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Synthesis, Antioxidant, and Anti-inflammatory Activity of Morpholine Mannich base of AMACs ((2*E*, 6*E*)-2-({4-hydroxy-3-[morpholin-4-yl-)methyl]phenyl}methylidene)-6-(phenylmethylidene) cyclohexan-1-one) and Its Analogs

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ABSTRACT

A series of asymmetrical mono-carbonyl analogs of curcumin (AMACs) containing morpholine Mannich base ((2*E*, 6*E*)-2-({4-hydroxy-3-[morpholin-4-yl-)methyl]phenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one) was synthesized. The title compounds and the parent compounds were evaluated for antioxidant and anti-inflammatory activity using 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenger method and protein denaturation method, respectively. Among the tested compounds, only compound **3d** showed potent antioxidant activity which was comparable to cyclovalone. All the AMACs exhibited lower anti-inflammatory activity than that of cyclovalone. However, compounds **4c** and **4d** exhibited a potent anti-inflammatory activity which was almost comparable to cyclovalone and the standard diclofenac sodium.

INTRODUCTION

Curcumin, ((1*E*, 6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione), is the active principle of *Curcuma sp*. The compound is well-documented to exhibit good biological activities with low toxicity as well as antioxidant, anti-inflammatory, and others. However, due to its low stability and bioavailability, its clinical application is limited (Anand *et al*., 2008; Wang *et al*., 1997; Rosemond *et al*., 2004; Grogan, 2005). Many curcumin analogs have been synthesized and investigated, such as mono-carbonyl analogs of curcumin (MACs), to improve the bioactivities, stability, and bioavailability. The compound and its analogs containing a cyclohexanone or cyclopentanone linker between the two phenyl rings exhibited greater anti-inflammatory and antioxidant activity and a higher stability and a better pharmacokinetic profile than that of curcumin (Zhao *et al*., 2013; Lamperti *et al*., 2014). The structures of the above MACs are generally symmetric. Nowadays, some asymmetrical mono-carbonyl of curcumin (AMACs) have been synthesized and among of the AMACs exhibited potent anti-inflammatory (Zhang *et al*., 2014a; Zhang *et al*., 2014b; Aluwi *et al*., 2016), antioxidant and antitumor (Li *et al*., 2015). The reports suggest that the AMACs are good lead compounds to be developed for discovering anti-inflammatory and antioxidant agents.

Introduction of aminoalkyl substituent via Mannich base reactions are known to increase the biological activity of compounds. The Mannich base serves as an important pharmacophore moiety to potentiate the biological activity of the drug (Bala *et al*., 2015). Ibuprofen-containing morpholine Mannich base substituent exhibited higher anti-inflammatory activity than that of diclofenac. The larger structure of the compound making it easier to interact with cyclooxygenase-2 having a larger active site (Sujith *et al*., 2009). Meanwhile, quercetin containing morpholine Mannich base substituent showed the highest antioxidant activity compared to quercetin, and quercetin containing piperazine

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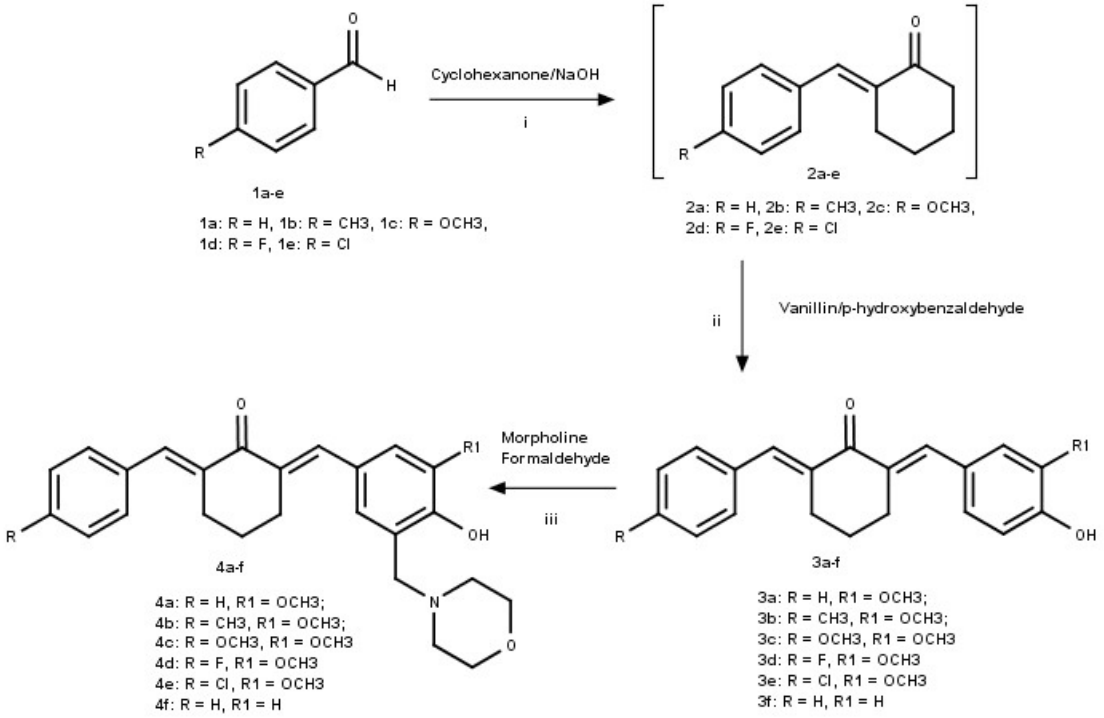
Mannich base, and quercetin containing dopamine Mannich base (Joshi *et al*., 2013). However, the study of the Mannich bases of AMACs as an antioxidant and anti-inflammatory agent has never been reported. Therefore, herein we report the synthesis, antioxidant and anti-inflammatory activity of morpholine Mannich base of AMACs.



Scheme 1: Structures and synthetic route of the title compounds. *Reaction conditions*: (**i**) cyclohexanone, NaOH, rt, stirred, 2h; (**ii**) EtOH, heated, 30 min, diluted HCl; (**iii**) EtOH, reflux, 3-7 h (TLC monitoring).

EXPERIMENTALS

Materials and instruments

All chemicals were reagent-grade and purchased commercially and used without purification. The purity of the synthesized compounds was tested by TLC method on silica gel 60 F254 plates (Merck). Melting points were determined in the capillary tube using melting point apparatus (Analogue Model SMP11, Stuart Scientific) and are uncorrected. The IR spectra were recorded on an FT-IR spectrophotometer (8400S, Shimadzu). The NMR spectra were recorded on NMR spectrometer (Agilent) at 500 MHz for ^{1}H and 125 MHz for ^{13}C using TMS as an internal standard. High-resolution mass spectra (HR-MS) were recorded on a Waters LCT Premier XE (ESI-TOF) system in negative mode.

Chemistry

The asymmetrical mono-carbonyl analogs of curcumin (AMACs) (**3a-f**) were prepared according to procedures reported by our research group previously (Wiji Prasetyaningrum *et al.,* 2017) (Scheme 1).

General procedure for the synthesis of morpholine Mannich base of AMACs ((2E, 6E)-2-({4-hydroxy-3-[morpholin-4-yl-)methyl]phenyl}methylidene)-6-(phenylmethylidene) cyclohexan-1-one) and its analogs (***4a-f***).

A solution of the AMACs (**3a-f**) (2 mmol) in ethanol (5 ml) was cooled in the ice bath, added morpholine (5-7 mmol) and formaldehyde solution 37% (5-7 mmol) and stirred for 30 min at room temperature. Subsequently, the mixture was refluxed for 3–7 h until the starting material disappeared, which was monitored by TLC. The solvent of the mixture was evaporated and methanol (40 ml) was added and subsequently evaporated. A colored precipitate obtained was filtered off, washed with cold methanol and dried at room temperature and purified by column chromatography to afford compound **4a-f**.

(2E,6E)-2-({4-hydroxy-3-methoxy-5-[(morpholin-4-yl)methyl] phenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one ***(4a)***

Bright yellow powder, 71.8% yield, mp 125-126°C. FT-IR (KBr) cm^{-1}: 3080 (H-C_{Ar}), 2913 (H-$C_{aliphatic}$), 1734 (C=O), 1601 and 1489 (C=C aromatic), 1663 (C=C), 1261 (C-N), 1140 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.81 (m, 2H, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 2.61 (s, 4H, CH_2-N-$CH_{2\ morpholine}$), 2.92 (t, 4H, =C-CH_2-$CH_{2\ cyclohexanone}$), 3.91 (s, 3H, CH3-O-Ar), 3.76 (s, 2H, Ar-CH_2-N, and 4H, CH_2-O-$CH_{2\ morpholine}$), 6.82 (1H, s, H-Ar), 6.98 (s, 1H, H-Ar), 7.39 (t, 2H, J = 7.4 Hz, H-Ar), 7.46 (d, 2H, J = 8.7 Hz, H-Ar), 7.33 (t, 1H, J = 8,3 Hz, H-Ar), 7.71 and 7.78 (s, 1H, and s, 1H, Ar-CH=C-$_{ethylenic}$). ^{13}C-NMR (100 MHz, CDCl3) δ/ppm: 23.16 (1C, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 28.48 and 28.81 (2C,

=C-CH_2-$CH_{2\ cyclohexanone}$), 53.00 (2C, CH_2-N-$CH_{2\ morpholine}$), 56.12 (1C, Ar-CH_2-N), 61.75 (1C, CH_3-O), 66.9 (2C, CH_2-O-$CH_{2\ morpholine}$), 124.16, 133.87, 113.8, 128.48, 130.43, 127.37, 128.6 (10C, C_{Ar}), 136.59, 136.40, 136.20, and 137.57 (4C, C=$C_{ethylenic}$), 147.87 (1C, C_{Ar}-O), 148.22 (1C, C_{Ar}-O), 190.20 (1C, C=O). HR ESI-MS m/z: 418.2061(M-H)-, calculated for $C_{26}H_{28}NO_4$: 418.2018.

*(2E,6E)-2-({4-hydroxy-3-methoxy-5-[(morpholin-4-yl)methyl] phenyl}methylidene)-6-[(4-methylphenyl)methylidene] cyclohexan-1-one (**4b**)*

Yellow powder, 50.2% yield, mp 130-131°C. IR (KBr) cm^{-1}: 3102 (H-C=aromatic), 2943 (C-H), 1734 (C=O), 1655 (C=C), 1601 and 1495 cm^{-1} (C=C aromatic), 1161. ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.8 (m, 2H, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 2.38 (s, 3H, CH_3-C3Ar), 2.61 (s, 4H, CH_2-N-$CH_{2\ morpholine}$), 2.90 (m, 4H, =C-CH_2-$CH_{2\ cyclohexanone}$), 3.90 (s, 3H, CH_3-O), 3.77 (s, 2H, Ar-CH_2-N, and 4H, CH_2-O-$CH_{2\ morpholine}$), 6.82 (s, 1H, H-Ar), 6.98 (s, 1H, H-Ar); 7.2 (d, 2H, *J* = 7.90 Hz, H-Ar), 7.37 (d, 2H, *J* = 7.9 Hz, H-Ar), 7.7 and 7.76 (s, 1H, and s, 1H, Ar-CH=C-$_{ethylenic}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 21.53 (1C, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 23.17 and 28.58 (2C, =C-CH_2-$CH_{2\ cyclohexanone}$), 28.79 (1C, CH_3-Ar), 53. 00 (2C, CH_2-N-$CH_{2\ morpholine}$), 56.12 (1C, Ar-CH_2-N), 61.76 (1C, CH_3-O), 66.88 (2C, CH_2-O-$CH_{2\ morpholine}$), 113.78, 120.74, 124.11, 127.42, 129.25, 130.56, 133.99, 133.37 (10C, C_{Ar}), 135.61, 136.77, 137.32, and 138.89 (4C, C=$C_{ethylenic}$), 148.14 and 147.86 (2C, C_{Ar}-O), 190.23 (1C, C=O). HR ESI-MS m/z: 432.2214(M-H)-, calculated for $C_{27}H_{30}NO_4$: 432.2175.

*(2E,6E)-2-({4-hydroxy-3-methoxy-5-[(morpholin-4-yl)methyl] phenyl}methylidene)-6-[(4-methoxyphenyl)methylidene]cyclo-hexan-1-one (**4c**)*

Bright yellow powder, 42.2% yield, m.p 144-146°C. FTIR (KBr) cm^{-1}: 3044 (C-H aromatic), 2930 (C-H aliphatic), 1734 (C=O), 1647 (C=C), 1597 and 1489 cm^{-1} (C=C aromatic), 1258 (C-N), 1161 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.81 (m, 2H, CH_2-CH_2-$CH_{2\ cyclohexanone}$); 2.61 (s, 4H, CH_2-N-$CH_{2\ morpholine}$), 2.90 and 2.93 (t, 2H and t, 2H, =C-CH_2-$CH_{2\ cyclohexanone}$); 3.9 (s, 3H, CH_3-O), 3.84 (s, 3H, CH_3-O), 3.77 (s, 2H, Ar-CH_2-N), 3.76 and (s, 4H, CH_2-O-$CH_{2\ morpholine}$), 6.81 (s, 1H, H-Ar), 6.93 (d, 2H, *J* = 8.8, H-Ar), 6.98 (s, 1H, H-Ar); 7.45 (d, 2H, *J* = 8,75 Hz, H-Ar), 7.7 and 7.75 (s, 1H, and s, 1H, Ar-CH=C-$_{ethylenic}$). ^{13}C NMR (100 MHz, $CDCl_3$), δ/ppm: 23.19 (1C, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 28.76 and 28.63 (2C, =C-CH_2-$CH_{2\ cyclohexanone}$), 66.89 (2C, CH_2-O-$CH_{2\ morpholin}$), 53.01 (2C, CH_2-N-$CH_{2\ morpholine}$), 55.46 (1C, Ar-CH_2-N), 56.12 (1C, O-CH_3), 61.7 (1C, O-CH_3)113.77, 124.08, 128.87, 127.47, 132.36 (9C, C_{Ar}), 134.07, 134.4, 136.66, 137.1 (4C, C=$C_{ethylenic}$), 147.86, 148.09 and 160.06 (3C, C_{Ar}-O), 190.16 (1C, C=O). HR ESI-MS m/z: 448.2126(M-H)-, calculated for $C_{27}H_{30}NO_5$: 448.2124.

*(2E,6E)-2-[(4-fluorophenyl)methylidene]-6-({4-hydroxy-3-methoxy-5-[(morpholin-4-yl)methyl] phenyl}methylidene) cyclohexan-1-one (**4d**)*

Bright yellow powder, 44.1% yield, mp 142-144°C. FTIR (KBr) cm^{-1}: 3065 (H-C aromatic), 2943 (C-H aliphatic), 1734 (C=O), 1659 (C=C), 1607 and 1489 cm^{-1} (C=C aromatic), 1221 (C-F), 1258 (C-N), 1157 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.81 (m, 2H, CH_2-CH_2-$CH_{2\ cyclohexanone}$) 2.58 (t, 4H, CH_2-N-$CH_{2\ morpholine}$), 2.92 and 2.97 (t, 2H, and t, 2H, =C-CH_2-$CH_{2\ cyclohexanone}$), 3.72 (s, 4H, CH_2-O-$CH_{2\ morpholine}$), 3.74 (s, 2H, Ar-CH_2-N), 3.88 (s, 3H, CH_3-O), 7.06 (s, 1H, H-Ar); 7.16 (t, 2H, *J* = 8.65 Hz, H-Ar), 7.52 (d-d, 2H, *J* = 5.45, 5.5 Hz, H-Ar), 7.67 and 7.68 (s, 1H, and s, 1H, Ar-CH=C-$_{ethylenic}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 24.12 (1C, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 29.28 and 29.61 (2C, =C-CH_2-$CH_{2\ cyclohexanone}$), 54.10 (2C, CH_2-N-$CH_{2\ morpholine}$), 56.6 (1C, Ar-CH_2-N), 60.39 (1C, CH_3-O), 67.77 (2C, CH_2-O-$CH_{2morpholin}$), 114.8, 116.35, 116.53, 123.07, 126.49, 128.33, 133.53, 133.59 (10C, C_{Ar}), 135.07, 136.38, 137.67, 139.11 (4C, C=$C_{ethylenic}$), 148.91 and 149.03 (2C, C_{Ar}-O), 191.82 (1C, C=O). HR ESI-MS m/z: 436.1927(M-H)-, calculated for $C_{26}H_{27}FNO_4$: 436.1924.

*(2E,6E)-2-[(4-chlorophenyl)methylidene]-6-({4-hydroxy-3-methoxy-5-[(morpholin-4-yl)methyl] phenyl}methylidene) cyclohexan-1-one (**4e**)*

Light yellow powder, 79.3% yield, mp 107-109°C. FTIR (KBr) cm^{-1}: 3080 (H-C aromatic), 2965 (C-H aliphatic), 1734 (C=O), 1659 (C=C), 1601 and 1497 (C=C aromatic), 1261 (C-N), 1159 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm : 1.81 (m, 2H, CH_2-CH_2-$CH_{2\ cyclohexanone}$); 2.61 (t, 4H, CH_2-N-$CH_{2\ morpholine}$), 2.87 and 2.93 (t, 2H, and t, 2H, =C-CH_2-$CH_{2\ cyclohexanone}$); 3.90 (s, 3H, CH_3-O), 3.75 (s, 2H, Ar-CH_2-N and 4H, CH_2-O-$CH_{2\ morpholine}$), 6.81 (s, 1H, H-Ar), 6.98 (s, 1H, H-Ar); 7.35 (d, 2H, *J* = 6.25 Hz, H-Ar), 7.38 (d, 2H, *J* = 10.95 Hz, H-Ar), 7.70 and 7.71 (s, 1H, and s, 1H, Ar-CH=C-$_{ethylenic}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 24.08 (1C, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 28.47 and 28.74 (2C, =C-CH_2-$CH_{2\ cyclohexanone}$), 53.00 (2C, CH_2-N-$CH_{2\ morpholine}$), 56,13 (1C, Ar-CH_2-N), 61.75 (1C, CH_3-O), 66.88 (2C, CH_2-O-$CH_{2morpholin}$), 113.83, 124.21, 128.76, 131.64, 133.64, 134.63 (10C, C_{Ar}), 134.52, 135.17, 136.85 and 137.86 (4C, C=$C_{ethylenic}$), 147.89 and 148.33 (2C, C_{Ar}-O), 189.92 (1C, C=O). HR ESI-MS m/z: 452.1618(M-H)-, calculated for $C_{26}H_{27}ClNO_4$: 452.1629.

*(2E,6E)-2-({4-hydroxy-3-[(morpholin-4-yl)methyl]phenyl} methylidene)-6-(phenylmethylidene) cyclohexan-1-one (**4f**)*

Light yellow powder, 21.3% yield, mp 115-117°C. FTIR (KBr) cm^{-1}: 3055 (C-H aromatic), 2872 (C-H aliphatic), 1732 (C=O), 1665 (C=C), 1610 and 1497 (C=C aromatic), 1265 (C-N), 1165 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.79 (m, 2H, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 2.58 (s, 4H, t, 4H, CH_2-N-$CH_{2\ morpholine}$), 2.94 (m, 4H, =C-CH_2-$CH_{2\ cyclohexanone}$), 3.73 (t, 4H, CH_2-O-$CH_{2\ morpholine}$), 3.75 (s, 2H, Ar-CH_2-N), 7.35 (s, 1H, H_{Ar}); 7.38 (t, 1H, *J* = 8.4 Hz, H_{Ar}), 7.36 (t, 1H, *J* = 8.8 Hz, H_{Ar}), 7,40 (d, 2H, *J* = 7.95 Hz, H_{Ar}), 7.47 (d, 2H, *J* = 9 Hz, H_{Ar}), 7.67 and 7.71 (s, 1H, and s, 1H, Ar-CH=C-$_{ethylenic}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 24.16 (1C, CH_2-CH_2-$CH_{2\ cyclohexanone}$), 29.64 and 29.36 (2C, =C-CH_2-$CH_{2\ cyclohexanone}$), 54.06 (2C, CH_2-N-CH_2), 61.26 (1C, 1C, Ar-CH_2-N), 67.79 (2C, CH_2-O-$CH_{2morpholin}$), 116.97, 131,38, 128.55, 129.8, 129.80, 129.53, 132.96 (10C, C_{Ar}), 133.72, 134.93, 137.62, and 137.87 (4C, C=$C_{ethylenic}$), 159.81 (1C, C_{Ar}-O), 192.08 (1c, C=O). HR ESI-MS m/z: 388.1925(M-H)-, calculated for $C_{25}H_{26}NO_3$: 388.1913.

Antioxidant Activity

The antioxidant activity of the title compounds (**4a-f**) and the parent compound AMACs (**3a-f**) were determined by measuring free radical scavenging capacity of the compound using the stable radical 2,2-diphenyl-2-picrylhydrazyl (DPPH),

according to the method reported previously (Rahmawati *et al*., 2010; Aksoy *et al*., 2013), with slight modification. The synthesized compounds at various concentrations were prepared in methanol and 0.5 ml of each concentration was added to 0.5 ml of 50 µg/ml methanolic solution of DPPH and incubated at room temperature in the darkroom for 30 min. The absorbance of the sample was measured at 517 nm. The synthesized compounds at various concentrations were served as blank because they showed a slight absorbance at 517 nm. Quercetin was used as a standard antioxidant. The experiment was done in triplicates. The percentage of inhibition was calculated as shown in the equation below:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance before reaction} - \text{Absorbance after reaction})}{\text{Absorbance before reaction}} \times 100$$

The free radical scavenging capacity of the compound was calculated by plotting percentage inhibition to control against concentration and expressed as an IC_{50} value.

Anti-inflammatory Activity

The title compounds (**4a-f**) and the parent compound AMACs (**3a-f**) were screened for anti-inflammatory activity using inhibition of albumin denaturation technique reported previously (Saso *et al*., 2001), with slight modification. Bovine Saline Albumin (BSA) solution 0.5% (w/v) was prepared in Tris-buffer saline and adjusted pH to 6.3 using glacial acetic acid. The reaction mixtures (5 ml) consisted of 0.5 ml of varying concentrations of standard diclofenac sodium or test compounds in methanol and 4.5 ml of the BSA solution were heated for 10 minutes at 70°C ± 2 in a test tube placed in water bath, then cooled and its turbidity measured at 660 nm and absorbance read using UV-Vis Spectrophotometer in 1 ml cuvettes in three replicates. The control was prepared as above and a similar volume of methanol was used instead of the test compounds. The percentage inhibition was calculated by using following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance the test compounds})}{\text{Absorbance control}} \times 100$$

The capacity of the compound to inhibit the denaturation was calculated by plotting percentage inhibition to control against concentration and expressed as an IC_{50} value.

RESULT AND DISCUSSION

Chemistry

The title compounds ((2*E*, 6*E*)-2-({4-hydroxy-3-[morpholin-4-yl-)methyl]phenyl} methylidene)-6-(phenylmethylidene)cyclohexan-1-one and its analogs (**4a-f**) were synthesized stepwise by the method summarized in **Scheme 1**. The benzaldehyde (**1a-e**) was condensed with cyclohexanone (**2**) in the presence of aqueous alkali to give (2*E*)-2-(phenylmethylidene) cyclohexan-1-one (**2a-e**) (Furniss *et al*., 1989). Condensation of **2a-e** with vanillin or p-hydroxybenzaldehyde in addition of diluted HCl/ethanol (1:1) under reflux conditions for 30 min gave asymmetrical mono-carbonyl of curcumin (AMACs) (**3a-f**) (Wiji Prasetyaningrum *et al*., 2017). Finally, the Mannich reaction of **3a-f** with morpholine and formaldehyde at reflux condition in ethanol afforded the title compounds **4a-f** (Geschickter and Meadow, 1969).

The synthesized compounds (**4a-f**) were characterized by IR, ^{1}H-NMR, ^{13}C-NMR dan mass spectra. The IR spectra of compounds **4a-f** showed the bands of C-H aromatic at 3,044-3,080 cm^{-1} and C-H aliphatic at 2913-2980. The α,β-unsaturated carbonyl groups, the C=C aromatic or ethylenic and C-O-C and C-N of the compounds are observed as strong bands at 1,732-1,734 cm^{-1}, 1,447-1,665 and 1,140–1,265 cm^{-1}, respectively. In the ^{1}H-NMR spectra, the two protons of the ethenyl chain of the compounds are observed as two singlets at a range of 7.67-7.78 ppm (^{1}H, respectively) indicating the asymmetrical of the structure. The protons of methylene connecting N of morpholine to phenyl ring (Ar-CH_2-N-) are observed as a singlet at 3.74-3.77 ppm, while the four protons of CH_2 connected to N (CH2-CH_2-N-) and the four protons of CH_2 connected to O (CH2-CH_2-O-) in the morpholine skeleton are observed as triplet at 2.58-2.61 ppm and 3.75-3.77 ppm, respectively (Silverstein *et al*., 2005). The structures were further supported by ^{13}C-NMR and HR-ESI-MS spectra of the compounds which showed the complete agreement with the desired molecular structures.

Antioxidant Activity

The antioxidant activity of the title compounds (**4a-f**) and the parent compound AMACs (**3a-f**) were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenger method. The choice of the method because it is fast, easy, sensitive, reliable and does not require a special reaction and device (Soare *et al*., 1977; Blois, 1958). The antioxidant compound reacts with DPPH radical through the hydrogen atom donation mechanism and producing the alteration of DPPH's color from violet to yellow measured by visible spectrophotometer (Aksoy *et al*., 2013; Blois, 1958). The result of the antioxidant activity of the synthesized compounds (**3a-f** and **4a-f**) are shown in **Table 1** and **Figure 1.** The data exhibited that the AMACs containing morpholine Mannich base group showed lower antioxidant activity than that of the parent compounds, AMACs, except for compound **4e** and **4f**. The result is in line with the effect of substitution of morpholine Mannich base to the antioxidant activity of cyclovalone reported previously (Hayun *et al*., 2017). All the synthesized compounds showed lower antioxidant activity than that of the symmetrical MAC, cyclovalone, and quercetin. Compound **4f**, the AMAC without methoxy group at the ortho position of the hydroxyl group, showed the lowest antioxidant activity. These results show the important role of the hydroxyl and the methoxy groups for antioxidant activity as earlier reported (Li *et al*., 2015). However, compound **3d**, the AMACs having a p-fluor group, showed antioxidant activity, which is comparable to cyclovalone (IC_{50} 68.9 µM).

Anti-inflammatory Activity

The anti-inflammatory activity of the title compounds (**4a-f**) and the parent compound AMACs (**3a-f**) were evaluated using inhibition of heat-induced albumin denaturation method.

Protein denaturation in vivo (occurred in certain rheumatic diseases) stimulates the production of autoantigens stimulating inflammation (Umapathy *et al*., 2010; Grant *et al*., 1970). Several anti-inflammatory drugs have shown the ability to inhibit heat-induced albumin denaturation (Grant *et al*., 1970). A fluorinated phenyl styryl ketone inhibited of heat-induced bovine serum albumin (BSA) denaturation and possess anti-inflammatory (Nargund *et al*., 1992). Although there was no complete correlation, several compounds of acetamido[(phenyl-4'-yl)-oxymethyl)]-2-(p-substituted phenylamino)-1,2,4-triazoles and -1,3,4-thiadiazoles which showed good inhibition of denaturation also showed significant anti-inflammatory activity by carrageenan-induced edema in the rat paw (Nargund *et al*., 1993). The results of the anti-inflammatory activity evaluation of the synthesized compound were presented in **Table 2** and **Figure 2.** The data revealed that all the synthesized compound exhibited inhibition of BSA denaturation with the range of IC_{50} = 25.3-80.1 µM. All the synthesized compounds showed lower activity than that of symmetrical MAC, cyclovalone. However, the compounds **4c** and **4d**, the AMACs having morpholine Mannich base substituent on the phenolic moiety and methoxy or fluoro substituent at para position on another phenyl ring of the compounds, exhibited a potent anti-inflammatory activity (IC_{50} = 25.3 µM and 26.3 µM, respectively), which almost comparable to cyclovalone (IC_{50} = 22.4 µM) and the standard diclofenac sodium (IC_{50} = 20.3 µM). All the AMACs containing morpholine Mannich base substituent showed inhibition of BSA denaturation activity higher than that of the parent compounds, AMACs; indicating that substitution of a morpholine Mannich base increased the activity of the parent compounds. In addition, the presence of methoxy at the ortho position of the hydroxyl group of the compound, revealed significant contribution towards anti-inflammatory activity as shown in compounds **3f** and **4f** compared to **3a** and **4a**, respectively.

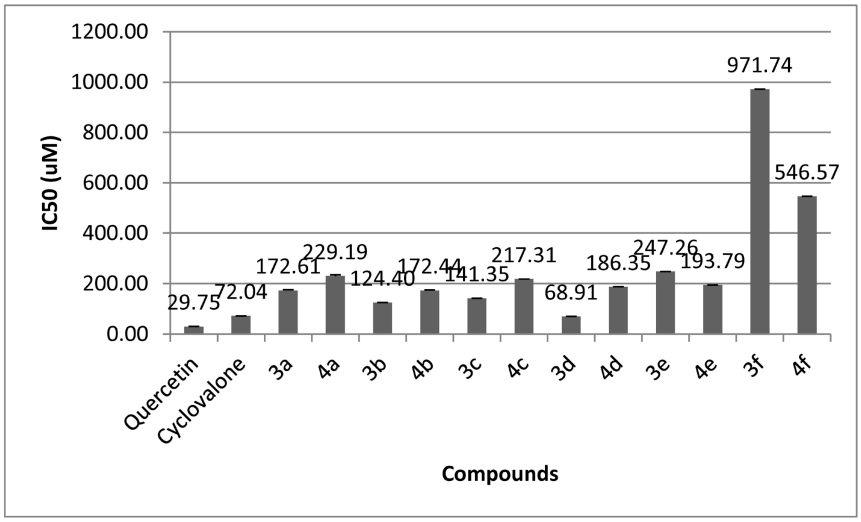


Fig. 1: The free radical scavenging activity (IC_{50}) of AMACs containing morpholine Mannich base (**4a-f**) and the parent compounds (AMACs, **3a-f**).

Table 1: The free radical scavenging activity (IC_{50}) of the title compounds (**4a-f**) and the parent compounds (AMACs, **4a-f**).

Comp	Substituents		IC_{50} ± SD (µM)*)	Comp	Substituents		IC_{50} ± SD (µM) *)
	R	R1			R	R1	
3a	H	OCH_3	172.61 ± 0.01	**4a**	H	OCH_3	229.20 ± 0.33
3b	CH_3	OCH_3	124.40 ± 0.40	**4b**	CH_3	OCH_3	171.57 ± 1.09
3c	OCH_3	OCH_3	141.37 ± 1.34	**4c**	OCH_3	OCH_3	217.31 ± 1.67
3d	F	OCH_3	68.91 ± 0.30	**4d**	F	OCH_3	186.35 ± 0.41
3e	Cl	OCH_3	247.26 ± 2.09	**4e**	Cl	OCH_3	193.63 ± 0.28
3f	H	H	971.73 ± 5.21	**4f**	H	H	546.56 ± 3.05
Cyclovalone			72.03 ± 0.06				
Quercetin			29.75 ± 0.05				

*)values are the mean ± SD (n = 3).

CONCLUSION

In summary, we successfully synthesized a series of asymmetrical mono-carbonyl analogs of curcumin (AMACs) containing morpholine Mannich base ((2*E*, 6*E*)-2-({4-hydroxy-3-[morpholin-4-yl-)methyl]phenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one). All the compounds and the parent compounds, AMACs, exhibited antioxidant and anti-inflammatory activity. The presence of morpholine Mannich

base substituent in the AMACs enhanced the inflammatory activity of the compounds but generally lowered their antioxidant activity. The results suggest that newly synthesized compounds are potential agents to be used in the treatment of inflammatory diseases. However, further studies are required to investigate the mechanism of the biological activity of the compound as an anti-inflammatory agent.

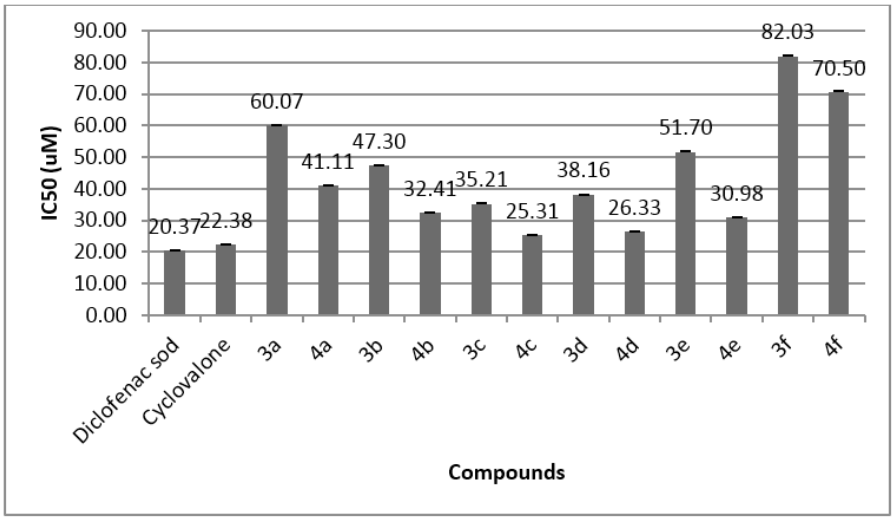


Fig. 2: The inhibition heat-induced BSA denaturation activity (IC_{50}) of AMACs containing morpholine Mannich base (**4a-f**) and the parent compounds (AMACs, **3a-f**).

Table 2: The inhibition of heat-induced BSA denaturation activity (IC_{50}) of the title compounds (**4a-f**) and the parent compounds (AMACs, **3a-f**).

Comp	Substituents		$IC_{50} \pm SD$ (µM) *)	Comp	Substituents		$IC_{50} \pm SD$ (µM)*)
	R	R1			R	R1	
3a	H	OCH_3	60.07 ± 0.22	**4a**	H	OCH_3	41.11 ± 0.08
3b	CH_3	OCH_3	47.30 ± 0.14	**4b**	CH_3	OCH_3	32.41 ± 0.07
3c	OCH_3	OCH_3	35.21 ± 0.08	**4c**	OCH_3	OCH_3	25.31 ± 0.08
3d	F	OCH_3	38.16 ± 0.11	**4d**	F	OCH_3	26.33 ± 0.13
3e	Cl	OCH_3	51.70 ± 0.26	**4e**	Cl	OCH_3	30.98 ± 0.06
3f	H	H	82.03 ± 0.05	**4f**	H	H	70.50 ± 0.51
Cyclovalone			22.38 ± 0.04				
Diclofenac Sodium			20.37 ± 0.03				

*)values are the mean ± SD (n = 3).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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