European Journal of Medicinal Chemistry 44 (2009) 3195–3200

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities

Ka-Heng Lee [a], Farida Haryani Ab. Aziz [a], Ahmad Syahida [a,b,*], Faridah Abas [b,c], Khozirah Shaari [b,d], Daud Ahmad Israf [b,e], Nordin Haji Lajis [b,d]

[a] Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[b] Institute of Biosciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[c] Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[d] Faculty of Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[e] Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 31 December 2008
Received in revised form
11 March 2009
Accepted 16 March 2009
Available online 26 March 2009

Keywords:
Anti-inflammatory
Antioxidant
Anti-tyrosinase
Diarylpentanoids
iNOS

ABSTRACT

A series of 46 curcumin related diarylpentanoid analogues were synthesized and evaluated for their anti-inflammatory, antioxidant and anti-tyrosinase activities. Among these compounds **2**, **13** and **33** exhibited potent NO inhibitory effect on IFN-γ/LPS-activated RAW 264.7 cells as compared to L-NAME and curcumin. However, these series of diarylpentanoid analogues were not significantly inhibiting NO scavenging, total radical scavenging and tyrosinase enzyme activities. The results revealed that the biological activity of these diarylpentanoid analogues is most likely due to their action mainly upon inflammatory mediator, inducible nitric oxide synthase (iNOS). The present results showed that compounds **2**, **13** and **33** might serve as a useful starting point for the design of improved anti-inflammatory agents.

1. Introduction

Nitric oxide (NO) is a free radical generated by nitric oxide synthase (NOS) and served as an important mediator that involved in the regulation of physiological and pathophysiological mechanism in cardiovascular, nervous and immunological systems [1]. However, abnormal expression of inducible nitric oxide synthase (iNOS) and excessive NO production may lead to chronic inflammation and also associated with number of diseases such as rheumatoid arthritis, diabetes, hypertension and septic shock [2]. Macrophages are known as major immune cells to express iNOS in response to stimuli such as lipopolysaccharide (LPS) or pro-inflammatory cytokine including tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interferon-gamma (IFN-γ) [3]. Studies have shown that NO biosynthesis is regulated by a variety of signaling mechanisms at the transcriptional and post-translation levels in activated macrophages. The upstream signaling protein or transcriptional factors such as nuclear factor kappa-B (NF-κB), activator protein-1 (AP-1) and mitogen activated protein kinase (MAPK) are the main regulators of iNOS expression [1]. Therefore, inhibition of iNOS expression or its activity as well as upstream signaling regulatory protein has been a prime target for development of new anti-inflammatory drugs.

In addition, NO can interact with reactive oxygen species (ROS) leading to formation of reactive nitrogen species (RNS) such as peroxynitrite [$OONO^-$], *S*-nitrosothiols [RSNO] and nitrogen dioxide [NO_2^-] [4]. It is well known that overproduction of ROS and RNS generated from activated macrophages are the potent oxidizing agents causing DNA fragmentation and lipid oxidation, thus resulting in oxidative stress and cell injury [5]. Moreover,

Abbreviations: AP-1, activator protein-1; DMEM, Dulbecco's modified eagle's medium; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; DPPH, diphenylpicrylhydrazine radical; HEMn, human epidermal melanocyte cell line; IC_{50}, inhibitory concentration at 50%; IFN-γ, interferon-gamma; IL-1β, interleukin-1beta; iNOS, Inducible nitric oxide synthase; L-DOPA, L-3,4-dihydroxyphenylalanine; L-NAME, NG-nitro-L-arginine methyl ester; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NF-κB, nuclear factor kappa-B; NO, nitric oxide; NO_2^-, nitrite; OD, optical density; PTIO, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide; RAW 264.7, murine monocytic macrophages cell line; RNS, reactive nitrogen species; ROS, reactive oxygen species; S.E.M., standard error of mean; SNP, sodium nitroprusside; TNF-α, tumour necrosis factor-alpha.

* Corresponding author. Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel.: +603 89466694; fax: +603 89430913.
E-mail address: syahida@biotech.upm.edu.my (A. Syahida).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.03.020

overproduction of ROS is associated with inflammatory response by regulating several activities of kinases, transcription factors and cell death machinery [6]. Therefore, compounds with radical scavenging activities can be used in treating or preventing various chronic inflammatory diseases.

On the other hand, tyrosinase is a copper containing enzyme involved in melanin synthesis and widely distributed in nature. Two reactions involved in melanin biosynthesis pathways catalyzed by tyrosinase are hydroxylation of monophenols to *o*-phenols and the oxidation of the *o*-phenols to *o*-quinones [7]. Tyrosinase catalyzed the formation of quinones and mediated the development of brown colour pigment through spontaneous polymerization of highly reactive quinones [8]. Elevated levels of epidermal pigmentation have been implicated in various dermatological disorders, such as melasma, age spots and sites of actinic damage. Hence, inhibitory action on tyrosinase activity is an important target in treating pigmentation-related disorders and new whitening agent development.

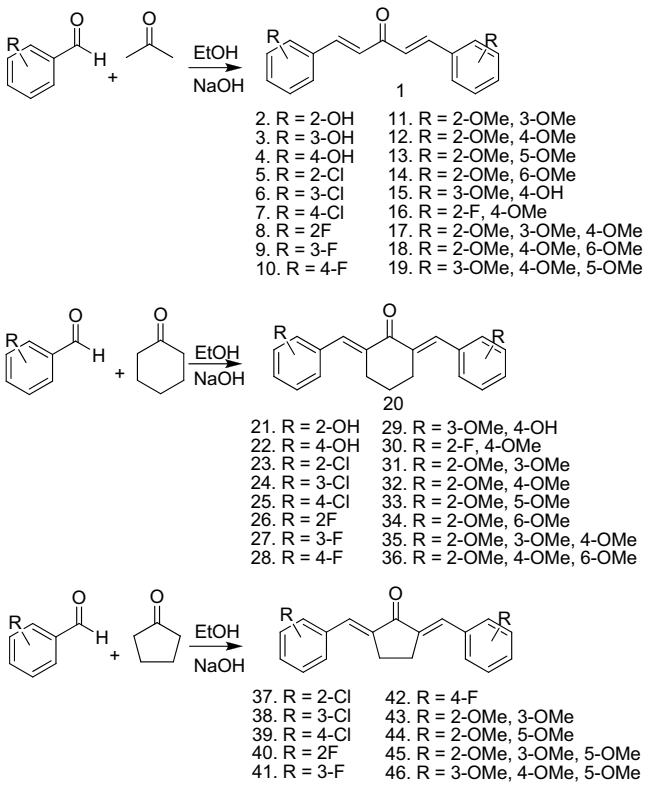
A number of dietary chemopreventive compounds such as curcumin and flavonoids have been shown beneficial in treating of inflammatory diseases or dermatological disorders respectively [9,10]. Recent studies have shown that curcumin analogues are compounds of great chemical and pharmacological interest due to their potential antioxidant, anti-proliferation [11], anti-angiogenesis [12], anti-tumour [13] and anti-inflammatory properties [14]. Furthermore, studies have suggested that two aromatic regions of curcumin might be critical for potential protein-ligand binding and became a rational design of potential inhibitor (ligand) of protein targets [12]. Therefore, a series of curcumin-like diarylpentanoid analogues with two identical aromatic ring region separated by five carbon spacers were synthesized and evaluated for their anti-inflammatory, antioxidant and anti-tyrosinase activities.

2. Chemistry

All compounds were synthesized by coupling the appropriate aromatic aldehyde with acetone (**1**–**19**), cyclopentanone (**20**–**36**) and cyclohexanone (**37**–**46**) under base catalyzed aldol condensation, using the ratio of ketone:aldehyde of 1:2. A straightforward approach to desired analogues is summarized in Scheme 1. All compounds were determined based on the analysis of spectroscopic data and comparison of these data with those of related compounds. Detailed spectroscopic data of the synthesized compounds can be obtained from us upon request.

3. Results and discussion

The results of anti-inflammatory, NO scavenging, antioxidant and anti-tyrosinase activities are shown in Table 1. Seven compounds (**2**, **13**, **16**, **26**, **28**, **31**, **33**) showed NO production inhibitory effect on IFN-γ/LPS-activated macrophage cells with IC_{50} values varying from 10.24 to 40.24 μM. Among the seven compounds, **2**, **13** and **33** exhibited potent NO inhibitory effect with IC_{50} value of 10.24 ± 1.05 μM, 13.64 ± 0.41 μM and 13.66 ± 0.61 μM, respectively as compared to positive control, L-NAME (IC_{50} value = 27.13 ± 5.58 μM) and curcumin (20.38 ± 0.28 μM). Compounds **2** (Fig. 1a) and **13** (Fig. 1b) showed NO inhibitory effect in a dose-dependent manner, but showed toxicity effect at concentrations of 25 μM (Fig. 1d) and 50 μM (Fig. 1e), respectively. Recently, Weber et al. [14] have shown that compounds **2** and **13** have attenuated TNF-α induced nuclear factor-kappa-B (NF-κB) activation from human embryonic kidney cells which transfected with stable NF-κB reporter. Transcriptional factor NF-κB is one of the upstream signaling proteins that involved in the regulation of iNOS expression and the inactivation of NF-κB indirectly caused the suppression of NO production by affecting the iNOS expression. In contrast, compound **33** (Fig. 1c) demonstrated potent inhibitory action of NO production without causing any cytotoxicity effect at all concentrations tested (Fig. 1f). Interestingly, compounds **13** and **33** shared same substitution groups, where methoxyl group was



Scheme 1. General synthesis of diarypentanoids analogues.

Table 1
The IC_{50} values of anti-inflammatory, NO-scavenging, DPPH radical scavenging and anti-tyrosinase activities of diarylpentanoid analogues.

Analogues	IC_{50} (μM)			
	Anti-inflammatory activity	Direct NO scavenging activity	DPPH radical scavenging activity	Anti-tyrosinase activity
2	10.24 ± 1.05	>500	>500	NA
4	NA	>500	>500	79.05 ± 3.92
10	NA	NA	NA	>250
13	13.64 ± 0.41	NA	NA	NA
15	NA	>500	257.35 ± 23.3	>250
16	33.13 ± 9.28	NA	NA	>250
22	NA	>500	>500	>250
25	NA	NA	NA	>250
26	42.04 ± 4.72	NA	NA	NA
28	42.01 ± 1.19	NA	NA	NA
29	NA	>500	316.64 ± 23.79	NA
31	36.95 ± 2.87	NA	NA	NA
33	13.66 ± 0.61	NA	NA	NA
Curcumin	20.38 ± 0.28	>500	109.74 ± 2.04	NA
L-NAME[a]	27.13 ± 5.58	NT	NT	NT
Quercetin[b]	NT	NA	41.79 ± 2.23	NT
PTIO[c]	NT	0.57 ± 0.27	NT	NT
Kojic acid[d]	NT	NT	NT	66.55 ± 0.19

NA: not active; NT: not tested.
[a] Positive control used for anti-inflammatory assay.
[b] Positive control used for DPPH radical scavenging assay.
[c] Positive control used for NO-scavenging assay.
[d] Positive control used for anti-tyrosinase assay.

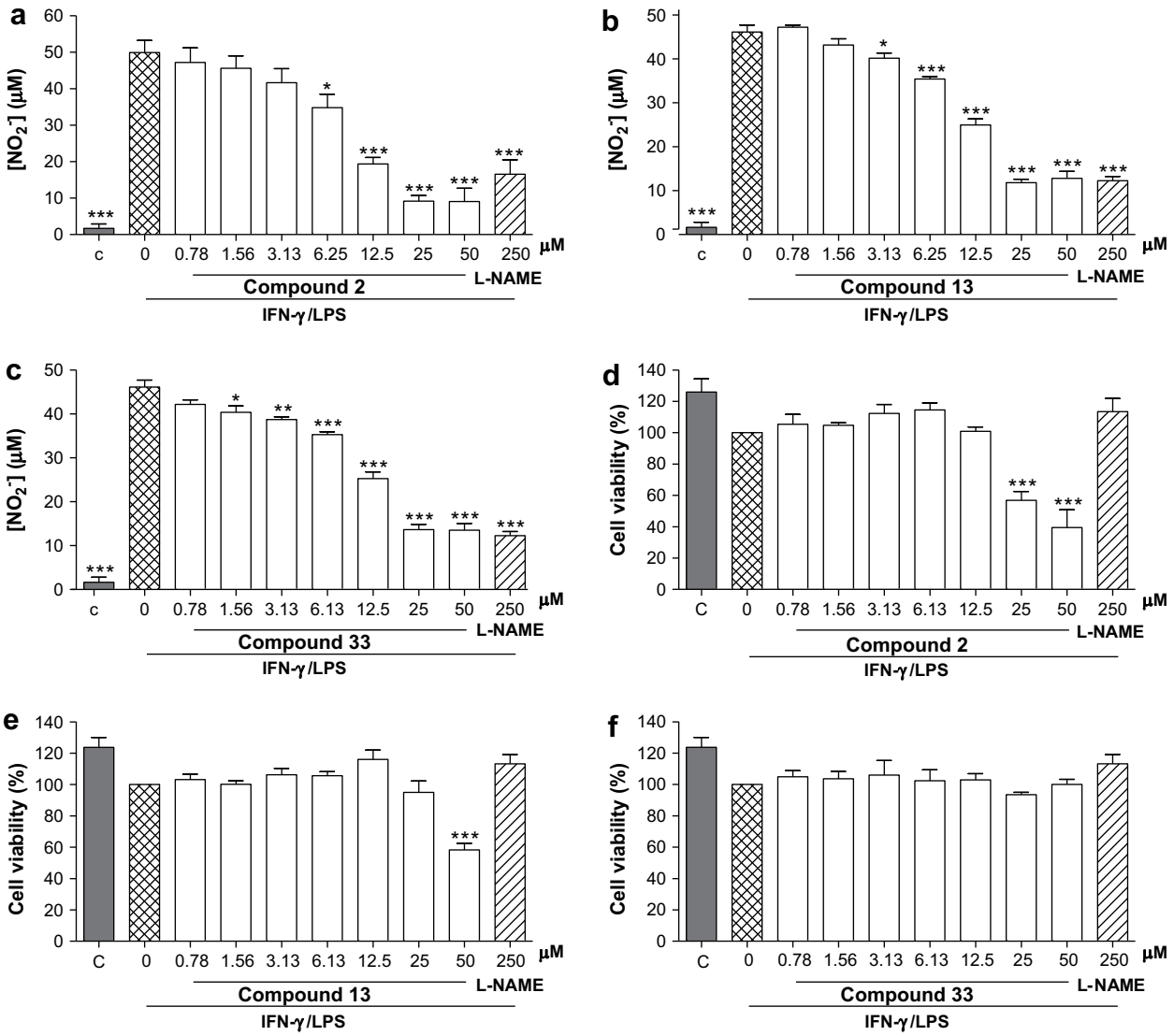


Fig. 1. Nitric oxide (NO) production level (a–c) and RAW 264.7 cell viability (d–f) in the presence of compounds **2**, **13** and **33**. C; basal level of nitrite concentration without IFN-γ/LPS-treatment. All values are mean ± S.E.M. of three different experiments. $^{*}P < 0.05$, $^{**}P < <0.01$, $^{***}P < 0.001$ significantly different from the IFN-γ/LPS-treated control group (second column).

present at positions 2 and 5 on both their phenyl ring structures. It was further supported by Ko et al. [15] and Rojas et al. [16] who showed that chalcone derivatives with methoxyl groups at positions 2 and 5 of their phenyl rings structure potently inhibiting NO production in activated RAW 264.7 cells. Therefore, it was suggested that 2,5-dimethoxyl substitution groups on phenyl rings are significant factors responsible for NO inhibitory activity.

It was found that excessive RNS and ROS generation takes place in many inflammation disorders [17]. In this study, the relationship of scavenging activity and anti-inflammatory properties of the analogues was studied. The stable free radical diphenylpicrylhydrazyl (DPPH) was used to estimate the antioxidant activity of the diarylpentanoid analogues. Compounds that can donate a hydrogen atom to the DPPH radical, then gives rise to the reduced form of DPPH which will be considered as potential antioxidant agents [18]. The present results showed that the majority of the analogues displayed poor radical scavenging activity. However, compounds **2**, **4** and **22** showed NO scavenging and DPPH radical scavenging activities less than 50% at highest concentration tested (IC_{50} values >500 μM) as compared to PTIO and quercetin, respectively. It was well known that the presence of hydroxyl group on phenyl ring is a critical factor contributing radical scavenging properties [19]. Concomitantly, these three analogues (**2**, **4**, **22**) have hydroxyl group at position either 2 or 4 of both their phenyl

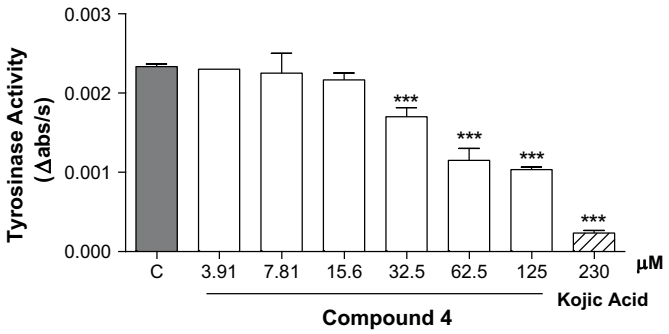


Fig. 2. Tyrosinase enzyme activity in the presence of compound **4**. C; tyrosinase enzyme activity without inhibitor. All values are mean ± S.E.M. of three different experiments. $^{***}P < 0.001$ significantly different from control group (first column).

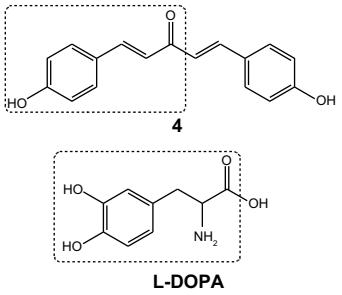


Fig. 3. The structure of tyrosinase substrates, L-DOPA and compound **4**.

rings, thus demonstrating their antioxidant effect. Moreover, the methoxyphenolic moiety has also been reported to be an essential structural feature contributing antioxidant activity [20]. Consistently, compounds **15** and **29** exhibited improved DPPH radical scavenging activity with IC_{50} values of 257.35 ± 23.3 μM and 316.64 ± 23.79 μM, respectively. The antioxidant activity of both **15** and **29** might be explained by the presence of 4-OH and 3-methoxy substitutions which enhanced their radical scavenging activity by the stabilized phenoxy radical.

On the other hand, tyrosinase plays a critical role in controlling melanin biosynthesis [21]. Hence, tyrosinase became an important target in treating pigmentation-related disorders and developing of new skin whitening agents. In the present study, most of the compounds did not show anti-tyrosinase property except for compound **4**, which has moderately inhibited tyrosinase enzyme activity in a dose-dependent manner (Fig. 2) with IC_{50} value of 79.05 ± 3.92 μM (Table 1). It was also suggested that the hydroxyl group at position 4 of both phenyl rings of compound **4** might be the important factor contributing to tyrosinase enzyme inhibition. Nerya et al. [8] had demonstrated that chalcone derivatives with hydroxyl group at position 4 (ring B) potentially inhibit tyrosinase enzyme activity. The tyrosinase inhibitory activity can also be explained by the resemblance of structure of compound **4** with tyrosinase substrate, L-DOPA (Fig. 3). Thus, compound **4** might be acting as a competitive inhibitor to L-DOPA. Further test has been performed to study the cytotoxicity effect of compound **4** on human epidermal melanocyte cell line (HEMn). Unfortunately, compound **4** exhibited high degree of cytotoxicity to HEMn (data not shown), thus denying the suitability of its use as cosmetic or therapeutic agent in humans. On the other hand, compounds **10**, **15**, **16**, **22**, and **25** inhibited less than 50% at highest concentration tested (IC_{50} values >250 μM).

4. Conclusion

The present results showed that a series of curcumin-like diarylpentanoid analogues were not significantly inhibiting NO scavenging, DPPH radical scavenging and tryrosinase enzyme activities. Among the **46** compounds tested, three curcumin-like diarylpentanoid analogues which are **2**, **13** and **33** showed more likely active in inhibitory action upon NO production in macrophage cells (RAW 264.7). The presence of 2,5-dimethoxylated phenyl rings on both compounds **13** and **33**, while 2-hydroxylated phenyl rings on compound **2** might become the main factors contributing to their potent anti-inflammatory property. It was suggested that the anti-inflammatory property of these compounds (**2**, **13**, **33**) might be due to the inhibition of target protein (iNOS) or its upstream regulatory protein [14], rather as radical scavengers or antioxidant agents. Thus, compounds **2**, **13** and **33** provide a useful starting point for the rational design of anti-inflammatory agents with improved pharmacokinetic and pharmacodynamic properties.

5. Experimental

5.1. Chemical syntheses

Analytical TLC was carried out on silica gel F_{254} precoated (0.2 mm thickness, Merck) plastic TLC sheets. The TLC plates were spotted with samples using a fine glass capillary tube and developed in a chromatographic tank saturated with solvent vapour at room temperature. Melting points were determined using a hot stage melting point apparatus equipped with a microscope, XSP-12 model 500X, and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity 500 spectrometer (Varian Inc., Palo Alto, CA) and measured at 500 MHz. Deuterated chloroform ($CDCl_3$), and acetone-d_6 were used as solvents. Chemical shifts were recorded in δ ppm. Mass spectra (MS) have been recorded on a Polaris Q ThermoFinnigan (San Jose, CA) with ionization induced by electron impacts (EIMS) at 70 eV.

5.1.1. General procedure for the preparation of analogues

A mixture of the aromatic aldehyde (20 mmol, 2 equiv) and the appropriate ketone (10 mmol, 1 equiv) were dissolved in 15 ml of ethanol in a single necked round bottomed flask and stirred for several minutes at 5 °C (ice bath). Into this solution 10 ml of a 40% NaOH solution in water was then added drop wise over several minutes. The mixture is then allowed to stir at room temperature for approximately 10 h. The reaction was neutralized with a dilute HCl solution to form a precipitate, which was then collected by suction filtration. The product, obtained after removal of the solvent under reduced pressure, was crystallized from an appropriate solvent.

5.1.2. 1,5-Bis(2-hydroxyphenyl)-1,4-pentadiene-3-one (Compound **2**)

Orange amorphous solid; Yield 75%; m.p. 155–157 °C; (lit. [13] m.p.155 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ in ppm: 8.09 (d, $J = 16.0$ Hz, 2H), 7.72 (dd, $J = 8.0$, 1.5 Hz, 2H), 7.34 (d, $J = 16.0$ Hz, 2H), 7.27 (td, $J = 8.5$, 1.5 Hz, 2H), 6.99 (d, $J = 8.5$ Hz, 2H), 6.93 (t, $J = 7.5$ Hz, 2H). MS (EI): $[M\text{-}H_2O]^+$ at m/z 248.

5.1.3. 1,5-Bis(4-hydroxyphenyl)-1,4-pentadiene-3-one (Compound **4**)

Orange amorphous solid; Yield 12%; m.p. 246–247 °C (lit. [13] m.p. 244–246 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ in ppm: 7.71 (d, $J = 16.0$ Hz, 2H), 7.63 (d, $J = 8.0$ Hz, 4H), 7.10 (d, $J = 16.0$ Hz, 2H), 6.93 (d, $J = 8.0$ Hz, 4H). MS (EI): $[M]^+$ at m/z 266.

5.1.4. 1,5-Bis(2,5-dimethoxyphenyl)-1,4-pentadiene-3-one (Compound **13**)

Yellow amorphous solid; Yield 90%; m.p. 104–105 °C (lit. [14] m.p. 105–106 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ in ppm: 7.98 (d, $J = 15.5$ Hz, 2H), 6.88 (d, $J = 15.5$ Hz, 2H), 6.84 (d, $J = 8.0$ Hz, 4H), 6.82 (s, 2H), 3.96 (s, 6H, OCH_3), 3.85 (s, 6H, OCH_3). MS (EI): $[M]^+$ at m/z 354.

5.1.5. 2,6-Bis(2,5-dimethoxybenzylidene)cyclohexanone (Compound **33**)

Yellow amorphous solid; Yield 75%; m.p. 118–120 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.88 (s, 2H), 7.01 (d, $J = 8{:}0$ Hz, 4H), 6.96 (s, 2H), 3.84 (s, 6H, OCH_3), 3.80 (s, 6H, OCH_3), 2.91 (t, $J = 6.0$ Hz, 4 H), 1.77 (q, $J = 6.0$ Hz, 2H). MS (EI): $[M]^+$ at m/z 394.

5.2. DPPH radical scavenging assay

The free radical scavenging activity assay was carried out according to Tagashira and Ohtake [22] with some modification.

Briefly, stock solution of test compound was prepared at 50 mM concentration in 100% DMSO. The solutions were serially diluted to different concentrations in a 96-well microtiter plate, from 7.81 to 500 μM. Then 5 μL of diphenylpicrylhydrazine radical (DPPH) solution (1 mg/ml dissolved in methanol) was then added to each well. The plate was shaken gently and incubated in the dark for 30 min. The absorbance was measured at 517 nm and percentage of total radical scavenging activity was calculated based on the formula:

$$\text{Percentage of total radical scavenging activity} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%$$

5.3. NO scavenging activity

The direct NO scavenging assay was carried out according to Mirkov et al. [23] with some modification. Briefly, 100 μL of SNP (10 mM) solution were incubated alone or in combination with different concentrations of test compounds (7.81–500 μM) for 60 min with light exposure. The nitrite levels of the mixture were then determined by Griess assay. NO scavenging activity was calculated following the formula:

$$\text{Percentage of NO scavenging activity} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%$$

5.4. Cell cultures and treatment

The RAW 264.7 cells (ATCC) were cultured in a plastic culture flask in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin under 5% CO_2 at 37 °C. Cells at confluency of 80–90% were scrapped out and seeded into 96-well plate at 5×10^4 cells/50 μL well. Attached cells were then induced with 100 U/ml of recombinant mouse interferon-gamma (IFN-γ) and 5 μg/ml of LPS (*Escherichia coli*, serotype 0111:B4) in the presence or absence of test compounds for 17–20 h. The NO production was then determined by Griess assay. NO inhibitory activity was calculated following the formula:

$$\text{NO inhibitory activity} = \frac{\left[\text{NO}_2^-\right]_{\text{control}} - \left[\text{NO}_2^-\right]_{\text{sample}}}{\left[\text{NO}_2^-\right]_{\text{control}}} \times 100\%$$

5.5. Griess assay

The NO scavenging and NO production in cell culture were determined by measuring the nitrite (NO_2^-) formation in the SNP solution mixtures and supernatants of spent cell culture media, respectively by using Griess assay [24]. Briefly, 50 μL of Griess Reagent (1% sulfanilamide and 0.1% naphtylethyenediamine dihydrochloride in 2.5% H_3PO_4) was added to 50 μL of SNP solution mixtures and/or cell culture supernatant, respectively. The colour density was measured at 550 nm using a microplate reader after 10 min incubation at room temperature.

5.6. Cell viability determination (MTT assay)

After removal of culture media, all wells were topped-up with 100 μL of DMEM, followed by the addition of 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 5 mg/ml). The cells were incubated under 5% CO_2 at 37 °C for 4 h. The formazan crystals formed were dissolved in DMSO and the absorbance was read at 570 nm using a microplate reader. Cell viability was calculated by following the formula:

$$\text{Percentage of cell viability} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%$$

5.7. Anti-tyrosinase assay

The inhibitory effect of diarylpentanoid derivatives on tyrosinase activity was carried out according to Khatib et al. [7] with some modification. Equal volume of phosphate buffer (50 mM) and tyrosinase (SIGMA) (333 units/ml) was added into 96-well microtiter plate. The test samples dissolved in absolute ethanol at concentration of 100 μM were then added into each well. After 5 min incubation, L-DOPA (6 mM) was added. The absorbance was measured at 492 nm at the intervals of 30 s for 10 min. Anti-tyrosinase activity was calculated by following the formula:

$$\text{Percentage of anti-tyrosinase activity} = \frac{\text{Slope}_{\text{control}} - \text{Slope}_{\text{sample}}}{\text{Slope}_{\text{control}}} \times 100\%$$

5.8. Statistical analysis

All the experiments are conducted three times and all data are presented as the mean ± S.E.M. Differences between groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett test. P values <0.05 were considered significant. The IC_{50} values were calculated using GraphPad Prism 5 software.

Acknowledgments

This study was financially supported by a grant from the Ministry of Higher Education, Malaysia under the Fundamental Research Grant Scheme (FRGS) (01-01-07-013FR). Ka-Heng Lee was a recipient of Graduate Research Fellowship (GRF) scheme supported by UPM. Post-Doctoral Fellowship awarded to Faridah Abas was highly appreciated.

References

[1] F. Aktan, Life Sci. 75 (2004) 639–653.
[2] A.B. Abramson, A.R. Amin, R.M. Clancy, M. Attur, Best Pract. Res. Clin. Rheumatol. 15 (2001) 831–845.
[3] J. Gao, D.C. Morrison, T.J. Parmely, S.W. Russell, W.J. Murphy, J. Biol. Chem. 272 (1997) 1226–1230.
[4] D. Sarkar, P.B. Fisher, Cancer Lett. 236 (2006) 13–23.
[5] M. Valko, D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, J. Telser, Int. J. Biochem. Cell Biol. 39 (2007) 44–48.
[6] D. Johar, J.C. Roth, G.H. Bay, J.N. Walker, T.J. Kroczak, M. Los, Rocz. Akad. Med. Bialymst. 49 (2004) 31–39.
[7] S. Khatib, O. Nerya, R. Musa, M. Shmuel, S. Tamir, J. Vaya, Bioorg. Med. Chem. 13 (2005) 433–441.
[8] O. Nerya, R. Musa, S. Khatib, S. Tamir, J. Vaya, Phytochemistry 65 (2004) 1389–1395.
[9] W.P. Steward, A.J. Gescher, Mol. Nutr. Food Res. 52 (2008) 1005–1009.
[10] A.K. Gupta, M.D. Gover, K. Nouri, S. Taylor, J. Am. Acad. Dermatol. 55 (2006) 10481065.
[11] Q.Y. Wei, J.P. Ma, Y.J. Cai, L. Yang, Z.L. Liu, J. Ethnopharmacol. 102 (2005) 177–184.
[12] H. Chandru, A.C. Sharada, B.K. bettadaiah, C.S. Ananda Kumar, K.S. Rangappa, Sunila, K. Jayashree, Bioorg. Med. Chem. 15 (2007) 7696–7703.
[13] B.K. Adam, E.M. Ferstl, M.C. Davis, S.M. Herold, S. Kurtkaya, R.F. Camalier, M.G. Hollingshead, G. Kaur, E.A. Sausville, F.R. Rickles, J.P. Snyder, D.C. Liotta, M. Shoji, Bioorg. Med. Chem. 12 (2004) 3871–3883.
[14] W.M. Weber, L.A. Hunsaker, N. Roybal, E.V. Bobrovnikova-Marjon, S.F. Abcouwer, R.E. Royer, L.M. Deck, D.L. Vander Jagt, Bioorg. Med. Chem. 14 (2006) 2450–2461.

[15] H.H. Ko, L.T. Tsao, K.L. Yu, C.T. Liu, J.P. Wang, C.N. Lin, Bioorg. Med. Chem. 11 (2003) 105–111.
[16] J. Rojas, J.N. Domínguez, J.E. Charris, G. Lobo, M. Payá, M.L. Ferrándiz, Eur. J. Med. Chem. 37 (2002) 699–705.
[17] V. Afonso, R. Champy, D. Mitovic, P. Collin, A. Lomri, Joint Bone Spine 74 (2007) 324–329.
[18] P. Molyneux, Songklanakarin, J. Sci. Technol. 26 (2) (2004) 211–219.
[19] M. Srinivasan, A.R. Sudheer, V.P. Menon, J. Clin. Biochem. Nutr. 40 (2007) 92–100.
[20] C. Selvam, S.M. Jachak, R. Thilagavathi, A.K. Chakraborti, Bioorg. Med. Chem. 15 (2005) 1793–1797.
[21] K. Iozumi, G.E. Hoganson, R. Pennella, M.A. Everett, B.B. Fuller, J. Invest. Dermatol. 100 (1993) 806–811.
[22] M. Tagashira, Y. Ohtake, Planta Med. 64 (1998) 555–558.
[23] S.M. Mirkov, A.N. Djordjevic, N.L. Andric, S.A. Andric, T.S. Kostic, G.M. Bogdanovic, M.B. Vojinovic-Miloradov, R.Z. Kovacevic, Nitric Oxide 11 (2004) 201–207.
[24] D. Tsikas, J. Chromatogr. B 51 (2007) 51–70.