Available online at www.sciencedirect.com
ScienceDirect
Procedia Chemistry 17 (2015) 224 – 229
Procedia Chemistry

3rd International Seminar on Chemistry 2014

Synthesis of Curcumin Analogue, *N*-H and *N*-Benzil-4-Piperidone and their Cytotoxic Activity

Yum Eryanti[a*], Rudi Hendra[a] ,Tati Herlina[b], Adel Zamri[a], and Unang Supratman[b]

[a]*Laboratory of Organic Synthesis, Department of Chemistry, Riau University, Pekanbaru 28293, Indonesia*
[b]*Laboratory of Natural Products Chemistry, Department of Chemistry, Universitas Padjadajaran*
Jl. Raya Bandung-Sumedang Km 21, Jatinangor 45363, Sumedang, Indonesia

Abstract

Four piperidone curcumin analogues, *N*-H-(3*E*,5*E*)-3,5-bis-(2,4,5-trimethoxybenzaldehyde)-4-piperidone (A1), *N*-H-(3*E*,5*E*)-3,5-bis-(3,4,5-trimethoxy benzaldehyde)-4-piperidone (A2), *N*-benzil-(3*E*,5*E*)-3,5-bis-(2,4,5- trimethoxybenzaldehyde)-4-piperidone (A3) and *N*-benzil-(3*E*,5*E*)-3,5-bis-(3,4,5-trimethoxybenzaldehyde)-4-piperidone) (A4) were synthesized from *N*-H-4-piperidone and *N*-benzil-4-piperidone with 2,4,5-trimethoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde. The Claisen-Schmidt condensation reaction was used in alkali condition with combinatorial approach. The compounds showed red brick crystal, yellow powder, brown powder, and light yellow powder with the yield of 88.8%, 44.5%, 84.7%, and 53.0% respectively. All structure of the compounds were confirmed by using UV, IR, ^{13}C-NMR, ^{1}H-NMR and mass spectroscopy. Cytotoxic activity against P-388 murine leukemia cell showed various IC_{50} values of >100, 92.62, 0.49 and 67.04 µg/mL, respectively.

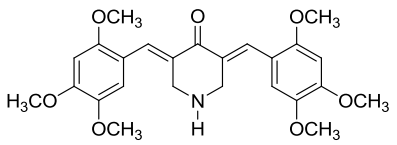
Keywords: Curcumin, Claisen-Schmidt ondensation, cytotoxicity activity, P-388 murine leukimia cell.

*Corresponding author: +62-8127515110
E-mail address: ym_eryanti@yahoo.com

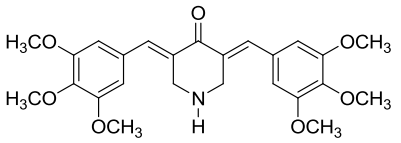
1. Introduction

Curcumin is naturally occurring compound isolated from *Curcuma* and the major pigment present in turmeric plan (*Curcuma longa*). Several curcuminoid compounds such as curcumin, 4-dimethoxycurcumin, bisdimethoxycurcumin (diarylheptanoid) and dihydrocurcumin (asymmetric) are found in turmeric plan[1]. Curcumin analog compounds have biological activities such as anti-inflammatory[2], antioxidant[3], anti-infective and anti-allergic[4], hepatoprotector agent[5], and HIV virus inhibition[6]. Encouraged by its biological activities, it becomes major interest as a model target for synthesis. Isolation of curcumin from the plant showed 3-5% and has limited structure variation[7].

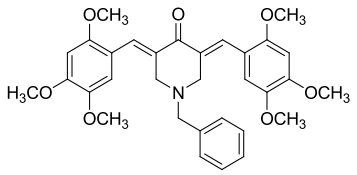
Peer-review under responsibility of Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University
doi:10.1016/j.proche.2015.12.136



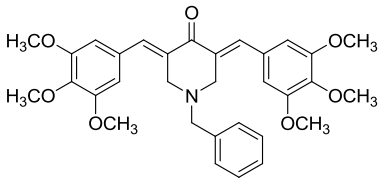
A1



A2



A3



A4

Claisen-Schmidt condensation is one of reaction which can be used to synthesis analogs of curcumin. This reaction is common used for carbon-carbon bonding formation because it's simple and environmental friendly. Along with those reasons, this reaction is able to do combinatorial chemistry approach.

In this study, analogs of curcumin were synthesized from *N*-H-4-piperidon, *N*-benzyl-4-piperidon, 2,4,5-trimethoxy-benzaldehyde and 3,4,5-trimethoxy-benzaldehyde with combinatorial approach. The reaction was done under irradiation with microwave. All the compounds were furthermore analyzed for their cytotoxicity against P-388 murine leukemia cell line *in vitro*.

2. Materials And Method

2.1. Materials

Chemicals were purchased from Merck except 2,5-dimethoxy bezaldehyde (Sigma). 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.

2.2. Syntesis of Curcumin Analogues.

All the compounds were synthesized by using aldol condensation reaction with sodium hydroxide as catalyst and ethanol as solvent (Carey and Sandberg, 1983)[8]. 0.01 mol of 4-piperidone derivative was mixed with sodium hydroxyde octahydrate (0.0055 mol) 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 minutes. Solid product obtained was cooled and 50 mL HCl 1N was added subsequently. The mixture was then filtered by using Buchner funnel and washed with 50 mL distilled water, 50 mL *n*-hexane respectively and finally was dried at 400 °C for 24 H.

3. Results And Discussion

3.1. Results

N-H-(3E,5E)-3,5-bis-(2,4,5-trimethoxybenziliden)-4-piperidon (A1), red powder, 88.80% yield, melting point: 246-248 °C, R_f = 0.67 (*n*-hexane : EtOAc = 2 : 8), HPLC, t_R = 11.5 min, UV (λ_{max} MeOH): 422 nm (ε 5,600), IR

(KBr) ν_{max}: 3432; 2997; 2942 and 2902 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz, Table 1), ^{13}C-NMR ($CDCl_3$,125 MHz) (Table 2). HR-ESI-TOFMS: *m/z* 456.2016 $[M+H]^+$, $C_{25}H_{29}NO_7$ *m/z* 455.1944.

N-H-(3E,5E)-3,5-bis-(3,4,5-trimethoxybenziliden)-4-peperidon (A2), brown powder, 84.70% yield, melting point: 46-48 °C, R_f = 0.7 (*n*-hexane : EtOAc = 4 : 6), HPLC, t_R = 6.2 min, UV (λ_{max} MeOH): 310 nm (ε 2,400), IR (KBr) ν_{max}: 2971; 2946; 1686; 1587; 1505 and 1237 cm^{-1}, HR-ESITOFMS: *m/z* 456.2043 $[M+H]^+$, $C_{25}H_{32}NO_7$ *m/z* 455.1944.

N-benzil-(3E,5E)-3,5-bis-(2,4,5-dimethoxybenziliden)-4-piperidon (A3), yellow powder, 44.53% yield, melting point: 177-178 °C, R_f = 0.51 ($CHCl_3$ 100%), HPLC, t_R = 13.8 min, UV (λ_{max} MeOH): 400 nm (ε 10,100) dan 278 nm (ε 6,600), IR (KBr) ν_{max}: 3629; 3139; 3053; 3001; 2945 dan 1656 cm^{-1}, HR-ESI-TOFMS: *m/z* 546.2516 $[M+H]^+$, $C_{32}H_{35}NO_7$ *m/z* 545.2414.

N-benzil-(3E,5E)-3,5-bis-(3,4,5-trimethoxybenziliden)-4-peperidon (A4), light yellow powder, 52.97% yield, melting point: 130-132 °C, R_f = 0.7 (*n*-hexane : EtOAc = 1 : 9), HPLC, t_R = 16.2 min, UV (λ_{max} MeOH): 345 nm (ε 3,800), IR (KBr) ν_{max}: 2953; 2839; 1686; 1270 dan 1230 cm^{-1}, HR-ESI-TOFMS: *m/z* 546.2421 $[M+H]^+$, $C_{32}H_{35}NO_7$ *m/z* 545.2414.

Table 1. Cytotoxic activity of Compounds **A1**-**A4** against P-388 murine leukemia cell line

Compound	IC_{50} (µg/mL)
N-H-(3*E*,5*E*)-3,5-bis-(2,4,5-trimethoxybenziliden)-4-piperidon (**A1**)	>100
N- H-(3*E*,5*E*)-3,5-bis-(3,4,5- trimethoxybenziliden)-4-piperidon (**A2**)	92.62
N-benzil-(3*E*,5*E*)-)-3,5-bis-(2,4,5-trimethoxybenziliden)-4-piperidon (**A3**)	0.49
N-benzil-(3*E*,5*E*)-)-3,5-bis-(3,4,5-dimethoxybenziliden)-4-piperidon (**A4**)	67.04
Curcumin	3.11

Discussion

Compound A1 was found as *N*-H-(3*E*,5*E*)-3,5-bis-(2,4,5-trimethoxybenziliden)-4-piperidon with chemical formula $C_{25}H_{29}NO_7$ and molecular weight as 456.2016 $[M+H]^+$ from HR-ESI-TOFMS spectra. This compound was assigned from ^{1}H-NMR and ^{13}C-NMR (Table 2). From ^{1}H-NMR spectra showed the presence of *N*-H signal (δ_H 10.19 (1H, s), two methylene protons at δ_H 4.39 (4H, s), two olefinic protons at down field at δ_H 8.0 (2H, s) and four aromatic protons from two substituted aromatic ring at δ_H 6.79 (2H, s), and δ_H 6.86 (2H, s), 18 proton from 3-OCH_3 at δ_H 3.89 (6H, s), δ_H 3.91 (6H, s) dan δ_H 3.92 (6H, s)]. ^{13}C-NMR spectra showed 25 carbon signals which was consist of two methylene carbons at δ_C 55.5), one conjugated carbon at δ_C 186.8), and six sp^2 carbons, five quaternary carbons, and three carbons from -OCH_3 at δ_C 55.9, 55.1 and 55.7 (Table 2).

Compound A2 was found as *N*-H-(3*E*,5*E*)-3,5-bis-(3,4,5-trimethoxybenziliden)-4-piperidon with chemical formula $C_{25}H_{32}NO_7$ and molecular weight as 456.2043 $[M+H]^+$ from HR-ESI-TOFMS spectra. This compound was assigned from ^{1}H-NMR and ^{13}C-NMR (Table 2). ^{1}H-NMR spectra from A2 showed *N*-H signal at δ_H 10,01 (1H, s), two methylene at δ_H 3.49 (4H, s), two olefinic protons at down field at δ_H 7.26 (2H, s), 18 protons from 6-OCH_3 at δ_H 3.89 (6H, s) and δ_H 3.90 (6H, s)] and δ_H 3.89 (6H, s), four aromatic protons which were from 2 substituted aromatic rings at δ_H 7.12 (2H, s) dan 7.12 (2H, s)]. ^{13}C-NMR showed 25 carbons which were 2 methylene carbons (δ_C 61,0), one conjugated carbonyl carbon at δ_C 191.0), and six sp^2 carbons, five quaternary carbons, three carbons from -OCH_3 at δ_C 56.3, 56.3 and 56.3 (Table 2). Unsaturated degree was counted as nine out of twelve for both compounds. The three remaining numbers of unsaturated degree was suitable with tricyclic from monoketone curcumin which had two symmetric part[9]. The methoxy position was found in 2,4,5 and 3,4,5 position respectively and this is in the same result which published by Gregory et al. (2013)[9].

Table 2. NMR Data of A1 and A2

Position	A1		A2	
	^{1}H NMR δ_H ppm [(ΣH, mult., *J* (Hz)]	^{13}C NMR δ_C ppm	^{1}H NMR δ_H ppm [(ΣH, mult., *J* (Hz)]	^{13}C NMR δ_C ppm
2/6	4.39 (4H, s)	55.5	3.94 (4H, s)	61.0
3/5	-	125.5	-	131.7
4	-	186.8	-	191.0
1'/1''	-	116.2	-	124.1
2'/2''	-	154.0	7.12 (2H, s)	106.7
3'/3''	6.79 (2H, s)	-	-	153.6
4'/4''	-	152.3	-	131.7
5'/5''	-	142.3	-	153.6
6'/6''	6.86 (2H, s)	114.1	7.12 (2H, s)	106.7
7'/7''	8.00 (2H, s)	134.6	7.26 (2H, s)	142.9
N-H	10.19 (H, s)	-	9.86 (1H, s)	-
2'/2''-OCH_3	3.89 (6H, s)	55.9	-	-
3'/3''-OCH_3	-	-	3.89 (s, 6H)	56.3
4'/4''-OCH_3	3.91 (6H, s)	55.1	3.90 (s, 6H)	56.3
5'/5''-OCH_3	3.92 (6H, s)	55.7	3.89 (s, 6H)	56.3

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

Compound A3 was found as *N*-H-(3*E*,5*E*)-3,5-bis-(3,4,5-trimetoksibenziliden)-4-piperidon with chemical formula $C_{25}H_{32}NO_7$ and molecular weight as 456.2043 $[M+H]^+$ from HR-ESI-TOFMS spectra. This compound was assigned from ^{1}H-NMR and ^{13}C-NMR (Table 3). From proton NMR was found *N*-benzyl signal at δ_H 3.68 (3H, s), two methylene signals at δ_H 3,80 (4H, s), one olefinic proton at δ_H 8.05 (2H, s), methoxy signals at δ_H 3.85 (6H, s), δ_H 3.77 (6H, s), and δ_H 3.93 (6H, s)], four aromatic protons which was from two substituted aromatic rings at δ_H 6.52 (2H, s), and δ_H 6.70 (2H, s), five aromatic protons from aromatic ring at δ_H 7.21 (5H, s)]. ^{13}C-NMR spectra showed 32 carbons which was consist of methylene at δ_C 54.5, a conjugated carbonyl at δ_C 187.4, *N*-benzyl at δ_C 61.2, siz metoxhyl signals at δ_C 56.4, δ_C 56.0 and δ_C 55.5, as well as 22 sp^2 carbons (Tabel 3). Apart from that, unsaturated degree was counted as 12 out of 16 which was meant 4 unsaturated remain suitable with tetracyclic form from monoketone curcumin[9]. Methoxyl position in both rings were determined based on their chemical shift and concluded as 2,4,5 position. The NMR data was compared with *N*-benzyl-(3*E*,5*E*)-3,5-bis-(2,4,5-trimethoxybenziliden)-4-piperidon and showed high similarity[9].

Compound A4 was found as *N*-benzyl-(3*E*,5*E*)-3,5-bis(3,4,5-trimethoxybenziliden)-4-piperidon with chemical formula $C_{32}H_{35}NO_7$ and molecular weight as 546.2421 $[M+H]^+$ from HR-ESI-TOFMS spectra. This compound was assigned from ^{1}H-NMR and ^{13}C-NMR (Table 3). From proton NMR was found *N*-benzyl signal at δ_H 3.94 (3H, s), olefinic proton at δ_H 7.38 (2H, s), methoxyl signals at δ_H 3.93 (6H, s), δ_H 3.93 (6H, s), δ_H 3.93 (6H, s), four aromatic protons which was from two substituted aromatic rings at δ_H 7.14 (2H, s), δ_H 7.27 (2H, s), five aromatic protons from aromatic ring at δ_H 7.90 (5H, s). ^{13}C-NMR spectra showed 32 carbons which was consist of methylene signal at δ_C 60.9, a conjugated carbonyl at δ_C 191.1, six metoxhyl signals at δ_C 56.2, δ_C 56.2 and δ_C 56.2 as well as 22 sp^2 carbons (Table 3). Apart from that, unsaturated degree was counted as 12 out of 16 which was meant 4 unsaturated remain suitable with tetracyclic form from monoketone curcumin[9]. Methoxy position in both rings were determined based on their chemical shift and concluded as 3,4,5 position. The NMR data was compared with *N*-benzyl-(3*E*,5*E*)-3,5-bis-(3,4,5-trimethoxybenziliden)-4-piperidon and showed high similarity[9].

Table. 3. NMR Data of A3 dan A4

	A3		A4	
Position	^{1}H NMR δ_H ppm [(ΣH, mult., *J* (Hz)]	^{13}C NMR δ_C ppm	^{1}H NMR δ_H ppm [(ΣH, mult., *J* (Hz)]	^{13}C NMR δ_C ppm
2/6	3.80 (4H, s)	54.5	3.94 (4H, d, 1)	60.9
3/5	-	131.4	-	
4	-	187.4	-	191.1
1’/1’’	-	116.1	-	124.1
2’/2’’	-	154.0	7.14 (2H, s)	107.4
3’/3’’	6.52 (2H, s)	-	-	152.9
4’/4’’	-	150.9	-	171.4
5’/5’’	-	142.4	-	152.9
6’/6’’	6.70 (2H, s)	113.7	7.27 (2H, s)	107.4
7’/7’’	8.05 (2H, s)	131.9	7.38 (2H, s)	142.9
2’/2’’-OCH_3	3.85 (6H, s)	56.4	-	-
3’/3’’-OCH_3	3.77 (6H, s)	56.0	3.93 (s, 6H)	56.2
4’/4’’-OCH_3	3.93 (6H, s)	56.5	3.93 (s, 6H)	56.2
5’/5’’-OCH_3	-	-	3.93 (s, 6H)	56.2
N-CH_2-Ph	3.68 (2H, s)	61.2	3.94 (2H, s)	-
1’’’	-	137.4	-	-
2’’’/6’’’	-	129.1	-	-
3’’’/5’’’	-	128.2	-	-
4’’’	-	127.3	-	
2’’’/3’’’/4’’’ /5’’’/6’’’	7.21(5H, obs)	-	7.90 (5H, br s)	-

[a]in $CDCl_3$ at 500 MHz for ^{1}HNMR and 125 MHz for ^{13}CNMR

Cytotoxic Activity against P-388 murine leukemia cell line (in vitro)

All the compounds were screened their cytotoxic activity against P-388 murine leukemia cell line by using MTT assay (Alley, 1988). The IC_{50} showed that A3 possessed high activity (0.49 µg/mL) compared with A1, A2, A4 with value >100; 92.62; 67.04 µg/mL (Table 1) respectively. It might be due to less steric around carbonyl and *N*-piperidon bonded with proton which did not has steric.

Conclusion

Four monoketone curcumin analogs had been synthesized by using aldol condensation reaction with base catalyst and irradiation from microwave. Compound A3 showed high cytotoxic activity compared to another compounds against P-388 murine leukemia cell line.

Acknowledgements.

The authors would like to thank to Directore General of Higher Education, Ministry of National Education, Indonesia for financial support.

References

1. Achmad, S.A., Hakim,E.H., Makmur.L., Syah.Y.M, Juliawaty.L.D. dan Mujahidin.D. 2007. “Ilmu kimia dan kegunaan tumbuh-tumbuan obat Indonesia” ITB. Bandung.
2. Kim,H.Y., Park,E.J. and Jou,I., 2003 “Curcumin suppresses janus kinase STAT inflammatory signaling through activation of SCC homology 2 domain-containing tyrosin phosphatase 2 in brain microglia” j.immunology 171 6072-6079.
3. Suzuki, M., Nakamura, T., Iyoki, S., Fujiwara, A., Watanabe, Y., Mohri, K., Isobe, K., Ono, K and Yano, S. “Elucidation of anti-allergic activities of curcumin-related compounds with a special reference to their anti-oxidative activities” j.pharm.Bull28: 1438-1443.
4. Handler, N., Jaeger, W., Puschacher, H., Leiser, K., Erker, T. 2007. “Synthesis of Novel Curcumin Anologues and Their Evaluation As Selective Cyloxigenase-1(cox-1) Inhibitors”. J. chem. Pharm. Bull55.64-71.

5. Liang, G., Yang, S., Jiang, L., Zhao, Y., Shao, L., Xiao, J., Ye, F.,Li, Y. and Li, X. "Synthesis and Antibacterial Properties of Mono-carbonyl Analogues of Curcumin" J.chem. Pharm. Bull56: 162-167.
6. Santo,D.R., Costi,R., Artico,M., Framontano,E., Colla,L.P., and Pani,A., 2003 "Replication in infected cells, planning synthetic derivatives from natural product" j.pure appl chem. 75 195-206 .
7. Stankovic, I. 2004. "Curcumin". Chemical and Technical Assement (CTA) JEFA.
8. Carey, F.A.;Sundberg, R.J (1983). "Advanced Organic Chemistry, Part B; Reactions and synthesis". second edition, Plenum, New York, 43-50.
9. Gregory, M., Dandavati, A., Lee, M., Tzou, S., Savagian, M., Kimberly, A., Brien, K.A., Vijay Satam, Pravin Patil, P. Synthesis, cytotoxic and structure-activity insight of NH-and N-methyl-3,5-bisarylidenyl-4-piperidone. *Med. Chem. Res*, 2013, 22, 5588-5597.