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Synthesis, Characterization of α-Cyanochalcone Compounds and Determination of Their Antibacterial, Antifungal, and Antioxidant Activities

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Abstract

In this study, the Knoevenagel condensation reactions of 3-(heteroaryl)-3-oxopropanenitriles and various aromatic/heteroaromatic aldehydes via L-proline were performed. In these reactions, α-cyanochalcone compounds were obtained in mid-good yields. Spectroscopic techniques were used to clarify the compounds' structures. Both the well-diffusion method and the minimum inhibitory concentration (MIC) method were used to assess the antibacterial and antifungal properties of all products. Compound 1c, containing the pyridin-3-yl group, exhibited broad-spectrum properties by acting against all bacteria and fungi tested. The antioxidant activities of the compounds were tested by a DPPH radical scavenging assay, and according to the results of antioxidant activities, the most effective compound among all was found to be 1c. It is possible to say that compounds 1b and 1d also have moderate antioxidant activity.

Keywords: α-cyanochalcone; Knoevenagel condensation; Antimicrobial activity; Antioxidant activity.

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α-Siyanokalkon Bileşiklerinin Sentezi, Karakterizasyonu ve Antibakteriyel, Antifungal ve Antioksidan Aktivitelerinin Belirlenmesi

Öz

Bu çalışmada, 3-(heteroaril)-3-oksopropannitriller ile çeşitli aromatik/heteroaromatik aldehitlerin L-prolin aracılığında Knoevenagel kondensasyon reaksiyonları gerçekleştirildi. Bu reaksiyonlarda orta-iyi verimlerde α-siyanokalkon bileşikleri elde edildi. Bileşiklerin yapıları spektroskopik yöntemler kullanılarak aydınlatıldı. Tüm ürünlerin antibakteriyel ve antifungal aktiviteleri hem kuyu- difüzyon yöntemi hem de minimum inhibisyon konsantrasyonu (MIC) yöntemiyle belirlendi. Piridin-3-il grubunu içeren bileşik 1c, hem Gram (+) hem de Gram (-) bakteri ve mantarlara karşı etki göstererek geniş spektrumlu özellikler sergiledi. Bileşiklerin antioksidan aktiviteleri DPPH radikal temizleme yöntemi ile test edildi ve antioksidan aktivite sonuçlarına göre en etkili bileşiğin 1c olduğu bulundu. 1b ve 1d bileşiklerinin de orta düzeyde antioksidan aktiviteye sahip olduğunu söylemek mümkündür.

Anahtar Kelimeler: α-Siyanokalkon; Knoevenagel kondensasyonu; Antimikrobiyal aktivite; Antioksidan aktivite.

1. Introduction

Chalcones, which are both found in natural products and are readily synthesized using a multitude of techniques, are an important compound class in organic chemistry because they show a broad and diverse biological activity and are the starting components of many important biologically active compound classes [1-3]. Among the wide range of biological activities shown by chalcones are antimicrobial [4, 5], antimitotic [6], antitumor [7, 8], anti-inflammatory [9], antioxidant [10], antihistamine [11], antidiabetic [12], antihypertensive [13], enzyme inhibition [14, 15], antiparasitic [16], and antiplasmodial [17] properties (Fig. 1).

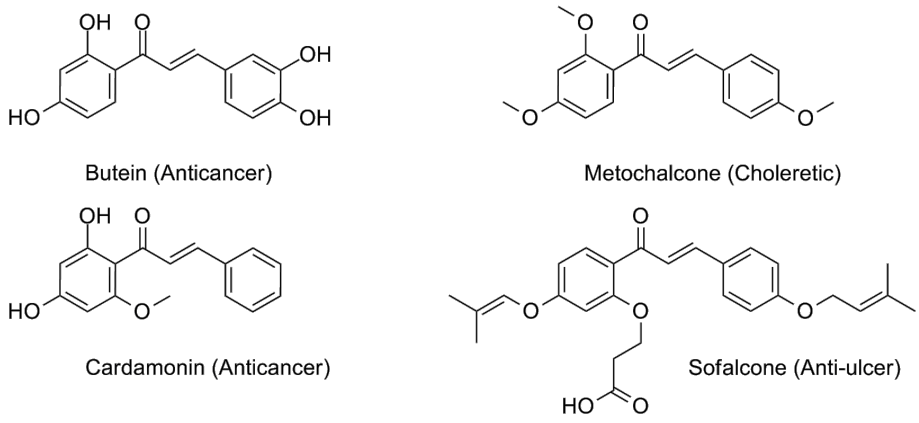


Figure 1: Some of the bioactive chalcone compounds.

Chalcones are mostly synthesized by base-catalyzed Claisen-Schmidt condensation but can also be synthesized by coupling reactions such as Heck, Suzuki, Suzuki-Miyaura, Wittik, and Sonogashira [2]. Additionally, chalcones can be converted to biologically important five-membered (pyrole, furan, benzofuran, pyrazole, imidazole, isoxazole, thiazole, triazole), six-membered (pyridine, pyrimidine), and seven-membered heterocycles (diazepine) [3].

α-Cyanocalcone compounds are mostly synthesized by Knoevenagel condensation and, like chalcones, exhibit a variety of biological functions [18]. For example, Kumar et al. [19] synthesized α-cyano chalcones, including bis(indolyl) groups, and examined the effectiveness of these compounds against lung (A549), prostate (PC3) and pancreas (PaCa2) cancer lines. They reported that the best results were obtained against lung cancer. Fahim and Farag [20] reported that they synthesized various α-cyanochalcone compounds. They also synthesized aminopyrazole compounds and pyrazolopyrimidine compounds, respectively, by reacting these compounds with hydrazine derivatives and various pyrazole derivatives. They examined the antimicrobial effects of both the α-cyanochalcones and the products they obtained from α-cyanochalcones. They found that both α-cyanochalcones and heterocyclic products showed moderate to good activity against bacteria and fungus.

In this study, α-cyanochalcone compounds were obtained by performing the Knoevenagel condensation reaction of 3-oxopropanenitriles and various aromatic/heteroaromatic aldehydes. Spectroscopic techniques were used to clarify the compounds' structures. The biological effects against bacteria, fungi, and antioxidant properties of the substances were investigated.

2. Materials and Methods

3-Oxo-propanenitriles (1-3) were synthesized according to the literature [21]. Aldehydes are commercially available and were not further purified before being used. An electrothermal capillary melting point device (Stuart SMP30) was used to measure melting points (mp). Infrared spectra were acquired using a SHIMADZU IRSpirit QATR-S in the 400-4000 cm^{-1} range. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III-400 MHz spectrophotometer. The coupling constants (*J*) are expressed in hertz (Hz), and chemical shifts are reported in parts per million (ppm) concerning Me_4Si as the internal standard. The following are the designations for splitting patterns: s stands for singlet, d for doublet, t for triplet, m for multiplet, and bs for broad singlet. An Agilent 6530 and an Agilent 6230 Accurate-Mass Q-TOF LC–MS apparatus were used to measure the mass spectra; m/z (rel. %). Using Merck silica gel 60 (230–400 mesh) as the stationary phase and EtOAc and hexane as eluents, column chromatography (cc) was utilized to purify the products.

2.1. General Procedure for the Synthesis of 3-Oxo-Propanenitriles (1-3)

A three-necked flask with a thermometer, dropping funnel, and condenser with a gas trap at the outlet; 60% sodium hydride (5.6 g; 140 mmol), ester (70 mmol), and 150 mL toluene are added and heated to 90 °C. To the mixture at this temperature, 6 mL of toluene solution of acetonitrile (140 mmol) is added dropwise over 30 minutes. The reaction continues until hydrogen gas is released. After gas evolution ends, the solution is cooled. The resulting solids are filtered and dried. These solids are then dissolved in water in an ice-salt bath and hydrolyzed dropwise with a dilute HCl (1:1) solution, preventing the temperature from rising above 5°C. The resulting crude product is purified by crystallization from methanol [21].

2.2. General Procedure for the Reactions of 3-Oxo-Propanenitriles (1-3) and Aldehydes (a-f) via L-Proline

3-aryl-3-oxo-propanenitrile (1 eq) and the L-proline (0.2 eq) are dissolved in 4 ml of ethanol. Aromatic aldehyde (1 eq) is added to the mixture. The chalcone derivative formed within 1-2 minutes from the solution mixed at room temperature on the magnetic stirrer begins to separate in solid form. The experiment is controlled by thin-layer chromatography and terminated at the appropriate time. Once the starting compounds are completed, cold water is added to the reaction medium. The solid undergoes filtration, a cold ethanol-water mixture wash, and a thorough water wash before drying. The product is purified by crystallization or cc [22].

2.3. Determination of Antimicrobial Activity

In this study, the techniques described in previous studies [23-24] were used to evaluate the compounds' minimal inhibitory concentrations (MIC) and agar well diffusion. In this study, 2 Gram (+) (*Staphylococcus aureus* ATCC 6538 and *Bacillus cereus* ATCC 7064), 3 Gram (-) (*Escherichia coli* ATCC 11293, *Klebsiella pneumonia* ATCC 27889, and *Pseudomonas aeuroginosa* ATCC 27853) and 2 fungi (*Candida krusei* ATCC 6258 and *Candida parapsilosis* 22019) reference microorganisms were used. DMSO, in which the chemicals were dissolved, was used as a negative control. Additionally, Bacteria were grown on Mueller-Hinton Agar (MHA) and Broth (MHB), while fungi were grown on Sabouraud-Dextrose Agar (SDA) and Broth (SDB).

2.4. Agar Well Diffusion Method

After being grown overnight in MHB and SDB, the bacterial and fungal cultures were adjusted using the McFarland 0.5 standard (~$1.5x10^8$ cfu/mL). Following this, approximately

10^6 cfu/mL of new cultures was added to recently prepared sterile MHA and SDA petri surfaces. Using a sterile cork borer, on the agar surface, wells with a diameter of 6 mm were created. 40 µL of the product's solution to be tested—2 mg of compounds coded 1c, 1d, and 1e and 0.1 mg of chemicals coded 1a, 1b, 2a, and 2b—was added to the wells. The petri dishes were then incubated for 18 to 24 hours at 37±4°C (for bacteria) and at 28±2°C (for fungi). At the end of the period, the zone diameter formed around the wells was measured with the help of a millimetric ruler and then transferred to Excel 2010 to obtain the Standard Deviations (±SD) of the three repeated tests. Commercial antibiotics were used as the positive control (pc), while DMSO was used as the negative control (nc).

2.5. Minimal inhibitory concentrations (MIC)

Minimum inhibition concentrations were determined by the tube dilution method followed by incubation techniques in an agar medium. For this, 1 mL of MHB and SDB was added to each tube, and after the sterilization process, serial dilutions (range 5 – 0.125 mg) of the compounds to be tested were prepared. Then, 50 µL of microorganism culture prepared based on the McFarland standard was inoculated into each tube and incubated at 37±4°C (for bacteria) and at 28±2°C (for fungi) for 18-24 hours. The results were visually inspected, and the dilution at which no growth or turbidity was observed was recorded as the MIC value. The sample taken from the dilution showing the MIC value was inoculated into sterile MHA and SDA petri dishes and incubated at 37±4°C and 28±2°C for 18-24 hours. Results were reported based on whether there was growth in the petri dishes.

2.6. Determination of antioxidant activities

Samples were analyzed for free radical scavenging activity using the DPPH radical scavenging test [25-26]. Different concentrations (1000 – 62.5 µg/mL) of the compounds to be tested were prepared. A 4 mL DPPH-ethanol solution (0.1 mM) was introduced to various component concentration solutions in 1 mL ethanol. Absorbance values at 517 nm were measured after the solutions were left in the dark for half an hour. The following formula was used to determine the DPPH radical's scavenging activity:

$$\% \, Inhibition = \frac{AB - AS}{AB} .100 \quad (1)$$

In this equation, AS represents the absorbance of the test substance, and AB represents the absorbance of the control reaction. IC_{50} stands for the concentration of a chemical needed to

block 50% of free radical scavenging action. The standard was ascorbic acid. Standard-free ethanol was used as a negative control, and the tests were also performed in triplicate.

3. Experimental

3.1. Physical and Spectral Data of the Substrates

3-Oxo-3-(furan-2-yl)-propanenitrile (1): Yield 76 %, brown solid, mp: 74-75 °C (Lit. [27] mp: 66-68 °C); IR (ATR, ν/cm): 3134 (ArH), 2950-2920 (RH), 2256 (C≡N), 1671 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.00 (s, 2H), 6.67 (dd, 1H, *J* = 3.6, 1.6 Hz), 7.42 (dd, 1H, *J* = 3.6, 0.8 Hz), 7.69 (t, 1H, *J* = 0.8 Hz). Similar to that previously reported [27].

3-Oxo-3-(thiophene-2-yl)-propanenitrile (2): Yield 81%, brown solid, mp: 114-115°C (Lit. [27] mp: 123-126 °C); IR (ATR, ν/cm): 3113-3091 (ArH), 2949-2918 (RH), 2256 (C≡N), 1664 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.00 (s, 2H), 7.20 (dd, 1H, *J* = 4.8, 4.4 Hz), 7.78-7.80 (m, 2H). Similar to that previously reported [27].

3-Oxo-3-(pyridine-2-yl)-propanenitrile (3): Yield 76%, claret red solid, mp 87-88°C; IR (ATR, ν/cm): 3101-3058 (ArH), 2953-2927 (RH), 2260-2186 (C≡N), 1711 (C=O); $_{1}$H-NMR (400 MHz, $CDCl_3$, δ): 4.74 (s, 2H), 7.76-7.73 (m, 1H), 8.09-8.00 (m, 2H), 8.75 (d, 1H, *J* = 3.2 Hz) ; ^{13}C-NMR (100 MHz, $CDCl_3$, δ): 29.73, 116.75, 122.30, 129.14, 138.35, 149.83, 151.27, 191.33; HRMS (m/z): calculated for $C_8H_6N_2O$ $[M]^+$: 146.0475, found: 146.9963.

3.2. Physical and Spectral Data of the Products

2-(furan-2-carbonyl)-3-(furan-2-yl)acrylonitrile (1a) : Yield 92%, yellow solid, mp: 143-144°C; IR (ATR, ν/cm): 3134-3117 (ArH), 2213 (C≡N), 1640 (C=O); ^{1}H-NMR (400 MHz, DMSO-d6, δ): 6.84-6.85 (m, 1H), 6.92-6.93 (m, 1H), 7.60 (d, 1H, *J* = 3.2 Hz), 7.70 (dd, 1H, *J* = 4.0, 0.8 Hz), 8.168-8.173 (m, 1H), 8.26 (s, 1H), 8.28 (d, 1H, *J* = 0.8 Hz); ^{13}C NMR (101 MHz, DMSO-d6, δ): 173.95 (C=O), 150.77 (CH), 150.29 (C), 149.59 (CH), 149.07 (CH), 139.89 (CH), 125.60 (CH), 121.82 (CH), 117.07 (C), 115.07 (CH), 113.52 (C), 103.20 (C); HRMS (m/z): calculated for $C_{12}H_7NO_3$ $[M+H]^+$: 214.04987, found: 214.9802.

2-(furan-2-carbonyl)-3-(thiophen-2-yl)acrylonitrile (1b) : Yield 90%, yellow solid, mp: 135-136°C; IR (ATR, ν/cm): 3113-3088 (ArH), 2214 (C≡N), 1644 (C=O); ^{1}H-NMR (400 MHz, DMSO-d6, δ): 6.84-6.87 (m, 1H), 7.39-7.42 (m, 1H), 7.70 (dd, 1H, *J* = 4.0, 0.8 Hz), 8.13 (d, 1H, *J* = 3.2 Hz), 8.17-8.18 (m, 1H), 8.26 (dd, 1H, *J* = 4.8, 0.8 Hz), 8.73 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6, δ) 173.81 (C=O), 150.33 (C), 149.58 (CH), 148.44 (CH), 141.33 (CH),

138.00 (CH), 136.56 (C), 129.41 (CH), 121.80 (CH), 117.61 (C), 113.55 (CH), 104.10 (C); HRMS (m/z): calculated for $C_{12}H_7NO_2S$ $[M+H]^+$: 230.027026, found: 230.9596.

2-(furan-2-carbonyl)-3-(pyridin-3-yl)acrylonitrile (1c) : Yield 62%, yellow solid, mp: 135-136°C; IR (ATR, ν/cm): 3137-3116 (C=CH), 2216 (C≡N), 1641 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ) 9.12 (s, 1H), 8.78 (d, *J* = 4.5 Hz, 1H), 8.55-8.48 (m, 2H), 8.22 (s, 1H), 7.79 (d, *J* = 3.6 Hz, 1H), 7.67 (dd, *J* = 8.0, 4.8 Hz, 1H), 6.88 (dd, *J* = 3.6, 1.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d6, δ) 174.04 (C), 153.48 (CH), 152.51 (CH), 152.49 (CH), 150.27 (CH), 149.89 (C), 136.93 (CH), 128.58 (C), 124.60 (CH), 123.04 (CH), 116.72 (C), 113.73 (CH), 111.36 (C); HRMS (m/z): calculated for $C_{13}H_8N_2O_2$ $[M+H]^+$: 225.065854, found: 225.06576.

3-(4-chlorophenyl)-2-(furan-2-carbonyl)acrylonitrile (1d) : Yield 79%, beige solid, mp: 125-126°C; IR (ATR, ν/cm): 3143 (C=CH), 3034 (ArH), 2207 (C≡N), 1651 (C=O); ^{1}H NMR (400 MHz, DMSO-d6, δ) 8.45 (s, 1H), 8.21 (d, *J* = 0.9 Hz, 1H), 8.11 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 3.5 Hz, 1H), 7.72 (d, *J* = 8.6 Hz, 2H), 6.87 (dd, *J* = 3.6, 1.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d6, δ) 174.28 (C), 154.02 (CH), 150.10 (CH), 150.00 (C), 138.32 (C), 132.94 (CHx2), 131.22 (C), 129.91 (CHx2), 122.75 (CH), 116.91 (C), 113.66 (CH), 109.74 (C); HRMS (m/z): calculated for $C_{14}H_8ClNO_2$ $[M+H]^+$: 258.031633, found: 258.03144.

2-(furan-2-carbonyl)-3-(4-hydroxyphenyl)acrylonitrile (1e) : Yield 63%, orange solid, mp: 181-182°C; IR (ATR, ν/cm): 3304 (OH), 3162 (C=CH), 2212 (C≡N), 1606 (C=O) ; ^{1}H NMR (400 MHz, DMSO-d6, δ) 10.92 – 10.87 (bs, 1H, OH), 8.33 (s, 1H), 8.16 (s, 1H), 8.06 (d, *J* = 8.5 Hz, 2H), 7.67 (s, 1H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.83 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6, δ) 174.75 (C), 163.52 (CH), 155.50 (CH), 150.37 (C), 149.41 (C), 134.74 (CHx2), 123.45 (CH), 121.64 (CH), 118.17 (C), 116.90 (CHx2), 113.42 (CH), 103.85 (C); HRMS (m/z): calculated for $C_{14}H_9NO_3$ $[M+H]^+$: 240.06552, found: 240.06524.

3-(furan-2-yl)-2-(thiophene-2-carbonyl)acrylonitrile (2a): Yield 87%, yellow solid, mp: 138-139°C; IR (ATR, ν/cm): 3085-3031 (ArH), 2210 (C≡N), 1629 (C=O); ^{1}H-NMR (400 MHz, DMSO-d6, δ): 6.92 (dd, 1H, *J* = 3.6, 1.6 Hz), 7.34 (dd, 1H, *J* = 5.2, 3.6 Hz), 7.59 (d, 1H, *J* = 3.6 Hz), 8.15 (dd, 1H, *J* = 4.0, 1.2 Hz), 8.18 (dd, 1H, *J* = 4.8, 1.2 Hz), 8.20 (s, 1H), 8.28 (d, 1H, *J* = 1.2 Hz); ^{13}C NMR (101 MHz, DMSO-d6, δ) 179.47 (C=O), 150.72 (CH), 149.00 (C), 141.52 (C), 140.08 (CH), 137.05 (CH), 135.42 (CH), 129.49 (CH), 125.66 (CH), 117.39 (C), 115.04 (CH), 103.53 (C); HRMS (m/z): calculated for $C_{12}H_7NO_2S$ $[M+H]^+$: 230.26242, found: 230.9593.

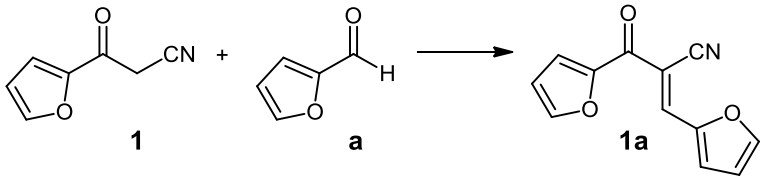
3-(thiophen-2-yl)-2-(thiophene-2-carbonyl)acrylonitrile (2b) : Yield 84%, yellow solid, mp: 150-151°C; IR (ATR, ν/cm): 3110-3083 (ArH), 2207 (C≡N), 1628 (C=O); ^{1}H-NMR (400

MHz, DMSO-d6, δ): 7.35 (dd, 1H, *J* = 4.8, 4.0 Hz), 7.40 (dd, 1H, *J* = 4.8, 4.0 Hz), 8.12 (d, 1H, *J* = 4.4 Hz), 8.15 (dd, 1H, *J* = 4.0, 0.8 Hz), 8.18 (dd, 1H, *J* = 4.8, 0.8 Hz), 8.26 (d, 1H, *J* = 4.8 Hz), 8.67 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d6, δ) 179.40 (C=O), 148.60 (CH), 141.55 (C), 141.31 (CH), 137.92 (CH), 137.04 (CH), 136.50 (C), 135.46 (CH), 129.54 (CH), 129.41 (CH), 117.89 (C), 104.56 (C); HRMS (m/z): calculated for $C_{12}H_7NOS_2$ $[MH]^+$: 246.00418, found: 246.0039.

4. Result and Discussion

Initially, the reactions of 3-Oxo-3-(furan-2-yl)-propanenitrile (1) and 2-furancarboxaldehyde (a) via different catalysts were investigated (Table 1). Inorganic bases, organic and inorganic acids, and L-proline were tested as catalysts in these reactions carried out in 80% ethanol solution, and the highest efficiency was obtained when L-proline was used as the catalyst. In reactions where absolute ethanol was used as a solvent, a significant increase in yield was observed due to the solubility of the compounds increased.

Table 1: Optimization conditions of Knoevenagel condensation reactions.



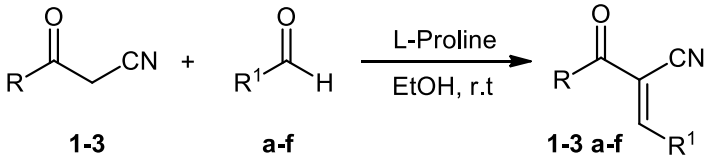
No	Reactive	Solvent	Time	1a Yield (%)[b]
1	Na_2CO_3	EtOH/H_2O[a]	o.n.	25
2	K_2CO_3	EtOH/H_2O[a]	o.n.	38
3	NaOH	EtOH/H_2O[a]	o.n.	45
4	KOH	EtOH/H_2O[a]	o.n.	48
5	der. H_2SO_4	EtOH/H_2O[a]	o.n.	5
6	p-Toluenesulfonic acid	EtOH/H_2O[a]	o.n.	45
7	Silica-Sulfuric acid	EtOH/H_2O[a]	o.n.	51
8	L-Proline	EtOH/H_2O[a]	o.n.	65
9	L-Proline	EtOH	1 h	89
10	L-Proline	EtOH	2.5 h	92

a: 80% Ethanole solution were used. b: All experiments were carried out at room temperature.

α-Cyano chalcone compounds were obtained in mid-good yields by performing L-proline catalyzed Knoevenagel condensation reactions of 3-oxo-propanenitriles (1, 2) and aldehydes (a-e) (Table 2). The structures of the compounds were clarified through the use of spectroscopic methods. However, the targeted α-cyanochalcones could not be synthesized from the reactions of pyridine-2-carbaldehyde with 3-aryl-3-oxo-propanenitriles and the reactions of 3-(pyridin-2-yl)-3-oxo-propanenitriles with aldehydes. In these experiments, compounds that were insoluble

in most solvents, dark colored, and had low light transmission were obtained, and their structures could not be elucidated due to solubility problems.

Table 2: Products of the Knoevenagel condensation reactions of 3-Oxo-propanenitriles (1-3) and aldehydes (a-f) via L-proline[a].



1-3 **a-f** **1-3 a-f**

No	R	R^1	Time	Product	Yield (%)[b]
1	Furan-2-yl	Furan-2-yl	2.5 h	1a	92
2	Furan-2-yl	Thiophene-2-yl	1 h	1b	90
3	Furan-2-yl	Pyridine-3-yl	o.n.	1c	62
4	Furan-2-yl	4-Cl-C_6H_4	o.n.	1d	79
5	Furan-2-yl	4-OH-C_6H_4	o.n.	1e	63
6	Thiophene-2-yl	Furan-2-yl	1 h	2a	87
7	Thiophene-2-yl	Thiophene-2-yl	o.n.	2b	84
8	Furan-2-yl	Pyridine-2-yl	o.n.	1f	-
9	Thiophene-2-yl	Pyridine-2-yl	4 h	2f	-
10	Pyridine-2-yl	Furan-2-yl	o.n.	3a	-
11	Pyridine-2-yl	Thiophene-2-yl	o.n.	3b	-
12	Pyridine-2-yl	Pyridine-2-yl	o.n.	3f	-

a: All the reactions were carried out in a 1: 1: 0.2 molar ratio of 3-Oxo-propanenitrile (1-3), aldehyde (a-f) and L-proline in EtOH at rt.
b: Isolated yield.
o. n. : overnight

When the IR spectra of the products were examined, the vibrations of the aromatic and vinylic hydrogens were observed in the range of 3162-3031 cm^{-1}, the vibrations of the cyano group were observed around 2210 cm^{-1}, and the vibrations of the conjugated carbonyl group were observed around 1640 cm^{-1} and all these vibrations are as expected (Table 3). 1e's phenolic O-H vibration was observed at 3304 cm^{-1}.

Table 3: Some selected IR vibrations.

Compound	O-H	C=C-H	C≡N	C=O
1a		3134-3117	2213	1640
1b		3113-3088	2214	1644
1c		3137-3116	2216	1641
1d		3143-3034	2207	1651
1e	3304	3162	2212	1606
2a		3085-3031	2210	1629
2b		3110-3083	2207	1628

In the ^{1}H-NMR spectra of the products except 1c, aromatic protons resonated at between 6.8 and 8.3 ppm. Due to the presence of the pyridine ring in compound 1c, resonance

occurred in a lower area according to the other compounds, and H-C2 in the pyridine ring gave a signal as a single peak at 9.1 ppm. While vinylic protons adjacent to the thiophene ring resonated at 8.7 ppm, vinylic protons in other compounds resonated in the range of 8.2-8.45 ppm. Similarly, in the ^{13}C-NMR spectrum, the carbon atom in the carbonyl group attached to the thiophene ring gives a peak at 179 ppm, while the carbonyl carbon attached to the furan ring gives a peak at 174 ppm.

Table 4:. Agar well diffusion test results of the samples.

Compound	*Staphylococcus aureus* ATCC 6538	*Bacillus cereus* ATTC 7064	*Escherichia coli* ATCC 11293	*Pseudomonas aeruginosa* ATCC 27853	*Klebsiella pneumonia* ATCC 27889	*Candida parapsilosis* ATCC 22019	*Candida krusei* ATCC 6258
1a	16±0.2	-	-	-	-	-	12±0.43
1b	13±0.05	-	-	-	-	-	-
1c	24±1	17.3±0.5	-	-	20±1	17.3±1.5	14±2
1d	-	-	-	-	-	-	-
1e	-	-	-	-	-	-	-
2a	-	14±0.45	-	-	-	-	-
2b	-	-	-	-	-	-	-
DMSO	-	-	-	-	-	-	*
Ipm 10	28	26	21	*	38	*	*
CL30	27	10	-	9	-	*	*
Te 30	11	21	21	13	16	*	*
Da 2	11	16	-	12	14	*	43
Cyc	*	*	*	*	*	40	*

(-) not effect, (*) not tested; negative control: DMSO; positive control: Imipenem (Ipm 10); Cephalexin (CL-30); Tetracycline (TE 30); Clindamycin (Da-2); Cycloheximide (Cyc); units of measurement are recorded in millimeters

Agar well diffusion test results of 7 different α-cyano chalcones tested are shown in Table 4. According to the results, compounds 1d, 1e, and 2b had no effect against microorganisms, while compound 1c had a significant effect (between 14±2 and 24±1 mm) against Gram-negative, Gram-positive, and fungi (Fig. 2). Based on the findings of the MIC test shown in Table 5, although the effects of 1d and 1e were observed above 2 mg concentration against two Gram-positive isolates and *C. krusei*, the effective result of 1c between 0.25 and 4 mg concentrations was remarkable.

Table 5: Minimal Inhibition Concentration (MIC) test results for the samples

Compound	*Staphylococcus aureus* ATCC 6538	*Bacillus cereus* ATTC 7064	*Escherichia coli* ATCC 11293	*Pseudomonas aeruginosa* ATCC 27853	*Klebsiella pneumonia* ATCC 27889	*Candida parapsilosis* ATCC 22019	*Candida krusei* ATCC 6258
1a	125	-	-	-	-	-	-
1b	125	500	-	500	-	-	-
1c	250	1000	4000	-	500	500	500
1d	2500	2500	-	-	-	-	2500
1e	2500	2500	-	-	-	-	5000
2a	-	-	-	-	-	-	-
2b	-	-	-	-	-	-	-
DMSO	-	-	-	-	-	-	-

(-) not effect; negative control: DMSO

Compounds 1d, 1e and 2b showed no antimicrobial effect against the tested microorganisms in the agar well-diffusion test. While compounds 1a, 1b, and 2a showed moderate to good activity only against Gram (+) bacteria, compound 1c showed a broad spectrum of activity against both Gram (+) and Gram (-) bacteria and fungi. In addition, the antioxidant activities of the compounds were measured by the DPPH method, and the most effective compound was found to be 1c (Table 6). It is possible to say that compounds 1b and 1d also have moderate antioxidant activity. On the other hand, samples coded 1a, 1e, 2a, and 2b were considered as having no activity since they showed antioxidant activity higher than 1000 µg/mL (Table 6).

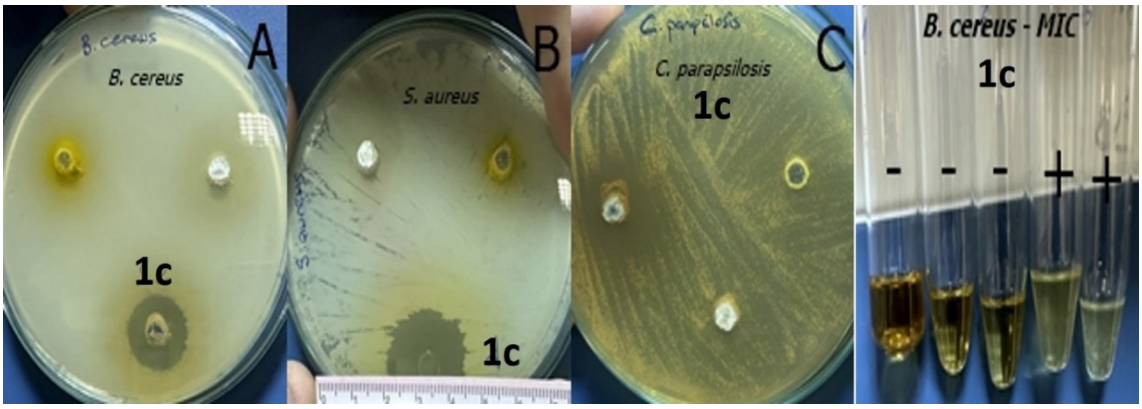


Figure 2: Agar well-diffusion test result images of (A) *B. cereus*, (B) *S. aureus*, (C) *C. parapsilosis* strains, (D) Five different MIC test result images of *B. cereus*, '-' on the tubes means no growth, '+' means growth (turbidity due to growth is seen in the last two concentrations).

Table 6: IC_{50} (µg/mL) antioxidant test results of the samples based on DPPH.

1a	-
1b	765.74±1.77
1c	553.14±2.38
1d	736.10±0.51
1e	-
2a	-
2b	-
A	110.96±0.58

A: Ascorbic acid; (-) no activity

When the antimicrobial and antioxidant activities of the synthesized α-cyanochalcone compounds from this study were examined, it was observed that different compounds had different effects (Tables 4-6). Although it is difficult to evaluate the synthesized α-cyanochalcone compounds among themselves due to their different chemical structures, there are still studies on α-cyanochalcone compounds in the literature. One of them, Fahim and Farag [20], synthesized α-cyanochalcone compounds in their study and reported that the compounds, including bis-substituted phenyl rings, showed antimicrobial activity in the range of 11.0 ± 0.11 - 19.2 ± 0.21 mm against Gram-positive and negative bacteria and some fungi. In another study conducted by El-Shenawy [28], in which the synthesis and antibacterial activities of quinazolinone substituted α-cyanochalcone compound was studied, it was determined that the compound was effective against Gram-positive (*S. epidermidis* and *S. aureus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria in the range of 14-22 mm. Apart from these, α-cyanochalcone compounds were studied not only in terms of antimicrobial activities but also in anticancer research, and it was determined that they have potential in the fight against lung cancer cells [19], colorectal carcinoma [29] and breast cancer cell lines [30]. To the best of our knowledge, there are only two studies involving the antioxidant properties of α-cyanochalcones [30, 31]. In the first of these, indole-pyrazole amalgamated α-cyano-substituted chalcones were synthesized, and it was stated that some of these compounds showed moderate DPPH free radical scavenging activity [30]. In the second study, compounds with α-cyano-substituted bis chalcone structure containing indolyl groups were synthesized, and their antioxidant properties were examined. According to the stated results, the synthesized compounds were found to have good to excellent DPPH free radical scavenging activity [31]. This study we have conducted, to the best of our knowledge, will be the third study involving the synthesis and antioxidant properties of α-cyano chalcones. Due to the scarcity of such studies, when we consider α-cyano chalcones as two different functional groups, chalcone, and acrylonitrile, there are many studies

in the literature showing that compounds with these structures also exhibit antioxidant activity [32-35].

5. Conclusion

In this study, α-cyanochalcone compounds were synthesized in mid-good yields by performing the Knoevenagel condensation reactions of 3-(heteroaryl)-3-oxopropanenitriles and various aromatic/heteroaromatic aldehydes. L-proline was found to be the most effective catalyst among those tested in these reactions. Spectroscopic techniques were used to clarify the compounds' structures. It was also observed that some of the synthesized α-cyanochalcone compounds were effective against Gram-positive and negative bacteria and fungi. In addition, they were evaluated for their antioxidant activity. Although one of the compounds had a five times lower effect than the tested positive control, it was observed to be promising. All these data show that the α-cyanochalcone compounds synthesized in this study are remarkable in terms of their antimicrobial and antioxidant capacities, and their inclusion in the literature shows the importance of this study.

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