Synthesis of N-methyl-4-piperidone Curcumin Analogues and Their Cytotoxicity Activity against T47D Cell Lines

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ABSTRACT

*Three piperidone curcumin analogues (N-methyl-(3E,5E)-3,5-bis-(2-chlorobenzylidene)-4-piperidone (**1**), N-methyl-(3E,5E)-3,5-bis-(3-bromobenzylidene)-4-piperidone (**2**) and N-methyl-(3E,5E)-3,5-bis-(4-chlorobenzylidene)-4-piperidone (**3**)) were synthesized from N-methyl-4-piperidone with halogenbenzaldehyde, 2-chlorobenzaldehyde, 3-bromobenzaldehyde and 4-chlorobenzaldehyde. The Claisen-Schmidt condensation reaction was used in alkali condition with combinatorial. All the compounds showed light yellow needle, light yellow powder, and yellow crystal form with percentage of yield 39, 66, and 40%, respectively. All the structure compounds were confirmed by using UV, IR, ^{13}C-NMR, ^{1}H-NMR and MS. Apart from that, the cytotoxicity results against breast cancer cell (T47D) showed strong to moderate activity with the IC_{50} value 8, 4, and 45 µg/mL, respectively.*

Keywords: *Curcumin; condensation; Claisen-Schmidt; cytotoxicity T47D*

ABSTRAK

*Tiga senyawa analog kurkumin turunan piperidon telah berhasil disintesis dari N-metil-4-piperidon dengan turunan halogenbenzaldehid, 2-klorobenzaldehid, 3-bromobenzaldehid dan 4-klorobenzaldehid melalui reaksi kondensasi Claisen-Schmidt dalam suasana basa secara kombinatorial. Analog kurkumin tersebut adalah N-metil-(3E,5E)-3,5-bis-(2,klorobenzaldehid)-4-piperidon (**1**), N-metil-(3E,5E)-3,5-bis-(3-bromobenzaldehid)-4-piperidon (**2**) dan N-metil-(3E,5E)-3,5-bis-(4-klorobenzaldehid)-4-piperidon (**3**). Ketiga senyawa tersebut memberikan bentuk dan warna berturut-turut kristal jarum kuning muda, serbuk kuning muda dan kristal kuning muda, rendemen 39, 66, dan 40. Semua senyawa tersebut telah dikonfirmasi strukturnya melalui analisis spektroskopi UV, IR, ^{13}C-NMR, ^{1}H-NMR dan MS. Uji aktivitas sitotoksisitas terhadap sel kanker payudara T47D memberikan nilai IC_{50} sebesar 8, 4, dan 45 µg/mL masing-masing untuk senyawa **1**, **2**, dan **3**.*

Kata Kunci: *kurkumin; kondensasi; Claisen-Schmidt; sitotoksisitas T47D*

INTRODUCTION

Curcumin is found in various types of *Curcuma* genera and is the major pigment contained in the turmeric plant (*Curcuma longa*). Curcumin, 4-demethoxy curcumin, bisdemethoxycurcumin, and dihydrocurcumin are found in turmeric [1]. Curcumin, a phenolic secondary metabolite, is believed to have anti-inflammatory activity [2], antioxidant [3], antiviral, anti-infective and anti-allergic [4], as well as anti-HIV [5], and anti-cancer [6], and [7] properties.

Based on its biological activities, curcumin 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-yield (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 [8]. Therefore, synthesis of curcumin derivatives might 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 methods one of which is through the condensation of an aldehyde with a ketone, either in acid or alkaline conditions. This method is known as aldol condensation 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

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a solvent such as ethanol [9], or without solvent. 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 *N*-methylpiperidin-4-one and (2-clorobenzaldehyde, 3-bromobenzaldehyde and 4-clorobenzaldehyde) for the synthesis of curcumin analogs under microwave conditions, we report herein the microwave-assisted synthesis of curcumin derivatives (**1**-**3**) and their cytotoxic activity against breast cancer cell (T47D). During the course of our continuing search for novel curcumin derivatives, we synthesized and characterized of 3,4-Bis-(2-hydroxybenzylidene)-piperidin-4-one [10].

EXPERIMENTAL SECTION

Materials

Chemicals were 4-methil-piperidone (Merck), 2-chloro-benzaldehyde, 3-bromo-benzaldehyde, 4-chloro-benzaldehyde (Aldrich), sodium hydroxide (Merck), and ethanol absolute (Merck).

Instrumentation

Microwave Mass II (Sineo Microwave Chemistry) was used for the synthesis condition. HPLC (Shimadzu Le Solution), FTIR (Shimadzu, IR Prestige-21), MS (Waters LCT Premier XE ES1-10F) were used for purification of the compounds. ^{1}H and ^{13}C-NMR were recorded by using Varian 500 MHz.

Procedure

Synthesis analogues of curcumin

All the compounds were synthesized by using aldol condensation reaction with sodium hydroxide as catalyst and ethanol as solvent (Carey and Sandberg, 1983). 0.01 mol 4 piperidone derivative was mixed with sodium hydroxide octahydrate 0.7 mL (40%) followed by 10 mL absolute ethanol in conical flask. Aldehyde derivative (0.02 mol in ethanol 2 mL) was added into mixture and finally placed into microwave and the reaction was run for 10 min. Solid product obtained was cooled and 50 mL HCl 1N was added subsequently. The mixture was than filtered by using Buchner funnel and washed with 50 mL distilled water, 50 mL hexane respectively and finally was dried at 40 °C for 24 H.

RESULT AND DISCUSSION

N-methyl-(3*E*,5*E*)-3,5-bis-(2-chlorobenzylidene)-4-piperidone (1)

Like yellow crystal, 38.90% yield, melting point: 138-140 °C, R_f = 0.67 (*n*-hexane : EtOAc = 6:4), HPLC, t_R = 16.9 min, UV (λ_{max} MeOH): 205 nm (e 5,600), 239 nm (e 3,800) and 310 nm (e 3,900). IR (KBr) ν_{max}: 3408; 3065; 2975; 1674 and 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) (Table 2). HR-ESI-TOFMS : *m/z* 358.0776 $[M+H]^+$, $C_{20}H_{17}Cl_2NO$ *m/z* 357.0657.

Compound **1** was found as *N*-methyl-(3*E*,5*E*)-3,5-bis-(2-chlorobenzylidene)-4-piperidone with chemical formula $C_{20}H_{17}Cl_2NO$ and molecular weight as 358.0776 $[M+H]^+$ from HR-ESI-TOFMS showed the presence of ion molecule peaks at 20 and 60% intensity with m/z 361 and 363 for ^{35}Cl and ^{37}Cl. This compound was assigned from ^{1}H-NMR and ^{13}C-NMR (Table 2). From ^{1}H-NMR spectra showed the presence of *N*-methyl signal [δ_H 2.37 (3H, s)], 2 methylene [δ_H 3.61 (4H, s)], 2 olefinic proton at down field [δ_H 8.0 (2H, s)] and 8 aromatic proton from 2 substituted aromatic ring [δ_H 7.24 (2H, dd, *J* = 8.0; 1.5 Hz), δ_H 7.30 (2H, d, *J* = 7.5 Hz), δ_H 7.31 (2H, dd, *J* = 8.0; 1.5 Hz) and δ_H 7.46 (dd, *J* = 8.0; 1.5 Hz). ^{13}C-NMR spectra showed 20 carbon which was consist of 2 methylene carbons (δ_C 56.7), 1 conjugated carbon (δ_C 186.1), *N*-methyl signal (δ_C 45.5), 10 sp^2 carbons, and 6 quaternary carbons (Table 2).

The saturated degree point was calculated as 9 out of 12 degrees. The remaining number was matched with tricyclic of symmetrical monoketone curcumin [11]. To determine the moiety position from the compound, HMBC was applied the results showed correlation between olefinic proton at δ_H 8.0 with C-4 (δ_C 186.1), C-2 (δ_C 56.7), C-3/5 (δ_C 134.3) and C-1′/1′′ (δ_C 133.6), while methylene proton (δ_H 3,61) showed correlation with C-3/5 (δ_C 134.3) and C-4 (δ_C 186.1). This result suggested α, β unsaturated ketone at C-7′, C-3/5 and C-4.

Chlorine position at both disubstitution aromatic rings was determined from correlation between olefinic

Table 1. Cytotoxic activity against breast cancer cell (T47D)

No	Compound	IC_{50} (µg/mL)
1	*N*-metil-(3*E*,5*E*)-3,5-bis-(2-klorobenziliden)-4-piperidon (**1**)	8
2	*N*-metil-(3*E*,5*E*)-3,5-bis-(3-bromobenziliden)-4-piperidon (**2**)	4
3	*N*-metil-(3*E*,5*E*)-3,5-bis-(4-klorobenziliden)-4-piperidon (**3**)	45

Table 2. NMR data of **1, 2** and **3**

No		1		2		3	
	Position	**^{1}H-NMR**	**^{13}C-NMR (δ_C ppm)**	**^{1}H-NMR**	**^{13}C-NMR (δ_C ppm)**	**^{1}H-NMR**	**^{13}C-NMR (δ_C ppm)**
1	2/6	3.61 (4H, s)	56.7	3.73 (4H, s)	56.8	3.71 (4H, d, 1.5)	56.9
2	3/5	-	134.3	-	134.0	-	133.4
3	4	-	186.1	-	186.4	-	186.4
4	1'/1''	-	133.6	-	137.2	-	133.6
5	2'/2''	-	135.2	7.52 (2H, s)	132.9	7.38 (2H, d, 8.5)	128.8
6	3'/3''	7.24 (2H, dd, 7.5;1.5)	130.3	-	122.7	7.31 (2H, d, 8.5)	131.6
7	4'/4''	7.30 (2H, d, 7.5)	130.0	7.5 (2H, dd, 9.75;2.5)	131.9	-	135.1
8	5'/5''	7.31 (2H, dd, 8.0; 1.5)	126.4	7.30 (2H, d, 6)	130.0	7.31 (2H, d, 8.5)	131.6
9	6'/6''	7.46 (2H, dd, 8.0; 1.5)	129.9	7.30 (2H, d, 6)	128.8	7.38 (2H, d, 8.5)	128.8
10	7'/7''	8.00 (2H, s)	134.0	7.71 (2H, s)	135.0	7.73 (2H, s)	135.1
11	*N*-CH_3	2.37 (3H, s)	45.5	2.47 (3H, s)	45.8	2.46 (3H, s)	45.9

[a]In $CDCl_3$ at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR

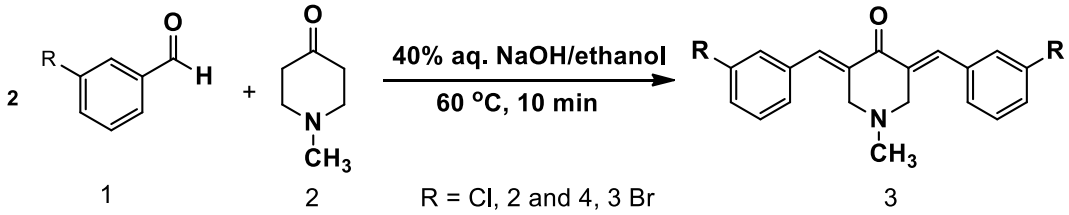

Fig 1. The synthesis of *N*-methyl-(3*E*,5*E*-bis(R-benzylidene)-4-piperidone

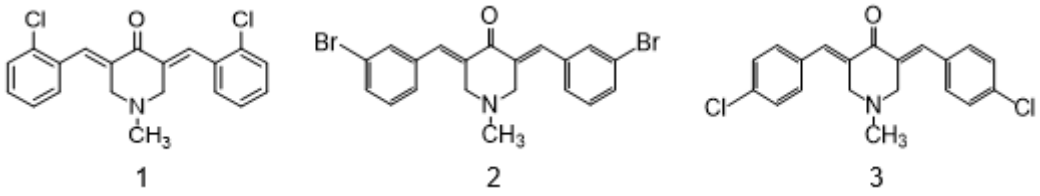

Fig 2. Structure of compound **1**, **2**, and **3**

proton at δ_H 8.0 with C-1´/1´´ (δ_C 133.6), C-2´/2´´ (δ_C 135.2), aromatic proton at δ_H 7.3 with C-1´/1´´ (δ_C 133.6) and C5´/5´´ (δ_C 126.4), aromatic proton at δ_H 7.24 with C-2´/2´´ (δ_C 135.2), and C-1´/1´´ (δ_C 133.6). These results suggested the chlorine position at *ortho* position. This suggestion was supported with coupling constant from aromatic proton. Compound **1** structure was confirmed with single X-ray crystal diffraction and ORTEP diagram (Fig. 2.).

N-methyl-(3*E*,5*E*)-3,5-bis-(3-bromobenzylidene)-4-piperidone (2)

Like yellow powder, 65.60% yield, melting point: 129-130 °C, R_f = 0.43 (*n*-hexane : EtOAc = 7:3), HPLC, t_R = 18.8 min, UV (λ_{max} MeOH): 324 nm (e 3,800), 238 nm (e 5,600) and 210 nm (e 5,600). IR (KBr) ν_{max}: 3432; 2927; 1668 and 1613 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) (Table 2). HR-ESI-TOFMS : *m/*z 447.9742 $[M+H]^+$, $C_{20}H_{17}Br_2NO$ *m/z* 447.9737.

Compound **2** was found as *N*-methyl-(3*E*,5*E*)-3,5-bis-(3-bromobenzylidene)-4-piperidone with chemical formula $C_{20}H_{17}Br_2NO$ and molecular weight as 467.9677 $[M+H]^+$ from HR-ESI-TOFMS and showed the presence of ion molecule peaks at 45 and 55% intensity with m/z 445 and 447 for ^{79}Br and ^{81}Br. This compound was assigned from ^{1}H-NMR and ^{13}C-NMR (Table 2). From ^{1}H-NMR spectra showed the presence of *N*-methyl signal [δ_H 2.47 (3H, s)], 2 methylene [δ_H 3.73 (4H, s)], 2 olefinic proton at down field [δ_H 7.71 (2H, s)] and 8 aromatic proton from 2 substituted aromatic ring [δ_H 7.52 (2H, s), δ_H 7.50 (2H, dd, *J* = 9.7; 2.5 Hz), δ_H 7.30 (2H, dd, *J* = 6 Hz) and δ_H 7.46 (dd, *J* = 8.0; 1.5 Hz). ^{13}C-NMR spectra showed 20 carbon which was consist of 2 methylene carbons (δ_C 56.7), 1 conjugated carbon (δ_C 186.4), *N*-methyl signal (δ_C 45.8), and 16 sp^2 carbons (Table 2).

The saturated degree point was calculated as 9 out of 12 degrees. The remaining number was matched with tricyclic of symmetrical monoketone curcumin [11]. The bromine at both aromatic rings was determined

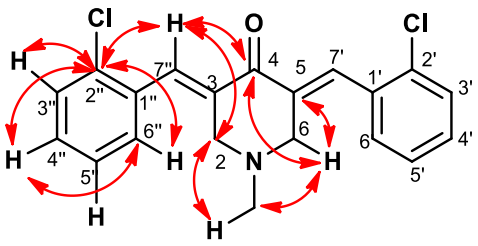

Fig 3. HMBC correlation of compound **1**

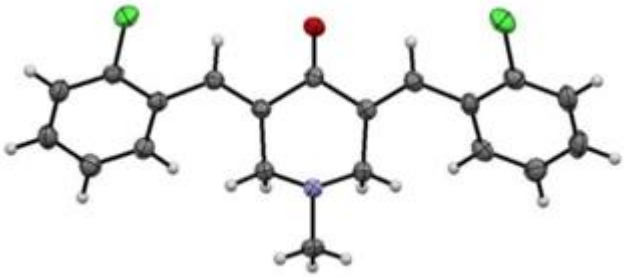

Fig 4. ORTEP of compound **1**

based on coupling constant as *meta* position. The comparison of NMR data from compound **2** with *N*-methyl-(3*E*,5*E*)-3,5-bis-(3-bromobenziliden)-4-piperidone [11-13] showed similarity.

N-methyl-(3*E*,5*E*)-3,5-bis-(4-chlorobenzylidene)-4-piperidone (3)

Yellow crystal, 39.80% yield, melting point: 180-182 °C, R_f = 0.40 (*n*-hexane : EtOAc = 8:2), HPLC, t_R = 17.8 min, UV (λ_{max} MeOH): 202 nm (e 5,600), 236 nm (e 5,600) and 333 nm (e 5,600). IR (KBr) ν_{max}: 3659; 3432; 2936; 1672 and 1613 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) (Table 2). HR-ESI-TOFMS : *m/z* 358.0776 $[M+H]^+$, $C_{20}H_{17}Cl_2NO$ *m/z* 357.0687.

Compound **3** was found as *N*-methyl-(3*E*,5*E*)-3,5-bis-(4-chlorobenzylidene)-4-piperidone with chemical formula $C_{20}H_{17}Cl_2NO$ and molecular weight as 358.0776 $[M+H]^+$ from HR-ESI-TOFMS showed the presence of ion molecule peaks at 20 and 60% intensity with m/z 361 and 363 for ^{35}Cl and ^{37}Cl. The comparison NMR spectra from compound **3** and **1** showed the similarity. The *para* position of chlorine in compound **3** was assigned from proton NMR at aromatic proton [δ_H 7.31 (4H, d, *J* = 8.5 Hz) and δ_H 7.38 (4H, d, *J* = 8.5 Hz)].

Cytotoxic Activity against Breast Cancer Cell Line (T47D)

All the compounds were screened their cytotoxic activity against breast cancer cell line (T47D) by using MTT assay [18]. The IC_{50} showed that **2** possessed high activities (4 µg/mL) compared with **1** and **3** with value 6 and 45 µg/mL (Table 1) respectively. It might be due to the electronegativity of halogen and the position of halogen where meta position was more active than ortho and para.

CONCLUSION

Three piperidone curcumin analogues have been synthesized by using aldol condensation reaction with base catalyst and irradiation from microwave. Compound **2** showed high cytotoxic activities compared to another compounds against breast cancer cell line (T47D).

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