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Original article

Bis-chalcone analogues as potent NO production inhibitors and as cytotoxic agents

M. Vijaya Bhaskar Reddy [a,1], Yuh-Chiang Shen [b,1], Emika Ohkoshi [c], Kenneth F. Bastow [d], Keduo Qian [c], Kuo-Hsiung Lee [c,e], Tian-Shung Wu [a,e,f,*]

[a] Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, ROC
[b] National Research Institute of Chinese Medicine, Taipei 112, Taiwan, ROC
[c] Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA
[d] Division of Medicinal Chemistry and Natural Products, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA
[e] Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan, ROC
[f] College of Pharmacy, China Medical University, Taichung, Taiwan, ROC

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ABSTRACT

Chalcones have a distinctive 1,3-diarylpropenone skeleton and exert numerous biological effects. Using a one-step Claisen–Schmidt condensation, we synthesized eleven bis-chalcones (**3**–**13**) and three acetyl chalcones (**14**–**16**) from substituted aldehydes and diacetylresorcinol. The compounds were tested for *in vitro* cytotoxic activity against four human cancer cell lines (A549, DU145, KB, and KB-VIN) and inhibition of NO production in lipopolysaccharide (LPS)-activated microglial cells. Among them, four compounds (**3**, **5**, **6**, and **13**) showed significant cytotoxic activity with EC_{50} values ranging from 1.57 to 5.14 μM, and seven compounds (**3**, **5**–**8**, **10**, and **13**) displayed potent anti-inflammatory activity by inhibiting NO production with IC_{50} values ranging from 0.95 to 8.65 μM. A mechanism of action study of active compounds **6** and **7** discovered that these compounds down-regulated iNOS expression by inhibiting p65 NF-κB activation/nuclear translocation due to prevention of IκBα degradation. Structure-activity relationship (SAR) findings are also discussed.

1. Introduction

Despite recent advances in our understanding of the biological processes leading to cancer development and the consequent improvement in drug development, new and more effective agents are still needed to help bring these diseases under control. Chronic inflammation and inflammatory mediators play critical roles in the carcinogenesis of cancers [1,2]. The interactions of tumor cells with their microenvironment and inflammation within the tumor environment are correlated with tumor growth and metastasis and poor prognosis in many cancer types [3,4]. Two major pathways for inflammation, the transcription factors nuclear factor *kappa* B (NF-κB) and signal transducer and activator of transcription 3 (STAT3), are constitutively active in most cancers; and most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by these two pathways [5]. Thus, the molecular and cellular pathways that connect inflammation and cancer have emerged as attractive targets for cancer prevention and therapy.

Chalcones, with a 1,3-diarylpropenone skeleton, constitute an important group of natural products as they possess a wide range of biological activities [6], including anti-inflammatory [7,8], antimicrobial (antibacterial, antifungal) [8-10], antiparasitic (antimalarial, antileishmanial) [8,11,12], antituberculosis [13], antioxidant [14,15], antimitotic [16], anti-invasive [17], anticancer [18-21], as well as modulation of P-glycoprotein-mediated multidrug resistance [22]. In a continued study of this important biologically active compound class, we report herein the synthesis and *in vitro* biological evaluation of nine new (**5**, **6**, **8**, **9**, and **11**–**15**) and five known (**3**, **4**, **7**, **10**, [23] and **16** [24]) bis-chalcones as NO production inhibitors and as cytotoxic agents. All 14 synthesized analogues were tested for cytotoxic activity against A549 (lung cancer), DU-145 (prostate cancer), KB (nasopharyngeal carcinoma), and KB-VIN (vincristine-resistant KB subline) cancer cell lines. In addition, representative biomarkers involved in the inflammatory or metastasis pathways

* Corresponding author. Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, ROC. Tel.: +886 6 2757575x65333; fax: +886 2 2740552.
E-mail address: tswu@mail.ncku.edu.tw (T.-S. Wu).
[1] These authors made equal contributions.

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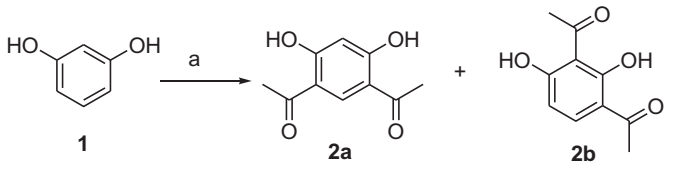


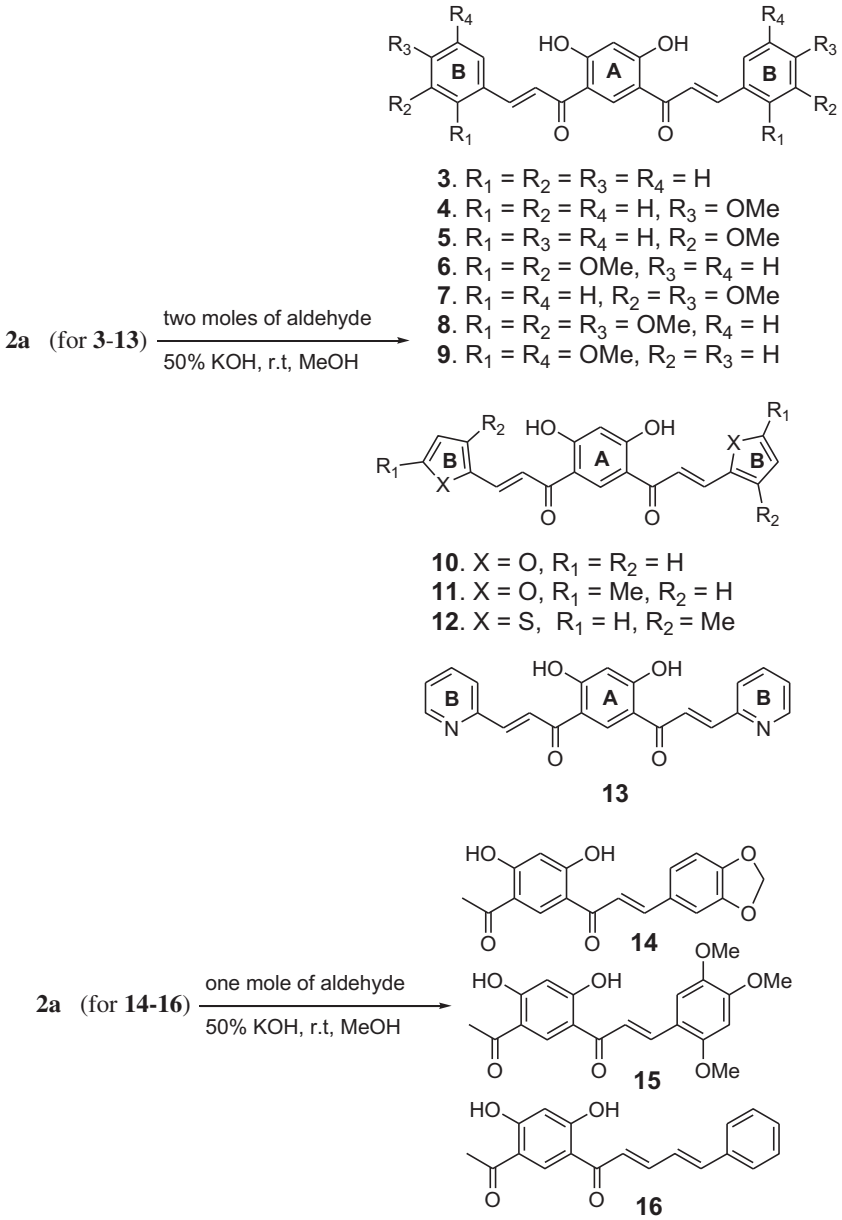
Scheme 1. Synthesis of precursor **2a**.

[e.g., NF-κB, nitric oxide (NO), etc.] were characterized after treatment with the newly synthesized bis-chalcone analogues. Specifically, we evaluated the compounds as inhibitors of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated microglial cells.

2. Chemistry

In a previously reported synthetic method, 2,4-diacetylphenol was prepared by acetylation of 4-hydroxyacetophenone followed by Fries rearrangement of the resulting 4-acetylphenyl acetate with aluminum chloride [25]. A similar synthetic approach [26] was applied to the reaction of resorcinol (**1**) with acetic anhydride in the presence of zinc chloride under acidic (HCl) conditions (140 °C, 24 h) to afford a mixture of diacetylresorcinols **2a** and **2b** in 72% and 9% yields, respectively (Scheme-1). Bis-chalcones **3**–**13** were prepared by reaction of **2a** with two equivalents of different substituted aldehydes in a 50% KOH solution. Reaction with one equivalent of aldehyde gave the mono-addition products **14**–**16** (Scheme 2).

The structures of all compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, EIMS, HREIMS and elemental analysis. A sharp two proton downfield signal was observed at δ 13.62 in the ^{1}H NMR spectrum



Scheme 2. Synthesis of target compounds **3**–**9**, **10**–**12** and **14**–**16**.

Table 1
Cytotoxic Activity Data for Compounds **3**–**16**.

Compound	EC_{50} (μM)			
	A549	DU145	KB	KB-VIN
3	4.08	1.70	1.62	1.57
4	NA	NA	NA	33.97
5	8.76	1.86	2.88	2.07
6	4.38	2.45	2.06	5.14
7	9.14	8.63	8.18	7.02
9	NA	NA	40.08	NA
13	13.66	2.85	2.93	11.04
14	19.38	16.32	17.18	17.21

NA: no activity (compounds did not reach 50% inhibition at 20 μg/mL). Compounds not listed in the table were not active against any cell line.

of **3**, suggesting the presence of two chelated hydroxy groups. This assignment was further supported by carbon resonances at δ 191.9 in the ^{13}C NMR spectrum of **3**. In addition, two sharp aromatic proton signals were observed with **3** at δ 8.53 and δ 6.53, and two sets of protons with a trans coupling constant by ($J = 15.3$ Hz) were present at δ 7.97 and δ 7.57.

3. Results and discussion

All synthesized compounds were tested for *in vitro* cytotoxic activity against four human cancer cell lines, (A549, DU145, KB, and KB-VIN), and the results are shown in Table 1. In addition, we also evaluated the compounds for inhibition of NOX-dependent ROS production and NOS dependent inhibition of NO production in microglial cells, as well as for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity. None of the compounds showed significant inhibition of NOX-dependent ROS production or direct radical scavenging in a cell-free DPPH solution. However, compounds **3**–**14** did inhibit NO production in microglial cells with IC_{50} values ranging from 0.95 to 43.27 μM, compared with an IC_{50} value of 13.35 μM for L-nitro-arginine methyl ester (L-NAME), a non-specific NOS inhibitor (Table 2).

Table 2
Summary of the effects of compounds **3**–**16** on iNOS[a] and NOX[b] activity in murine microglial cells.

Compound	IC_{50} (μM) iNOS[a]	IC_{50} (μM) NOX[a]
3	5.44 ± 0.17	121.84 ± 11.36
4	25.69 ± 5.18	149.59 ± 11.76
5	8.09 ± 0.72	127.05 ± 13.19
6	1.82 ± 0.01	89.02 ± 7.92
7	0.95 ± 0.01	49.08 ± 0.97
8	7.04 ± 0.16	70.92 ± 3.18
9	23.29 ± 3.4	94.97 ± 12.86
10	4.00 ± 0.43	49.61 ± 1.74
11	36.31 ± 3.36	60.42 ± 2.60
12	43.27 ± 2.18	81.41 ± 7.70
13	8.65 ± 0.40	76.80 ± 0.36
14	17.74 ± 2.54	72.05 ± 3.91
15	NA	72.97 ± 15.56
16	NA	120.06 ± 1.14
L-NAME	13.35 ± 0.81	NT
DPI	NT	0.65 ± 0.01

NT: Not tested; NA: Not active.

[a] iNOS activity was measured by NO production in the presence of 1–50 μM of test drug. L-NAME (*N*-nitro-*l*-arginine methyl ester, a non-specific NOS inhibitor) was included as a positive control.

[b] NOX activity was measured by ROS production (chemiluminescence), and DPI (a NOX inhibitor) was included as a positive control. Data were calculated as 50% inhibitory concentration (IC_{50}) and expressed as mean ± SEM from three experiments performed on different days using microglial cells from different passages. Compounds were also tested in DPPH assay, but none of the compounds showed significant activity. For the DPPH assay, Trolox (an antioxidant) was the positive control (IC_{50} 30.24 ± 3.2 μM).

Compound **3,** which has two un-substituted phenyl B-rings, showed the highest potency against DU145, KB, and KB-VIN tumor cell lines. It was slightly less potent against A549 cells (Table 1). Bis-chalcone **5**, which has 3-methoxyphenyl B-rings, also showed significant cytotoxic activity. In contrast, bis-chalcone **4,** which has 4-methoxyphenyl B-rings, was inactive against all cancer cell lines. Thus, the position of the methoxy group on the B-ring had great importance; position 3 was favourable, while position 4 was unfavourable for cytotoxic activity.

Three of the new bis-chalcone analogues have dimethoxy substituted phenyl B-rings, **6** (2,3-dimethoxy), **7** (3,4-dimethoxy), and **9** (2,5-dimethoxy). Compound **6** was significantly active against DU145 and KB cell lines, while 7 showed only weak activity, and **9** was inactive. The 2,3,4-trimethoxy bis-chalcone **8** was also inactive. These findings demonstrate that the positions and numbers of the methoxy substituents affect cytotoxic activity.

When the B-rings were changed from phenyl to heteroaromatic rings, only analogue **13**, with pyridine B-rings, showed significant potency against DU145 and KB cancer cell lines, although it was less potent than **3**. Compounds **10**–**12**, which have five-membered pyrrole or thiophene B-rings, did not show appreciable cytotoxic activity. Finally, compounds **14**–**16,** which have only one arylpropenone group, were essentially inactive in the cytotoxicity assays.

Regarding inhibition of NO production, bis-chalcone **3** was more potent than the positive control L-NAME. The 3-methoxyphenyl analogue **5** also showed significant inhibition of NO production, while the 4-methoxy analogue **4** exhibited only weak inhibition. These results paralleled those found in the cytotoxicity assay. The most potent inhibitors of NO production were the 2,3-dimethoxyphenyl analogue **6** and 3,4-dimethoxyphenyl analogue 7. These two compounds were more potent than the corresponding mono-methoxy substituted analogues **4** and **5**, and control compound L-NAME. Although bis-chalcone **7** was less potent than **6** in the cytotoxicity assay, it was more potent in the NO production inhibition assay. Three additional compounds (**8**, **10**, **13**) showed greater potency than L-NAME, while **14** showed slightly lower potency. In mechanistic studies, compounds **6** and **7**

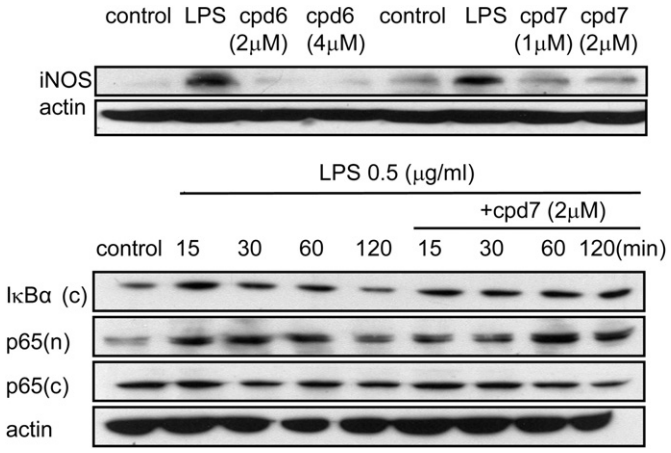


Fig. 1. Effects of compounds **6** and **7** on the lipopolysaccharide (LPS)-induced iNOS expression, degradation of cytosolic IκBα, and nuclear translocation of p65NF-κB in murine microglial BV2 cells. Upper panel, representative immunoblots of LPS-induced iNOS up-expression; lower panel, degradation of cytosolic IκBα [IkBα(c)] and the time-dependent translocation of p65NF-κB from the cytosol [p65(c)] to the nucleus [p65(n)] in microglial cells pre-treated with solvent only (LPS) or compound **6** (cpd **6**) (2, 4 μM) and cpd**7** (1, 2 μM) followed by stimulation with LPS (0.5 μg/mL) for different times (from 15 to 120 min). Cells in control group (control) received solvent only (drug free). The β-actin signal was used as a reference for normalization. At least three independent experiments per compound were examined.

down-regulated iNOS expression by inhibiting p65NF-κB activation/nuclear translocation due to prevention of IκBα degradation (Fig. 1). These results indicate that dimethoxy substitution on the phenyl B-rings leads to the highest potency for the inhibition of NO production of these compounds, most likely due to inhibition of IκBα-dependent NF-κB activation.

4. Conclusions

In this paper we described the synthesis and screening of bis-chalcones against four human cancer cell lines (A549, DU-145, KB, and KB-VIN) and for inhibition of NO production in LPS-activated microglial cells. The results showed that compounds with un-substituted (**3**) and 3-methoxy substituted (**5**) phenyl rings generally exhibited the most potent cytotoxicity, and compounds **3**, **5-8**, **10**, and **13** were more potent inhibitors of cellular NO production in LPS-activated microglial cells than the control drug L-NAME, a non-specific NOS inhibitor. Active compounds **6** and **7** exerted their potent anti-inflammatory effects by inhibiting p65NFκB-dependent iNOS expression, and thus, reducing NO production. Based on the above mentioned results, these active bis-chalcones may be useful for further development as anticancer and anti-inflammatory agents.

5. Experimental section

5.1. General procedure

Melting points were determined using a Yanagimoto MP-S3 micro-melting point apparatus and are uncorrected. IR spectra were determined on a Shimadzu FT-IR Prestige 21 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer, using tetramethylsilane (TMS) as internal standard; all chemical shifts are reported in parts per million (ppm, δ). EIMS and HREIMS spectra were obtained on a VG-70-250S mass spectrometer. Elemental analyses were determined by Elementer Vario EL III and reported as combustion values for C, H, N, and S. Column chromatography was performed on silica gel (70–230 mesh, 230–400 mesh). TLC was conducted on precoated Kieselgel 60 F254 plates (Merck), and the spots were detected by examination under a UV lamp.

5.2. Synthesis of diacetylresorcinol (*2a*)

A mixture containing resorcinol **1** (22 g, 200 mmol), acetic anhydride (20.4 mL, 200 mmol), zinc chloride (13.6 g, 100 mmol) and a drop of concentrated hydrochloric acid was heated to reflux at 140 °C for 24 h. Additional excess acetic anhydride (5.1 mL, 50 mmol) was added during the reaction. The progress of the reaction was monitored by TLC. After the reaction was complete, the residue was purified by column chromatography ($CHCl_3$:hexane, 1:1) to yield **2a** (28.0 g, 72%) and **2b** (3.5 g, 9%). **2a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.88 (2H, s), 8.17 (1H, s), 6.36 (1H, s), 2.59 (6H, s). **2b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.79 (2H, brs), 7.71 (1H, d, J = 8.4 Hz), 6.50 (1H, d, J = 8.4 Hz), 2.57 (6H, s).

5.3. General procedure for synthesis of Bis-chalcones (*3*–*13*)

A solution of 50% KOH (40 mL) was added drop-wise to a well-stirred mixture of **2a** (5.0 mmol) and different substituted aldehydes (10.0 mmol) in MeOH at room temperature. After 24–36 h, the solvent was removed under reduced pressure, and 5% HCl (50–70 mL) was added to the residue. After extraction with EtOAc, the organic layer was washed with brine, dried (Na_2CO_3), and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 50-50% EtOAc in hexanes, to afford bis-chalcones **3**–**13.** Reaction of **2a** with one equivalent of aldehyde under the above conditions gave compounds **14**–**16**.

5.3.1. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-phenylprop-2-en-1-one) (*3*)

Pale yellow solid (1.08 g), 58% yield, mp 190–192 °C. IR (neat) ν_{max} 2916, 1630, 1553, 1485, 1345, 1300, 1279, 1245, 1193, 1031, 973, 841, 760, 732, 711 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.62 (2H, s, OH), 8.53 (1H, s), 7.97 (2H, d, J = 15.3 Hz), 7.69 (4H, m), 7.57 (2H, d, J = 15.3 Hz), 7.48 (6H, m), 6.53 (1H, s).^{13}C NMR (75 MHz, $CDCl_3$): δ 191.9, 169.9, 146.0, 134.3, 134.0, 131.1, 129.1, 128.7, 119.3, 113.8, 105.4.EIMS *m/z* 370 $[M]^+$ (5), 282 (100), 281 (78), 205 (65), 196 (16), 163 (63), 129 (17), 103 (20), 77 (23).HREIMS *m/z* 370.1205 (calcd for $C_{24}H_{18}O_4$, 370.1205). Elemental analysis: Found C 76.68%, H 4.80%; Calcd for C 77.82%, H 4.90%.

5.3.2. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(4-methoxyphenyl)prop-2-en-1-one) (*4*)

Bright yellow solid (1.1 g), 53% yield, mp 187–189 °C. IR (neat) ν_{max} 2934, 2838, 1627, 1569, 1509, 1422, 1356, 1308, 1286, 1198, 1166, 1031, 974, 844, 704 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.73 (2H, s), 8.48 (1H, s), 7.93 (2H, d, J = 15.3 Hz), 7.63 (4H, d, J = 9.0 Hz), 7.43 (2H, d, J = 15.3 Hz), 6.96 (4H, d, J = 9.0 Hz), 6.49 (1H, s), 3.88 (6H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 191.9, 169.8, 162.2, 145.8, 133.6, 130.6, 127.1, 116.8, 114.6, 113.8, 105.2, 55.4.EIMS *m/z*: 430 $[M]^+$ (81), 429 (29), 325 (16), 163 (20), 134 (100), 121 (65), 119 (17), 91 (14).HREIMS *m/z* 430.1413 (calcd for $C_{26}H_{22}O_6$, 430.1416). Elemental analysis: Found C 71.68%, H 5.10%; calcd for C 72.55%, H 5.15%.

5.3.3. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(3-methoxyphenyl)prop-2-en-1-one) (*5*)

Bright yellow solid (1.34 g), 62% yield, mp 144–146 °C. IR (neat) ν_{max} 2917, 2835, 1632, 1565, 1483, 1432, 1345, 1316, 1266, 1192, 1049, 1031, 841, 775, 729 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.56 (2H, s, OH), 8.48 (1H, s), 7.88 (2H, d, J = 15.3 Hz), 7.52 (2H, d, J = 15.3 Hz), 7.35 (2H, dd, J = 9.0, 9.0 Hz), 7.24 (2H, d, J = 8.4 Hz), 7.16 (2H, d, J = 1.8 Hz), 7.00 (2H, dd, J = 1.8, 7.8 Hz), 6.49 (1H, s), 3.85 (6H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 169.9, 160.0, 145.7, 135.7, 134.1, 130.1, 121.3, 119.5, 116.8, 113.7, 113.5, 105.3, 55.2.EIMS *m/z* 430 $[M]^+$ (100), 429 (47), 399 (11), 323 (51), 312 (43), 295 (25), 281 (15), 205 (30), 163 (40).HREIMS *m/z* 430.1415 (calcd for $C_{26}H_{22}O_6$, 430.1416). Elemental analysis: Found C 72.12%, H 5.12%; calcd for C 72.55%, H 5.15%.

5.3.4. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(2,3-dimethoxyphenyl)prop-2-en-1-one) (*6*)

Yellow solid (1.72 g), 70% yield, mp 168–170 °C. IR (neat) ν_{max} 2938, 2836, 1634, 1566, 1480, 1428, 1342, 1299, 1267, 1229, 1200, 1073, 999, 849, 783, 737, 694 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$) δ 13.62 (2H, s), 8.55 (1H, s), 8.18 (2H, d, J = 15.3 Hz), 7.68 (2H, d, J = 15.3 Hz), 7.26 (2H, dd, J = 9.0 Hz, 2.1 Hz), 7.11 (2H, t, J = 9.0 Hz), 7.01 (2H, dd, J = 9.0, 2.1 Hz), 6.51 (1H, s), 3.90 (12H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 192.5, 169.9, 153.2, 149.2, 141.0, 134.3, 128.5, 124.2, 121.0, 120.2, 114.8, 113.9, 105.3, 61.2, 55.9.EIMS *m/z* 490 $[M]^+$ (32), 459 (100), 295 (35), 214 (11), 163 (14).HREIMS *m/z* 490.1631 (calcd for $C_{28}H_{26}O_8$, 490.1628). Elemental analysis: Found C 68.22%, H 5.30%; calcd for C 68.56%, H 5.34%.

5.3.5. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(3,4-dimethoxyphenyl)prop-2-en- 1-one) (*7*)

Bright yellow solid (1.58 g), 65% yield, mp 188–190 °C. IR (neat) ν_{max} 2937, 2839, 1632, 1557, 1510, 1463, 1422, 1338, 1311, 1267, 1235, 1196, 1160, 1139, 1022, 973, 796, 701 .^{1}H NMR (300 MHz, $CDCl_3$)

δ 13.66 (2H, s, OH), 8.51 (1H, s), 7.88 (2H, d, $J = 15.3$ Hz), 7.42 (2H, d, $J = 15.3$ Hz), 7.28 (2H, dd, $J = 9.0$ 1.2 Hz), 7.14 (2H, d, $J = 1.2$ Hz), 6.91 (2H, d, $J = 9.0$ Hz), 6.56 (1H, s), 3.95 (12H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 169.8, 152.0, 149.3, 145.8, 133.8, 127.4, 123.0, 117.3, 113.7, 111.2, 110.9, 105.2, 56.06, 56.01.EIMS *m/z* 490 $[M]^+$ (100), 489 (16), 326 (10), 191 (13), 164 (67), 163 (19), 151 (73), 149 (15).HREIMS *m/z* 490.1625 (calcd for $C_{28}H_{26}O_8$, 490.1628). Elemental analysis: Found C 67.94%, H 5.31%; calcd for C 68.56% H 5.34%.

*5.3.6. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one) (**8**)*

Pale yellow solid (1.4 g), 51% yield, mp 161–163 °C. IR (neat) ν_{max} 2941, 2840, 1634, 1556, 1493, 1464, 1414, 1365, 1287, 1201, 1098, 1060, 990, 945, 842, 800, 696 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.77 (2H, s), 8.58 (1H, s), 8.06 (2H, d, $J = 15.3$ Hz), 7.76 (2H, d, $J = 15.3$ Hz), 7.38 (2H, d, $J = 9.0$ Hz), 6.76 (2H, d, $J = 9.0$ Hz), 6.51 (1H, s), 3.93–3.97 (18H, m).^{13}C NMR (75 MHz, $CDCl_3$) δ 192.5, 169.8, 156.3, 154.1, 142.5, 141.6, 134.0, 125.5, 121.5, 118.8, 113.9, 107.6, 105.1, 61.2, 60.9, 56.1.EIMS *m/z* 550 $[M]^+$ (11), 519 (100), 325 (32), 179 (8), 163 (13).HREIMS *m/z* 550.1837 (calcd for $C_{30}H_{30}O_{10}$, 550.1839). Elemental analysis: Found C 64.58%, H 5.45%; calcd for, C 65.45%, H 5.49%.

*5.3.7. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(2,5-dimethoxyphenyl)prop-2-en-1-one) (**9**)*

Yellow solid (1.32 g), 54% yield, mp 205–207 °C. IR (neat) ν_{max} 2936, 2834, 1625, 1543, 1492, 1314, 1244, 1220, 1188, 1047, 1020, 982, 951, 877, 848, 798 .^{1}H NMR (300 MHz, DMSO-d_6) δ 13.81 (2H, s, OH), 8.99 (1H, s), 8.21 (2H, d, $J = 15.9$ Hz), 8.15 (2H, d, $J = 15.9$ Hz), 7.69 (2H, d, $J = 1.8$ Hz), 7.09 (4H, m), 6.44 (1H, s), 3.83–3.80 (12H, m).^{13}C NMR (75 MHz, DMSO-d_6) δ 192.2, 168.8, 153.2, 152.9, 138.8, 136.0, 123.2, 121.0, 118.6, 114.1, 113.0, 112.9, 104.0, 56.1, 55.6.EIMS *m/z* 490 $[M]^+$ (50), 459 (100), 295 (39), 163 (20).HREIMS *m/z* 490.1631 (calcd for $C_{28}H_{26}O_8$, 490.1628). Elemental analysis: Found C 68.19%, H 5.33%; calcd for C 68.56%, H 5.34%.

*5.3.8. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(furan-2-yl)prop-2-en-1-one) (**10**)*

Orange solid (1.42 g), 81% yield, mp 203–205 °C. IR (neat) ν_{max} 3031, 1631, 1583, 1550, 1499, 1473, 1360, 1301, 1283, 1259, 1236, 1204, 1190, 1015, 978, 924, 882, 758 .^{1}H NMR (300 MHz, DMSO-d_6) δ 13.34 (2H, s, OH), 8.67 (1H, s), 7.95 (2H, s), 7.76 (2H, d, $J = 15.0$ Hz), 7.63 (2H, d, $J = 15.0$ Hz), 7.16 (2H, d, $J = 3.3$ Hz), 6.71 (2H, t, $J = 1.5$ Hz), 6.44 (1H, s).^{13}C NMR (75 MHz, DMSO-d_6) δ 190.9, 167.6, 151.2, 146.6, 135.4, 130.6, 119.0, 117.9, 115.0, 113.3, 103.8.EIMS *m/z* 350 $[M]^+$ (100), 256 (43), 228 (11), 163 (20), 121 (15), 95 (10), 65 (17).HREIMS *m/z* 350.0790 (calcd for $C_{20}H_{14}O_6$, 350.0790). Elemental analysis: Found C 68.31%, H 4.00%; calcd for C 68.57%, H 4.03%.

*5.3.9. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(5-methylfuran-2-yl)prop-2-en-1-one) (**11**)*

Yellow solid (1.28 g), 68% yield, mp 185–187 °C. IR (neat) ν_{max} 2919, 2849, 1633, 1557, 1494, 1366, 1259, 1193, 1025, 960, 785, 771, 696, 636, 596 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.87 (2H, s), 8.57 (1H, s), 7.67 (2H, d, $J = 15.0$ Hz), 7.47 (2H, d, $J = 15.0$ Hz), 6.73 (2H, d, $J = 3.0$ Hz), 6.49 (1H, s), 6.21 (2H, d, $J = 3.0$ Hz), 2.45 (6H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 191.5, 169.9, 156.6, 150.1, 133.7, 131.3, 119.7, 114.9, 113.8, 109.9, 105.1, 14.0.EIMS *m/z* 378 $[M]^+$ (100), 270 (58), 255 (11), 163 (19), 108 (42), 95 (29).HREIMS *m/z* 378.1106 (calcd for $C_{22}H_{18}O_6$, 378.1103). Elemental analysis: Found C 69.49%, H 4.72%; calcd for C 69.84%, H 4.79%.

*5.3.10. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(3-methylthiophen-2-yl)prop-2-en-1-one) (**12**)*

Yellow solid (1.52 g), 74% yield, mp 229–231 °C. IR (neat) ν_{max} 2917, 2849, 1626, 1554, 1486, 1384, 1341, 1298, 1273, 1243, 1193, 1036, 968, 871, 844, 712, 682 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.71 (2H, s), 8.45 (1H, s), 8.14 (2H, d, $J = 15.0$ Hz), 7.39 (2H, d, $J = 5.1$ Hz), 7.28 (2H, d, $J = 15.0$ Hz), 6.95 (2H, d, $J = 5.1$ Hz), 6.48 (1H, s), 2.44 (6H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 191.2, 169.9, 143.8, 136.5, 134.2, 133.6, 131.7, 128.4, 116.9, 113.6, 105.2, 14.4.EIMS *m/z* 410 $[M]^+$(100), 395 (15), 374 (16), 286 (17), 271 (25), 268 (54), 260 (21), 163 (48), 151 (32), 124 (72), 111 (53), 95 (30).HREIMS *m/z* 410.0645 (calcd for $C_{22}H_{18}O_4S_2$, 410.0647). Elemental analysis: Found C 64.10%, H 4.43%, S 15.53%; calcd for C 64.37%, H 4.42%, S 15.62%.

*5.3.11. (2E, 2′E)-1,1′-[4,6-Dihydroxy-1,3-phenylene]bis(3-(pyridin-2-yl)prop-2-en-1-one) (**13**)*

Pale yellow solid (1.2 g), 64% yield, mp 145–147 °C. IR (neat) ν_{max} 3057, 2952, 1649, 1574, 1468, 1423, 1350, 1312, 1260, 1224, 1212, 976, 869, 843, 777, 740, 591 .^{1}H NMR (300 MHz, DMSO-d_6) δ 12.96 (2H, s, OH), 8.71 (2H, d, $J = 4.2$ Hz), 8.63 (1H, s), 8.27 (2H, d, $J = 15.3$ Hz), 7.92 (4H, m), 7.77 (2H, d, $J = 15.3$ Hz), 7.45 (2H, m), 6.62 (1H, s).^{13}C NMR (75 MHz, DMSO-d_6) δ 191.0, 166.9, 152.5, 150.0, 142.4, 137.3, 135.8, 126.2, 125.4, 124.9, 115.9, 103.8.EIMS *m/z* 372 $[M]^+$ (100), 354 (24), 327 (15), 294 (27), 239 (30), 154 (11), 132 (31), 106 (22), 104 (30), 78 (22).HREIMS *m/z* 372.1113 (calcd for $C_{22}H_{16}N_2O_4$, 372.1110). Elemental analysis: Found C 70.61%, H 4.28%, N 7.45%; calcd for C 70.96%, H 4.33%, N 7.52%.

*5.3.12. (E)-1-[5-Acetyl-2,4-dihydroxyphenyl]-3-(benzo[d] [1,3] dioxol-5-yl)prop-2-en-1-one (**14**)*

Yellow solid (980 mg), 63% yield, 166–168 °C. IR (neat) ν_{max} 3012, 2911, 1635, 1553, 1489, 1447, 1356, 1313, 1243, 1206, 1104, 1063, 1035, 929, 753, 662, 588 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.68 (1H, s, OH), 12.95 (1H, s, OH), 8.34 (1H, s), 7.86 (1H, d, $J = 15.0$ Hz), 7.34 (1H, d, $J = 15.0$ Hz), 7.14 (2H, dd, $J = 9.0$, 3.0 Hz), 6.88 (1H, d, $J = 9.0$ Hz), 6.46 (1H, s), 6.06 (2H, s), 2.68 (3H, s).^{13}C NMR (75 MHz, $CDCl_3$): δ 202.4, 191.6, 170.0, 168.7, 148.5, 145.8, 134.8, 128.7, 125.9, 116.8, 113.7, 113.5, 108.6, 106.6, 105.1, 101.8, 26.1.EIMS *m/z*: 326 $[M]^+$ (100), 325 (37), 205 (11), 179 (10), 163 (15), 148 (76), 135 (59), 89 (15).HREIMS *m/z* 326.0792 (calcd for $C_{18}H_{14}O_6$, 326.0790). Elemental analysis: Found C 66.65%, H 4.21%; calcd for, C 66.26%, H 4.32%.

*5.3.13. (E)-1-[5-Acetyl-2,4-dihydroxyphenyl]-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**15**)*

Yellow solid (1.2 g), 65% yield, mp 206–208 °C. IR (neat) ν_{max} 2934, 2836, 1632, 1584, 1548, 1438, 1384, 1353, 1307, 1275, 1213, 1046, 1029, 998, 833, 760 .^{1}H NMR (300 MHz, $CDCl_3$) δ 13.85 (1H, s, OH), 12.89 (1H, s, OH), 8.35 (1H, s), 8.11 (1H, d, $J = 15.3$ Hz), 7.53 (1H, d, $J = 15.3$ Hz), 7.07 (1H, s), 6.52 (1H, s), 6.43 (1H, s), 3.96 (3H, s), 3.94 (3H, s), 3.90 (3H, s), 2.65 (3H, s).^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 192.4, 170.1, 168.5, 155.3, 153.2, 143.3, 141.7, 135.0, 117.3, 114.9, 114.0, 113.4, 113.1, 104.9, 96.6, 56.8, 56.2, 56.1, 25.9.EIMS *m/z* 372 $[M]^+$ (43), 341 (100), 181 (16), 179 (13).HREIMS *m/z* 372.1212 (calcd for $C_{20}H_{20}O_7$, 372.1209). Elemental analysis: Found C 64.16%, H 5.39%; calcd for C 64.51%, H 5.41%.

*5.3.14. (2E, 4E)-1-[5-Acetyl-2,4-dihydroxyphenyl]-5-phenylpenta-2,4-dien-1-one (**16**)*

Yellow solid (750 mg), 49% yield, mp 178–180 °C. IR (neat) ν_{max} 2918, 2842, 1630, 1554, 1487, 1451, 1353, 1323, 1241, 1230, 1199, 1150, 1063, 1000, 953, 844, 758, 745 .^{1}H NMR (300 MHz, Me_2CO-d_6) δ 13.69 (1H, s, OH), 12.95 (1H, s, OH), 8.32 (1H, s), 7.76 (1H, dd, $J = 15.0$, 15.0 Hz), 7.64 (3H, m), 7.43 (3H, d, $J = 15.0$ Hz), 7.25 (2H, m), 6.35 (1H, s), 2.78 (3H, s).^{13}C NMR (75 MHz, Me_2CO-d_6) δ 204.7, 193.2, 170.7, 146.4, 143.8, 137.2, 137.0, 130.2, 129.7, 128.2, 127.6, 124.2, 114.3, 104.7, 26.4.EIMS *m/z* 308 $[M]^+$ (100), 307 (20), 265 (12), 231 (12), 179 (24), 163 (13), 152 (7).HREIMS *m/z* 308.1046 (calcd for $C_{19}H_{16}O_4$, 308.1049). Elemental analysis: Found C 73.21%, H 5.20%; calcd for C 74.01%, H 5.23%.

5.4. Cytotoxic activity assay

All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500–7500 cells per well with compounds added from DMSO-diluted stock. After 3 days in culture, attached cells were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbance at 562 nm was measured using a microplate reader after solubilizing the bound dye. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions; and it is the average from at least three independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: DU-145 (prostate cancer), A549 (lung cancer), KB (nasopharyngeal carcinoma) and KB-VIN (vincristine-resistant KB subline). All cell lines were obtained from the Lineberger Comprehensive Cancer Center (UNC-CH) or from ATCC (Rockville, MD) and were cultured in RPMI-1640 medium supplemented with 25 μM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin [27].

5.5. Measurement of NADPH oxidase activity

NADPH oxidase activity was measured as described previously [28]. Test compounds were added to the wells of a bioluminescence plate and incubated with 50 μg of cell homogenate for 20 min at 37 °C in the dark. $O_2^{\cdot}$ production was stimulated with 200 μM NADPH, and the chemiluminescence was monitored for 30 min, after which the AUC (area under the curve) was calculated to represent reactive oxygen species production (NADPH activity).

5.6. Microglial cell culture and measurement of nitric oxide (NO)

A murine microglial cell line (BV2) was cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, USA) supplemented with 5% fetal bovine serum (Hyclone, USA). The production of NO was determined by measuring the accumulation of nitrite in the culture medium 24 h after stimulation with LPS (0.5 μg/mL) by the Griess reagent as in our previous report [28].

5.7. Western immunoblot analysis of iNOS, IκBα and NF-κB p65

Equal amounts of protein (50 μg) at different time points from 0.5 μg/mL, LPS-treated samples in the absence or presence of compounds **6** (2.0–4.0 μM) and **7** (1.0–2.0 μM) was subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) and electro-transferred to a hydrophobic polyvinylidene difluoride (PVDF) membrane. After blocking with 5% nonfat milk in phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST) at 4 °C for 1 h, the membrane was washed three times with PBST and incubated overnight at 4 °C with an antibody against iNOS (BD Pharmingen, BD Biosciences, San Diego, CA, USA), IκBα and NF-κB p65 (BD Transduction Laboratories, BD Biosciences, San Diego, CA, USA) at a properly titrated dilution (1:1000–2500). After additional washes with PBST, the membrane was incubated with a second antibody IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The immunoblot on the membrane was visible after development with an enhanced chemiluminescence (ECL) system (Perkin–Elmer, Wellesley, MA, USA) and was quantitated using an image program [29] (Multi Gauge v2.2 software, Fujifilm, Tokyo, Japan).

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References

[1] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (2002) 860–867.
[2] L.F. Fecker, E. Stockfleth, I. Nindl, C. Ulrich, I. Forschner, J. Eberle, The role of apoptosis in therapy and prophylaxis of epithelial tumours by nonsteroidal anti-inflammatory drugs (NSAIDs), Br. J. Dermatol. 156 (Suppl 3) (2007) 25–33.
[3] J.E. Goldberg, K.L. Schwertfeger, Proinflammatory cytokines in breast cancer: mechanisms of action and potential targets for therapeutics, Curr. Drug Targets 11 (2010) 1133–1146.
[4] L.M. Postovit, E.A. Seftor, R.E. Seftor, M.J. Hendrix, Influence of the microenvironment on melanoma cell fate determination and phenotype, Cancer Res. 66 (2006) 7833–7836.
[5] B.B. Aggarwal, R.V. Vijayalekshmi, B. Sung, Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe, Clin. Cancer Res. 15 (2009) 425–430.
[6] D.I. Batovska, I.T. Todorova, Trends in the utilization of the pharmacological potential of chalcones, Curr. Clin. Pharmacol. 5 (2010) 1–29.
[7] C. Kontogiorgis, M. Mantzanidou, D. Hadjipavlou-Litina, Chalcones and their potential role in inflammation, Mini Rev. Med. Chem. 8 (2008) 1224–1242.
[8] Z. Nowakowska, A review of anti-infective and anti-inflammatory chalcones, Eur. J. Med. Chem. 42 (2007) 125–137.
[9] V. Opletalová, D. Sedivý, Chalcones and their heterocyclic analogs as potential antifungal chemotherapeutic agents, Ceska Slov. Farm. 48 (1999) 252–255.
[10] V. Opletalová, Chalcones and their heterocyclic analogs as potential therapeutic agents in bacterial diseases, Ceska Slov. Farm. 49 (2000) 278–284.
[11] M.L. Go, Novel antiplasmodial agents, Med. Res. Rev. 23 (2003) 456–487.
[12] M. Liu, P. Wilairat, S.L. Croft, A.L. Tan, M.L. Go, Structure-activity relationships of antileishmanial and antimalarial chalcones, Bioorg. Med. Chem. 11 (2003) 2729–2738.
[13] Y.M. Lin, Y. Zhou, M.T. Flavin, L.M. Zhou, W. Nie, F.C. Chen, Chalcones and flavonoids as anti-tuberculosis agents, Bioorg. Med. Chem. 10 (2002) 2795–2802.
[14] S. Padhye, A. Ahmad, N. Oswal, F.H. Sarkar, Emerging role of garcinol, the antioxidant chalcone from Garcinia indica Choisy and its synthetic analogs, J. Hematol. Oncol. 2 (2009) 38.
[15] V. Opletalová, L. Jahodár, D. Jun, L. Opletal, Chalcones (1,3-diarylpropen-1-ones) and their analogs as potential therapeutic agents in cardiovascular system diseases, Ceska Slov. Farm 52 (2003) 12–19.
[16] N.J. Lawrence, A.T. McGown, The chemistry and biology of antimitotic chalcones and related enone systems, Curr. Pharm. Des. 11 (2005) 1679–1693.
[17] Y. Kimura, New anticancer agents: in vitro and in vivo evaluation of the antitumor and antimetastatic actions of various compounds isolated from medicinal plants, In Vivo 19 (2005) 37–60.
[18] H.L. Liu, W.B. Jiang, M.X. Xie, Flavonoids: recent advances as anticancer drugs, Recent Pat. Anticancer Drug Discov. 5 (2010) 152–164.
[19] A.M. Katsori, D. Hadjipavlou-Litina, Chalcones in cancer: understanding their role in terms of QSAR, Curr. Med. Chem. 16 (2009) 1062–1081.
[20] V.R. Yadav, S. Prasad, B. Sung, B.B. Aggarwal, The role of chalcones in suppression of NF-κB-mediated inflammation and cancer, Int. Immunopharmacol 11 (2011) 295–309.
[21] M.L. Go, X. Wu, X.L. Liu, Chalcones: an update on cytotoxic and chemoprotective properties, Curr. Med. Chem. 12 (2005) 481–499.
[22] F. Bois, A. Boumendjel, A. Mariotte, G. Conseil, A. Di Petro, Synthesis and biological activity of 4-alkoxy chalcones: potential hydrophobic modulators of P-glycoprotein-mediated multidrug resistance, Bioorg. Med. Chem. 7 (1999) 2691–2695.
[23] B. Sreenivasulu, P.N. Sarma, A facile synthesis of 3,7-diphenyl-4,6-distyryl-2,8-dioxo-2H,8H-benzo[1,2-b:5,4-b′]dipyrans and their antifeedant activity, Syn. Comm. 27 (1997) 2281–2287.
[24] E.T. Oganesyan, A.S. Saraf, A.V. Simonyan, I.N. Shiryaev, Structure-activity relationship in flavonoids. 9. Antiallergic activity of chalcones, Pharm. Chem. J. 25 (1991) 526–530.
[25] N.K. Sangwan, B.M. Verma, K.S. Dhindsa, 2,4-Bis(substituted cinnamoyl/pyrazol-3-yl)phenoxyacetic acid esters and hydrazides as potential antimicrobial agents, Indian J. Chem. 25B (1986) 672–674.
[26] A.S.R. Anjaneyulu, U.V. Mallavadhani, Y. Venkateswaralu, A.V.R. Prasad, Reaction of acetic anhydride/zinc chloride reagent with phenols: improved yields of hydroxyacetophenones, Indian J. Chem. Sect. B. 26 (1987) 823–826.

[27] X. Wang, K.F. Bastow, C.M. Sun, Y.L. Lin, H.J. Yu, M.J. Don, T.S. Wu, S. Nakamura, K.H. Lee, Antitumor Agents. 239. Isolation, structure elucidation, total synthesis, and anti-breast cancer activity of neo-tanshinlactone from Salvia miltiorrhiza, J. Med. Chem. 47 (2004) 5816–5819.
[28] Y.H. Wang, W.Y. Wang, C.C. Chang, K.T. Liou, Y.J. Sung, J.F. Liao, C.F. Chen, S. Chang, Y.C. Hou, Y.C. Chou, Y.C. Shen, Taxifolin ameliorates cerebral ischemia-reperfusion injury in rats through its anti-oxidative effect and modulation of NF-kappaB activation, J. Biomed. Sci. 13 (2006) 127–141.
[29] Y.H. Wang, Y.C. Shen, J.F. Liao, C.H. Lee, C.Y. Chou, K.T. Liou, Y.C. Chou, Anti-inflammatory effects of dimemorfan on inflammatory cells and LPS-induced endotoxin shock in mice, Br. J. Pharmacol. 154 (2008) 1327–1338.