DOI:10.25092/baunfbed. 1119869 *J. BAUN Inst. Sci. Technol., 25(1), 293-304, (2023)*

Thiophenyl-chalcone derivatives: Synthesis, antioxidant activity, FMO energies and molecular parameters

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Geliş Tarihi (Received Date): 23.05.2022.
Kabul Tarihi (Accepted Date): 02.01.2023

Abstract

In this study, a series of thiophenyl-chalcones derivatives was synthesized and their DPPH and ABTS activities were evaluated. All thiophenyl-chalcones exhibited high antioxidant activity. Among them, 4e ((E)-5-(3-(4-(chlorosulfonyl)-3-hydroxyphenyl)-3-oxoprop-1-en-1-yl)thiophene-2-sulfonyl chloride) have higher ABTS activity (IC_{50} = 13.12 μM) than quercetin (IC_{50} = 15.49 μM), well-known as antioxidant agent and used as a standard. The structure-activity relationship results revealed that most of synthesized sulfonyl chloride derivatives (4a-e) have higher antioxidant activity than the sulphonamide derivatives (5a-c) and also 4d and 4e including hydroxyl group, exhibited the strongest antioxidant activity as expected. Additionally, the frontier molecular orbitals (FMOs) energies and molecular parameters of the synthesized molecules were calculated to support experimental results. The quantum chemical calculation results indicated that the strongest antioxidant compounds, in this study, had the lowest LUMO energies and the highest electronegativity, electron affinity and electrophilicity index.

***Keywords**: Thiophene, chalcone, antioxidant activity*

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Tiyofenil-kalkon türevleri: Sentez, antioksidan aktivite, FMO enerjileri ve moleküler parametreler

Öz

Bu çalışmada bir dizi tiyofenil-kalkon türevi sentezlendi ve bunların DPPH ve ABTS aktiviteleri incelendi. Tüm tiyofenil-kalkonlar yüksek antioksidan aktivite sergilemiştir. Bunlardan, 4e ((E)-5-(3-(4-(klorosülfonil)-3-hidroksifenil)-3-oksoprop-1-en-1-il)tiyofen-2-sülfonil klorür) antioksidan ajan olarak iyi bilinen ve standart olarak kullanılan quersetinden (IC_{50} = 13.12 μM) daha yüksek ABTS aktivitesine sahiptir. Yapı-aktivite ilişkisi sonuçları, sentezlenen sülfonil klorür türevlerinin (4a-e) sülfonamid türevlerinden (5a-c) daha yüksek antioksidan aktiviteye sahip olduğunu ve ayrıca hidroksil grubu içeren 4d ve 4e'nin beklendiği gibi en güçlü antioksidan aktiviteyi sergilediğini ortaya koymuştur. Ayrıca, deneysel sonuçları desteklemek için sentezlenen bileşiklerin sınır moleküler orbital (FMO) enerjileri ve moleküler parametreleri hesaplandı. Kuantum kimyasal hesaplama sonuçları, bu çalışmada en güçlü antioksidan bileşiklerin en düşük LUMO enerjilerine ve en yüksek elektronegatiflik, elektron afinitesi ve elektrofilik indeksine sahip olduğunu göstermiştir.

Anahtar kelimeler: *Tiyofen, kalkon, antioksidan aktivite*

1. Introduction

Preservation of the oxidant-antioxidant balance of the organism is necessary for maintaining a healthy life [1, 2]. Free radicals are produced endogenously during the normal metabolic process [3]. Moreover, exogenous factors such as radiation, sun rays, environmental pollution and cigarettes also cause the formation of free radicals [4]. Due to their reactivity, free radicals have the potential to damage and interact with all cell components, especially lipids, nucleic acids and proteins [5]. Oxidative stress can develop in the organism due to the increase in free radical formation and/or the deficiency in the antioxidant defence system [6]. Oxidative stress, which is one of the factors that cause many common diseases such as diabetes, cancer and aging, arises from the imbalance between reactive oxygen species (ROS) and the antioxidant defence system of the cell [7, 8]. The antioxidant system is activated to reduce free radical toxicity [9,10]. However, exceeding the antioxidant defence system capacity and excessive presence of superoxide radical result in the formation of ROS [11, 12]. Antioxidants act an active role in the prevention of many diseases by catching free radicals in the living body [13, 14]. Therefore, the design and synthesis of effective new antioxidants continues to be the focus of interest for scientists.

Chalcones (1,3-diphenyl-2-propen-1-one) are known as open-chain flavonoids in which two aromatic rings are joined by a three-carbon α,β unsaturated carbonyl system [15, 16]. Natural and synthetic chalcones have widespread biological activity [17-20]. Heteroaryl chalcones are obtained by modifications of phenyl rings [21, 22]. Several studies have shown that heteroaryl chalcones have widespread biological potential such as anti-fungal, antibacterial, antimalarial, anticancer, anti-inflammatory, anti-angiogenic and anti-HIV activities [23, 24].

Revealing different natural or synthetic bioactive molecules is important in terms of being an alternative to the drugs used for the treatment of various diseases. Moreover, the determination and correlation of the structure-activity relationships of novel bioactive molecules with theoretical calculations provides ideas for future studies involving molecular design. In the lights of all above findings, this study aimed to investigate the antioxidant potential of newly designed heteroaryl-chalcones and to support their activity result with calculated molecular parameters. In this study, eight thiophenyl-chalcones were synthesized and their DPPH and ABTS activities were evaluated as antioxidant property. Furthermore, the quantum chemical energies of the synthesized compounds were calculated for contributing to the determination of structure-activity relationships.

2. Materials and methods

2.1. General methods

The chemicals and solvents were purchased from Sigma-Aldrich and Merck. Varian Infinity Plus spectrometer were used for ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) analysis. Leco CHNS-932 instrument and BioTek Power Wave XS were used for the elemental analyses and antioxidant assay.

2.2. General procedures and spectral data

Synthesis of thiophenyl-chalcone derivatives (3a-e): 1.5 mmol of 2-thiophenaldehyde (1) and 1 mmol of acetophenone derivatives (2a-e) were dissolved in 30 mL of ethanol. 5 mL of 10% aqueous NaOH was added to this mixture. It was stirred for 5 hours at room temperature and the mixture was poured onto 100 mL of ice water acidified with 5 mL of 2 M HCl. The obtained solids were filtered, washed with cold water and dried in vacuum oven.

(E)-3-(thiophen-2-yl)-1-(p-tolyl)prop-2-en-1-one (3a): Yellow powder, 92% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 2.43 (3H, s), 7.07-7.10 (1H, dd, J_1=1.2 Hz, J_2=3.8 Hz), 7.26-7.36 (4H, m), 7.41 (1H, d, J=5.0 Hz), 7.90-7.96 (3H, m); ^{13}C NMR ($CDCl_3$,75 MHz) δ/ppm: 21.9, 120.9, 128.6, 128.8, 128.9, 129.6, 132.2, 135.7, 137.1, 140.7, 143.9, 189.6.

(E)-1-(4-methoxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3b): Cream powder, 96% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 3.88 (3H, s), 6.98 (2H, d, J=8.8 Hz), 7.07-7.10 (1H, dd, J_1=1.2 Hz, J_2=3.8 Hz), 7.26-7.37 (2H, m), 7.40 (1H, d, J= 5.0Hz), 7.93 (1H, d, J= 15.2 Hz), 8.02 (2H, d, J= 8.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm: 55.7, 114.1, 120.8, 128.5, 128.7, 130.9, 131.2, 132.1, 136.7, 140.8, 163.6, 188.3.

(E)-1-(4-chlorophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3c): Yellow powder, 95% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.10 (1H, t, J=4.4 Hz), 7.28 (1H, d, J=14.7 Hz), 7.37 (1H, d, J=3.5 Hz), 7.44 (1H, t, J=5.5 Hz), 7.47 (2H, d, J=8.5Hz), 7.93-7.98 (3H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm: 120.3, 128.7, 129.2, 129.4, 130.1, 132.7, 136.6, 137.9, 139.4, 140.4, 188.7.

(E)-1-(4-hydroxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3d): Cream powder, 88% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 6.90 (2H, d, J= 8.5 Hz), 7.19 (1H, t, J=5.0 Hz), 7.54 (1H, d, J= 15.2 Hz), 7.66 (1H, d, J=3.5 Hz), 7.76 (1H, d, J= 5.0 Hz), 7.85 (1H, d, J=15.2 Hz), 8.01 (2H, d, J= 8.5 Hz), 0.43 (1H, s, OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 116.1, 121.0, 129.3, 129.6, 130.5, 131.7, 133.0, 136.2, 140.6, 162.8, 187.2.

(E)-1-(3-hydroxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3e): Cream powder, 90% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.06 (1H, D, *J*= 6.4Hz), 7.20 (1H, t, *J*=4.1 Hz), 7.37 (2H, m), 7.47 (1H, d, *J*=15.5 Hz), 7.54 (1H, d, *J*= 7.6 Hz), 7.70 (1H, d, *J*=3.3 Hz), 7.79 (1H, d, *J*= 5.0Hz), 7.89 (1H, d, *J*= 15.5 Hz), 9.83 (1H, s, OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 115.1, 120.0, 120.9, 121.0, 129.4, 130.6, 131.1, 133.6, 137.2, 139.5, 140.3, 158.4, 189.2.

Synthesis of thiophenylchalcone-sulfonylchloride derivatives (4a-e): 1 mmol of thiophenyl-chalcone derivatives (3a-e) was placed in the reaction flask in an ice bath and 5 mL of chlorosulfonic acid was slowly added. The mixture was stirred at room temperature for 15 hours. Then, it was poured dropwise onto 100 g of ice and stirred until the ice melted. The precipitated products were filtered, washed with cold water and dried in vacuum oven [25].

(E)-5-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)thiophene-2-sulfonyl chloride (4a): Brown powder, 68% yield. ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz) δ/ppm: 2,46 (3H, s), 7.26-7.35 (3H, m), 7.53 (1H, s, *J*= 15.5 Hz), 7.82-7.88 (2H, m), 7.94 (2H, d, *J*= 8.0Hz); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz) δ/ppm: 22.0, 125.7, 128.9, 129.9, 130.1, 134.1, 134.8, 135.4, 144.1, 145.0, 150.0, 188.3. Anal. Calcd. for $C_{14}H_{11}ClO_3S_2$: C, 51.45; H, 3.39; found: C, 51.82; H, 3.11.

(E)-5-(3-(3-(chlorosulfonyl)-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)thiophene-2-sulfonyl chloride (4b): Brown powder, 80% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 4.19 (3H, s), 7.28 (1H, d, *J*=8.8 Hz), 7.42 (1H, d, *J*=4.1 Hz), 7.49 (1H, d, *J*=15.5 Hz), 7.84-7.86 (1H, dd, J_1= 0.6 Hz, J_2= 4.1 Hz), 7.92 (1H, d, *J*= 15.3 Hz), 8.39-8.42 (1H, dd, J_1= 1.57 Hz, J_2=8.8 Hz), 8.61 (1H, d, *J*= 0.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm: 57.6, 113.8, 123.9, 129.6, 130.7, 130.8, 132.0, 135.4, 135.6, 137.7, 144.8, 149.1, 161.1, 185.2. Anal. Calcd. for $C_{14}H_{10}Cl_2O_6S_3$: C, 38.10; H, 2.28; found: C, 38.68; H, 2.12.

(E)-5-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)thiophene-2-sulfonyl chloride (4c): Dark brown powder, 80% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.12 (1H, d, J=3.5 Hz), 7.48 (1H, d, *J*=3.3 Hz), 7.54-7.60 (3H, m), 7.83 (1H, d, *J*= 15.2 Hz), 8.00 (2H, d, *J*= 7.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm: 125.0, 129.5, 130.2, 130.6, 134.9, 135.4, 135.6, 140.4, 144.5, 149.5, 187.6. Anal. Calcd. for $C_{13}H_8Cl_2O_3S_2$: C, 44.97; H, 2.32; found: C, 44.55; H, 2.62.

(E)-5-(3-(3-(chlorosulfonyl)-4-hydroxyphenyl)-3-oxoprop-1-en-1-yl)thiophene-2-sulfonyl chloride (4d): Black powder, 68% yield. ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz) δ/ppm: 7.02 (1H, d, *J*= 8.8 Hz), 7.25 (1H, d, *J*= 3.2 Hz), 7.34 (1H, d, *J*= 15.5 Hz), 7.58-7.63 (2H, m), 7.95-9.99 (1H, dd, J_1= 2.0 Hz J_2= 8.8), 8.26 (1H, d, *J*=2.0 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 117.5, 121.0, 127.6, 128.9, 129.2, 131.5, 132.5, 132.8, 136.8, 140.4, 154.3, 158.4, 187.0. Anal. Calcd. for $C_{13}H_8Cl_2O_6S_3$: C, 36.54; H, 1.89; found: C, 36.66; H, 1.78.

(E)-5-(3-(4-(chlorosulfonyl)-3-hydroxyphenyl)-3-oxoprop-1-en-1-yl)thiophene-2-sulfonyl chloride (4e): Black powder, 62% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.02 (1H, d, *J*= 8.0 Hz), 7.11 (1H, d, *J*=2.4 Hz), 7.32 (1H, d, *J*= 8.0 Hz), 7.36-7.45 (3H, m), 7.53 (1H, d, *J*= 7.3 Hz), 7.78 (1H, d, *J*= 15.3 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 115.1, 120.1, 121.0, 121.4, 127.5, 130.6, 132.7, 137.4, 139.5, 140.2, 154.9, 158.4, 189.1. Anal. Calcd. for $C_{13}H_8Cl_2O_6S_3$: C, 36.54; H, 1.89; found: C, 36.74; H, 1.82.

Synthesis of thiophenylchalcone-sulfonamide derivatives (5a-e): 1 mmol of thiophenylchalcone-sulfonylchloride derivatives (4a-e) were dissolved in 20 mL of ethanol and 15 mL ethanolic solution of methylamine was added dropwise to it in ice bath. The mixture was stirred at room temperature for 2 hours. The solution was evaporated under vacuum. The solid products were washed with chloroform (15 mL), filtered and dried in vacuum oven [25].

(E)-N-methyl-5-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)thiophene-2-sulfonamide (5a): Brown powder, 90% yield. ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz) δ/ppm: 2.34 (3H, s), 2.60 (3H, s), 7.20-7.26 (3H, m), 7.30 (1H, d, *J*= 15.2 Hz), 7.42 (1H, d, *J*= 3.8 Hz), 7.73 (1H, d, *J*=15.2 Hz), 7.81 (2H, d, *J*=8.2 Hz), ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz) δ/ppm: 21.9, 29.4, 123.5, 128.8, 129.7, 130.9, 132.3, 135.1, 135.4, 142.1, 144.4, 145.6, 188.9. Anal. Calcd. for $C_{15}H_{15}NO_3S_2$: C, 56.05; H, 4.70; N, 4.36; found: C, 56.77; H, 4.42; N, 4.54.

(E)-5-(3-(4-methoxy-3-(N-methylsulfamoyl)phenyl)-3-oxoprop-1-en-1-yl)-N-methylthiophene-2-sulfonamide (5b): Brown powder, 84% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 2.41 (3H, d, *J*= 4.7 Hz), 2.53 (3H, d, *J*= 4.7 Hz), 4.00 (3H, s), 7.27 (1H, q, *J*=4.6 Hz), 7.36 (1H, d, *J*= 8.8 Hz), 7.59 (1H, d, *J*= 3.8 Hz), 7.75 (1H, d, *J*= 3.8 Hz), 7.77-7.93 (2H, m), 8.37 (1H, s), 8.49 (1H, d, *J*= 8.5Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 29.4, 29.5, 57.6, 113.5, 123.5, 127.8, 129.8, 131.1, 132.9(x2), 136.1, 136.2, 142.7, 145.3, 160.7, 186.8. Anal. Calcd. for $C_{16}H_{18}N_2O_6S_3$: C, 44.64; H, 4.21; N, 6.51; found: C, 44.22; H, 4.47; N, 6.13.

(E)-5-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-N-methylthiophene-2-sulfonamide (5c): Dark brown powder, 73% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 2.56 (3H, s), 7.61-7.70 (3H, m), 7.77-7.82 (3H, m), 7.94 (1H, d, *J*= 15.5Hz), 8.18 (2H, d, *J*= 8.2 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 29.5, 123.7, 129.6, 131.2, 131.3, 132.8, 132.9, 136.4, 139.1, 142.9, 145.2, 188.1. Anal. Calcd. for $C_{14}H_{12}ClNO_3S_2$: C, 49.19; H, 3.54; N, 4.10; found: C, 49.55; H, 3.32; N, 4.44.

(E)-5-(3-(4-hydroxy-3-(N-methylsulfamoyl)phenyl)-3-oxoprop-1-en-1-yl)-N-methylthiophene-2-sulfonamide (5d) and (E)-5-(3-(3-hydroxy-4-(N-methylsulfamoyl)phenyl)-3-oxoprop-1-en-1-yl)-N-methylthiophene-2-sulfonamide (5e) could not be purified.

2.3. Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was used for DPPH assay according to literature [26].

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) radical solution was used for ABTS assay according to the literature [27].

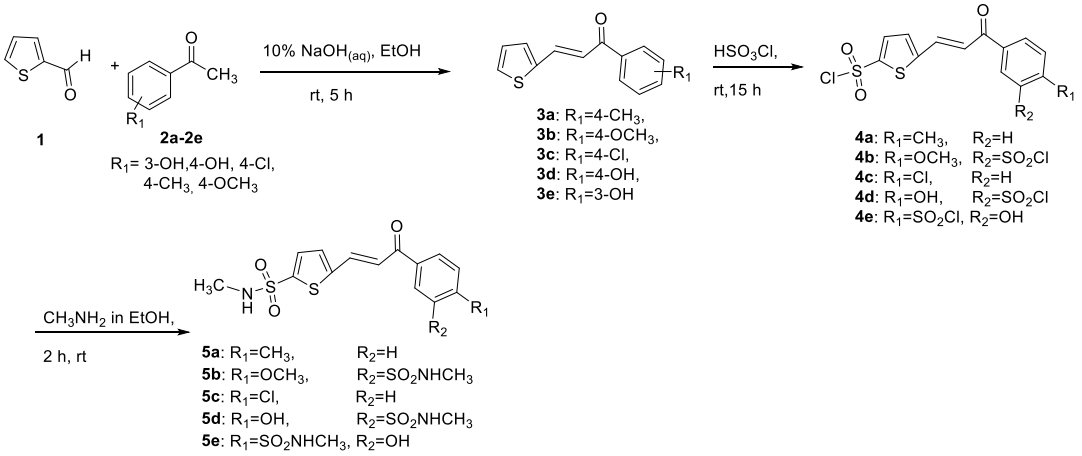
2.4. Quantum chemical calculations

The ground state calculations were performed by using the Q-CHEM 4.3 and IQmol programs [28, 29]. The molecular structures of synthesized compounds were optimized in the ground state at DFT (density functional theory) based on the TPSSTPSS/6-311G(d,p) [30, 31] level. Furthermore, the HOMO (the highest occupied molecular orbitals) and LUMO (the lowest unoccupied molecular orbitals) energies were calculated at the same level [32-34].

3. Results and Discussions

3.1. Chemistry

The synthesis route is shown in Scheme 1.



Scheme 1. Synthesis of heteroaryl chalcone derivatives

The heteroaryl-chalcones were obtained by reacting 2-thiophenealdehyde (1) with various acetophenones (2a-e) in alcoholic bases known as Claisen-Smith condensation. The thiophenyl-chalcones (3a-e) were treated with excess chlorosulfonic acid to obtain sulfonyl chloride derivatives (4a-e). In this step for compounds 3b, 3d and 3e (R_1 was methoxy or hydroxyl, which are strong electron donating groups), two sulfonyl chlorides were attached both heteroaryl ring and phenyl ring of them. These compounds were treated with small amount of chlorosulfonic acid to attach sulfonyl chloride to only heteroaryl ring; however, compounds 4b, 4d and 4e (having two sulfonyl chloride groups) were obtained. These results indicated that the reaction mechanism occurred through simultaneous sulfonation of both rings. 4a-e was reacted with the methylamine in EtOH solution at room temperature for 2h to give sulphonamide derivatives. In this step, compounds 5d and 5e were obtained as black sticky gel-like crude products in low yields and they could not be purified.

According to ^{1}H NMR data of the obtained molecules, the double bond peaks of the α, β unsaturated system observed at 7-8 ppm with *J* value of 15.5 Hz indicates that the obtained compounds are *E* isomers.

3.2. Antioxidant Activities

The DPPH and ABTS results of compounds are given in Table 1. All compounds showed the antioxidant activity. The IC_{50} values of them were between 18.32 μM and 99.15 μM for DPPH activity, while they were between 13.12 μM and 124.22 μM for ABTS activity.

Among them, 4e and 4d showed the strongest DPPH activity with the IC_{50} value of 18.32 μM and 20.86 μM, respectively, which are almost 2-fold lower than that of quercetin (IC_{50} = 8.69 μM) used as a standard. Furthermore, 4e and 4d had the highest ABTS activity with the IC_{50} value of 13.12 μM and 16.47 μM, respectively, which are almost similar with that of quercetin (IC_{50} = 15.49 μM).

From Table 1, the structure–activity relationship (SAR) can be observed as follows:

(i) Generally, most of synthesized sulfonyl chloride derivatives (4a-e) exhibited stronger both DPPH and ABTS activity than the sulphonamide derivatives (5a-c). Additionally, most of them showed similar DPPH activity with ABTS.

(ii) As expected, compounds 4d and 4e, containing hydroxyl group, have the highest antioxidant activity among the synthesized compounds. Changing the position of hydroxyl with sulfonyl chloride moiety did not significantly alter antioxidant activity (comparing 4d (R_1 = OH, R_2 = SO_2Cl; IC_{50} = 20.86 μM and 16.47 μM, in DPPH and ABTS assays, respectively) with 4e (R_1 = SO_2Cl, R_2 = OH; IC_{50} = 18.32 μM and 13.12 μM, in DPPH and ABTS assays, respectively)).

(iii) Compounds 4c and 5c, having -Cl as R_1 substituent, showed the weakest antioxidant activity in their category (thiophenyl-chalcones including sulfonyl chloride moiety or sulphonamide moiety).

(iv) Compounds 4b and 5b, having –OCH_3 as R_1 substituent, showed the second most effective antioxidant activity after those containing hydroxyl group in their category (thiophenyl-chalcones including sulphonamide or sulfonyl chloride moiety).

Table 1. The results of DPPH and ABTS assays

Comp.	X	R_1	R_2	DPPH (IC_{50}, μM)[a]	$ABTS^{\cdot+}$ (IC_{50}, μM)[a]
4a	S	CH_3	H	62.72 ± 1.04	66.18 ± 0.65
4b	S	OCH_3	SO_2Cl	47.95 ± 0.92	61.98 ± 0.66
4c	S	Cl	H	70.24 ± 1.14	79.36 ± 0.24
4d	S	OH	SO_2Cl	20.86 ± 0.70	16.47 ± 0.12
4e	S	SO_2Cl	OH	18.32 ± 0.52	13.12 ± 0.20
5a	S	CH_3	H	76.45 ± 0.74	77.26 ± 0.82
5b	S	OCH_3	SO_2NHCH_3	64.13 ± 0.85	78.98 ± 0.65
5c	S	Cl	H	99.15 ± 1.18	124.22 ± 1.10
Quercetin	-	-	-	8.69 ± 0.04	15.49 ± 2.33

[a] IC_{50} values are given according to three parallel measurement results.

Many synthetic and natural antioxidants have been reported in recent years [26, 35, 36]. Most of synthesized chalcones, in this study, exhibited stronger DPPH activity than bis-carbohydrazones (IC_{50} values of them ranging from 51.82 μM to not active) [35] and isatin derivatives (IC_{50} = 64.03-204.90 μM) [26], whereas they showed lower ABTS activity than bis-thiocarbohydrazones (IC_{50} = 2.69-5.32 μM) [35] and isatin derivatives (IC_{50} = 0.39-6.83 μM) [26]. On the other hand, some synthesized chalcones, especially 4d and 4e, have higher ABTS activity than some reported natural extracts (IC_{50} = 40.22-106.01 μM) [36] .

It is well-known that the DPPH and ABTS mechanisms involve an electron transfer process [37-39]. The proposed antioxidant mechanism of compound 4e, the best antioxidant agent in this study, is given in Figure 1. As can be seen in Figure 1, it is considered that 4e can oxidize to quinone product, known as one of the anticancer agents. Quinones act antitumor activity via reversible enzymatic oxidation and reduction [40]. Therefore, the conversion of compound 4e to quinone may provide a distinct advantage in terms of its use in the treatment of some diseases.

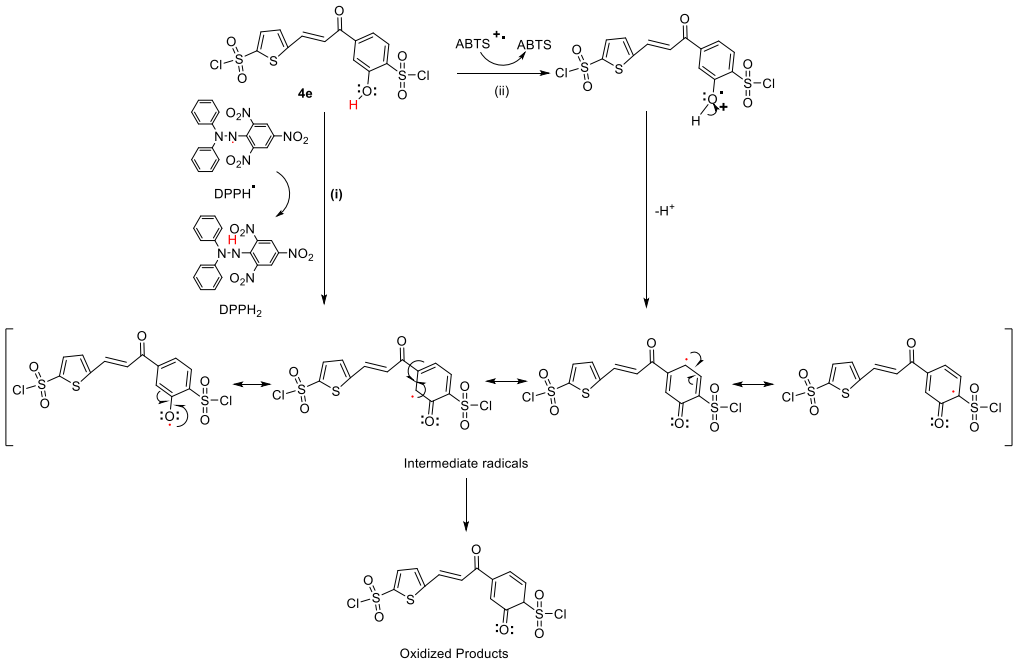


Figure 1. The estimated mechanism of compounds 4e for antioxidant assays; (i) DPPH, (ii) ABTS

3.3. Frontier Molecular Orbital (FMO) energies and Molecular Parameters

The frontier molecular orbital (FMO) energies and molecular parameters (electron affinity, chemical hardness and softness, electronegativity, electrophilicity index, nucleophilicity index, and dipole moment) were calculated to explain and support the antioxidant activity and SAR results. The FMO energies and molecular parameters of the obtained compounds are shown in Table 2.

The chemical reactivity and stability of the molecules are related with the frontier molecular orbitals (FMOs). It is known that electron affinity and also reactivity of the compounds are associated with HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energies. HOMO and LUMO energies are related to susceptibility to electrophilic and nucleophilic attack, respectively [41].

Table 2. The frontier molecular orbital (FMO) energies and molecular parameters of the synthesized compounds

Comp.	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	A (eV)	χ (eV)	ω (eV)	φ $(eV)^{-1}$	μ (D)
4a	-6.1498	-4.2450	1.9048	4.2450	5.1974	14.1815	0.0705	5.4109
4b	-6.6396	-4.4082	2.2314	4.4082	5.5239	13.6746	0.0731	8.2363
4c	-6.3675	-4.3538	2.0137	4.3538	5.3607	14.2701	0.0701	3.7551
4d	-6.6940	-4.4443	2.2497	4.4443	5.5692	14.4776	0.0690	5.0413
4e	-6.7212	-4.4443	2.2769	4.4443	5.5828	14.4844	0.0690	5.0387
5a	-5.8232	-3.4014	2.4218	3.4014	4.6123	8.7841	0.1138	2.5675
5b	-5.9865	-3.5647	2.4218	3.5647	4.7756	9.4171	0.1062	6.0485
5c	-6.0409	-3.5919	2.4490	3.5919	4.8164	9.4723	0.1056	2.8845

A: Electron affinity, χ: Electronegativity, ω: Electrophilicity Index, φ : Nucleophilicity Index, μ: Dipole Moment.

As can be seen in Table 2, generally, chalcones binding sulfonyl chloride moiety (4a-4e) have lower E_{HOMO} (between -6.1498 eV and -6.7212 eV), E_{LUMO} (between -4.2450 eV and -4.4443 eV) and higher electron affinity (between 4.2450 eV and 4.4443 eV), electronegativity (between 5.1974 eV and 5.5828 eV) and electrophilicity index (between 13.6746 eV and 14.4844 eV) than chalcones binding sulphonamide moiety (5a-5c; E_{HOMO} ranging from -5.8232 eV to -6.0409 eV, E_{LUMO} ranging from -3.4014 eV to -3.5919 eV, electron affinity ranging from 3.4014 eV to 3.5919 eV, electronegativity ranging from 4.6123 eV to 4.8164 eV, and electrophilicity index ranging from 8.7841 eV to 9.4723 eV).

In addition, compounds 4d and 4e, which are the strongest antioxidant agents in this study, have the lowest LUMO energies (E_{LUMO} = -4.4443 eV). It has been reported that the decreasing LUMO energy rises the acceptor features of compounds [41], so they may pick up electrons more easily.

Furthermore, 4d and 4e have the highest electronegativity (χ = 5.5692 eV and 5.5828 eV, respectively), electron affinity (A= 4.4443 eV) and electrophilicity index (ω = 14.4776 eV and 14.4844 eV, respectively). It is considered that the high electron affinity, electrophilicity index and electronegativity rise ability to gain and hold electrons of the molecules and thus their antioxidant property increase. Additionally, the radicals formed as a result of electron transfer gain stability thanks to high electronegativity of the molecules.

It can be said that all these results support to antioxidant activity results and structure-activity relationships.

4. Conclusion

In conclusion, eight thiophenyl-chalcones derivatives were synthesized as antioxidant agents. All of them showed good antioxidant activity. Compound 4e and 4d exhibited the highest antioxidant activity with the IC_{50} values of 18.32 μM and 20.86 μM for DPPH and 13.12 μM and 16.47 μM for ABTS activity, respectively. As expected, among the synthesized compounds, 4d and 4e, including hydroxyl group, exhibited the strongest antioxidant activity. These results indicated that the hydroxyl groups attached the phenyl ring can act a critical role for antioxidant agents.

Moreover, the calculated frontier molecular orbital (FMO) energies and molecular parameters support to antioxidant activity results. Low LUMO energies and high electronegativity, electron affinity and electrophilicity index can also increase the antioxidant features of molecules.

Acknowledgements

Thanks to Prof. Dr. Davut AVCI, from Sakarya University, for quantum chemical calculations. This study was produced from the MSc thesis entitled "Tiyofenilşalkon Türevlerinin Sentezi" by Sakarya University, which was accepted in 2019.

References

[1] Mccord, J. M., Human disease, free radicals, and the oxidant/antioxidant balance, **Clinical Biochemistry**, 26, 351-357, (1993).

[2] Dai, Y., Shao, C., Piao, Y., Hu, H., Lu, K., Zhang, T., Zhang, X., Jia, S., Wang, M. and Man, S., The mechanism for cleavage of three typical glucosidic bonds induced by hydroxyl free radical, **Carbohydrate Polymers**, 178, 34-40, (2017).

[3] Oberley, L. W., Free radicals and diabetes, **Free Radical Biology and Medicine**, 5, 113-124, (1988).

[4] Fang, Y. Z., Sheng, Y. and Guoyao, W., Free radicals, antioxidants, and nutrition, **Nutrition**, 18, 872-879, (2002).

[5] Moskovitz, J., Moon, B. Y. and Chock, P. B., Free radicals and disease, **Archives of Biochemistry and Biophysics**, 397, 354-359, (2002).

[6] Jensen, S. J. K., Oxidative stress and free radicals, **Journal of Molecular Structure: THEOCHEM**, 666-667, 387-392, (2003).

[7] Hayes, J. D., Dinkova-Kostova, A.T. and Tew, K. D., Oxidative stress in cancer, **Cancer Cells**, 38, 167-197, (2020).

[8] Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G. and Serban, A. I., Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status, **European Journal of Medicinal Chemistry**, 209, 112891, (2021).

[9] Sies, H. and Jones, D. P., Reactive oxygen species (ROS) as pleiotropic physiological signalling agents, **Nature Reviews Molecular Cell Biology**, 21, 363-383, (2020).

[10] Zhang, N., Hu, P., Wang, Y., Tang, Q., Zheng, Q., Wang, Z. and He, Y., A reactive oxygen species (ROS) activated hydrogen sulfide (H_2S) donor with self-reporting fluorescence, **ACS Sensors**, 5, 319-326, (2020).

[11] Irazabal, M. V. and Torres, V. E., Reactive oxygen species and redox signaling in chronic kidney disease, **Cells**, 9, 1342, (2020).

[12] Kirtonia, A., Sethi, G. and Garg, M., The multifaceted role of reactive oxygen species in tumorigenesis, **Cellular and Molecular Life Sciences**,77, 4459–4483, (2020).

[13] Kurt, B. Z., Gazioglu, I., Kandas, N. O. and Sonmez, F., Synthesis, anticholinesterase, antioxidant, and anti-aflatoxigenic activity of novel coumarin carbamate derivatives, **ChemistrySelect**, 3, 3978–3983, (2018).

[14] Kahriman, N., Yeni 3,5-disübstitüe-2-pirazolin türevlerinin sentezi ve biyolojik aktivitelerinin incelenmesi, **Journal of Balıkesir University Institute of Science and Technology**, 22, 1, 34-47, (2020).

[15] Sahu, N., Balbhadra, S., Choudhary, J. and Kohli, D., Exploring pharmacological significance of chalcone scaffold: a review, **Current Medicinal Chemistry**,19, 209-225, (2012).

[16] Gaonkar, S. L. and Vignesh, U. N., Synthesis and pharmacological properties of chalcones: a review, **Research on Chemical Intermediates**, 43, 6043-6077, (2017).

[17] Singh, P., Anand, A. and Kumar, V., Recent developments in biological activities of chalcones: A mini review, **European Journal of Medicinal Chemistry**, 85, 758-777, (2014).

[18] Sonmez, F., Sevmezler, S., Atahan, A., Ceylan, M., Demir, D., Gencer, N., Arslan, O. and Kucukislamoglu, M., Evaluation of new chalcone derivatives as

polyphenol oxidase inhibitors, **Bioorganic and Medicinal Chemistry Letters**, 21, 7479–7482, (2011).

[19] Dan, W. and Dai, J., Recent developments of chalcones as potential antibacterial agents in medicinal chemistry, **European Journal of Medicinal Chemistry**, 187, 111980, (2020).

[20] Rani, A., Anand, A., Kumar, K. and Kumar, V., Recent developments in biological aspects of chalcones: the odyssey continues, **Expert Opinion Drug Discovery**, 14, 249-288, (2019).

[21] Dandawate, P., Ahmed, K., Padhye, S., Ahmad, A. and Biersack, B., Anticancer active heterocyclic chalcones: recent developments, **Anti-Cancer Agents in Medicinal Chemistry**, 21, 558-566, (2021).

[22] Basappa V. C., Ramaiah, S., Penubolu, S. and Kariyappa, A. K., Recent developments on the synthetic and biological applications of chalcones-A review, **Biointerface Research in Applied Chemistry**, 12, 180-195, (2021).

[23] Salotra, R. and Utreja, D., A comprehensive appraisal of chalcones and their heterocyclic analogs as antimicrobial agents, **Current Organic Chemistry**, 24, 2755-2781, (2020).

[24] Kesari, C., Rama, K. R., Sedighi, K., Stenvang, J., Björkling, F., Kankala, S. and Thota, N., Synthesis of thiazole linked chalcones and their pyrimidine analogues as anticancer agents, **Synthetic Communications**, 51, 1406-1416, (2021).

[25] Gür, T., Tiyofenilşalkon Türevlerinin Sentezi, Yüksek Lisans Tezi, Sakarya Üniversitesi, Fen Bilimleri Enstitüsü, Sakarya, (2019).

[26] Sonmez, F., Gunesli, Z., Kurt, B. Z., Gazioglu, I., Avci, D. and Kucukislamoglu, M., **Molecular Diversity**, 23, 829–844, (2019).

[27] Yakan, H., Cavus, M. S., Kurt, B. Z., Muglu, H., Sonmez, F. and Güzel, E., **Journal of Molecular Structure**, 1239, 130495, (2021).

[28] Shao, Y., Gan, Z., Epifanovsky, E., Gilbert, A.T.B., Wormit, M., Kussmann, J., Lange, A. W., Behn, A., Deng, J., Feng, X., Ghosh, D., Goldey, M., Horn, P.R., Jacobson, L. D., Kaliman, I., Khaliullin, R.Z., Kús, T., Landau, A., Liu, J., Proynov, E.I., Rhee, Y. M., Richard, R.M., Rohrdanz, M.A., Steele, R.P., Sundstrom, E.J., Woodcock, H. L., Zimmerman, P.M., Zuev, D., Albrecht, B., Alguire, E., Austin, B., and Chen, Y., **Q-Chem 4.3**, Pleasanton, CA, (2015).

[29] Shao, Y. H., Gan, Z. T., Epifanovsky, E., Gilbert, A. T. B., Wormit, M., Kussmann, J., Lange, A. W., Behn, A., Deng, J. and Feng, X. T., Advances in molecular quantum chemistry contained in the Q-Chem 4 program package, **Molecular Physics**, 113, 184–215, (2015).

[30] Tao, J., Perdew, J. P., Staroverov, V. N. and Scuseria, G. E., Climbing the density functional ladder: Nonempirical meta–generalized gradient approximation designed for molecules and solids, **Physical Review Letters**, 91, 146401, (2003).

[31] Liu, F., Proynov, E., Yu, J. G., Furlani, T. R. and Kong, J., Comparison of the performance of exact-exchange-based density functional methods, **Journal of Chemical Physics**, 137, 114104, (2012).

[32] Chattaraj, P. K. and Roy, D.R., Update 1 of: Electrophilicity index, **Chemical Reviews**, 107, 46-74, (2007).

[33] Eryılmaz, S., Gül, M. and Inkaya, E., Synthesis, spectral characterization, t heoretical analysis and antioxidant activities of aldol derivative isophorone structures, **Journal of Balıkesir University Institute of Science and Technology**, 19, 3, 89-104, (2017).

[34] Dege, N., Ozge, O., Avci, D., Basoglu, A., Sonmez, F., Yaman, M., Tamer, O., Atalay, Y. and Kurt, B. Z., Concentration effects on optical properties, DFT,

crystal characterization and α-glucosidase activity studies: Novel Zn(II) complex, **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**, 262, 120072, (2021).

[35] Muğlu, H., Kurt, B. Z., Sönmez, F., Güzel, E., Çavuş, M. S. and Yakan, H., Preparation, antioxidant activity, and theoretical studies on the relationship between antioxidant and electronic properties of bis(thio/carbohydrazone) derivatives, **Journal of Physics and Chemistry of Solids**, 164, 110618, (2022).

[36] Gazioglu, I., Kurt, B. Z., Sevgi, E. and Sonmez, F., Anticholinesterase, antioxidant, antiaflatoxigenic activities of ten edible wild plants from Ordu area, Turkey, **Iranian Journal of Pharmaceutical Research**, 17, 1047-1056, (2018).

[37] Belkheiri, N., Bouguerne, B., Bedos-Belval, F., Duran, H., Bernis, C., Salvayre, R., Negre-Salvayre, A. and Baltas, M., Synthesis and antioxidant activity evaluation of a syringic hydrazones family, **European Journal of Medicinal Chemistry**, 45, 3019-3026, (2010).

[38] Campos, A. M. and Lissi, E. A., Kinetics of the reaction between 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) derived radical cations and phenols, **International Journal of Chemical Kinetics**, 29, 219-224, (1997).

[39] Herraiz, T. and Galisteo, J., Endogenous and dietary indoles: A class of antioxidants and radical scavengers in the ABTS assay, **Free Radical Research**, 38, 323-331, (2004).

[40] Powis, G., Free radical formation by antitumor quinones, **Advances in Free Radical Biology and Medicine**, 6, 63-101, (1989).

[41] Soares, M. A., Lessa, J. A., Mendes, I. C., Da Silva, J. G., dos Santos, R. G., Salum, L. B., Daghestani, H., Andricopulo, A. D., Day, B. W., Vogt, A., Pesquero, J. L., Rocha, W. R. and Beraldo, H., N^4-Phenyl-substituted 2-acetylpyridine thiosemicarbazones: Cytotoxicity against human tumor cells, structure–activity relationship studies and investigation on the mechanism of action, **Bioorganic and Medicinal Chemistry**, 20, 3396-3409, (2012).