006.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-3,3'-DIYL)-BIS-[1-(2-METHOXYPHENYL)PROP-2-EN-1-ONE], (11)**

Yield: 12 %, oily, $R_f$: 0.55 (hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3065 (=CH), 1597 (C=C, aromatic ring), 1242 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 238 (3.2), 306 (3.3); $^1$H-NMR (400 MHz, $CDCl_3$ ($\delta$, ppm): Ar-H and CH=CHCO: 6.9–7.8 (m, 20H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 193.0 (2C=O), 120.4 ($2CH_2$), 143.2 ($2CH_2$), ar-C [129.1 (2CH), 158.0 (4CH), 111.5 (2CH), 132.8 (2CH), 126.9 (2CH), 130.2 (2CH), 128.8 (2-CH), 134.9 (2C-C), 130.4 (2CH), 132.8 (2CH), 128.3(2CH), 55.6 (2-$OCH_3$)]. HRESI-TOF-MS: (*m*/*z*, %) $[M-CH_3+K+H]^+$: 499.1996 (100), calc. 499.1927.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-4,4'-DIYL)-BIS-[1-(2-METHOXYPHENYL)PROP-2-EN-1-ONE], (12)**

Yield: 16 %, oily, $R_f$: 0.54 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3060 (=CH), 1598 (C=C, aromatic ring), 1242 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 252 (3.1), 305 (3.3); $^1$H-NMR (400 MHz, $CDCl_3$ ($\delta$, ppm): Ar-H and CH=CHCO: 7.0–7.7 (m, 20H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 192.8 (2C=O), 120.7 ($2CH_2$), 143.2 ($2CH_2$), ar-C [129.2 (2CH), 158.1 (2CH), 111.5 (2CH), 132.9 (2CH), 127.0 (2-CH), 130.2 (2-CH), 135.3 (2-CH), 128.8 (4-CH), 128.4 (4CH), 55.7 (2-$OCH_3$)]. HRESI-TOF-MS: (*m* / *z*, %) $[M+H]^+$: 475.1913(50), calc. 475.1917, $[M-CH_3+K+H]^+$: 499.2193 (100), calc. 499.1927.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-2,2'-DIYL)-BIS-[1-(3-METHOXYPHENYL)PROP-2-EN-1-ONE], (13)**

Yield: 17 %, oily, $I_f$: 0.47 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3056 (=CH), 1590 (C=C, aromatic ring), 1181 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 252 (3), 306 (3.0); $^1$H-NMR (400 MHz, $CDCl_3$ ($\delta$, ppm): Ar-H and CH=CHCO: 7.1–7.9 (m, 20H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 190.1 (2C=O), 121.9 ($2CH_2$), 144.8 ($2CH_2$), ar-C [137.2 (2CH), 111.1 (2CH), 159.8 (2CH), 119.3 (2CH), 130.4 (2CH), 120.7 (2CH), 139.5 (2-CH), 136.9 (2C-C), 129.5 (2CH), 133.5 (2CH), 128.3 (2CH), 131.9 (2CH), 55.5 (2-$OCH_3$)]. HRESI-TOF-MS: (*m* / *z*, %) $[M-CH_3+H_2O]^+$: 477.2193(100), calc. 477.2130, $[M-CH_3+K+H]^+$: 499.2007 (100), calc. 499.1927.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-3,3'-DIYL)-BIS-[1-(3-METHOXYPHENYL)PROP-2-EN-1-ONE], (14)**

Yield: 20 %, oily, $R_f$: 0.47 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3061 (=CH), 1576 (C=C, aromatic ring), 1255 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 241 (3.2), 306 (3.4); $^1$H-NMR (400 MHz, $CDCl_3$ ($\delta$, ppm): Ar-H and CH=CHCO: 7.1–7.9 (m, 20H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 190.2 (2C=O), 121.0 ($2CH_2$), 144.8 ($2CH_2$), ar-C [139.5 (2CH), 112.7 (2CH), 159.8 (2CH), 119.3 (2CH), 130.5 (2CH), 122.0 (2CH), 159.8 (2-CH), 128.9 (-2C), 134.8 (2CH), 128.1 (2CH), 129.5 (2CH), 128.4 (2CH), 55.5 (2-$OCH_3$)]. HRESI-TOF-MS: (*m* / *z*, %) $[M+H]^+$ 475.1913(50), calc. 475.1917, $[M-CH_3+K+H]^+$: 499.1987(12), calc. 499.1927, $[M-CH_3+H_2O]^+$: 477.2158 (43), calc. 477.2130.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-4,4'-DIYL)-BIS-[1-(3-METHOXYPHENYL)PROP-2-EN-1-ONE], (15)**

Yield: 15 %, oily, $R_f$: 0.46 (hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3061 (=CH), 1576 (C=C, aromatic ring), 1255 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 243 (2.6), 317 (3.0); $^1$H-NMR (400 MHz, $CDCl_3$ ($\delta$, ppm): Ar-H and CH=CHCO: 7.1–7.9 (m, 20H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 190.2 (2C=O), 121.9 ($2CH_2$), 144.8 ($2CH_2$), ar-C [139.4 (2CH), 112.7 (2CH), 159.8 (2CH), 119.2 (2CH), 129.5 (2CH), 120.9 (2CH), 134.7 (2-CH), 128.9 (-4CH), 128.4 (4CH), 55.4 (2-$OCH_3$)].] HRESI-TOF-MS: (*m* / *z*, %) $[M+H]^+$: 475.20.42(11), calc. 475.1917.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-2,2'-DIYL)-BIS-[1-(4-METHOXYPHENYL)PROP-2-EN-1-ONE], (16)**

Yield: 18 %, oily, $R_f$: 0.55 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, cm$^{-1}$): 3056 (=CH), 1597 (C=C, aromatic ring), 1184 (O-$CH_3$); UV-vis $\lambda$ nm (logε): 240 (3.2), 317 (3.7); $^1$H-NMR (400 MHz, $CDCl_3$-$CD_3OD$ (20:1, $\delta$, ppm): Ar-H: 6.9 (d, $J$ = 8.6 Hz, 4H), 8.1 (d, $J$ = 8.6 Hz, 4H), 7.2-7.4 (m, 8H), and CH=CHCO: 7.5 (d, $J$ = 15.6 Hz, 2H), 7.8 (d, $J$=15.6 Hz, 2H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 188.4 (2C=O), 121.7 ($2CH_2$), 143.9 ($2CH_2$), ar-C [130.2 (2CH), 130.1 (4CH), 113.8 (4CH), 163.3 (2CH), 135.0 (2CH), 135.0 (2C-C), 128.9 (2CH), 130.8 (2CH), 128.3 (2-CH), 130.3 (2CH), 55.2 (2-$OCH_3$)]. HRESI-TOF-MS: (*m*/*z*, %) $[M+H]^+$: 475.1910(10), calc. 475.1917, $[M-CH_3+K+H]^+$: 499.1929(50), calc. 499.1927.

**(2E,2'E)-3,3'-(BIPHENYL-3,3'-DIYL)-BIS-[1-(4-METHOXYPHENYL)PROP-2-EN-1-ONE], (17)**

Yield: 20 %, oily, $R_f$: 0.68 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, $cm^{-1}$): 3056 (=CH), 1572 (C=C, aromatic ring), 1178 (O-$CH_3$); UV-vis $\lambda$ nm (log$\epsilon$): 240 (2.5), 319 (3.1); $^1$H-NMR (400 MHz, $CDCl_3$-$CD_3OD$ (20:1, $\delta$, ppm): Ar-H: 7.0 (d, *J*=8.6 Hz, 4H), 8.1 (d, *J* = 8.6 Hz, 4H), 7.3–7.6 (m, 8H), and CH=CHCO: 7.6 (d, *J* = 15.6 Hz, 2H), 7.8 (d, *J* = 15.6 Hz, 2H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 188.6 (2C=O), 121.7 ($2CH_2$), 143.9 ($2CH_2$), ar-C [130.7 (4CH), 130.9 (4CH), 113.8 (4CH), 163.4 (2CH), 126.9 (2CH), 163.6 (2CH), 130.3 (2-CH), 134.9 (-2C-C), 128.8 (2CH), 55.4 (2-$OCH_3$)]. HRESI-TOF-MS: (*m* / *z*, %) $[M+H]^+$: 475.1900(20), calc. 475.1917, $[M-CH_3+K+H]^+$: 499.1950(50), calc. 499.1927.

**(2*E*,2'*E*)-3,3'-(BIPHENYL-4,4'-DIYL)-BIS-[1-(4-METHOXYPHENYL)PROP-2-EN-1-ONE], (18)**

Yield: 18 %, oily, $R_f$: 0.67 (*n*-hexane-diethyl ether, 2 : 1); IR (ATR, $cm^{-1}$): 3057 (=CH), 1574 (C=C, aromatic ring), 1224 (O-$CH_3$); UV-vis $\lambda$ nm (log$\epsilon$): 239 (2.9), 319 (3.4); $^1$H-NMR (400 MHz, $CDCl_3$-$CD_3OD$ (20:1, $\delta$, ppm): Ar-H: 7.0 (d, *J* = 8.6 Hz, 4H), 8.1 (d, *J*=8.6 Hz, 4H), 7.4–7.8 (m, 8H), and CH=CHCO: 7.5 (d, *J* = 15.6 Hz, 2H), 7.8 (d, *J* = 15.6 Hz, 2H), -$OCH_3$: 3.9 (s, 6H); $^{13}$C-NMR (50 MHz, $CDCl_3$-$CD_3OD$ (20 : 1, $\delta$, ppm): 188.4 (2C=O), 121.8 ($2CH_2$), 143.9 ($2CH_2$), ar-C [130.3 (4CH), 131.0 (2CH), 113.8 (4CH), 163.4 (2CH), 135.0 (2CH), 128.9 (4CH), 128.3 (4CH), 55.5 (2-$OCH_3$)]. HRESI-TOF-MS: (*m*/*z*, %) $[M+H]^+$: 475.1946(12), calc. 475.1917, $[M-CH_3+K+H]^+$: 499.1980(50), calc. 499.1927, $[M-CH_3+H_2O]^+$: 477.2063(100), calc. 477.2006.

## Antimicrobial Activity

The antimicrobial activity tests conducted for the synthesized compounds (**10**–**18**) determined by MIC measurements revealed that the compounds generally had very low activity, but they were generally effective on *C. tropicalis*, a fungal disease agent (**Table 3**). All these efficacy values were lower than the efficacy of the control antibiotic drug. These compounds generally exhibited very low or no activity against G(–) bacteria, and activity was observed in compounds **10** and **12** only against *E. coli* as a G(–) bacterium. No effect of the control antibiotic on this microorganism was detected. The antimicrobial activity tests of bichalcone compounds determined that the MIC values on the bacteria used were generally very low. Compounds **10** and **12,** 2-methoxy-substituted bichalcone compounds, showed activity only against *E. coli* with a MIC value of 100 μg $mL^{-1}$. Compounds **15** and **17,** 3-methoxy-substituted bichalcone compounds, displayed activity against *B. subtilis* with a MIC value of 100 μg $mL^{-1}$ as a G(+) bacterium. It was determined that bichalcone compounds **11**, **12** and **17** were highly effective against *C. tropicalis*, a fungal disease agent. Especially the MIC values of bichalcone derivatives **11**, **12** and **17** of 1.56 μg $mL^{-1}$ were quite remarkable. Compounds **10** and **14** also showed a high effect on the fungus with a MIC value of 6.25 μg $mL^{-1}$.

## Antioxidant Activity

Table 4 contains results of antioxidant tests (DPPH and FRAP) for bichalcones. Antioxidant activity was observed for all tested compounds **10**–**18**. When the results of the

**Table 3.** $^{13}$C NMR (50 MHz, $CDCl_3$) data of bichalcones (**10**–**18**).

| No | Microorganisms[a] (MIC, μg $mL^{-1}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Bc* | *Ef* | *Sa* | *Se* | *Ec* | *Kp* | *Pv* | *St* | *Yp* | *Ecl* | *Ct* |
| 10 | – | – | – | – | 100 | – | – | – | – | – | 6.25 |
| 11 | – | – | – | – | – | – | – | – | – | – | 1.56 |
| 12 | – | – | – | – | 100 | – | – | – | – | – | 1.56 |
| 13 | – | – | – | – | – | – | – | – | – | – | 100 |
| 14 | – | – | – | – | – | – | – | – | – | – | 6.25 |
| 15 | 100 | – | – | – | – | – | – | – | – | – | 100 |
| 16 | – | – | – | – | – | – | – | – | – | – | – |
| 17 | 100 | – | – | – | – | – | – | – | – | – | 1.56 |
| 18 | – | – | – | – | – | – | – | – | – | – | – |
| **Kan[b]** | 0.19 | 6.25 | 0.78 | 0.39 | 1.56 | 0.39 | 0.19 | 1.56 | 0.78 | 1.56 | |

*Bc: Bacillus subtilis; Ef: Enterococcus faecalis; Sa: Staphylococcus aureus; Se: Staphylococcus epidermidis; Ec: Escherichia coli; Kp: Klebsiella pneumonia; Pv: Proteus vulgaris; St: Salmonella typhimirium; Yp: Yersinia pseudotuberculosis; Ecl: Enterobacter cloacae; Ct: Candida tropicalis.*

[a] Inhibition zone in diameter ≥ 6 mm around the disks.

[b] Kanamycin.

two different antioxidant activity determination methods are compared, there is a general agreement, but there are also differences. In both methods, it was observed that the methoxy functional group (15 and 16) increased the antioxidant effect with the increase in the distance from each other in the bischalcone compounds. In other words, bichalcone compounds with methoxy at 3 and 4 positions increased the activity, while the compounds containing 3-methoxy were more active in the FRAP test than in the DPPH method. The results of the two methods were evaluated by graphing in order to determine the consistency between the methods. Looking at the distribution of the graph points obtained, it is seen that the two methods show a certain degree of agreement. However, the correlation coefficient of 0.23 of the line in the graph indicates that the fit is not very good. It was determined that the activity of the **16**–**18** compounds, which are expected to show the highest activity in terms of steric compatibility, generally showed lower activity than the 3-substituted biscalcone compounds. The basis of these differences is thought to be due to the different reaction mechanisms of the two methods and the steric hindrance in the DPPH radical scavenging test.

## CONCLUSION

In conclusion, nine new C–C bichalcones were synthesized in the present research. The preparation of the key intermediate for C–C bichalcones was made by one-pot biaryl synthesis via the Ullmann C–C cross-coupling reaction using bromo-substituted chalcones. The antimicrobial activities of the synthesized compounds were studied on eleven microorganisms, and it was found that they exhibited high activity against the fungus, especially against *C. tropicalis.* While compounds **10**–**12** (2-methoxy bichalcone derivatives) displayed antimicrobial activities against the Gram-negative bacteria and fungus (*E. coli* and *C. tropicalis*), compounds **15**–**17** (3-methoxy and 4-methoxy bichalcone derivatives) showed antimicrobial activity against G(+) and fungus (*B. subtilis* and *C. tropicalis*). The MIC values for *E. coli*, *B. subtilis*, and *C. tropicalis* ranged from 1.56 to 100 μg/mL, respectively. This study identified bichalcones as potential *in vitro* antimicrobial agents against *C. tropicalis,* which may serve as leading compounds for future research.

Thus, there is still a need for synthesizing new bichalcones and evaluating their biological activities.

**Table 4.** Antioxidant activities.

| No | FRAP / μmol | DPPH (SC50 mg mL$^{-1}$) |
|---|---|---|
| **10** | 6.247±2.106 | 19.263±1.201 |
| **11** | 1.235±0.147 | 35.095±1.211 |
| **12** | 23.905±1.429 | 8.977±0.142 |
| **13** | 12.080±0.326 | 7.378±0.823 |
| **14** | 4.641±0.327 | 17.409±1.003 |
| **15** | 30.701±1.774 | 4.386±0.623 |
| **16** | 30.072±0.869 | 4.594±0.513 |
| **17** | 17.132±1.025 | 8.607±0.567 |
| **18** | 23.502±3.547 | 8.575±0.478 |
| Trolox[(a)]* | - | 0.0034±0.000 |

[(a)] Reference compound.

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