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SYNTHESIS AND CYTOTOXIC ACTIVITY OF CURCUMIN ANALOGUES

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ABSTRAK

Tiga senyawa analog kurkumin (1-3) disintesis melalui reaksi 1-metilpiperidin-4-one dan turunan metoksi-benzaldehida pada iradiasi gelombang micro. Natrium hidroksida digunakan sebagai katalis. Struktur kimia dari analog kurkumin tersebut telah ditetapkan berdasarkan interpretasi data spektroskopi. Semua senyawa analog dievaluasi efek sitotoksiknya terhadap sel murine leukemia P-388. Senyawa 1-3 menunjukan aktivitas sitotoksik yang lemah dengan nilai IC_{50} berturut-turut 6,5; 6,0 dan 18,3 mg/ mL.

Kata kunci: kurkuminoid, aktivitas sitotoksik, 1-metil-piperidin-14-satu, methoxybenzaldehyde, iradiasi gelombang mikro.

ABSTRACT

Three curcumin analogues (1-3) were synthesized via the reaction of 1-methylpiperidin-4-one and methoxybenzaldehyde derivatives under microwave irradiation. Sodium hydroxide was used as a catalyst. The chemical structures of the curcumin analogeus were elucidated on the basis of spectroscopic data interpretation. All of the analog compounds were evaluated for their cytotoxic effects against P-388 murine leukemia cells. Compounds 1-3 displayed weak cytotoxic activity with IC_{50} values of 6.5, 6.0 and 18.3 µg/mL, respectively.

Keywords: curcuminoid, cytotoxic activity, 1-methyl-piperidine-14-one, methoxybenzaldehyde, microwave irradiation.

INTRODUCTION

Curcumin is found in various types of *Curcuma*-genera and is the major pigment contained in the turmeric plant (*Curcuma longa*). Some of the compounds found in turmeric curcuminoids include curcumin, 4-demethoxycurcumin and bisdemethoxy-curcumin all of which are diarylheptanoid analogues compounds. Also present is a curcuminoid derivative that is not symmetric, called dihydrocurcumin (Rostom et al., 2009). Chemically, curcumin is a phenolic secondary metabolite known to possess anti-inflammatory activity (Dandia et al., 2012), antioxidant (Kumar et al., 2007), antiviral, anti-infective and anti-allergic (Suzuki et al., 2005), as well as anti-HIV (Handler et al., 2007) and anti-cancer (Babasaheb et al., 2010 and Wichitnithad et al., 2011) properties.

Based on the potent biological activities, crucumin is attracting more interest as a model for new target compounds to be synthesized. Isolation of curcuminoids from natural materials found in small quantities is low-yeilding (3-5% of the dry-weight), and the curcuminoids thus obtained possess limited structural variation. Indeed, this method presents an obstacle to optimize the function of curcumin (Rajesh et al., 2012).Therefore, synthesis of curcumin derivatives should be conducted in the laboratory to obtain a reasonable amount of material as well as a wider variety of structures.

In general, curcumin can be made in a variety of method some of which is through the condensation of an aldehyde with a ketone, either in acid or alkaline conditions. This method is known as aldolcondensation reaction or, more specifically called the Claisen-Schmidt condensation reaction. Aldol condensation reaction are very popular and widely used in the formation of carbon-carbon bonds, because the reaction is simple, with readily available materials and is also known as environmentally friendly. Acid catalysts are commonly used in the aldol condensation reaction, such as dilute hydrochloric acid, while the base catalyzed conditions use sodium hydroxide, sodium methoxide, or lithium methoxide, either by used a solvent such as ethanol (Carey 1983), or without solvent (Palleros, 2000). Besides the above reasons, another attractive feature of this method is that it allows a combinatorial chemistry approach. Thus, this method can be used to create derivatives of curcumin with a variety of substituents on the benzene ring so that curcumin analog libraries can be rapidly produced. In continuation of our interest in the reaction of 1-methylpiperidin-4-one and methoxybenzaldehyde for the synthesis of curcumin analogs under microwave conditions, we report herein the microwave-assisted synthesis of curcumin analogues (**1-3**) and their cytotoxic activity against P-388 murine leukemia cells.

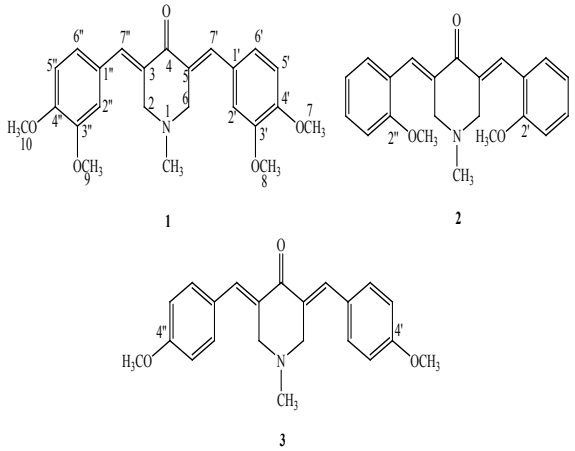


Figure 1. Chemical structures of curcumin analogues, **1–3**.

MATERIAL AND METHOD

General

Melting points were measured on an Electro-thermal melting point apparatus and are uncorrected. The UV spectra were obtained on a UV Ultraspec 3000 Pro spectrophotometer. The IR spectra were recorded on a Perkin-Elmer 1760X FT-IR in KBr. The mass spectra were recorded with a JEOL JMS-700 and a SynaptG2 mass spectrometer and a Mariner Biospectrometry-Finnigan and a Waters LCT Premier XE mass spectrometer instruments. ^{1}H and ^{13}C NMR spectra were recorded with an Agilent DD2 system operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual (δ_H 7.26) and deuterated solvent (δ_C 77.0) peaks of $CDCl_3$ as reference standards. For synthesis used Mas-II Sineo Microwave. Chromatographic separations were carried out on silica gel 60 (Merck). TLC plates were precoatedwithsilica GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating.

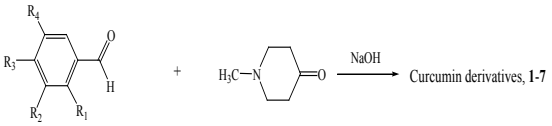
Procedure for preparation of curcumin derivatives

*(3E,5E)-3,5-bis(3,4-dimethoxybenzyliden)-1-methylpiperidin-4-one (**1**)*: A total of (0.01 mol) 1-methyl-4-piperidinone mixed with 3,4-dimethoxybenzaldehyde (0.02 mol), add 40% sodium hydroxide (0.7 mL) and 10 mL of ethanol absolute into round bottom flask that has been include magnetic stirrer and condenser. The reaction mixture was put in the microwave on the condition of the temperature 60 ^{o}C for ten minutes. The reaction product was cooled and added to 50 mL of distilled water then filtere d using a Buchner funnel. Further more the precipitate was washed with 50 mL of distilled water and 50 mL of cold *n*-hexane then dried at 40^{0}C for 24 hours.The crude product was recrystalized in *n*-hexane-ethyl acetate to yield a curcumin derivative **1**. The purity of the synthetic product **1**, were analyzed by TLC and HPLC analysis. Compound **1** was obtained as yellowish needle-like crystals; Yield 64%; m.p. 161-162 ^{o}C; UV (λ_{max} in MeOH) 260 nm (ε 4,700), 380 nm (ε 9,600); IR (KBr) ν_{max} 1,727, 1,599 and 1,252 cm^{-1}.^{1}H-NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-ESI-TOFMS calculated for $C_{24}H_{27}NO_5[M+H]^+$*m/*z410.1967, found *m/*z 409.1957.

*(3E,5E)-3,5-bis(2-methoxybenzylidene)-1-methylpiperidin-4-one(**2**)*: A total of (0.01 mol) 1-methyl-4-piperidinone mixed with 2-methoxybenzaldehyde (0.02 mol), add 40% sodium hydroxide (0.7 mL) and 10 mL of ethanol absolute into round bottom flask that has been include magnetic stirrer and condenser. The reaction mixture was worked up as described for compound **1** and yield synthetic product **2**. Compound 2 was obtained as *y*ellowish needle-like crystals; Yield 61%; m.p. 86-88^{o}C; UV (λ_{max} in MeOH) 270 nm (ε 3,800), 350 nm (ε 5,600); IR (KBr) ν_{max} 1,720, 1,596 and 1,250 cm^{-1}.^{1}H-NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HRESI-TOFMS calculated for $C_{24}H_{27}NO_5[M+H]^+$ *m/z* 350.1756, found *m/z* 349. 721 *(3E,5E)-3,5-bis(4-methoxybenzylidene)-1-methylpiperidin-4-one(**3**)*:A total of (0.01 mol) 1-methyl-4-piperidinon mixed with 4-methoxybenzaldehyde (0.02 mol), add 40% sodium hydroxide (0.7 mL) and 10 mL of ethanol absolute into round bottom flask that has been include magnetic stirrer and condenser. The reaction mixture was worked up as described for compound **1** and yield synthetic product **3**. Compound **3** was obtained as yellowish needle-crystals; Yield 59%; m.p. 184-186 ^{o}C; UV (λ_{max} in MeOH) 270 nm (ε 3,900), 380 nm (ε 7,400); IR (KBr) ν_{max} 1,710, 1,570 and 1,210 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C-NMR ($CDCl_3$,125 MHz), see Table 1; HR-ESI-TOFMScalculated for $C_{24}H_{27}NO_5[M+H]^+$ *m/z* 350.1956, found *m/z* 349.1721.

In vitro Cytotoxicity Activity against P-388 Murine leukimia cells

The P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3 x 10^{4} cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30 - 7.65). Control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h incubation was conducted. Optical density was read by using a micro plate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (μg/mL). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.



1. R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=H
2. R_1=OCH_3, R_2=H, R_3=H, R_4=H
3. R_1=H, R_2=H, R_3=OCH_3, R_4=H
4. R_1=OCH_3, R_2=H, R_3=OCH_3, R_4=OCH_3
5. R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3
6. R_1=OCH_3, R_2=OCH_3, R_3=H, R_4=H
7. R_1=H, R_2=OCH_3, R_3=H, R_4=H

Figure 2. Reaction for the preparation of curcuminanalogs

RESULTS AND DISCUSSION

The curcumin analogs, **1-3**, were synthesized by microwave-assisted reaction of 1-methylpiperidin-4-one with the methoxy-substituted benzaldehyde in the presence of sodium hydroxide as the catalyst using

combinatorial synthesis as shown in Figure 2. The general experimental procedure involved irradiating the mixture with microwaves at a temperature of 60 °C for 10 minutes.

The reaction product was cooled and added to50 mL of distilled water then filtered using a Buchner funnel. Furthermore the precipitate was washed with 50 mL of distilled water and 50 mL of cold *n*-hexane then dried at 40^{0}C for 24 hours.The crude product was recrystalized in *n*-hexane-ethyl acetate to yield a curcumin analogues **1**-**3**. The purities of the synthetic product were analyzed by TLC and HPLC.

Curcumin analog **1** was obtained as a yellowish needle-like crystals, m.p. 161–162 °C, from *n*-hexane-EtOAc. Its molecular formula was established to be $C_{24}H_{27}NO_5$ by HR-ESI-TOFMSdata (*m/*z 410.1957, $[M+H]^+$), which combined with the ^{1}H- and ^{13}C-NMR spectral data (Table 1), thus indicated 12 degrees of unsaturation. The UV spectrum showed an absorption maximum at 260 (ε 4,700) and 380 nm (ε 9,600), indicating the presence of curcumin skeleton. The IR spectrum showed bands which were ascribable to α, β-unsaturated ketone (ν_{max} 1,727 cm^{-1}), conjugated double bond(ν_{max}1,599 cm^{-1}) and an ether (ν_{max}1,252 cm^{-1}) functionalities. The ^{1}H-NMR spectrum showed the presence of a singlet signal at δ_H 2.47, (3H) from *N*-methyl group, a singlet signal at δ_H 3.75 (4H) from two methylene groups and two singlet signals at δ_H 3.89 (6H) and 3.91(6H) from four methoxyl groups. A singlet appeared in the downfield region at δ_H 7.75 (2H) that was ascribed to the two olefinic protons. A detailed analysis of ^{1}H-NMR spectrum showed characteristic signals due to two trisubstituted benzene rings at δ_H 7.00 (d, 2H, *J*=6.2 Hz), 6.98 (d, 2H, *J*=2.2 Hz), and δ 6.91 (dd, 2H, *J*=2.2, 6.2 Hz). The ^{13}C-NMR spectra, revealed 12 carbon signals, including characteristic signals due to trisubstituted benzene ring at [δ_C 123.7, 128.2, 131.5, 136.2, 148.7, and 149.9], a conjugated ketone (δ_C186.6), two methoxyl groups (δ_C 55.9 and 57.1), one methylene signal at δ_C 55.8, and one *N*-methyl signal at δ_C 45.7, thus suggesting that **1** possesed a tricyclic structure with a 1-methylpiperidin-4-one moiety and two similar unit structure. These observations along with the similarity of spectral data between **1** and curcumin analog previously reported (Syam et al., 2012; Elias et al., 2008; and Mohamed et al., 2012), let us determine**1**to be a (3*E*,5*E*)-3,5-bis(3,4-dimethoxybenzyliden)-1-methylpiperidin-4-one).

Curcumin analogues **2** was obtained as a yellowish needle-like crystals, m.p. 86-88^{o}C, from *n*-hexane-EtOAc. Its molecular formula was established to be $C_{22}H_{23}NO_3$ by HR-ESI-TOFMS data (*m/*z 350.1721, $[M+H]^+$), which combined with the ^{1}H- and ^{13}C-NMR spectral data (Table 1), thus indicated 12 degrees of unsaturation. The UV spectrum showed an absorption maximum at 270 (ε 3,800) and 350 nm (ε 5,600), indicating the presence of on α, β-unsaturated ketone from the curcumin skeleton. The IR spectrum showed bands which were ascribable to α, β-unsaturated ketone (ν_{max} 1,720 cm^{-1}), conjugated double bond (ν_{max}1,596 cm^{-1}) and an ether ($_{max}$1,250 cm^{-1}) functionalities. The ^{1}H- and ^{13}C-NMR (Table 1) spectra of **2** were quite similar to those of **1**, except for the absence of one of the methoxy signal at [δ_C 57.1, δ_H 3.91 (6H, s)] and appearance of two substituted aromatic proton signals at δ_H7.20 (2H, d, 7.2), 7.33 (2H, t, 6.4), 6.98 (2H, t, 7.2), and 6.91 (2H, d, 6.4), suggesting that compound **2** was a 2-methoxy derivative of curcumin and it was thus named (3*E*,5*E*)-3,5-bis(2-methoxybenzylidene)-1-methylpiperidin-4-one).

Table 1. NMR spectral data for compounds **1-3**[a]

Position	1		2		3	
	^{13}C NMR δ_C (ppm)	^{1}H NMR δ_H (integral mult., *J* Hz)	^{13}C NMR δ_C(ppm)	^{1}H NMR δ_H (integralmult.,*J* Hz)	^{13}C NMR δ_C (ppm)	^{1}H NMR δ_H (integralmult., *J* Hz)
2/6	55.8	3.77 (4H, s)	55.5	3.65 (4H, s)	55.3	3.70 (2H, s)
3/5	131.5	-	132.6	-	132.3	-
4	186.6	-	187.0	-	186.8	-
1'/1''	128.2	-	129.6	-	131.3	-
2'/2''	110.9	7.00 (2H, d, 2.2)	158.5	-	128.0	6.94 (4H, d, 6.7)
3'/3''	149.9	-	110.8	6.92 (2H, d, 6.4)	114.1	7.37 (4H, d, 6.7)
4'/4''	148.7	-	130.4	7.33 (2H, t, 6.4, 7.2)	160.2	-
5'/5''	113.8	6.92 (2H, d, 6.2)	124.5	6.98 (2H, t, 7.2)	114.1	7.37 (4H, d, 6.7)
6'/6''	123.7	6.98 (2H, dd, 2.2, 6.2)	130.3	7.20 (2H, d, 7.2)	128.0	6.94 (4H, d, 6.7)
7'/7''	136.2	7.75 (2H, s)	133.0	8.06 (2H, s)	116.1	8.02 (2H, s)
N-CH_3	45.7	2.47 (3H, s)	45.5	2.40 (3H, s)	45.2	2.40 (3H, s)
2'/2''-OCH_3	-	-	57.1	3.85 (6H, s)	56.0	3.84 (6H, s)
3'/3''-OCH_3	57.1	3.91(6H, s)	-	-	-	-
4'/4''-OCH_3	55.9	3.89 (6H, s)	-	-	56.8	3.85 (6H, s)
5'/5''-OCH_3	-	-	-	-	57.5	3.93 (6H, s)

[a]Taken in $CDCl_3$ at 500 MHz for ^{1}H and at 125 MHz for ^{13}C.

Curcumin analog **3** was obtained as a yellowish needle-like crystals, m.p. 120-122°C, from *n*-hexane-EtOAc. Its molecular formula was established to be $C_{26}H_{31}NO_7$ by HR-ESI-TOFMS data (*m/z* 350.1721, $[M+H]^+$), which combined with the NMR spectral data (Table 1), thus indicated 12 degrees of unsaturation. The UV spectrum showed an absorption maximum at 270 (ε 3,900) and 380 nm (ε 7,400), indicating the presence of α,β-unsaturated ketone from the curcumin skeleton. The IR spectrum showed bands which were ascribable to α,β-unsaturated ketone (ν_{max} 1,710 cm^{-1}), conjugated double bond (ν_{max}1,570 cm^{-1}) and an ether (ν_{max}1,210 cm^{-1}) functionalities. The NMR spectra of **3** (Table 1) were quite similar to those of **1**, except for the absence one of the methoxy signal at [δ_C 57.1, δ_H 3.91 (6H, s)] and appearance of an AB aromatic proton signals at δ_H6.94 (4H, d, 6.7), and 7.37 (4H, d, 6.4), suggesting that compound **3** was a 4-methoxy analog of curcumin and named (3*E*,5*E*)-3,5-bis(4-methoxybenzylidene)-1-methylpiperidin-4-one).

The cytotoxicity of three curcumin analogs (**1-3**) were assessed on P-388 murine leukimia cells using a 48 h continuous exposure MTT assay technique (Sahidin et al., 2005 and Alley et al., 1988). The experiments were performed in triplicate and the concentrations at which 50% of cell growth was inhibited (IC_{50}, µg/mL) were determined for each compound. The results are presented in Tables 1. For comparison, the IC_{50} values of artonin E was obtained under same conditions, was 0.3 µg/mL.Compounds **1-3** displayed weak cytotoxic activity with IC_{50} values of 6.5, 6.0 and 18.3 µg/mL, respectively.

CONCLUSIONS

Three curcumin analogues have been synthesized by microwave irradiation. This work showed a rapid and efficient method for synthesis of some curcumin analogues via the reaction of 1-methylpiperidin-4-one and methoxybenzaldehyde analogues. All of analogues compounds showed weak activity against P-388 murine leukimia cell and curcumin analog compound having a 2-methoxyl group showed strongest activity.

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