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Thieny/Furanyl-hydroxyphenylpropenones as Inhibitors of LPS-induced ROS and NO Production in RAW 264.7 Macrophages, and Their Structure-Activity Relationship Study

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Twelve thienyl/furanyl-hydroxyphenylpropenones were systematically designed and synthesized, and evaluated for their inhibitory effect on LPS-induced ROS and NO production in RAW 264.7 macrophages. Compound **11** displayed the most significant inhibitory activity of LPS-induced ROS and NO production in RAW 264.7 macrophages. Structure-activity relationship study indicated that *para*-hydroxyphenyl moiety plays an important role for inhibitory activities on both LPS-induced ROS and NO production as well as 3-thienyl moiety on molecule.

Key Words : Thieny/Furanyl-hydroxyphenylpropenones, LPS-induced ROS and NO, Inhibition, Structure-activity relationship study

Introduction

Reactive oxygen species (ROS), the molecules containing highly reactive oxygen, actively interact with various intracellular molecules. Excessive ROS production results in alterations of diverse intracellular signaling pathways and causes significant damages to the target cells, collectively known as oxidative stress. Abnormally high production of ROS contributes to the development of diverse pathological events, such as DNA mutation, carcinogenesis, aging, cell death and inflammation,[1] although it is required for normal physiological processes as well. Macrophages have been considered as one of the main cellular sources responsible for ROS production in various pathophysiological conditions. Excessive ROS production by macrophages leads to the damage in various target tissues. Therefore, development of the pharmacological agent interferes with excessive ROS production could be considered as a useful strategy for the treatment of various pathological conditions.

Inflammation is a complex biological response to the harmful stimuli and associated with many pathophysiological conditions. In response to inflammatory stimuli, macrophages produce and secret various pro-inflammatory molecules, including the short-lived free radical nitric oxide (NO).[2] During inflammation, the NO level is rapidly increased and plays a role as a defense system against different types of various pathogens.[3] In fact, appropriate levels of NO plays an important role in regulating a number of physiological functions, such as blood vessel relaxation mainly modulated by endothelial nitric oxide synthase (eNOS), neurotransmission modulated by neuronal nitric oxide synthase (nNOS) and immune response. However, in many cases, abnormal excess production of NO is produced by inducible nitric oxide synthase (iNOS) during inflammation and acts as a toxic radical that contributes to the development of damage in target tissue.[4] Therefore, regulation of NO production could be a promising strategy for the treatment of diseases associated with inflammation.

Chalcones (1,3-diphenyl propenone) known as precursors of flavonoids and isoflavonoids, are versatile in nature.[5] Many structural modifications of the chalcone moiety by introducing different substituents in the phenyl rings or replacing the phenyl rings with heterocyclic rings exhibited a wide range of pharmacological activities[5,6] such as antibacterial,[7,8] antifungal,[9] antimalarial,[10] antileishmanial,[11] antioxidant,[12,13] anticancer activity,[14-17] antiangiogenesis,[18] and anti-inflammatory[19] activities as shown in (Figure 1). In our previous study it has been reported that isosteres of chalcone substituted by aromatic (heteroaromatic) rings of two phenyl moiety of chalcone exhibited considerable COX/5-LOX inhibitory activities for the development of safer anti-inflammatory agents.[20] In addition, chalcone and 1,3-diarylpropenones (Figure 2) were previously synthesized in our research group and reported to have anti-angiogenic activity.[21-23]

Many natural and synthetic derivatives bearing the hydroxyl group or phenol have exhibited a wide spectrum of biological activities with potential for application as pharmaceutical drugs. Numerous polyphenolic phytochemicals such as resveratrol, curcumin, flavonoids, epigallocatechin gallate, and chalcones have been well studied and reported to interfere with specific stages of the carcinogenic process.[24-27] In our previous results, we observed that introduction of hydroxyl group on phenyl moiety increased bio-

[a]These authors contributed equally to this work.

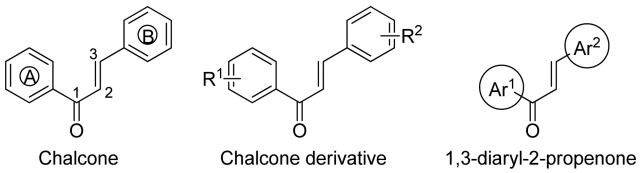


Figure 1. Structures of chalcone, chalcone derivative and 1,3-diaryl-2-propenone.

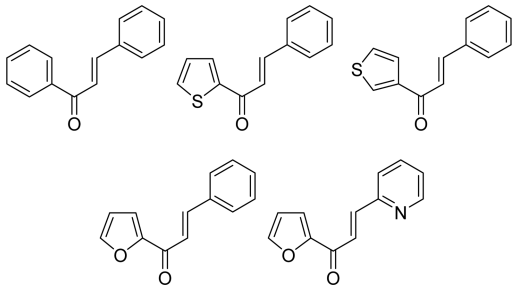


Figure 2. Structures of chalcone and 1,3-diaryl-2-propenones.[21-23]

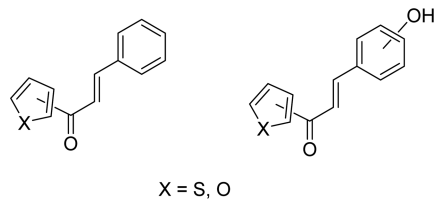
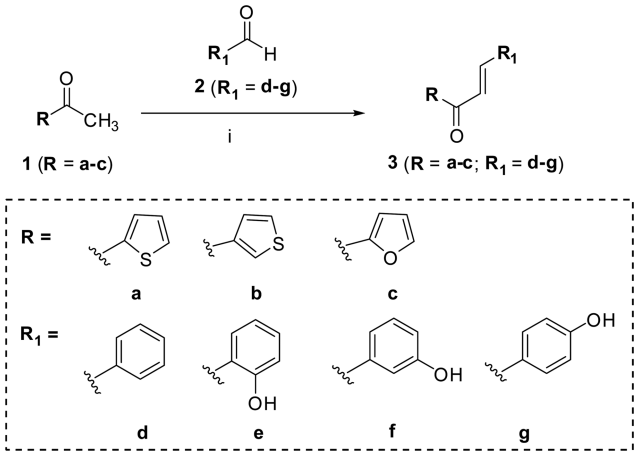


Figure 3. Design of Thienyl/furanyl-phenylpropenones and thienyl/furanyl-hydroxyphenylpropenones.

logical activities.[28-30]

In this study, based on the thienyl/furanyl-phenylpropenones we incorporated the hydroxyl group on phenyl moiety to investigate the effect on LPS-induced ROS and NO production in RAW 264.7 macrophages inhibitory activities, and performed structure-activity relationship study. We have systematically designed and synthesized twelve thienyl/furanyl-phenylpropenone derivatives containing hydroxyl groups at different positions (none, ortho, meta, or para) of the phenyl ring (Scheme 1 and Figure 3).



Scheme 1. General synthetic scheme of thienyl/furanyl-phenylpropenones and thienyl/furanyl-hydroxyphenylpropenones. Reagents and conditions: (i) aryl aldehyde **2** (**R**$_1$ = **d-g**) (1.0 eq.), KOH (1.2 eq.), MeOH/H_2O (5:1), 10 min to 3 h, 0 °C, 29.4-76.3%.

Experimental

Material and Methods. Compounds used as starting materials and reagents were obtained from Aldrich Chemical Co., Junsei or other chemical companies, and utilized without further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Burdick and Jackson, USA. Thin-layer chromatography (TLC) and column chromatography (CC) were performed with Kieselgel 60 F_{254} (Merck) and silica gel (Kieselgel 60, 230-400 mesh, Merck) respectively. Since all the compounds prepared contain aromatic ring, they were visualized and detected on TLC plates with UV light (short wave, long wave or both). NMR spectra were recorded on a Bruker AMX 250 (250 MHz, FT) for ^{1}H NMR and 62.5 MHz for ^{13}C NMR, and chemical shifts were calibrated according to TMS. Chemical shifts (δ) were recorded in ppm and coupling constants (*J*) in hertz (Hz). Melting points were determined in open capillary tubes on electrothermal 1A 9100 digital melting point apparatus and were uncorrected.

ESI LC/MS analyses were performed with a Finnigan LCQ Advantage® LC/MS/MS spectrometry utilizing Xcalibur® program. For ESI LC/MS, LC was performed with 10 μL injection volume on a Waters × Terra® 3.5 μm reverse-phase C_{18} column (2.1 × 100 mm) with a gradient elution: from 10% to 95% of B in A for 10 min followed by 95% to 10% of B in A for 10 min at a flow rate of 200 μL, where mobile phase A was 100% distilled water with 20 mM AF and mobile phase B was 100% ACN. MS ionization conditions were: Sheath gas flow rate: 70 arb, aux gas flow rate: 20 arb, I spray voltage: 4.5 KV, capillary temp.: 215 °C, capillary voltage: 21V, tube lens offset: 10 V. Retention time is given in minutes.

General Method for Preparation of 3. To an ice cold solution of 85% KOH (1.2 eq.) in methanol (10 mL) and H_2O (2 mL) aryl acetyl **1** (1.0 eq.) was added. After dissolution, aryl aldehyde **2** (1.0 eq.) was added dropwise. The reaction mixture was then stirred for 10 min to 3 h at 0 °C. The mixture was neutralized with 4 M aqueous HCl solution (pH adjusted to 2). The mixture was extracted with ethyl acetate (100 mL), washed with water (75 mL × 2) and saturated NaCl solution (50 mL). The organic layer was dried with magnesium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was purified by silica gel column chromatography with a gradient elution of ethyl acetate/*n*-hexane to afford a solid compound **3** in 29.4 to 76.3% yield. Following the same procedure, twelve compounds were synthesized.

3-Phenyl-1-(thiophen-2-yl)prop-2-en-1-one (4): White solid (76.3%), R_f (EtOAc/*n*-Hexane = 1:5, v/v): 0.33, mp 80.5-81.1 °C, ESI LC/MS: Retention time: 17.09 min, $[MH]^+$: 215.1. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 4.1, 0.9 Hz, 1H, 1-thiophene H-5), 7.81 (d, *J* = 15.2 Hz, 1H, -CO-

C=CH-), 7.68 (d, J = 5.1 Hz, 1H, 1-thiophene H-3), 7.65-7.61 (m, 2H, 3-phenyl H-2, H-6), 7.44 (d, J = 15.5 Hz, 1H, -CO-CH=C-), 7.42-7.39 (m, 3H, 3-phenyl H-4, H-3, H-5), 7.17 (t, J = 4.6 Hz, 1H, 1-thiophene H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 182.04, 145.50, 144.08, 134.68, 133.89, 131.80, 130.59, 128.95, 128.47, 128.23, 121.60.

3-(2-Hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (5): Brown solid (29.4%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.37, mp 165.4-167.8 °C, ESI LC/MS: Retention time: 15.11 min, $[MH]^+$: 231.1. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.32 (s, 1H, 3-phenyl 2-OH), 8.23 (d, J = 3.4 Hz, 1H, -CO-C=CH-), 8.06-8.01 (m, 2H, 1-thiophene H-5, H-3), 7.87 (d, J = 6.7 Hz, 1H, 3-phenyl H-6), 7.78 (d, J = 15.7 Hz, 1H, -CO-CH=C-), 7.31-7.23 (m, 2H, 1-thiophene H-4, 3-phenyl H-4), 6.94-6.84 (m, 2H, 3-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 182.04, 157.50, 145.96, 138.72, 135.48, 133.41, 132.44, 129.18, 128.76, 121.40, 120.77, 119.65, 116.48.

3-(3-Hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (6): Light yellow solid (62.9%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.45, mp 163.6-165.3 °C, ESI LC/MS: Retention time: 17.12 min, $[MH]^+$: 231.1. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.88 (br, 1H, 1-thiophene H-5) 7.78 (s, 1H, 3-phenyl H-2), 7.69 (dd, J = 4.9, 1.1 Hz, 1H, 1-thiophene H-3), 7.40 (d, J = 15.7 Hz, 1H, -CO-C=CH-), 7.33-7.17 (m, 4H, 1-thiophene H-4, -CO-CH=C-, 3-phenyl H-4, H-6), 6.92 (br, 1H, 3-phenyl H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 182.32, 156.26, 145.30, 144.13, 136.16, 134.23, 132.12, 130.17, 128.34, 121.85, 120.99, 117.91, 115.13.

3-(4-Hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (7): Light yellow solid (48.4%), R_f (EtOAc/*n*-Hexane = 1:1 v/v): 0.51, mp 204.9-206.1 °C, ESI LC/MS: Retention time: 15.19 min, $[MH]^+$: 231.1. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.05 (s, 1H, 3-phenyl 4-OH), 8.24 (d, J = 3.7 Hz, 1H, 1-thiophene H-5), 7.89 (d, J = 4.9 Hz, 1H, 1-thiophene H-3), 7.71 (d, J = 8.5 Hz, 2 H, 3-phenyl H-2, H-6), 7.64-7.58 (m, 2 H, -CO-C=CH-, -CO-CH=C-), 7.28 (t, J = 4.2 Hz, 1H, 1-thiophene H-4), 6.83(d, J = 8.5 Hz, 2H, 3-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 181.46, 160.12, 145.81, 143.52, 134.80, 132.84, 130.94, 128.70, 125.56, 118.33, 115.77.

3-Phenyl-1-(thiophen-3-yl)prop-2-en-1-one (8): White solid (58.4%), R_f (EtOAc/*n*-Hexane = 1:5, v/v): 0.37, mp 103.2-104.5 °C, ESI LC/MS: Retention time: 16.98 min, $[MH]^+$: 215.1. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.16 (dd, J = 2.7, 1.1 Hz, 1H, 1-thiophene H-2), 7.83 (d, J = 15.7 Hz, 1H, -CO-C=CH-), 7.67 (dd, J = 5.2, 1.0 Hz, 1H, 1-thiophene H-5), 7.63-7.60 (m, 2H, 3-phenyl H-2, H-6), 7.42-7.39 (m, 3H, 3-phenyl H-4, H-3, H-5), 7.41 (d, J = 15.7 Hz, 1H, -CO-CH=C-), 7.37 (dd, J = 4.3, 3.0 Hz, 1H, 1-thiophene H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 183.96, 144.12, 143.04, 134.76, 132.08, 130.51, 128.94, 128.41, 127.43, 126.51, 122.65.

3-(2-Hydroxyphenyl)-1-(thiophen-3-yl)prop-2-en-1-one (9): Dark green solid (34.9%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.34, mp 166.7-168.4 °C, ESI LC/MS: Retention time: 16.78 min, $[MH]^+$: 231.1. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.29 (s, 1H, 3-phenyl 2-OH), 8.73 (br, 1H, 1-thiophene H-2), 7.99 (d, J = 15.7 Hz, 1H, -CO-C=CH-), 7.85 (dd, J = 6.5, 1.2 Hz, 1H, 3-phenyl H-6), 7.75 (d, J = 15.7 Hz, 1H, -CO-CH=C-), 7.68-7.61 (m, 2H, 1-thiophene H-5, 3-phenyl H-4), 7.26 (td, J = 8.37, 1.4 Hz, 1H, 1-thiophene H-4), 6.93-6.83 (m, 2H, 3-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 183.52, 157.40, 143.32, 138.53, 134.09, 132.30, 128.53, 127.90, 127.38, 128.97, 121.57, 119.64, 116.48.

3-(3-Hydroxyphenyl)-1-(thiophen-3-yl)prop-2-en-1-one (10): Light yellow solid (61.7%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.47, mp 188.1-189.3 °C, ESI LC/MS: Retention time: 13.68 min, $[MH]^+$: 231.1. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.17 (br, 1H, 1-thiophene H-2), 7.78 (d, J = 15.7 Hz, 1H, -CO-C=CH-), 7.67 (d, J = 5.1 Hz, 1H, 1-thiophene H-5), 7.41-7.20 (m, 4H, 1-thiophene H-4, -CO-CH=C-, 3-phenyl H-5, H-6), 7.13 (s, 1H, 3-phenyl H-2), 6.91 (d, J = 7.6 Hz, 1H, 3-phenyl H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 183.22, 157.93, 143.48, 143.07, 136.13, 134.51, 130.06, 127.87, 127.30, 123.13, 120.04, 117.93, 115.44.

3-(4-Hydroxyphenyl)-1-(thiophen-3-yl)prop-2-en-1-one (11): Light yellow solid (31.8%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.54, mp 175.9-176.6 °C, ESI LC/MS: Retention time: 14.58 min, $[MH]^+$: 231.1. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.3 (s, 1H, 3-phenyl 4-OH), 8.71 (br, 1H, 1-thiophene H-2), 7.69 (d, J = 8.5 Hz, 2H, 3-phenyl H-2, H-6), 7.64-7.57 (m, 4H, 1-thiophene H-4, H-5, -CO-C=CH-, -CO-CH=C-), 6.84 (d, J = 8.56 Hz, 2H, 3-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 182.92, 159.98, 143.35, 143.13, 133.37, 130.79, 127.74, 127.06, 125.71, 190.63, 115.71.

1-(Furan-2-yl)-3-phenylprop-2-en-1-one (12): White solid (62.9%), R_f (EtOAc/*n*-Hexane = 1:2, v/v): 0.43, mp 84.3-86.1 °C, ESI LC/MS: Retention time: 15.40 min, $[MH]^+$: 199.1. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.89 (d, J = 15.8 Hz, 1H, -CO-C=CH-), 7.65-7.61 (m, 3H, 1-furan H-5, 3-phenyl H-2, H-6), 7.46 (d, J = 15.8 Hz, 1H, -CO-CH=C-), 7.40-7.38 (m, 3H, 3-phenyl H-4, H-3, H-5), 7.32 (d, J = 3.5 Hz, 1H, 1-furan H-3), 6.58 (dd, J = 3.4, 1.6 Hz, 1H, 1-furan H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 178.01, 153.70, 146.50, 143.99, 134.73, 130.59, 128.93, 128.52, 121.15, 117.48, 112.53.

1-(Furan-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (13): Light brown solid (53.3%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.38, mp 169.2-169.9 °C, ESI LC/MS: Retention time: 14.30 min, $[MH]^+$: 215.6. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.29 (s, 1H, 3-phenyl 2-OH), 8.04-7.99 (m, 2H, 1-furan H-5, -CO-C=CH-), 7.80 (d, J = 7.7 Hz, 1H, 3-phenyl H-6), 7.70-7.61 (m, 2H, 1-furan H-3, -CO-CH=C-), 7.26 (td, J = 8.6, 1.5 Hz, 1H, 3-phenyl H-4), 6.94-6.68 (m, 2H, 3-phenyl H-3, H-5), 6.76 (dd, J = 3.5, 1.6 Hz, 1H, 1-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 177.26, 157.46, 153.34, 148.27, 138.46, 132.30, 128.82, 121.32, 121.87, 119.60, 119.02, 116.46, 112.92.

1-(Furan-2-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one (14): Light yellow solid (58.9%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.33, mp 162.3-163.4 °C, ESI LC/MS: Retention time: 15.33 min, $[MH]^+$: 215.6. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.64 (s, 1H, 3-phenyl 3-OH), 8.06 (br, 1H, 1-furan H-5), 7.81 (d, J = 3.6 Hz, 1H, 1-furan H-3), 7.62 (br, 2H, -CO-C=CH-, -CO-CH=C-), 7.28-7.19 (m, 3H, 3-phenyl H-6, H-5, H-2),

6.86 (br, 1H, 3-phenyl H-4), 6.77 (dd, J = 3.6, 1.6 Hz, 1H, 1-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 176.84, 157.94, 153.11, 148.59, 143.26, 135.89, 130.11, 121.92, 120.03, 119.74, 118.05, 115.34, 112.96.

1-(Furan-2-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (15): Light yellow solid (69.8%), R_f (EtOAc/*n*-Hexane = 1:2 v/v): 0.23, mp 180.1-181.3 °C, ESI LC/MS: Retention time: 16.35 min, $[MH]^+$: 215.6. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.06 (s, 1H, 3-phenyl 4-OH), 8.01 (br, 1H, 1-furan H-5), 7.70-7.68 (m, 2H, 1-furan H-3, -CO-C=CH-), 7.66 (d, J = 7.6 Hz, 2H, 3-phenyl H-2, H-6), 7.49 (br, 1H, -CO-CH=C-), 6.82 (d, J = 7.9 Hz, 2H, 3-phenyl H-3, H-5), 6.74 (m, 1H, 1-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 176.91, 160.31, 153.31, 148.01, 143.36, 131.01, 125.68, 118.73, 118.54, 116.00, 112.73.

Cell Culture. The RAW 264.7 macrophage cell line was purchased from the Korean cell line bank (Seoul, Korea) and routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin at 37 °C in an incubator with a humidified atmosphere of 5% CO_2.

Measurement of Cell Viability. Cell viability was assessed using the CellTiter 96 Aqueous One kit (Promega, Madison, WI, USA). Briefly, RAW 264.7 macrophages were seeded in 96-well plates at 5 × 10^4 cells/well. After overnight incubation, cells were treated with different concentrations of compounds. Then, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2*H*-tetrazolium (MTS) solution was added and the cells were incubated for 2 h at 37 °C. Cell viability was monitored *via* a SPECTROstar Nano microplate reader (BMG Labtech Inc., Ortenberg, Germany) by measuring absorbance at 490 nm.

Measurement of ROS Production. Intracellular ROS production was determined by measuring the changes in the fluorescence of 5-chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) essentially as described previously.[31] Briefly, cells were initially seeded in 4-well culture slides at a density of 2 × 10^5 cells/ well. After overnight culture, cells were pretreated with the compounds for 1 h and further incubated with LPS for 24 h. ROS production was measured using the FLUOstar OPTIMA fluorometer and analyzed by FLUOstar OPTIMA software from BMG. Excitation and emission wavelengths were set to 485 nm and 520 nm, respectively.

Measurement of Total Nitric Oxide Production. Nitric oxide production was assessed spectrophotometrically as a formed nitrite (NO_2). In brief, RAW 264.7 macrophages were seeded at a density of 5 × 10^4 cells/well in 96-well plates. Cells were pretreated with indicated concentration of compounds for 1 h followed by treatment with 100 ng/mL LPS for additional 24 h. The 50 μL of culture medium was reacted with 50 μL of sulfanilamide and 50 μL of *N*-1-napthylethylenediamine dihydrochloride (NED). Then, the absorbance was measured at 540 nm using a SPECTROstar Nano microplate reader (BMG Labtech Inc., Ortenberg, Germany).

Results and Discussion

Synthetic Chemistry. Thienyl/furanyl-hydroxyphenylpropenones were synthesized by earlier reported methodology.[28-30] Base catalyzed reactions were performed without protection of hydroxyl group. Base catalyzed method employed 85% KOH (1.2 eq.) in methanol (10 mL) and H_2O (2 mL) to the solution of equimolar amounts of aryl methyl ketone **1** and hydroxyphenyl aldehyde **2** to obtain compounds **3** in 29.4 to 76.3% yield.

Total twelve compounds (**4-15**) were synthesized as shown

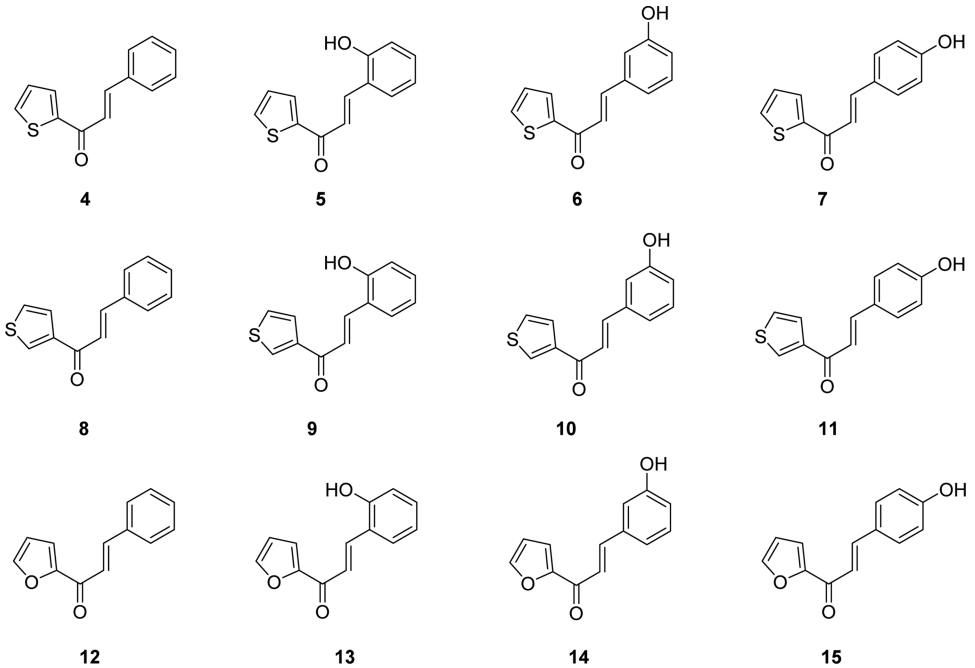


Figure 4. Prepared Thienyl/furanyl-phenylpropenones and thienyl/furanyl-hydroxyphenylpropenones.

Table 1. Inhibitory activity of LPS-induced ROS and NO production in RAW 264.7 macrophages of prepared compounds **4-15**

Compounds	Inhibition (IC_{50}, μM)	
	ROS	NO
4	55	2.7
5	7.3	5.5
6	toxic[a]	toxic
7	4.8	2.8
8	9.0	5.0
9	3.7	3.2
10	toxic	2.2
11	2.2	0.70
12	12	5.9
13	5.1	4.7
14	12	5.4
15	toxic	toxic
Malvidin[33]	9.0	
KB-34[b,34]		0.95

[a]Less than 90% cell viability at 1 μM. [b]KB-34: 3-phenyl-1-(2,4,6-tris(methoxymethoxy)phenyl)prop-2-yn-1-one

in Figure 4. Among them, compounds **4-7** contain 2-thienyl moiety and phenyl, *o*-, *m*-, *p*-hydroxyphenyl moiety, respectively. Compounds **8-11** contain 3-thienyl moiety and phenyl, *o*-, *m*-, *p*-hydroxyphenyl moiety, respectively. Likewise, compounds **12-15** contain 2-furanyl moiety and phenyl, *o*-, *m*-, *p*-hydroxyphenyl moiety, respectively. Structure-activity relationship (SAR) was determined according to 2-, 3-thienyl and/or 2-furanyl moiety, and position of hydroxyl group in the phenyl ring as well as absence of hydroxyl group in the phenyl moiety.

Inhibitory Effects on LPS-induced ROS and NO Production in RAW 264.7 Macrophages. Inhibitory activity of LPS-induced ROS and NO production in RAW 264.7 macrophages of prepared compounds is shown in Table 1.

Among compounds **4-7**, which possess 2-thienyl-phenylpropenone as a basic skeleton without hydroxyl group on phenyl ring (**4**), and with a single hydroxyl group at *ortho*, *meta* or *para* position on phenyl ring (**5**, **6**, **7**, respectively), compound **7** displayed the most significant inhibitory activity of LPS-induced ROS production in RAW 264.7 macrophages (4.8 μM of IC_{50}), and compound **4** and **7** displayed the most significant inhibitory activity of LPS-induced NO production in RAW 264.7 macrophages (2.7 and 2.8 μM of IC_{50}, respectively), which indicated that *para*-hydroxyphenyl moiety is important for inhibitory activities on both LPS-induced ROS and NO production. Among compounds **8-11**, which possess 3-thienyl-phenylpropenone as a basic skeleton without hydroxyl group on phenyl ring (**8**), and with a single hydroxyl group at *ortho*, *meta* or *para* position on phenyl ring (**9**, **10**, **11**, respectively), compound **11** displayed the most significant inhibitory activity of LPS-induced ROS and NO production in RAW 264.7 macrophages (2.2 and 0.70 μM of IC_{50}, respectively), which also indicated that *para*-hydroxyphenyl moiety is important for inhibitory activities on both LPS-induced ROS and NO production. Among compounds **12-15**, which possess 2-furanyl-phenylpropenone as a basic skeleton without hydroxyl group on phenyl ring (**12**), and with a single hydroxyl group at *ortho*, *meta* or *para* position on phenyl ring (**13**, **14**, **15**, respectively), unfortunately compound **15** which possess *para*-hydroxyphenyl moiety displayed cytotoxicity in RAW 264.7 macrophages on both LPS-induced ROS and NO production. From those results it was observed that thienyl/furanyl-phenylpropenones which do not possess hydroxyl group on phenyl moiety did not exhibited significant inhibitory activity of LPS-induced ROS production in RAW 264.7 macrophages. As several compounds showed cytotoxicity, concrete structure-activity relationship could not be determined. However, the compounds possessing *para*-hydroxyphenyl moiety generally exhibited significant inhibitory activities both on LPS-induced ROS production in RAW 264.7 macrophages, and 3-thienyl-possessing compound generally showed better inhibitory activities on both LPS-induced ROS and NO production in RAW 264.7 macrophages than those of 2-thienyl- or 2-furanyl-possessing compounds. Also thienyl/furanyl-phenylpropenones which do not possess hydroxyl group on phenyl moiety did not exhibited condsiderable inhibitory activity of LPS-induced ROS production in RAW 264.7 macrophages. It would be concluded that *para*-hydroxyphenyl moiety plays important role for inhibitory activities on both LPS-induced ROS and NO production as well as 3-thienyl moiety on molecule. In addition, hydroxyphenyl-containing compounds possess drug-like properties on the basis of Lipinski's rule of five.[32] Introduction of hydroxyl group decrease the Log P value of compounds that helps to further optimize the basic thienyl/furanyl-phenylpropenone template.

Conclusions

Twelve thienyl/furanyl-hydroxyphenylpropenones were systematically designed and synthesized using *Claisen-Schmidt* condensation reaction. Prepared compounds were evaluated for their inhibitory effect on LPS-induced ROS and NO production in RAW 264.7 macrophages. Among them compound **11** displayed the most significant inhibitory activity of LPS-induced ROS and NO production in RAW 264.7 macrophages. Structure-activity relationship study indicated that *para*-hydroxyphenyl moiety plays important role for inhibitory activities on both LPS-induced ROS and NO production as well as 3-thienyl moiety on molecule.

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