Journal of Applied Pharmaceutical Science Vol. 9(08), pp 018-024, August, 2019
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2019.90803
ISSN 2231-3354

Synthesis and anticancer potential of aminomethyl derivatives of methyl-substituted asymmetrical curcumin mono-carbonyl

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ARTICLE INFO

Received on: 06/02/2019
Accepted on: 11/06/2019
Available online: 03/08/2019

Key words:
Curcumin, asymmetrical curcumin mono-carbonyl, aminomethyl derivatives, anticancer potential, cytotoxicity, selectivity index

ABSTRACT

A series of new aminomethyl derivatives of methyl-substituted asymmetrical curcumin mono-carbonyl was synthesized and evaluated for their anticancer potential by means of cytotoxicity and selectivity determination against MCF-7, WiDr, Hela, A549, PLC/PRF/5, and Chang Liver cells lines using the methyl thiazolyl tetrazolium proliferation assay method. All the synthesized compounds (**3a–f**) exhibited high cytotoxic against WiDr cells lines, but only **3a–e** had high cytotoxic against MCF-7 cells lines, and only **3b** showed high cytotoxic against HeLa, A549, and PLC/PRF/5 cell lines. However, **3b** and **3c** exhibited high cytotoxic against Chang Liver (normal liver) cells lines. Further evaluations showed that compounds **3d**, **3e**, and **3f** exhibited a potent and selective cytotoxic agent (IC_{50} = 5.70, 5.55, and 2.97 µM) against WiDr (colorectal carcinoma) cells lines with selectivity index (SI) = 4.43, 2.69, and 2.04, respectively. The compounds performed better cytotoxic activity than curcumin and 5-fluorouracil (IC_{50} = 8.29 and >100 µM and SI = 1.28 and <1). So, compounds **3d**, **3e**, and **3f** were potential as an anticancer agent for colorectal carcinoma and should be further studied for investigating their mechanism of action and their effectivity in preclinical studies using an animal model..

INTRODUCTION

The prevalence of cancer worldwide continues to increase significantly. International Agency for Research Cancer estimated that in 2018 there are 18,100,000 new cancer patients and 9,600,000 cancer deaths. Lung cancer, breast cancer, and colorectal cancer are the types of cancer that have the most incidence (Press Release, 2018). For more than six decades, cancer chemotherapeutic agents have been developed and used as one approach for cancer treatment. Unfortunately, the use of chemotherapeutic agents generally may produce irreversible chronic and delayed toxicities against many vital organs, such as kidneys, heart, and lungs, because of low specificity for cancer cells (Roche, 2012). Moreover, some patients develop resistance to anticancer drugs, such as 5-fluorouracil (5-FU) (Chibaudel *et al*., 2008). Therefore, there is a significant need to develop a new anticancer agent with better efficacy and selectivity.

Curcumin was well known to possess many biological activities, such as anti-inflammatory inhibition, growth inhibition in various tumor cells, and chemopreventive effects on certain cancers with low toxicity (Anand *et al*., 2008). The curcumin's antitumor mechanism is multiple, involving apoptosis induction, proliferation inhibitory, G1/S arrest, and the mitotic block (Kunnumakkara *et al*., 2017; Srivastava *et al*., 2007). Although curcumin has evidence as anti-cancer, its therapeutical usage of curcumin is restricted by low of water solubility, chemical and metabolical stability, and relatively poor *in vivo* bioavailability (Anand *et al*., 2008). The chemical structure of curcumin has been modified intensively to find the analogs had better physical and chemical properties, as well as better biological activity. New analogs that show an inhibitory activity of cancer cells growth 30 times than curcumin and other drugs often used to treat cancer were identified (Ohori *et al*., 2006). Monocarbonyl analogs of curcumin (MACs) with cyclohexanone as central can inhibit

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the growth of colon, ovarian, and breast cancer cells better than cisplatin (Adams *et al*., 2004; Liang *et al*., 2009; Yerdelen *et al*., 2015). Recently, our research group reported that methoxy- and methyl-substituted of asymmetrical mono-carbonyl analogs of curcumin (AMACs) (Fig. 1) showed moderate cytotoxicity against MCF-7 (Prasetyaningrum *et al*., 2018). Aminomethylation is one of the feasible and cost-efficient procedures for drug development (Biersack *et al*., 2018). Several aminomethyl derivatives had been synthesized and reported to have better anticancer activity than the parent analogs, such as aminomethyl derivatives of chalcones, acetophenones, benzylidenecyclohexanones, carbazoles, 4,11-dihydroxynaphthol[2,3-f]indole-5,10-dione, gatifloxacin, 8-hydroxyquinoline, benzothiazoles, 2-propoxybenzylidene-isonicotino hydrazide, fluoroquinolones, 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones, and MACs (Bala *et al*., 2014; Dimmock *et al*., 1992; Roman, 2015; Subramaniapillai, 2013; Yerdelen *et al*., 2015). The phenol derivatives having quaternary ammonium group are bioactive compounds. They act as DNA interstrand cross-linking agent to inhibit transcription and furthermore the apoptosis of tumor cells (Song *et al*., 2006). Diethylaminomethyl derivatives of methyl-substituted of AMACs (Fig. 1) exhibited moderate cytotoxicity against MCF-7 but low selectivity against normal cells (Prasetyaningrum *et al*., 2018). Thereby, as continuation study of our research group, we synthesized a series of new aminomethyl derivatives of methyl-substituted asymmetrical curcumin mono-carbonyl [2-(4-hydroxy-3-methoxy-benzylidene)-6-(4-methyl-benzylidene)-cyclohexanone] and evaluated their anticancer potential.

MATERIAL AND METHODS

General procedures

The measurement of melting points was performed using analog melting point apparatus (Model SMP11, Stuart Scientific) and the values obtained are uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel Si 60 F254 plates (Merck). Infrared spectral data were attained by an FT-IR spectrophotometer (8400S, Shimadzu). Proton Nuclear Magnetic Resonance (NMR) and Carbon NMR spectra were obtained on NMR spectrometer (Agilent), and Mass spectra were recorded in positive mode on High Resolution Mass Spectrometer (LCT Premier XE-TOF) (Waters Corp.). The known compounds: 2-(4-methyl-benzylidene)-cyclohexanone (**1**), 2-(4-hydroxy-3-methoxy-benzylidene)-6-(4-methyl-benzylidene)-cyclohexanone (**2**), 2-(3-diethylaminomethyl-4-hydroxy-5-methoxy-benzylidene)-6-(4-methyl-benzylidene)-cyclohexanone (**3e**), and 2-(4-hydroxy-3-methoxy-5-morpholin-4-ylmethyl-benzylidene)-6-(4-methyl-benzylidene)-cyclohexanone (**3f**) were obtained from earlier reasearcher (Prasetyaningrum *et al*., 2018; Putri *et al*., 2018).

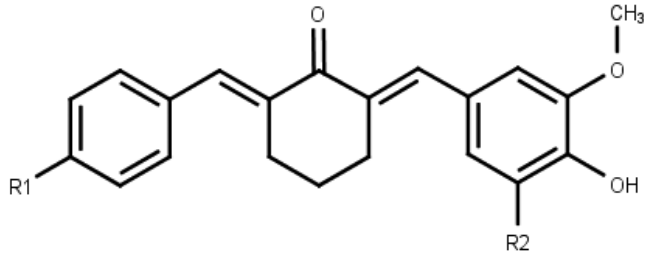


Figure 1. (A) Methoxy-substituted, (B) Methyl-substituted, and (C) Diethylaminomethyl derivatives of methyl-substituted of AMACs (Prasetyaningrum *et al*., 2018).

Synthesis of compounds 3a–d

The compounds were prepared according to the synthesis method of compound **3e** and **3f** reported earlier with little modifications (Prasetyaningrum *et al*., 2018; Putri *et al*., 2018). To a cold solution of compound **2** and appropriate secondary amine compound (2,6-dimethylmorpholine/diethylamine/pyrrolidine/1-methylpiperazine) in ethanol, formaldehyde solution was added dropwise while stirring in an ice bath. After stirring for 30 minutes at r.t., the reaction mixture was refluxed for 7–11 hours (TLC monitoring). Upon completion, evaporation of the solvent and residue dissolution in methanol was done twice, then the solution warmed and poured gradually into cold distilled water (with constant stirring) to obtain the precipitate product. The product was separated by means of decantation, filtration, washing with cold distilled water, and drying at room temperature. Purification was done by column chromatography to obtain pure **3a–d.**

2-[3-(2,6-Dimethylmorpholin-4-ylmethyl)-4-hydroxy-5-methoxy-benzylidene]-6-(4-methyl-benzylidene)-cyclohexanone (3a)

Yellow powder, yield 64.5%, mp 103°C–105°C. FT-IR (KBr) cm^{-1}: 2,933–2,860 (C-H aliphatic), 1,737 (carbonyl), 1,662, 1,600, 1,494 (C=C), 1,271 (C-N), and 1,157 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 1.17 ppm (6H, d, J = 6 Hz, two $C\underline{H}_3CH$-, 2,6-dimethylmorpholine), 1.89 and 2.85 ppm (4H, t, J = 10 Hz, and d, J = 12 Hz, two $CHC\underline{H}_2$-N 2,6-dimethylmorpholine), 4.08 and 3.70 ppm (2H, m, two N-$CH_2C\underline{H}(CH_3)$-O 2,6-dimethylmorpholine), 3.90 and 3,91 ppm (3H, s, 3-CH_3-O) (Untung *et al*., 2017), 1.80 ppm (2H, p, J = 7 Hz, $CH_2C\underline{H}_2CH_2$ cyclohexanone), 2.37 ppm (3H, s, 4-CH_3Ar); 2.90 and 2.94 ppm (4H, t overlap, J = 8 Hz, two $CH_2C\underline{H}_2C$ cyclohexanone), 3.72 ppm (2H, s, $ArCH_2$-N), 6.82 ppm (1H, d, J = 2 Hz, H phenyl), 6.99 ppm (1H, d, J = 2 Hz, H phenyl), 7.20 ppm (2H, d, J = 8 Hz, two H phenyl), 7.38 ppm (2H, d, J = 8 Hz, two H phenyl), 7.71 and 7.77 ppm (1H, s, and 1H, s, two H methylidene). ^{13}C-NMR ($CDCl_3$, 125 MHz), δ: 19.1 ppm (2C, two CH_3-, 2,6-dimethylmorpholine), 21.5 ppm (1C, 4-CH_3Ar), 23.2, 28.6 and 29.8 ppm (3C, three $C\underline{H}_2$ cyclohexanone), 56.1 ppm (1C, CH_2-N-), 58.5 (2C, CH_2-N- 2,6-dimethylmorpholine), 61.6 ppm (1C, 4-CH_3-O), 71.81 ppm (2C, CH_2-O- 2,6-dimethylmorpholine), 113.7, 120.8, 124.0, 127.3, 129.2, 130.6, 137.4, and 138.9 ppm (8C, CAr), 133.4, 133.9, 135.6, and 136.8 ppm (4C, -C=C methylidene), 147.8 and 148.3 ppm (2C, C-O), 190.2 ppm (1C, carbonyl) (Silverstein *et al*., 2005). Calcd masses for $C_{29}H_{35}NO_4$: 461.5925, HR-ESI-MS (m/z) found 462.2637 ($[M+H]^+$).

2-(3-Dimethylaminomethyl-4-hydroxy-5-methoxy-benzylidene)-6-(4-methyl- benzylidene)-cyclohexanone (3b)

Red caramel-like solid, yield 63.2%, mp 96.97°C. FT-IR (KBr) cm^{-1}: 2,945–2,829 (C-H aliphatic), 1,662 (carbonyl), 1,597, 1,489 (C=C), 1,255 (C-N), and 1,159 (C-O-C). ^{1}H-NMR (CD_3OD, 500 MHz), δ: 1.77 ppm (2H, p, *J = 6 Hz*, $CH_2C\underline{H}_2CH_2$ cyclohexanone), 2.34 ppm (3H, s, 4-CH_3Ar); 2.38 ppm (6H, s, two CH_3-N), 2.86 and 2.92 ppm (4H, t, *J = 6 Hz*, two $CH_2C\underline{H}_2C$ cyclohexanone), 3.72 ppm (2H, s, Ar-CH_2-N), 3.85 ppm (3H, s, 3-CH_3-O), 6.92 ppm (1H, s, H phenyl), 7.02 ppm (1H, d, *J* = 2 Hz, H phenyl), 7.21 ppm (2H, d, *J* = 8 Hz, two H phenyl), 7.34 ppm (2H, d, *J* = 6 Hz, two H phenyl), 7.64 and 7.65 ppm (1H, s, and 1H, s, two H methylidene). ^{13}C-NMR (CD_3OD, 125 MHz), δ: 21.4 ppm (1C, 4-CH_3-Ar), 24.1, 29.4, and 29.6 ppm (3C, three $\underline{C}H_2$ cyclohexanone), 44.4 ppm (2C, two CH_3-N-, dimethylamine), 56.5 ppm (1C, $ArCH_2$-N), 61.5 ppm (1C, 3-CH_3-O), 114.9, 123.0, 126.6, 127.3. 130.2, 131.6, 139.3, and 140.2 ppm (8C, CAr), 134.3, 134.5, 137.0, and 137.7 ppm (4C, -C=C- methylidene), 149.3 and 151.2 ppm (2C, C-O), 191.8 ppm (1C, carbonyl) (Silverstein *et al*., 2005). Calcd masses for $C_{25}H_{29}NO_3$: 391.507, HR-ESI-MS (m/z) found 392.2222 ($[M+H]^+$).

2-[4-Hydroxy-3-methoxy-5-(pyrrolidin-1-ylmethyl)-benzylidene]-6-(4-methyl- benzylidene)-cyclohexanone (3c)

Red caramel-like solid, yield 52.08%, mp 82°C–84°C. FT-IR (KBr) cm^{-1}: 2,937–2,833 (C-H aliphatic), 1,654 (carbonyl), 1,566, 1,415 (C=C), 1,255 (C-N), and 1,155 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 1.80 ppm (4H, t, J = 6 Hz, $C\underline{H}_2C\underline{H}_2$ pyrrolidine), 1.86 ppm (2H, p, *J = 6 Hz*, $CH_2C\underline{H}_2CH_2$ cyclohexanone), 2.37 ppm (3H, s, 4-CH_3Ar), 2.68 ppm (4H, t, *J = 6 Hz*, two $C\underline{H}_2$-N pyrrolidine), 2.89 and 2.95 ppm (4H, t overlap, *J = 5 Hz,* two $CH_2C\underline{H}_2C$ cyclohexanone), 3.88 ppm (3H, s, 3-CH_3O), 3.90 ppm (2H, s, $ArCH_2$-N), 6.82 ppm (1H, s, H phenyl), 6.98 ppm (1H, d, *J* = 2 Hz, H phenyl), 7.20 ppm (2H, d, *J* = 8 Hz, two H phenyl), 7.36 ppm (2H, d, *J* = 8 Hz, two H phenyl), 7.72 and 7.76 ppm (1H, s, and 1H, s 2H methylidene). ^{13}C-NMR ($CDCl_3$, 125 MHz), δ: 21.5 ppm (1C, 4-CH_3Ar), , 23.8 ppm (2C, $\underline{C}H_2\underline{C}H_2$ pyrrolidine), 23.2, 28.6, and 28.8 ppm (3C, three $\underline{C}H_2$ cyclohexanone), 53.6 ppm (2C, CH_2N- pyrrolidine), 56.1 ppm (1C, $ArCH_2N$), 58.6 ppm (1C, 3-CH_3-O), 113.5, 122.3, 123.6, 126.7, 129.2, 130.5, 137.7, and 138.8 ppm (8C, CAr), 133.4, 133.6, 135.7, and 136.6 ppm (4C, -C=C- methylidene), 147.8 and 149.0 ppm (2C, C-O), 190.3 ppm (1C, carbonyl) (Silverstein *et al*., 2005). Calcd masses for $C_{27}H_{31}NO_3$: 417.2304, HR-ESI-MS (m/z) found 418.2379 ($[M+H]^+$).

2-[4-Hydroxy-3-methoxy-5-(4-methylpiperazin-1-ylmethyl)-benzylidene]-6-(4-methyl-benzylidene)-cyclohexanone (3d)

Orange powder, yield 67.76%, mp 134°C–136°C. FT{IR (KBr) cm^{-1}: 2,937–2,837 (C-H aliphatic), 1,658 (carbonyl), 1,602, 1,562, and 1,492 (C=C), 1,253 (C-N) and 1,157 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 1.79 ppm (2H, p, *J = 6 Hz*, $CH_2C\underline{H}_2CH_2$ cyclohexanone), 2.29 ppm (3H, s, 4-CH_3-N methylpiperazine), 2.36 ppm (3H, s, 4-CH_3-Ar); 2.60 ppm (8H, m, two -N-$C\underline{H}_2C\underline{H}_2$-N methylpiperazine), 2.89 and 2.92 ppm (4H, t, *J = 6 Hz*, $CH_2C\underline{H}_2C$ cyclohexanone), 3.75 ppm (2H, s, $ArCH_2$-N), 3.89 ppm (3H, s, 3-CH_3-O), 6.81 ppm (1H, d, *J* = 2 Hz, H phenyl), 6.96 ppm (1H, d, *J* = 2 Hz, H phenyl); 7.20 ppm (2H, d, *J* = 8 Hz, two H phenyl); 7.37 ppm (2H, d, *J* = 8 Hz, two H phenyl), 7.69 and 7.75 ppm (1H, s, and 1H, s, two H methylidene). ^{13}C-NMR ($CDCl_3$, 125 MHz), δ: 21.5 ppm (1C, 4-CH_3-Ar), 23.5, 28.5, and 28.8 ppm (3C, three $\underline{C}H_2$ cyclohexanone), 45.9 ppm (1C, 4-CH_3-N-piperazine), 52.5 and 54.9 ppm (4C, -N-$\underline{C}H_2\underline{C}H_2$-N- piperazine), 56.1 ppm (1C, $ArCH_2$-N), 61.2 ppm (1C, 3-CH_3-O), 113.7, 121.1, 123.9, 127.2, 129.2, 130.5, 137.5, and 138.8 ppm (8C, CAr), 133.4, 133.8, 135.6, and 136.7 ppm (4C, -C=C- methylidene), 147.8 and 148.47 ppm (2C, C-O), 190.2 ppm (1C, carbonyl) (Silverstein *et al*., 2005). Calcd masses for $C_{28}H_{34}N_2O_3$: 446.2569, HR-ESI-MS (m/z) found 447.2652 ($[M+H]^+$).

Cytotoxicity evaluation

Screening

The synthesized compounds (**3a–f**) was screened for their cytotoxic activity against five cancer cell lines: estrogen-dependent breast carcinoma (MCF-7), Colon carcinoma (WiDr), cervix carcinoma (HeLa), lung carcinoma (A549), and hepatoma (PLC/PRF/5) and one normal cell lines: normal liver (Chang Liver) using the methyl thiazolyl tetrazolium (MTT) method conducted according to the protocol of MTT Assay for cell viability reported earlier (Stockert *et al*., 2012). The cell lines were purchased from American Type Culture Collection, the cells were grown with a density of 5,000 cells in 100 µl growth media consisting of Roswell Park Memorial Institute 1640, Dulbecco's Modified Eagle's Medium (D-MEM), Fetal Bovine Serum (FBS) 5%, Penicillin 100 U/ml, and Streptomycin 100 µg/ml. After 50% confluent cell (24 hours), the tested compounds and 5-fluorouracil (positive control) solutions were added to each well to the final concentration of 12.5 µg/ml. The MTT test was carried out on day 3. The culture medium was replaced by complete D-MEM and then added 10 µl of a fresh solution of MTT (5 mg/ml). After the cells were incubated for 4 hours at 37°C, the medium was removed and the culture was washed with phosphate buffer saline. The dissolved formazan product in ethanol was measured spectrophotometrically at 595 nm. The experiment was conducted in triplicate. The formula used to calculate the percentage of proliferation inhibition:

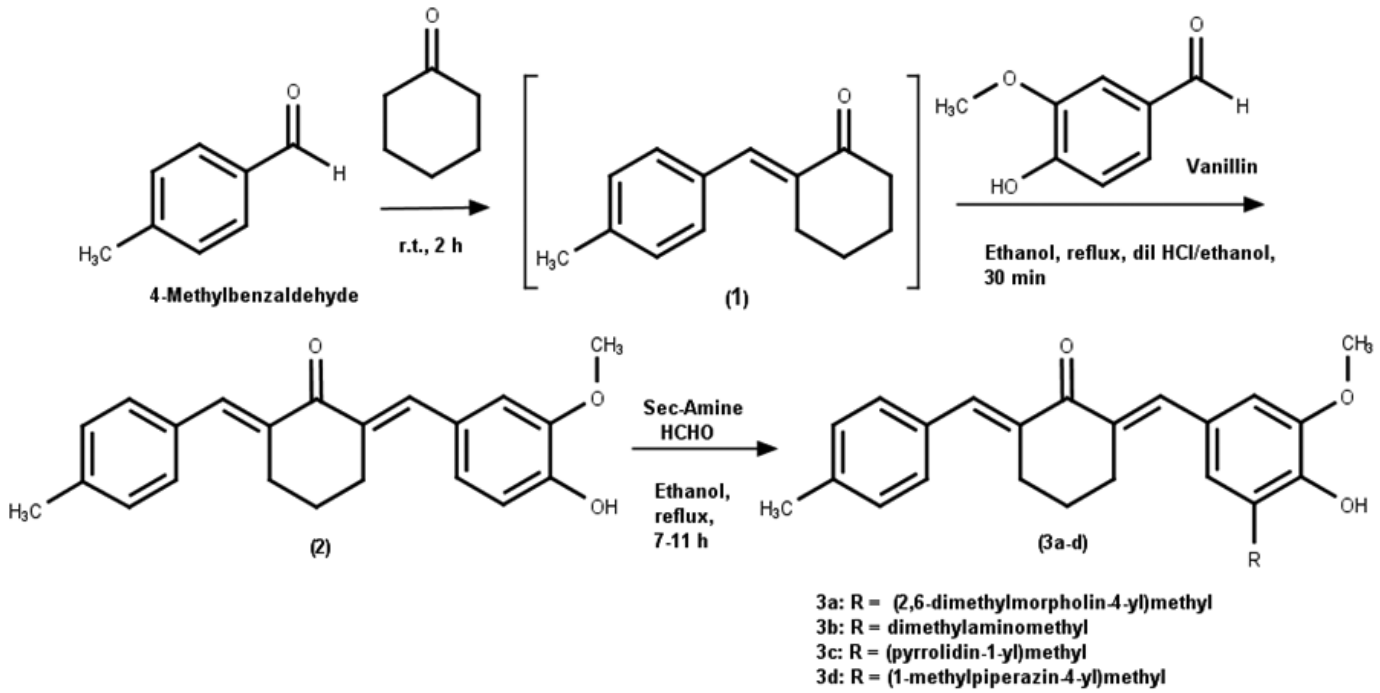
$$\text{Growth cells inhibition } (\%) = 100 - \frac{(\text{At} - \text{Ab})}{(\text{Ac} - \text{Ab})} \times 100$$

At, Ab, and Ac = Absorbance of test, blank, and control solution

The compounds showed growth inhibition against cancer cells more than 80% and the ratio between the inhibition to cancer and normal cells more than 1.5 were continued to determine the IC_{50} values.

IC_{50} determination

The selected cancer cells and Chang cells were grown with a density of 5,000 cells in 100 µl growing media consisting



Scheme 1. Synthesis of the target compounds

of D-MEM, FBS 5%, Penicillin 100 U/ml, and Streptomycin 100 µg/ml. After the cell reaches 50% confluent (24 hours), a series of concentrations of selected compounds, 5-fluorouracil and curcumin solutions was added to each well to the final concentration of 1.56–100 µg/ml. Furthermore, the MTT test was carried out as described in the screening.

The IC50 values were obtained by analyzing the relationship between the concentrations of the tested compounds and their percent (%) inhibitions using GraphPad Prism 7 (La Jolla, CA, www.graphpad.com). The ratio between the IC50 value of the compounds in normal cells and selected cancer cells shows the value of the selectivity index (SI).

RESULTS AND DISCUSSION

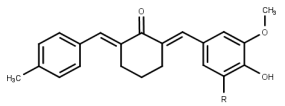
Chemistry

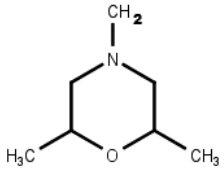
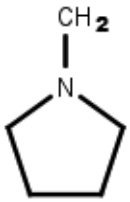
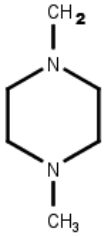
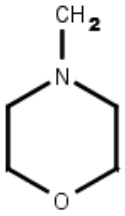
A series of new aminomethyl derivatives of methyl-substituted asymmetrical curcumin mono-carbonyl (**3a–d**) were synthesized stepwise summarized in Scheme 1 in a good yield. The FTIR spectra of **3a–d** showed the appearance of C-O-C and C-N bands at 1,155–1,271 cm^{-1} and the disappearance of OH phenolic group. In the ^{1}H-NMR spectra, the two singlet peaks at 2.34–2.37 and 3.85–3.90 ppm (3H) correspond to protons of methyl groups of Ar-CH_3 and Ar-OCH_3, respectively. While the protons of methylene group linking the amine to the phenyl ring appeared as a singlet peak at 3.72–3.90 ppm. The two protons of the two methylidene chain (1H, respectively) appeared as two singlet peaks and more downfield in range of 7.64–7.71 ppm indicated that the structures of the synthesized compounds are asymmetrical and E-configuration (Silverstein *et al.*, 2005). Furthermore, the structures were completed with ^{13}C-NMR and HR-MS data, which showed the full conformity of the structures assigned.

Cytotoxicity and selectivity

The synthesized compounds were screened against five cancer cell lines: MCF-7, WiDr, HeLa, A549, and PLC/PRF/5 and one normal cell lines: Chang Liver using MTT assay at a final concentration of 12.5 µg/ml. The results showed that all the synthesized compounds (**3a–f**) exhibited high cells growth inhibition (more than 80%) against WiDr cells lines, but only compounds **3a–e** had high cytotoxic activity against MCF-7 cells lines, and only compound **3b** showed high cytotoxic activity against HeLa, A549, and PLC/PRF/5 cell lines. Unfortunately, compound **3b** and **3c** exhibited high cells growth inhibition against Chang Liver (normal liver) cells lines (Table 1). Based on the above screening's results, then further anticancer potential evaluation only performed for compounds **3a**, **3d**, **3e**, and **3f** by IC_{50} values determination. Compounds **3a**, **3d**, and **3e** were evaluated against MCF-7 and WiDr cells lines, while compound **3f** was evaluated against WiDr cells lines. Curcumin and 5-fluorouracil were used as compared and positive control. The compounds also were tested against Chang Liver cell lines to evaluate their selectivity. The results showed that all the compounds possessed better cytotoxic activity against MCF-7 and WiDr cells lines than curcumin and 5-fluorouracil (Table 2, Fig. 2). The low cytotoxic activity of 5-fluorouracil indicated that MCF-7 and WiDr cells lines have been resistance

Table 1. The percentage of growth inhibition (% GI) of the various cell lines due to the synthesized compounds (**3a–f**) at 12,5 µg/ml.



Compounds	R	% Growth inhibition (mean, n = 3)1					
		MCF7	WiDr	HeLa	A549	PLC/PRF/5	Chang Liver
3a		85.78	80.89	40.25	31.07	58.05	50.95
3b	(CH3)2NCH2	96.64	94.17	97.58	88.40	94.63	94.05
3c		80.91	87.13	55.88	35.10	78.53	80.48
3d		89.48	83.32	54.75	35.50	72.03	50.53
3e	(CH3CH2)2NCH2	85.63	81.86	30.70	8.65	58.19	41.78
3f		49.64	80.24	20.74	28.52	62.85	43.32
5-Fluorouracil	-	54.36	50.93	14.09	41.30	27.26	29.25

[1]Mean, *n* = 3: mean of three experiments.

Table 2. The cytotoxicity (IC50 values) of compound **3a**, **3d**, **3e**, **3f**, curcumin, and 5-fluorouracil against MCF-7, WiDr, and Chang Liver cells

Compounds	IC50 (µM) (mean, n = 3)1			SI2	
	MCF-7	WiDr	Chang Liver	MCF-7	WiDr
3a	4.18	3.98	1.79	0.43	0.45
3d	18.29	5.70	25.27	1.38	4.43
3e	15.85	5.55	14.91	0.94	2.69
3f	-	2.97	6.05	-	2.04
Curcumin	51.06	8.29	10.60	0.21	1.28
5-Fluorouracil	64.31	>100	17.53	0.27	0.04

[1]Mean, *n* = 3: mean of experiment. [2]SI = Selectivity index = ratio of IC_{50} value in normal cell (Chang) and cancer cell.

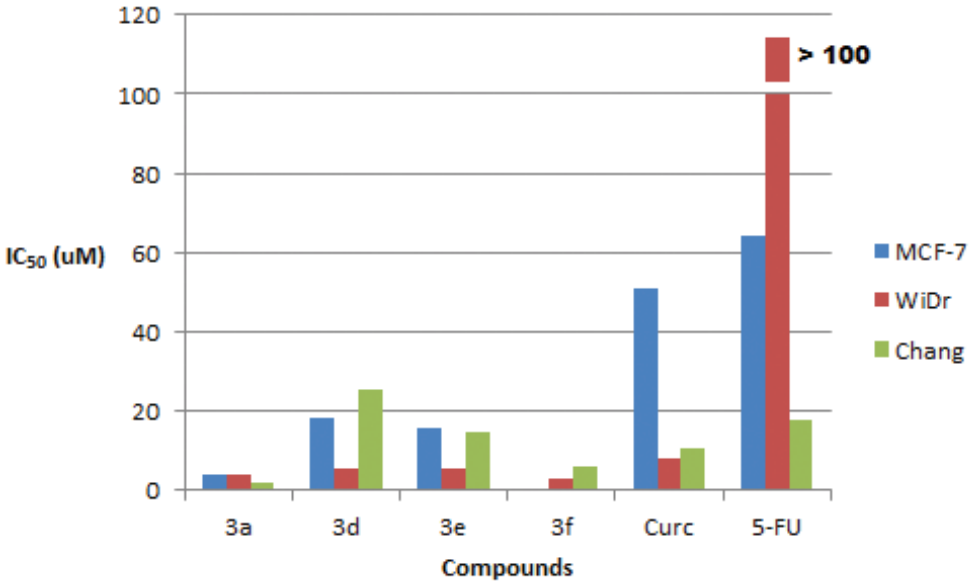


Figure 2. Cytotoxicity of compounds **3a**, **3b**, **3e**, and **3f**, curcumin (Curc) as a comparative compound, and 5-fluorouracil (5-FU) as a positive control, against MCF-7, WiDr, and Chang Liver cells. **3f** was not tested against MCF-7 cells

to the compound (Chibaudel *et al.*, 2008). Compounds **3a**, **3d**, and **3e** exhibited moderate-to-high cytotoxicity against MCF-7 cells lines, (IC_{50} values = 4.18, 18.29, and 15.85 µM), but no one of the compounds showed high selectivity index (SI= 0.43, 1.38, and 0.94). These results were consistent to reported previously (Prasetyaningrum *et al.*, 2018). Compounds **3a**, **3d**, **3e**, and **3f** exhibited high cytotoxicity against WiDr cells lines (IC_{50} values = 3.98, 5.70, 5.55, and 2.97 µM), but compound **3a** was not selective (SI = 0.45), while compounds **3d**, **3e**, and **3f** showed moderate-to-high selectivity index (SI = 4.43, 2.69, and 2.04).

The standard used previously for pure compounds considered to be further tested as anticancer agents in preclinical tests using experimental animals should possess IC_{50} values equal or less than 10 µM (4 ppm) in cell cultures with SI value more than 2 (Burger and Fiebig, 2004). Therefore, compounds **3d**, **3e**, and **3f** were potential as an anticancer agent for colorectal carcinoma and fulfilled the requirements for further evaluated *in vivo* pre-clinical studies. The compounds should also be further study to explore their mechanism action for justifying their cytotoxic activity.

CONCLUSION

A series of new aminomethyl derivatives of methyl-substituted asymetrical curcumin mono-carbonyl was successfully synthesized. The synthesized compounds exhibited low to high cytotoxicity against MCF-7, WiDr, HeLa, A549, and PLC/PRF/5 cells. Further evaluations showed that compound **3d**, **3e**, and **3f** exhibited a potent and selective cytotoxic agent (IC_{50} < 10 µM, SI > 2) against colorectal carcinoma (WiDr) cells. The compounds should be considered for further evaluation for investigating their mechanism of action and their effectivity *in vivo* pre-clinical studies.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Research, Technology, and Higher Education, Republic of Indonesia for the financial support (PDUPT Research Grant, 2018), and to Chemistry Study Program, Institut Teknologi Bandung (ITB), Indonesia for recording NMR and mass spectra.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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How to cite this article:
Kurnia A, Saputri FC, Hayun H. Synthesis and anticancer potential of aminomethyl derivatives of methyl-substituted asymmetrical curcumin mono-carbonyl. J Appl Pharm Sci, 2019; 9(08):018–024.